MICROBIOLOGY AND MOLECULAR BIOLOGY REVIEWS, Mar. 2004, p. 1–108 Vol. 68, No. 1
1092-2172/04/$08.00+0 DOI: 10.1128/MMBR.68.1.1–108.2004
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Lessons from the Genome Sequence of *Neurospora crassa*: Tracing the Path from Genomic Blueprint to Multicellular Organism

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INTRODUCTION

Neurospora crassa: a Model Filamentous Fungus

The kingdom Fungi contains an estimated 1.5 million species, the majority of which are filamentous (321). The impact of this group of organisms on human affairs and the ecosystem rivals that of plants and bacteria. Fungi are probably the most biotechnologically useful group of organisms and are used to synthesize a wide range of economically important compounds, enzymes, and secondary metabolites, including antibiotics and other pharmaceuticals (841). Fungi are the primary agents of decay on this planet (225) and play critical ecological roles in nutrient recycling. This biodegradation activity can also be deleterious, leading to decomposition of economically useful products, including building timber, man-made materials, and food (841).

Fungi are often found in association with other organisms. Numerous diseases of humans and other animals are caused by fungi, and the incidence of life-threatening fungal infections is on the rise, in parallel with the increased number of immunocompromised patients (453). Fungi are the most important group of plant pathogens, causing significant and often devastating losses in crop yield worldwide (8). Mycorrhizal fungi form symbiotic associations with the roots of higher plants (744) and effectively determine what type of plant ecosystem develops (see, e.g., reference 389).

The fungi have played a major role in the progress of biochemistry, genetics, and molecular biology. George W. Beadle and Edward L. Tatum (55) defined the role of genes in metabolism, and this led quickly to the mid-century revolution in genetics. Their work took advantage of the filamentous ascomycete *Neurospora crassa* (hereafter referred to as *Neurospora*), which was first described in 1843 as the causative agent of an orange mold infestation in French bakeries; (Fig. 1) (607, 610). *Neurospora* was later domesticated as an experimental organism by Bernard O. Dodge (725) and Carl C. Lindegren (see, e.g., reference 476). Beadle and Tatum sought an organism displaying Mendelian genetics that could be grown on simple media and might display additional nutrient requirements arising by mutation. Their success emboldened others to use bacteria, algae, and other fungi in similar studies. Together with the elucidation of the structure of DNA in 1953, molecular biology as we know it was born.

Neurospora soom became a popular experimental model organism (185). Diverse research programs centered on *Neurospora* have ranged from formal, population, and molecular genetics, biochemistry, physiology, and molecular cell biology to more recent studies of development, photobiology, circadian rhythms, gene silencing, ecology, and evolution. Substantial genetic and molecular information has been obtained about species differences and intraspecific variation, building on the efforts of David Perkins, who has sampled natural iso-

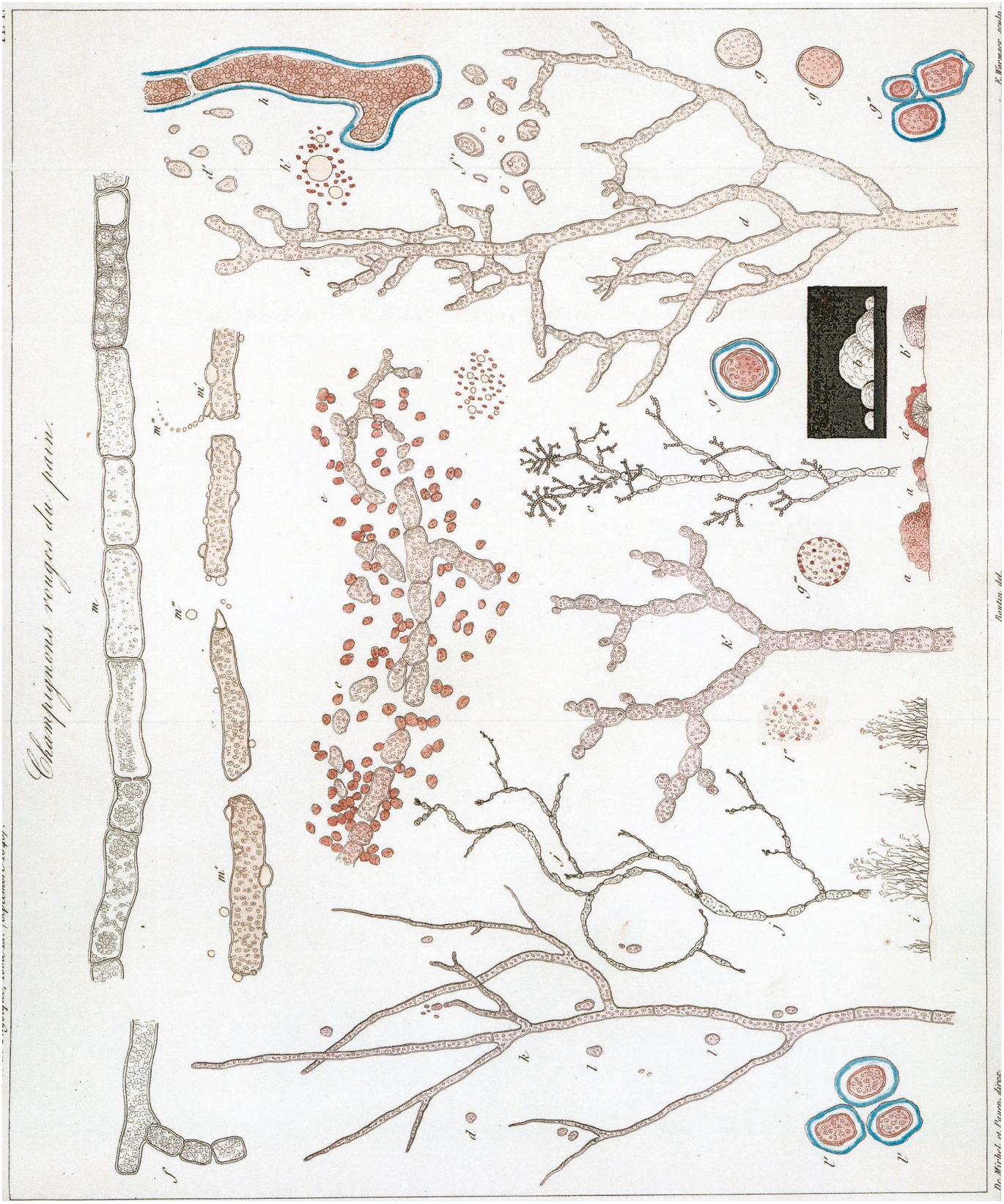
Champignons rouges du pain.

FIG. 1. Plate from the first published scientific study of *Neurospora*. Plate 1 from Payen (607). The following translated legend for the portions of the figure labeled a, a′, b′, c, g″, i, and k′ is taken from reference 610. "a. Colonies of the red-orange fungus *Oidium aurantiacum* as they appear to the naked eye in the cavities of infected bread. a′. A similar colony cut in two, showing in the red area a thick layer composed of innumerable small spores formed at the end of radiating filaments. The latter are yellowish white. b. Similar colonies that have grown up completely in the dark, with the result that the red color has not developed. b′. One of the colonies in b seen after exposure to light for one hour. Color begins to appear and then pigmentation progresses rapidly. c. Branching filament, about 150× g″. Spore treated successively, under the microscope, with a dilute solution of potassium hydroxide, and aqueous alcoholic solution of iodine, then with gradually more concentrated solutions of sulfuric acids. This acid, which separates parts of the cellulose envelope that contains less nitrogenous substance, results in a blue color turning to purple, which is characteristic of the state intermediate between cellulose and dextrin. i. Normal vegetative growth as seen with the naked eye; well-developed, especially under conditions of high humidity. k′. Termini of well developed filaments, showing spores and young cells."

zlates from all over the world (809). The legacy of 70 years of intense research with this organism continues to be driven by a large and interactive research community that has also served to draw together a wider group of scientists working with other filamentous fungi.

One of the attractive features of *Neurospora* as a model organism is its complex yet genetically and biochemically tractable life cycle (Fig. 2). *Neurospora* is multicellular and produces at least 28 morphologically distinct cell types (82), many of which are derived from hyphae. *Neurospora* vegetative hyphae are tip-growing cellular elements that undergo regular branching (294, 798, 800, 812) and are multinucleate. These hyphae contain incomplete cross walls (septa) (315) that allow the movement of organelles between compartments. Frequent fusion among hyphal filaments produces a complex hyphal network (the mycelium) (336) and promotes the formation of heterokaryons in which multiple genomes can contribute to the metabolism of a single mycelium. Specialized aerial hyphae are differentiated from vegetative hyphae in response to nutrient deprivation, desiccation, or various stresses, and these form chains of asexual spores (the multinucleate macroconidia) for dispersal (752). The timing of macroconidiation is controlled by a circadian rhythm, which in turn is modulated by exposure to blue light. Another type of asexual spore, the uninucleate microconidium, is differentiated from microconidiophores or directly from the vegetative hypha (82, 495, 752). Limiting nitrogen induces a type of hyphal aggregation that leads to generation of multicellular female sexual organs (protoperithecia) (564, 642). Mating is accomplished by chemotropic growth of a specialized female hypha from the protoperithecium toward the male cell (typically a conidium) in a process involving pheromones (81). Fertilization and meiosis result in development of the female structure into a beaked fruiting body (the perithecium) within which asci, each containing eight ordered sexual spores (ascospores), are formed (638).

The genome sequence of *Neurospora* was recently reported (269, 498). Here we provide a more detailed analysis, with the annotation of approximately 1,100 genes, or more than 10% of the total predicted in the genome. Several themes emerge from this study. First, the multicellular *Neurospora* possesses a large number of genes without homologues in *Saccharomyces cerevisiae*, suggesting that *Neurospora* will be a better model for higher eukaryotes in many aspects of cell biology, including multicellularity (579). Among the unshared genomic equipment is an expanded group of sugar transporters, transcription factors, and environmental sensing pathways, plus a diversified metabolic machinery. Second, *Neurospora* displays a number of gene-silencing mechanisms acting in the sexual or the vegetative phase of the life cycle. Best studied is repeat-induced point mutation (RIP), an effective defense against duplicated sequences such as those arising from the multiplication of transposons (see "Genome defense, DNA repair, and recombination" below) (709). However, RIP appears to impose constraints on gene and genome evolution, raising the question of whether *Neurospora* currently is able to utilize gene duplication as a means of gene diversification. Finally, *Neurospora*, a generalist species in its life cycle, genetic system, and growth requirements, provides a basis for comparison with the highly diversified plant pathogens and other specialized fungi of narrow habitat. For example, the number of

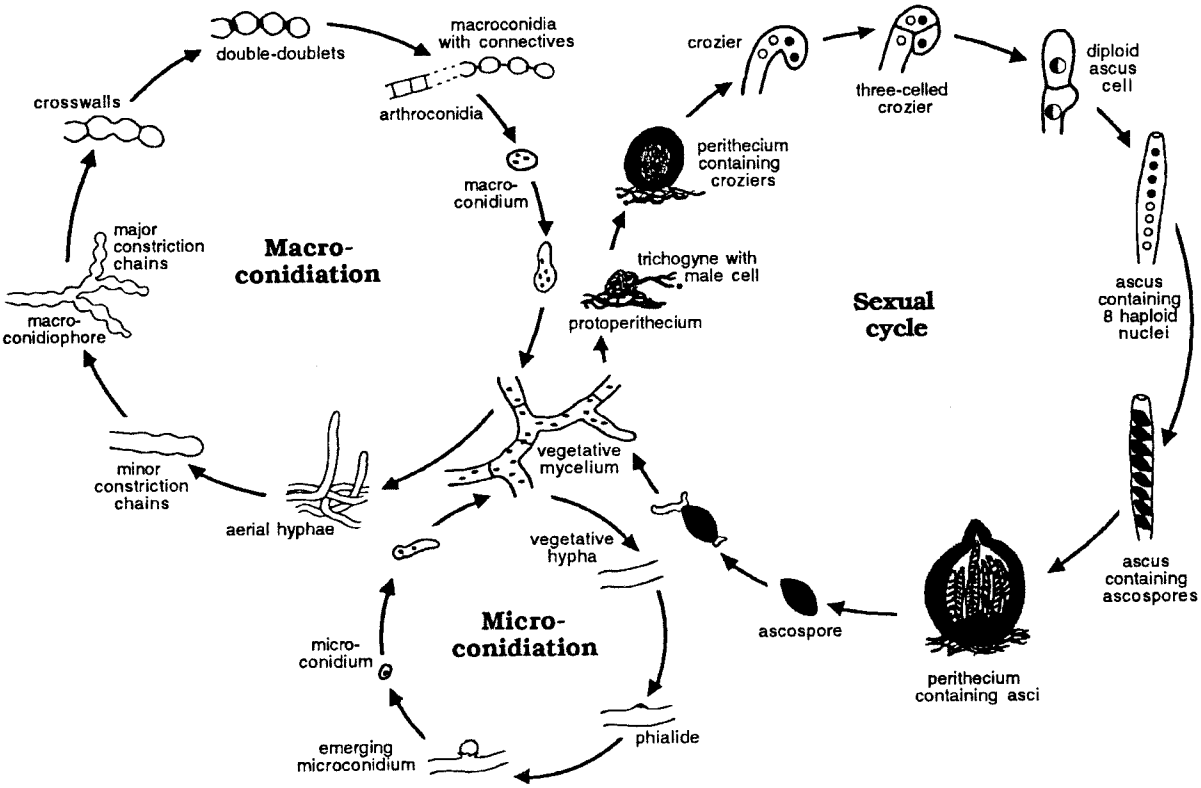

FIG. 2. Life cycle of *Neurospora*. "Depending on surrounding conditions, the vegetative mycelium can undergo the asexual sporulation processes of macroconidiation and microconidiation. It can enter the sexual cycle by forming protoperithecia; which, upon fertilization, can initiate development leading to the production of meiotically-derived ascospores." Reprinted from reference 751 with permission from the publisher.

homologues it shares with pathogens offers a starting point for study of evolutionary gene recruitment in these specialized organisms. Conversely, genes found in pathogenic fungi but absent from *Neurospora* provide potential targets to exploit for development of antifungal drugs and fungicides.

Basic Features of the *Neurospora* Genome

Sequencing and assembly. The *Neurospora* genome was sequenced using a whole-genome shotgun strategy (a summary of its general features is given in Table 1). Paired end reads were acquired from a variety of clone types, including plasmids, fosmids, cosmids, bacterial artificial chromosomes (BACs), and jumping clones. In all, greater than 20-fold sequence coverage and greater than 98-fold physical converage were generated. These data were assembled into a draft sequence by using the Arachne whole-genome assembler (50, 378). The resulting assembly consists of 958 sequence contigs (contiguous stretches of sequence derived from reads that overlap with high sequence similarity) with a total length of 38.6 Mb and an average N50 length of 114 kb (meaning that 50% of all bases are contained in contigs of at least 114 kb). Contigs were assembled into 163 scaffolds (sets of contigs that are ordered and oriented with respect to each other by using paired read information) with an N50 length of 1.6 Mb.

The long-range continuity of the assembly was confirmed by comparisons with previously described BAC physical maps for linkage groups II and V (11), in which only one discrepancy was noted. Confirmation of long-range continuity was also provided by comparisons with the *Neurospora* genetic map (see below). The assembly also has high accuracy, with 99.5% of the sequence having Arachne quality scores of ≥30. A comparison with 17 Mb of finished BAC sequence (http://mips.gsf.de/proj/Neurospora/) confirmed the sequence accuracy. Only 12 discrepancies were identified in this comparison. Five lie at the end of contigs and are most probably caused by misaligned or low-quality terminal reads. Four are short insertions or deletions, ranging from 9 to 559 bp. The remaining three discrepancies appear to be instances in which the finished sequence does not correctly represent the genome, owing to chimerism in the BACs and in which the whole-genome assembly is correct.

The total genome size can be approximated from the draft assembly by estimating the size of gaps between contigs and scaffolds. The size of gaps between adjacent contigs in a scaffold can be derived from the size of clones spanning the gap. When these gap sizes are included, the total physical length of all scaffolds is estimated to be 39.9 Mb. The size of gaps between scaffolds is more difficult to estimate since spanning clones are not available. In addition, these gaps include difficult-to-sequence regions of the genome including the ribosomal DNA (rDNA) repeats, centromeres, and telomeres. A total of ~1.7 Mb of additional sequence (251) is probably accounted for by these regions. Based on these considerations, the genome size is estimated to be 41 Mb. The most recent

TABLE 1. Features of the *Neurospora* genome

Feature	Value	No. (%) of genes with BLASTP hits[a]	No. (%) of genes with BBH hits[b]
General			
Size (bp)	38,044,345		
No. of chromosomes	7		
%G+C	50		
Protein-coding genes	10,082		
Protein-coding genes >100 aa[c]	9,200		
Introns	17,118		
tRNA genes	424		
5S rRNA	74		
Percent coding	44		
Avg gene size (bp)	1,673 (481 aa)		
Avg intron size (bp)	135.4		
Avg intergenic distance (bp)	1,953		
Predicted protein-coding sequences			
Identified by similarity to known sequences	1,336 (13%)		
Conserved hypothetical proteins	4,606 (46%)		
Predicted proteins (no similarity to known sequences)	4,140 (41%)		
Organism comparison			
Schizosaccharomyces pombe		3,883 (39)	2,225 (22)
Saccharomyces cerevisiae		3,722 (37)	2,209 (22)
Drosophila melanogaster		3,120 (31)	1,619 (16)
Caenorhabditis elegans		2,986 (30)	1,521 (15)
Arabidopsis thaliana		3,291 (33)	1,756 (17)
Homo sapiens		3,379 (34)	1,350 (13)

[a] At e < 1e-5.
[b] BBH, best bidirectional hit.
[c] aa, amino acid.

estimate of the genome size based on pulsed-field gel electrophoresis of intact *Neurospora* chromosomes is 42.9 Mb (www.fgsc.net/fgn39/oneline.html). The size predicted from the sequence (41 Mb) is well within the limits of resolution for pulsed-field gel electrophoresis measurements of such large molecules.

Integration of the sequence with the genetic map. Approximately 1,000 genetic markers exist for *Neurospora*. The majority have been ordered on the genetic maps for the seven linkage groups and are described in a recent compendium (612). The *Neurospora* assembly was correlated with the genetic maps by using a subset of 252 markers for which there is sequence in *Neurospora* (or other closely related fungi). The marker sequences were located on the current assembly by using BLASTN and filtering for unique high-quality alignments (http://www.ogi.edu/satacad/ase [269]). The 243 (96% of the total) markers that aligned were then used to place contigs and scaffolds on the physical map, according to the genetic marker order. In all, 95% of the assembly was assigned to a linkage group; 85% of this sequence was further ordered and oriented within a linkage group.

Only a handful of discrepancies were noted between the physical and genetic maps. There were three cases where gene order differs between the two maps. Five markers were located in more than one contig, indicating places where the assembly failed to merge contigs. Twelve markers failed to be located within the physical map, indicating sequence gaps within the current assembly. Finally, nine scaffolds contained markers on different linkage groups, indicating either misplaced markers on the genetic map or contigs incorrectly linked within a scaffold.

Nuclear and mitochondrial genes. An automated annotation of the *Neurospora* draft genome sequence was performed by the Whitehead Institute Center for Genome Research (WICGR) by using the Calhoun annotation and analysis system. A combination of three gene prediction algorithms (FGenesH, FGenesH+, and Genewise) was combined with available protein homology to predict protein-coding genes. Gene predictions were compared with BLAT alignments of available expressed sequence tags (ESTs) (63, 565, 900) to assess accuracy. A total of 10,082 protein-coding genes were predicted. Eliminating proteins shorter than 100 amino acids that lack protein or EST similarity reduces this number to 9,200. This number of genes is within the range of 9,200 to 13,000 estimated by previous authors (56, 409, 442, 565). An additional 26 protein-coding genes reside in the mitochondrial genome (see "Metabolic processes and transport" below).

Consistent with the greater biological complexity of filamentous fungi compared to both fission and budding yeast, *Neurospora* possesses nearly twice as many genes as *Schizosaccharomyces pombe* (~4,800) and *S. cerevisiae* (~6,300). *Neurospora* contains almost as many as genes as *Drosophila melanogaster* (~14,300), despite the relative developmental complexity of the latter. In addition, 41% of the predicted *Neurospora* proteins do not have significant similarity to known or predicted proteins in other organisms and 57% do not have good matches to proteins in either *S. cerevisiae* or *S. pombe* (269). The *Neurospora* gene complement also displays greater structure complexity than those of the two yeasts. *Neurospora* genes possess a predicted 17,118 introns (1.7 introns per gene), compared to roughly 286 (0.04 intron per gene) and 4435 (0.95 intron per gene) in *S. cerevisiae* and *S. pombe*, respectively. However, as with the yeasts and other simple eukaryotes, *Neurospora* introns do appear to be biased toward the 5′ regions of genes.

A total of 413 tRNA genes were identified using tRNAscan (487), including 234 (57%) with introns. Of this number, 396 are predicted to decode all standard amino acids and one could potentially decode UAG termination codons. Ten tRNA pseudogenes were identified, two of which were inferred to be mutated by RIP. An additional six tRNAs were predicted with undetermined specificity; one of these was inferred to be a relic of RIP.

All annotation data are available at the WICGR *Neurospora crassa* website (http://www-genome.wi.mit.edu/annotation/). In addition, a manually curated annotation of the *Neurospora* gene set is available at the Munich Information Center for Protein Sequences (MIPS) *Neurospora crassa* database (MNCDB; http://mips.gsf.de/proj/neurospora) (498). At present, MNCDB contains 8,500 *Neurospora* proteins; this number is expected to increase as manual gene prediction and annotation progress. MIPS protein codes were chosen according to the cosmid, BAC, and DNA shotgun contigs from which they were derived. Linkage was established with their respective counterparts in the WICGR database that were identified using automated gene prediction tools. The proteins in the WICGR database may differ from those in MNCDB, due to manual

correction, but the proteins in the different databases are linked as long as partial matches are found.

Codon bias. The mRNA expression level is influenced by synonymous codon usage in a number of organisms. In particular, increasing codon bias is correlated with greater expression level in *Escherichia coli* (362), *S. cerevisiae* (156, 266, 606 [although see also reference 305]), as well as *Caenorhabditis elegans*, *D. melanogaster*, and *Arabidopsis thaliana* (208). Correspondence between tRNA gene copy number and codon usage has also been demonstrated for highly expressed genes in *S. cerevisiae* (816) and *E. coli* (362). It has been proposed, based on these and other data, that codon bias reflects coadaptation between codon usage and tRNA abundance in order to maximize the efficiency of protein translation for highly expressed genes. However, in mammalian genomes, codon bias has been attributed to regional variations in genomic G+C content (i.e., isochores). In support of this, it has been shown that in mammals the G+C content of regions flanking genes (816) and the GC content of introns (207) covary with the G+C content in the third position of codons.

Although *Neurospora* genes display significant variation in codon bias, the determinants of this bias are not known for filamentous fungi. To determine whether this variation might reflect mutational selection for translational efficiency, EST sequences from a number of previously characterized libraries were used to estimate relative transcript levels (63, 565, 900). In particular, a count of the number of distinct EST clones that align with a given gene (or flanking region) was used as an estimate of the relative transcript level for that gene. Two different measures of codon bias were used: the codon bias index (CBI) (65) and the effective number of codons (Nc). CBI is a measure of the amount of bias toward a particular set of favored codons, with a large CBI indicating greater bias; for this analysis, the set of favored codons from reference 481 was used. Nc is a measure of codon bias away from uniform codon usage, with a smaller Nc indicating greater bias.

A statistically significant correspondence between estimated transcript level and codon bias was detected using both CBI (Spearman rank correlation coefficient $R = 0.30$, $n = 10{,}082$, $P < 1\text{e-}197$) and Nc (Spearman rank correlation coefficient $R = -0.25$, $n = 10{,}082$, $P < 1\text{e-}138$). Furthermore, a significant correspondence between estimated transcript level and the degree of correlation of codon usage with synonymous tRNA copy number was detected. In other words, more highly expressed genes showed a strong tendency to display a codon usage that was more closely aligned with a synonymous tRNA gene copy number. A significant correspondence between codon third-position G+C content in genes and estimated transcript levels was also detected; however, there was no significant relationship between intron G+C content and estimated transcript levels. These data suggest that, similar to the situation in *S. cerevisiae*, codon usage and tRNA abundance in *Neurospora* have coevolved to maximize the efficiency of protein translation for highly expressed genes.

Comparative multigene family and domain analysis. Despite the presence of RIP, *Neurospora* possesses 527 multigene families, including 118 families expanded relative to their counterparts in *S. cerevisiae*. In addition, *Neurospora* possesses numerous Interpro protein domains that display expansions in number relative to other sequenced eukaryotes. Particularly surprising is the abundance of cytochrome P450 domains, which are numerous in plants and in *Neurospora* but very scarce in both *S. cerevisiae* and *S. pombe*. The cytochrome P450 enzyme domain, including the E-class P450 group 1 domain and E-class P450 group IV domain subclasses, are represented by 38 proteins in *Neurospora*. In contrast, *S. cerevisiae* and *S. pombe* contain only two to four proteins with these domains. Accounting for genome size, this represents a six- to eightfold increase in genes with these domains in *Neurospora*. Cytochrome P450s are known for playing roles in both detoxification and secondary metabolism, and the implications of their high representation in *Neurospora* have been discussed previously (269).

Other domains abundant in *Neurospora* include the zinc finger C2H2-type domain, the *S*-adenosylmethionine (SAM) binding motif domain, the short-chain dehydrogenase/reductase (SDR) superfamily domain, and the flavin adenine dinucleotide-dependent pyridine nucleotide-disulfide oxidoreductase domain. Interestingly, a number of domains involved in signaling appear underrepresented compared to other fungi and plants. These include the eukaryotic, serine/threonine, and protein tyrosine kinase domains. Other underrepresented domains include certain helicases, RNA binding protein motifs, and the AAA-ATPase superfamily domain.

In the following sections, different variations of the BLAST program (17) were used to search DNA or protein databases using DNA or protein sequences. The resulting e value is dependent on the database size, and various databases are of different sizes and many are increasing in size over time. Hence, the magnitude of e should be treated as an indication and not as absolute measure of the similarity between two sequences.

CHROMATIN ASSEMBLY AND GENE REGULATION

Centromere Organization and Kinetochore Complexes

Centromeres and centromere-associated proteins are necessary to accomplish the movement of chromosomes on microtubule spindles during cell division (for a recent review, see reference 154). Conventional wisdom holds that a genetic locus and the information it conveys are defined by its DNA sequence. Most eukaryotic centromeres, however, run counter to this concept. In fact, they represent one of the best examples of how cells make use of so-called "junk DNA", because centromeric DNA in most eukaryotes is an assembly of satellite DNA and transposons or transposon relics. While the function of the small centromere of the budding yeast *S. cerevisiae* can be disrupted by point mutations, the much larger centromeres of other eukaryotes, from the fission yeast *S. pombe* to metazoans, appear to be functionally redundant (497). The hunt for centromere sequence elements has been supplanted by the realization that redundant, nonconserved DNA segments can act as a "scaffold" for the assembly of a specialized centromere nucleosome complex. The defining unit in this complex is a variant of histone H3, encoded by CENP-A in mammals, *cid* in *Drosophila*, *CSE4* in *S. cerevisiae*, and *cnp1* in *S. pombe* (331). Cse4-containing nucleosomes are found predominantly or exclusively at the core of centromeres, surrounded by stretches of transcriptionally inactive heterochromatin (520). In mammals

TABLE 2. Contigs with centromeric sequences ordered by linkage group[a]

LG	Scaffold	Size (kb)	Cen contigs	Cen (kb)	Nearest locus/marker
I	54	213	522–528	210	Uncertain
II L	93	68	680	28	*vma*, 41-kDa subunit
II R	20	566	303–304	215	
III L	22	565	323–329	271	*acr-2* (2 kb)
IV R	29	418	373–374	20	Uncertain, *pyr-1* (60 kb)
IV L	17	667	273–277	200	On 3.273: NCU04963.1 (1 kb) On 3.277: NCU04984.1 (1 kb)
V L	82	110	628	11	Uncertain, NCU09669.1; *ccg-8*
V R	15	769	246–254	300	NCU04676.1
VI L	24	534	335–348	125	NCU06013.1
VI R	12	862	206–208	8	Uncertain, NCU03846.1 (*rib-1*?)
VII C	6	1,266	119–130	336	*CenVII* (contig 129); *hH2A* (contig 119)

[a] Putative centromeric sequences were detected by BLAST searches with *Tcen*, *Tgl* (121), and *Tad* (122) elements. The edge of the centromere (Cen) is defined as the beginning of uninterrupted AT-rich repeat segments. One large scaffold containing most of the centromere was found for each linkage group (LG). Contigs for LG IIL, IVC, VR, and VI were mapped based on sequencing data available at http://www.mips.biochem.mpg.de/proj/neurospora/ and ordered cosmid libraries at http://gene.genetics.uga.edu/. Additional putative core centromeric pieces are contained on scaffolds 96, 98, 101, 103, 111, and 112, but cannot be mapped using available data. Based on assembly 3, the LG IV centromere may be contained in its entirety on scaffold 17.

and *Drosophila*, centromeric DNA associated with CENP-A and Cid, respectively, is interspersed with histone H3-containing nucleosomes (86). This assembly forms multiple foci for kinetochore subunits to create microtubule attachment points, an arrangement also predicted for *Neurospora*, where centromeres are rich in inactive transposons (121) and where a CENP-A homologue, *hH3v*, has been identified (322). The centromere binding proteins and kinetochore complexes previously isolated in *S. cerevisiae*, *S. pombe*, and animal systems have few counterparts in *Neurospora* (with the exception of the Ndc80 complex), lending support to the idea that centromeric regions and their associated complexes undergo "accelerated evolution" (330).

Organization of centromeres. *S. cerevisiae* has the simplest eukaryotic centromere known, only ~125 bp of DNA associated with a single nucleosome. This short region is divided into three centromere DNA elements (CDEs) which are conserved on all 16 chromosomes and serve as binding sites for the sequence-specific DNA binding protein Cbf1 and the essential CBF3 complex (457). The sequence of CDE II is not conserved, but the length (~80 bp) and A+T content (~90%) is similar at all yeast centromeres. CDE III is associated with the histone H3 variant Cse4/CENP-A and the kinetochore chromatin binding protein Mif2/CENP-C. In contrast to *S. cerevisiae, S. pombe* centromeres are much larger (40 to 100 kb) and are composed of two inverted repeats surrounding a nonconserved core. The inner inverted repeats and the core sequence are associated with Cnp1/CENP-A nucleosomes and the Ctf19 homologues Mis6 and Mis12 (603, 772). The flanking regions are assembled into heterochromatin in part by the histone methyltransferase, Clr4, and the heterochromatin protein Swi6 (see "Genome defense, DNA repair, and recombination" below). *Drosophila* centromeres are large (400 to 500 kb) and are composed of 5-bp satellite sequences interspersed with transposons or transposon relics, while human centromeres are 0.5 to 5 Mb long and are homogenously composed of 171-bp long α-satellites (for a review, see reference 154). In most animals and in plants, the repeat sequences of the satellite arrays are not conserved but the array repeats usually approximate the length of a nucleosome repeat of DNA sequence (330).

The seven centromeres of *Neurospora* remain largely uncharacterized, even after the cloning of *Cen VII* (137) and detailed analysis of a 17-kb segment (121). It is clear, however, that *Neurospora* centromeres are large (~200 to 400 kb) and AT rich, like those in *Drosophila* (Table 2). As in flies, they appear to consist of an accumulation of complete or fragmented and rearranged transposon relics, in particular the *gypsy*-type *Tgl1* or *copia*-type *Tcen* retrotransposon relics, the LINE-like element *Tad*, and a homolog of the *S. cerevisiae Ty3* transposon, *Tgl2* (121). All such transposon relics have been inactivated by the genome defense mechanism of RIP (see "RIP" below), which introduces CG-to-TA transition mutations and thus renders the DNA highly AT rich. Micro- and minisatellites and homopolymeric stretches can be identified in centromeric regions (498). No specific accumulation of tRNA genes has been noted close to the centromeres. Assembly 3 of the *Neurospora* genome sequence contains the sequence of most of the centromeres of all chromosomes, but some of the putative centromeric regions cannot yet be assigned to supercontigs on specific chromosomes. Similarly, the German sequencing project (498) has not yielded complete sequence information for the centromeres of linkage groups (LG) II and V. Curiously, no significant increase in the ratio of physical to genetic distance between known markers has been observed in regions near the centromeres of LG II and V (498), in contrast to that previously reported for LG III (183).

Comparison of centromeric DNA sequences in eukaryotes suggests that epigenetic factors, rather than simply DNA sequence, determine the activity of the locus. Both centromeres and the specialized histone H3, CENP-A, evolve quickly and show no obvious sequence conservation across species or even at different centromeres of the same species (330). DNA se-

TABLE 3. Putative *Neurospora* homologues of proteins involved in centromere binding and kinetochore assembly

Protein	Locus (gene)	Match found by BLAST				
		Best match[c]	*S. cerevisiae*	*S. pombe*	Animal[a]	Plant[b]
*Hs*CENP-B	0996.1	*A. thaliana*	No match	8e-39	1e-25	1e-40
*Sc*Cbf1	8999.1	*P. carinii*, 2e-33	2e-20	4e-29	2e-7	3e-4
*Hs*CENP-C/*Sc*Mif2	1635.1	*S. pombe*	2e-6	2e-7	1e-6	No match
*Sc*CBF3d (=Skp1)	8991.1 (*scon-3*)	*E. nidulans*, 5e-44	4e-35	1e-45	7e-36	5e-34
*Sc*Ndc80/*Hs*Hec1	3899.1	*S. pombe*	1e-52	2e-56	2e-27	3e-9
*Hs*Nuf2	6568.1	*S. pombe*	0.013	6e-21	3e-5	0.32
*Sc*Spc24	5312.1	*S. pombe*	2e-4	6e-6	No match	No match
*Sc*Spc25	0965.1	*S. pombe*	2e-6	1e-6	4e-5	1.1
*Sc*Dam1	6878.1	*S. pombe*	0.11	8e-6	No match	No match
*Sc*Okp1	0367.1	*S. cerevisiae*	0.16	No match	No match	No match
*Sc*Mtw1/*Sp*Mis12	9120.1	*S. cerevisiae*	1e-4	0.002	No match	No match
*Sp*Mis6	4131.1	*S. pombe*	No match	3e-32	3e-27	No match
*Sc*Ch14	3537.1	*S. cerevisiae*	2e-6	No match	No match	No match
*Sc*Sli15/*Hs*INCE NP	5211.1	*S. cerevisiae*	0.025	No match	No match	No match
*Sc*Bir1/*Hs*Survivin	6621.1	*S. pombe*	No match	3e-10	1e-8	No match
*Sc*Ip11/*Hs*Aurora B	0108.1	*S. pombe*	1e-64	1e-102	1e-87	4e-80

[a] *Caenorhabditis elegans*, *Drosophila melanogaster*, *Anopheles gambiae*, *Mus musculus*, or *Homo sapiens* (*Hs*).
[b] *Arabidopsis thaliana* or *Oryza sativa*.
[c] Species listed as best match are *Saccharomyces cerevisiae* (*Sc*), *Schizosaccharomyces pombe* (*Sp*), *Pneumocystis carinii*, and *Emericella (Aspergillus) nidulans*.

quences characteristic of centromeres are by themselves unable to direct centromere function, and in rearranged chromosomes where bona fide centromeric DNA sequences have been deleted, novel sequences can aquire centromere function (154).

Kinetochore complexes and motors that move chromosomes. To move chromosomes during cell division, centromeres are attached to spindle microtubules via the kinetochore and various motor complexes. In *S. cerevisiae*, the Cse4p/CENP-A nucleosome is bound by a centromere protein clamp, consisting of a homodimer of Cbf1p, the essential CBF3 complex, and Mif2/CENP-C (274, 519). Interestingly, *Neurospora* has putative homologues to both, presumably functionally equivalent, *S. cerevisiae* helix-loop-helix Cbf1p and the animal-type CENP-B proteins (Table 3). Curiously, alignments of putative *Neurospora* CENP-B homologues with *S. pombe* proteins (Cbh1, Cbh2, and Abp1) involved in centromere binding revealed that the *Neurospora* CENP-B homologues contain RIP-type mutations, including numerous nonsense mutations. Because CENP-B and Cbh1-like proteins are also related to the *Drosophila Pogo* and human *Tigger* transposons, most of the *Neurospora* Cbh1 homologues have been considered to be transposon relics (717). CBF3 is an octameric complex composed of four subunits (four Ndc10p, two Ctf13p, and one each of Cep3p and Skp1p). *Neurospora* has one Skp1p homolog, SCON-3, which is part of an SCF complex (E3 ubiquitin ligase see "Sulfur metabolism" below) involved in sulfur regulation (741), but there are no matches to the other three essential CBF3 subunits.

In *S. cerevisiae*, the Okp1/Ctf3 (577) and Ndc80 (379, 853) complexes connect the core centromere to distal spindle components and serve as kinetochore "glue," while the Dam/Duo complex is important for direct microtubule binding (141, 142, 380). *Neurospora* has all four previously identified components of the Ndc80 complex, and all of them are most closely related to the *S. pombe* homologues. *Neurospora* contains only three poor matches to proteins of the *S. cerevisiae* Okp1 complex, however (Table 3). Similarly, there is one Dam1p homologue but no good match to the other subunits (i.e., Dad1p-4p, Duo1p, Spc19p and Spc34p, Ask1p) of the yeast Dam/Duo complex in *Neurospora*. The activity of Dam1p is itself regulated by the conserved Ipl1p/Sli15p (Aurora B/INCENP) kinase complex (351, 613). It appears that the Ndc80 complex and proteins important for regulation of microtubule capture and proper segregation (Dam1p and its kinase, Ipl1p) are evolutionary conserved while functional homologues of the CBF3, Okp1p and Dam/Duo complexes are possibly subject to the same "accelerated evolution" that has been suggested for the CENP-A protein family and the centromeric DNA substrate (330).

In mammals, the kinesin CENP-E, cytoplasmic dynein, microtubule tracking proteins, and disassemblases are involved in chromosome movement (see "Growth and Reproduction" below); whereas in yeast, dynein positions the spindle but is not involved in actual chromosome movement (154). It remains to be seen whether dynein is involved in chromosome segregation in *Neurospora;* nevertheless, effects similar to those in mammals have been observed in *Tetrahymena* and *Drosophila* (154). It is likely that both active motor movement and microtubule flux contribute to anaphase movements of chromosomes.

Chromosomes move through checkpoints. The mitotic (or spindle assembly) checkpoint blocks the entry into anaphase until the two kinetochores of duplicated chromatid pairs have attached to spindle microtubules. This ensures accurate segregation (655). It appears that a combination of unattached kinetochores coupled to lack of tension acting on both kinetochores of a chromatid pair causes the block (154). Genetic dissection in *S. cerevisiae* has identified seven components of the mitotic checkpoint (552), all of which appear conserved in *Neurospora*. The molecular interactions between kinetochores and all checkpoint proteins are not established, but it appears that Mad2p and Cdc20p play central roles in signaling the unattached kinetochore, either directly or through a signal-amplifying cascade which inactivates or sequesters Cdc20p. Loss or reduced levels of checkpoint proteins in metazoans

TABLE 4. *Neurospora* histones and histone variants involved in nucleosome assembly and nucleosome spacing

Protein	Locus (gene)	Match found by BLAST[c]				
		Best match	*S. cerevisiae*	*S. pombe*	Animal[a]	Plant[b]
Core histones						
Histone H2A	2437.1 (*hH2A*)	*P. anserina*, 3e-52	1e-48	1e-50	3e-45	4e-41
Histone H2B	2435.1 (*hH2B*)	*P. anserina*, 2e-32	2e-32	1e-32	6e-41	3e-29
Histone H3	1635.1 (*hH3*)	*A. nidulans*, 7e-53	3e-50	1e-51	8e-53	7e-50
Histone H4-1	1634.1 (*hH4-1*)	*A. nidulans*, 1e-31	9e-30	3e-30	7e-32	3e-29
Histone H4-2	212.1 (*hH4-2*)	Same as H4-1				
H2Az	5347.1 (*hH2Az*)	*S. pombe*	3e-37	1e-42	1e-35	1e-32
H3v/*Sc*Cse4	0145.1 (*hH3v*)	*S. pombe*	3e-28	2e-30	3e-24	4e-24
H4v	4338.1 (*hH4v*)	*P. polycephalum*, 3e-18	2e-16	4e-16	1e-16	1e-15
Linker histones						
Histone H1	6863.1 (*hH1*)	*A. nidulans*, 2e-14	1e-9	No match	6e-5	No match
Histone-like proteins						
HMG1.2/*Sc*Nhp6	9995.1	*V. faba*	1e-17	2e-10	2e-11	1e-21
HMG2A	2819.1	*M. musculus*	4e-9	1e-5	2e-13	3e-13
HMG4	2695.1	*P. anserina*, 3e-11	0.008	No match	3e-5	2e-6
MATA-3	1960.1 (*matA-3*)	*S. macrospora*, 1e-37	No match	1e-4	No match	No match
MAT**a**-1	No ID (*mata-1*)	*S. macrospora*, 1e-135	No match	2e-14	2e-11	No match
S. pombe Ste11-like	2326.1	*N. crassa*, 1e-10, 9387.1	No match	0.011	No match	No match
S. pombe Ste11-like	9387.1	*N. crassa*, 9e-11, 2326.1	No match	1e-7	4e-6	No match
*Sc*Rox1/Rfg1/S RY	3481.1	*C. albicans*, 4e-14	2e-13	6e-12	4e-9	No match
TBF (TATA-bind.)	2017.1	*S. pombe*	2e-15	6e-29	1e-18	4e-16
HAP (CAAT-bind)	9248.1	*H. jecorina*, 3e-49	2e-26	1e-30	3e-31	4e-26
CHRAC17/POL II	3073.1	*S. pombe*	4e-4	1e-11	2e-6	6e-5
Polyamine oxidase with HMG box	9120.1	*H. sapiens*	No match	8e-25	4e-69	9e-54

[a] *Caenorhabditis elegans*, *Drosophila melanogaster*, *Strongylocentrotus purpuratus*, *Mus musculus*, or *Homo sapiens*.
[b] *Arabidopsis thaliana*, *Vicia faba*, or *Oryza sativa*.
[c] Histones and histone-like proteins are short; therefore BLAST scores are comparatively low and e values are high. Species listed as best matches are *Podospora anserina*, *Aspergillus nidulans*, *Physarum polycephalum*, *Vicia faba*, *Sordaria macrospora*, *Candida albicans*, and *Hypocrea jecorina*.

causes chromosome missegregation, tumorigenesis, and apoptosis (154).

Chromatin Structure and Gene Regulation

Nucleosome assembly and histone modification. (i) Nucleosome assembly and nucleosome spacing. *(a) Core histones.* Nucleosomes are assembled into an octamer from dimers of the core histones H2A, H2AB, H3, and H4. Except for H4, which is encoded by two unlinked genes, *hH4-1* and *hH4-2*, all *Neurospora* core histones are encoded by single genes (Table 4) (322, 860). This is similar to the situation in other fungi but is in contrast to that in plants and metazoans, which have numerous histone genes (322). The distribution and small number of histone genes, as well as the presence of introns within the histone genes of filamentous ascomycetes other than yeasts, may reflect the operation of the genome surveillance systems RIP and MIP (322). Introns are absent from the histone genes of most other eukaryotes.

(b) Core histone variants. Three core histone variants are present in the *Neurospora* genome (Table 4) (322). The H2Az variant is closely related to *S. cerevisiae* Htz1p and *S. pombe* Pht1, proteins involved in maintaining chromatin integrity, recruiting polymerase II to specific promoters, and protection from telomeric silencing (4, 130, 374, 521). The H3v variant (see "Centromere organization and kinetochore complexes" above) is a homolog of Cse4p from *S. cerevisiae* (143, 520, 759), CENP-A from humans (21, 827, 895), and *cid* from *D. melanogaster* (85, 331). There are no good matches to the H4 variant (H4v) in other fungi; this may be a pseudogene (322).

(c) Linker histones. Similar to other fungi (403), *Neurospora* has only one histone H1 gene (Table 4) (242). Studies with *Neurospora*, *S. cerevisiae*, *Tetrahymena*, *Aspergillus nidulans*, and *Ascobolus immersus* revealed that H1 is not essential in any of these organisms (44, 252, 640, 729, 817) but, rather, is implicated in the regulation of nitrogen and carbon metabolism (242, 329, 727).

(d) Histone fold motifs and HMG proteins. Short proteins with histone fold motifs are involved in transcriptional regulation in all eukaryotes (271, 639). The histone fold motif in CBFD/NF-YB/HMF is similar to domains found in archaebacteria (608). Three predicted *Neurospora* proteins containing histone folds are related to general transcription factors (Table 4): TATA binding factor (TBF) (NCU02017.1); CHRAC17 (NCU03073.1), a putative subunit of RNA polymerase II with homology to a subunit of the CHRAC chromatin remodeling factor from *Drosophila;* and HAP (NCU09248.1), a homologue of the CCAAT binding proteins Hap3p from *S. cerevisiae*, HAP-C from *A. nidulans*, Php3 from *S. pombe*, and NF-YB from humans.

Two sex-determining region, Y chromosome (SRY)-related high-mobility group (HMG) transcription factors, MATA-3 and MATa-1, have been characterized in *Neurospora* (233, 615). Three additional proteins have homology to mating peptides or carry a sterile alpha motif and an HMG-1-like box (Table 4). NCU03481.1 is related to a repressor of hypoxic genes (Rox1p) in *S. cerevisiae* and a virulence factor (Rfg1) in *Candida albicans*. NCU09387.1 and NCU02326.1 are related to *S. pombe* Ste11. Interestingly, the Ste11-related proteins from *Neurospora* are each other's closest homologues, which is unusual in

Neurospora. HMG-like proteins are typically short, such as HMG1.2 (NCU09995.1; related to *S. cerevisiae* recombination proteins Nhp6A and Nhp6B) and HMG2A (NCU02819). The most unusual HMG protein predicted in *Neurospora* (NCU09120.1) has a HMG box at the C terminus and is most similar to human polyamine oxidases.

(ii) Histone modifications. Core and linker histones are extensively modified at the posttranscriptional level (826). Histone modifications have been studied both for their effects on the regulation of specific genes and for their importance to global regulatory phenomena. Recent work has uncovered an epigenetic "histone code" (384, 750, 761, 811) involved in transcriptional regulation (for reviews, see references 67 and 104) and other DNA transactions (for recent reviews, see references 245, 361, 691, and 810). Histone residues can be actively modified by acetylation and deacetylation of lysine; methylation of lysine and arginine; phosphorylation and dephosphorylation of serine, threonine, and histidine; ADP-ribosylation of glutamic acid, and ubiquitylation of the entire proteins (384, 826). Because the interrelationships between histone modifications are essential for the formation and maintenance of silent chromatin states (384, 445, 559), genes involved in *Neurospora* histone modification are described below (see "Genome defense, DNA repair, and recombination"). The combinatorial possibilities of the histone code are staggering, even without considering the facts that different modification states are possible on the same residue and that lysine and arginine can be mono-, di-, or trimethylated (see, e.g., reference 778).

(a) HATs. Histone acetyltransferases (HATs) transfer acetyl groups from acetyl coenzyme A acetyl-CoA to lysines, most often located within the amino-terminal tail of the core histones. *Neurospora* has representatives of many of the HATs involved in transcriptional activation and gene silencing (e.g., TAFII250, Gcn5p, Sas2p, Sas3p, Esa1p, and Elp3p [J. Dobosy and E. Selker, unpublished results]) but lacks homologues to *S. cerevisiae* Hpa1p and metazoan CBP and SRC (Table 5). The homologue of *S. cerevisiae* Gcn5p lacks a locus identifier because the predicted protein lies at the beginning of contig 3.38 (Table 5). *Neurospora* has three N-terminal acetyltransferases that correspond to the budding yeast proteins Nat1p, Nat3p, and Mak3p, all of which are involved in cell cycle control (Table 5) (415). Interestingly, three of the putative GNAT/RIM1-type acetyltransferases have bacterial proteins as their closest relatives. The range of substrates of the predicted *Neurospora* acetyltransferases remains unknown.

(b) HDACs. Histone deacetylases (HDACs) remove acetyl groups from lysine residues, e.g., histone tails. They are separated into three families (190). *Neurospora* has 11 predicted proteins related to known or putative HDACs in two of the families (Table 5). Four proteins (HDA-1 to HDA-4) have homologs in the Rpd3/Hos/Hda group (Dobosy and Selker, unpublished), compared to five such proteins in budding yeast and seven and nine in animals and plants, respectively. Seven predicted proteins (NST-1 to NST-7) are related to the NAD-dependent HDAC Sir2 and related proteins ("sirtuins" [G. Kothe, M. Freitag, and E. Selker, unpublished results]). *Neurospora* has homologues of all four *S. cerevisiae* sirtuins as well as three additional sirtuins most closely related to those in animals. *Arabidopsis* has just one sirtuin but contains a class of unrelated HDACs, called HD2 type, which is not found in *Neurospora* or animals (593).

(c) HMTs. Histone methyltransferases (HMTs) add methyl groups supplied by SAM to lysines (Lys) or arginines (Arg) on core histones. *Neurospora* has a single member of all known HMT subfamilies (Table 5), whereas metazoans, plants, and in some cases *S. cerevisiae* and *S. pombe* have multiple proteins for each. *Neurospora* is predicted to have nine proteins (SET-1 to SET-8 and DIM-5; M. Freitag, K. Adhvaryu, and E. Selker, unpublished data) with SET domains, a motif first found in the *Drosophila* Su(var) 3–9, Enhancer of zeste, and Trithorax proteins. SET domains are characteristic of lysine protein methyltransferases (for a review, see reference 445), although not all SET domain proteins are HMTs. *Neurospora* lacks some SET proteins identified in *S. cerevisiae* (e.g., Set5p and Set6p) and in humans but has two proteins (SET-6 and SET-8) that are either novel or restricted to only a subset of fungi. Both proteins align well by their Zn finger and Jumanji (JmJi) domains, but the pairing of these domains with the SET motif is rare. Like its homologues in *S. pombe*, animals, and plants (641, 690), *Neurospora* DIM-5 is a histone H3 Lys9 HMT (776, 778). Budding yeast Set1p and Set2p methylate histone H3 on Lys4 (106, 553, 660) and Lys36 (762), respectively, and *Neurospora* has striking homologues (SET-1 and SET-2) to these two proteins. *Neurospora* SET-3 is related to *Drosophila* ASH1, which methylates histone H4 on Lys20 and histone H3 on Lys4 and Lys9 (59), and SET-7 is related to E(z), which methylates histone H3 Lys9 and Lys27 (546). Human G9A also methylates histone H3 on Lys9 and Lys27 (770, 771), and *Neurospora* SET-5 appears related to the G9A subgroup.

Neurospora has three putative arginine methyltransferases (PRMTs) (for reviews, see references 432 and 511) that are homologous to PRMT1, PRMT3 and PRMT5 of humans, respectively (Table 5). Interestingly, *Neurospora* does not have a recognizable homologue of either PRMT2 or PRMT4 (CARM1). CARM1 methylates histone H3 Arg2, Arg17, and Arg26 (51, 180). PRMT3 has not yet been shown to methylate histones (249, 781), but PRMT1 methylates histone H4 Arg3 in vivo (696, 843) and PRMT5 and its homologues can methylate both histone H2A and H4 in vitro (103, 227). PRMT5 is a homolog of *S. pombe* Skb1 and *S. cerevisiae* Hsl7p, which methylate the protein kinases Shk1 (43) and Swe1p (150), respectively. The *Neurospora* homolog is called PP-2 (P. Bobrowicz and D. Ebbole, unpublished data).

Neurospora has one homologue of *S. cerevisiae* Dot1p (M. Freitag, C. Matsen, J. Murphy, G. Kothe and E. Selker, unpublished data) (Table 5), an HMT which methylates Lys79 within the globular domain of histone H3 and which is important in telomeric silencing in *S. cerevisiae* (740) and humans (232, 568).

(d) Histone kinases. Like histone acetylation and methylation, histone phosphorylation has been intensively studied, and found to be important for chromosome codensation, the signaling of active versus silent chromatin states, transcription, regulated cell death, and DNA repair (145). All core and linker histone H1 can be phosphorylated in vitro (826), and all histone kinases that act on histones in vivo are involved in the control of cell cycle progression. Histone H3 Ser10 can be phosphorylated by at least two different kinases in *S. cerevisiae*, Snf1p (483) and Ipl1p/Aurora B (172, 351). The *S. cerevisiae*

TABLE 5. *Neurospora* homologues of proteins involved in histone modifications[a]

Protein	Locus (gene)	Match found by BLAST				
		Best match[d]	*S. cerevisiae*	*S. pombe*	Animal[b]	Plant[c]
Histone acetyltransferases						
*Sc*Hat1	6472.1 (*hat-1*)	*S. cerevisiae*	4e-28	4e-14	4e-22	3e-15
*Hs*TAFII250	2556.1 (*hat-2*)	*S. pombe*	No match	1e-109	1e-49	3e-27
*Sc*Elp3	1229.1 (*elp-3*)	*S. cerevisiae*	0.0	0.0	0.0	0.0
*Sc*Gcn5	No ID (*ngf-1*)	*Y. lipolytica*; 1e-138	1e-135	1e-116	7e-78	6e-79
*Sc*Esa1	5218.1 (*hat-4*)	*S. cerevisiae*	1e-122	1e-122	9e-96	2e-90
*Sc*Sas2	2249.1 (*hat-5*)	*S. cerevisiae*	2e-34	1e-25	3e-34	2e-34
*Sc*Sas3	4782.1 (*hat-6*)	*S. pombe*	7e-78	1e-95	2e-84	1e-79
Protein acetyltransferases						
*Sp*Ard1/*Sc*Nat3	1276.1	*S. pombe*	1e-26	2e-44	6e-40	1e-32
*Sc*Mak3	2417.1	*P. anserina*; 3e-75	1e-33	4e-35	6e-41	1e-33
*Sp*Ats1	7589.1	*P. aeruginosa*; 8e-25	No match	7e-22	2e-14	4e-20
GNAT-type	9914.1	*B. iaponicum*; 2e-5	No match	No match	No match	No match
GNAT-type	4583.1	*B. cereus*; 1e-13	No match	No match	No match	No match
Deacetylases						
*Sc*Hda1/*Sp*Clr3	1525.1 (*hda-1*)	*S. pombe*	1e-137	1e-145	4e-80	4e-67
*Sc*Hos2	2795.1 (*hda-2*)	*A. nidulans*; 1e-143	1e-104	1e-106	1e-100	2e-95
*Sc*Rpd3	0824.1 (*hda-3*)	*A. nidulans*; 1e-178	1e-161	1e-144	1e-146	1e-127
*Sc*Hos3	7018.1 (*hda-4*)	*S. cerevisiae*	9e-83	No hits	3e-18	8e-25
*Sc*Hst1/*Hs*Sirtuin1	4737.1 (*nst-1*)	*S. pombe*	6e-80	5e-88	7e-62	No match
*Sc*Hst2/*Hs*Sirtuin2	0523.1 (*nst-2*)	*S. pombe*	5e-56	7e-71	1e-65	No match
*Sc*Hst4	3059.1 (*nst-3*)	*S. pombe*	5e-61	4e-73	5e-21	No match
*Sc*Hst3/*Hs*Sirtuin3	4859.1 (*nst-4*)	*S. cerevisiae*	7e-28	3e-15	2e-6	0.001
*Hs*Sirtuin4	0203.1 (*nst-5*)	*A. thaliana*	No match	No match	5e-35	8e-42
*Hs*Sirtuin5	5973.1 (*nst-6*)	*M. musculus*	No match	No match	1e-35	No match
*Hs*Sirtuin7	7624.1 (*nst-7*)	*A. thaliana*	No match	No match	3e-43	1e-49
Protein methyltransferases						
Lysine, SET domain						
*Sp*Clr4	4402.1 (*dim-5*)	*S. pombe*	No match	2e-43	7e-36	2e-26
*Sc*Set1	1206.1 (*set-1*)	*S. cerevisiae*	3e-64	2e-53	3e-53	7e-45
*Sc*Set2	0269.1 (*set-2*)	*S. pombe*	1e-104	1e-116	3e-50	3e-43
*Dm*Ash1	1932.1 (*set-3*)	*R. norvegicus*	1e-29 (set 2)	3e-25	2e-37	2e-31
*Sc*Set3/Set4	4389.1 (*set-4*)	*H. sapiens*	9e-10	8e-8	9e-13	0.027
*Hs*G9a	6119.1 (*set-5*)	*C. elegans*	No match	No match	8e-12	No match
*Nc*SET-6	9495.1 (*set-6*)	*S. pombe*	2e-6	7e-36	9e-6	3e-6
*Dm*En(z)/*At*Medea	7496.1 (*set-7*)	*M. musculus*	No match	No match	4e-29	1e-25
*Nc*SET-8	1973.1 (*set-8*)	*H. sapiens*	5e-4	No match	4e-13	4e-6
Lysine, non-SET						
*Sc*Dot1	6266.1	*S. cerevisiae*	3e-38	No match	5e-22	No match
Arginine						
*Hs*PRMT1/*Sc*Hmt1	7459.1 (*prm-1*)	*S. pombe*	1e-111	1e-116	1e-100	3e-98
*Hs*PRMT3	1669.1 (*prm-3*)	*S. pombe*	4e-62	6e-86	6e-67	2e-62
*Hs*PRMT5/*Sc*Hs17/*Sp*Skb1	1613.1 (*pp-2*)	*S. pombe*	4e-69	1e-107	5e-86	3e-77
Kinases						
*Sc*Snf1	4566.1	*F. oxysporum*; 0	1e-134	1e-123	1e-103	1e-103
*Sc*Snf4	1471.1	*K. lactis*; 2e-94	4e-92	4e-88	7e-48	8e-24
*Sc*Gal83	3937.1	*S. cerevisiae*	1e-27	5e-23	5e-19	2e-10
*Sc*Ip11/*Hs*Aurora B	0108.1	*S. pombe*	1e-64	1e-102	1e-87	4e-80
*En*Nim-A	3187.1 (*nim-1*)	*E. nidulans*; 1e-172	6e-68 (Kin3)	3e-66 (Fin1)	8e-63 (Nek2)	1e-36
Ubiquitylases						
*Sc*Rad6	9731.1 (*mus-8*)	*N. haematococca*; 4e-75	2e-63	4e-64	3e-57	3e-53
ADP-Ribosylases						
PARP	8852.1 (*parp*)	*A. thaliana*	No match	No match	3e-60	3e-67

[a] Histone phosphatases and histone kinases are listed in Tables 48 and 56, respectively. Note: Based on sequence comparisons with fungal Snf1p homologues, the Snf1 locus is misannotated at the WICGR but correct at MNCDB (CAD70761).

[b] *Caenorhabditis elegans*, *Drosophila melanogaster*, *Strongylocentrotus purpuratus*, *Xenopus laevis*, *Mus musculus*, or *Homo sapiens*.

[c] *Arabidopsis thaliana*, *Vicia faba*, or *Oryza sativa*.

[d] Species listed as best matches are *Podospora anserina*, *Aspergillus nidulans*, *Physarum polycephalum*, *Vicia faba*, *Sordaria macrospora*, *Fusarium oxysporum*, *Yarrowia lipolytica*, *Candida albicans*, *Nectria haematococca*, and *Hypocrea jecornia*.

TABLE 6. *Neurospora* homologues of proteins involved in nucleosome and chromatin assembly

Protein	Locus (gene)	Match found by BLAST				
		Best match[c]	*S. cerevisiae*	*S. pombe*	Animal[a]	Plant[b]
CAF-1						
*Sc*Cac1 (*Hs*p150)	4198.1 (*cac-1*)	*S. pombe*	0.026	0.002	No match	No match
*Sc*Cac2 (*Hs*p60)	8357.1 (*cac-2*)	*S. pombe*	1e-39	1e-52	7e-37	1e-31
*Sc*Cac3 (*Hs*p48)	6679.1 (*cac-3*)	*D. rerio*; 1e-113	2e-45	1e-105	1e-113	1e-105
RCAF						
*Sc*Asf1	9436.1 (*asf-1*)	*S. cerevisiae*	2e-58	3e-52	8e-48	5e-43
NAP						
*Nc*NAF-1	1438.1 (*naf-1*)	*S. pombe*	6e-55	1e-67	5e-39	6e-34
*Nc*NAF-2	3769.1 (*naf-2*)	*S. pombe*	No match	5e-12	2e-8	3e-5
Accessory factors						
*Hs*PCNA	9239.1 (*pcn*)	*S. pombe*	2e-62	7e-96	5e-76	3e-80

[a] *Drosophila melanogaster*, *Anopheles gambiae*, *Caenorhabditis elegans*, *Strongylocentrotus purpuratus*, *Danio rerio*, *Mus musculus*, or *Homo sapiens*.
[b] *Arabidopsis thaliana*, *Daucus carota*, *Vicia faba*, or *Oryza sativa*.
[c] Species listed as best match is *Danio rerio*.

Snf1p kinase is a heterotrimer, composed of the Snf1p α subunit, the Snf4p γ subunit and three different β subunits, Sip1p, Sip2p, or Gal83p (474). *Neurospora* has homologues to Snf1p and Snf4p but has only one β subunit, most closely related to Gal83p (Table 5). Phosphorylation of H3 Ser10p is a prerequisite of acetylation at histone H3 Lys14 and is usually required for gene activation (482, 572). Like H3, the centromeric H3 variant CENP-A can be phosphorylated, albeit at Ser7 (895). In addition to homologues of Snf1p and Ip1p/Aurora B, filamentous fungi have a cell cycle kinase, Nim-A (*Neurospora* NIM-1 [635]), that phosphorylates histone H3 Ser10 (192).

In animals, histone H3 Thr13 can also be phosphorylated by the Dlk/Zip kinase in vivo (629). This modification is found on both H3 and CENP-A at assembled centromeres, suggesting that Thr11 phosphorylation rather than Ser10 phosphorylation may be involved in the maintenance of silent chromatin and kinetochore attachment. Curiously, these threonine DAP (death-associated protein kinase)-like enzymes seem restricted to animals, since no homologue has been found in any fungus, including *Neurospora*.

Candidate histone phosphatases from *Neurospora* (PPP-1, PPH-1, and PZL-1) have been isolated by biochemical means, and their genes have been identified (see "Environmental sensing" below).

(e) Histone ubiquitylases. Our understanding of histone ubiquitylation is still fragmentary. Ubiquitylated histones H2A and H2B are the most abundant ubiquitylated proteins in eukaryotes (381). Ubiquitin is linked to histone H2A Lys119 (91) and H2B Lys120 or Lys123 in animals and *S. cerevisiae*, respectively (658, 789). All histone H2 variants known in animals can be ubiquitylated (381). *Neurospora* has several predicted subunits of ubiquitin-activating (E1) and ubiquitin-ligating (E2) proteins that are predicted to be involved in histone ubiquitylation. For example, the *Neurospora mus-8* gene encodes a Rad6p-like H2B ubiquitin-ligase (Table 5) (658, 749). This enzyme affects gene silencing via histone H3 methylation states in *S. cerevisiae*, an example of "trans-tail" regulation of histone modifications (765).

(f) Histone ADP-ribosylases. Of the histone modifications, ribosylation is currently the least well understood. One class of enzymes thought to be involved in ribosylation and histone turnover are the poly(ADP-ribose) polymerases (PARPs) (16). In contrast to mammals, which have multiple PARPs, *Neurospora* has a single *parp* gene (G. Kothe and E. Selker, unpublished results; (Table 5). Whether any of the small GTP binding proteins with ADP ribosylation activity have effects on histones in vivo is unknown.

Chromatin assembly and remodeling. (i) CAFs. Eukaryotes use histone chaperones or chromatin assembly factors such as CAF and RCAF to guide histones prior to assembly into nucleosomes (for a review, see reference 517). As in other eukaryotes, *Neurospora* CAF-1 is predicted to be composed of three subunits (CAC-1 to CAC-3) (M. Freitag and E. Selker, unpublished data) (Table 6). Disruption mutants with mutations in the gene encoding the largest, least well conserved subunit, *cac-1*, are viable (Freitag and Selker, unpublished); this is similar to the situation in *S. cerevisiae*, where neither CAF nor the RCAF subunit of the antisilencing factor (Asf1p) are essential. These findings suggest the presence of additional chromatin assembly factors. CAC-2 and CAC-3 contain WD40 domains and are conserved among all eukaryotes studied. *Neurospora* CAC-3 is more closely related to retinoblastoma binding protein 48 (RBBP4; p48) from mammals than it is to *S. cerevisiae* Cac3p (Msi1p/Ira1p). In mammals, RBBP4/p48 also associates with HDAC complexes (653, 840).*Neurospora* contains one homologue of *S. cerevisiae* Asf1p (Table 6), a component of an alternative chromatin assembly factor first described in *Drosophila* as RCAF (813). *asf1* mutants exhibit more drastic silencing effects in *S. cerevisiae* than do *cac1* mutants, and the severity is further enhanced in double-knockout strains (740, 813). *Drosophila* ASF1 cooperates with the brahma chromatin-remodeling complex (see below), and mutation of ASF1 results in derepression of heterochromatic regions (541). These results suggest that both CAF and RCAF play a role in heterochromatin silencing in eukaryotes.

Neurospora has two predicted proteins with domains characteristic of nucleosome assembly proteins (NAPs) (Table 6). All other eukaryotes with sequenced genomes have at least two paralogues. NAF-1 (nucleosome assembly factor 1) is a canonical NAP, which probably functions as a chaperone for the

TABLE 7. *Neurospora* homologues of predicted chromatin-remodeling factors

Protein	NCU no. (gene)	Match found by BLAST				
		Best match[c]	*S. cerevisiae*	*S. pombe*	Animal[a]	Plant[b]
INO80/DOMINO						
*Sc*Swr1	9993.1 (*crf1-1*)	*S. cerevisiae*	0.0	0.0	1e-121	0.00
*Sc*Ino80	8919.1 (*crf2-1*)	*S. cerevisiae*	0.0	1e-146	1e-117	1e-164
Swi2/Snf2/BRM						
*Sc*Swi2/Snf2	6488.1 (*crf3-1*)	*S. pombe*	0.0	0.0	0.0	00
ISWI						
*Dm*ISWI	3875.1 (*crf4-1*)	*S. cerevisiae*	0.0	0.0	0.0	0.0
*Sc*Ioc4	2684.1 (*crf4-3*)	*S. pombe*	0.035	6e-6	No match	No match
*Sc*Itc1	164.1 (*crf4-2*)	*S. cerevisiae*	7e-21	No match	4e-6	5e-7
Lsh/*Sc*YFK8						
*Hs*LSH	6306.1 (*crf5-1*)	*A. fumigatus*; 9e-95	1e-59	No match	2e-64	2e-56
Mi-2						
*Sc*Chd1/*Hs*CHD2	3060.1 (*crf6-1*)	*S. pombe*	0.0	0.0	1e-173	1e-160
*Hs*CHD3/Mi-2	1406.1 (*crf7-1*)	*S. pombe*	1e-96 (Chd1)	1e-155	1e-111	1e-115
*At*GYMNOS						
TAF172/Mot1-like						
*Hs*TAF172	7556.1 (*crf8-1*)	*S. pombe*	1e-69	0.0	0.0	0.0
Ris1						
*Sc*Ris1	0631.1 (*crf9-1*)	*S. pombe*	6e-70	1e-118	2e-47	2e-59
*Sc*Ris1-like/*Dm* LODESTAR-like	4786.1	*S. pombe*	5e-43 (Ris1)	4e-70	4e-50	3e-50
Role in transcription?						
POLII term. factor	7358.1	*S. pombe*	No match	1e-13	5e-9	3e-12
POLII term. factor	4445.1	*H. sapiens*	4e-21 (Ris1)	1e-39 (Rad8, Rhp16)	1e-39	9e-37 (Rad5)
*Hs*ETL-like	7975.1	*A. thaliana*	3e-66 (Rad16)	1e-72 (Rad8)	2e-85	1e-110
*Hs*ETL-like	5246.1	*A. thaliana*	No match	4e-57 (Rhp16)	9e-73	1e-84
Viral activator	2913.1	*C. fumiferana* MNPV; 3e-42	No match	4e-31 (Rhp16)	9e-73	1e-33
Activator protein	4424.1[d]	*A. thaliana*	No match	No match	3e-24	6e-32
Fun30/ETL1						
*Sc*Fun30/*Hs*ETL1	9106.1 (*crf10-1*)	*S. pombe*	1e-104	1e-122	1e-105	1e-74

[a] *Drosophila melanogaster*, *Anopheles gambiae*, *Mus musculus*, or *Homo sapiens*.
[b] *Arabidopsis thaliana*, *Zea mays*, or *Oryza sativa*.
[c] Species listed as best match are *Aspergillus fumigatus* and *Choristoneura fumiferana* multicapsid nucleopolyhedrovirus (MNPV).
[d] NCU04424.1 may be misannotated (the 5′ region is too short), and a homologue of *A. nidulans sonB*, a nucleoporin (NCU04784.1) is annotated as containing a SNF2 domain.

histone H2A-H2B tetramer (139, 368). NAF-2 is related to phosphatase 2A inhibitor 2 (471), which is involved in chromatin decondensation in *S. pombe* and humans and may help balance competing kinase and phosphatase activities at histone H3 Ser10 (572).

Like *S. cerevisiae* and *S. pombe*, *Neurospora* has a single homologue of the proliferating-cell nuclear antigen protein (PCNA) (Table 6). The homotrimeric PCNA complex serves to mark newly replicated DNA at the replication foci to which the CAF complex localizes. PCNA is involved in many activities involving DNA, including replication, repair, and silencing (517).

(ii) CRFs. Chromatin-remodelling factors (CRFs) use the energy generated by their ATPase subunits to remove or position nucleosomes relative to the DNA substrate (for recent reviews, see references 58, 240, and 452). *Neurospora* has 24 predicted proteins related to the SWI/SNF ATPase/helicase domain (Table 7). In organisms in which they have been studied, these proteins have been implicated in chromatin remodeling, DNA repair, and activation of transcription and are typically found in large complexes. Ten of the predicted *Neurospora* proteins are homologues of previously identified CRFs. The remaining 14 may be involved in ATPase-dependent repair processes and transcription. One of these, MUS-25, is the previously identified *Neurospora* RAD54 homolog (312). The *Neurospora* complement of SWI/SNF ATPases is a subset of those found in *S. cerevisiae*, *S. pombe*, *Arabidopsis*, and animals.

The SWI/SNF domain is homologous to viral and prokaryotic helicases. Therefore, all proteins in this group were previously called helicases (217, 290), even though helicase activity had not been demonstrated. Several members of this family have now been shown to function in large chromatin complexes such as ATPases without apparent helicase activity (58). In combination, CRFs may be expected to be involved in regulating the activity of many, if not most, genes by either activation or repression. All known CRFs have at least two com-

ponents in vivo, but some ATPase subunits can remodel chromatin in vitro alone, albeit with altered specificity (210).

CRFs were categorized by their putative conserved ATPase subunits alone. Usually, ATPase subunits of various CRFs from different organisms are more closely related to each other than to different CRF ATPases from the same organism. Compared to CRFs from the yeasts, *Drosophila*, and human, relatively few homologues to non-ATPase subunits can be identified in *Neurospora* (e.g., to the yeast RSC, SNF2, and ISW complexes, the *Drosophila* CHRAC and NURF complexes, and the human BRG and BRM complexes). While there are fewer CRF ATPases in the *Neurospora* genome than in the *S. cerevisiae* genome, it appears likely that one ATPase subunit may associate with different accessory proteins to form specific complexes, analogous to the situation in *Drosophila*, where ISWI is present in three complexes: ACF, NURF, and CHRAC (452).

Similar to *S. cerevisiae*, *Neurospora* has two ATPase homologues in the INO80 group of CRFs, CRF1-1 and CRF2-1 (Table 7). Ino80p is the only CRF that has helicase activity (728). CRF1-1 is a homologue of Swr1p, while CRF2-1 is a homolog of Ino80p. Both are related to DOMINO CRFs of *Drosophila* (667).

Arabidopsis, metazoans, *S. pombe*, and *S. cerevisiae* have several CRFs that are generally associated with global gene activation. In *Drosophila*, for example, the distribution of RNA polymerase II and the BRAHMA complex largely coincide (27). Like humans (BRG and BRM), *S. cerevisiae* has two such complexes (SNF2 and RSC) with the bromodomain ATPases Snf2p and Sth1p, respectively (392, 542). Strikingly, *Neurospora* has only one predicted bromodomain ATPase, CRF3-1 (Table 7). Similar to their homologues, *Neurospora* CRF3-1 and two polybromodomain proteins (PBD-1 and PBD-2 [Table 7]) predicted to be in RSC- or SNF2-like complexes contain bromodomains, which bind to acetylated lysine residues (581).

Most eukaryotes have more than one heterochromatin-associated complex of the Imitation Switch (ISWI) type. In *S. cerevisiae*, at least four ISWI complexes exist, two each with specific ATPase, Isw1p and Isw2p, whereas in *Drosophila*, the single ISWI protein is present in three separate complexes: ACF, NURF, and CHRAC (for a review, see reference 452). This may be similar to the situation in *Neurospora*, where only one ISWI homologue, CRF4-1, exists but where subunits related to a yeast ISW2 component and *Drosophila* CHRAC subunits (Table 4, "Histone-like proteins") can be identified (Table 7). CRF4-2, for example, is predicted to be a DNA binding protein with a DDT domain, similar to *Drosophila* ACF1, which is present in both ACF and CHRAC. As in *S. cerevisiae*, and in contrast to *Drosophila*, disruption of *crf4-1*is not lethal in *Neurospora* (Freitag and Selker, unpublished). ISWI complexes are generally thought to serve as global repressors of gene expression because they are colocalized with silent heterochromatin in *Drosophila* polytene chromosomes and excluded from transcriptionally active regions (193).

Neurospora CRF5-1, a relatively short (882-amino-acid) CRF ATPase (Table 7), is related to similar-length proteins involved in DNA methylation: DDM1 from *Arabidopsis* (382) and LSH from humans (189). DDM1-like CRF subunits may represent a subgroup of the ISWI CRFs, since CRF5-1 is closely related to CRF4-1.

Arabidopsis and animals have two chromodomain CRF ATPase groups with at least two representatives in each group, while *S. cerevisiae* apparently has a single Mi-2-like chromodomain protein, Chd1p (796). *Neurospora* has one representative for each of the two groups (Table 7). CRF6-1 is a homologue of *S. pombe* Hrp1 (888) and Hrp3 (377), *Drosophila* KISMET (179), and mammalian CHD2 (859), while the ATPase domain of CRF7-1 is more closely related to human Mi-2/CHD3 (794, 840) and *Arabidopsis* GYMNOS/PICKLE (223). In animals, Mi-2-containing NuRD complexes can bind to methylated DNA via proteins with methyl binding domains (560, 657, 839). While the ATPase domain of CRF7-1 most closely matches Mi-2, the chromodomain is a poor match to the chromodomain consensus sequence.

Three additional predicted ATPases may be subunits of CRFs involved in transcription in *Neurospora* (Table 7). CRF8-1, CRF9-1, and CRF10-1 are similar to Mot1p, Ris1p, and Fun30p from *S. cerevisiae*, respectively. Mot1p-like proteins repress genes by interaction with TATA binding factor but can also be involved in gene activation, presumably by chromatin remodeling during transcription preinitiation (178, 543). The *Neurospora* protein is more closely related to plant and animal homologues of Mot1, such as human TAF172. Ris1p has a "role in silencing" (286). Two RING finger domain-containing Ris1p homologs can also be found in *S. pombe* and *Arabidopsis*. Fun30p may be important for chromosome stability (580).

Seven poorly characterized yet closely related predicted *Neurospora* ATPases may be involved in transcription (Table 7). These proteins are similar to human RNA polymerase termination factor, plant transcription factors, and viral activator proteins. An additional seven putative helicases or SWI/SNF ATPases are known or predicted to be involved in DNA repair in *Neurospora* (Table 8). *Neurospora* has a single Rad5p homologue and two members each of the Rad16p, Rad26p and Rad54p groups (discussed in more detail in "Genome defense, DNA repair, and recombination below). Human Rad26 homologues have chromatin-remodeling activity (151). One of the RAD54 proteins has homology to ATRX, a protein involved in DNA methylation (281).

Transcription Factors

With the availability of genome sequences from many diverse organisms, it is now possible to conduct comparative genomics on proteins required for gene transcription and regulation. This analysis will contribute to our understanding of promoter evolution by providing information about the regulation of organismal complexity. A recent review addressing the gene number conundrum, which is exemplified by the fact that a simple nematode worm, *C. elegans*, has ~20,000 genes whereas a more complex organism, *Drosophila*, has ~14,000 protein-coding genes, suggests that it is not gene number but the multitude of regulatory combinations that determine complexity. This is accomplished by the increased elaboration of *cis*-regulatory elements controlling gene expression coupled with more complex transcription machinery. A simple eukaryotic promoter found in unicellular eukaryotes is contrasted with complex metazoan transcriptional control modules (470).

This section is a compilation of the sequence-specific DNA

TABLE 8. *Neurospora* homologues of predicted ATPases or helicases involved in DNA repair

Protein	Locus (gene)	BLAST match				
		Best match[c]	*S. cerevisiae*	*S. pombe*	Animal[a]	Plant[b]
*Sc*Rad5/*Sp*Rad8						
*Sc*Rad5	9516.1 (*rad-5*)	*S. pombe*	1e-124	1e-167	1e-75	1e-118
*Sc*Rad26/*Sp*Rhp26						
*Hs*CSA/*Sc*Rad26	4229.1 (*csa*)	*M. musculus*	6e-54	4e-58 (Rhp54, Rhp26)	1e-115	1e-118
Sp *Rhp26*/Hs*CSB*	7837.1 (*csb*)	*S. pombe*	9e-64 (Sth1)	0 (Rhp26)	1e-153	1e-157
*Sc*Rad16						
*Sc*Rad16	3650.1 (*rad-16*)	*S. cerevisiae*	0.0	0.0	3e-61	1e-114
*Sc*Rad16-like	3652.1	*S. pombe*	2e-74	1e-134	2e-31	4e-31
*Sc*Rad54						
*Sc*Rad54	2349.1 (*mus-25*)	*M. grisea*; 0.00	0.0	9e-91	0.0	1e-135
*Sc*Rad54-like	6190.1 (*atrx*)	*O. sativa*	1e-48 (Rad54)	7e-55 (Rhp54)	4e-71	2e-72

[a] *Drosophila melanogaster*, *Anopheles gambiae*, *Mus musculus*, or *Homo sapiens*.
[b] *Arabidopsis thaliana* or *Oryza sativa*.
[c] Species listed as best match is *Magnaporthe grisea*.

binding transcriptional regulatory proteins encoded by the *Neurospora* genome. This preliminary examination reveals that *Neurospora* gene regulation shares elements of both the simple and more complex metazoan models (Fig. 3).

$Zn(II)_2Cys_6$ fungal binuclear cluster family. The largest class of transcription factors in the *Neurospora* genome belongs to the fungus-specific $Zn(II)_2Cys_6$ fungal binuclear cluster family. Gal4p, a transcriptional activator that regulates galactose utilization in *S. cerevisiae*, is the prototype for this transcription factor family. Structure studies revealed that Gal4p is a $Zn(II)_2Cys_6$ binuclear cluster protein (591). Three functional domains are characteristic of this transcription factor family: a C_6 zinc cluster, which is involved in DNA binding, a middle homology region, which is thought to be necessary for in vivo DNA binding specificity (685), and a third, less well understood activation domain.

The *Neurospora* genome contains 77 open reading frames (ORFs) with the $Zn(II)_2Cys_6$ motif. Twenty of these factors have no match to proteins in yeasts (Table 9). There are nine ORFs with homologues in other filamentous fungi. Interestingly, half of the putative $Zn(II)_2Cys_6$ motif proteins are most similar to other *Neurospora* $Zn(II)_2Cys_6$ fungal binuclear cluster proteins, a rare finding in the *Neurospora* genome. This result suggests a regulatory mechanism involving sets of factors and several novel mechanisms for gene regulation. The factor set may recognize the same promoter elements, but with different binding affinities, depending on activation or repression requirements. Alternatively, the factors may interact with one another, thus preventing DNA binding.

Only two of the $Zn(II)_2Cys_6$ proteins, NIT-4 and QA1F, have been characterized at the protein level in *Neurospora* (see below). NIT-4, the most extensively studied of the two, is required for nitrate assimilation and interacts with NIT-2, a GATA factor (see below), to activate expression of nitrate and nitrite reductases (230, 257, 892). The QA1F factor, which regulates quinic acid utilization, is another example where protein function and DNA binding properties have been determined (52). The *fluffy* gene product (FL), required for macroconidiation, is also a member of the $Zn(II)_2Cys_6$ family (40) but has not been characterized at the protein level. Of interest, the closest homologue of FL is a hypothetical protein (NCU09205.1) from *Neurospora*.

C2H2 zinc fingers. There are 43 C2H2 zinc finger transcription factors, making this the second largest class encoded in the genome (Table 10). Unlike the $Zn(II)_2Cys_6$ factors, which are unique to fungi, the C2H2 factors are found in both prokary-

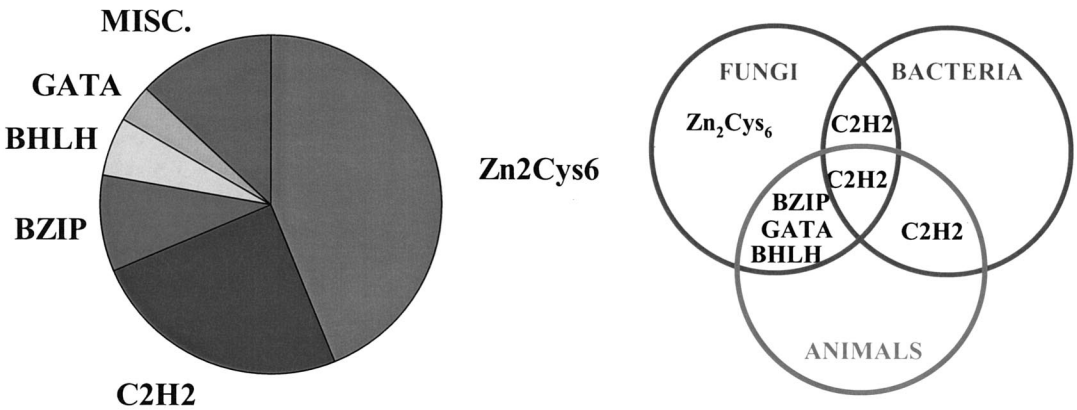

FIG. 3. Transcription factor distribution. (Left) proportion of analyzed *Neurospora* transcription factors in each of the indicated classes. (Right) Venn diagram showing the distribution of Zn_2Cys_6, C2H2, BZIP, and bHLH transcription factors in bacteria, fungi, and animals.

TABLE 9. $Zn(II)_2Cys_6$ fungal binuclear cluster family

NCU no.	Characterized *Neurospora* protein	Best BLAST match		
		Overall	Yeast[a]	Comment(s)
08042.1		*Arabidopsis thaliana*; 3.00e-27	*S. pombe* NP_5953118.1; 1.00e-12	
06799.1		*S. cerevisiae*; 9.00e-11	Best match	*A. thaliana*; 5.00e-10
07669.1		*A. nidulans* positive regulator of purine utilization; 0.00	*S. cerevisiae* positive regulator of URA1&3; 5.00e-21	
00054.1		*S. pombe* NP_593605; 3.00e-12	Best match	*A thaliana*; 1.00e-09
01478.1		*S. pombe* NP_592804; 1.00e-22		*Aspergillus niger* FacB; 6.00e-19
02934.1		*N. crassa* NCU07669.1; 3e-21	*S. cerevisiae*; 7.00e-11	
08000.1		*N. hocca* cutinase T F 1; 0.00		*Aspergillus oryzae* AmdR; 1.00e-56
07007.1		*S. pombe* NP_588286; 1.00e-07	Best match	
08294.1	NIT-4	*T. inflatum* CAB71797 0.00	*S. pombe* NP_587679; 4.00e-30	
08652.1		*Collectotrichum lindemuthianum*; 1.00e-110	*S. pombe* NP_595318; 9.00e-07	
08651.1		*N. crassa* NCU07669.1; 2.00e-23	*S. cerevisiae* NP_013114; 1.00e-11	
06656.1	ACU-15	FacB homolog from *Aspergillus niger*; 1.00e-153	*S. cerevisiae* CAT8; 2.00e-23	
02752.1		*N. crassa* NCU00217.1; 6.00e-64	*S. pombe* NP_595318; 4.00e-14	EAA27366
07374.1		*N. crassa* NCU07705.1; 1.00e-11	*S. cerevisiae* CAT8; 5.00e-05	
06407.1		*S. pombe* CAA22853; 2.00e-51	Best match	
05383.1		*S. cerevisiae* NP_012329; 2.00e-13	Best match	
00217.1		*N. crassa* NCU02752.1; 3.00e-56	None	
04866.1		*N. crassa* NCU02752.1; 5.00e-48	*S. cerevisiae* CAA63906; 7.00e-07	
03110.1		*N. crassa* NCU02752.1; 2.00e-11	None	
05994.1		*Aspergillus oryzae* TamA; 1.00e-108	*S. cerevisiae* Dal81p; 2.00e-80	
09033.1		*S. pombe* NP_587679; 7.00e-12	Best match	
05536.1		*S. pombe* NP_594098; 1.00e-05	Best match	
07705.1		*N. crassa* NCU00808.1; 4.00e-34	None	
04827.1		*S. pombe* NP_587726; 4.00e-22	Best match	
07788.1		*Aspergillus oryzae*; 3.00e-40	*S. cerevisiae* Malp; 3.00e-22	
06990.1		*S. pombe* NP_594000; 1.00e-23	Best match	
06028.1	QA1F	*Podospora anserina*; 1.00e-140	*S. pombe* BAA87112; 4.00e-08	WD repeats; quinic acid utilization activator
03120.1		*A. nidulans*; 9.00e-15	None	
01097.1		*N. crassa* NCU08049.1; 3.00e-53	None	
05411.1		*N. crassa* NCU06407.1; 5.00e-12	*S. pombe* NP_594160; 3.00e-05	EAA26713
07392.1		*Sordaria macrospora* Pro1 0.00	*S. cerevisiae* Ume6p; 5.00e-06	*S. macrospora* gene required for fruiting-body formation
08848.1		*N. crassa* NCU06407; 6.00e-08	None	
08726.1	Fluffy (FL)	*N. crassa* NCU09205.1; 2.00e-20	*S. pombe* NP_593001; 4.00e-06	
04359.1		*A. nidulans*; 2.00e-10	*S. pombe* NP_595318; 1.00e-05	
08049.1		*N. crassa* NCU01097.1 3.00e-55	None	
08899.1		*N. crassa* NCU02896.1; 6.00e-27	*S. pombe*; 2.00e-09	
03643.1		*Nectria haematococca*; 1.00e-153	*S. pombe*; 1.00e-14	Cutinase transcription factor 1 beta
01386.1		*N. crassa* NCU06407.1; 8.00e-06	*S. pombe*; 1.00e-05	
05767.1		*Fusarium pseudograminearum*; 2.00e-17	*S. cerevisiae* Ume6p/Car80p; 3.00e-06	EAA30506
04390.1		*N. crassa* NCU02896.1; 9.00e-28	*S. pombe* NP_593170; 2.00e-16	
03931.1		*N. crassa* NCU05993.1; 6.00e-07	*S. cerevisiae*; 3.00e-06	Upc2p involved in sterol uptake
2307.1		*S. pombe* NP_593160; 1.00e-10	Best match	*N. crassa* NCU04001.1; 9.00e-09
07139.1		*S. pombe* NP_593605; 2.00e-09		Some homology to *A. nidulans* PrnA (3.00E-06)
07945.1		*S. pombe* NP_592804; 9.00e-12	Best match	*A. nidulans* PrnA; 9.00e-11
03320.1		*Aspergillus parasiticus* Apa-2; 2e-04	None	
08658.1		*A. nidulans* ArcA; 2.00e-32	None	Regulatory gene in the arginine catabolic pathway
02094.1		*N. crassa* NCU00344.1; 5.00e-17	*S. cerevisiae* Leu3p; 5.00e-25, top hit	Leu3p regulates the levels of Leu1, Leu2, and Leu4
00808.1		*N. crassa* NCU07705.1; 8.00e-27	*S. pombe* NP_596765; 5.00e-06	
08443.1		*Aspergillus niger* AmyR; 6.00e-24	*S. cerevisiae* NP_116603; 6.00e-06	
09739.1		*N. crassa* NCU00017.1; 3.00e-39	*S. pombe* NP_593160; 5e-12	
06068.1		*N. crassa* NCU08294.1; 7.00e-15	*S. pombe* NP_595069; 1.00e-14	
00945.1		*N. crassa* NCU03489.1; 2.00e-05	None	
09829.1		*S. pombe* NP_5960; 4e-04	Best match	*N. crassa* NCU01478.1; 5e-05
06971.1		*Hypocrea jecorina* xylanase reg 1; 0.00	*S. pombe* NP_012136; 1.00e-06	Found in *A. niger* and *A. oryzae*
02214.1		*N. crassa* NCU00054.1; 7.00e-15	*S. pombe* NP_588286.1; 2.00e-08	
02896.1		*N. crassa* NCU04390.1; 1.00e-46	*S. pombe* NP_593170; 3.00e-14	

Continued on following page

TABLE 9—*Continued*

NCU no.	Characterized *Neurospora* protein	Best BLAST match		
		Overall	Yeast[a]	Comment(s)
02142.1		*Candida albicans* Fcr1p; 1.00e-08	*S. pombe* Ntf1; 3.00e-07	
02576.1		*S. pombe* NP_593467; 3.00e-04	Best match	
04001.1		*S. pombe* NP_593160; 3.00e-13	Best match	EAA32829
05294.1		*Bacillus subtilis* acetyltransferase; 2.00e-28		Wbbj, acetyltransferase domain
00017.1		*N. crassa* NCU09739.1; 2.00e-37	*S. pombe*; 5.00e-10	EAA27768
03686.1		*N. crassa* NCU03931.1; 1.00e-12	*S. cerevisiae* Upc2p; 4.00e-08	CAD11364
05993.1		*S. pombe* NP_59361; 4.00e-07	Best match	EAA29565
09804.1		*S. pombe* NP_594497; 8.00e-18	Best match	EAA3482
03417.1		*N. crassa* NCU09549.1; 4.00e-19	*S. pombe* NP_593170; 5.00e-08	EAA27628
09549.1		*N. crassa* NCU04851.1; 2.00e-38	*S. pombe* NP_593170; 4.00e-10	EAA29532.1
00289.1		*Aspergillus fumigatus*; 2.00e-54	None	EAA28534
08901.1		*N. crassa* NCU09804.1; 8.00e-05	None	EAA29603.1
04851.1		*N. crassa* NCU09549.1; 4.00e-34	*S. cerevisiae* Rdr1p; 5.00e-06	EAA28676
02768.1		*N. crassa* NCU02896.1; 2e-32	*S. pombe* NP_593170; 3.00e-24	EAA36342
08063.1		*Nectria haematococca*; 2.00e-06	None	
07535.1		*N. haematococca* flanks pea pathogenicity (PEP) cluster; 9.00e-12	*S. pombe* Ntf1; 1.00e-06	EAA29812.1
03489.1			*S. cerevisiae* YKR064wp; 2.00e-05	EAA26640
09205.1		*A. nidulans* NIRA; 2.00e-45	*S. pombe* NP_595069; 6.00e-12	EAA29913.1
05051.1		*N. crassa* NCU03417.1; 5e-25	*S. pombe* NP_593170; 1e-16	
09529.1		*N. crassa* NCU07575.1; 8E-04	*S. cerevisiae* NP_013610; 0.003	EAA28818
08289.1		None below 0.003	None below 0.003	EAA33266

[a] *S. cerevisiae* or *S. pombe*.

otic and eukaryotic organisms (99). What is striking about this class of transcriptional regulators is that they can be easily separated into two distinct groups. About half are most homologous to *S. cerevisiae* and *S. pombe* protein sequences, while the remainder are most similar to proteins from filamentous fungi and animals. Several members of the latter group have homology to Krüpple-type ZNF transcription factors from mice, rats, and humans (Table 10). None of the C2H2 zinc finger transcription factor proteins have yet been characterized in *Neurospora*.

The *Neurospora* Ste12p-like transcription factor (NCU00340.1) has a C2H2 domain as well as a homeodomain, and it has five homologs in available databases: *A. nidulans* SteA (819), *Magnaporthe grisea* Mst12 (597), *Gibberella zeae* Fst12, *Colletotrichum lagenarium* Cst1 (805), and *Penicillium marneffei* St1A (96). Ste12p from *S. cerevisiae* has been extensively characterized and shown to play regulatory roles during mating for haploid cells (200, 222, 234) and filamentous growth of diploids (489). The *S. cerevisiae* protein lacks a C2H2 domain. The conservation of this protein in filamentous fungi is remarkable, suggesting that this regulatory element is critical and necessary for mating and development. Three of the genes encoding fungal homologues (SteA, CST1, and Mst12) have been deleted in their respective organisms. The *steA* deletion strain is sterile and exhibits defects in ascogenous tissue and fruiting-body development, but no effect on vegetative growth or morphology was detected (819). The *cst1* deletion mutant is impaired in the production of infectious hyphae from appressoria, rendering it nonpathogenic (805). When the *mst12* deletion mutants were tested on onion epidermal cells, appressoria appeared normal but could not penetrate and carry out infectious growth. These phenotypes reveal important roles for Ste12p-like proteins in developmental pathways and pathogenesis.

The dichotomous results found with C2H2 transcription factors, half with homology to proteins from unicelluar yeasts and the other half with sequences from filamentous fungi and animals, suggest that these factors represent a point of divergence in promoter evolution and gene regulation. A recent study of human and mouse C2H2 factors offers evidence supporting continuing evolution of C2H2 zinc finger proteins. There are several familial gene clusters encoding C2H2 zinc finger proteins in these vertebrates. A careful analysis of a syntenic region in humans and mice suggested that the analyzed clusters arose from tandem duplications with eventual divergence, resulting in a large assortment of this type of factor (722). Unlike other gene family clusters in mammals, specifically those involved in immunity and smell, these did not contain pseudogenes. This suggests that C2H2 zinc finger gene family is still evolving in mice and humans.

GATA factors. The founding member of the extensive family of GATA transcription factors, GATA-1, was cloned in 1989 and shown to regulate erythroid cell differentiation in vertebrates (226, 804). GATA transcription factors bind (A/T)GAT A(A/G) motifs and have one or two Cys-X_2-Cys-X_{17}-Cys-X_2-Cys zinc fingers. Unlike the C2H2 zinc finger proteins, GATA factors are found only in eukaryotic organisms. Although only six have been identified in the *N. crassa* genome, all but one has been cloned, making this class of transcription factors the most extensively characterized in this organism (Table 11). *Neurospora* GATA factors regulate critical processes, ranging from nitrogen utilization and iron uptake to light regulation and ascospore development.

TABLE 10. C2H2 zinc finger transcription factors

NCU no.	Best BLAST match		
	Overall	Yeast[a]	Comment(s)
03975.1	*Mus musculus*; 2.00e-33	None	Three domains
10025.1	*Mus musculus*; 2.00e-33	None	Identical to above
02699.1	*S. cerevisiae*; 6.00e-24	Best match	Zap1p DNA binding protein; zinc ion homeostasis
02666.1	*S. cerevisiae*; 2.00e-15	Best match	Crz1p calcineurin responsive
04179.1	*S. cerevisiae* NP_014756; 1.00e-49	Best match	Asparagine-rich zinc finger
03421.1	*Homo sapiens* locus AAH07307; 4.00e-27	None	Two domains
06907.1	*S. pombe* NP_594109; 4e-25	Best match	*S. cerevisiae* Ace2p; 2.00e-16
00038.1	*S. pombe* NP_594670; 4.00e-36	Best match	TFIIIA from frog
01629.1	Zebrafish NP_571798; 4.00e-12	None	Krupple-like factor
07952.1	*S. cerevisiae* NP_014371; 2.00e-37	Best match	One domain; Crz1p calcineurin responsive
02853.1	*Colletotrichum lagenarium* CMR1; 2.00e-12	*S. cerevisiae* NP_012661; 8.00e-08	
02671.1	*Nectria haematococca* MPV1; 3.00e-65	*S. cerevisiae* YER130cp; 6.00e-20	Cutinase G-box binding protein
02173.1	*Colletotrichum lagenarium* CMR1; 1.00e-12	*S. cerevisiae* Adr1p; 3.00e-07	Two domains
06503.1	*Emericella nidulans* AmdA; 3.00e-16	S. cerevisiae YM1081Wp; 2.00e-06	Two domains; positive-acting TF
04561.1	*Colletotrichum lagenarium* CMR1; 6.00e-23	*S. pombe* NP_592812; 9.00e-08	
08807.1	*Trichoderma reesei* Cre1; 4.00e-82	*Debaryomyces occidentalis* Mig1 protein; 5E-23	Two domains; CRE-1 carbon catabolite repressor
05064.1	*S. cerevisiae* NP_015094; 7e-13	Best match	Up-regulated during starvation in *S. cerevisiae*
10006.1	*Homo sapiens* BAA91019; 4.00e-11	None	
00694.1	*D. willistoni* AAO01096; 5.00e-06	None	Two domains
06487.1	*Rattus norvegicus* XP_234852; 1.00e-06	None	Two domains
09576.1	*Drosophila* sp. Sp1/egr-like; 8.00e-08	*S. cerevisiae* MET32; 3.00e-06	Two domains; regulator of sulfur metabolism
03043.1	*Podospora anserina* CAD12881; 1.00e-75	None	Two domains; *fle* gene coordinates male and female sexual differentiation
02994.1	*Ascobolus immersus* CAA67549; 5.00e-45	None	
05285.1	*Emericella nidulans* AA024631; 1.00e-29	*S. pombe* 0.018	
03184.1	*S. pombe* CAB61785; 8.00e-10	Best match	Four domains
01122.1	*S. pombe* NP_594996; 9.00e-28	Best match	Two domains
03699.1	*Ascobolus immersus* CAA67549; 4.00e-62	None	Four domains
05242.1	*S. pombe* NP_594996; 7.00e-47	Best match	
03206.1	*S. pombe* CAB59682; 4.00e-35	Best match	COG5048 SFP: putative transcriptional repressor regulating G_2/M transition
00340.1	*C. langenarium* BAC11803.1; 0.00	*S. cerevisiae* NP_011952; 2.00e-54	STE12 has both a homeodomain and the C2H2 domain
	M. grisea Mst12; 0.0		
	G. zeae AF509440; 0.0		
	E. nidulans SteA; 0.0		
	P. marneffei AF284062; 0.0		
02713.1	*S. cerevisiae* NP_009622; 2.00e-09	Best match	RG1 transcriptional repressor—invasive growth
05909.1	*Homo sapiens* NP_114124.1; 4.00e-11	None	Two domains; BTE binding protein 4
09333.1	*Hypocrea jecorina* AAF35286; 1.00e-180	None	*ace1* gene regulates activity of the cellulase promoter *chb1* in *Trichoderma reesei*
09252.1	*N. crassa* NCU04628.1; 1.00e-07	None	Three domains
00090.1	*Colletotrichum sublineolum*; Pac C 5.00e-67	*S. cerevisiae* RIM1; 5.00e-28	Regulates meiosis in yeast
03552.1	*Mus musculus* LOC244674; 3.00e-06	None	Two domains
04619.1	*Aspergillus fumigatus* CAD28447; 2.00e-31	None	
00385.1	*N. crassa* CAD37059; 8e-14	None	
06186.1	*Aspergillus nidulans* AAF15889; 1e-33	*S. pombe* NP_594670; 4.00e-05	Four domains
04628.1	*N. crassa* NCU09252.1; 2.00e-07	None	Two domains
00285.1	*N. crassa* NCU04619.1; 9.00e-14	None	
06919.1	*Rattus norvegicus* NP_579846; 7.00e-09	None	Six domains

[a] *S. cerevisiae*, *S. pombe*, or *Debaryomyces occidentalis*.

NIT-2, a positive regulator of nitrate assimilation and nitrogen utilization, was shown to have one DNA binding motif (262). Careful analysis of NIT-2 binding to promoters of nitrogen-regulated genes revealed that two closely spaced GATA sites were required for high-affinity binding by this factor to the DNA (146, 231). Another GATA factor, SRE, was isolated using a probe generated from a PCR with degenerate primers to the conserved zinc finger region (899). This GATA factor has two zinc fingers and sequence similarity to several fungal proteins, SreP from *P. chrysogenum*, UrbS from *U. maydis*, and SreA from *A. nidulans*. All are negative regulators of siderophore synthesis (317, 318, 835). SRE negatively regulates siderophore production in *Neurospora* (898). Analysis of a *sre* null mutant (898) revealed the existence of other regulators involved in maintaining iron homeostasis. However, these components have not been identified. The cloning and sequencing of the genes that encode White Collar 1 (WC-1) and White Collar 2 (WC-2) revealed that they both contain a single GATA transcription factor motif (42, 478). These GATA factors mediate responses, including input for the circadian clock and light-induced expression of genes that regulate blue light processes, as well as functioning exclusively in

TABLE 11. GATA factors

NCU no.	Characterized *Neurospora* protein	Best BLAST match		
		Overall	Yeast[a]	Comment(s)
09068.1	NIT-2	*Colletotrichum lindemuthiannum*; 1.00e-139	*S. cerevisiae* YFL021w BAA09217; 8.00e-14	One GATA domain; Major nitrogen regulatory protein
07039.1	ASD-4	*Penicillium chrysogenum*; 7.00e-41	*S. cerevisiae* Gzf3p/Dal80; 4.00e-19	One GATA domain
07728.1	SRE	*Botryotinia fuckeliana*; 3.00e-44	*S. cerevisiae* UGA43; 9.00e-09	Two domains; siderophore regulation protein
00902.1	WC-2	*Nectria haematococca*; 1.00e-153	None	One GATA domain; one PAS domain; palindrome-binding protein (PBP)
02356.1	WC-1	*Podospora anserina*; 0.00	None	One GATA domain; three PAS domains; two PAC motifs
01154.1		*Penicillium chrysogenum*; 2.00e-11	None	One GATA domain

[a] *S. cerevisiae* or *S. pombe*.

the dark as the positive elements of the core feedback loop of the circadian clock (see "Photobiology and circadian rhythms" below). Each factor has PAS domains; WC-1 has three, and WC-2 has only one. One of the PAS domains in WC-1 is also a LOV (for "light, oxygen, or voltage") domain, a motif that was recently shown to be required for light sensing in *Neurospora* (144). The final characterized GATA factor is ASD-4, which is required for ascospore development (229). The exact role of ASD4 in this developmental pathway is not known.

There are four characterized GATA factors in *S. cerevisiae;* Gln3p, Nil1p/Gat1p, Dal80p, and Gfz3p/Nil2p (155, 175, 531, 753). All four participate in nitrogen regulation of gene expression, with the first two acting as positive regulators and the last two acting as negative regulators. It appears that the fungal GATA factors regulate processes that require exquisite balance in maintaining metabolic homeostasis. In nitrogen utilization, it is suboptimal for an organism to expend cellular energy taking up metabolites that it does not need. However, since nitrogen is essential, a critical level must be sustained. In addition to regulating nitrogen utilization in *Neurospora*, GATA factors regulate genes required for iron homeostasis and adaptation to blue light. Iron and light are necessary for normal growth and development in this organism; however, excesses are known to be deleterious.

bHLH transcription factors. The basic helix-loop-helix (bHLH) motif was first described in mammals (551) and is unique to eukaryotes. This motif consists of conserved bipartite domains that dictate DNA binding and protein dimerization (551). A phylogenetic analysis based on 122 divergent bHLH sequences revealed that bHLH factors fall into four groups based on the DNA binding and protein interaction domains (32). Ten predicted bHLH or HLH proteins have been identified in the *Neurospora* genome sequence (Table 12); only one has been cloned and characterized, the *nuc-1* gene product, which was shown to regulate phosphorous utilization (399). All predicted *Neurospora* bHLH proteins are more similar to other fungal bHLH proteins than to animal bHLH proteins, and all are members of the B group (32). Interestingly, this family of transcription factors represents one of the largest found in the *A. thaliana* genome, with over 133 members (647).

B-ZIP transcription factors. The defining B-ZIP motif is a bipartite α-helix between 60 and 80 amino acids in length, with a DNA binding region of two basic clusters at the N terminus and a dimerization domain, formed by an amphipathic helix with a leucine every 7 amino acids, at the C terminus (833). The heptads, which comprise the leucine zipper, can be of different lengths and can participate in dimerization. These factors can homodimerize, heterodimerize, or homo- and heterodimerize, giving each transcription factor the capability of multiple functions.

TABLE 12. bHLH transcription factors

NCU no.	Characterized *Neurospora* protein	Best BLAST match		
		Overall	Yeast[a]	Comment(s)
02957.1		*Aspergillus nidulans*; 2.00e-21	*S. pombe* NP_593230; 2.00e-08	bHLH sexual differentiation protein Esc1p
06744.1		No hits below 1.00e-05	None	BHLH; CAD70402
01871.1		*N. crassa* XP_328825; 9.00e-56	*S. pombe* NP_596545; 7.00e-44	HLH/MCM; Mcm7p
00749.1		*Fusarium culmorum* ANN73248; 8.00e-10	*S. pombe* NP_595229; 0.075	HLH I domain; USF protein
03077.1		*N. crassa* XP_331923; 3.00e-06	*S. pombe* NP_595229; 0.002	HLH; *A thaliana* H84860 1e-05
02724.1		*A. nidulans* AAG49357; 1.00e-24	*S. cerevisiae* NP_009447; 2.00e-06	bHLH; GLCD beta An; Rtg3pSc
05970.1		*N. crassa* XP_324088; 1.00e-13	*S. pombe* NP_595694; 1.00e-13	HLH
04731.1		*N. crassa* XP329061; 3.00e-23	*S. pombe* NP_595694; 5.00e-23	HLH
00144.1		*Fusarium culmorum* ANN73248; 1.00e-14	*S. cerevisiae* NP_014989; 0.42	HLH
09315.1	NUC-1	None	None	HLH; controls phosphorus aquisition

[a] *S. cerevisiae* or *S. pombe*.

TABLE 13. B-ZIP transcription factors

NCU no.	Characterized *Neurospora* protein	Best BLAST match		
		Overall	Yeast[a]	Comment(s)
04050.1	CPC-1	*Cryphonectria parasitica*; 5.00e-18	None	BZIP; general amino acid control
03905.1		*S. pombe* NP_593662; 1.00e-05	Best match	BZIP; AP-1-like transcription factor
08055.1		*Cladosporium fulvum*; 2.00e-09	None	BZIP
01345.1		*Claviceps purpurea*; 7.00e-87	*S. pombe* Atf CREB family; 6.00e-10	BZIP; Cptf1 involved in oxidative stress
01459.1		*S. pombe* Atf CREB family; 4.00e-11	4.00E-36	BZIP
00499.1		None less than 1e-05	None	BZIP
08744.1		None less than 1e-05	None	BZIP
07900.1		None less than 1e-05	None	BZIP
05637.1		None less than 1e-05	None	BZIP
00329.1		None less than 1e-05	*S. cerevisiae* Adr1p; 3.00e-07	BZIP
00233.1		*Apergillus nidulans* MeaB; 3e-41	None	BZIP
08891.1		*Apergillus oryzae*; 2.00e-05	None	BZIP
06399.1		None less than 1e-05	None	BZIP plus Rho guanine nucleotide exchange domain; EAA33001
07379.1		None less than 1e-05	None	BRLZ; EAA29636
04211.1		*N. crassa* NCU03905.1 5e-05	*Candida albicans;* 0.001	BZIP
01994.1		None less than 1e-05	*Candida albicans;* 0.002	BZIP; EAA35623

[a] *S. cerevisiae*, *S. pombe*, or *C. albicans*.

A total of 17 predicted B-ZIP proteins are present in the *Neurospora* genome sequence (Table 13). This number includes CYS-3, a well-characterized B-ZIP protein involved in regulating sulfur uptake and utilization (260, 396, 585), which escaped identification by the automated gene-calling program in the present WICGR assembly. Based on the reported number of unique B-ZIP proteins for other sequenced genomes, 53 for human (832), 27 for *Drosophila*, and 17 for *S. cerevisiae* (228), it appears that the majority of this class of transcription factors has been identified in *Neurospora*.

In addition to CYS-3, the *Neurospora* B-ZIP protein CPC-1, a Gcn4p ortholog, has been previously characterized. Gcn4p and CPC-1 were first identified as regulators of genes expressed during amino acid starvation or imbalance (46, 339, 589). The global nature of this regulation in *S. cerevisiae* was reported previously (28), and a recent review illustrates the central position of Gcn4p in regulating responses to environmental signals (340).

Half of the other identified *Neurospora* B-ZIP proteins have no significant homology (E values greater than 1e-05) to any protein sequences presently in GenBank. The remainder are most similar to proteins from other filamentous fungi and *S. pombe;* only NCU04050.1 (CPC-1) has highest homology to the *S. cerevisiae* Gcn4p protein (Table 13).

Miscellaneous factors. An additional 23 putative DNA binding proteins are listed in Table 14; they include 4 CBF CAAT binding factors, 3 forkhead domain proteins, 6 homeodomain, and 6 RING finger and WD repeat proteins. These factors play important roles in the biology of eukaryotic organisms. They link transcription to the cell cycle, RNA metabolism, meiosis, mitosis, cell death, DNA repair, chromatin remodeling, and nucleosome assembly. *Neurospora* shares four CBF CAAT binding factors with yeast. However, it has three winged-helix forkhead factors, one more than identified in *S. cerevisiae* (Table 14). Included in this list is RCO-1 (869), a protein similar to *S. cerevisiae* Tup1p, which plays a role in transcriptional repression. The identified homeodomain proteins have higher homology to filamentous fungi and metazoans than to unicellular yeasts. This is also true of proteins with the RING finger motif; of this group, only *Neurospora* UVS-2, similar to *S. cerevisiae* Rad18p, has been characterized (792).

Translation Factors

Many polypeptide components comprise the factors important for translation initiation, elongation, and termination (747). Analysis of the genome sequence shows that hypothetical *Neurospora* proteins homologous to cytoplasmic translation factors in other organisms (Table 15) are often incorrectly annotated (e.g., the predicted polypeptides contain N-terminal extensions, or the genes from which they are conceptually translated have incorrectly predicted intron structures). Nevertheless, several conclusions can be reached concerning the nature of eukaryotic initiation factors (eIFs), elongation factors (eEFs), and release (termination) factors (eRFs) in *Neurospora*.

The *Neurospora* genome contains essentially the same complement of translation factors as do the *S. cerevisiae* and *S. pombe* genomes. Each contains a gene for eEF3, while the animal and plant genomes lack a closely related polypeptide (20). Unlike animals and/or plants, these fungi lack eIF3j, eIF3k, and eEF1Bβ. One difference between *Neurospora* and the two yeasts is that the former has a protein similar to the eIF3l polypeptide that is known to be in the plant eIF3 complex, which is present in animals and plants but lacking in the yeasts (118). The function of this protein is unknown.

The predicted *Neurospora* translation factors more closely resemble those of *S. pombe* than those of *S. cerevisiae*. In the majority of cases, the *Neurospora* translation factor has a primary sequence more similar to the *S. pombe* factor. The *Neurospora* and *S. pombe* genomes, as well as animal and plant genomes, contain sequences for eIF3d-f and eIF3h, while *S. cerevisiae* lacks similar polypeptides. The *Neurospora* and *S. pombe* genomes both lack apparent homologues of eIF4B. This

TABLE 14. Miscellaneous transcription factors

NCU no.	Characterized *Neurospora* protein	Best BLAST match		
		Overall	Yeast[a]	Comment(s)
03033.1		*Hypocrea jecorina* HapB; 9.00e-34	*S. cerevisiae* Hap2p; 1.00e-17	CBF CAAT binding factor
09248.1		*Hypocrea jecorina* Hap3; 3.00e-49	*S. cerevisiae* Hap3p; 2.00e-26	CBF CAAT binding factor/HMF
03073.1		*Ratius norvegicus*; 2.00e-06	*S. pombe*; 1.00e-11	CBF CAAT binding factor/HMF; EAA34930
02017.1		*R. norvegicus* TBP binding NC2; 1.00e-18	*S. pombe* NP_596283; 6.00e-29	CBF CAAT binding factor/HMF; EAA35646
00019.1		*S. cerevisiae* Fkhp1; 5.00e-41	Best match	Two forkhead domains
06173.1		*S. pombe* Hnf-3; 2.00e-28	Best match	One forkhead domain; regulation of septation
08634.1		*S. cerevisiae* AAA99643; 1.00e-07	Best match	One forkhead domain
03244.1		*Branchiostoma floridae*; 2.00e-72	*S. pombe* NP_595227; 8.00e-46	WD repeats; *D. melanogaster* (4.00e-71) will die slowly
06205.1	RCO-1	*Penicillium marneffei*; 1.00e-167	*S. pombe* NP_592873; 1.00e-117	WD repeats; Transcriptional Repression
06411.1		No hits below 1e-05	None	RING-type zinc finger
03593.1		*Podospora anserina* CAC16792; 0.00	*S. cerevisiae* NP_010177/Pho2p; 9e-09	Homeobox
03070.1		*D. melanogaster* AAD38649; 1.00e-07	*S. cerevisiae* CAA44264; 9.00e-04	Homeobox
03266.1		*M. musculus* ACC53336; 7.00e-06	None	Homeobox
05257.1		*D. discoideum* AA052126; 7.00e-10	None	Homeobox; *Lycopersicon esculentum;* 3e-08
00097.1		*H. sapiens* AAC51243; 5.00e-13	*S. cerevisiae* NP_015148/Cup9p; 1.00e-08	Homeobox
05250.1		*Coprinus cinereus* S71461; 2.00e-65	*S. pombe* NP_592917.1; 3.00e-66	Homeobox; sister chromatid cohesion molecule
03962.1		*H. sapiens* BAB14697; 5.00e-06	*S. pombe* NP_594050; 1.00e-09	Human herpesvirus 6 immediate early protein
05210.1	UVS2	*A. nidulans* CAA90033; 1.00e-63	*S. pombe* NP_595423; 1.00e-32	RING finger UVS2
06213.1		*S. pombe* NP_593123; 6.00e-38	*S. cerevisiae* NP_010697; 1.00e-34	MIZ zinc finger RING finger
07561.1		*H. sapiens* BAA96066; 2.00e-12	*S. pombe* NP_588450; 2.00e-17	PHD-finger
00631.1		*S. pombe* NP_596602; 1.00e-118	Best match	SNF2/RING/Helicases; *A. thaliana* NP_564568; 1.00e-52
01954.1		*H. sapiens* CAC42525; 1.00e-109	*S. pombe* NP_596357; 6.00e-79	RING finger; C3HC4
03356.1		*M. musculus* P70191; 9.00e-05	None	Related to TRAF5; RING-type zinc finger

[a] *S. cerevisiae* or *S. pombe*.

polypeptide is poorly conserved between *S. cerevisiae* and higher eukaryotes and thus may have gone undetected because of low sequence similarity, since the closest *S. pombe* match to eIF4B, Sce3p, does not have an obvious homologue in *Neurospora*.

GENOME DEFENSE, DNA REPAIR, AND RECOMBINATION

Genome Defense Mechanisms

A basic tenet of classical genetics is the notion that the structure and behavior of an organism are determined by the structure and workings of its genome. In contrast, the idea that the structure and behavior of a genome may reflect, at least in part, the structure and behavior of the organism is relatively new. Some of the earliest and strongest evidence for active processes that can shape genomes has come from studies of *Neurospora*. This organism provided the first example of a eukaryotic genome defense system, RIP (Fig. 4) (709, 712). Like most filamentous fungi, plants, and many animals, but in contrast to *S. cerevisiae* and *S. pombe*, *Neurospora* can methylate its DNA (712). More recently, studies have shown that *Neurospora* employs at least two additional epigenetic systems that appear well suited to help maintain its streamlined genome: quelling and meiotic silencing (Fig. 4) (24, 131, 732). Repeated sequences are detected and inactivated during haploid, vegetative growth by a vegetative RNA silencing mechanism called quelling (157, 158, 162). Quelling produces diffusible signals, small interfering RNAs, that interfere with the propagation of the repeated element within nuclei in the same cytoplasm (132). During meiosis, *Neurospora* cells use a process related to RNA interference to check for unpaired sequences; this process is called meiotic transvection (24, 25) and meiotic silencing (732). The many convenient features of *Neurospora*, including its streamlined genome, facile genetics, and well-developed molecular tools, have made this organism a preferred model for the study of genomic surveillance processes.

Heterochromatin silencing and DNA methylation. The terms "gene silencing," "transcriptional silencing," "silent chromatin," and "heterochromatic silencing" are often used interchangeably to describe processes that, in contrast to promoter- or gene-specific transcriptional regulation, act on larger chromosomal regions (for reviews, see references 34, 299, and 535). Stably inherited gene silencing is called "epigenetic" because phenotypes are maintained over many generations but

TABLE 15. *Neurospora* translation factors

Protein factor class	NCU no.	BLAST matches				
		Closest[c]	*S. cerevisiae*	*S. pombe*	Animal[a]	Plant[b]
Initiation factors						
eIF1	01981.1	Sc	2e-31	7e-28	3e-25	4e-25
eIF1A	07437.1	Animal	2e-32	2e-37	2e-39	1e-29
eIF2α	08277.1	Sp	2e-88	3e-89	2e-62	4e-67
eIF2β	04640.1	Sc	4e-49	9e-49	2e-45	2e-43
eIF2γ	02810.1	Sc	0	0	0	0
eIF2Bα	04344.1	Sp	2e-30	6e-32	7e-20	6e-16
eIF2Bβ	04640.1	Sc	1e-49	2e-49	5e-46	4e-44
eIF2Bγ	03548.1	Sp	2e-18	2e-19	2e-16	
eIF2Bδ	01468.1	Sp	3e-31	4e-43	5e-31	1e-33
eIF2Bε	02414.1	Sp	2e-80	4e-95	4e-59	6e-67
eIF3a	00040.1	Sp	4e-87	e-167	e-122	e-103
eIF3b	02208.1	Sp	e-114	e-167	e-120	e-114
eIF3c	07831.1	Sp	e-108	e-180	e-102	e-135
eIF3d	07380.1	Sp		e-136	e-87	e-90
eIF3e	05889.1	Animal		3e-85	3e-91	7e-85
eIF3f	01021.1	Sp		5e-62	7e-30	4e-26
eIF3g	08046.1	Animal	6e-19	2e-19	3e-27	8e-22
eIF3h	07929.1	Sp		3e-59	2e-41	9e-54
eIF3i	03876.1	Sp	1e-99	e-119	4e-93	8e-73
eIF3j						
eIF3k						
eIF3l	06279.1	Animal			e-113	8e-73
eIF4A	07420.1	Sp	e-146	e-177	e-162	e-149
eIF4B						
eIF4E	02076.1	Sp	3e-37	1e-48	5e-27	4e-22
eIF4G	07868.1	Sp	3e-47	3e-82	4e-42	8e-26
eIF5	00366.1	Sp	4e-83	8e-88	7e-62	4e-34
eIF5A	05274.1[d]	Sc	2e-51	2e-46	3e-48	2e-40
eIF5B	05270.1	Sc	0	0	e-166	0
eIF6	09004.1	Sc	e-103	e-100	5e-97	7e-94
PABP	04799.1	Sp	e-130	e-157	e-125	e-116
Elongation factors						
eEF1A	02003.1	Sp	0.00	0.00	0.00	0.00
eEF1Bα	06035.1	Sp	2e-26	5e-49	2e-44	1e-30
eEF1Bβ						
eEF1Bγ	03826.1	Sc	2e-81	2e-71	7e-54	9e-43
eEF2	07700.1	Sc	0.00	0.00	0.00	0.00
eEF3	07922.1	Sc	0.00	0.00	7e-44	1e-42
Termination factors						
eRF1	00410.1	Animal	e-176	e-166	0.00	e-174
eRF3	04790.1	Sp	e-159	e-164	e-147	e-133

[a] *Anopheles gambiae*, *Drosophila melanogaster*, *Homo sapiens*, *Mus musculus*, *Oryctolagus cuniculus*, *Rattus norvegicus*, or *Xenopus laevis*.
[b] *Arabidopsis thaliana*, *Oryza sativa*, *Triticum aestivum*, or *Zea mays*.
[c] Sc, *S. cerevisiae;* Sp, *S. pombe*.
[d] BLAST results obtained using Protein Accession AAA61707.1.

are not determined by the DNA component alone. Two major, evolutionarily distinct mechanisms for chromatin-associated gene silencing that operate through posttranslational histone modifications have emerged, currently best characterized in the yeasts *S. cerevisiae* (Sir-mediated silencing) and *S. pombe* (HP1/Swi6-mediated silencing) (reviewed in references 34, 275, 299, 353, 384, 535, and 651). *Neurospora* contains homologues of most proteins previously implicated in gene silencing. Some proteins considered key in the silencing pathways of *S. cerevisiae*, *S. pombe*, plants, and *Drosophila* are absent from the *Neurospora* proteome, however. In contrast to *S. cerevisiae*, *S. pombe*, and *Drosophila*, *Neurospora* also uses DNA methylation to inactivate genes.

In *S. cerevisiae*, some chromosomal domains are transcriptionally silenced by the action of complexes that contain Sir proteins (275, 353). *Neurospora*, like *S. pombe*, plants, and metazoans, does not have homologues of *S. cerevisiae* Sir1p, Sir3p, Sir4p, and Net1p. Sir2p-like deacetylases (sirtuins) are the only members of Sir complexes conserved from bacteria to eukaryotes, and seven putative *Neurospora* sirtuins have been identified (see "Chromatin assembly and gene regulation" above).

Formation of heterochromatin in *S. pombe*, plants, and metazoans is achieved by HP1/Swi6-mediated silencing (reviewed in references 34, 299, 353, 535, and 651). As outlined in "Chromatin assembly and gene regulation" (above), *Neurospora* has components of this pathway, e.g., well-conserved HATs of the GNAT and MYST type, HDACs of the yeast Rpd3/Hos/Hda group, bromodomain proteins which are involved in the recognition of acetylated lysines, at least 3 putative histone kinases and 3 phosphatases, and 12 known or putative HMTs. Most Su(var)3-9 lysine methyltransferases contain a chromodomain within their amino terminus, but *Neurospora* DIM-5 lacks this domain (776). Curiously, *Neurospora* has two short chromodomain proteins (CDP-1 and CDP-2 [Table 16]) that bear Su(var)3-9-like chromodomains. *Neurospora* also has a single HP1/Swi6 homologue, heterochromatin protein 1, (HP1), characterized by an N-terminal chromodomain and C-terminal chromoshadow domain. In fission yeast, the formation of heterochromatin at the silent mating-type locus and the centromeres is dependent on homologues of the RNA interference machinery (307, 837), a process that, at least in plants, may involve DNA methylation (277, 524).

Over the last 15 years, *Neurospora* has been developed as a model to investigate the control and function of DNA methylation in eukaryotes, an area which attracted increased attention with the realization that abnormal methylation is commonly associated with cancer and other diseases (54, 73, 387). *Neurospora* has proven to be particularly useful in efforts to elucidate the establishment of methylation. In *Neurospora*, about 1.5% of the cytosines (C's) are methylated (246, 669), but no methylation has been reported for any of the protein-coding genes that have been studied. Three naturally methylated regions have been characterized in great detail: the tandemly arranged rDNA (611), the 1.6 kb zeta-eta (ζ-η) region (526, 714–716), and the psi-63 (ψ63) region (246, 500, 525, 527). The last two regions are relics of RIP. It is possible that almost all DNA methylation in the wild-type *Neurospora* genome, including the limited methylation of the rDNA, is a result of RIP. Indeed, a survey of methylated *Neurospora* sequences isolated by affinity chromatography, using the methyl binding domain of mammalian MeCP2, revealed clear evidence of RIP in nearly all sequenced fragments (717). It is clear, however, that sequences not exposed to RIP can be methylated in the genome. For example, some transforming sequences are subject to de novo methylation without going through the sexual cycle (114, 575, 594, 662, 706, 714, 715). Detailed analyses of short DNA segments constructed in vitro revealed that the methylation machinery preferentially recognizes AT-rich regions as short as 75 bp (526, 777). Methylation indirectly blocks transcription elongation in *Neurospora* (664)

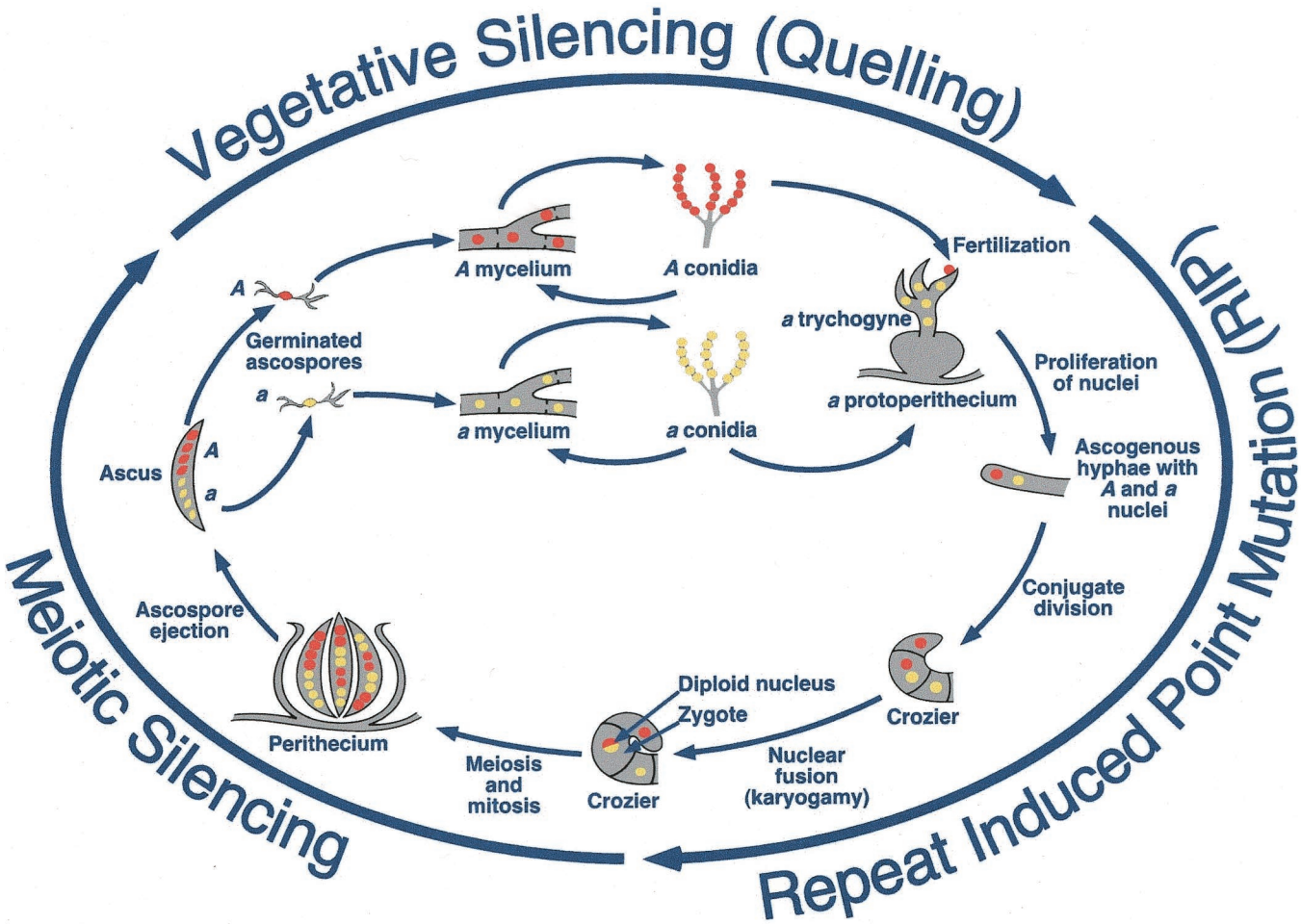

FIG. 4. *Neurospora* silencing pathways. The stages of the life cycle of *Neurospora* are presented, indicating points where the various gene silencing pathways are active. Quelling is a post-transcriptional gene silencing (PTGS) pathway that is active in the vegetative phase of the life cycle, from germination of ascospores or conidia to formation of the mycelium and differentiation of conidiophores and conidia. RIP and meiotic silencing are silencing pathways that are specific to the sexual cycle but differ in their molecular mechanisms. RIP scans for the presence of duplicated copies of DNA fragments present in the genomes destined to participate in meiosis. Duplicated regions are inundated with a series of transition mutations, and most of the remaining nonmutated cytosine bases are methylated. This process occurs in the heterokaryotic ascogeneous tissue formed following fertilization but prior to karyogamy. Meiotic silencing, like quelling, is a PTGS-like mechanism that is activated when a discrete region of DNA fails to sense (i.e., *trans*-sense) an equivalent region in the opposite chromosome. This failure of *trans*-sensing in turn triggers the silencing of all genes contained in the loop of unpaired DNA.

and can silence transforming DNA (365). Hence, DNA methylation may serve as a genome defense system (707) distinct from quelling (131) and meiotic silencing of unpaired DNA (24, 732).

A connection between one of the steps in HP1/Swi6-mediated silencing, histone H3 methylation, and genome-wide DNA methylation was first shown in *Neurospora* (776). More recently, this connection has been established in *Arabidopsis* (375, 386, 496) and in animals (39, 264, 465). *Neurospora* has two cytosine DNA methyltransferase (DMT) homologues, DIM-2 and RID, but no homologues of plant chromomethylases, plant domain rearranged methylases (DRMs), or the *S. pombe* PMT1 and human DNMT2 candidates for DMT pseudogenes. DIM-2 is required for all known DNA methylation in *Neurospora* (247, 433, 712), whereas RID, a member of a family of putative DMTs known only in filamentous fungi, is required for RIP (253). *Neurospora* does not have proteins that bear canonical methyl-binding domains (MBDs), suggesting the existence of alternative proteins involved in the recognition and maintenance of methylated DNA, compared to the situation for *Arabidopsis* and mammals (712). As indicated above, SWI/SNF-like putative CRFs from *Arabidopsis* (DDM1) and humans (LSH) are involved in DNA methylation (for reviews, see references 100 and 651). DDM1 is essential to silence transposons and transgenes via control of histone H3 lysine 9 methylation (277). While *Neurospora* has a putative homolog of LSH (CRF5-1), another SWI/SNF-like *Arabidopsis* protein involved in silencing, MOM (19), is apparently absent from the *Neurospora* proteome.

In conclusion, the picture emerging from a combination of experimental and bioinformatic analyses predicts that *Neurospora* relies on a variation of the HP1/Swi6 pathway to achieve a silenced chromatin state that is coupled directly or indirectly to de novo and/or maintenance DNA methylation (712). Whether DNA methylation is involved in all heterochromatin silencing remains to be determined.

TABLE 16. *Neurospora* bromodomain and chromodomain proteins[a]

Protein	Locus (gene)	Match found by BLAST				
		Best match	*S. cerevisiae*	*S. pombe*	Animal[b]	Plant[c]
Bromodomain proteins						
*Sc*Rsc1/Rsc2	2354.1 (*pbd-1*)	*P. anserina*; 0	6e-76	4e-68	2e-20	No match
*Sp*Brd1	2078.1 (*pbd-2*)	*M. musculus*	No match	1e-6	1e-8	No match
*Sp*Bdf1-like	8809.1 (*bdp-1*)	*D. rerio*	No match	4e-12	8e-14	2e-12
*Sc*Spt7	2276.1 (*bdp-2*)	*S. pombe*	4e-38	1e-42	4e-14	No match
*Sc*Bdf1	8423.1 (*bdp-3*)	*S. pombe*	1e-18	3e-19	4e-18	2e-14
Chromodomain proteins						
*Dm*HP1/*Sp*Swi6	4017.1 (*hpo*)	*S. pombe*	No match	3e-8	0.005	No match
CDP-1	8362.1 (*cdp-1*)	*H. sapiens*	No match	0.001	5e-7	No match
CDP-2	738.1 (*cdp-2*)	*H. sapiens*	No match	No match	0.087	No match
CDP-3	1522.1 (*cdp-3*)	*D. melanogaster*	No match	No match	0.001	No match

[a] This table excludes CRF3-1 (bromodomain) and CRF6-1 and CRF7-1 (chromodomains). There are also several RIP-mutated relics of retrotransposons whose inactive POL genes match chromodomains in BLAST searches.
[b] *Drosophila melanogaster*, *Mus musculus*, *Homo sapiens*, *Danio rerio*, or *Caenorhabditis elegans*.
[c] *Arabidopsis thaliana* or *Oryza sativa*.

RIP. The prototypical genome defense system RIP was discovered in 1986 as a result of a detailed analysis of progeny from crosses of *Neurospora* transformants (Fig. 4) (298, 707, 708, 710, 711, 713). RIP detects duplicated sequences in the haploid genomes of special dikaryotic cells resulting from fertilization (711) and riddles both copies of the duplicated sequence with GC-to-AT mutations (123). In a single passage through the sexual cycle, up to ~30% of the GC pairs in duplicated sequences can be changed to AT pairs (124). Because RIP shows some sequence preference — the C-to-T mutations occur principally at CpA dinucleotides, thus generating an excess of TpA dinucleotides (708) — most genomic regions that have been subjected to RIP are readily recognizable (500). Frequently, but not invariably, cytosines in sequences altered by RIP become methylated de novo. It is possible that the mutations induced by RIP occur by enzymatic deamination of 5-methylcytosines, but they could also occur in other ways, such as by deamination of cytosines followed by DNA replication (306, 509, 708, 845). As a result of the *Neurospora* genome sequencing projects, a putative DNA methyltransferase (RID) has been recently identified and demonstrated to be essential for RIP (253). RIP appears tailor-made to counter "selfish" DNA such as transposons. Hence, it is not surprising that the great majority of *Neurospora* strains appear devoid of active transposable elements (417, 419) and that *Neurospora* contains numerous examples of nonfunctional transposable elements bearing hallmarks of RIP (418, 500, 717).

RNA-dependent silencing. In *Neurospora*, several genes involved in RNA silencing have been identified through a combination of mutant screens and candidate gene approaches. Three genes, *qde-1*, *qde-2*, and *qde-3*, coding respectively for an RNA-dependent RNA polymerase (RdRP), an Argonaute-like protein, and a RecQ-like helicase (131, 132, 159–161, 163), provided the backbone of the vegetative silencing pathway called quelling (Fig. 4). Analysis of these genes was fundamental to our understanding of the RNA silencing machinery in all organisms. A second RdRP, *Suppressor of ascus dominance 1* (*Sad-1*), was identified (732) as a suppressor of a meiotic phenomenon called meiotic silencing (24, 459). Recently, Argonaute-like (related to translation initiation factors) and Dicer-like (double stranded-specific endonuclease related to the SFII helicase-RNase III fusion protein of the carpel factory) genes, *Suppressor of meiotic silencing 2* (Sms-2) (458) and *Suppressor of meiotic silencing 3* (*Sms-3*) (M. McLaughlin and R Aramayo, unpublished data), respectively, have been implicated in meiotic silencing. These observations suggested the existence of more than one RNA silencing pathway in *Neurospora*.

Quelling belongs to the broad category of posttranscriptional gene silencing (PTGS) mechanisms (132, 295), and all existing experimental evidence suggests a similar molecular mechanism for meiotic silencing (Fig. 4) (458, 459, 732). These two pathways are thought to be derived from ancestral natural defense systems directed against invading nucleic acids (858). Surprisingly, mutations in all known components of the meiotic silencing pathway (i.e., *sad-1, sms-2*, and *sms-3*) and in some genes of the quelling pathway (e.g., *qde-1*) affect sexual development and/or meiosis, suggesting that in addition to being a defense system, RNA silencing plays a critical role in controlling development.

Pathways similar to quelling and meiotic silencing are also found in plants (PTGS) and animals (RNA interference or RNAi [309]), where they participate in controlling transposon mobilization and development (302, 376, 769). Cosuppression, RNAi, quelling, and meiotic silencing are all related mechanisms that share similar molecular components. These include RdRPs and Argonaute-like and Dicer-like proteins, in addition to many other ATP-dependent DNA and ATP-dependent RNA helicases and double-stranded RNA binding proteins, whose specific function in the silencing pathways remain to be elucidated (359).

This two-pathway hypothesis predicts (i) that there are paralogues in the *Neurospora* genome for genes involved in RNA silencing and (ii) that these paralogues, if functioning in different pathways, should have diverged from a presumed ancestral pathway into phylogenetically distinguishable clades. This hypothesis was tested by searching the *Neurospora* genome for homologues of known components of the RNA silencing machinery. Three RdRPs, two Argonaute-like translation initiation factors, two Dicer-like RNases, and two RecQ-like helicases were identified (Table 17). The presence of these

TABLE 17. RNA-silencing pathways

Predicted protein[a]	*N. crassa*[b]	*A. fumigatus*[c]	*S. pombe*[d]	Pathway[e]
RNA-directed RNA polymerase	*qde-1* (NCU07534.1)	*rrpA* (contig158)		Quelling
	Sad-1 (NCU02178.1)	*rrpB* (contig472)	*rdp1*$^+$ (SPAC6F12.09)	Meiotic silencing
	rrp-3 (NCU08435.1)			Unknown
Argonaute-like, related to translation initiation factors	*qde-2* (NCU04730.1)	*ppdA* (contig720)		Quelling
	Sms-2 (NCU09434.1)	*ppdB* (contig196)	*ago1*$^+$ (SPCC736.11)	Meiotic silencing
Dicer-like, related to SFII-RN aseIII RNase of the carpel factory	*dcl-2* (NCU06766.1)	*dclB* (contig618)		Quelling
	Sms-3 (NCU08270.1)	*dclA* (contig310)	*dcr1*$^+$ (SPCC584.10C)	Meiotic silencing
RecQ helicase-like, related to Bloom's and Werner syndrome helicases	*qde-3* (NCU08598.1)	*rqhA* (contig443)		Quelling
	rqh-2 (NCU03337.1)[f]	*rqhB* (contig58)[f]	*hus2*$^+$ (SPAC2G11.12)	Unknown

[a] Function predicted based on protein homology.
[b] *Neurospora crassa* Genome Project at http://www-genome.wi.mit.edu.
[c] Unfinished *Aspergillus fumigatus* Genome Project at http://www.tigr.org.
[d] *Schizosaccharomyces pombe* Genome Project at http://www.genedb.org.
[e] Pathway assigned based on either known experimental data for *qde-1*, *qde-2*, and *qde-3* (quelling pathway); *Sad-1*, *Sms-2*, and *Sms-3* (meiotic silencing pathway); or predicted based on phylogenetic analysis.
[f] *Rec*Q helicase-like (rqh).

paralogues fulfills the first prediction of the two-pathway hypothesis.

Alignment of the *Neurospora* RdRP, Argonaute-like, and Dicer-like paralogues to their homologues in plants, animals, and other fungi (using neighbor joining [672]) and the Bayesian or most posterior probability methods (354, 355) resulted in trees in which all of the *Neurospora* paralogues clustered with different fungal homologs into two separate clades. This result is consistent with a clade representing independent quelling and meiotic silencing pathways and was used to predict a role for the other fungal homologues (Table 17).

Surprisingly, the predicted *A. fumigatus* homologues also separate into the different clades, suggesting that two mechanisms similar to quelling and meiotic silencing also exist in this organism. This observation is striking for an organism that is not known to have a sexual phase during its life cycle and suggests that perhaps a sexual stage does exist but has missed detection or that such ability was recently lost. *S. pombe* appears to have only one pathway, which clustered consistently with the known *Neurospora* meiotic silencing genes, *Sms-2* and *Sad-1*. Extrapolating this observation onto the phylogenetic results for the Dicer homologs, the genes *Sms-3* and *dcl-2* were predicted to be part of the meiotic silencing and quelling pathways, respectively (Table 17).

One of the *Neurospora* RdRPs (RRP-3) could not be clearly placed in either the quelling or the meiotic silencing pathways. Its presence suggests either that this protein might function in a yet to be discovered silencing pathway or that it forms the remains of an ancestral silencing pathway that may be present in other fungi but that has been lost in *Neurospora* (Table 17).

Thus, *Neurospora* contains paralogues for the fundamental RNA silencing machinery present in plants and animals and these genes have evolved into at least two separate pathways: quelling (vegetative) and meiotic silencing (developmental).

DNA Repair

Repair of DNA damage is essential for the maintenance of genome integrity. DNA damage results from exposure to UV, ionizing radiation, and various chemical mutagens and from errors in DNA replication. Isolation and characterization of strains sensitive to mutagens has led to an understanding of the range of DNA repair mechanisms. These include excision repair, recombination repair, photoreactivation repair, post-replication repair, and mismatch repair, all of which exist in *Neurospora* (287, 364, 692). Another mechanism, known as checkpoint control, affects the efficiency of DNA repair. Checkpoint control mutants are also sensitive to DNA damage.

This section covers all of the above repair systems (recombination repair is described in more detail in the section on meiotic recombination, below). Some of the genes involved in DNA repair were cloned by isolating DNA fragments that complement the sensitivity of damage-sensitive mutants. Other genes were identified by searches of the genome database by using candidate genes from other organisms. In some cases, roles in repair have been confirmed by reverse genetics, while some genes remain as tentative orthologues.

Photoreactivation. Photoreactivation repair of UV damage to DNA is carried out by DNA photolyase (674). The *Neurospora phr* gene encodes a photolyase specific for the cyclobutane pyrimidine dimer (CPD 867). Unlike *Drosophila*, *Xenopus*, and zebrafish, *Neurospora* does not have a photolyase that repairs a TC(6-4) photoproduct (730).

Excision. The eukaryotic excision repair system has been characterized primarily in *S. cerevisiae* and in humans and is one of the most fundamental systems that utilizes double-stranded DNA in the repair process (254). Two different excision repair systems have been reported: nucleotide excision repair (NER) and base excision repair (BER). NER, which deals with UV damage and the bulky DNA lesions produced by many chemical mutagens, is processed by a sequentially assembled protein complex. This system is highly conserved across kingdoms from bacteria to humans. *Neurospora* has two NER systems (319); one is the system conserved in all organisms, and the other is found only in *Neurospora* and the fission yeast *S. pombe*. BER, in which a DNA glycosylase specific for a particular lesion produces an apurinic or an apyrimidic site (AP-site) as a first repair step, has been characterized in many organisms.

(i) NER. In *S. cerevisiae* and human cells, mutants defective in NER genes show extremely high sensitivity to UV and to some chemicals. *Neurospora* NER mutants have been difficult to characterize because *Neurospora* has an additional NER system that is represented by the *mus-18* gene. *Neurospora*

TABLE 18. Nucleotide excision repair genes

S. cerevisiae gene	Function	*Neurospora* NCU no. (gene)	Contig	E value	Best match to *Neurospora* protein in SP + TrEMBL	E value (BLASTP)
RAD1	5′ endonuclease	07440.1 (*mus-38*)	3.434	e-140	*S. pombe* Rad16	0.0
					M. musculus XPF	e-141
					Human XPF	e-140
					S cerevisiae Rad1p	e-134
RAD2	3′ endonuclease	07498.1 (*mus-40*)	3.439	1e-72	*S. pombe* Rad13	1e-87
					S. cerevisiae Rad2p	9e-68
					Human XPG	5e-55
RAD3	5′-3′ helicase	01625.1	3.67	0.0	*S. pombe* Rad15	0.0
					S. cerevisiae Rad3p	0.0
					Human XPD	0.0
RAD4	GGR-specific damage recognition factor	06585.1	3.381	2e-28	*S. pombe* Rad4 homologue	2e-62
					Drosophila MUS210	5e-26
					Human XPC	8e-22
					S. cerevisiae Rad4p	3e-20
RAD7	DNA damage recognition	03649.1	3.199	4e-11	*S. pombe* Rad7 homologue	2e-41
					S. cerevisiae Rad7p	4e-14
					Human FBL6	3e-06
RAD10	5′ endonuclease	07066.1 (*mus-44*)	3.413	8e-16	*S. pombe* Swi10	3e-53
					Rice Rad10 homologue	2e-43
					Human ERCC1	5e-42
					S. cerevisiae Rad10p	2e-13
RAD14	Damaged DNA binding activity	08742.1 (*mus-43*)	3.541	3e-34	*S. pombe* Rad14 homologue	1e-42
					S. cerevisiae Rad14p	4e-31
					Human XPA	3e-15
RAD16	DNA damage recognition, DNA-dependent ATPase activity	03650.1	3.199	0.0	*S. cerevisiae* Rad16p	0.0
					S. pombe Rhp16	0.0
					Human Rad16 homologue	2e-50
RAD23	DNA damage recognition	07542.1	3.442	8e-31	*S. pombe* Rad23 homologue	9e-58
					A. thaliana Rad23 homologue	1e-39
					Human HHR23B	8e-32
					Human HHR23A	3e-21
					S. cerevisiae Rad23p	2e-29
RAD25/SSL2	DNA helicase activity, TFIIH complex	06438.1	3.371	0.0	*S. pombe* Rad25 homologue	0.0
					S. cerevisie Rad25p	0.0
					Human XPB	0.0
RAD26	DNA-dependent ATPase activity	07837.1	3.471	0.0	*S. pombe* Rhp26	0.0
					S. cerevisie Rad26p	0.0
					Human ERCC6/CSB	e-154
RAD28	Unknown	None				
S. pombe uve1^{+}	UV damage-dependent endonuclease	08850.1 (*mus-18*)	3.550	8e-99	*S. pombe* Uve1	0.0
					Bacillus UVSE	1e-30
Human *DDB1*	UV-damaged DNA binding protein	06605.1	3.382	1e-92	*O. sativa* OSUVDDB	e-122
					A. thaliana AT4G05420	e-108
					Green monkey DDB1	7e-87
					Human DDB1	2e-86

MUS18 and *S. pombe* UVDE are UV damage-specific endonucleases able to nick DNA to the 5′ side of UV damage to initiate excision repair (868, 887). After nicking of single-stranded DNA, a polynucleotide, including the damaged base, is removed by a flap endonuclease, Fen1, in *S. pombe* (889). In *S. pombe*, UVDE is involved in repair of DNA damage in mitochondria, as well as in the nucleus (880). However, the roles of Fen1 in excision repair and of MUS18 in repair of mitochondrial DNA damage remain to be established in *Neurospora*.

Genes in the primary NER pathway have been characterized in *Neurospora* only in the last 10 years, after the discovery of the second, *mus-18* NER pathway. *mus-38* and *mus-40* respectively encode homologues of the *S. cerevisiae* genes *RAD1* and *RAD2*. A mutation in either of these genes results in mild sensitivity to UV radiation in a normal genetic background but a synergistic sensitivity to UV light in *mus-18* strains. All other NER genes that comprise the conserved pathway are present in *Neurospora*, and their amino acid sequences are similar to those of the two sequenced yeasts, particularly *S. pombe* (Table 18).

(ii) BER. BER has not been studied in *Neurospora*, since no suitable mutants have been isolated. *Neurospora* orthologues of BER genes have been identified on the basis of sequence homology, although homologies are usually not high (Table 19). Indeed, sequences of these genes appear highly divergent from organism to organism. Confirmation of a role in BER for the *Neurospora* orthologues awaits the analysis of disruption mutants.

Recombination repair. Recombination repair genes are separated into two groups: homologous recombination (HR) and nonhomologous/end joining (NHEJ). Homologous recombination is covered in the Meiotic Recombination section below.

TABLE 19. Base excision repair genes

S. cerevisiae gene	Function	*Neurospora* NCU no.	Contig	E value	Best match to *Neurospora* protein in SP + TrEMBL	E value (BLASTP)
APN1	AP endonuclease	10044.1	3.748	2e-78	*S. cerevisiae* Apn1p	9e-81
					Plasmodium Apn1	5e-69
MAG1	3-Methyladenine DNA glycosylase	08938.1	3.559	6e-21	*O. sativa* Mag1 homologue	1e-18
					Halobacterium Mag1 homologue	7e-18
					S. cerevisiae Mag1p	6e-12
NTG1	DNA *N*-glycosylase	06654.1	3.385	2e-44	*S. pombe* Nth1	5e-62
					Drosophila Nth1 homologue	6e-57
					Human NTH1	2e-50
OGG1	8-Oxo-guanine DNA glycosylase	03040.1	3.154	3e-33	Rat OGG1	3e-39
					M. musculus OGG1	2e-38
					Human OGG1	6e-38
					S. cerevisiae Ogg1p	3e-31
UNG1	Uracil *N*-glycosylase	07482.1	3.439	4e-65	*S. pombe* Ung1	1e-86
					S. cerevisiae Ung1p	5e-68
					Human UNG	1e-52
Human *PARP-1*	Poly(ADP-ribose) polymerase activity, protein modification	08852.1	3.550	2e-63	*A. thaliana* app	8e-66
					Dictyostelium ADPRT2	2e-62
					Human ADPRT	4e-60

The human Nijmegen breakage syndrome (NBS1) protein has weak homology to the *XRS2* gene product of *S. cerevisiae* (508). While a possible *Neurospora* orthologue of human *NBS1* was identified, the similarity is very poor (see Table 24), consistent with the observation that Xrs2 orthologues are highly divergent between species. *mus-45* mutants have a similar phenotype to that of *uvs-6* (yeast *rad50* homologue) and *mus-23* (*S. cerevisiae mre11* homologue) mutants, and *mus-45* is likely to be a true orthologue of *NBS1* (H. Inoue, unpublished data). Orthologues of the KU70 and KU80 proteins, which bind to DNA double-strand breaks (DSBs), are also found in *Neurospora*. However, the DNA protein kinase (DNA-PKcs) gene involved in NHEJ in humans is not present in *Neurospora* or yeasts.

Postreplication repair. The postreplication repair pathway includes two ubiquitin-conjugating enzymes and two ubiquitin ligases which have ring finger motifs, suggesting that the main function of this repair system may be related to a ubiquitin-control system. *Neurospora* has orthologues of the budding

TABLE 20. RAD6 DNA repair genes

S. cerevisiae gene	Function	*Neurospora* homologue	Contig	E value	Best match to *Neurospora* protein in SP + TrEMBL	E value (BLASTP)
RAD5	RING finger protein, DNA helicase	NCU09516.1 *mus-41*	3.611	e-120	*S. pombe* Rad8	0.0
					S. cerevisiae Rad5	e-143
					Human HIP116	9e-76
RAD6	Ubiquitin-conjugating enzyme, required for histone ubiquitination, protein monoubiquitination, and ubiquitin-dependent protein catabolism	NCU09731.1 *mus-8*	3.634	7e-67	*N. haematococca* NhRAD6	6e-83
					E. nidulans UVSJ	5e-80
					S. pombe Rhp6	2e-70
					S. cerevisiae Rad6	5e-69
					Human HHR6B	4e-61
					Human HHR6A	2e-60
RAD18	RING finger protein, ATPase activity, single-stranded DNA binding activity	NCU05210.1 *uvs-2*	3.293	7e-31	*E. nidulans* NUVA	1e-74
					S. pombe Rhp18	3e-40
					S. cerevisiae Rad18	4e-29
					Human hRAD18	8e-27
UBC13	Ubiquitin-conjugating enzyme, heterodimer with Mms2	NCU02113.1	3.95	9e-63	*Catharanthus* CrUBIE2	2e-62
					A. thaliana AY049261	3e-62
					S. cerevisiae Ubc13	3e-61
					S. pombe Spu13	3e-60
					Human UBE2N	4e-60
MMS2	Ubiquitin-conjugating enzyme, heterodimer with Ubc13	No number	Excluded contig 3.72		*K. delphensis* MMS2	2e-39
					S. pombe Spm2	1e-37
					S. cerevisiae Mms2	2e-35
					Human hMMS2	4e-34
					Human CROC-1B	4e-34

TABLE 21. DNA replication-related repair genes

S. cerevisiae gene	Function	*Neurospora* homologue	Contig	E value	Best match to *Neurospora* protein in SP + TrEMBL	E value (BLASTP)
SRS2	DNA helicase; recombination suppression, DSBR via NHEJ	NCU04733.1	3.260	3e-76	*S. pombe* Srs2	e-117
					L. citreum PcrA	7e-93
					E. coli UVRD	1e-78
					S. cerevisiae Srs2	4e-70
MGS1	DNA-dependent AAA(+) ATPase; helicase activity	NCU08706.1	3.541	5e-95	*S. pombe* putative protein	e-107
					A. thaliana AC002396	3e-94
					Human WHIP	9e-92
					S. cerevisiae Mgs1	1e-82
POL30/PCNA	DNA polymerase processivity factor	NCU09239.1	3.579	1e-67	*S. pombe* PCNA	e-100
					D. carota PCNA	9e-83
					Human PCNA	1e-78
					S. cerevisiae Pol30	4e-60
Human *p66*	DNA polymerase δ subunit	NCU07998.1	3.481	1e-07	Human p66	7e-06
					M. musculus p66	2e-05
					S. pombe Cdc27	0.28
CTF4	Polα accessory subunit, DNA binding activity	NCU08484.1 *mus-27*	3.513	1e-50	*E. nidulans* SepB	0.0
					S. pombe Ctf4 homolog	e-154
					Human AND1	1e-40
					S. cerevisiae Ctf4	2e-36
RAD27	Flap endonuclease activity, Okazaki fragment processing	NCU02288.1	3.108	e-113	*S. pombe* Rad2	e-126
					Xenopus Rad27	e-113
					S. cerevisiae Rad27	e-113
					Human FEN1	e-108

yeast *RAD6* and *MMS2/UBC13* genes, which encode ubiquitin-conjugating enzymes, and of *RAD18* and *RAD5*, which are ubiquitin ligase genes (Table 20). An orthologue of budding yeast Srs2p, a helicase that functions in postreplication repair, is present in *S. pombe* and *Neurospora* but not in plants and animals (Table 21).

The postreplication repair group also includes specific DNA polymerases that function in translesion DNA synthesis. Among these are human polymerase κ (kappa), polymerase ζ (zeta), polymerase ε (epsilon), and polymerase ι (iota) (628). Although an orthologue of DNA polymerase κ is absent from *S. cerevisiae* (but present in *S. pombe*) and both yeast species lack polymerase ι, orthologues of these polymerases exist in *Neurospora*. Some *Neurospora* polymerase orthologues are more similar to those of human than those of *S. cerevisiae* (Table 22). Except for the *polζ* homologue *upr-1* (673), the functions of *pol* gene products are not well understood in *Neurospora*.

Checkpoint control. *Neurospora* has orthologues of almost all yeast DNA damage checkpoint genes, although some of them do not show a high degree of sequence similarity (Table 23). Some of the mutagen-sensitive *Neurospora* mutants that have not yet been characterized at the sequence level may be included in this group. The multinucleate nature of *Neurospora* cells makes it difficult to detect damage checkpoint defects, since nuclear divisions are not synchronized, even in the same cellular compartment. However, the similarity between deduced amino acid sequences and observed mutant phenotypes made it possible to designate *Neurospora mus-9*, *uvs-3*, and *mus-21* as orthologues of *MEC1*, *DDC2*, and *TEL1*, respectively, in *S. cerevisiae* (C. Ishii, unpublished data).

In conclusion, many *Neurospora* repair proteins are more similar to those of *S. pombe* than to *S. cerevisiae*, while others are more closely related to proteins from higher organisms such as humans. In some cases, the phenotype of a *Neurospora* mutant deficient in repair differs from that of a yeast strain carrying a mutation in the orthologous gene (for example, *srs-2*, *fen1*, and *mus-21* [H. Inoue, unpublished]). Some members of repair gene families that are found in other organisms are not found in *Neurospora* (for example, Rad59p of *S. cerevisiae*), likely due to constraints imposed upon gene duplication by RIP.

Meiotic Recombination

S. cerevisiae exhibits very high rates of recombination and has a highly tractable genome; much of what is known about both the mechanism and enzymology of meiotic recombination derives from studies of this species. Indeed, organisms other than *S. cerevisiae* appear to harbor very few meiotic recombination genes that it does not also possess (Table 24). The reverse is not true, however, since *S. cerevisiae* appears to contain quite a few genes not found in other organisms. Moreover, it is likely that numerous recombination genes remain to be identified in nonyeast organisms because, in relative terms, less effort has gone into their cloning.

Meiotic recombination is thought to begin with a DSB in the DNA duplex. This is followed by 5′-to-3′ degradation either side of the DSB, yielding single-stranded tails. These single-stranded tails then invade the intact homologue, and the formation of a recombination intermediate, which holds homologues together, is thought to follow. The formation of a proteinaceous structure, the synaptonemal complex, between homologues signals synapsis. Sometime prior to the separation of homologues at anaphase I, the recombination intermediate is resolved. Resolution may yield homologues with or without a crossover. While all of this is going on, mismatched bases are detected and usually corrected. Although this account of meiotic recombination is not necessarily chronologically accurate, it does provide a framework around which a discussion of the numerous genes known to be involved in meiotic recombina-

TABLE 22. Repair-related DNA polymerases

S. cerevisiae gene	Function	*Neurospora* NCU no.	Contig	E value	Best match to *Neurospora* protein in SP + TrEMBL	E value (BLASTP)
POL1	Polα catalytic subunit, priming activity in DNA replication	07870.1	3.473	0.0	*S. pombe* Poll	0.0
					C. cinereus Poll	0.0
					S. cereviciae Pollp	0.0
POL4	Polβ catalytic subunit, BER in nuclear DNA	07461.1	3.453	3e-11	Human POLL	5e-52
					M. musculus POLL	1e-49
					S. cerevisiae Pol4p	2e-09
	Polλ meiosis-associated DNA repair	01321.1	3.51	9e-07	*S. pombe* Polβ-like	1e-39
					Human POLM	5e-36
					S. cerevisiae Pol4p	0.002
MIP1	Polγ, BER in mitochondrial DNA	00279.1	3.12	0.0	*S. pombe* Mip1	0.0
					P. pastoris Mip1	0.0
					S. cerevisiae Mip1p	0.0
					Human POLG	e-118
POL3	Polδ catalytic subunit, NER and MMR	01192.1	3.45	0.0	*S. pombe* Pol3	0.0
					C. albicans Pol3	0.0
					S. cerevisiae Pol3p	0.0
					Human POL3	0.0
POL2	Polε catalytic subunit, NER and MMR	04548.1	3.233	0.0	*E. nidulans* NimP	0.0
					S. pombe Cdc20	0.0
					S. cerevisiae Pol2p	0.0
					Human POLE1	0.0
REV1	dCTP transferase, TLS	02053.1 *mus-42*	3.90	3e-63	Human REV1	e-100
					M. musculus REV1	4e-95
					S. cerevisiae Rev1p	6e-57
	Polκ, TLS	02457.1	3.131	1e-09	*P. anserina* CAD60612	0.0
					S. pombe O74944	2e-92
					Human POLK	1e-43
REV3	Polζ catalytic subunit, TLS	01951.1 *upr-1*	3.86	1e-62	*E. nidulans* UvsI	0.0
					S. cerevisiae Rev3p	0.0
					Human REV3	0.0
REV7	Polζ accessory subunit	06577.1 *mus-26*	3.381	0.017	*S. pombe* Mad2-like	7e-13
					M. musculus Mad2L	3e-08
					Human REV7	2e-07
RAD30	Polη, TLS	01936.1 *polh*	3.84	2e-54	*S. pombe* Eso1	2e-95
					S. cerevisiae Rad30p	9e-53
					Human XPV	2e-40
	Polτ, TLS	06757.1 *poli*	3.390	5e-07	Human POLI	4e-32
					M. musculus POLI	4e-31
					S. pombe O74944	2e-15
Human *POLQ*	Polθ, DNA cross-link repair	07411.1	3.432	5e-85	*Drosophila* Mus308	e-112
					Human POLQ	e-106
					M. musculus POLQ	e-104
TRF4	Polσ, nucleotidyltransferase, sister chromotid cohesion	05588.1	3.312	6e-38	*S. cerevisiae* Trf4	3e-37
					S. cerevisiae Trf5p	2e-33
					Human TRF4-1	2e-26
	Polσ, *TRF5?*	00538.1	3.20	3e-09	*S. pombe* Cid13	1e-77
					S. pombe AL031154	5e-60
					Human KIAA0191	1e-27
					S. cerevisiae Trf5p	4e-8

tion can be organized. However, it should be borne in mind that the influence of some genes is not restricted to a given step.

Before the DSB. While the generation of DSBs is thought to be the initiating event of meiotic recombination, it appears that in *S. cerevisiae*, premeiotic DNA replication is a necessary precondition for DSB genesis. Smith et al. (743) showed that mutation of the B-type cyclins *CLB5* and *CLB6* prevented premeiotic DNA replication, which in turn blocked the formation of DSBs. The situation seems to be similar in *Arabidopsis*. Mutation of a meiosis-specific cyclin Solo dancers (*sds*) in *Arabidopsis* results in defects in homologue pairing and in recombination (36). While both of the *S. cerevisiae* cyclins (Clb5/Clb6) and the sds protein have limited homology to several hypothetical *Neurospora* proteins (Table 24), homology is largely restricted to mitosis-specific cyclins from other organisms. A fully functional Spo22 appears to be required for premeiotic DNA synthesis in *Coprinus* (523). Thus, it remains to be established as to whether one or more of the hypothetical *Neurospora* cyclins has any effect on meiotic recombination.

DSB generation. Spo11p is a novel type II topoisomerase (68) that is thought to cleave DNA, generating the DSBs considered to be the universal initiating event of meiotic recombination. *SPO11* was first identified in *S. cerevisiae* (408) and has orthologues in fission yeast (*rec-12*), *Coprinus*, *Neurospora*, *Sordaria*, nematodes, *Drosophila* (*mei-W68*), *Arabidopsis* (*Spo11-1*), mice, and humans. In fact, it seems that wherever *SPO11* has been sought in eukaryotes, it has been found. It is

TABLE 23. DNA damage checkpoint genes

S. cerevisiae gene	Function	*Neurospora* homologue	Contig	E value	Best match to *Neurospora* protein in SP + TrEMBL	E value (BLASTP)
RAD17	DNA damage checkpoint, control protein, 3′-5′ exonuclease (putative)	NCU00942.1	3.37	9e-05	*Xenopus* RAD1	9e-17
					M. musculus REC1	4e-16
					S. pombe Rad1	5e-15
					S. cerevisiae Rad17	0.009
MEC1	DNA damage checkpoint, inositol/phosphatidylinositol kinase activity	NCU00625.1 *mus-9*	3.23	e-135	*E. nidulans* UVSB	0.0
					S. pombe Rad3	0.0
					Human ATR	e-146
					S. cerevisiae Mec1	e-130
DPB11	S-phase checkpoint, epsilon DNA polymerase activity	NCU09503.1	3.610	8e-04	*S pombe* Rad4	2e-55
					Human KIAA0259	6e-15
					S. cerevisiae Dpb11	1e-08
S. pombe rad9^{+}	Cell cycle checkpoint	NCU00470.1	3.15	2e-18	*S pombe* Rad9	5e-16
					S. octosporus homolog	7e-16
					Human RAD9 homolog	5e-09
RAD24	DNA damage checkpoint, DNA clamp loader activity	NCU00517.1	3.18	2e-22	*M. musculus* RAD17	7e-2
					Green monkey RAD17	2e-22
					Human RAD17	3e-22
					S pombe Rad17	2e-14
					S. cerevisiae Rad24	1e-09
E. nidulans uvsD (DDC2/PIE1/LCD1)	DNA damage checkpoint, damaged DNA binding activity, protein binding activity	NCU09644.1 *uvs-3*	3.627	8e-30	*E. nidulans* UVSD	4e-22
CHK1	DNA damage checkpoint, protein kinase activity	NCU08346.1 *un-1*	3.501	2e-68	*S. pombe* Chk1	1e-82
					S. cerevisiae Chk1	5e-63
					Human CHK1	3e-43
RAD9	DNA damage checkpoint, protein binding activity	NCU08879.1	3.553	1e-06	*S. pombe* RHP9	1e-12
					Leishmania PPG3	2e-07
					S. cerevisiae Rad9	6e-05
RAD53/MEC2/SPK1/LSD1	DNA repair, protein threonine/tyrosine kinase	NCU02751.1	3.140	6e-50	*S. cerevisiae* Rad53	1e-46
					S. pombe Cds1	4e-36
					Human CAMK1	2e-32
DUN1	DNA damage checkpoint, protein kinase activity	NCU02814.1	3.146	9e-72	*S. cerevisiae* Dun1	7e-72
					Human CHK2	2e-71
S. pombe hus1^{+}	DNA damage checkpoint, DNA binding activity	NCU03820.1	3.203	8e-30	*A. nidulans* NUV101	2e-56
					S. pombe Hus1	9e-44
					M. musculus HUS1	1e-10
TEL1	Response to DNA damage, inositol/phosphatidylinositol kinase activity	NCU00274.1 *mus-21*	3.12	e-112	*S. pombe* Tell	e-158
					Xenopus ATM	e-116
					Human ATM	e-104
					S. cerevisiae Tel1	5e-97
PTC2	DNA damage checkpoint, protein phosphatase type 2C activity	NCU04600.1	3.239	2e-79	*S. pombe* Ptc2	e-121
					S. pombe Ptc3	e-117
					S. cerevisiae Ptc3	4e-79
					Human PPM1A	4e-51
RFX1/CRT1	DNA damage checkpoint, specific transcriptional repressor activity	NCU06701.1	3.387	6e-29	*Cephalosporium* CPCR1	e-169
					Penicillium RFX1	e-102
					S. cerevisiae Rfx1	2e-36
					Human RFX1	2e-30
ASF1	Histone binding, DNA damage response, signal transduction	NCU09436.1	3.602	8e-65	*S. cerevisiae* Asf1	1e-61
					S. pombe Cia1	2e-54
					Human HSPC146	2e-45

perhaps a little surprising that with such a widely conserved and important function the amino acid sequence is not highly conserved. *Neurospora spo-11* is quite similar to its *Coprinus*, *S. pombe*, and human orthologues but has limited homology to budding yeast Spo11p (Table 24). However, all Spo11 orthologues possess five conserved motifs containing a number of invariant amino acids. While the predicted amino acid sequence for the *Neurospora* orthologue appears to lack important residues in motif 3, this is probably an artifact of the gene prediction algorithm. Sequencing of part of the *Neurospora* cDNA indicates that these "missing" residues are in fact present (F. J. Bowring, P. J. Yeadon, R. G. Stainer, and D. E. A. Catcheside, unpublished data).

S. cerevisiae Spo11p requires a number of accessory proteins such as Mei4p, Mer2p, Rec102p, Rec104p, and Rec114p (reviewed in reference 407) for the formation of DSBs. Apart from modest similarity between part of the Rec114p protein sequence and *S. pombe* Rec7, these accessory proteins do not appear to have counterparts in any of the other Spo11p-containing organisms. Possibly some of the *S. cerevisiae* Spo11p function is carried out by one or more of these accessory proteins, or perhaps one or more of these proteins is responsible for the rather high recombination frequencies typical of this yeast. Rec103p, probably also required for DSB generation (273), does have orthologues in *Neurospora* and other organisms (Table 24).

TABLE 24. Genes involved in DSB initiation and processing[a]

S. cerevisiae gene[b]	*Neurospora* homologue	E value	Best match to protein in SP + TrEMBL	E value (BLASTP)
Before the DSB				
CLB5	NCU02758.1 hyp protein	3e-51	*A. nidulans* G_2/mitosis-specific cyclin B	2.1e-161
CLB6		5e-59	*S. pombe* G_2/mitosis-specific cyclin *cdc13*	3.6e-129
sds (Arabidopsis)		2e-11	*S. cerevisiae* G_2/mitosis-specific cyclin 2	1.0e-106
			Human G_2/mitosis-specific cyclin B2	7.9e-75
DSB generation				
HOP2	None		*S. pombe MEI13*	8e-08
			P. falciparum hyp. protein	8e-04
			Human *BRCA1*-associated protein	9e-04
MEI4	None		None	
MEK1/MRE4	NCU02814.1	2e-36	*S. cerevisiae DUN1*	1e-72
			Human *CHK2*	4e-72
			S. cerevisiae MEK1	1.5e-27
MER1	None		None	
MER2/REC107	None		Similarity only to myosin heavy-chain genes	
MER3	NCU09793.1 hyp. protein	e-148	*S. cerevisiae HFM1/MER3*	7.4e-170
			A. thaliana genomic DNA	7.1e-93
			D. melanogaster GH18520 full-length cDNA	1.3e-65
			Human mRNA for putative RNA helicase, 3′ end	1.0e-52
MRE2/NAM8	NCU00768.1	8e-55	*A. thaliana* at5g54900	3e-67
			Nicotiana tabacum putative RNA binding protein	4e-65
			S. cerevisiae NAM8	2e-58
			Human hyp. protein flj20503	5e-37
REC102	None		None	
REC103/SK18	NCU03517.1 hyp. protein	2.00e-17	*S. pombe rec14*	1.0e-71
			Human *REC14*	1.2e-25
REC104	None		None	
REC114	None		Some homology to *S. pombe Rec7*	
RED1	None		*Kluyveromyces lactis red1*	2e-70
			Human KIAA0874 protein	4e-06
SPO11	NCU01120.1 *S. pombe rec12*-related protein	2e-10	*Coprinus cinereus spo11*	3e-29
			S. pombe rec12	2e-28
			Human *SPO11*	3e-25
			S. cerevisiae SPO11	1e-08
Removal of Spo11 protein from DNA				
MRE11	NCU08730.1 *mus-23* (AB002530)	e-123	*Magnaporthe grisea* (NK73)	e-178
			A. nidulans meiotic recombination protein	e-158
			S. cerevisiae MRE11	e-118
			Human *MRE11*	e-103
RAD50	NCU00901.1 *uvs-6* (AB055069)	e-175	*S. cerevisiae RAD50*	e-175
			Mouse *RAD50*	e-159
			Human *RAD50*	e-159
SAE2/COM1	None		None	
Resection of ends				
XRS2	None		*P. falciparum* e1-e2 putative ATPase/hydrolase	8e-06
			Human neurofilament, heavy polypeptide	4e-05
Human *NBS1*	NCU04329.1	3e-05	*A. fumigatus* hyp. protein	e-116
			S. cerevisiae LP19	3e-15
Strand invasion				
DMC1	None		Widely conserved, including mammals	
RAD51	NCU02741.1 *mei-3*	e-123	*A. nidulans uvsC*	e-178
			Penicillium paxilli pp *rad51*	e-174
			Human *RAD51*	e-135
			S. cerevisiae RAD51	e-131
RAD52	NCU04275.1 *mus-11*	5e-46	*M. grisea NK72*	4e-95
			A. nidulans radC	2e-67
			S. cerevisiae RAD52	6e-28
			Human *RAD52*	2e-22
RAD54	NCU02348.1 (AB032901) *mus-25*	0.0	*M. grisea NK74*	0.0
			S. pombe RAD54	0.0
			S. cerevisiae RAD54	0.0
			Human *RAD54*	0.0

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TABLE 24—*Continued*

S. cerevisiae gene[b]	*Neurospora* homologue	E value	Best match to protein in SP + TrEMBL	E value (BLASTP)
RAD55	NCU08806.1	7e-05	*Methanopyrus kandleri rada*	7e-04
			S. pombe rhp55	0.001
			S. cerevisiae RAD55	0.003
RAD57	NCU01771.1 *RAD57* homologue	2e-23	*S. pombe RHP57*	2e-32
			S. cerevisiae RAD57	1e-16
			Human *XRCC3*	6e-12
RDH54/TID1	NCU02348.1 *RAD54* homologue	e-107		
	NCU07837.1	1e-57	*S. pombe RHP26*	0.0
			S. cerevisiae RAD26	0.0
			Human *ercc-6*	e-156
			S. cerevisiae RDH54	6.8e-51
RFA1	NCU03606.1	e-115	*S. pombe RFA1*	e-170
			Human *RFA1*	e-119
			S. cerevisiae RFA1	e-114
RFA2	NCU07717.1	2e-23	*A. fumigatus* possible Rfa	2e-34
			S. pombe RFA2	1e-28
			Human *RFA2*	2e-16
			S. cerevisiae RFA2	7e-13
RFA3	None		None	
SAE3	None		None	
Synapsis and synaptonemal complex formation				
HOP1	None		*K. lactis HOP1*	1e-93
			Poorer matches (6e-14 to e-07) to proteins involved in synapsis in plants and animals	
MND1			Human *GAJ*. Also mouse, *A. thaliana*, and *S. pombe*	1e-17 to 1e-08
ZIP1	NCU00658.1	8e-24	Rat Golgi complex-associated protein	2e-75
			Human centromeric protein	3e-69
			S. cerevisiae ZIP1	2e-21
ZIP2	None		None	
Regulation of crossover frequency				
MEI5	None		Human ninein (*GSK3B*-interacting protein)	2e-04
MLH1	NCU08309.1	e-159	*S. cerevisiae MLH1*	e-168
			Human *MLH1*	e-167
MLH3	NCU08309.1 *mlh-1*	5e-23		
	NCU08020.1 *pms-1*	9e-18		
	NCU09373.1 *mlh-2*	5e-10		
	NCU05385.1	2e-09	*S. cerevisiae MLH3*	3.4e-13
			A. thaliana PMS1	2e-09
			Mouse *PMS2*	3e-08
			Human *PMS2*	2e-07
MSH4	NCU02230.1 *msh-2*	1e-40		
	NCU08115.1 *msh-3*	8e-32		
	NCU08135.1 *msh-6*	8e-27		
	NCU09384.1 *msh-5*	3e-22		
	NCU07407.1 *msh-1*	6e-18		
	Contig 3.27 (scaffold 2)	2e-36 (TBLASTN)	Mouse *MSH4*	4e-39
			Thermotoga maritima MutS	4e-39
			S. cerevisiae MSH4	2e-35
			Human *MSH4*	3e-34
MSH5	NCU09384.1	1e-58	*S. cerevisiae MSH5*	1e-55
			Human *MSH5*	4e-42
TAM1/NDJ1	None		None	
Drosophila mei-9	NCU07440.1 *mus-38*	e-119	*S. pombe* chromosome III cosmid	2e-270
			Mouse DNA repair endonuclease *XPF*	5.3e-199
			Human excision repair	2.2e-197
			S. cerevisiae RAD1	5.9e-170
			A. thaliana 5′ repair endonuclease	1.5e-139
Drosophila mei-218	None		None	
Mismatch repair				
MLH2	NCU08020.1 *pms-1*	6e-19		

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TABLE 24—*Continued*

S. cerevisiae gene[b]	*Neurospora* homologue	E value	Best match to protein in SP + TrEMBL	E value (BLASTP)
	NCU08309.1 *mlh-1*	6e-16		
	NCU09373.1 *mlh-2*	4e-15	Human *PMS1*	4e-24
			Phaeosphaeria nodorum hyp. protein	5e-24
			S. cerevisiae MLH2	5.0e-15
MSH2	NCU02230.1 *msh-2*	0.0	*S. cerevisiae MSH2*	0.0
			Human *MSH2*	0.0
MSH3	NCU08115.1 *msh-3*	e-115	Human *MSH3*	e-168
			Mouse *MSH3*	e-164
			S. cerevisiae MSH3	e-105
MSH6	NCU08135.1 *msh-6*	0.0	*S. pombe MSH6*	0.0
			S. cerevisiae MSH6	0.0
			Human *MSH6*	e-157
PMS1	NCU08020.1 *pms-1*	8e-82	*S. pombe PMS1*	5e-83
			S. cerevisiae PMS1	8e-81
			Human *PMS2*	4e-80
Resolution of recombination intermediates				
MMS4/SLX2	NCU04047.1	2e-04	*Xenopus laevis* nucleolar phosphoprotein	5e-04
			Human nucleolar phosphoprotein	0.002
SLX1	NCU01236.1	2e-26	*S. cerevisiae SLX1*	2e-23
			S. pombe hyp. protein	5e-17
			Human hyp. protein MGC5178	7e-12
SLX3/MUS81	NCU07457.1	2e-52	*S. cerevisiae MUS81*	8.2e-50
			S. pombe hyp. protein	5e-76
			Human *MUS81*	2e-36
SLX4	None		Human *SMC4*	2e-05
SLX8	NCU03872.1	2e-07	*S. pombe* zinc finger protein	1e-69
			S. cerevisiae Chr XII sequence	7e-39
			Human *RNF10*	6e-17
			S. cerevisiae SLX8	4e-05
HEX3/SLX5	None		None	
TOP1/MAK1/MAK17	NCU09118.1	0.0	*A. nidulans TOP1*	0.0
			S. pombe TOP1	0.0
			S. cerevisiae TOP1	0.0
			Human *TOP1*	e-114
TOP2/TOR3/TRF3	NCU06338.1	0.0	*A. niger TOP2*	0.0
			Penicillium chrysogenum TOP2	0.0
			S. cerevisiae TOP2	0.0
			Human *TOP2*	0.0
TOP3/EDR1	NCU00081.1	e-114	*S. pombe* topoisomerase 3	e-115
			C. elegans topoisomerase I	e-105
			Human topoisomerase III	e-104
			S. cerevisiae TOP3	e-101
Nonhomologous end joining				
LIF1	None		Human hyaluronan-mediated motility receptor (RHAMM) isoform A	4e-05
LIG4	NCU06264.1	7e-97	*S. pombe* DNA ligase 4	e-143
			C. albicans LIG4	1e-94
			Human ligase IV	3e-89
			S. cerevisiae LIG4	1e-88
YKU70/HDF1/NES24	NCU08290.1	1e-27	*S. pombe* putative DNA helicase	3e-91
			Gallus gallus ku70	1e-58
			Human *ku70*	6e-51
			S. cerevisiae YKU70	4e-25
YKU80/HDF2	NCU00077.1	2e-14	*S. pombe* putative DNA helicase	3e-73
			Mouse *Ku80*	2e-26
			Human DNA helicase	9e-26
			S. cerevisiae HDF2	8e-12

[a] Searches of the literature and of the Saccharomyces database (http://genome-www4.stanford.edu/cgi-bin/SGD/SAGE/querySAGE) were used to identify candidate genes. The resulting amino acid sequences were used to find orthologues in the *Neurospora* predicted protein database. Where this approach yielded nothing, a TBLASTN search of the whole *Neurospora* genome sequence was performed, thus allowing us to locate *msh-4*. The amino acid sequence of the best match for each gene was used to search the Swiss Prot + TrEMBL nonredundant protein databases. For *msh-4*, the *Neurospora* nucleotide sequence was used in a BLASTS search against Swiss Prot + TrEMBL. *S. cerevisiae* genes with no *Neurospora* orthologue were used to search Swiss Prot + TrEMBL or the human genome protein database (http://www.ncbi.nih.gov/genome/seq/HsBlast.html).

[b] Unless otherwise specified.

Red1p, a component of the synaptonemal complex, and Hop2p, a protein that discourages recombination between nonhomologous chromosomes, are both required for normal levels of DSB formation in *S. cerevisiae*. Neither has an orthologue in *Neurospora* or higher eukaryotes. Mek1p is a kinase that phosphorylates Red1p and is also required for full levels of DSBs in *S. cerevisiae*. Curiously, while both *Neurospora* and humans have a Mek1p equivalent, both apparently lack an orthologue of its substrate, Red1p.

As indicated above, Mer2p appears to be unique to *S. cerevisiae*. Splicing of *MER2* and *MER3* RNA is regulated by two genes, *MER1* and *MRE2* (534, 555). *MER3* codes for a putative helicase thought to be involved in the processing of DSBs and in interference (555) and has orthologues in *Neurospora* and other organisms (Table 24). Although *MER1* appears to be unique to budding yeast, there are *MRE2* orthologues in *Neurospora*, *Arabidopsis*, and humans (Table 24). It is possible that Mre2p plays a role in the splicing of transcripts from their *MER3* equivalents in *Neurospora* and other organisms lacking *MER2*.

Removal of Spo11 protein from DNA. The *S. cerevisiae* Rad50p, Mre11p, and Sae2p/Com1p proteins are implicated in the removal of Spo11p from DNA following DSB genesis. While Rad50p and Mre11p are widely conserved (Table 24), Sae2p/Com1p appears to be unique to *S. cerevisiae*.

Resection of ends. Rad50p and Mre11p are also required for post-scission 5′-to-3′ degradation, yielding 3′ single-stranded tails on either side of the DSB. While it is not known whether Xrs2p participates in resection, Rad50p, Mre11p, and Xrs2p are known to form a complex. Apart from weak homology to a putative protein from *Plasmodium*, Xrs2p does not appear to have potential orthologues in other organisms. Human NBS1 and budding yeast Xrs2p have little sequence homology, but NBS1 is similar in size to Xrs2p and also forms a complex with the human Rad50 and Mre11 proteins. Partly because of this, the possibility that NBS1 is functionally equivalent to Xrs2p has been considered. NBS1 shows weak homology to a putative *Neurospora* protein (Table 24).

Strand invasion. During meiosis, the *E. coli* RecA orthologues Dmc1p and Rad51p colocalize on chromosomes at foci that are the probable sites of recombination in *S. cerevisiae*. Both are thought to play a role in strand invasion, possibly via different pathways (596). While many organisms possess both Dmc1p and Rad51p orthologues, it appears that only Rad51p is represented in the *Neurospora* genome. Although budding yeast Dmc1p has homology to the hypothetical *Neurospora* protein NCU02741.1 (2.00E-83), NCU02741.1 is a closer match to *S. cerevisiae* Rad51p (Table 24). *dmc1* and *sae3* mutants have a similar phenotype and are thought to act in the same pathway (596). *SAE3* appears to be unique to budding yeast. Because Tid1p/Rdh54p is generally needed for *S. cerevisiae* sporulation, affects spore viability, and interacts with Dmc1p in two-hybrid experiments, it is thought that it may be part of the Dmc1p pathway. It is possible that *Neurospora* also lacks an orthologue of this protein, since the two putative *Neurospora* proteins with Tid1p/Rdh54p homology are more similar to *S. cerevisiae* Rad54p and Rad26p, respectively (Table 24). Given the constraints imposed by RIP on the evolution and maintenance of gene families, it would not be surprising if *Neurospora* lacked this putative Dmc1p/Sae3p/Rdh54p pathway.

Localization of Dmc1p and Rad51p to chromosomal foci during meiosis appears to require Rad52p, Rad55p, and Rad57p, and *Neurospora* has an orthologue of each (Table 24). Rfalp forms a trimeric single-stranded DNA binding protein with Rfa2p/Rfa3p and is known to colocalize with Rad52p at chromosomal foci. While other organisms, including *Neurospora*, carry both *RFA1* and *RFA2* (Table 24), *RFA3* appears to be unique to *S. cerevisiae*.

Synapsis and SC formation. Synaptonemal complex (SC) formation in *S. cerevisiae* depends on recombination. It is thought that this is also the case for mammals but not for the worm or the fly. Zip1p and Zip2p are required for synaptonemal complex formation (148) but also modulate crossover frequency in *S. cerevisiae* (806).

Of the *S. cerevisiae* genes required for synapsis and SC formation, *Neurospora* has an orthologue only of *ZIP1* (Table 24). Although *S. cerevisiae* Zip1p is not highly similar to *Neurospora* ZIP-1, each matches human Zip1 to the same extent (Table 24), suggesting that this protein diverges between species. In support of this, human and mouse Zip proteins are only 65% identical. Of the other proteins required for synapsis in *S. cerevisiae* (Hop1p, Hop2p, Red1p, Mnd1p, and Mer1p) (186, 346, 469, 742, 855), all but Red1p and Hop1p have no close orthologues in any sequenced species. Red1p and Hop1p have close orthologues only in other yeasts, suggesting that these proteins may be species or lifestyle specific, with functions performed by different proteins in different groups of organisms.

Regulation of crossover frequency. *S. cerevisiae MSH4* and *MSH5* are *E. coli mutS* homologues with no role in mismatch repair (MMR). *S. cerevisiae MLH1* is an *E. coli mutL* homologue with MMR function. Mutation in any of these three genes decreases crossover frequency in *S. cerevisiae* (26, 570). The *S. cerevisiae MER3* gene, whose transcript is spliced by Mre2p/Mer1p, encodes a novel helicase required for transition of DSBs to intermediates (554). *mer3* mutants have fewer crossovers, and those that remain are randomly spaced along chromosomes (555), indicating a requirement for Mer3p in interference. A mutation in *TAM1/NDJ1* also abolishes interference in *S. cerevisiae* (167). Zip1p and Zip2p, as described above, are components of the SC, itself required for crossover regulation, and *S. cerevisiae mei5* mutants have normal levels of gene conversion but no crossovers (280).

It has been suggested (97) that a heterodimer of Msh4 and Msh5 proteins binds to Holliday junctions and that a heterodimer of Mlh1p and another MutL protein, possibly Mlh3p, subsequently binds to the Mshp-DNA complex. The complex of Mlh and Msh proteins influences resolution of the conversion intermediate and is required for crossover formation.

Neurospora has close orthologues of the *S. cerevisiae MSH4*, *MSH5*, *MLH1*, and *MLH3* genes. Of these, Mlh1p is highly conserved in all sequenced eukaryotes (Table 24). Msh4 and Msh5 are moderately conserved, with the *Neurospora* MSH-4 protein somewhat more closely related to the mouse than to the *S. cerevisiae* Msh4p, and Mlh3p much less so (Table 24). Although the *Neurospora* orthologues of *S. cerevisiae PMS1* and *MSH5* are unambiguously identifiable, it is difficult to determine which coding sequences are orthologous to *MSH4* or *MLH3* (Table 24). Therefore, relating *S. cerevisiae* phenotypes to the specific roles of these genes in crossing over in *Neurospora* is not likely to be useful.

Neurospora has a close Mer3p orthologue (Table 24). Orthologous DNA helicases exist in other eukaryotes including humans, although both *Neurospora* and *S. cerevisiae* Mer3 proteins are closer matches to other eukaryotic RNA helicases than to DNA helicases (Table 24).

All other *S. cerevisiae* proteins required for crossing over and interference (Tam1p/Ndj1p, Rec102p, Rec104p, Rec107p/Mer2p, and Mei5p/Lph6p) (77, 167, 270, 659) have no orthologues in any sequenced organism and thus may be species specific.

In addition, there are two genes in *Drosophila*, *mei-9*, encoding an orthologue of *S. cerevisiae RAD1* (703), and *mei-218* (515), each of which reduces crossing over by 95% but has no effect on gene conversion. Hence, these proteins may also play a role in resolution of conversion intermediates. Mei-218p appears to be *Drosophila* specific, since it has no orthologue in any sequenced species, but *S. cerevisiae rad1* mutants have only 1-h delay in crossing over (166), clearly a different phenotype from *mei-9*. MUS-38 is the *Neurospora* Rad1p/Mei-9 orthologue, superficially a better match to Rad1p (Table 24) than to Mei-9 (Table 24). However, MUS-38 and Mei-9 proteins are more similar in length, each lacking both the amino and carboxy termini of Rad1p, and share more similarities in the carboxy termini of the proteins. It is possible, therefore, that MUS-38 has a function closer to that of *Drosophila* Mei-9 than of *S. cerevisiae* Rad1p.

Mismatch repair. Msh and Mlh proteins function in a highly conserved pathway for removing mismatches in DNA duplexes. Msh2p, as a heterodimer with Msh3p or Msh6p, binds to the mismatch. The protein-DNA complex attracts a heterodimer of Mlh proteins, Pms1p-Mlh1p, Pms1p-Mlh2p, or Mlh1p-Mlh2p. The identity of the Msh and Mlh proteins in the complex determines the type of mismatch recognized (97) and possibly influences the direction of correction (596). Mlh1 may be the only MMR protein responsible for restoration, if it ever occurs.

Mlh1p, Msh2p, Msh3p, and Msh6p are very highly conserved in eukaryotes, including *Neurospora*, while Pms1p is slightly less so (Table 24). In contrast, Mlh2p is much less highly conserved and cannot be unambiguously identified in *Neurospora* (Table 24). This suggests that although the pathway for correction of single-base mismatches, involving Msh2p-Msh6p and Mlh1p-Pms1p (97), is probably invariant across higher organisms, the way in which other types of mismatches are corrected may vary between species and must be determined by analysis of null mutations in each organism.

Interestingly, *Neurospora* MSH-3 is much more similar to the human than to the budding yeast orthologue (Table 24). If the Msh3p heterodimers interact with those including the less highly conserved Mlh2p, as suggested (97), MMR in *Neurospora* may have more similarities to MMR in mammals than in *S. cerevisiae*.

Resolution of recombination intermediates. Of the *S. cerevisiae* proteins thought to be involved in resolution of recombination intermediates (Slx1p, Mms4p/Slx2p, Slx3p/Mus81p, Slx4p, Hex3p/Slx5p, and Slx8p), *Neurospora* has orthologues of Slx1p and Slx3p/Mus81p (Table 24). In *S. cerevisiae*, Mus81p forms a complex with Mms4p to yield an endonuclease with roles in both meiotic recombination and in resolution of stalled replication forks (395). Slx1p forms a heterodimer with Slx4p, while Slx5p forms a heterodimer with Slx8p (544).

Mus81p shows a moderate level of conservation in eukaryotes, but its partner in the complex, Mms4p, has no close orthologue either in *Neurospora* (Table 24) or in other organisms. Slx1p may be somewhat conserved, although only in fungi, but its partner, Slx4p, has no orthologue in sequenced species (Table 24). The closest match to Slx8p in *Neurospora* may be another DNA binding protein with a different function, since it is very similar to a *S. pombe* zinc finger protein and a closer match to a different yeast chromosomal region than that of the *SLX8* sequence (Table 24). Once again, the partner, Slx5p, has no identifiable orthologue. In conclusion, because in each case only one member of each *S. cerevisiae* heterodimeric protein has an orthologue in *Neurospora*, the processes involved in this stage of recombination in other species cannot be predicted using knowledge gained from experiments with *S. cerevisiae*.

If recombination proceeds by a synthesis-dependent strand-annealing mechanism (596), recombination intermediates may be resolved by a topoisomerase (282). All three *S. cerevisiae* topoisomerases (Top1p/Mak1p/Mak17p, Top2p/Tor3p/Trf3p, and Top3p/Edr1p) belong to a highly conserved protein family, and *Neurospora* is no exception (Table 24).

Nonhomologous end joining. NHEJ is a mechanism for the repair of DSBs without homologous recombination. Of the *S. cerevisiae* proteins required for this process (Lig4p, Yku80p/Hdf2p, Lif1p, and Yku70p/Hdf1p/Nes24p [326, 334, 855]), only Lif1p is not conserved in eukaryotes, and it is also the only protein lacking a *Neurospora* orthologue.

METABOLIC PROCESSES AND TRANSPORT

Extracellular Digestion

Neurospora is a saprophyte, obtaining nutrition from a substrate that is, for the most part, polymeric and insoluble. To assimilate this resource, polymers must first be broken into small, soluble units: monomers or small oligomers. Carbon nutrition is derived from polysaccharides, proteins, lipids, and nucleic acids; proteins and nucleic acids are nitrogen sources, sulfur is obtained from proteins, and phosphorus sources are predominantly nucleic acids. To exploit these resources, *Neurospora* possesses a number of genes for glycosyl hydrolases, proteases, lipases, nucleases, and phosphatases. In spite of the existence of RIP, the glycosyl hydrolases, proteases, and lipases are present in families; however, as with the sugar transporter family (269) (see "Transporters" below), there is no evidence of recent duplication. Therefore, it is probable that these families arose by gene duplication prior to the evolution of RIP.

Secreted cellulases can be detected by clearing of a cellulose halo and staining with Congo red (786), a starch halo is seen after iodine staining (760), proteases are detected by clearing of an opaque casein halo (10), and a nucleic acid halo is assayed by staining with α-naphthyl phosphate and Diazo blue B (393). Using such methods following mutagenesis, strains with altered halo sizes have been obtained, but many of these are regulatory mutants, affecting whole classes of secreted enzymes. For example, general carbon catabolite repression affects glycosyl hydrolases, lipases, proteases, and nucleases, the

TABLE 25. Glycosyl hydrolases

Enzyme	EC no.	NCU no.	Cloned gene
Family 3			
Xylan 1, 4-β-xylosidase	3.2.1.37	00709.1	
1,4-β-Glucosidase	3.2.1.21	03641.1	
Family 5			
Endoglucanase	3.2.1.4	00762.1	
Family 6			
Cellobiohydrolase	3.2.1.91	03996.1	
		07190.1	
		09680.1	
Family 7			
Cellobiohydrolase	3.2.1.91	04854.1	
		05104.1	
		05955.1	
		07340.1	*cbh-1*
Endoglucanase	3.2.1.4	05057.1	
		08227.1	
Family 10			
Endo-1,4-β-xylanase	3.2.1.8	04997.1	
		05924.1	
		07130.1	
		08189.1	
Family 11			
Endo-1,4-β-xylanase	3.2.1.8	02855.1	
		07225.1	
Family 13			
α-Amylase	3.2.1.1	08131.1	
		09805.1	
Family 15			
Glucoamylase	3.2.1.3	01517.1	*gla-1*
Family 16			
Mixed linked glucanase	3.2.1.-	01353.1	
1,3-,1,4-β-glucanase	3.2.1.8/73	08746.1	
Family 18			
Endochitinase	3.2.1.14	02814.1	
		04500.1	
Family 25			
Lysozyme	3.2.1.17	00701.1	
Family 28			
Endo-polygalacturonase	3.2.1.15	02369.1	
Exo-polygalacturonase	3.2.1.67	06961.1	
Family 31			
α-Glucosidase	3.2.1.20	02583.1	
		04674.1	
		09281.1	
Family 32			
Invertase	3.2.1.26	04265.1	*inv*
Family 35			
β-Galactosidase	3.2.1.23	00642.1	
		04623.1	
Family 45			
Endoglucanase	3.2.1.4	05121.1	
Family 55			
β-1,3-Exoglucanase	3.2.1.58	04850.1	
		04947.1	
		05105.1	
		07523.1	
		08097.1	
		09791.1	
Family 61			
Cellobiohydrolase	3.2.1.91	00836.1	
Cellobiohydrolase	3.2.1.91	02240.1	
Endoglucanase	3.2.1.4	02916.1	
Cellobiohydrolase	3.2.1.91	07760.1	
Endoglucanase	3.2.1.4	08760.1	
Cellobiohydrolase	3.2.1.91	09764.1	
Family 81			
Glucan 1,3-β-glucosidase	3.2.1.58	07076.1	

nitrogen metabolite repression genes *nit-2* and *nmr* regulate proteases and nucleases, and the transcriptional activator *nuc-1* regulates nucleases and phosphatases.

Glycosyl hydrolases. Halo methods have demonstrated the presence of extracellular cellulase and amylase activities in *Neurospora*. Four endoglucanases, three cellobiohydrolases, and one β-glucosidase were identified in spent medium (886); only one extracellular cellobiohydrolase had been cloned and sequenced prior to the completion of the genome sequence (775). An extracellular glucoamylase has also been cloned and sequenced (760), and an extracellular invertase has been identified (680). RIP inactivation of both the cellobiohydrolase and the glucoamylase left significant residual activity, suggesting the existence of isozymes of both. Mutants with quantitatively altered halos have demonstrated regulatory genes, e.g., *exo-1* (296) and *sor-4* (548).

Analysis of the genome predicted genes for approximately 100 glycosyl hydrolases. Those with the required function, the necessary secretion signal sequence, and no other predicted destination have resulted in the list of candidate extracellular glycosyl hydrolases shown in Table 25. This group includes the previously sequenced *gla-1* glucoamylase, *cbh-1* cellobiohydrolase, and *inv* invertase. The enzymes are categorized according to the glycosyl hydrolase classification of Henrissat and Bairoch (332).

Proteases. Some mutational work has been done on extracellular proteases; the PTS-1 extracellular alkaline protease was identified based on electrophoretic variants in wild isolates (3, 313). Three inducible extracellular acid proteases have also been described (475). The genome sequence predicts a total of approximately 55 proteases. Analysis of these with regard to the required function and possession of the necessary signal sequence to permit secretion yields a total of 1 putative extracellular serine protease, 10 aspartyl proteases, and 1 metalloprotease (Table 26).

Nucleases and phosphatases. Several extracellular phosphatases have been characterized, including the PHO-3 repressible

TABLE 26. Proteases

Enzyme	NCU no.	EC no.
Serine protease		
Subtilase (lactocepin)	00263.1	3.4.21.96
Aspartyl protease		
Endothiapepsin	00994.1	3.4.23.22
	02059.1	3.4.23.22
Aspergillopepsin	00338.1	3.4.23.-
Podosporapepsin	09484.1	3.4.23.-
	07533.1	3.4.23.-
Candidapepsin	03168.1	3.4.23.24
	09155.1	3.4.23.24
	07063.1	3.4.23.24
Pepsin?	02956.1	3.4.23.-
	00249.1	3.4.23.-
Metalloprotease		
Thermolysin	05756.1	3.4.24.-

TABLE 27. Nucleases and phosphatases

Enzyme	NCU no.	EC no.	Cloned gene
Nonspecific endo-RNase S1	09194.1	3.1.30.1	
Nonspecific endo-RNase S1	08648.1	3.1.30.1	
DNase 1	01173.1	3.1.-.-	
Guanyl-specific RNase N1	01045.1	3.1.27.3	
Acid phosphatase	08643.1	3.1.3.3	*pho-3*
Alkaline phosphatase	01376.1	3.1.3.1	*pho-2*

acid phosphatase and the PHO-2 repressible alkaline phosphatase. Two secreted phosphate-repressible alkaline DNases have been described (394), as well as a DNase A and a single-strand-specific endonuclease (250). There are more than 30 nucleases and phosphatases in the *Neurospora* genome. These were further analyzed to identify those with the necessary function and signal sequence (Table 27). The group includes two non-specific S1 RNases, a DNase, a guanyl-specific N1 RNase, the PHO-2 alkaline phosphatase, and the PHO-3 acid phosphatase.

Lipases. A single extracellular triacylglycerol lipase associated with conidia has been described to date (441). Examples of extracellular lipases have been characterized in a number of other fungal species. Analysis of the genome and predicted genes for lipases reveals a total of 19, of which 7 triacylglycerol lipases have a secretion signal sequence, but 4 of these are probably membrane proteins (see also "Lipids" below). The remaining three candidates for extracellular lipases are listed in Table 28.

Transporters

Based on genome analysis, *Neurospora* encodes approximately 25% more transporter systems than does *S. cerevisiae* (605), primarily due to the presence of an increased number of major facilitator superfamily (MFS) and ATP binding cassette (ABC) family transporters (Fig. 5). A complete listing of predicted *Neurospora* membrane transporters categorized by gene family and function is available at http://www.membranetransport.org/. *Neurospora* appears to possess an enhanced capacity in terms of predicted drug efflux genes. It encodes more than twice as many ABC and MFS drug efflux systems as *S. cerevisiae* or *S. pombe* and also encodes two RND family efflux proteins, a type of efflux pump not seen in either *S. cerevisiae* or *S. pombe*. This expanded inventory of efflux systems relative to other fungi could conceivably play roles in (i) secretion of secondary metabolites, (ii) mediation of resistance to plant-produced secondary metabolites or other toxic compounds, or (iii) secretion of signaling molecules perhaps related to hypha morphogenesis. The first two possibilies correlate with the described repertoire of *Neurospora* secondary metabolite biosynthesis genes and cytochrome P450 detoxification genes and with the known abilities of plants and soil organisms to secrete a broad range of toxic compounds.

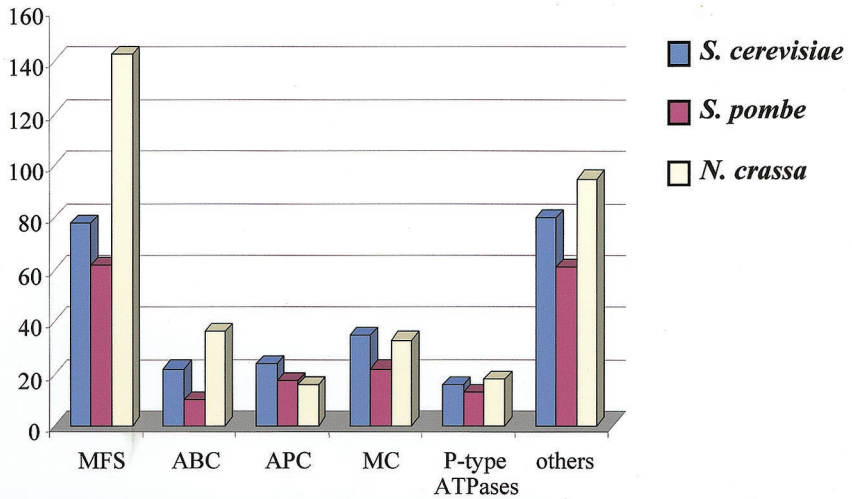

FIG. 5. Relative numbers of *Neurospora*, *S. cerevisiae*, and *S. pombe* transporters in various families. *Neurospora* was compared to *S. cerevisiae* (605) and *S. pombe* (http://www.membranetransport.org) with respect to transporters in the major facilitator superfamily (MFS), ATP binding cassette (ABC) superfamily, amino acid/polyamine/choline (APC) superfamily, mitochondrial carrier (MC) family, P-type ATPase family, and other families.

In terms of metabolite uptake, the capabilities of *Neurospora* appear to resemble those of *S. cerevisiae*, although *Neurospora* does encode an increased number of MFS uptake transporters of unknown specificity. Speculatively, these may be involved in the usage of plant-derived aliphatic and aromatic compounds. *Neurospora* has fewer predicted amino acid transporters than does *S. pombe* or *S. cerevisiae. Neurospora* has a similar number of predicted sugar MFS transporters as *S. cerevisiae*. However, phylogenetic analysis of fungal sugar transporters (269) indicates that the *Neurospora* sugar transporters are more divergent than those of *S. pombe* or *S. cerevisiae*. Consistent with the effects of RIP, there are no close paralogues of *Neurospora* sugar transporters, whereas the majority of the *S. cerevisiae* HXT hexose transporters and the *S. pombe* GHT transporters represent two relatively recent and independent expansions and include very recent duplicated genes such as HXT15 and HXT16. Hence, the *Neurospora* sugar transporters are much more divergent, and few, if any, are clear paralogues of characterized *S. cerevisiae* sugar transporters. This may indicate that the *Neurospora* predicted sugar transporters are more functionally diverse and able to utilize a much broader range of sugars, which would correlate with the expanded number of sugar polysaccharide hydrolases encoded in the *Neurospora* genome (605).

Glycolysis, Fermentation, and Gluconeogenesis

Glycolysis and the pentose phosphate cycle. Glycolysis is the process whereby sugars are metabolized to acetyl-CoA or pyruvate before oxidation during the citric acid cycle or fermentation to ethanol or lactate (reviewed in references 184, 241, and 300). Three glycolytic pathways have been described: the Embden-Meyerhof-Parnas (EM), hexose monophosphate (HM), and Entner-Doudoroff (ED) pathways (reviewed in reference 300). The EM and HM pathways are prevalent in fungi, while evidence for the ED pathway has been demonstrated for only a few species (Fig. 6) (300). The EM and HM pathways share glucose-6-phosphate, fructose-6-phosphate, and glyceraldehyde-3-phosphate. However, these two pathways play very different roles during metabolism (184, 241, 300). The EM pathway utilizes NAD as an electron acceptor to produce NADH, which can then be reoxidized by fermentation or oxidative phosphorylation. In contrast, HM glycolysis reduces NADP to provide NADPH for a host of biosynthetic reactions. EM glycolysis produces ATP and pyruvate that can be converted to acetyl-CoA for entry into several key pathways, including the citric acid cycle and fatty acid biosynthesis. The latter portion of the HM pathway, termed the pentose phosphate cycle, consists of a series of freely reversible sugar-phosphate interconversions that provide glyceraldehyde-3-phosphate and fructose-6-phosphate to enter the EM pathway, as well as precursors of sugar alcohols, nucleic acids, and aromatic amino acids (184, 241, 300).

Consistent with the action of RIP, *Neurospora* usually possesses one copy of each gene in a given pathway, in contrast to the two (or more) often found in the *S. cerevisiae* genome (Table 29; Fig. 6). In addition, with the exception of a single transaldolase (see below), the predicted *Neurospora* proteins had other fungal enzymes as their best match. Of these, the highest similarity was to corresponding proteins from other filamentous fungi; in only one case (an alcohol dehydrogenase) was the closest homologue another *Neurospora* protein. Finally, only two enzymes in the pathways analyzed, phosphoglucomutase and glucose-6-phosphate isomerase, appear to be represented by known mutations in *Neurospora* (see below).

TABLE 28. Lipases

Enzyme	NCU no.	EC no.
Triacylglycerol lipase	03639.1	3.1.1.3
Triacylglycerol lipase	03301.1	3.1.1.3
Triacylglycerol lipase	08752.1	3.1.1.3

(i) Hexose phosphorylation. The glycolytic process begins with the phosphorylation of hexoses. Glycogen phosphorylation and cleavage to form glucose-1-phosphate is accomplished by glycogen (starch) phosphorylase. Similar to yeasts (241), *Neurospora* contains one form of this enzyme (Table 29). Glucose and fructose monomers can be phosphorylated by hexokinase at the C-6 position, while glucokinase phosphorylates glucose but not fructose. *S. cerevisiae* contains two hexokinases and one glucokinase (241). Evidence had previously been presented for four hexokinase activities in *Neurospora* (reviewed in reference 184). Analysis of the genome sequence is superficially consistent with these observations, since *Neurospora* has one good match each to glucokinase and hexokinase and two other genes with similarity to hexokinases. However, the protein corresponding to one of these other genes (NCU06996.1) does not appear to function as a hexokinase in *Aspergillus nidulans* but, instead, somehow regulates extracellular proteases during carbon starvation (404).

(ii) EM glycolysis. Previous work had suggested that the predominant route for glycolysis in *Neurospora* is entry of glucose-6-phosphate into the EM pathway (80 to 90%), with the remainder diverted to the HM pathway (reviewed in reference 184). The analysis of the genome sequence is consistent with intact EM and HM glycolytic pathways in *Neurospora* (Table 29).

To begin the EM pathway, the glucose-1-phosphate produced by the action of glycogen phosphorylase on endogenous glycogen is converted to glucose-6-phosphate by phosphoglucomutase; mutation of the *Neurospora rg* gene (533) causes deficiencies in this enzyme. Similar to *S. cerevisiae*, *Neurospora* contains one phosphoglucomutase gene. The presumed allelic *gpi-1* and *gpi-2* mutants (547) are lacking in glucose-6-phosphate isomerase, the enzyme that converts glucose-6-phosphate into fructose-6-phosphate. Analysis of the genome sequence showed that *Neurospora*, like yeasts (241), contains one glucose-6-phosphate isomerase gene.

Phosphofructokinase is a key regulatory enzyme in glycolysis, catalyzing the nonreversible phosphorylation of fructose-6-phosphate to produce fructose-1,6-bisphosphate, consuming a molecule of ATP in the process. *Neurospora* possesses one phosphofructokinase gene, in contrast to the two found in *S. cerevisiae* (241). *Neurospora* and yeasts both contain one fructose-bisphosphate aldolase gene (241), encoding the next enzyme in the pathway, which cleaves fructose-1,6-bisphosphate to form one molecule each of glyceraldehyde-3-phosphate and dihydroxyacetone phosphate.

Triose-phosphate isomerase interconverts glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. Analysis of the

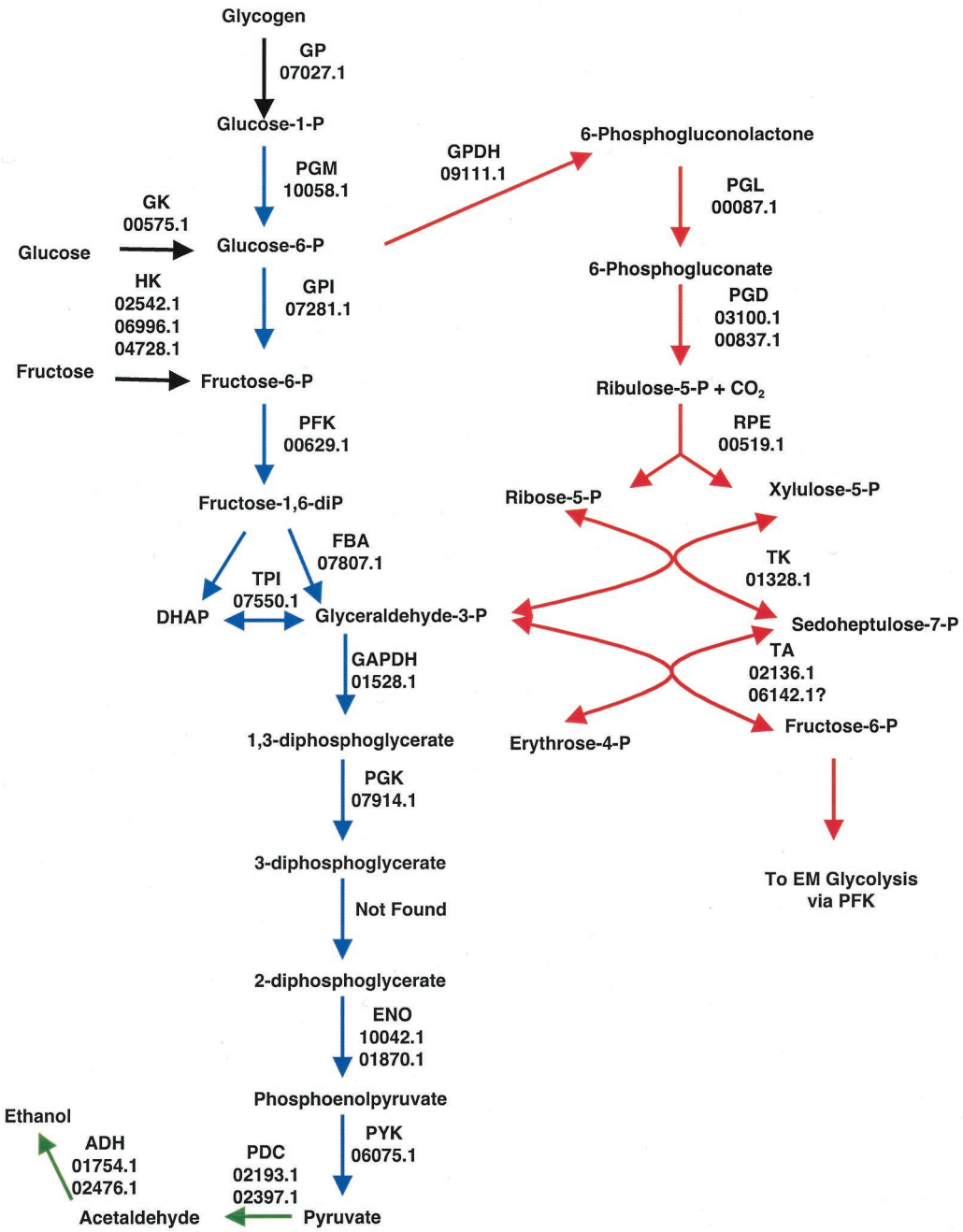

FIG. 6. Glycolysis and alcoholic fermentation. *Neurospora* contains genes encoding enzymes of the EM (blue arrows) and HM (red arrows) pathways of glycolysis. Enzymes required for hexose phosphorylation are indicated above the black arrows, while those involved in the fermentation pathway are indicated by green arrows. Abbreviations for enzyme names are presented in Table 29. NCU numbers for predicted *Neurospora* proteins corresponding to each enzyme are indicated alongside each arrow.

genome sequence shows that *Neurospora* possesses one good match to this gene. The protein encoded by another gene (NCU04399.1) shows some similarity to triose-phosphate isomerases in the carboxy terminus but is most similar to a putative ribose 5-phosphate isomerase from *Streptomyces coelicolor*. The weaker similarity, coupled with a large intron in the amino terminal region, lessens the likelihood that this gene is a true triose-phosphate isomerase. In addition, *S. cerevisiae* possesses only one triose-phosphate isomerase gene (241).

Glyceraldehyde-3-phosphate dehydrogenase converts glyceraldehyde-3-phosphate into 1,3-diphosphoglycerate, with accompanying reduction of NAD to NADH and H^+. For glycolysis to continue, the reduced NADH must be reoxidized (241). *Neurospora* contains one glyceraldehyde-3-phosphate dehydrogenase gene, in contrast to the three found in *S. cerevisiae* (241). It has been shown that glyceraldehyde-3-phosphate dehydrogenase is allelic to *ccg-7*, a clock-controlled gene in *Neurospora* (63, 731).

TABLE 29. Glycolysis, the pentose phosphate cycle, alcoholic fermentation, and gluconeogenesis

Enzyme	EC no.	NCU no.	BLAST match				
			Best overall	*S. cerevisiae*	*S. pombe*	Animal[a]	Plant[b]
Hexose phosphorylation							
Glycogen phosphorylase (GP)	2.4.1.1	07027.1	*A. fumigatus* GP; 0.00	Gph1p; 0.00	None	Liver GP; 0.00	Os α-1,4-glucan phosphorylase; 0.00
Hexokinase (HK)	2.7.1.1	02542.1	*A. oryzae* HxkA; 1.00e-167	Hxk2p; 2.00e-117	HK 1; 3.00e-112	Dm HK-t2; 1.00e-63	Os HK II; 8.00e-54
Hexokinase-like		06996.1	*A. nidulans* XprF; 1.00e-100	Glk1p; 1.00e-17	HK 1; 6.00e-20	Hs MGC:29452; 5.00e-20	Os putative HK 1; 4.00e-22
Hexokinase-like		04728.1	*K. lactis* RAG5 hexokinase; 4.00e-34	Hxk2p; 2.00e-31	HK 1; 7.00e-32	Dm HK-t2; 5.00e-26	At HK; 2.00e-25
Glucokinase (GK)	2.7.1.2	00575.1	*A. niger* GK; 1.00e-155	Glk1p; 3.00e-77	HK 2; 1.00e-76	Dm HK-t2; 1.00e-51	Os putative HK 1; 3.00e-60
EM glycolysis							
Phosphoglucomutase (PGM)	5.4.2.2	10058.1	*A. oryzae* PgmA; 0.00	Pgm2p/Gal5 p; 0.00	NP_59615 3.1; 0.00	Hs PGM1; 1.00e-158	At PGM; 1.00e-155
Glucose-6-phosphate isomerase (GPI)[c]	5.3.1.9	07281.1	*A. oryzae* PgiA; 0.00	Pgi1p; 0.00	NP_59663 5.1; 0.00	Dm AAA6367 2.1; 1.00e-168	At BAB1763 7.1; 1.00e-111
6-Phosphofructokinase (PFK)	2.7.1.11	00629.1	*A. niger* PFK; 0.00	Pfk2p; 0.00	NP_59594 6.1; 0.00	Hs 6-PFK; 0.00	At BAB5549 9.1; 1.00e-10
Fructose-bisphosphate aldolase (FBA)[c]	4.1.2.13	07807.1	*P. brasiliensis* AAL34519.2; 1.00e-135	Fbalp; 1.00e-128	FBA NP_59569 2.1; 1.00e-118	Ag EAA0269 2.1; 5.00E-88 (not in mammals)	AT T10O22.2 4; 2.00e-08
Triose-phosphate isomerase (TPI)[c]	5.3.1.1	07550.1	*P. brasiliensis* TPI; 2.00e-83	Tpi1p; 5.00e-79	TPI; 7.00e-72	Hs TPI 1; 2.00e-77	Os TPI; 3.00e-76
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)[c]	1.2.1.12	01528.1	*S. macrospora* Gpd; 1.00e-178	Tdh1p; 1.00e-119	GAPDH; 1.00e-127	Mm NP_03211 0.1; 2.00e-135	At NP_17807 1.1; 1.00e-136
Phosphoglycerate kinase (PGK)[c]	2.7.2.3	07914.1	*T. viride* PGK; 0.00	Pgk1p; 1.00e-154	NP_59673 0.1; 8.00e-163	Hs PGK1; 1.00e-160	At NP_17601 5.1; 1.00e-105
Enolase (ENO)[c]	4.2.1.11	10042.1	*A. oryzae* ENO; 0.00	Eno1p; 1.00e-173	NP_59590 3.1; 1.00e-155	Dm NP_72272 1.1; 2.00e-149	At ENO F2P9.10; 1.00e-140
Enolase[c]	4.2.1.11	01870.1	*S. japonicum* ENO; 8.00e-129	Eno1p; 1.00e-120	NP_59590 3.1; 1.00e-120	Mm ENO 1; 1.00e-123	At ENO F2P9.10; 1.00e-122
Pyruvate kinase (PYK)	2.7.1.40	06075.1	*T. reesei* PYK; 0.00	Pyk1p; 4.00e-173	PYK NP_59434 6.1; 9.00e-179	Ag EAA1055 5.2; 2.00e-140	At PYK NP_19436 9.1; 4.00e-107
HM glycolysis/pentose phosphate pathway							
Glucose-6-phosphate 1-dehydrogenase (GPDH)	1.1.1.49	09111.1	*A. niger* GPDH; 0.00	Zwf1p; 1.00E-160	Zwf1; 1.00E-147	GPDH X-linked; 1.00e-138	At GPDH; 1.00e-132
6-Phosphogluconol aconase (PGL)	3.1.1.31	00087.1	*S. pombe* Sol1; 8.00e-65	Sol1p; 4.00e-57	Top hit	Mm PGL; 4.00e-42	At PGL-like; 4.00e-39
6-Phosphogluconic dehydrogenase (PGD)	1.1.1.44	03100.1	*A. niger* PGD; 0.00	Gnd1p; 0.00	PGD; 0.00	Hs PGD protein; 0.00	At PGD; 1.00e-115
6-Phosphogluconic dehydrogenase (PGD)	1.1.1.44	00837.1	*A. niger* PGD; 1.00e-120	Gnd2p; 2.00e-66	SPBC660 16; 5.00e-67	Mm PGD; 1.00e-62	At PGD-related; 9.00e-72
Ribulose-phosphate 3-epimerase (RPE)	5.1.3.1	00519.1	*S. cerevisiae* Rpe1p; 8.00e-51	Top hit	SPAC31G 5.05c; 5.00e-48	Hs MGC:226 37; 9.00e-44	RPE; 9.00e-48
Transketolase (TK)	2.2.1.1	01328.1	*A. niger* TK; 0.00	Tk11p; 0.00	SPBC2G5.05; 0.00	Hs TK; 2.00e-42	At TK precursor related; 0.00
Transaldolase (TA)	2.2.1.2	02136.1	*S. kluyveri* Tal1p; 1.00e-106	Tal1p; 3.00e-95	Tal1; 1.00e-106	Ag XP_32071 5.1; 2.00e-88	At ToTAL2; 7.00e-32
Transaldolase?	2.2.1.2	06142.1	*H. sapiens* transaldolase; 1.00e-15	Tal1p; 5.00e-10	Tal1; 2.00e-09	Top hit	None
Fermentation							
Pyruvate decarboxylase (PDC)	4.1.1.1	02193.1	*S. pombe* SPAC186.09; 0.00	Pdc6p; 6.00e-57	Top hit	Dm CG11208-PA; 2.00E-11	At Pdc1; 1.00e-119
Pyruvate decarboxylase (PDC)	4.1.1.1	02397.1	*P. anserina* CAD60727.1; 0.00	Pdc1p; 1.00e-141	C13A11.06; 1.00e-163	Ce 2-hydroxyphytanoyl-CoA lyase; 1.00e-11	At PDC-related protein; 2.00e-63
Alcohol dehydrogenase (ADH)	1.1.1.1	01754.1	*A. flavus* Adh1; 1.00e-133	Adh3p; 1.00e-105	Adh1; 9.00e-97	Ce probable ADH; 1.00e-73	Os CAD3990 7.1; 3.00e-26
Alcohol dehydrogenase (ADH)	1.1.1.1	02476.1	*N. crassa* NCU01754.1; 1.00e-126	Adh3p; 6.00e-97	Adh1; 1.00e-87	Ce ADH; 2.00e-70	At cinnamyl-ADH; 8.00e-30
Gluconeogenesis							
Fructose-bisphosphatase (FBP)	3.1.3.11	04797.1	*A. nidulans* FBP; 5.00e-163	Fbp1p; 1.00e-112	P09202; 1.00e-102	Dm CG31692-PA; 1.00e-83	At 1g43670; 6.00e-93
Phosphoenolpyruvate carboxykinase (PEPCK)	4.1.1.49	09873.1	*A. nidulans* PEPCK; 0.00	Pck1p; 0.00	None	None	At 4g37870; 0.00

[a] *Caenorhabditis elegans* (Ce), *Drosophila melanogaster* (Dm), *Anopheles gambiae* (Ag), *Mus musculus* (Mm), or *Homo sapiens* (Hs).
[b] *Arabidopsis thaliana* (At) or *Oryza sativa* (Os).
[c] Also a component of gluconeogenic pathway.

A high-energy phosphate from 1,3-diphosphoglycerate is transferred to ADP to produce ATP and 3-phosphoglycerate by the enzyme phosphoglycerate kinase. This is the first of two ATP-generating steps in glycolysis (241). Like *S. cerevisiae*, the *Neurospora* genome sequence contains one phosphoglycerate kinase gene. *S. cerevisiae PGK1* is abundantly expressed; there are also numerous ESTs for the corresponding *Neurospora* gene.

There were no good matches in the genome database to phosphoglycerate mutase, the enzyme that allows the interconversion between 3-phosphoglycerate and 2-phosphoglycerate. The best match to both the rat and fungal enzymes, NCU01921.1, is interrupted by several introns and is most similar to an uncharacterized ORF from *S. cerevisiae*. Because phosphoglycerate mutase is a highly conserved enzyme, it is likely that the true phosphoglycerate mutase gene is absent from the current sequence assembly. *S. cerevisiae* contains one phosphoglycerate mutase gene (241).

Enolase catalyzes the production of phosphoenolpyruvate from 2-phosphoglycerate. *Neurospora* and *S. cerevisiae* (241) each contain two enolase genes. The two *S. cerevisiae* genes are differentially regulated (241); in this regard, it may be significant that NCU10042.1 is encoded by several ESTs while NCU01870.1 is represented by only one EST in available *Neurospora* databases.

The final step of EM glycolysis is the conversion of phosphoenolpyruvate and ADP to pyruvate and ATP by pyruvate kinase. This is the both the second ATP-generating and irreversible step of the EM pathway. *Neurospora* possesses one pyruvate kinase gene, in contrast to the two found in *S. cerevisiae* (241).

(iii) HM and ED glycolysis and the pentose phosphate cycle. The HM and ED pathways begin with glucose-6-phosphate being converted to glucono-1,5-lactone-6-phosphate by glucose-6-phosphate dehydrogenase. Similar to *S. cerevisiae* (241), *Neurospora* contains a single version of this enzyme (Table 29). The second step of the HM and ED pathways, production of 6-phosphogluconate from glucono-1,5-lactone-6-phosphate, is catalyzed by 6-phosphogluconolaconase. *S. cerevisiae* possesses genes encoding four such proteins, while *Neurospora* contains only one.

At this point, the HM and ED pathways diverge. The next reaction in the ED pathway is the conversion of 6-phosphogluconate to 2-dehydro-3-deoxygluconate-6-phosphate by phosphogluconate dehydratase. This enzyme is not found in *S. cerevisiae* (http://www.yeastgenome.org/). The best *Neurospora* match to the *E. coli* phosphogluconate dehydratase is an enzyme of amino acid biosynthesis, a dihydroxy acid dehydratase (Ilv3p; http://www.yeastgenome.org/) found in both *S. cerevisiae* and *S. pombe*. Similarly, the final enzyme in ED glycolysis, 2-dehydro-3-deoxyphosphogluconate aldolase, is not present in the *Neurospora, S. cerevisiae*, or *S. pombe* genome sequences (http://www.yeastgenome.org/; http://www.sanger.ac.uk/Projects/S_pombe/). This enzyme catalyzes the production of glyceraldehyde-3-phosphate and pyruvate from 2-dehydro-3-deoxygluconate-6-phosphate. Thus, *Neurospora*, like the two sequenced yeasts, lacks the final two (and the only pathway-specific) enzymes of ED glycolysis.

Continuing along the HM glycolytic route is the enzyme 6-phosphogluconate dehydrogenase, which catalyzes the production of ribulose-5-phosphate and carbon dioxide from 6-phosphogluconate. Like *S. cerevisiae* and *S. pombe*, *Neurospora* possesses two genes encoding this enzyme. Similarly, *S. cerevisiae* (241) and *Neurospora* each contain one gene encoding ribulose-phosphate 3-epimerase, the enzyme that converts ribulose-5-phosphate to xylulose-5-phosphate.

The next two enzymes in the HM pathway, transketolase and transaldolase, comprise the pentose phosphate cycle. Transketolase interconverts ribose-5-phosphate and xylulose-5-phosphate to produce sedoheptulose-7-phosphate and glyceraldehyde-3-phosphate. In some species, this enzyme can utilize erythrose-4-phosphate and xylulose-5-phosphate to produce fructose-6-phosphate and glyceraldehyde-3-phosphate. *Neurospora* has one transketolase gene, in contrast to the two found in *S. cerevisiae* (241).

Transaldolase reacts sedoheptulose-7-phosphate and glyceraldehyde-3-phosphate to produce erythrose-4-phosphate and fructose-6-phosphate. *S. cerevisiae* and *S. pombe* contain one transaldolase gene (241). *Neurospora* has two matches to transaldolases; one is most similar to fungal isozymes (NCU02136.1), while the other (NCU06142.1) shows highest identity to transaldolase from humans but still exhibits significant similarity to transaldolases from fungi. Further analysis is needed to determine whether this second protein possesses transaldolase activity and contributes to pentose phosphate metabolism in *Neurospora*.

Alcoholic fermentation. Pyruvate produced from the glycolytic pathway can be oxidized to CO_2 through the action of the citric acid cycle, or it can be converted to ethanol by the alcoholic fermentation pathway (184, 241). Fermentation in fungi begins with the decarboxylation of pyruvate to yield acetaldehyde and CO_2 by the enzyme pyruvate decarboxylase (184). The *Neurospora* genome contains one pyruvate decarboxylase gene, while *S. cerevisiae* possesses at least three such genes (241).

To complete fermentation, acetaldehyde is reduced to ethanol using NADH + H^+ by alcohol dehydrogenase (184). *Neurospora* possesses two proteins with high similarity to *S. cerevisiae* alcohol dehydrogenases (yeast has a total of four [241]), as well as several other predicted proteins with lower BLASTp scores (Table 29; Fig. 6). One of the two *Neurospora* alcohol dehydrogenases has the other *Neurospora* protein as its closest homologue.

Gluconeogenesis. Gluconeogenesis allows organisms to utilize noncarbohydrates as energy sources (Fig. 6). The gluconeogenic pathway is essentially the reversal of EM glycolysis and shares several enzymes that catalyze freely reversible reactions, including enolase, phosphoglycerate kinase, glyceraldehyde-3-phosphate dehydrogenase, triose-phosphate isomerase, fructose-bisphosphate aldolase, and glucose-6-phosphate isomerase. However, two of the reactions catalyzed by EM glycolytic enzymes, pyruvate kinase and 6-phosphofructokinase, are not reversible due to unfavorable levels of reactants in the cytoplasm (241). Thus, organisms must utilize phosphoenolpyruvate carboxykinase which catalyzes the conversion of oxaloacetate to phosphoenolpyruvate with net consumption of ATP) and fructose bisphosphatase (which hydrolyzes fructose-1,6-diphosphate to form fructose-6-phosphate) to circumvent the two nonreversible steps. *Neurospora* and *S. cerevisiae*

(241) each contain one copy of the phosphoenolpyruvate carboxykinase and fructose bisphosphatase genes (Table 29).

Mitochondrion and Energy Metabolism

The mitochondrial genome of *Neurospora* is a circle of 64,840 bp. It contains two rRNAs, at least 27 tRNAs, and 26 ORFs. Three tRNAs (Met-1, Met-2, and Cys) have been duplicated. Thus, including tRNA Met-3, a total of five methionine tRNAs are present. The duplicated tRNAs Met-2 and Cys are part of a 3-kb duplication and are located upstream and downstream, respectively, of two duplicated concatenated fragments of the *nd2* gene. The entire duplicated region gives rise to one polycistronic mRNA (9). As in most eukaryotes, 14 subunits of complexes involved in oxidative phosphorylation are encoded in the mitochondrial DNA. A gene for ATPase subunit 9 is present in the nucleus as well as in the mitochondrial genome (823). The nuclear gene accounts for the bulk of the subunit in vegetative cells (373, 699, 814), but the mitochondrial version seems to be expressed in germinating spores (83). As mentioned above, part of the *nd2* gene is duplicated, resulting in an additional ORF of unknown significance.

A total of 10 intronic ORFs are present in the single introns of the *atp6*, *nd1*, *nd3*, *nd4*, and *nd4L* genes, the two introns in each of *nd5* and *cob*, and the intron in the *rnl* gene that encodes the large subunit rRNA. The latter intronic ORF codes for ribosomal protein S5, a homologue of the *S. cerevisiae* Var1p protein (115). All introns in the protein-coding genes are group I introns that encode putative maturases/endonucleases (136). The ORFs in the introns of the *atp6* and *nd1* genes are free-standing, while the other intron ORFs are continuous, in frame with the preceding exon. The ORF in intron 1 of the *cob* gene shows an exci-endo-N domain encoding a putative GIY-YIG motif endonuclease. All other introns encode putative endonucleases of the LAGLIDADG (dodecapeptide) type (448). However, the ORF in the *nd3* gene is truncated by a stop codon interrupting what may have been the coding sequence for a maturase/endonuclease.

The only ORF in the *Neurospora* mitochondrial genome lacking a homologue in other organisms encodes a hypothetical 70-kDa protein. The ORF is located upstream of the *cox1* gene and is cotranscribed with the latter (117). No experimental data are available regarding its expression. Reviews of studies of the content and expression of the *Neurospora* mtDNA have been published elsewhere (301, 410).

Mitochondrial proteins encoded in the nucleus have to be imported into mitochondria. The majority of mitochondrial proteins contain N-terminal presequences that serve as targeting signals that are cleaved after import. Others lack a cleavable N-terminal import signal, and their targeting information is part of the mature protein sequence (567, 646, 838). Sequence features conserved in N-terminal targeting sequences have been used to predict the mitochondrial localization of proteins deduced from DNA sequence. However, the predictions are hampered by poor conservation of these targeting signals. Target P, a widely used software tool for the sequence based identification of N-terminal presequences (220), predicts that 18%, or 1,962, of the 10,900 deduced *Neurospora* ORFs would be mitochondrial. To judge the usefulness of this prediction for compiling a set of mitochondrial ORFs in *Neurospora*, the performance of Target P was tested using 1,096 ORFs assigned to various cellular compartments based on their close relationship to known proteins from other organisms (Table 30) (498). Of 269 ORFs predicted by Target P to be mitochondrial, 95 (36%) had been assigned to a nonmitochondrial compartment. Only 58% of the 301 ORFs assigned to mitochondria were identified by Target P (Table 30). ORFs not identified by Target P to be mitochondrial include those lacking an N-terminal targeting signal, those containing an N-terminal targeting signal not recognized by Target P, and those incorrectly suggested to contain an ER targeting signal by the program. When the prediction of Target P is adjusted by considering these two counteracting sources of error, the number of different mitochondrial proteins predicted for *Neurospora* reaches about 2,200. We are not aware of any bias in the set of 1,096 ORFs used for the comparison that would result in a significant error when extrapolated to the entire gene set. Estimates for the number of mitochondrial proteins in other organisms cover a wide range. For example, for *S. cerevisiae*, an estimate based largely on the results of a high-throughput immunolocalization screen suggested that 13%, or 793, of the 6,100 predicted yeast proteins would be mitochondrial (439). Based on protein phylogenetic profiles, Marcotte et al. (499) calculated about 630 mitochondrial proteins for yeast and 660 for *C. elegans*. Larger numbers were calculated for *A. thaliana* (2,000) (437) and humans (1,500) (784), based on data obtained from two-dimensional (2D) gel electrophoresis.

TABLE 30. *Neurospora* ORFs assigned to mitochondria by different means

ORF	Total no.	No. (%) assigned to mitochondria by Target P
Manually annotated	8,350	1,515 (18)
Manually annotated assigned to any cell compartment	1,096	269 (25)
Manually annotated assigned to mitochondria	301	174 (58)

An alternative approach to the identification of mitochondrial proteins deduced from the genomic sequence of *Neurospora* relies on sequence comparison. This works quite well for generally known mitochondrial proteins, since the classification can be drawn from the comparison to homologs from various organisms. Thus, by manual annotation of 8,350 of the 10,900 *Neurospora* ORFs predicted in the genome sequence, 301 *Neurospora* ORFs were assigned to the mitochondrion due to their similarity to known mitochondrial proteins (Table 30). Significantly less reliable are conclusions drawn from comparisons to mitochondrial proteins from single, distantly related organisms. This is illustrated by a comparison to a large set of mitochondrial proteins compiled from data obtained by 2D gel electrophoresis of human mitochondria (785). Of 615 proteins in this set, 178 proteins did not match ($E > 10^{-5}$) a *Neurospora* ORF. Among these were ribosomal proteins and subunits of complexes involved in protein import and oxidative phosphorylation which probably did not yield a *Neurospora* homologue due to weak sequence conservation. Furthermore, detection of high similarity to human proteins identified by the 2D gel approach can also be misleading. Among 71 known *Neurospora*

proteins that are closely related to human proteins in the data set, 22 have been shown experimentally to exist outside mitochondria. This could be due to contamination of the human mitochondria that were analyzed or it could be because some proteins that show a relationship to mitochondrial proteins are actually localized to a different cellular compartment.

More reliable conclusions can be drawn from comparisons to closely related organisms. In the *S. cerevisiae* database, a set composed of 412 protein sequences is annotated as mitochondrial (MIPS database: http://mips.gsf.de/proj/yeast/CYGD/db; SGD database: http://genome-www.stanford.edu/Saccharomyces). By searching the entire *Neurospora* gene set deduced from the genomic sequence for close homologues, we identified 350 *Neurospora* ORFs. When these were combined with the 301 ORFs attributed to mitochondria by manual annotation, a set of 446 *Neurospora* ORFs resulted. For 321 of the predicted *Neurospora* mitochondrial protein ORFs, a 1:1 match to an ORF of *S. cerevisiae* was found. A total of 36 *Neurospora* ORFs were found in a 2:1 ratio relative to *S. cerevisiae*. That is, 18 pairs of *Neurospora* ORFs were found where both members of the pair were most closely related to a single *S. cerevisiae* ORF. In one case, a set of three *Neurospora* ORFs was most closely related to a single *S. cerevisiae* ORF (3:1 ratio). Conversely, 17 *Neurospora* ORFs were found in a 1:2 ratio relative to yeast ORFs. That is, 17 pairs of *S. cerevisiae* ORFs were found where both members of the pair most closely matched one *Neurospora* ORF. Four *Neurospora* ORFs each matched three different ORFs of *S. cerevisiae* (1:3 ratio). There were 65 *Neurospora* mitochondrion-assigned ORFs and 46 *S. cerevisiae* mitochondrion-assigned ORFs that were not detected in the deduced sequences of the other organism.

ORFs identified as mitochondrial in *S. cerevisiae* but lacking a significant *Neurospora* match are involved mainly in the expression of mitochondrial genes and the processing of gene products. A considerable number are likely to have a *Neurospora* homologue that was not detected in this analysis due to insufficient sequence conservation. For example, there are nine subunits of *S. cerevisiae* mitochondrial ribosomes that lack a matching ORF in the *Neurospora* genome. Similarly, 22 proteins involved in the processing and translation of mitochondrial mRNAs and the assembly of respiratory complexes, four subunits of respiratory complexes, and two subunits of the translocation channel of the outer membrane are known in yeast, but a *Neurospora* homologue has not yet been identified. Three *S. cerevisiae* proteins, Imp1p, Imp2p, and Som1p, are involved in the processing of proteins in the inner membrane. The other nonmatched *S. cerevisiae* proteins include a protoporphyrinogen oxidase (Hem14p), a protein required for autophagy (Apg14p), RNase P (Rpm2p), a 3′–5′ exonuclease (Rex2p), and Lag2p, which is involved in determining longevity.

Most of the *Neurospora* mitochondrial proteins not found in *S. cerevisiae* are involved in respiration. *S. cerevisiae* and other fermentative yeasts lack respiratory complex I, and so the 31 subunits of complex I (24 nuclear and 7 mitochondrially encoded) are not present. In addition, five intron-located ORFs in *nd* genes of the mitochondrial genome and two assembly factors for complex I identified in *Neurospora* (438) are absent from yeast. Also missing in *S. cerevisiae* is the alternative oxidase that catalyzes the oxidation of ubiquinol by oxygen, thus bypassing respiratory complexes III and IV. Alternative oxidase is present in the mitochondria of all higher plants, many fungi, many eukaryotic algae, and some protists (828). In *Neurospora*, the enzyme is present only under conditions that compromise normal mitochondrial function (450, 451). The *aod-1* gene (4nc285_080 [Table 31]) codes for the *Neurospora* alternative oxidase (472). Mutant screens also identified the *aod-2* gene, which is thought to encode an as-yet-unidentified regulatory factor required for *aod-1* expression (72, 449). A search of the *Neurospora* genome revealed a close relative of *aod-1*. This gene, termed *aod-3* (xnc010_210 [Table 31]) specifies a second alternative oxidase, but the conditions required for its expression are unknown (782). Finally, yeast mitochondria lack a transhydrogenase for coupling the redox state of the internal and external NAD^+-NADH pool, which is found in *Neurospora* and many other organisms (13e11_040 [Table 31] (446).

A number of ORFs assigned to mitochondria in *Neurospora* but not found in *S. cerevisiae* are involved in the degradation of amino acids. A probable isovaleryl-CoA dehydrogenase and a probable β -subunit of methylcrotonyl-CoA carboxylase are found adjacent to each other on linkage group I (1nc356_060 and 1nc356_070) in a head-to-head orientation, while the α subunit of methylcrotonyl-CoA carboxylase (b22i21_180) is located on linkage group II. All three participate in the degradation of leucine. In addition, ORF 29e8_120 is related to 3-hydoxybutyrate dehydrogenase, involved in the degradation of valine. The amino acids valine, leucine, and isoleucine share related degradation pathways. Enzymes involved in the degradation are found in mitochondria as well as the cytosol. *Neurospora* uses the same enzymes as plants and animals for this purpose, while *S. cerevisiae* and *S. pombe* lack these enzymes.

Other ORFs found in *Neurospora* but not in *S. cerevisiae* are rather distantly related to known proteins, and their function is therefore still unresolved (Table 31). These include another two acyl-CoA dehydrogenases. Also listed are seven mitochondrial carrier proteins of unknown specificity for which a close yeast homologue has not been identified. This is in contrast to the majority of mitochondrial carrier proteins, for which a 1:1 ratio to a yeast homologue is apparent. The closest *S. cerevisiae* homologues listed in Table 31 are significantly more closely related to a different *Neurospora* protein.

A peculiarity of filamentous fungi appears to be the presence of a specific succinyl-CoA synthetase (SCS). The known SCS is a heterodimer of two subunits (105). The genome of *Neurospora* reveals two ORFs for these subunits (bj10_140 and 8d4_130 [Table 31]), as well as a third ORF (1nc250_090 [Table 31]) in which the N-terminal half is related to the beta subunit, while the C-terminus is homologous to the alpha subunit. This additional SCS is also found in *A. nidulans* and *M. grisea* but has not yet been found in yeast, plants, and animals.

As yet, there is no comprehensive set of mitochondrial proteins compiled for *Neurospora* or any other organism. Since fewer than 500 known or deduced proteins have been assigned to mitochondria, this suggests that only one-quarter of the expected 2,000 different mitochondrial proteins can be extracted using the genomic sequence information alone. Although the proteins required for well-characterized mitochon-

TABLE 31. Known and deduced *Neurospora* mitochondrial proteins lacking a close homologue in *S. cerevisiae*[a]

Gene name	NCU no.	MIPS code	BLAST match				
			Best overall	*S. cerevisiae*	*S. pombe*	Animal	Plant
aod-3	04874.1	xnc010_210	2.0e-140, T:AF321004_1	None	None	None	1e-53, PIR:T07947
Alternative oxidase (*aod-1*)	07953.1	4nc285_080	1.6e-251, TN:AY140655_1	None	None	None	1e-50, PIR:T07947
Probable isovaleryl-CoA dehydrogenase	02126.1	1nc356_060	1.0e-145, PIR:D95929	None	None	5.7e-130, PIR:A37033	9.0e-143, SP:IVD2_SOLTU
Probable methylcrotonyl-CoA carboxylase beta chain	02127.1	1nc356_070	3.2e-234, TN:AE003790_19	None	None	2.0e-221, SN:MCCB_HUMAN	1e-159, T:AF386926_1
Probable methylcrotonyl-CoA carboxylase alpha chain	00591.1	b22i21_180	2.5e-165, TN:AE003779_43	None	None	1.3e-122, SN:MCCA_HUMAN	7.4e-161, SN:MCCA_ARAT H
Probable dienoyl-CoA isomerase (ECH1)	06647.1	18a7_070	4e-62, PIR:T16494	None	None	4e-62, PIR:T16494	2e-49, T:AB017070_10
Related to 3-hydroxyisobutyrate dehydrogenase	ND[b]	29e8_120	6.6e-52, PIR:G96013	None	None	7.4e-22, T:AC007130_1	8.1e-52, PIR:D86317
Related to long-chain-specific acyl-CoA dehydrogenase	08924.1	20h10_010	1.1e-109, T:AY033936_1	None	None	6.0e-26, TN:AB083302_1	None
Related to short-chain 3-hydroxy-acyl-CoA dehydrogenase	08058.1	1nc100_110	1e-22, T:AP000996_69	None	None	5e-19, SP:HCDH_RAT	4e-4, PIR:T08956
CoxI translation protein CYA5	08692.1	4nc677_080	1.0e-10, T:AB008268_15	None	None	None	1.0e-10, T:AB008268_15
Related to nicotinamide nucleotide transhydrogenase	01140.1	13e11_040	0.0, PIR:T15521	None	None	0.0, T:BTNAD_1	None
Related to light-induced alcohol dehydrogenase Bli-4	01107.1	b13o8_020	5.2e-29, TN:CEE04F6_10	None	3.3e-23, PIR:T41570	None	9.9e-27, T:AC020666_24
Related to kinetoplast-associated protein KAP	04650.1	5f3_190	3.6e-30, PIR:A44937	None	None	None	None
Related to lysophosphatidic acid phosphatase	06460.1	3nc220_480	1.6e-55, PIR:T40420	None	1.6e-55, PIR:T40420	1.5e-08, PIR:JH0610	None
Related to succinate-CoA ligase alpha and beta chains	09810.1	1nc250_090	1.7e-78, PIR:S61696	1.7e-78, PIR:S61696	None	1.8e-68, SP:SUCA_PIG	5.0e-70, PIR:T51816
Mitochondrial carrier protein	06662.1	100h1_060	3.4e-34, PIR:T38879	None	3.4e-34, PIR:T38879	1.7e-33, SN:MCAT_MOUSE	3.7e-33, T:AC060755_11
Mitochondrial carrier protein	03556.1	b7n14_120	6.0e-67, PIR:S50283	None	None	1.7e-31, SP:PM34_HUMAN	6.1e-51, PIR:F84823
Mitochondrial carrier protein	01564.1	b21o8_150	3.9e-136, T:AF419344_1	3.9e-136, T:AF419344_1	2.4e-97, T:SPBC12D12_6	4.3e-62, PIR:T50686	6.9e-81, PIR:T49871
Mitochondrial carrier protein	07578.1	ND	2.5e-21, PIR:F84823	1.3e-16, PIR:S69019	None	7.7e-13, T:XLA289240_1	2.5e-21, PIR:F84823
Mitochondrial carrier protein	04792.1	ND	2.3e-169, PIR:T11614	1.5e-92, PIR:S64101	2.3e-169, PIR:T11614	6.6e-114, TN:BC024043_1	6.5e-79, PIR:T07405
Mitochondrial carrier protein	01810.1	b8b8_140	7.0e-44, PIR:T40082	8.8e-18, PIR:S60997	7.0e-44, PIR:T40082	1.1e-22, T:BC037680_1	1.4e-24, T:AC060755_11
Mitochondrial carrier protein	02352.1	7nc520_030	5.0e-27, PIR:B96830	1.1e-19, PIR:S61660	7.4e-21, PIR:T38879	1.4e-26, TN:BC037680_1	5.0e-27, PIR:B96830

[a] Not included are 40 proteins related to respiratory complex I (subunits, assembly proteins and intron coded proteins). E-values and codes of BLAST hits are given (T, TREMBL; TN, TREMBLNEW; SP, SWISSPROT; SN, SWISSNEW).

[b] ND, not determined.

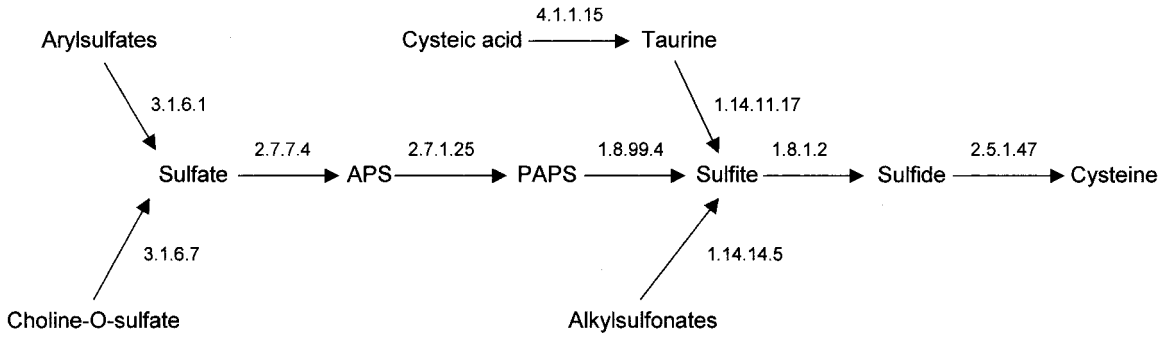

FIG. 7. Pathway of sulfur acquisition leading to sulfur assimilation and cysteine biosynthesis in *Neurospora*. Potential sulfur sources from the environment or internal stores are indicated. The alkylsulfonate and cysteic acid conversions are predicted from the genome analysis. EC designations are shown; in some cases, putative multiple forms are indicated from the analysis (see Tables 32 and 35). Corresponding NCU numbers are as follows: EC 3.1.6.1 (arylsulfatase, NCU06041.1), EC 3.1.6.7 (choline sulfatase, NCU08364.1), EC 2.7.7.4 (ATP sulfurylase, NCU01985.1), EC 2.7.1.25 (adenylyl sulfate kinase, NCU0896.1), EC 1.8.99.4 (PAPS reductase, NCU02005.1), EC 4.1.1.15 (cysteic acid decarboxylase, NCU06112.1), EC 1.14.11.17 (taurine dioxygenase, NCU07610.1, NCU07819.1, NCU09738.1, NCU09800.1), EC 1.14.14.5 (alkanesulfonate monooxygenase, NCU05340.1, NCU10015.1), EC 1.8.1.2 (sulfite reductase, NCU04077.1, NCU05238.1), and EC 2.5.1.47 (cysteine synthase, NCU02564.1, NCU03788.1, NCU06452.1). Abbreviations: APS, adenosine-5′-phosphosulfate; PAPS, 3′-phosphoadenosine-5′-phosphosulfate.

drial functions have been assigned, these represent only a subset of mitochondrial metabolism.

Sulfur Metabolism

Sulfur acquisition and processing. When a nutrient such as sulfur is limiting, fungal cells will exhibit responses targeted to specifically alleviate the nutrient deficiency. In *Neurospora*, the responses include induction of transport systems (permeases) and enzymes that can release sulfur from internal or external sources (506, 584). These responses allow *Neurospora* to scavenge sulfur from a variety of possible sources. Previously unknown capabilities related to *Neurospora* sulfur acquisition are revealed from the genomic data. Of particular interest are two putative alkanesulfonate monooxygenases that can use aliphatic sulfonates (e.g., ethanesulfonic acid and isethionic acid but not compounds such as taurine) to generate sulfite, which can be used in the synthesis of cysteine (Fig. 7). The two putative alkanesulfonate monooxygenases show similarities only to bacterial alkanesulfonate monooxygenases (Table 32) (214) and have no significant matches to known eukaryotic proteins, they may represent a case of horizontal gene transfer. The presence of these putative alkanesulfonate monooxygenases would give *Neurospora* the versatility to use an important class of sulfur compounds typically found in the environment. Sulfonates and sulfate esters represent the bulk of the sulfur content of aerobic soils (411).

Both cysteic acid and taurine are also potential sulfur sources for *Neurospora*. Analysis of the *Neurospora* genome suggests that the metabolism of cysteic acid is likely to occur by conversion into taurine. Extensive iterative database searching did not reveal other metabolic routes for either cysteic acid and taurine besides those shown in Fig. 7 (e.g., no homologues of sulfinoalanine decarboxylase, cysteine lyase, hypotaurine dehydrogenase, or other related enzymes involved in taurine and hypotaurine metabolism in a variety of other organisms were identifiable). Cysteic acid decarboxylase (also known as glutamate decarboxylase), which can convert cysteic acid to taurine, is present. Surprisingly, the putative *Neurospora* cysteic acid/glutamate decarboxylase is most homologous to human GDC isoform 67 and has no identifiable homologs in yeast or plants (Table 32). This area of metabolism is relatively unexplored in *Neurospora*. In mammalian systems, taurine is an abundant

TABLE 32. Sulfur acquisition and processing

Enzyme	EC no.	NCU no.	BLAST match				
			Best overall	*S. cerevisiae*	*S. pombe*	Animal[a]	Plant[b]
Arylsulfatase (ARS-1)	3.1.6.1	06041.1	*P. anserina* Arylsulf./0.0	—[c]	—	2e-49	—
Choline sulfatase	3.1.6.7	08364.1	*P. aeruginosa* Chol. Sulf./e-162	—	2e-15	1e-29	—
Alkanesulfonate monooxygenase	1.14.14.5	10015.1	*P. putida* MsuD/7e-126	—	—	—	—
Alkanesulfonate monooxygenase	1.14.14.5	05340.1	*R. solanacearum* SsuD/3e-36	—	—	—	—
Cysteic acid decarboxylase	4.1.1.15	06112.1	*H. sapiens* Gdc67/3e-74	—	—	3e-74	3e-17
Taurine dioxygenase	1.14.11.17	09738.1	*S. pombe* SCPB1C11.04C/6e-73	—	6e-73	—	—
Taurine dioxygenase	1.14.11.17	09800.1	*Y. pestis* Tau. Diox./3e-28	5e-27	—	—	—
Taurine dioxygenase	1.14.11.17	07610.1	*E. coli* TauD/3e-27	4e-26	—	—	—
Taurine dioxygenase	1.14.11.17	07819.1	*S. cerevisiae* Y11057cp/4e-36	4e-36	—	—	—
Cysteine dioxygenase	1.13.11.20	06625.1	*H. sapiens* cys. diox./3e-37	—	—	3e-37	—
Sulfite oxidase	1.8.3.1	04474.1	*H. sapiens* SUOX/1e-48	—	—	1e-48	1e-47
Sulfite oxidase	1.8.3.1	06931.1	*C. elegans* XQ117/9e-88	—	—	9e-88	3e-46

[a] *Caenorhabditis elegans*, *Drosophila melanogaster*, *Mus musculus*, or *Homo sapiens*.
[b] *Arabidopsis thaliana* or *Oryza sativa*.
[c] Values below e-10 are represented as —.

TABLE 33. Sulfur transporters

Transporter	TC no.	NCU no.	BLAST match				
			Best overall	*S. cerevisiae*	*S. pombe*	Animal[a]	Plant[b]
Sulfate permease I (CYS-13)	2A.53.1.2	03235.1	*P. chrysogenum* SutB/0.0	e-137	e-158	2e-56	8e-52
Sulfate permease II (CYS-14)	2.A.53.1.2	04433.1	*P. chrysogenum* SutB/e-179	e-112	e-134	2e-30	1e-27
Sulfate permease	2.A.53.1.2	09642.1	*S. cerevisiase* Ypr003cp/e-112	e-112	3e-80	4e-24	3e-41
Sulfate permease	2.A.53.1.2	02632.1	*S. pombe* SPAC24H6.11c/0.0	e-146	0.0	—[c]	—
Methionine permease	2.A.3.8.4	02195.1	*S. cerevisiae* Mup1p/e-75	1e-75	—	4e-17	—
Methionine permease	2.A.3.8.4	04942.1	*S. cerevisiae* Mup1p/4e-94	4e-94	—	3e-25	—
Methionine Permease	2.A.3.8.4	07754.1	*S. cerevisiae* Mup1p/6e-87	6e-87	—	4e-32	—
Chromate resistance efflux	2.A.51.1.3	01055.1	*A. aceti* ChrA/7e-22	—	—	—	—

[a] *Caenorhabditis elegans*, *Drosophila melanogaster*, *Mus musculus*, or *Homo sapiens*.
[b] *Arabidopsis thaliana* or *Oryza sativa*.
[c] Values below e-10 are represented as —.

intracellular free amino acid with incompletely defined and disparate roles in brain development, osmolarity, and bile function (244). Taurine metabolism in *Neurospora* appears to be routed solely by conversion into sulfite by taurine dioxygenase. The sulfite can be subsequently converted to sulfide by sulfite reductase, and the sulfide can be used by cysteine synthase to generate cysteine (Fig. 7). An unusual finding is that four putative *Neurospora* taurine dioxygenases appear to be present (Table 32), two with best matches to bacterial enzymes and two with best matches to fungal enzymes (213, 815, 824). No matches for the putative taurine dioxygenases were found in animals or higher plants (Table 32).

Interestingly, the *Neurospora* genome encodes only two sulfatases. Arylsulfatase (ARS-1; NCU06041.1) (584) releases sulfate from aromatic sulfate compounds, while a choline sulfatase (NCU08364.1) releases sulfate from choline-*O*-sulfate (which also may serve as a sulfur storage compound). Homologues to the two *Neurospora* sulfatases were not observed in *S. cerevisiae, S. pombe*, or higher plants (Table 32). The *Neurospora* arylsulfatase could serve as a unique and useful model for the homologous multiple and specialized sulfatases in mammalian systems.

Sulfate can be transported into the cell by the sulfate permeases encoded by *cys-13* (primarily conidial expression,) and *cys-14* (primarily mycelial expression) (412). Since *cys-13 cys-14* double mutants cannot use sulfate for growth (504), it was unexpected that the genomic data reveals the presence of two additional sulfate permeases that are homologous to those in other fungal and yeast species (Table 33). Presumably, the two additional putative sulfate permeases have specialized functional roles during other phases of the *Neurospora* life cycle that have not yet been studied with regard to sulfate transport. By comparison, only two genes encoding sulfate permeases in *S. cerevisiae* are known (*SUL1* and *SUL2*) and have been found to encode high-affinity sulfate transporters (788). In contrast, *A. thaliana* has 14 isoforms in the sulfate transporter family, probably reflecting a variety of specific roles in plants (320).

The identification of a *Neurospora* gene showing homology to the ChrA chromate resistance gene of *Pseudomonas aeruginosa* (and *Acetobacter aceti*), which functions by chromate efflux (616), is intriguing. No significant homology to any eukaryotic genes is observed for this putative member of the chromate ion transporter (TC 2.A.51.1.3) family (Table 33), and this may represent another potential case of horizontal gene transfer. The capacity for chromate efflux in eukaryotes is currently unknown. The connection to sulfur metabolism is that both sulfate and chromate (which is toxic) are transported into the cell by sulfate permeases (504). On a related note, the bacterial ChrA family homologues are sulfur regulated (569).

Finally, analysis of the *Neurospora* genome reveals three methionine permeases (Table 33). The three putative methionine permeases show significant homology to *MUP1* of *S. cerevisiae*, which encodes a high-affinity methionine permease (366). Available data from studies of *S. cerevisiae* suggest that *MUP1* is also involved in cysteine uptake (429). *MUP2* and *MUP3* in yeast encode low-affinity permeases (788). Thus, *Neu-*

TABLE 34. Sulfur regulatory proteins

Enzyme	NCU no.	BLAST match				
		Best overall	*S. cerevisiae*	*S. pombe*	Animal[a]	Plant[b]
bZIP activator (CYS-3)	None	*A. nidulans* MetR/3e-19	—[c]	1e-19	—	—
F-box/WD-40 (SCON-2)	None	*A. nidulans* SconB/0.0	3e-114	3e-86	5e-37	3e-21
Skp1 homologue (SCON-3)	08991.1	*M. canis* SconC/5e-73	4e-35	3e-45	2e-36	5e-34
Rbx1 homologue	06224.1	*S. salar* Shop21/2e-43	4e-28	7e-38	2e-43	2e-40
Cullin[d]	05204.1	*A. nidulans* Cu1A/0.0	9e-107	2e-170	0.0	2e-91
Cullin[d]	00272.1	*D. melanogaster* Cu14/3e-118	7e-40	4e-95	3e-118	2e-107
Cullin[d]	00512.1	*H. sapiens* Apc2/1e-32	1e-15	1e-30	1e-32	6e-28
Cullin[d]	02498.1	*H. sapiens* Cu1-3/e-126	2e-45	1e-115	2e-128	3e-120

[a] *Caenorhabditis elegans*, *Drosophila melanogaster*, *Mus musculus*, or *Homo sapiens*.
[b] *Arabidopsis thaliana* or *Oryza sativa*.
[c] Values below e-10 are represented as —.
[d] All cullins are listed that might be involved in the sulfur-related SCF complex.

TABLE 35. Generation of sulfide and cysteine

Enzyme	EC no.	NCU no.	BLAST match				
			Best overall	*S. cerevisiae*	*S. pombe*	Animal[a]	Plant[b]
ATP sulfurylase (CYS-11)	2.7.7.4	01985.1	*A. fumigatus* ATP sulf./0.0	e-152	3e-95	5e-28	2e-28
Adenylyl sulfate kinase	2.7.1.25	0896.1	*A. nidulans* APS kin./3e-75	2e-74	1e-73	5e-60	3e-51
PAPS reductase (CYS-5)	1.8.99.4	02005.1	*P. chrysogenum* ParA/4e-92	5e-69	8e-71	—[c]	—
Sulfite reductase (Cys-2, beta chain)	1.8.1.2	04077.1	*S. cerevisiae* Met10p/e-167	e-167	e-162	7e-36	5e-27
Sulfite reductase (CYS-4, alpha chain)	1.8.1.2	05238.1	*S. pombe* SPAC4C5.05c/0.0	0.0	0.0	—	2e-99
Serine acetyl-transferase	2.3.1.30	00536.1	*A. nidulans* CysA/0.0	4e-32	5e-145	—	—
Cysteine synthase	2.5.1.47	06452.1	*A. nidulans* CysB/5e-167	2e-50	1e-122	3e-47	6e-39
Cysteine synthase	2.5.1.47	02564.1	*S. pombe* SPAC3A12.17c/2e-92	2e-88	2e-92	1e-36	2e-72
Cysteine synthase	2.5.1.47	03788.1	*S. coelicolor* Cys.Syn./2e-15	5e-11	1e-13	4e-10	7e-12

[a] *Caenorhabditis elegans*, *Drosophila melanogaster*, *Mus musculus*, or *Homo sapiens*.
[b] *Arabidopsis thaliana* or *Oryza sativa*.
[c] Values below e-10 are represented as —.

rospora appears to encode three high-affinity methionine transporters for which individual specialized functions or patterns of expression are currently unknown. Low-similarity animal genes exist, but no homologues are found in higher plants (Table 33). On sulfur starvation in *Neurospora*, there is the coordinate induction of an extracellular protease along with methionine permease activity. In yeast, Ssy1p is involved in sensing external amino acids and couples amino acid availability to transcriptional regulation of transporters (426). A homologue to Ssy1p cannot be identified in the *Neurospora* genome.

Generation of sulfide and cysteine. Analysis of the genomic data has allowed for the identification of all genes encoding the proteins necessary for the assimilation of sulfur as sulfate, sulfite, or sulfide (Fig. 7; Table 35). These sulfur assimilation enzymes confer on *Neurospora* the ability to synthesize cysteine from a variety of sulfur compounds. The findings from the genomic data are in agreement with biochemical evidence for the pathway (612), as follows: (i) ATP sulfurylase (*cys-11*) produces adenosine-5′-phosphosulfate (APS), (ii) adenylyl sulfate kinase produces 3′-phosphoadenosine-5′-phoshosulfate (PAPS), (iii) PAPS reductase (*cys-5*) generates sulfite, (iv) sulfite reductase (*cys-4* alpha subunit; and *cys-2* beta subunit) converts the sulfite into sulfide, and finally, (v) cysteine synthase uses the sulfide and *O*-acetyl serine (provided by serine acetyltransferase) for the synthesis of cysteine. The last step in the pathway provided an interesting finding in that there are three putative cysteine synthase homologues (NCU02564.1, NCU03788.1, and NCU06452.1) that each contain an identical or close match to the cysteine synthase motif (557), including a lysine residue which binds to pyridoxal 5′-phosphate. Each putative cysteine synthase has its most similar match to a different organism (i.e., *A. nidulans, S. pombe*, and *Streptomyces coelicolor* [Table 35]). The presence of two isoforms of cysteine synthase (cytoplasmic and mitochondrial) would be predicted. The presence of multiple cysteine synthases is known to occur in higher plants; rice has four genes encoding cysteine synthases that encode functionally distinct isoforms (557).

In relation to internal sulfur cycling, two key genes identified from the *Neurospora* genomic data suggest similarities to animal sulfur metabolism. The degradation of cysteine appears to occur by conversion to 3-sulfino-L-alanine catalyzed by cysteine dioxygenase. Significant similarity was observed only for mammalian cysteine dioxygenase (443, 602); no matches were observed for yeasts or higher plants (Table 32). The subsequent conversion to 3-sulfino-pyruvate is probably carried out by aspartate transaminase. Finally, 3-sulfino-pyruvate yields sulfite and pyruvate in an apparent nonenzymatic reaction. Sulfite can be used as in Fig. 7 or in a reaction catalyzed by sulfite oxidase to generate sulfate. Sulfite oxidase is regarded as a terminal enzyme in the degradation pathway of sulfur amino acids and can eliminate endogenously produced sulfite or detoxify exogenously added sulfite. Sulfite oxidase is present in *Neurospora* (Table 32), with the highest homology to *H. sapiens* and *C. elegans* and less similarity to higher plants. The presence of sulfite oxidase has only been recently established in higher plants (e.g., *A. thaliana* [216]). No matches were observed with *S. cerevisiae* or *S. pombe* for the putative *Neurospora* sulfite oxidases.

Homocysteine and methionine metabolism. Analysis of the *Neurospora* genomic data reveals homologues that cover every needed step for the interconversion of methionine and cysteine and for the SAM cycle (Table 36). Cystathionine is generated by cystathionine gamma-synthase (NCU08117.1; NCU05093.1; and *met-7*, NCU02430.1) from cysteine (derived as in Fig. 7) and *O*-acetyl homoserine (derived from aspartate-β-semialdehyde by homoserine dehydrogenase and homoserine *O*-acetyl transferase, *met-5*). Cystathionine β-lyase (*met-2*) then cleaves cystathionine to generate homocysteine. Methionine is generated by methionine synthase, which utilizes homocysteine and 5-methyl tetrahydrofolate as a methyl donor. An unanticipated finding is the presence of three putative methionine synthases. The known *met-8* locus is homologous to the typical eukaryotic cobalamin-independent methionine synthases (e.g., *A. nidulans MetH/D*). Two additional putative methionine synthases have the closest similarity to bacterial methionine synthases (e.g., *Rhodopseudomonas palustris*) and are also cobalamin independent. No significant matches to *S. cerevisiae, S. pombe*, animals, or higher plants were observed for these two proteins, and they may represent potential cases of horizontal gene transfer. Since *met-8* results in a phenotype of sulfur auxotrophy under typical vegetative growth, the role(s) of the bacterium-like putative methionine synthases cannot be defined at present. The 5-methyl tetrahydrofolate is supplied for methionine synthesis by 5,10-methylene tetrahydrofolate reductase, which is encoded by two homologues, *met-1* and a previously unknown gene (NCU09545.1). *met-1* would appear

TABLE 36. Homocysteine and methionine metabolism

Enzyme	EC no.	NCU no.	BLAST match				
			Best overall	*S. cerevisiae*	*S. pombe*	Animal[a]	Plant[b]
Cystathionine γ-synthase	2.5.1.48	08117.1	*S. pombe* SPBC1504.09c/1e-42	1e-29	1e-42	—[c]	—
Cystathionine γ-synthase	2.5.1.48	05093.1	*P. anserina* CAC6069/2e-50	1e-43	2e-29	—	—
Cystathionine γ-synthase (MET-7)	2.5.1.48	02430.1	*P. anserina* Pa5D0012/0.0	7e-92	1e-131	3e-16	1e-15
Homoserine dehydrogenase	1.1.1.3	03935.1	*S. pombe* SPBC776.03/1e-86	4e-85	1e-86	—	1e-70
Homoserine *O*-acetyltransferase (met-5)	2.3.1.31	07001.1	*A. nidulans* MetE/0.0	2e-142	9e-147	—	—
Cystathionine β-lyase (MET-2)	4.4.1.8	07987.1	*B. fuckeliana* meC/0.0	2e-97	3e-81	3e-74	1e-100
Methionine synthase (MET-8)	2.1.1.13	06512.1	*A. nidulans* MetH/D/0.0	0.0	0.0	1e-95	0.0
Methionine synthase	2.1.1.13	08434.1	*R. palustris* ZP8831/8e-44	—	—	—	—
Methionine synthase	2.1.1.13	10020.1	*R. palustris* ZP8831/8e-44	—	—	—	—
O-Acetyl-homoserine sulfhydrolase (bifunctional)	2.5.1.49	01652.1	*A. nidulans* CysA/0.0	e-141	2e-148	9e-43	1e-39
Folyl polyglutamate synthase (met-6)	6.3.2.17	00892.1	*S. cerevisiae* Met7p/4e-104	4e-104	4e-98	1e-75	3e-60
Folyl polyglutamate synthase	6.3.2.17	01337.1	*S. cerevisiae* Fo13p/5e-65	5e-65	3e-64	2e-29	4e-27
Serine hydroxymethyl transferase (for)	2.1.2.1	02274.1	*C. albicans* SHM2/0.0	0.0	0.0	4e-151	1e-153
Glycine hydroxylmethyl transferase	2.1.2.1	05805.1	*S. pombe* Shm2/3e-168	3e-164	3e-168	1e-153	3e-167
5,10-Methylene tetrahydrofolate reductase (MET-1)	4.2.1.22	07690.1	*S. pombe* MTHFR2/4e-174	3e-168	4e-174	1e-135	4e-138
5,10-Methylene tetrahydrofolate reductase	4.2.1.22	09545.1	*S. cerevisiae* Met12p/8e-143	8e-143	2e-142	3e-83	9e-94
SAM synthetase (ETH-1)	2.5.1.6	06512.1	*S. pombe* Sam1/1e-155	e-145	1e-155	2e-141	4e-118
SAM:3-amino-3-carboxylpropyl transferase	2.5.1.-	03032.1	*R. sphaeroides* btaA/2e-18	—	—	—	—
Adenosyl homocysteinase	3.3.1.1	07930.1	*S. cerevisiae* Sah1p/0.0	0.0	0.0	1e-80	5e-142
Cystathionine β-synthase	4.2.1.22	08216.1	*M. grisea* CBS1/0.0	1e-118	2e-47	5e-107	5e-49
Cystathionine γ-lyase (CYS-16)	4.4.1.1	09230.1	*A. chrysogenum* MecB/3e-178	2e-129	5e-44	4e-96	1e-65

[a] *Caenorhabditis elegans*, *Drosophila melanogaster*, *Mus musculus*, or *Homo sapiens*.
[b] *Arabidopsis thaliana* or *Oryza sativa*.
[c] Values below e-10 are represented as —.

to function during typical vegetative growth, while a role for the other version of tetrahydrofolate reductase awaits determination. The balance of folate metabolism appears typical and complete (Table 36) (612).

SAM synthetase (*eth-1*) generates SAM from methionine and ATP. Subsequently, SAM is used for a wide variety of methyltransferase reactions (e.g., DNA methylation). After donating a methyl group, SAM is converted into *S*-adenosylhomocysteine. A typical array of eukaryotic methyltransferases, about twice the number found in yeasts, are represented in the *Neurospora* genome. An interesting side note is that a biosynthetically rare SAM-dependent 3-amino-3-carboxypropyltransferase of the MAPEG (membrane-associated proteins in eicosanoid and glutathionine metabolism) class is present and shows similarity to a *Rhodobacter sphaeroides* protein with no significant hits to eukaryotic proteins (Table 36) (425).

The SAM cycle can be completed by conversion of *S*-adenosylhomocysteine back to homocysteine (adenosylhomocysteinase), followed by regeneration of methionine by methionine synthase. Additionally, homocysteine can converted to cystathionine by β-cystathionine synthase. Cystathionine γ-lyase (*cys-16*) then converts the cystathionine into cysteine, thus completing the conversion of methionine into cysteine.

Additional aspects of sulfur metabolism. Glutathione metabolism has not been studied in detail in *Neurospora*. Glutathione *S*-transferases (GSTs) represent an important group of enzymes involved primarily in detoxification reactions (197). Three GSTs are of particular interest and do not show homology to yeast GSTs (Table 37). A putative microsomal GST is closest to animal and plant GSTs of that class, while a putative mitochondrial GST (NCU06494.1) is most similar to a human GST. The third predicted protein (NCU02888.1) is similar only to fungal GSTs (e.g., *Gibberella fujikuroi*).

TABLE 37. Additional aspects of sulfur metabolism

Enzyme	EC no.	NCU no.	BLAST match				
			Best overall	*S. cerevisiae*	*S. pombe*	Animal[a]	Plant[b]
Glutathione *S*-transferase (microsomal)	2.5.1.18	01320.1	*O. sativa* Gst/3e-15	—[c]	—	2e-12	3e-15
Glutathione *S*-transferase (mito.)	2.5.1.18	06494.1	*H. sapiens* GST13/5e-13	—	—	5e-13	—
Glutathionine *S*-transferase	2.5.1.18	02888.1	*G. fujikuroi* GST/2e-55	—	—	—	—
MET-10 (unknown function)		09311.1	*S. pombe* SPAPB18E9.01/5e-73	9e-61	5e-73	4e-54	4e-43
Thioredoxin reductase (CYS-9)	1.6.4.5	08352.1	*P. chrysogenum* TrxB/e-132	e-120	e-113	—	e-103
Thioredoxin		00598.1	*S. pombe* SPC57.08c/1e-16	1e-15	1e-16	8e-14	3e-13
Thioredoxin		06556.1	*P. anserina* Trx2/1e-27	1e-19	6e-21	1e-16	8e-113
Halotolerance PAPS phosphatase (CYS-1?)	3.1.3.7	04069.1	*S. pombe* SPCC1753.04/1e-83	2e-62	1e-83	—	4e-59
Sulfide dehydrogenase	1.8.5.-	07112.1	*A. oryzae* BAC55902/3e-175	—	7e-102	1e-78	—
Cysteine desulfurase	4.4.1.-	04636.1	*C. albicans* NFS1/0.0	—	5e-180	2e-177	1e-157
Methionine sulfoxide	1.8.4.6	100029.1	*S. pombe* SPAC30.09c/4e-41	6e-38	4e-41	2e-27	1e-29

[a] *Caenorhabditis elegans*, *Drosophila melanogaster*, *Mus musculus*, or *Homo sapiens*.
[b] *Arabidopsis thaliana* or *Oryza sativa*.
[c] Values below e-10 are represented as —.

TABLE 38. Nitrogen assimilation and regulation

NCU no.	Cloned gene	BLAST score				
		Best	*S. cerevisiae*	*S. pombe*	Animal[a]	Plant[b]
Regulatory genes						
09068.1	*nit-2*	*C. lindemuthianum* AAN65464.1; 1.00e-139	Gat1p; 9.00e-14	AAC35593.1; 1.00e-13	Mm GATA-2; 3.00e-08	None
08294.1	*nit-4*	*T. inflatum* CAB71797.1; 0.00	Tea1p; 3.00e-17	SPCC757.04; 4.00e-30	None	None
04158.1	*nmr*	*G. fujikuroi* CAA75863.1; 1.00e-154	None	None	Hs XP 293629.1; 8.00e-06	None
07669.1	*pco-1*	*A. nidulans* uaY; 0.00	Ppr1p; 5e-21	SPBC530.05; 4e-09	None	None
Structural genes						
05298.1	*nit-3*/nitrate reductase	*M. anisopliae* CAA04554.1; 0.00	Yml125cp; 3.00e-28	SPCC970.03; 4e-24	Dm CG7280-PA; 1.00e-42	At nitrate reductase; 1.00e-156
04720.1	*nit-6*/nitrite reductase	*P. nodorum* CAA08856.1; 0.00	None	None	Ag XP 306456.1; 1.00e-166	None
07205.1	Nitrate permease	*A. fumigatus* CAD28427.1; 7.00e-77	None	None	None	NP_172754.1; 3.00e-20
01816.1	*alc*/allantoicase	*S. pombe* NP_594495.1; 1.00e-104	Dal2p; 3.00e-94	Best match	Mm allantoicase; 1.00e-44	None
03350.1	*xdh*/xanthine dehydrogenase	*A. nidulans* Xdh; 0.00	None	None	Hs NP_000370.1; 0.00	At NP_195215.2; 0.00

[a] *Caenorhabditis elegans* (Ce), *Drosophila melanogaster* (Dm), *Anopheles gambiae* (Ag), *Mus musculus* (Mm), or *Homo sapiens* (Hs).
[b] *Arabidopsis thaliana* (At) or *Oryza sativa* (Os).

A number of additional genes that are involved in routine sulfur metabolic activities and that do not show unusual homology patterns are also included in Table 37.

Components of the regulatory machinery for sulfur metabolism. The complex regulatory network involved in the control of sulfur metabolism provides a fascinating model for understanding how fungi (and other organisms) adjust to a constantly changing nutrient environment in order to maintain sustaining levels of key elements (e.g., carbon, nitrogen, phosphorous, and sulfur) (586). An important aspect of the sulfur regulatory system of *Neurospora* is the role of the F-box protein *sulfur controller-2* (scon-2) (440). F-box proteins assemble with Skp-1p, Cdc53p, and Rbx1p to form a complex known as the SCF (Skp1p/Cullin/F-box) (171). SCF complexes act as E3 ubiquitin ligases to target proteins for ubiqitin-mediated proteolysis by the proteasome (see "Proteasome" below). The SCF complex is represented in the *Neurospora* genome by SCON3 (741) as the Skp1p homolog, SCON2 as the F-box protein, NCU06224.1 as the Rbx1p (ring box) homologue, and four potential cullin components (Table 34). Additional F-box proteins, probably unrelated to sulfur metabolism, are also present in the *Neurospora* genome. Extensive searches did not reveal homologues of known sulfur regulatory proteins found in other organisms (e.g., the *sac1*, *sac2*, and *sac3* genes of *Chlamydomonas reinhardtii* [182]). Major unanswered regulatory questions remain with regard to the identity of the sulfur sensor and subsequent signal transduction pathway. Functional genomic studies will provide an important future means of identifying other regulatory proteins in the sulfur control system.

Nitrogen Metabolism

A basic understanding of nitrogen metabolism and its regulation exists for *Neurospora* and includes the identification and characterization of many of the genes that participate in this system (Table 38). *Neurospora* utilizes ammonia and glutamine as preferred nitrogen sources but is also capable of using many different secondary nitrogen sources (505). The use of various alternative nitrogen sources, e.g., nitrate, purines, amides, amino acids, and proteins, requires the expression of specific sets of structural genes which encode enzymes for catabolism of a particular nitrogenous source. A globally acting regulatory gene, *nit-2*, encodes a DNA binding protein, a member of the family of GATA transcription factors (522) (see "Chromatin assembly and gene regulation" above). NIT-2 acts in a positive fashion to activate the expression of structural genes of diverse nitrogen pathways under conditions of nitrogen source limitation. A major negatively acting regulatory protein, NMR (for "nitrogen metabolite repression") mediates nitrogen repression and acts by directly binding to NIT-2 and inhibiting its function (590). Activation of the genes of specific pathways requires inducers and is mediated by minor control genes. A pathway-specific gene, *nit-4*, codes for a positively acting protein with a binuclear zinc finger DNA binding motif (257, 892) (see also "Chromatin assembly and gene regulation" II). NIT-4 mediates the induction of enzymes specific for assimilation of inorganic nitrate. The regulatory genes (*nit-2*, *nmr*, and *nit-4*) and the structural genes encoding nitrate reductase (*nit-3*), nitrite reductase (*nit-6*), and allantoicase (*alc*) were cloned by complementation of mutants (258, 259, 261, 460). These genes have been extensively characterized and demonstrated to have homologues in *A. nidulans* and other filamentous fungi.

Several new genes involved in nitrogen metabolism have recently been identified by analysis of the *Neurospora* genome sequence (Table 38). The gene encoding a nitrate permease (*nit-10*), which had never been revealed by conventional genetics despite extensive work in this pathway, was identified in the *Neurospora* genome sequence and its function was demon-

TABLE 39. *Neurospora* proteasome components

Role	Subunit	*Neurospora* gene	Alias(es)	Symbol	Gene name
Catalytic	alpha-1	EAA28671, NCU10061.1	C7	*pca-1*	Proteasome catalytic alpha-1
Catalytic	alpha-2	EAA28122, NCU06764.1	Y7	*pca-2*	Proteasome catalytic alpha-2
Catalytic	alpha-3	EAA29550, NCU05942.1	Y13	*pca-3*	Proteasome catalytic alpha-3
Catalytic	alpha-4	EAA28095, NCU06440.1	PRE6	*pca-4*	Proteasome catalytic alpha-4
Catalytic	alpha-5	EAA32830, NCU05295.1	PUP2	*pca-5*	Proteasome catalytic alpha-5
Catalytic	alpha-6	EAA31656, NCU06712.1	PRE5	*pca-6*	Proteasome catalytic alpha-6
Catalytic	alpha-7	EAA35851, NCU02493.1	C1	*pca-7*	Proteasome catalytic alpha-7
Catalytic	beta-1	EAA28906, NCU09290.1	PRE3	*pcb-1*	Proteasome catalytic beta-1
Catalytic	beta-2	EAA34801, NCU08605.1	PUP1	*pcb-2*	Proteasome catalytic beta-2
Catalytic	beta-3	EAA35245, NCU03304.1	PUP3	*pcb-3*	Proteasome catalytic beta-3
Catalytic	beta-4	EAA31484, NCU01368.1	PRE1 (C11)	*pcb-4*	Proteasome catalytic beta-4
Catalytic	beta-5	EAA29213, NCU09309.1	PRE2	*pcb-5*	Proteasome catalytic beta-5
Catalytic	beta-6	EAA34540, NCU09366.1	C5	*pcb-6*	Proteasome catalytic beta-6
Catalytic	beta-7	EAA28757, NCU07365.1	PRE4	*pcb-7*	Proteasome catalytic beta-7
Regulatory	RPN1	EAA32735, NCU07721.1	Non-ATPase subunit	*rpn-1*	Regulatory particle, non-ATPase-like-1
Regulatory	RPN2	EAA28800, NCU09450.1	Non-ATPase subunit	*rpn-2*	Regulatory particle, non-ATPase-like-2
Regulatory	RPN3	EAA30401, NCU02224.1	Non-ATPase subunit	*rpn-3*	Regulatory particle, non-ATPase-like-3
Regulatory	RPN4	EAA26772, NCU01640.1	Non-ATPase subunit	*rpn-4*	Regulatory particle, non-ATPase-like-4
Regulatory	RPN5	EAA36187, NCU02650.1	Non-ATPase subunit	*rpn-5*	Regulatory particle, non-ATPase-like-5
Regulatory	RPN6	EAA27271, NCU01596.1	Non-ATPase subunit 4	*rpn-6*	Regulatory particle, non-ATPase-like-6
Regulatory	RPN7	EAA28375, NCU03972.1	Non-ATPase subunit	*rpn-7*	Regulatory particle, non-ATPase-like-7
Regulatory	RPN8	EAA27014, NCU01547.1	Non-ATPase subunit	*rpn-8*	Regulatory particle, non-ATPase-like-8
Regulatory	RPN9	EAA30559, NCU02374.1	Non-ATPase subunit 7	*rpn-9*	Regulatory particle, non-ATPase-like-9
Regulatory	RPN10	EAA36132, NCU02982.1	Non-ATPase subunit	*rpn-10*	Regulatory particle, non-ATPase-like-10
Regulatory	RPN11	EAA35130, NCU00823.1	Non-ATPase subunit 2, Pad1, Sks1	*rpn-11*	Regulatory particle, non-ATPase-like-11
Regulatory	RPN12	EAA28646, NCU10067.1	Non-ATPase subunit	*rpn-12*	Regulatory particle, non-ATPase-like-12
Regulatory	RPT1	EAA34894, NCU02840.1	AAA ATPase subunit 7, Cim5	*rpt-1*	Regulatory particle, ATPase-like-1
Regulatory	RPT2	EAA32354, NCU01224.1	AAA ATPase subunit	rpt-2	Regulatory particle, ATPase-like-2
Regulatory	RPT3	EAA30668, NCU02260.1	AAA ATPase subunit 6B, Ynt1	rpt-3	Regulatory particle, ATPase-like-3
Regulatory	RPT4	EAA29624, NCU07367.1	AAA ATPase subunit	rpt-4	Regulatory particle, ATPase-like-4
Regulatory	RPT5	EAA28255, NCU04414.1	AAA ATPase subunit 6A, Tbp1	rpt-5	Regulatory particle, ATPase-like-5
Regulatory	RPT6	EAA34118, NCU05363.1	AAA ATPase subunit 8, Sug1, Cim3	rpt-6	Regulatory particle, ATPase-like-6

strated by creation of a RIP mutant and parallel biochemical and molecular studies (272). In the purine catabolic pathway, *xdh*, which encodes xanthine dehydrogenase, was readily identified. A new gene, *pco-1*, which codes for a binuclear zinc DNA binding protein that regulates purine metabolism, was identified in the *Neurospora* genome sequence by its regions of homology to *uaY*, the corresponding factor of *A. nidulans* (T.-W. Liu and G. A. Marzluf, unpublished data). A *pco-1* RIP mutant was created; analysis of phenotypes, in combination with DNA binding studies of the expressed protein, demonstrate that PCO-1 serves as a positive activator to induce expression of multiple enzymes of the purine catabolic pathway.

It appears that it should be possible to discover most, perhaps even all, of the genes involved in nitrogen metabolism or its regulation by careful analysis of the *Neurospora* genome sequence and subsequent functional studies. Significant questions in nitrogen metabolism remain. A postulated, but unknown, factor that senses glutamine as the initial step in nitrogen repression has yet to be identified. Similarly, other proteins in the signaling pathway for nitrogen repression that may converge on NIT-2 and NMR have yet to be identified. Additional factors that control specific metabolic pathways, as well as the way in which they interact with inducers and the globally acting NIT-2 protein, remain to be explored. Investigation of these areas and similar features will clearly be enhanced by the availability of the *Neurospora* genome sequence.

Proteasome

The proteasome is one of the most complex oligomeric protein structures within the cell, and its function is the proteolysis of ubiquitin-tagged cellular proteins. Proteasomes are found within eukaryotic cells free in the cytoplasm, associated with the endoplasmic reticulum (ER), and in the nucleus. The structure and component polypeptide identities of the 26S proteasome and its 20S catalytic and 19S regulatory subunits have been studied (88). The subunits of both regulatory (285) and catalytic (327) components have been identified. The crystallographic structure of the *S. cerevisiae* proteasome has also been determined (303).

To date, none of the *Neurospora* genes encoding the subunits of the proteasome have been identified, although some proteins of the ubiquitin modification pathway have been isolated as regulatory genes in various pathways (e.g., "Sulfur metabolism", above). However, using the yeast polypeptide sequences as probes, all 14 polypeptides of the catalytic component and all 18 polypeptides of the regulatory component of the proteasome of *Neurospora* have now been identified (Table 39).

Lipids

Most work on *Neurospora* lipids has focused on understanding factors that control the synthesis and composition of the most common fatty acids and fatty acid-containing (acyl) lipids. These acyl lipids include both membrane lipids (phospholipids,

TABLE 40. Lipid metabolism

Enzyme	EC no.	NCU no.	BLAST match			
			Best overall	*S. cerevisiae*	Animal	Plant
Fatty acid synthesis						
Acetyl-CoA carboxylase	6.4.1.2	08535.1	0.0; *E. nidulans*	0.0		
Fatty acid synthase (alpha)	2.3.1.85	07308.1	0.0; *E. nidulans*	0.0		
Fatty acid synthase (beta)	2.3.1.86	07307.1	0.0; *E. nidulans*	0.0		
Fatty acid elongase	2.3.1.41	06694.1	4e-75; *S. pombe*	4e-73		
Fatty acid desaturation						
Fatty acid hydroxylase	1.14.15.	03492.1	1e-93; *S. cerevisiae*	Best match	3e-33; *M. musculus*	8e-35; *O. sativa*
Stearoyl-CoA desaturase	1.14.99.5	05259.1	0.0; *Ajellomyces capsulatus*	8e-121		
Oleate Δ12 desaturase	1.14.99.	02209.1	e-180; *E. nidulans*	5e-1		2e-80; *S. oleracea*
Oleate Δ12 desaturase	1.14.99.	09497.1	1e-78; *A. parasiticus*	3e-1		1e-68; *S. oleracea*
Fatty acid Δ6 desaturase	1.14.19.1	02408.1	0.0; *P. anserina*	2e-3		7e-54; *T. aestivum*
Fatty acid degradation						
Multifunctional beta-oxidation protein	1.1.1.35 5.1.2. 4.2.1.17	08828.1	0.0; *Y. lipolytica*	0.0	e-141; *H. sapiens*	2e-52; *A. thaliana*
Acyl group transfer						
Glycerol-3-phosphate acyltransferase	2.3.1.15	05985.1	e-150; *S. pombe*	e-132		
Lysophosphatidic acid acyltransferase	2.3.1.-	00168.1	2e-71; *E. nidulans*	1e-44	3e-24; *M. musculus*	
Diacylglycerol acyltransferase	2.3.1.20	02665.1	2e-89; *M. ramanniana*	1e-65	3e-68; *H. sapiens*	
Diacylglycerol acyltransferase	2.3.1.20	00035.1	3e-58; *B. napus*	2e-33	2e-51; *D. melanogaster*	Best match
Phospholipid acyltransferase	2.3.1.-	02416.1	0.0; *P. anserina*	e-137		3e-52; *A. thaliana*
Sterol acyltransferase	2.3.1.43	04144.1	2e-69; *S. pombe*	7e-56		
Sterol acyltransferase	2.3.1.43	03991.1	4e-65; *S. kluyveri*	4e-79		
Serine palmitoyltransferase subunit 1	2.3.1.50	06870.1	0.0; *E. nidulans*	7e-85	4e-81; *A. gambiae*	3e-83; *A. thaliana*
Serine palmitoyltransferase subunit 2	2.3.1.50	00447.1	0.0; *E. nidulans*	e-157	e-139; *M. musculus*	e-131; *S. tuberosom*
Phospholipid synthesis						
Phosphatidylserine synthase	2.7.8.8	01141.1	e-107; *S. cerevisiae*	Best match	7e-66; *D. melanogaster*	
Phosphatidylserine synthase	2.7.8.8	02381.1	2e-76; *T. aestivum*	4e-59		Best match
Phosphatidylserine decarboxylase	4.1.1.65	01004.1	e-135; *S. pombe*	3e-96		7e-72; *A. thaliana*
Phosphatidylserine decarboxylase	4.1.1.65	03695.1	4e-94; *S. cerevisiae*	Best match	3e-52; *H. sapiens*	7e-54; *A. thaliana*
Phosphatidylserine decarboxylase	4.1.1.65	02302.1	8e-67; *B. fungorum*	3e-11		2e-21; *A. thaliana*
Phosphatidylethanolamine methyl-transferase	2.1.1.17	08045.1	e-168; *S. pombe*	e-102		
Phosphatidyl-*N*-methylethanolamine methyltransferase	2.1.1.71	04699.1	8e-48; *S. cerevisiae*	Best match	3e-40; *D. rerio*	
Choline phosphotransferase	2.7.8.2	03223.1	8e-38; *S. pombe*	2e-32		1e-33; *A. thaliana*
Diacylglycerol choline/ethanolamine transferase	2.7.8.1/2	01993.1	5e-70; *S. pombe*	3e-62		3e-45; *B. napus*
CDP-alcohol transferase (probable cardiolipin synthase)	2.7.8.-	00135.1	2e-36; *D. melanogaster*	8e-29	3e-29; *M. musculus*	
Phosphatidylinositol synthase	2.7.8.11	09192.1	1e-49; *S. pombe*	2e-41	2e-42; *R. norvegicus*	
Choline kinase	2.7.1.32	03176.1	2e-58; *S. pombe*	2e-46		5e-28; *P. sativum*
Ethanolamine kinase	2.7.1.82	02726.1	2e-42; *R. norvegicus*		Best match	
Cholinephosphate cytidylyltransferase	2.7.7.15	03880.1	6e-80; *S. pombe*	1e-74		2e-59; *P. sativum*
Phosphoethanolamine cytidylyltransferase	2.7.7.14	04289.1	1e-63; *D. melanogaster*	3e-53	2e-60; *R. norevgicus*	
Inositol-3-phosphate synthase	5.5.1.4	06666.1	e-170; *T. aestivum*	e-291	e-169; *H. sapiens*	Best match
Phosphatidylcholine/-inositol exchange protein (sec14)		02263.1	e-112; *A. capsulatus*	3e-80		
Phosphatidylcholine/-inositol exchange protein (sec14)		07320.1	7e-29; *S. cerevisiae*	Best match		

sphingolipids, and sterol esters) and storage lipids (triacylglycerols). *Neurospora* is an excellent model system for studying the roles of lipids: it synthesizes a broad range of lipids de novo, its lipid composition can be influenced by culture conditions, and many mutant strains have altered lipid metabolism (514, 612). Because of these traits, the biochemistry and genetics of *Neurospora* lipids are still among the best understood of any organism; in the fungal kingdom, more extensive studies have only been done for the yeast *S. cerevisiae* (128, 181). Characteristic of *Neurospora* is the accumulation of high levels of polyunsaturated fatty acids (linoleate and α-linolenate) and triacylglycerols under appropriate conditions (184, 485). The de novo synthesis of these lipids is typical of plants and some other filamentous fungi (485, 563); *S. cerevisiae* does not synthesize detectable levels of polyunsaturates, and triacylglycerols are not as readily accumulated (181).

The major fatty acids of *Neurospora* are palmitate, stearate, oleate, linoleate, and α-linolenate; the relative proportion of these fatty acids in phospholipids and triacylglycerols is similar to that in many higher plants and is not typical of most fungi (289, 485, 825). The 16- and 18-carbon saturated fatty acids palmitate and stearate are synthesized from malonyl-CoA by a multifunctional fatty acid synthase, which consists of two subunits, α and β (219). In *S. cerevisiae*, the subunits are encoded

on different chromosomes (694); however, in *Neurospora*, the genes are adjacent to each other on the same chromosome, oriented in opposite directions (Table 40), as in *A. nidulans* (108). The *cel-1* mutant, which synthesizes only small amounts of fatty acids de novo due to impaired pantotheine binding activity of the α subunit, has been exploited extensively to study the effects of supplemental fatty acids on *Neurospora* physiology (612). The *cel-2* mutants, obtained by RIP mutation of the β subunit, are blocked in fatty acid synthesis (288). *S. cerevisiae* deletion mutants with mutations of genes for either fatty acid synthase subunit require supplemental fatty acids for growth, and deletions of genes for acetyl-CoA carboxylase (which carries out the committed step in fatty acid biosynthesis, conversion of acetyl-CoA to malonyl-CoA) are lethal (181). A few predicted *Neurospora* proteins have homology to proteins carrying out individual enzymatic activities of fatty acid synthases (Table 40). Some of these proteins (for example, that encoded by NCU000563) are likely to be involved in mitochondrial β-oxidation or in the minor amount of fatty acid synthesis that takes place in mitochondria (528), which, similarly to bacterial cells, utilize individual enzymes to carry out fatty acid biosynthesis. Some seven other predicted proteins can be identified as probable polyketide synthases. Polyketides, common secondary metabolites in filamentous fungi, are synthesized by a pathway that resembles fatty acid biosynthesis (348). Although a polyketide synthase synthesizes polyunsaturated fatty acids de novo in a few organisms (842), biochemical studies indicate that *Neurospora*, like higher plants and other fungi that synthesize polyunsaturated fatty acids, derives its polyunsaturates from stearate and oleate precursors utilizing a phospholipid substrate (41, 289).

A desaturase first converts stearoyl-CoA to oleoyl-CoA (41), as in other fungi and animals (563). Membrane-bound Δ12 and Δ15 desaturases carry out the final two consecutive desaturation steps, to linoleate and α-linolenate, respectively, after oleate has become incorporated into phosphatidylcholine (41), the major phospholipid in *Neurospora* and most other eukaryotes. The relative levels of α-linolenate, particularly in phospholipids, are affected by many factors, including growth temperature, developmental stage, and the circadian clock (612). Regulation of the desaturase genes is therefore of interest. *Neurospora* has at least three loci that control formation of oleate (612); however, a single candidate gene for a stearoyl-CoA desaturase has been identified based on homology, and in BLAST searches the sequence appears to be enriched in an EST library from sequences expressed during the evening phase of the circadian cycle (900). In common with the *S. cerevisiae* protein (588), the *Neurospora* enzyme contains a cytochrome *b* sequence. Membrane-bound desaturases have been best characterized in higher plants, most extensively in *A. thaliana* (60); to date, only a few fungal desaturases have been identified, and they form different polyunsaturates, generally in the Δ6 fatty acid series (including γ-linolenate). The highest homologies of candidate *Neurospora* proteins to membrane-bound desaturase sequences are to fungal Δ12 desaturases (which form linoleate) and to a putative fungal desaturase that itself has highest homology to a Δ6 sphingolipid desaturase (Table 40). In plants, Δ6, Δ12, and Δ15 desaturases and hydroxylases have high homology (107, 516); given that other fungal Δ15 desaturases have not been identified and that *Neurospora* does not synthesize detectable levels of Δ6 fatty acids, it is likely that either the putative Δ6 or one of the Δ12 desaturase sequences actually encodes a Δ15 desaturase, which would form α-linolenate. If, indeed, *Neurospora* does contain two Δ12 desaturase genes, this could account for the fact that known mutants are only partially blocked in the synthesis of polyunsaturated fatty acids (612). In *Neurospora*, longer-chain fatty acids are almost exclusively the 18- to 24-carbon 2-hydroxy fatty acids found in sphingolipids (1, 514); a sequence with homology to fatty acid hydroxylases and another with homology to fatty acid elongases have also been identified (Table 40).

Lipid composition (both fatty acid composition and relative proportion of individual lipid classes) has many possible points of control. Membrane lipid composition is particularly important because it influences fluidity and related physical properties important to membrane function (563). Most acyl lipids can be synthesized through multiple routes, and modified by exchange of acyl groups (563). Degradative pathways are also important for control of lipid composition as well as for fatty acid utilization; many products of phospholipases and other lipases are also important signaling molecules (see "Calcium signaling" below). In addition to mitochondrial proteins probably involved in β-oxidation, *Neurospora* contains an inducible multifunctional peroxisomal β-oxidation protein (248) and many lipase candidates (see "Extracellular digestion" above). Most of the complex biochemical pathways involved in synthesis, remodeling, and turnover of membrane and storage lipids in *Neurospora* have not been well characterized. However, genes homologous to those encoding many enzymes involved in these processes in other organisms have been identified in the *Neurospora* genome database (Table 40). As expected, given the importance of acyl group transfer in these processes, the genome contains several candidate genes for acyltransferases, including glycerol-3-phosphate acyltransferase and lysophosphatidic acid acyltransferase, which carry out the two acylation steps leading to the synthesis of CDP-diacylglycerol and diacylglycerol (the precursors for the synthesis of phospholipids and triacylglycerols), genes representing two families of diacylglycerol acyltransferases, which form triacylglycerols, and genes for both subunits of serine palmitoyltransferase, which catalyzes the committed step for synthesis of sphingolipids.

The genome also includes candidate genes encoding a set of enzymes in the major pathway of phosphatidylcholine synthesis (two for phosphatidylserine synthase, three for phosphatidylserine decarboxylase [phosphatidylethanolamine synthase], and one each for phosphatidylethanolamine methyltransferase and phosphatidylmonomethylethanolamine methyltransferase). One of the phosphatidylserine synthase sequences includes a domain homologous to phospholipase D, and one of the phosphatidylserine decarboxylase sequences includes a domain homologous to protein kinase C. In addition, the genome includes candidate sequences for enzymes in the minor pathway of phosphatidylethanolamine and phosphatidylcholine synthesis via the CDP-alcohols (128, 181, 563). A similar pathway is used for synthesis of phosphatidylserine, phosphatidylinositol, and phosphatidylglycerol. A total of six CDP-alcohol transferase gene homologues, the same number as in *S. cerevisiae* (128, 588), are identifiable. Although the CDP-

alcohol pathway is the major pathway in plants and animals (563), in *Neurospora* it is a minor pathway, except in mutants impaired in the methylation pathway such as the choline-requiring *chol-1* and *chol-2* mutants, which are blocked at the final steps leading to phosphatidylcholine formation (612). Another mutant, the *inl* mutant, requires inositol and is blocked in the formation of *myo*-inositol-l-phosphate, the rate-limiting step in the synthesis of both phosphatidylinositol and sphingolipids (612). All three mutants, the *chol-1*, *chol-2*, and *inl* mutants, have altered phospholipid composition and abnormal morphology at suboptimal levels of the growth supplement (612). In *S. cerevisiae*, regulation of the homologues of these genes and *sec14*, which encodes a phosphatidylcholine/phosphatidylinositol transfer protein, are key points for control of membrane lipid composition (128). Two genes in *Neurospora* have homology to *sec14* (Table 40). Some enzymes and pathways in *S. cerevisiae* are specialized for cytoplasmic versus mitochondrial phospholipid synthesis (128, 181), consistent with a need for duplication of function in *Neurospora* as well. However, in *Neurospora*, three phosphatidylserine decarboxylase homologues are present, as in *A. thaliana* (60), while *S. cerevisiae* contains only two (128, 181). The reason for this is unclear, but one reason for apparent duplication of function may be a need for one enzyme that preferentially uses polyunsaturated fatty acids (822).

Over the past 15 years, the amenability of two model systems, *S. cerevisiae* and *A. thaliana*, to genetic and molecular studies has been exploited for systematic identification and characterization of genes involved in lipid metabolism, leading to a wealth of literature and a more complete understanding of the roles of lipids in these and other organisms. The diversity of *Neurospora* lipids and the well-studied effects of various developmental and environmental factors on their composition suggest an excellent opportunity for new insights from studies of this organism and productive future comparative genomics studies.

Protein Glycosylation, Secretion, and Endocytosis

N-linked protein glycosylation (dolichol) pathway. Most proteins present in the fungal cell wall are glycoproteins, and some of these are enzymes involved in structural modifications of the wall required for growth, morphogenesis, and nutrition. In addition, glycoproteins bear the most significant antigenic determinants of fungal cells (668). The biosynthesis of N-linked polysaccharides is rather complex and involves multiple steps. Thus, identification of the relevant components and determination of the overall degree of conservation of the pathway is an important advance in establishing the ability to structurally and functionally dissect the glycosylation process in *Neurospora*.

N glycosylation involves the assembly and transfer of oligosaccharide-P-P-dolichol, which is then processed and forms an N-glycosidic bond with one or more specific Asn residues of an acceptor protein emerging from the luminal surface of the membrane of the endoplasmic reticulum (ER). The commonality of glycoproteins throughout evolution suggests the presence of highly conserved components of the multistep process involved in glycoprotein biosynthesis. Hence, it is not surprising that the key elements of this pathway are present in *Neurospora*, even though some components of this pathway were previously not detected, most probably due to the technical shortcomings of heterologous probing (902).

Burda and Aebi (116) reviewed the dolichol pathway of N-linked glycosylation and suggested that N-linked glycosylation in eukaryotes and in archaea have a common evolutionary origin. The *S. cerevisiae* dolichol pathway was used as a basis for comparison while scanning the *Neurospora* genome for similar components. A very high degree of conservation was found in the machinery involved in the stepwise production of the core oligosaccharide $Glc_3Man_9GlcNAc_2$. However, the en bloc transfer of the core oligosccharide catalyzed by the ER-resident enzyme *N*-oligosaccharyltransferase (OST) complex appears to be more streamlined in *Neurospora* (Fig. 8; Table 41). This is evident on the basis of the apparent absence of a good match to the *S. cerevisiae* Ost4p and Ost5p proteins in the *Neurospora* sequence database (homologues of these proteins are also not found in the *M. grisea* genome sequence [http://www-genome.wi.mit.edu]). Interestingly, a similar degree and pattern of streamlining of this part of the pathway is also evident in the human OST complex. In *S. pombe*, there is an apparent homologue to Ost4p but not to Ost5p. Even though these differences may have evolutionary significance, it should be noted that Ost4p and Ost5p are very short proteins (36 and 86 amino acids, respectively), a feature that may impose limitations on the efficacy of the standard BLAST-based searches.

Apart from evolutionary implications, the structural similarity of the individual components, as well as the fact that the two major modules (oligosaccharide production and transfer complexes) are highly conserved, also has practical implications. For example, it is clear that much of the success in the harnessing of filamentous fungi for heterologous expression of proteins (383) has stemmed from the presence of proper post-translational protein modification processes, including glycosylation.

Secretory and endocytic pathways. The high elongation rates and high secretory capacities observed in filamentous fungi pose special requirements on their endocytic/exocytotic pathways. Small GTPases of the Rab/Ypt and ARF branches of the Ras superfamily play central roles during secretory and endocytic trafficking. ARF subfamily proteins are involved in the formation of carrier vesicles from the donor organelle, while Rab proteins are required for vesicle targeting and fusion with the acceptor organelle (140, 187). Due to their high specificity for a given transport step, these proteins can be used as markers to monitor the different trafficking routes that are present in *Neurospora* and unicellular yeasts (Table 42) (304).

The *S. cerevisiae* secretory pathway is defined by the Rab proteins Ypt1p, Sec4p, and the paralogues Ypt31p/32p, while Ypt6p, Ypt7p, and the paralogues Ypt51p/52p/53p regulate the endocytic pathway (Table 42; Fig. 9). These proteins represent the minimal set of Rab-type GTPases present in all fungi examined. Deletion of any of the Rab genes of the secretory pathway is lethal in *S. cerevisiae*, *C. albicans*, and *S. pombe*, while mutations in the endocytic pathway result in viable *S. cerevisiae* and *S. pombe* mutants. In addition to this minimal set, *Neurospora* and other filamentous fungi possess Rab proteins that are not present in unicellular yeasts but do exist in mammals, implying that the more complex growth behavior also requires more complex organization of the ve-

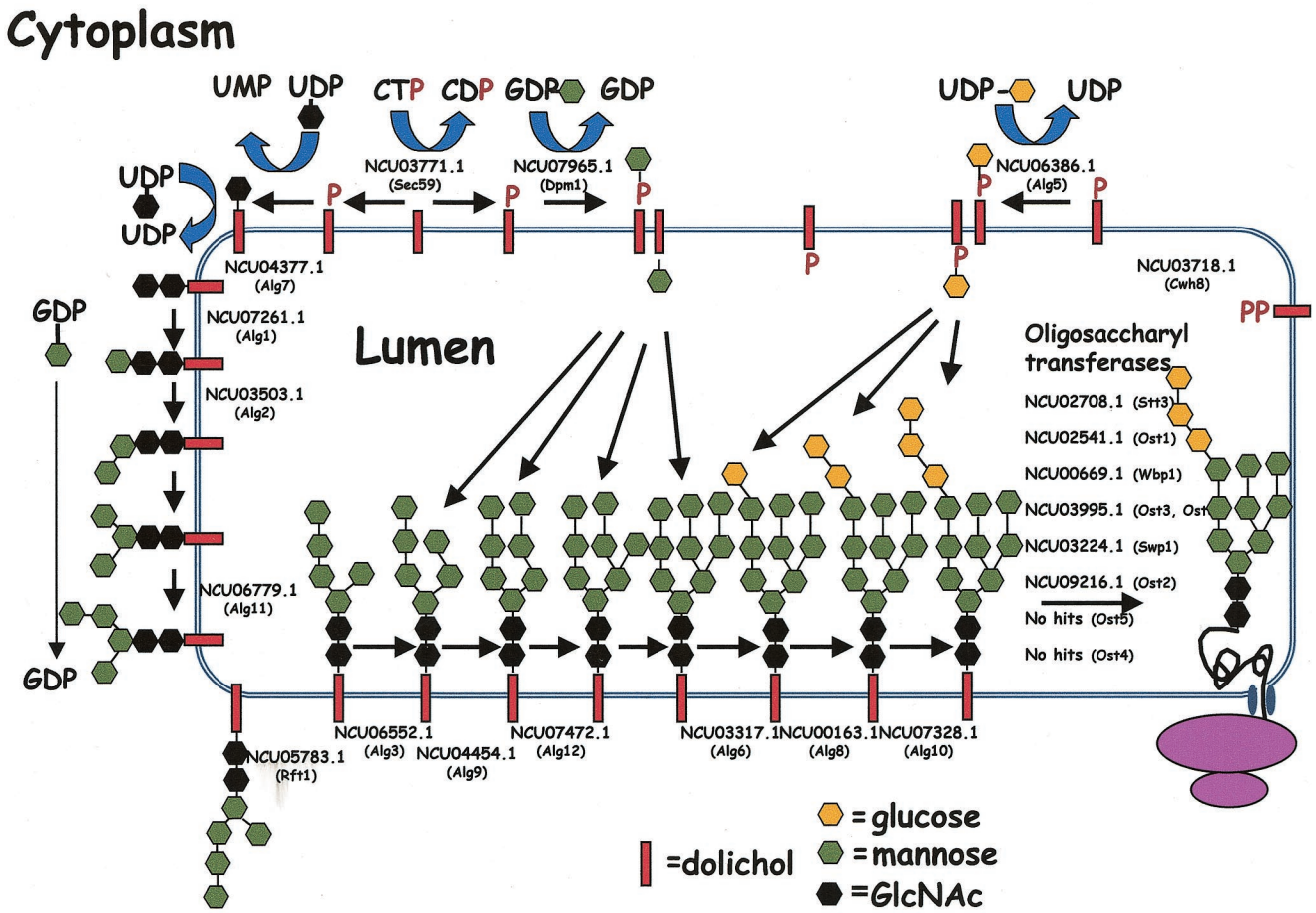

FIG. 8. *Neurospora* dolichol pathway. The mechanism for sequential glycosylation of proteins in the ER is shown. *S. cerevisiae* homologues are presented in parentheses. GlcNAc, *N*-acetylglucosamine. Modified with permission from Markus Aebi (personal communication, 2003)

sicular transport system. Filamentous fungi are known for their high secretory capacity, and this may have required an expansion in the number of the secretion-related GTPases. In addition to an Ypt1p orthologue, *Neurospora* contains a Rab2-related protein that is likely to function in the transport from endoplasmic reticulum to the Golgi apparatus. Surprisingly, in contrast to yeasts, deletion of *srgA* (the orthologue of Sec4p) was not lethal in *A. niger* (636). Also, *srgA* did not complement the *S. cerevisiae sec-4* defect, arguing for differences in the organization of the secretory pathway between filamentous fungi and yeasts that are not apparent at the genome level. The *A. niger srgA* phenotype further suggested that two different secretory routes might exist: a major SrgA/Sec4 dependent route for the constitutive transport of cell wall proteins and a second inducible pathway that is less dependent on SrgA function.

Endocytosis provides a mechanism for plasma membrane proteins and lipids, and extracellular molecules, to be internalized by cells. It is generally regarded as an essential process of eukaryotic cells serving many functions, including recycling of membrane proteins and lipids, removal of membrane proteins and lipids for degradation, and the uptake of signal molecules. Endocytosis has been well characterized in *S. cerevisiae* (276)

TABLE 41. *Neurospora* oligosaccharyl transferase complex components

Enzyme	NCU no.	BLAST match				
		Best overall	*S. cerevisiae*	*S. pombe*	Animal[a]	Plant[b]
Oligosaccharyltransferase subunit	02708.1	*S. cerevisiae* Stt3/0.00	0.00	4e-25	0.00; *C. elegans*	0.00
Dolichyl-diphosphooligosaccharide protein glycosyltransferase	02541.1	*H. sapiens* Rib1/3e-49	e-44	6e-45	3e-49; *H. sapiens*	2e-52
Dolichyl-diphosphooligosaccharide protein glycotransferase	00669.1	*S. pombe* 7e-55	9e-43	7e-55	3e-48; *C. elegans*	8e-46
Oligosaccharyl transferase delta subunit	03224.1	*H. sapiens* QPCT/2e-44	2e-47	2e-54	2e-44; *H. sapiens*	No match
Oligosaccharyl transferase gamma subunit	03995.1	*R. norvegicus* AIG2/6e-26	8e-23	No match	5e-25; *H. sapiens*	3e-7
Oligosaccharyl transferase epsilon subunit	09216.1	*H. sapiens* DAD1/5e-26	4e-12	2e-15	5e-26; *H. sapiens*	2e-13

[a] *Caenorhabditis elegans*, *Drosophila melanogaster*, *Mus musculus*, or *Homo sapiens*.
[b] *Arabidopsis thaliana*.

TABLE 42. Distribution and proposed role of transport-related small GTPases[a]

Neurospora[b] protein	Proposed transport role	*S. cerevisiae*	Mammals
Rab proteins			
Minimal set required in all fungi			
NCU08477.1	ER–GA, IGA, GA–ER	Ypt1p[c]	Rab1
NCU06404.1	LGA–PM	Sec4p[c]	Rab8
NCU01532.1	IGA, LGA–PM?	Ypt31/32p[c]	Rab11
NCU05234.1	IGA, LGA–LE, LE–LGA	Ypt6p[c]	Rab6
NCU03711.1	LE–V	Ypt7p[c]	Rab7
NCU00895.1, NCU06410.1	PM–EE, EE–LE	Ypt51/52/53p[c]	Rab5
Specific for *S. cerevisiae* or *S. pombe*			
	?	Ypt10p	
	?	Ypt11p	
	EE–LE, LE–V		Rab7
Lost in *S. cerevisiae*, but present in *S. pombe*, filamentous fungi and animals			
NCU00889.1	EE–PM		Rab4
Present in filamentous fungi and animals, but not *S. cerevisiae*			
NCU01647.1	ER–GA, GA–ER		Rab2
NCU01453.1	PM–EE?, EE–PM?		Rab18?
NCU08271.1 (unusually long protein)	PM–EE, EE–LE		Rab5
ARF/ARF-like proteins			
Minimal set required in all fungi			
NCU08340.1	GA–ER, IGA, LGA, PM	Arf1/2p[c]	Arf1
NCU08989.1	LGA	Arl1p[c]	Arl1
NCU00333.1	LGA	Arl3p[c]	Arl3
NCU00218.1	?	Arf3p (=Arl2p)[c]	Arl2
NCU00381.1	ER–GA	Sar1p[c]	Sar1
Present in filamentous fungi and animals, but not *S. cerevisiae*			
NCU07173.1	PM–EE		Arf6
NCU08618.1	?		Hypothetical protein

[a] Abbreviations: GA, Golgi apparatus; LGA, late Golgi apparatus; IGA, intra-Golgi transport; PM, plasma membrane; ER, endoplasmic reticulum; EE, early endosome; LE, late endosome; V, vacuole.
[b] Orthologues are also present in *M. grisea* and *A. fumigatus*.
[c] Orthologues are also present in *S. pombe* and *C. albicans*.

but is still considered controversial for filamentous fungi. Nevertheless, there is a growing body of evidence for endocytosis occurring in filamentous fungi, and particularly *Neurospora* (644).

The *Neurospora* genome database was searched for homologues of 29 key proteins involved in endocytosis in *S. cerevisiae*. Each of these proteins was highly similar to one or more predicted proteins in the *Neurospora* genome (644). These

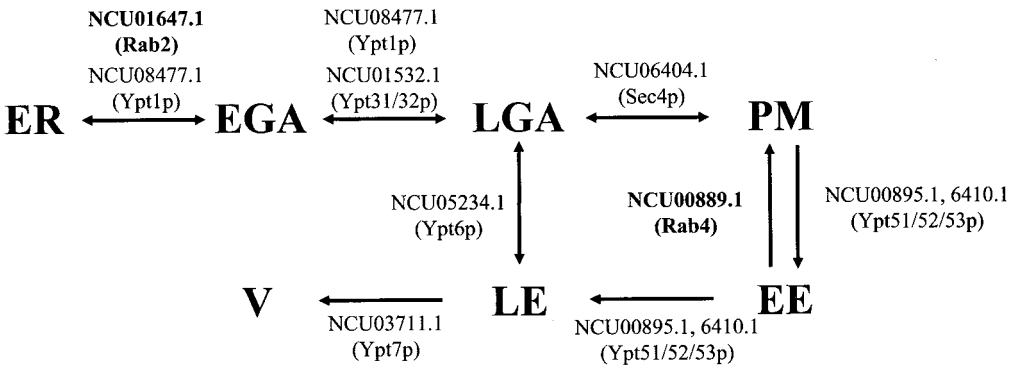

FIG. 9. Proteins of the secretory pathway. *Neurospora* predicted proteins homologous to those involved in the various steps of protein sorting to membranous organelles are shown. Abbreviations: EGA, early Golgi apparatus; LGA, late Golgi apparatus; PM, plasma membrane; V, vacuole; LE, late endosome, and EE, early endosome. NCU numbers for putative Rab proteins are indicated using boldface type.

TABLE 43. Proteins involved in endocytosis in *Neurospora*

NCU no.	BLAST match				
	Best overall match in other organisms	*S. cerevisiae*	*S. pombe*	Animal[a]	Plant[b]
10073.1	7e-23 *H. sapiens* cervical SH3P7	2e-11	1e-16	7e-23	8e-04
07989.1	8e-54 *A. thaliana* clathrin coat assembly protein AP17	1e-34	2e-49	2e-44	8e-54
07171.1	1e-167 *S. cerevisiae* actin-like protein ACT2	1e-167	1e-166	1e-153	1e-129
01756.1	1e-158 *S. pombe* actin-like protein 3	1e-154	1e-158	1e-121	1e-124
02510.1	0.0 *S. pombe* clathrin heavy chain	0.0	0.0	0.0	0.0
04115.1	3e-24 *S. pombe* clathrin light chain	5e-09	3e-24	6e-06	4e-03
09808.1	0.0 *S. cerevisiae* Dnm1p	0.0	0.0	1e-157	1e-163
04100.1	0.0 *A. nidulans* VpsA	0.0	0.0	1e-149	1e-158
01255.1	1e-143 *S. pombe* dynamin-related protein	1e-137	1e-143	5e-47	1e-51
06347.1	1e-116 *A. nidulans* SAGA	1e-34	9e-63	8e-20	3e-06
04783.1	1e-32 *H. sapiens* ENTH domain of epsin	6e-31	2e-30	1e-32	7e-26
03298.1	1e-162 *S. pombe* synaptojanin-like protein	1e-146	1e-162	6e-77	4e-36
03792.1	4e-44 *C. elegans* synaptojanin UNC-26A	6e-39	2e-43	4e-44	2e-38
00896.1	1e-125 *S. cerevisiae* RSD1 (SAC1)	1e-125	1e-109	1e-90	2e-65
01330.1	3e-75 *H. sapiens* KIAA0966 protein	4e-51	2e-51	3e-75	2e-43
07438.1	2e-61 *A. thaliana* putative protein	3e-36	2e-34	9e-10	2e-61
02111.1	0.0 *A. nidulans* myosin 1 myoA	0.0	0.0	0.0	1e-135
06397.1	4e-29 *S. pombe* profilin	2e-22	4e-29	2e-14	7e-13
06171.1	7e-71 *S. cerevisiae* Pan1p	7e-71	3e-53	6e-16	3e-35
06777.1	2e-08 *S. cerevisiae* Pep12 p	2e-08	4e-05	2e-04	2e-07
06397.1	5e-29 *S. pombe* profilin	2e-22	5e-29	3e-14	No hits
01069.1	4e-96 *S. pombe* Hob3	6e-70	4e-96	2e-15	No hits
04637.1	2e-89 *S. pombe* RVS167 homologue	5e-83	2e-89	8e-10	8e-07
02978.1	3e-17 *S. cerevisiae* Sla1p	3e-17	9e-16	7e-06	6e-14
01956.1	0.0 *Y. lipolytica* SLA2-like protein	5e-75	4e-95	3e-42	9e-14
04119.1	4e-38 *S. pombe* t-SNARE complex subunit	3e-26	4e-38	2e-22	3e-19
06192.1	0.0 *A. nidulans* VpsB	1e-122	1e-148	1e-148	1e-117
06410.1	2e-61 *S. cerevisiae* Ypt51p	2e-61	6e-46	7e-51	2e-46
00895.1	5e-68 *S. pombe* Ypt5 protein	3e-52	5e-68	1e-61	2e-55
01523.1	2e-83 *D. melanogaster* Rab11	No match	1e-75	2e-83	1e-77

[a] *Bos taurus*, *Caenorhabditis elegans*, *Drosophila melanogaster*, *Homo sapiens*, *Mus musculus*, or *Rattus norvegieus*.
[b] *Arabidopsis thaliana*.

results strongly suggest that *Neurospora* possesses the complex protein machinery required to conduct endocytosis and that this machinery is well conserved in both budding yeast and filamentous fungi. This hypothesis is supported by experiments that have shown that the membrane-selective markers of endocytosis, FM4-64 and FM1-43, are internalized by *Neurospora* hyphae in an energy-dependent manner (239, 336, 643, 795). Sequences of the 29 *Neurospora* endocytic proteins were then used to search for homologues in budding and fission yeast, animal, and *A. thaliana* genomes (Table 43). The highest homology was found to endocytic proteins in *S. pombe* (Table 43; Fig. 10). In a comparison with just animals and plants, 68% of the proteins showed higher homology to animal homologues, while 32% were better matches to plant homologues.

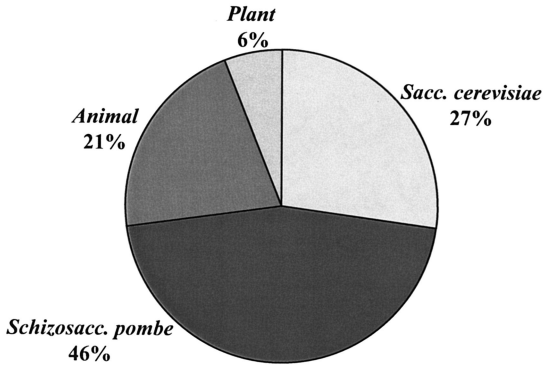

FIG. 10. *Neurospora* endocytosis proteins. The percentage of *Neurospora* proteins with the greatest homology to proteins in *S. cerevisiae*, *S. pombe*, animals, and plants is shown.

The analysis of the different coat complexes and adaptors required for the generation of vesicles from various membrane compartments revealed significant differences in the vesicular transport system in *Neurospora* and *S. cerevisiae*. All components that constitute the core COPI, COPII, and clathrin complexes are highly conserved in *Neurospora*, but, interestingly, the *Neurospora* genome encodes an additional pair of proteins corresponding to the large subunit of the AP-1 adaptor complex (NCU01992.1, γ-subunit; NCU04404.1, β-subunit) that are not found in *S. cerevisiae* but are present in mammals (89, 90, 420). The functional significance of this duplication is unknown. Clathrin-coated vesicles and pits, which are commonly visualized with the electron microscope in animal and plant cells, are often indicative of clathrin-mediated endocytosis. However, there is no ultrastructural evidence for their occurrence in fungal cells, including those of *N. crassa* (644). Nevertheless, both heavy- and light-chain clathrin and a clathrin coat assembly protein are present in *Neurospora* (Table 43). Clathrin-coated vesicles are also involved in other parts of the vesicle trafficking network of eukaryotic cells. Whether clathrin-mediated endocytosis occurs in *N. crassa* or other filamentous fungi is, as yet, unclear.

An indirect route through the late endosomal compartment and back to the Golgi apparatus functions to recycle plasma membrane components in yeast. The high growth rate of *Neurospora* places a much higher demand on the recycling of material toward the growing tip (643), and several proteins that function in this recycling step have been identified. Homologues of mammalian Rab4p and ARF6 are probably acting during the transport from early endosomes to the plasma membrane. Interestingly, ARF6 function is thought to be linked to Rac (which is absent from yeasts) in vertebrate cells (587). Sequence analysis of NCU001453.1 showed that this protein is distantly related to Rab18p proteins, which are involved in the recycling to the plasma membrane, but also has significant homology to Sec4p. Therefore, this protein may act in the late secretory pathway, perhaps in parallel with Sec4. Finally, NCU08271.1 and NCU08618.1 encode unusually long proteins with homology to Rab5p and a hypothetical but highly conserved ARF that is found in mammalian cells.

ENVIRONMENTAL SENSING

Filamentous fungi are able to grow in more diverse environments than yeasts and presumably are confronted with a wider variety of environmental stimuli. Therefore, it is expected that this group of organisms should possess an extensive array of sensing and signaling capabilities. Analysis of genes implicated in signal transduction and stress responses demonstrates that *Neurospora* possesses classes of sensing molecules not found in the two sequenced yeasts. The expansion of upstream signaling proteins is often coupled with a conserved core of downstream components, suggesting the presence of extensive networking and/or new signaling interactions not found in *S. cerevisiae* and *S. pombe*.

Major Signal Transduction Pathways

Two-component regulatory systems. Two-component signal transduction systems are minimally composed of two proteins: a sensor histidine kinase and a response regulator (sometimes called a receiver) (600). The histidine kinase is autophosphorylated in an ATP-dependent manner on a conserved histidine residue, and this autophosphorylation activity is regulated by an environmental signal sensed by the kinase. Stimuli include osmolarity, nutrient levels, oxygen levels, cellular redox status, and light. Phosphate transfer occurs from the histidine kinase to a conserved aspartate residue in the response regulator. This differential phosphorylation of the response regulator results in an altered cellular response such as activation of enzyme activity, modulation of transcription, or altered protein-protein interactions. Two-component signal transduction pathways are extensively used to mediate prokaryotic signaling events. In recent years it has been discovered that these signaling systems are also found in eukaryotes, including plants, yeasts, filamentous fungi, and slime molds (679, 758, 857). Their presence has not yet been demonstrated in mammals, and since some two-component systems are involved in virulence responses in fungi, they present attractive antifungal targets.

Histidine kinase (HK) and response regulator (RR) domains can be easily recognized in proteins by using sequence alignments (600). The HK domain contains several conserved sequence elements, including the H box, which is the site of autophosphorylation, and the N, G_1, F, and G_2 boxes, which mediate binding of ATP, as shown by the recent structures of two bacterial HK domains (79, 80, 793). As with most signal transduction proteins, the HK and RR domains are modular; that is, they can be combined in various arrangements with each other and with other protein domains to yield the desired signaling protein.

A more complex version of the two-component pathway is the phosphorelay. In a phosphorelay system, the sensor is typically a "hybrid" HK, containing both HK and RR domains. In this pathway, there are two layers of His-to-Asp phosphotransfer. The sensor autophosphorylates in response to a signal, and the phosphate is subsequently transferred to the aspartate of its own response regulator domain. Transfer of the phosphate from the sensor aspartate to the histidine of a separate Hpt (histidine phosphotransfer domain) protein follows. The phosphate is then ultimately transferred to another aspartate in a separate response regulator, resulting in a His-Asp-His-Asp phosphorelay. It has been speculated that the additional phosphotransfer steps allow for more complex regulation of signaling events in a cell, since there are more opportunities to modulate phosphorylation activity. In addition, multiple HK inputs can be channeled into common downstream signaling proteins (Hpt and RR). This scenario is reminiscent of multiple receptor inputs into a single HK in bacterial chemotaxis.

Interestingly, although the hybrid kinases comprise only a small fraction of the sensor kinases found in prokaryotes, they are the major form found in eukaryotes. Experimental confirmation of the phosphorelay system has been obtained for some of these systems, but it is only inferred in others. The best-characterized example of a eukaryotic phosphorelay is the SLN1-YPD1-SSK1 system that regulates the HOG1 mitogen-activated protein kinase (MAPK) cascade in *S. cerevisiae* (624, 626). Sln1p is a transmembrane hybrid HK whose phosphorylation is regulated in response to changes in extracellular osmolarity, although the actual signal sensed remains unknown. Under conditions of low osmolarity, the Sln1p HK autophosphorylates on its histidine residue and transfers this phosphate to its RR domain. The phosphate is subsequently transferred to the histidine of the Hpt called Ypd1p. Finally, the phosphate is transferred to the aspartate of an RR domain of Ssk1p. It is the phosphorylated form of Ssk1p that is unable to activate the MAPKKKs Ssk2/22p. Ssk2/22p are a pair of redundant MAPKKKs that activate the MAPKK, Pbs2p, which ultimately regulates the Hog1p MAPK. Under conditions of increased osmolarity, Sln1p is not phosphorylated; hence, there is no phospho transfer to Ypd1p and Ssk1p. The unphosphorylated form of Ssk1p is able to activate Ssk2/22 and ultimately Hog1p, which then modulates transcription to allow glycerol synthesis in the cell. Sln1p also mediates a phosphorelay to a second response regulator in the cell, Skn7p. Skn7p regulation by Sln1p results in control of expression from of a *lacZ* reporter construct that contains an Mcm1p binding site in its promoter (473, 891). The physiological relevance of this has yet to be determined. However, the Skn7p response regulator is able to mediate responses to oxidative stress independent of its role in the phosphorelay with Sln1p (435, 637).

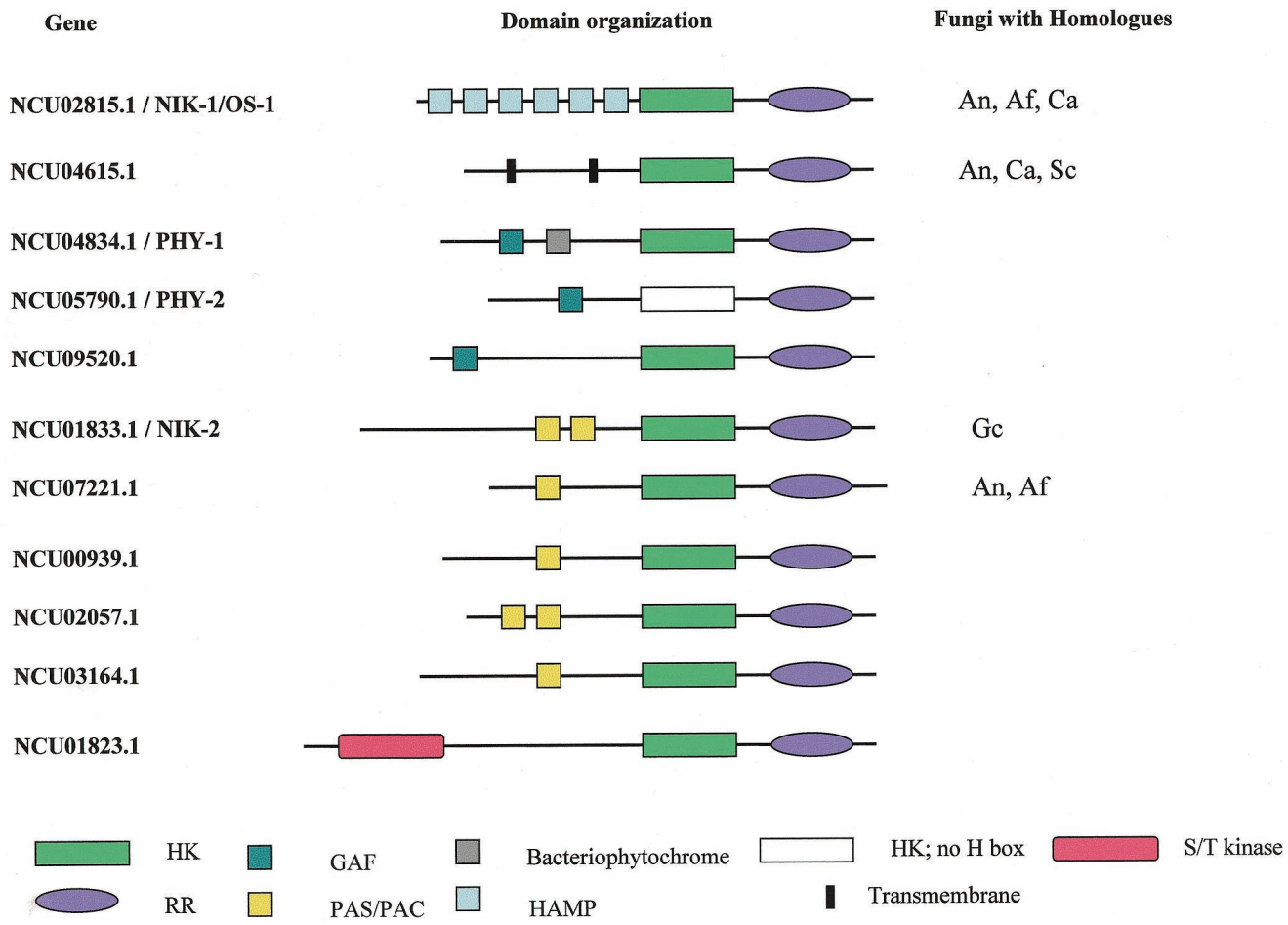

FIG. 11. Domain organization of *Neurospora* HKs. Abbreviations: An, *A. nidulans;* Af, *A. fumigatus;* Ca, *C. albicans;* Sc, *S. cerevisiae;* Gc, *Glomerella cingulata*. Note that assignment of the total number of PAS/PAC domains in the relevant proteins is somewhat subjective, since it depends on the threshold values used during BLAST analyses.

The genome sequence of *Neurospora* reveals that it has 11 putative HK containing proteins, all of them hybrid kinases (Fig. 11). Only one of these (NCU04615.1) is predicted to span the membrane and appears to be most similar to Sln1p from *S. cerevisiae* and TcsB from *A. nidulans*. Two of the HKs have been previously characterized. Nik-1/Os-1 (NCU02815.1) has been shown to play a role in hyphal development (13, 695). Deletion mutants exhibit multiple morphological defects including aberrant hyphal development and reduced conidiation. These defects become exacerbated when cells are grown in media of high osmolarity; this may be the result of a defective cell wall structure. Nik-1/Os-1 has a homologue, Cos-1, in the opportunistic pathogen *C. albicans*, where it plays a role in hyphal development under various conditions on solid surfaces (14). Cos-1 also plays a role in virulence (704). Nik-2 (NCU01833.1) is a hybrid HK that also contains a PAS domain, which suggests that it may be involved in redox or light sensing (783) (see also "Photobiology and circadian rhythms" below). However, *nik-2* deletion mutants exhibit no obvious phenotype when grown under a variety of conditions (L. Alex and M. Simon, unpublished results).

Interestingly, two of the HK proteins appear to be light sensors. NCU05790.1 and NCU04834.1 contain domains common to plant phytochromes and bacteriophytochromes, respectively (see "Photobiology and circadian rhythms" below). Deletion of these genes and their respective phenotypes is currently under investigation. Although there is no evidence that *Neurospora* growth and development are sensitive to red light, the presence of these two genes suggests that this may need to be readdressed.

There is also an HK that is similar to Fos-1 from *A. fumigatus* and TcsA from *A. nidulans*. These HKs play a role in conidiation in their respective species, but the phenotypes are slightly different (627, 834). The remaining 7 of the 11 *Neurospora* HKs have some similarity to other known HK sequences; however, the function of these HKs remains to be elucidated.

A comparison of HK numbers among a variety of fungi shows that the filamentous fungi have a larger complement of these proteins than any of the sequenced yeasts or *C. albicans*. *S. cerevisiae* has only 1 HK, Sln1p; *S. pombe* has 3 HKs, Mak1, Mak2, and Mak3; *C. albicans* has 3 HKs, Chk1, CaNik1/Cos-1; and CaSln1; and *Neurospora* has 11 HKs (269). Sln1p homologues appear in *S. cerevisiae*, *C. albicans*, and *Neurospora* but are absent from *S. pombe*. The three HKs from *S. pombe* do not have good homologues in *Neurospora*, *S. cerevisiae*, or *C. albicans*.

The downstream components of two-component pathways are also present in *Neurospora*. There is one Hpt protein (NCU01489.1) that is most similar to *S. pombe* Spy1/*S. cerevisiae* Ypd1p. Two response regulators are present; NCU01895.1 and NCU02413.1. These are most similar to *S. pombe* Mcs4 (*S. cerevisiae* Ssk1p) and *S. pombe* Prr1 (*S. cerevisiae* Skn7p), respectively. The conservation of a few downstream signaling elements, i.e., the Hpt and RR proteins, suggests that these proteins may act to integrate multiple signaling inputs from the many HKs to evoke the proper cellular response. Alternatively, some of the HKs may not act through phosphorelays. These various possibilities await testing.

Heterotrimeric G proteins. Seven-transmembrane helix, G-protein-coupled receptors (GPCRs) are used to detect diverse environmental stimuli in eukaryotes (198). GPCRs regulate the activity of second messengers through their interaction with heterotrimeric G proteins (562). A heterotrimeric G protein consists of a Gα subunit, which binds and hydrolyzes GTP, and a tightly associated Gβ and Gγ subunit. Ligand binding to the GPCR leads to exchange of bound GDP for GTP on the Gα subunit and to dissociation of the heterotrimer into Gα-GTP and Gβγ units. Gα-GTP and Gβγ can both interact with effectors to generate changes in cellular physiology and development.

Prior to the completion of the genome sequence, the total number of G-protein subunits in a filamentous fungal species was not known. This analysis (Table 44). showed that *Neurospora* possesses more heterotrimeric Gα subunits than do budding and fission yeasts (three versus two) but the same number of Gβ and Gγ genes (one each) (Fig. 12). Previous work has demonstrated that these subunits are important for hyphal growth, conidiation, female fertility, and stress responses in *Neurospora* (37, 369, 406, 873).

It has recently been shown that protein "mimics" for Gβ (Gpb1p and Gpb2p) and Gγ (Gpg1p) subunits in *S. cerevisiae* are coupled to the Gpa2p Gα protein and regulate filamentous growth in this organism (314). The two Gβ mimics each contain seven Kelch repeats and may assume a tertiary structure similar to the β-propeller of Gβ proteins. However, analysis of the *Neurospora* genome sequence did not reveal any proteins with seven Kelch repeats or with high similarity to either the Gβ or Gγ mimics.

Analysis of the genome sequence shows that *Neurospora* possesses at least 10 predicted seven-transmembrane helix proteins that are potential GPCRs (Fig. 12). These proteins fall into five families: microbial opsins, pheromone receptors, glucose sensors, nitrogen sensors, and a novel class, not previously identified in fungi. The first class consists of an opsin (*nop-1*) and an opsin-related protein (*orp-1*) that were identified during prior EST projects (78); this family has three members in *S. cerevisiae* (Hsp30p, Yro2p and Mrh1p) (78, 297, 592, 861). In the second family are two predicted pheromone receptors, similar to *S. cerevisiae* α-factor and **a**-factor pheromone receptors (reviewed in reference 199). The expression patterns of the putative *Neurospora* pheromone receptor genes have been published (622). The third class of GPCRs is represented by a protein similar to putative glucose sensors in both *S. cerevisiae* and *S. pombe* (847, 866), while the fourth group consists of two proteins similar to the Stm1 protein from *S. pombe*, implicated in nitrogen sensing (149).

The fifth class contains three related GPCR-like genes. This group is not present in the genomes of *S. cerevisiae* or *S. pombe*. The encoded proteins are most similar to known and predicted GPCRs from the protists *Dictyostelium discoideum* (423) and *Polysphodylium pallidum* (TasA) and also to predicted proteins in *C. elegans* and *A. thaliana* (388); similarity is highest in the predicted seven transmembrane helices. Functions for this group of proteins have been elucidated only in *D. discoideum*, where four related cAR receptors sense cyclic AMP (cAMP) levels during multicellular development and sporulation (reviewed in reference 33).

In both fungi and higher organisms, phosducin and Regulator of G-Protein Signaling (RGS) proteins regulate G-protein signaling pathways by modulating the activity of Gβγ dimers and the GTPase activity of Gα subunits, respectively (402, 734). *S. cerevisiae* possesses two phosducin and two RGS protein genes, while *Neurospora* contains two phosducin genes and one RGS genes (Fig. 12).

Ras-like GTPases. The superfamily of monomeric small GTPases (20 to 40 kDa) function as molecular switches that control a wide variety of cellular processes including signal transduction, cell polarity, the cytoskeleton, and the identity and dynamics of membranous compartments (reviewed in references 654 and 773).

A total of five Ras/Ras-related small GTPases are present in the *Neurospora* genome (Table 44). *Neurospora* is similar to *S. cerevisiae* in that it has two Ras proteins (Fig. 12), in contrast to the one in *S. pombe*. The two *S. cerevisiae* Ras proteins regulate adenylyl cyclase activity (791), while the sole *S. pombe* Ras, Ras1, regulates the activity of a Cdc42 guanine exchange factor to control morphogenesis, as well as a MAPK cascade that regulates mating in this organism (595). The *Neurospora* RAS-1 (18) and RAS-2/SMCO-7 (397) proteins have already been defined. Mutation of *ras-2/smco-7* leads to severe defects in hyphal growth and conidiation (397). The function of *Neurospora* RAS-1 is unknown.

The three *Neurospora* Ras-related proteins include one that is most similar to Rheb, which is evolutionarily conserved and controls arginine uptake in *S. cerevisiae* and *S. pombe* (reviewed in reference 649). The second, the previously identified KREV-1 protein (367), is similar to mammalian Rap (which can antagonize Ras signaling [reviewed in reference 649]) and *S. cerevisiae* Rsr1p (which controls bud site selection and cell polarity [598]). *Neurospora krev-1* null mutants are phenotypically indistinguishable from the wild type; however, analysis of mutationally activated alleles suggests involvement of *krev-1* in the sexual cycle (367). The third Ras-related *Neurospora* protein was not characterized previously but is most similar to a predicted Ras protein from the yeast *Yarrowia lipolytica*.

cAMP signaling. As in the two sequenced yeasts, *Neurospora* contains one gene encoding adenylyl cyclase, *cr-1* (427), one cyclase-associated protein gene, *cap*, and one protein kinase A (PKA) regulatory subunit gene (*mcb*) (Table 44; Fig. 12) (113). In contrast, the genome sequence predicts two distinct PKA-cat subunits in *Neurospora*. One is most similar to PkaC from *Colletotrichum trifolii* but is also related to the three *S. cerevisiae* Tpk PKA-cat genes (790). Mutation of the *Neurospora* gene causes a phenotype similar to that of the adenylyl cyclase mutant *cr-1* (M. Plamann, unpublished data). The second protein is most closely related to Pka-cat from *M. grisea* and is also

TABLE 44. Heterotrimeric G-protein signaling, cAMP metabolism, and Ras proteins

Protein class	*Neurospora crassa* protein	NCU no.	BLAST match				
			Best overall	*S. cerevisiae*	*S. pombe*	Animal[a]	Plant[b]
Heterotrimeric Gα	GNA-1	06493.1	*Cryphonectria parasitica* Cpg-1; 0.00	Gpa2p; 1e-71	Gpa1; 5e-70	Gαi-3; e-102	GPA1; 1e-48
	GNA-2	06729.1	*Magnaporthe grisea* MAGC; 1e-137	Gpa2p; 3e-46	Gpa1; 1e-59	G-α65A; 3e-63	GPA1; 2e-34
	GNA-3	05206.1	*M. grisea* MAGA; 1e-178	Gpa2p; e-102	Gpa2; 4e-85	G-αi-3; e-102	GPA1; 1e-48
Heterotrimeric Gβ	GNB-1	00440.1	*M. grisea* Gβ; 1e-164	Ste4p; 5e-51	Git5; 1e-60	GPB-1; e-108	Gβ; 2e-73
Heterotrimeric Gγ	GNG-1	00041.1	*Botrytis cinerea* CNS01BJB (Gγ); 9e-20	None	None	None	None
Pheromone receptor (GPCR)	PRE-1	00138.1	*Sordaria macrospora* Pre-1; 1e-138	Step3; 0.063	None	None	None
	PRE-2	05758.1	*S. macrospora* Pre2; 0.00	None	None	None	None
Putative cAMP GPCR	GPR-1	00786.1	*Dictyostelium discoideum* CrlA; 3e-15	None	None	None	GPCR; 0.002
	GPR-2	04626.1	*Arabidopsis thaliana* GPCR CAA72145.1; 2e-09	None	None	None	GPCR; CAA72145.1; 2e-09
	GPR-3	09427.1	*D. discoideum* AA062367; 3e-07	None	None	None	GPCR CAA72145.1; 1e-04
Putative glucose sensor GPCR	GPR-4	06312.1	*S. cerevisiae* Gpr1p; 3e-14	Gpr1p; 3e-14	None	None	None
Stm1-like GPCR	GPR-5	00300.1	*S. cerevisiae* YOL092w; 1e-29	YOL092w; 1e-29	Stm1; 1e-18	NP_493686.1; 3e-13	AT4g36850; 2e-14
	GPR-6	09195.1	*S. cerevisiae* YBR147w; 4e-32	YBR147w; 4e-32	Stm1; 8e-14	Y43H11AL2; 2e-14	NP_568009.1; 9e-10
Microbial opsin	NOP-1	10055.1	*Leptosphaeria maculans* opsin; 2e-75	Yro2p; 1e-08	SPCC31H12-02e; 9e-07	None	None
Opsin-related protein	ORP-1	01735.1	*Coriolus versicolor* Hsp30; 5e-31	Yro2p; 8e-23	SPCC31H12.02c; 8e-12	None	None
Phosducin		00441.1	*C. parasitica* Bdm-1; 2e-57	None	None	None	None
		00617.1	*S. cerevisiae* Plp2p; 2e-42	Plp2p; 2e-42	SPBC2A9; 1e-34	IAP-associated factor VIAF1; 8e-17	F18O22.30; 9e-12
Regulator of G-protein signaling		08319.1	*A. nidulans* FlbA; e-139	Sst2p; 1e-14	C22F3.12c; 9e-27	RGS16; 9e-05	None
Adenylyl cyclase	CR-1	08377.1	*M. grisea* MAC1; 0.00	Cyr1p; 3e-160	Adenylyl cyclase; e-111	Scribbled; 1e-27	F3M18.12; 1e-21
Cyclase-associated protein		08008.1	*Candida albicans* CAP; 2e-67	Srv2p/CAP; 2e-65	CAP; 1e-66	CAP2; 1e-59	Cap1; 1e-36
PKA regulatory subunit	MCB	01166.1	*Colletotricum lagenarium* Rpk-1; e-142	Bcy1p; 2e-67	Cgs1; 2e-58	PKA regulatory, type II α; 3e-55	None
PKA catalytic subunit		06240.1	*Colletotricum trifolii* pkaC; e-164	Tpk1p; e-129	KapB; e-110	PKA C-beta; 4e-93	ATPK19; 2e-58
		00682.1	*M. grisea* PKA-ct; e-142	Tpk1p; 5e-74	KapB; 1e-67	PKX1; 1e-81	ATPK19; 2e-58
High-affinity cyclic nucleotide phosphodiesterase		00478.1	*C. albicans* high-affinity cAMP phosphodiesterase; 2e-45	Pde2p; 4e-23	None	XP319716.1; 3e-25	None
Low-affinity cyclic nucleotide phosphodiesterase		00237.1	*S. pombe* Pde1; 3e-16	Pde1p; 4e-15	Pde1; 3e-16	None	None
Ras	RAS-1	08823.1	*Sclerotinia sclerotiorum* hypothetical Ras; 3e-63	Ras1p; 1e-24	Ras1; 1e-26	rRas2; 9e-28	BAB78669.1; 1e-06
	RAS-2/SMCO-7	03616.1	*U. maydis* Ras2; 2e-57	Ras2p; 2e-38	CAA27399.1; 4e-39	BAB27790.1; 2e-42	Ric1; 9e-19
Ras-related	KREV-1	02167.1	*H. sapiens* Raichu404X; 6e-49	Rsr1p; 3e-47	CAA27399.1; 5e-33	Raichu404X; 6e-49	Ran1; 7e-18

[a] *Caenorhabditis elegans*, *Drosophila melanogaster*, *Anopheles gambiae*, *Mus musculus*, or *Homo sapiens*.
[b] *Arabidopsis thaliana* or *Oryza sativa*.

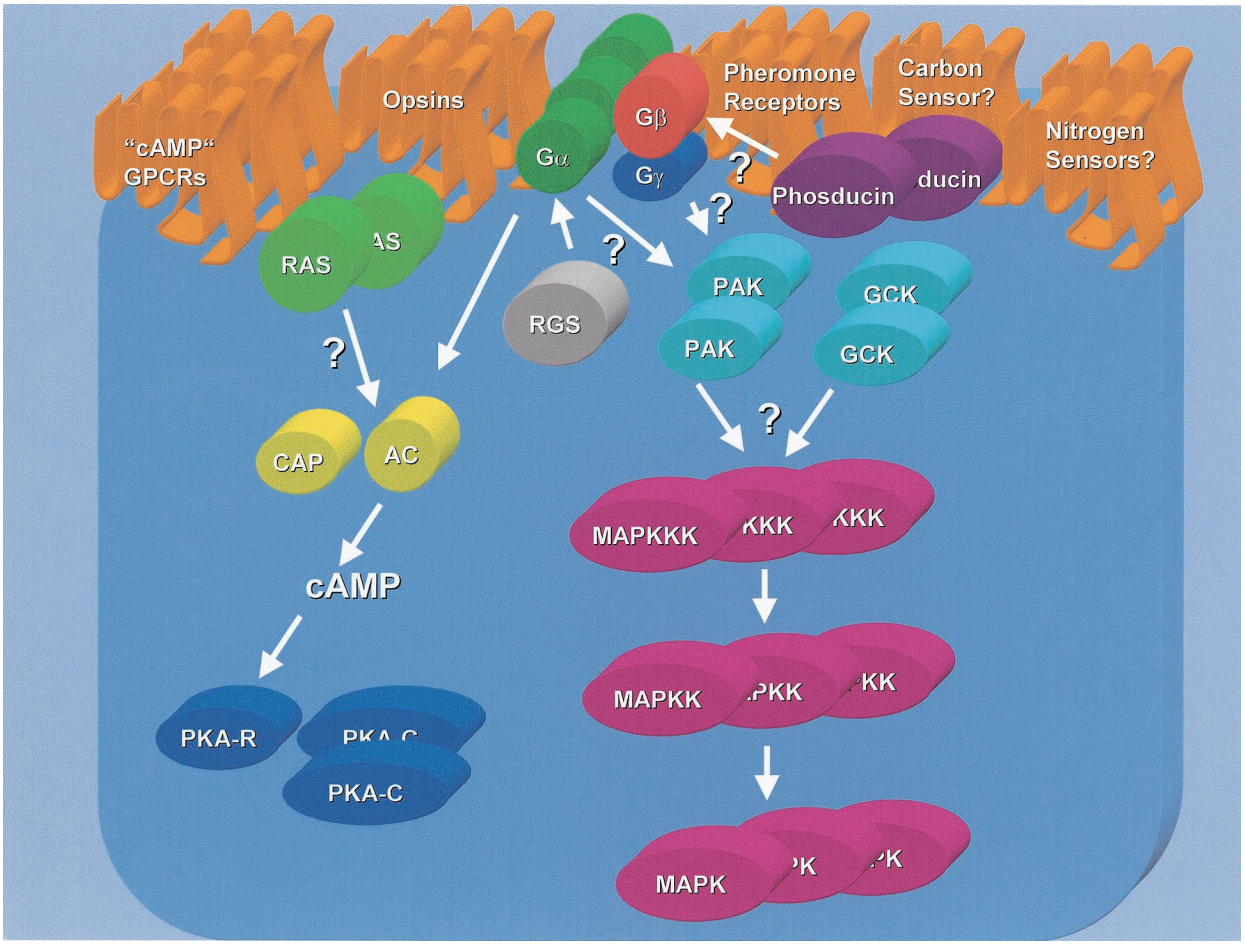

FIG. 12. Known and predicted heterotrimeric G-protein, Ras, cAMP, and PAK/MAPK signaling pathways in *Neurospora*. The number of images for each signaling protein class in the cartoon represents the number of *Neurospora* predicted gene products in each group. Arrows depict interactions that are supported by evidence from other systems but have not yet been demonstrated in *Neurospora*. The MAPK cascade(s) may also receive input from two-component signaling pathways (Fig. 11). Various Rho GTPase superfamily members (Fig. 18) may regulate certain signaling events downstream of Ras and upstream of PAK proteins. Abbreviations: GPCR, G-protein-coupled receptor; RGS, regulator of G-protein signaling; PAK, p21-activated kinase; GCK, germinal-center kinase; AC, adenylyl cyclase; CAP, cyclase-associated protein; PKA-R, regulatory subunit of cAMP-dependent protein kinase; PKA-C, catalytic subunit of cAMP-dependent protein kinase; MAPK, MAPKK, and MAPKKK, mitogen-activated protein kinase, kinase kinase, and kinase kinase kinase, respectively.

similar to Uka1 from *Ustilago maydis* (209) but is less similar to *S. cerevisiae* PKA-cat subunits than the first. Two predicted cAMP phosphodiesterase genes are most similar to the high- and low-affinity forms, respectively, found in *S. cerevisiae* (491, 682).

cAMP accumulates in the extracellular medium of wild-type *Neurospora* cultures (371, 724), and it is has recently been shown that loss of the heterotrimeric Gα gene, *gna-1*, blocks the ability of strains lacking a functional adenylyl cyclase or *gna-3* Gα gene to respond to cAMP supplementation (370, 405). These data, coupled with the presence of GPCRs similar to slime mold cAMP receptors, suggest that cAMP or a related molecule may serve as an environmental signal and GPCR ligand in *Neurospora*. The existence of such a pathway has not previously been demonstrated in any fungal system.

PAKs and GCKs. (i) PAKs. p21-activated kinases (PAKs) are serine/threonine protein kinases that can be activated by binding to GTP-bound Rho-like GTPases of the Cdc42/Rac family. PAKs have been found in most eukaryotes, where they regulate a wide variety of processes including modulation of MAPK pathways, cytoskeletal dynamics, cell cycle progression, and apoptosis (92). PAKs have a common arrangement of protein domains, a CRIB domain at the N terminus and the serine/threonine kinase domain at the C terminus. The CRIB domain is able to bind to the kinase domain and inhibit its activity. On binding of a GTP-bound Rho-like GTPase to the CRIB domain, inhibition of kinase activity is relieved (reviewed in references 92 and 177).

The most well-characterized member of the PAK family in fungi is Ste20p from *S. cerevisiae*. Ste20p activity is important for the response to pheromone, osmostress, filamentation, and polarized growth. Of these, the best understood is the pheromone response. On pheromone binding to its GPCR, the heterotrimeric G-protein is activated. This, in turn, results in recruitment of the Ste20p-Cdc42p complex to the mating MAPK module and allows phosphorylation of the MAPKKK Ste11p. *S. cerevisiae* also has a second PAK, Cla4p, that is involved in budding and cytokinesis (66, 176). In addition to

TABLE 45. p21-Activated and germinal center kinases

NCU no.	Kinase type	Best BLAST match[a]			
		S. cerevisiae	Animal	Other fungi	Plant
03894.1	PAK	Ste20p[b]; 1e-109	Pak1 (Hs); 1e-108	Um; 1e-107	Putative S/T kinase (At); 1e-55
00406.1		Cla4p; 9e-99	Pak1 (Hs); 2e-92	CHM1[b] (Mg); 1e-153	Putative S/T kinase (Hv); 1e-47
00772.1	GCK	Sps1p; 5e-65	Severin kinase[b] (Dd); 1e-109	Ste20-like kinase (Um); 6e-81	Putative S/T kinase (Os); 9e-74
04096.1		Sps1p; 1e-67	Severin kinase[b] (Dd); 1e-102	Ste20-like kinase (Um); 1e-101	Putative protein kinase (At); 5e-82

[a] Hs, *Homo sapiens*; Um, *Ustilago maydis*; Mg, *Magnaportha grisea*; At, *Arabidopsis thaliana*; Hv, *Hordeum vulgare*; Os, *Oryza sativa*; Dd, *Dictyostelium discoideum*.
[b] Best overall hit.

the CRIB and kinase domains present in all PAKs, Cla4p also has a PH (plekstrin homology) domain adjacent to the CRIB domain. PH domains have been implicated in protein-protein interactions, binding to phosphatidylinositol-4,5-bisphosphate, and membrane association (467).

The genome sequence of *Neurospora* indicates that there are two PAKs, one a Ste20p homolog and one a Cla4p homolog (Table 45; Fig. 12). Many of the components that are known to interact with Ste20p and Cla4p in *S. cerevisiae* are present in *Neurospora* (Ste50p, Cdc42p, and Ste11p). Therefore, it can be expected that these two PAKs will control similar responses in *Neurospora* but that there will definitely be differences, given the difference in growth processes for these two organisms. For example, NcCla4p may play a role in conidiophore production by analogy to budding yeast. The physical characterization of the PAKs and their functions awaits further investigation.

(ii) GCKs. A second group of kinases that are similar to PAKs are the germinal center kinases (GCKs). The domains and their organization in GCKs differ from those in PAKs, in that the serine/threonine kinase domain is present at the N terminus of the protein and this is followed by a sequence that is not conserved with PAKs or between many GCKs themselves (444, 719). There are no CRIB or PH domains present. It is thought that the C-terminal sequences act as autoinhibitory to the kinase activity and that binding of other components to the C-terminal sequences probably relieves this inhibition. GCKs are responsible for mediating some stress responses (reviewed in reference 444). In yeast, there appears to be two GCK-like proteins, Sps1p and Kic1p. Sps1p is a developmentally regulated kinase that is necessary for later events in sporulation (255). Kic1p is a kinase whose activity is dependent on the yeast centrin-like protein Cdc31p and is necessary for cell wall integrity (764). *Neurospora* has two predicted GCK-like proteins (Table 45; Fig. 12) that show the highest homology to severin kinase from *D. discoideum*. The *D. discoideum* severin kinase phosphorylates the actin binding protein severin, leading to rapid rearrangement of the actin cytoskeleton during amoeboid movement (215). The role of these two GCK-like proteins in *Neurospora* is currently unknown, but the roles of similar proteins in other eukaryotic microbes suggest involvement in cytoskeletal dynamics and/or ascospore development.

MAPKs. MAPK pathways consist of three serine/threonine protein kinases (MAPKKK, MAPKK, and MAPK) that act sequentially, culminating in phosphorylation of target proteins that regulate transcription, the cell cycle, or other cellular processes (138, 604, 625). MAPK modules are regulated by a wide variety of signaling proteins in other fungi, including GPCRs, PAKs, histidine phosphorelays, and Cdc42p (138, 604, 625).

A total of nine MAPKKK/MAPKK/MAPK protein-encoding genes were found in the *Neurospora* genome sequence (Table 46; Fig. 12). The similarity scores demonstrate that these nine proteins could form three pathways corresponding to those for pheromone response/filamentation, osmosensing/

TABLE 46. MAPKs

Protein class	*Neurospora* protein	NCU no.	BLAST match				
			Best overall	*S. cerevisiae*	*S. pombe*	Animal[a]	Plant[b]
MAPKKK (osmosensing/stress?)		03071.1	*S. pombe* Win1/Wak1/Wis4; 0.00	Ssk2p; 1e-128	Win1/Wak1/Wis4; 0.00	PRO0412; 9e-54	NPK1-related protein kinase3; 2e-49
MAPKK		00587.1	*S. cerevisiae* Pbs2p; 1e-95	Pbs2p; 1e-95	Wis1/Sty2; 6e-92	*Drosophila* Dsor1; 2e-60	MKK2; 6e-44
MAPK	OS-2	07024.1	*M. grisea* osmotic stress MAPK; 0.00	Hog1p; 1e-165	Sty1; 1e-170	EAA00104.1; 1e-99	MPK3; 2e-91
MAPKKK (pheromone response?)	NRC-1	06182.1	*A. nidulans* MAPKKK; 1e-179	Ste11p; 1e-82	NP595714.1; 5e-84	CAD38973.1; 1e-58	NPK1-related protein kinase2; 1e-69
MAPKK		04612.1	*Glomerella cingulata* EMK1; e-165	Ste7p; 6e-66	Byr1; 4e-80	EAA01212.2; 1e-74	MKK6; 2e-50
MAPK	MAK-2	02393.1	*Podospora anserina* CAD60723.1; 0.00	Fus3p; 2e-120	Spk1; 1e-133	CAA77753.1; e-108	MPK4; 3e-95
MAPKKK (cell integrity?)		02234.1	*P. anserina* AAL77223.1; 0.00	Bck1p; 4e-80	Mkh1; 1e-100	MEKK 3; 2e-49	NPK1-related protein kinase3; 3e-59
MAPKK		06419.1	*M. grisea* Mkk1; 1e-176	Mkk1p; 3e-85	Skh1; 4e-79	EAA01212.2; 3e-50	MKK2; 8e-43
MAPK		09842.1	*C. lagenarium* AAL50116.1; 0.00	Slt2p; 1e-136	Spm1; 1e-140	BMK1 kinase; 6e-85	MPK6; 9e-90

[a] *Caenorhabditis elegans*, *Drosophila melanogaster*, *Anopheles gambiae*, *Mus musculus*, or *Homo sapiens*.
[b] *Arabidopsis thaliana* or *Oryza sativa*.

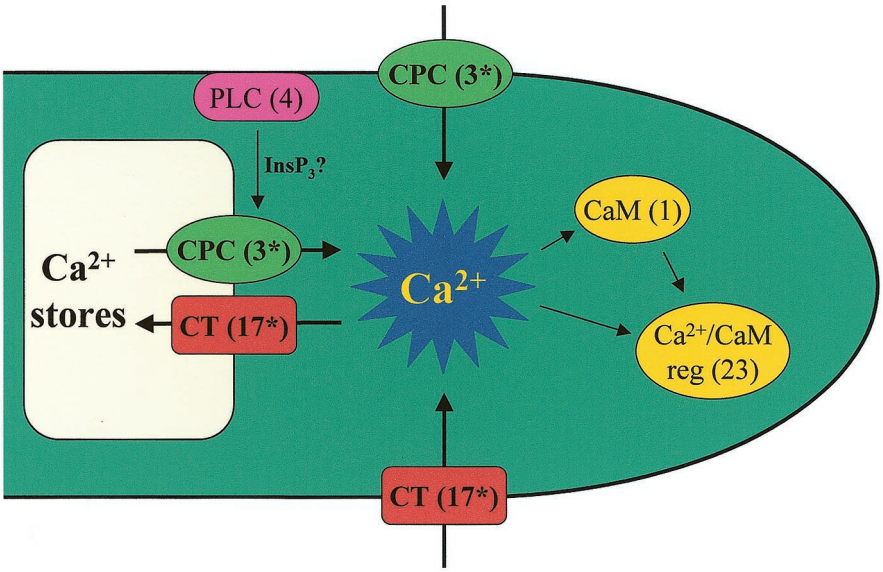

FIG. 13. Calcium signaling proteins in *Neurospora*. The numbers of each gene in a particular class are in parentheses. An asterisk indicates that the location in the plasma membrane and/or intracellular calcium store membrane has not been determined. CPC, Ca^{2+}-permeable channel; CT, Ca^{2+}-transporter (Ca^{2+}-ATPases, cation-ATPases, Ca^{2+}/H^{+} exchangers, and Ca^{2+}/Na^{+} exchangers); CaM, calmodulin; reg, regulated.

stress, and cell integrity pathways in *S. pombe* and *S. cerevisiae* (reviewed in reference 625). Two of the MAPKs and one MAPKKK had been previously identified in *Neurospora*. The MAK-2 MAPK is related to the class of proteins represented by *S. cerevisiae* Fus3p and Kss1p, required for the pheromone response and filamentous/invasive growth pathways, respectively (494). Mutations in *mak-2* result in inappropriate conidiation, female sterility, and loss of hyphal fusion (D. J. Ebbole and N. L. Glass, unpublished data). The OS-2 MAPK, related to the MAPK Hog1p from *S. cerevisiae*, regulates osmosensitivity and resistance to phenylpyrrole fungicides in *Neurospora* (897). NRC-1 is a MAPKKK that is most similar to *Pneumocystis carinii* mekk and is also related to *S. cerevisiae* Ste11p, which participates in multiple MAPK pathways. Similar to *mak*-2 mutants, *nrc-1* mutants show inappropriate conidiation, female sterility, and inability to undergo hyphal fusion (431); N. L. Glass, unpublished data). The *Neurospora* genome also contains a protein similar to Sho1p, which operates upstream of the osmosensing MAPK pathway in *S. cerevisiae* (reviewed in reference 625).

The third *Neurospora* MAPK, similar to *Magnaporthe grisea* Mps1 (864) and *S. cerevisiae* Slt2p/Mpk1p (462), had not been identified prior to completion of the genome sequence. The existence of this sequence argues for the presence of a cell integrity-type MAPK pathway in *Neurospora*.

In contrast to *S. cerevisiae* and *S. pombe*, *Neurospora* does not possess additional MAPK components outside the three conserved modules found in all three species. This finding, coupled with the diversification of GPCRs and histidine kinases, suggests greater integration of signals from multiple upstream sensory proteins in *Neurospora* than in yeasts. Alternatively, these nine basic parts may be used to create multiple MAPK modules, leading to even more signaling diversity and cross talk than observed in yeasts. The latter hypothesis is consistent with the observation that loss of proteins similar to those involved in yeast pheromone response/filamentation (MAK-2 and NRC-1) leads to multiple phenotypic defects in *Neurospora*.

Calcium signaling. Calcium plays a central role as an intracellular signal in eukaryotic cells (70, 676), yet little is known about Ca^{2+} signaling in filamentous fungi compared to animals and plants. Much evidence, particularly from pharmacological studies, has indicated that Ca^{2+} signaling is involved in regulating numerous processes in filamentous fungi, including secretion, cytoskeletal organization, hyphal tip growth, hyphal branching, sporulation, infection structure differentiation, and circadian clocks (267, 723). However, information about the main proteins involved in any one Ca^{2+}-mediated signal response pathway is lacking for *Neurospora* or, indeed, any other filamentous fungus. Initial analysis of the *Neurospora* genome indicated that more than 25 proteins are likely to be involved in Ca^{2+} signaling (269). This has now been updated with the identification of 22 additional Ca^{2+}-signaling proteins in *Neurospora* (Table 47) (http://fungalcell.org/fdf/). These results indicate that *Neurospora* possesses a complex Ca^{2+}-signaling machinery and that Ca^{2+} signaling is a significant component of its signal transduction network (Fig. 13).

Significantly, more Ca^{2+}-signaling proteins are present in *Neurospora* than in *S. cerevisiae* (Table 47) (http://fungalcell.org/fdf; M. Bencina, B. J. Bowman, O. Yarden, and N. D. Read, unpublished data). Three new Ca^{2+} channel proteins, which have close homologues to the three Ca^{2+} channel proteins (Mid1p, Cch1p, and Yvc1p [238, 545, 583]) in *S. cerevisiae*, have been identified in *Neurospora*. Eight P-type Ca^{2+}-ATPases have been identified in *Neurospora*, of which four are new (64). All of these have close homologues to the four P-type Ca^{2+}-ATPases in *S. cerevisiae* (135). Another P-type cation-ATPase has also been identified in *Neurospora*. It is homologous to an *S. cerevisiae* hypothetical ORF of unknown function. Six recognizable Ca^{2+}/H^{+} exchangers are present in

TABLE 47. Calcium signaling proteins in *Neurospora*

NCU no.	Name	Type of protein	BLAST match				
			Best overall	*S. cerevisiae*	*S. pombe*	Animal	Plant
02762.1		Ca^{2+}-permeable channel	0; *Aspergillus nidulans* (AAL37946)	0.0	0.0	0.0	None
06703.1		Ca^{2+}-permeable channel	*A. nidulans* (Mid1)	9e-27	1e-48	None	None
07605.1		Ca^{2+}-permeable channel	6e-77; *Saccharomyces cerevisiae* (Yvc1p)	6e-77	None	None	None
03305.1	NCA1	Ca^{2+}-ATPase	0; *Caenorabditis elegans* (SCA-1)	None	None	0.0	0.0
04736.1	NCA2	Ca^{2+}-ATPase	0; *Schizosaccharomyces pombe* (NP_593890)	0.0	0.0	e-120	e-155
05154.1	NCA3	Ca^{2+}-ATPase	0; *S. pombe* (SPAPB2B4)	0.0	0.0	e-170	e-165
03292.1	PMR1	Ca^{2+}-ATPase	0; *Aspergillus niger* (PmrA)	0.0	0.0	0.0	e-105
08147.1	PH-7	Ca^{2+}-ATPase	0; *S. pombe* (SPBC839)	0.0	0.0	3e-98	1e-94
04898.1		Ca^{2+}-ATPase	0; *S. cerevisiae* (SCE9871)	0.0	0.0	0.0	0.0
03818.1		Ca^{2+}-ATPase	0; *S. cerevisiae* (Neo1p)	0.0	0.0	0.0	0.0
07966.1		Cation-ATPase	0; *S. pombe* (Cta3p)	0.0	0.0	e-100	e-102
01437.1		Cation-ATPase	0; *S. pombe* (NP_587882)	0.0	0.0	0.0	9e-77
07075.1	CAX	Ca^{2+}/H^{+} exchanger	1e-80; *S. cerevisiae* (Mnr1p)	1e-80	2e-73	None	2e-58
00916.1		Ca^{2+}/H^{+} exchanger	1e-43; *S. cerevisiae* (Vex1p)	1e-43	8e-41	None	1e-32
00795.1		Ca^{2+}/H^{+} exchanger	3e-43; *S. cerevisiae* (Vex1p)	3e-43	1e-38	None	4e-30
06366.1		Ca^{2+}/H^{+} exchanger	1e-43; *S. cerevisiae* (Mnrp)	1e-43	7e-41	None	6e-43
07711.1		Ca^{2+}/H^{+} exchanger	2e-64; *S. cerevisiae* (Hum1p)	2e-64	2e-36	None	3e-50
05360.1		Ca^{2+}/H^{+} exchanger	e-128; *S. cerevisiae* (Rpd3p)	e-128	4e-80	None	2e-11
02826.1		Ca^{2+}/Na^{+} exchanger	2e-23; *S. pombe* (SPAC3A12)	9e-08	2e-23	2e-16	3e-12
08490.1		Ca^{2+}/Na^{+} exchanger	6e-07; *Anopheles gambiae* (EAA01911)	None	None	6e-05	None
01266.1		Phospholipase C	0; *Magnaporthe grisea* (MPLC1)	5e-87	1e-61	1e-64	5e-28
06245.1		Phospholipase C	6e-53; *Botryotinia fuckeliana* (BCPLC1)	1e-47	1e-47	1e-43	2e-23
09655.1		Phospholipase C	2e-38; *S. cerevisiae* (YSCL9606)	2e-38	3e-24	6e-26	3e-22
02175.1		Phospholipase C	1e-30; *S. cerevisiae* (Plc1p)	1e-30	1e-30	7e-28	2e-13
04120.1	CaM[a]	Calmodulin	3e-52; synthetic construct (CAD79597)	4.8e-36	1.1e-45	8e-47	8e-57
05225.1		Ca^{2+} and/or CaM binding protein	2e-90; *Solanum tuberosum* (CAB52797)	1e-79	1e-48	None	2e-90
02115.1		Ca^{2+} and/or CaM binding protein	2e-7; *Homo sapiens* (HERC2)	None	3.2e-05	2e-7	7e-5
01564.1		Ca^{2+} and/or CaM binding protein	8e-73; *S. cerevisiae* (YNL083W)	8e-73	2.5e-55	9e-38	3e-45
03804.1	CNA-1	Calcineurin catalytic subunit	0; *A. nidulans* (cna^{+})	e-165	0.0	0.0	0.0
03833.1	CNB-1	Calcineurin regulatory subunit	1e-73; *B. cinerea* (CNS018VZ)	4e-49	7.9e-52	3e-47	5e-18
09265.1		Calnexin	e-168; *A. niger* (ClxA)	2e-40	e-100	9e-86	7e-76
06948.1		Ca^{2+} and/or CaM binding protein	2e-40; synthetic construct (AF084415)	1.3e-35	9.8e-38	None	1e-41
04379.1		Ca^{2+} and/or CaM binding protein	5e-91; *M. grisea* (MgNCS1)	1e-59	2e-66	2e-64	2e-13
02738.1		Ca^{2+} and/or CaM binding protein	1e-37; *Mus musculus* (AK008610)	4e-20	5.4e-9	1e-37	2e-26
09871.1		Ca^{2+} and/or CaM binding protein	9e-27; *B. cinerea* (CNS01CAX)	3e-24	9.5e-24	3e-23	5e-21
01241.1		Ca^{2+} and/or CaM binding protein	e-133; *H. sapiens* (SLC25A12)	e-100	5.3e-32	e-133	4e-29
06347.1		Ca^{2+} and/or CaM binding protein	e-125; *A. nidulans* (SagA)	3e-43	5e-41	7e-23	None
06617.1		Ca^{2+} and/or CaM binding protein	5e-68; *B. cinerea* (CNS019V4)	3.6e-24	3e-32	2e-27	1e-27
03750.1		Ca^{2+} and/or CaM binding protein	2e-53; *B. cinerea* (CNS01AXV)	1.8e-11	2.5e-13	3e-5	2e-05
08980.1	NDE-1	Ca^{2+} and/or CaM binding protein	e-174; *Yarrowia lipolytica* (NDH2)	e-124	e-134	None	5e-92
02283.1		Ca^{2+} and/or CaM binding protein	0; *Glomerella cingulata* (cmk)	9e-61	e-105	7e-67	3e-52
09123.1		Ca^{2+} and/or CaM binding protein	e-158; *A. nidulans* (CmkA)	2e-94	1.4e-63	4e-68	3e-49
02814.1		Ca^{2+} and/or CaM binding protein	6e-71; *S. cerevisae* (SCYDL101C)	6e-71	3e-53	2e-70	1e-50
09212.1		Ca^{2+} and/or CaM binding protein	e-134; *S. pombe* 9SPCC13220	7e-97	e-134	1e-57	1e-42
06650.1		Ca^{2+} and/or CaM binding protein	1e-32; *Helicosporium* sp. (BAB70714)	None	None	None	None
02411.1		Ca^{2+} and/or CaM binding protein	0; *Podospora anserina* (CAD60740)	7.4e-20	1.9e-23	1e-13	2e-25
06177.1		Ca^{2+} and/or CaM binding protein	e-112; *A. nidulans* Ca^{2+}/CaM-dependent protein kinase C (AAD38851)	4.6e-42	1.5e-55	2e-67	4e-50
04265.1		Ca^{2+} and/or CaM binding protein	3e-71; *Bacillus megaterium* β-fructosidase (FruA)	1.3e-35	1e-36	None	1e-33

[a] CaM, calmodulin.

Neurospora, of which only one (CAX [501]) was previously known. With one exception, all of these proteins have the same homologue in *S. cerevisiae* (Vax1p/Hum1p [532]). The exception is homologous to a hypothetical ORF of unknown function in *S. cerevisiae*. Our analysis also revealed two novel putative Ca^{2+}/Na^{+} exchangers in both *Neurospora* and *S. cerevisiae*. It is interesting that animals possess Ca^{2+}/Na^{+} exchangers but not Ca^{2+}/H^{+} exchangers, plants contain Ca^{2+}/H^{+} exchangers but not Ca^{2+}/Na^{+} exchangers, while fungi possess both. As with *S. cerevisiae*, only one calmodulin is present in *Neurospora* (125, 518).

Calcium signaling in animal and plant cells normally involves Ca^{2+} release from internal stores (71, 676). This is commonly mediated by the second messengers inositol-1,4,5-trisphosphate ($InsP_3$) or cADP ribose or by Ca^{2+}-induced Ca^{2+} release, although other second messengers (e.g., sphingolipids and NAADP) also exist (95).

Four novel phospholipase C-δ subtype (PLC-δ) proteins (which synthesize $InsP_3$) have been identified in *Neurospora*, in contrast to one in *S. cerevisiae* (22). Furthermore, $InsP_3$ is present within *Neurospora* hyphae (447), and physiological evidence suggests that it plays a role in Ca^{2+} signaling (170, 737, 738). In spite of this, *Neurospora* (and *S. cerevisiae*) lack recognizable $InsP_3$ receptors. Whether $InsP_3$ signaling in *Neurospora* involves $InsP_3$ receptors that differ from those found in animal cells remains to be determined. Neither ADP-ribosyl cyclase (which synthesizes cADP ribose or NAADP) nor ryanodine receptor proteins (which are also key components of Ca^{2+} release mechanisms in animal cells) are recognizable in *Neurospora*. Furthermore, no homologues of either sphingo-

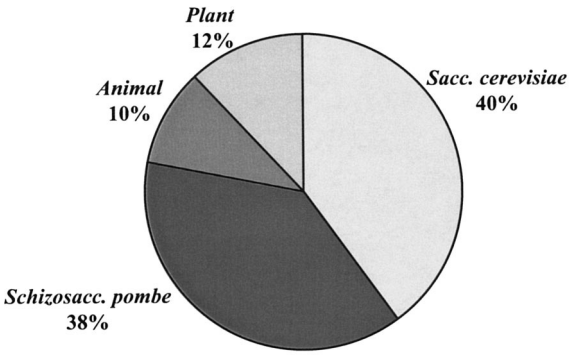

FIG. 14. Calcium signaling proteins. The percentage of *Neurospora* proteins with the greatest homology to proteins in *S. cerevisiae*, *S. pombe*, animals, and plants is shown.

sine kinase, which synthesizes the second-messenger sphingosine-1-phosphate, or the sarcoplasmic reticulum Ca^{2+} release channel, SCaMPER, which is a possible target of sphingolipids (70), could be indentified in this fungus. These observations raise the intriguing question of whether other, perhaps novel second-messenger systems responsible for Ca^{2+} release from internal stores remain to be discovered in filamentous fungi.

The reason for the diversification of PLC- isoforms is unclear. Besides $InsP_3$, PLC also synthesizes diacylglycerol, a second messenger which regulates protein kinase C (PKC). Two PKCs have been identified in *Neurospora*, but neither possesses a C2 domain with Ca^{2+} binding sites (689).

Another Ca^{2+}-signaling protein which has not been identified in the *Neurospora* genome is the external calcium-sensing receptor described in animal cells (109).

In terms of downstream elements involved in Ca^{2+} signaling, 23 Ca^{2+}/calmodulin-regulated proteins have been found. Very few of these proteins have been analyzed with respect to the biological responses that they regulate in *Neurospora*. One of these proteins is the Ca^{2+}/calmodulin-dependent Ser/Thr phosphatase calcineurin, which is composed of a catalytic subunit, calcineurin A (CNA), and a regulatory subunit, calcineurin B (CNB) (337, 430). One function of *Neurospora* calcineurin is regulation of hyphal tip growth and branching (631). Another downstream element is a Ca^{2+}/calmodulin-dependent protein kinase (CAMK-1) that is involved in regulating growth and the circadian clock (875). A third downstream element is a Ca^{2+}-dependent neutral trehalase (TREB), which is responsible for trehalose mobilization at the onset of conidial germination (188).

The *Neurospora* Ca^{2+}-signaling proteins (Table 47) showed a markedly greater homology to Ca^{2+}-signaling proteins in both *S. cerevisiae* and *S. pombe* than to those in animals or plants (Fig. 14). In comparison with just animals and plants, 58% of the *Neurospora* proteins exhibited greater similarity to animal homologues whereas 42% showed higher homology to plant proteins.

Protein phosphatases. Protein phosphorylation and dephosphorylation are essential elements of signal transduction pathways in eukaryotic cells. Protein phosphatases (PPs) are classified as Ser/Thr, Tyr, dual-specificity, or His PPs on the basis of substrate specificity. More recently, a two-family gene classification has been proposed (164, 202). The two gene families, designated PPP and PPM, are defined by distinct amino acid sequences and three-dimensional atomic structures. The PPP family includes the signature phosphatases PP1, PP2A, PP2B, and PP5 (also known as PPT), while the PPM family comprises the Mg^{2+}-dependent protein phosphatases, which include PP2C and pyruvate-dehydrogenase phosphatase.

PP activity in *Neurospora* was first reported by Tellez de Inon and Torres (787), and more recently, the biochemical and genetic analyses of *Neurospora* phosphatases (337, 430, 631, 766, 767, 881–884, 893, 896) has been complemented, in part, with the isolation of several phosphatase-encoding genes and their functional analysis (56, 768, 885) (Table 48). Within the PP1 and PP2A subfamilies, several novel PPs have been identified that show less than 65% sequence identity to PP1 and PP2A. These novel-type phosphatases also possess properties indicating that they perform cellular functions distinct from PP1 and PP2A (164) and are also present in *Neurospora* (Table 48). In addition to the structural features of the catalytic subunits, it is important not to underestimate the involvement of PP regulatory subunits in phosphatase specificity and function (883). Stark (754) has listed some 25 *S. cerevisiae* proteins associated with phosphatase function and suggests that there are still considerably more PP regulatory polypeptides to be identified. Although in some instances, structural conservation between such polypeptides in various organisms may assist in identifying some of these polypeptides in filamentous fungi (196), the structural diversity of PP regulatory proteins is likely to be much greater than that of the catalytic counterparts. In accordance, only a few of the more highly conserved regulatory PP subunits have likely structural homologues in *Neurospora*, while the presence of proteins considered to be associated with PPs in other organisms cannot readily be detected on the basis of structural similarity searches.

Mammalian signaling proteins not found in *Neurospora*. Although the SH2 domain (reviewed in reference 821) is present in some proteins, *Neurospora* does not contain recognizable tyrosine kinases, including c-Src (reviewed in references 308 and 821) and nuclear hormone receptors (reviewed in reference 645). In addition, *Neurospora* lacks proteins with scores less than 2e-10 to the SH2- or SH3-containing Grb2, Shc, and Sck (556) tyrosine kinase adaptor proteins. Raf kinases (reviewed in reference 614) are apparently absent from the genome, as are β-arrestin homologues (reviewed in reference 308). There are no good matches to the *S. cerevisiae* Ste5p MAPK scaffold (218) or to mammalian scaffold proteins, such as MP-1 (683) or JIP-1 (879). There is no GTPase protein homologous to Gh (558), which regulates PLC-δ activity in mammals.

Photobiology and Circadian Rhythms

Neurospora is equipped to respond to, and anticipate predictible changes in, a variety of environmental stimuli. Aside from the obvious role of nutrition, the most prominent and pertinent environmental factors are ambient light and temperature, both of which affect the circadian system of the organism. Acutely, light is known to affect the expression of a large number of genes influencing carotenogenesis, conidiation,

TABLE 48. *N. crassa* Ser/Thr phosphoprotein phosphatas

Enzyme	NCU no.	BLAST match				
		Best overall	*S. cerevisiae*	*S. pombe*	Animal[a]	Plant[b]
PPP1: type 1 serine/threonine-specific protein phosphatase	00043.1	*A. nidulans* BimG/e-178	e-166	e-171	e-161; *M. musculus*	e-148
PPH1: type 2A serine/threonine-specific protein phosphatase, catalytic subunit	06630.1	*H. sapiens* P2AA/e-142	e-138	e-141	e-149; *H. sapiens*	e-142
CNA: serine/threonine protein phosphatase 2B catalytic subunit (calmodulin-dependent calcineurin A subunit)	03804.1	*A. nidulans* CnaA/0.00	e-167	0.00	0.00; *M. musculus*	9e-61
Protein phosphatase 2C	00434.1	*S. pombe* p2c1/4e-70	2e-65	4e-70	2e-22; *H. sapiens*	7e-19
PPE-like serine/threonine-specific protein phosphatase	03436.1	*S. pombe* ppe1/e-112	e-108	e-112	e-101; *H. sapiens*	e-91
PPG-like serine/threonine-specific protein phosphatase	06563.1	*S. pombe* YD44/e-120	e-110	e-120	2e-95; *H. sapiens*	4e-97
PPT (type 5) serine/threonine-specific protein phosphatase	01433.1	*R. norvegicus* PPP5/e-136	e-111	e-55	e-136; *M. musculus*	5e-57
Protein phosphatase X	08301.1	*H. sapiens* PPP4/e-41	4e-38	2e-38	e-41; *H. sapiens*	e-41
PZL1: type Z serine/threonine protein phosphatase	07489.1	*S. cerevisiae* Ppz2p/e-141	e-141	e-116	e-120; *H. sapiens*	e-119
Pyruvate dehydrogenase phosphatase, catalytic subunit 1 and similar to type 2C protein phosphatase	01767.1	*H. sapiens* PDP1/3e-44	2e-12	5e-13	3e-44	2e-9
RGB1: type 2A phosphatase regulatory B subunit	09377.1	*S. pombe* 2aba/e-178	e-149	e-178	e-132; *M. musculus*	e-141
Putative type 2A protein phosphatase B56 regulatory subunit	03786.1	*S. pombe* e-150	e-148	e-150	e-142; *H. sapiens*	e-114
Putative Type 2A phosphatase-associated protein	08268.1	*S. pombe* putative protein/4e-39	5e-28	4e-39	2e-21; *H. sapiens*	e-24
CNB1: calcineurin regulatory subunit	03833.1	*S. pombe*, probably calcineurin B subunit/5e-54	e-51	5e-54	3e-47	5e-18
Putative phosphatase-associated protein	10018.1	*S. cerevisiae* Sit4p-associated protein/3e-82	3e-82	2e-42	4e-35; *H. sapiens*	2e-21
Putative phosphatase-interacting protein	08779.1	*S. cerevisiae* Gip2p/2e-10	2e-10	6e-9	6e-8; *H. sapiens*	No hits

[a] *Caenorhabditis elegans*, *Drosophila melanogaster*, *Mus musculus*, or *Homo sapiens*.
[b] *Arabidopsis thaliana*.

perithecial neck bending, and directionality in ascospore shooting (184, 477). A major, but less immediate, effect of light derives from its central role in phasing the circadian clock, which, even in the absence of light, can influence the expression of several percent of the genome. In published work on *Neurospora* light sensing extending back over 100 years (38, 184), mention is made only of its ability to sense blue light; no red-light responses are known. An unexpected aspect of the *Neurospora* genome is the presence of novel genes whose products reflect environmental sensing of light stimuli.

Perhaps largely reflecting the fact that circadian rhythmicity has been intensively studied in *Neurospora* for the past 15 to 20 years (61, 206, 484), there were no notable additions to the list of proteins involved in the core regulatory feedback loop. Known central clock components (Fig. 15; Table 49) that function in the core circadian oscillator in continuous darkness include FRQ (29, 30), WC-1 and WC-2 (173), and the kinases that act on them in a circadian context, including casein kinases 1 (291) and 2 (874) and calmodulin-regulated kinase (875). It is perhaps interesting that WC-1 retains the dark function as a positive element in the circadian feedback loop, similar to its mammalian counterpart BMAL1, but also has acquired a light-based activity as the blue light photoreceptor, as described below. There were no unidentified duplicates of any core clock genes found, nor were there sequence homologues to the *Drosophila* or vertebrate *period* or *CLOCK* genes, although a gene similar to *Drosophila vrille* is found; the strong sequence homology between *Neurospora* WC-1 and the vertebrate clock protein BMAL1 has already been noted (461). Likewise, the PAS protein VVD, which acts to link circadian output with light input/output, was already identified (328, 697, 733). In *Drosophila*, the *shaggy* gene, encoding a GSK-3 kinase homologue, plays an important role in regulating nuclear movements of the key negative element TIM (503). *Neurospora* does contain a GSK-3 homologue, whose significance in the circadian feedback loops has yet to be appraised.

In spite of intensive study for two decades (478), and in contrast to the analysis of clock components, the genome sequence revealed a surprising number of homologues to novel light-sensing genes (Fig. 16; Table 49). DNA photolyase (see "Genome defense, DNA repair, and recombination" above), previously characterized as a photoresponsive molecule, plays no role in light responses beyond DNA repair (730). Known elements in light sensing include the circadian blue-light photoreceptor WC-1 (42, 256, 323), WC-2 (478), and VVD (328, 698); however, no homologues of plant photoresponse proteins such as phytochromes or cryptochromes have ever been identified, despite intense genetic screening. Nonetheless, they are present, including clear phytochrome and cryptochrome homologues and a gene whose product, like VVD, contains a single PAS/LOV-type domain of the type associated with light sensing. There is also a homologue of the *Aspergillus velvet* gene, implicated in regulation of both red- and blue-light responses (537).

Perhaps the biggest surprise to emerge from the genome analysis is the appearance of a cryptochrome, which had pre-

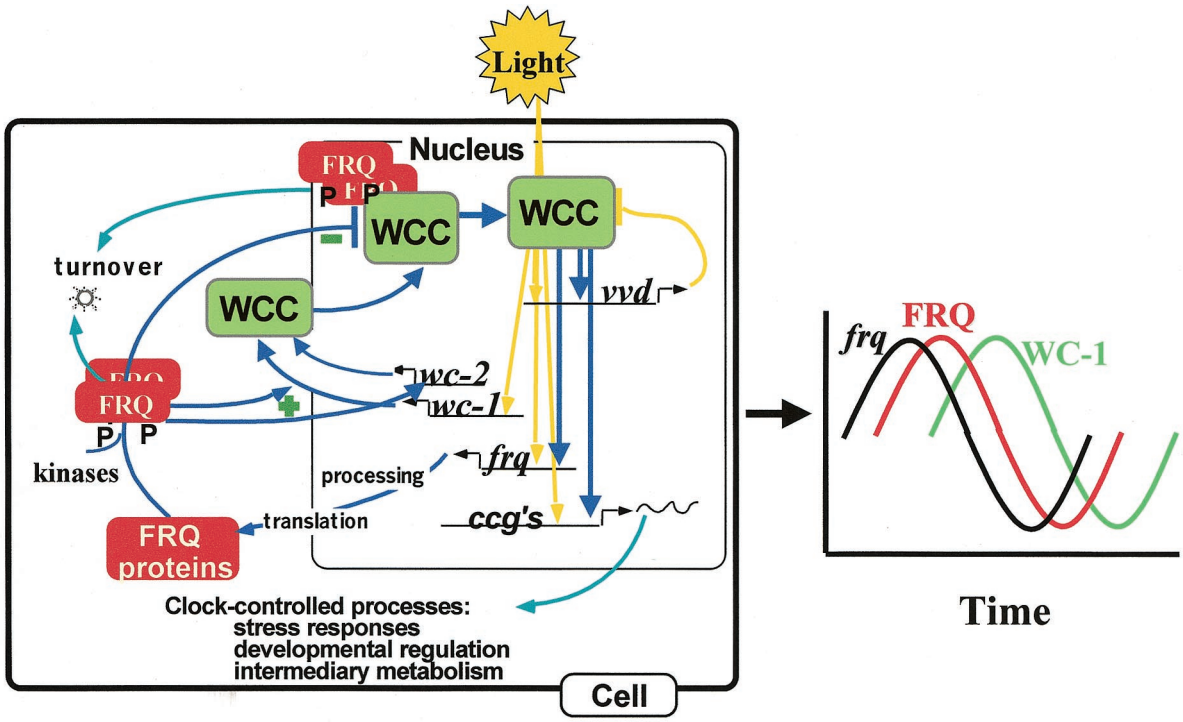

FIG. 15. Known molecular components in the coupled feedback loops of the *Neurospora* circadian system. The WC-1 and WC-2 proteins form a White Collar Complex (WCC) that activates *frq* gene expression and also *clock-controlled gene* (*ccg*, output) and *vvd* expression in the dark. The WCC also mediates light-induced transcription from *frq*, *ccg* genes, *vvd*, and *wc-1* (gold arrows). VVD expression is strongly light induced, and VVD in turn is a photoreceptor that mediates light adaptation responses, transiently turning down the WCC activity. In the circadian cycle in the dark, *frq* mRNA is translated to make FRQ proteins which dimerize and play two roles: (i) FRQ feeds back into the nucleus to rapidly block the activity of the WCC in driving *frq* transcription, and (ii) FRQ acts to promote the synthesis of new WC-1 and *wc-2* mRNA, thus making more WCC, which is held inactive by FRQ. Phosphorylation of FRQ by several kinases, including casein kinases 1 and 2 and CAMK-1, triggers its turnover mediated by an interaction with the ubiquitin ligase encoded by *fwd-1;* the kinetics of phosphorylation-mediated turnover is a major determinant of period length in the clock. When FRQ is degraded in the proteasome, the pool of WCC is released to reinitiate the cycle. See the text for details. Adapted from reference 461

viously been extensively sought by other molecular methods, along with two phytochromes. At first glance, the appearance of two phytochromes is unexpected, given that these molecules in plants are chiefly known in the context of red/far-red sensing, and to date, no red-light photobiology has been described in *Neurospora*. However, since *Arabidopsis* phytochromes have recently been shown to associate with cryptochromes and to play a role in blue-light sensing and signaling (194), this aspect of photobiology would be a likely candidate for the *Neurospora* phytochromes. The same might be said for the *velvet* homologue.

The *Neurospora* genome contains nearly a dozen proteins containing PAS/PAC domains (compared to four in *S. cerevisiae* and five in *S. pombe*). PAS and PAC proteins as a class are associated with light, oxygen, and voltage sensing and are often associated with protein-protein interaction domains, such as those in the heterodimers that act as positive elements in fungal and vertebrate circadian systems (783). In some cases, the PAS domains are juxtaposed to HK domains. These domain associations are characteristic of bacterial and plant two-component regulatory systems and have been associated with light responses in the cyanobacterium *Synechococcus* (385) (see also Two-component regulatory systems above).

Heat Shock and Stress Responses

All classes of the major heat shock-induced proteins are encoded in the genome of *Neurospora* (Table 50). These stress proteins, which are ubiquitously and abundantly synthesized in response to supraoptimal temperature and other protein-denaturing stresses, function as chaperones that guide and stabilize the conformation of other proteins. Most heat shock proteins (Hsps) are also synthesized constitutively, and many are essential.

DnaK/Hsp70, the first Hsp to be characterized, is strongly conserved among many organisms. It has an amino-terminal ATPase domain and a carboxyl-terminal peptide binding domain. The release of peptides by Hsp70, allowing them to refold, is linked to ATP hydrolysis (278). Homologues of the chief cytosolic classes of Hsp70 (*S. cerevisiae* nomenclature) are present in *Neurospora*: the major heat-induced Ssap, which was previously sequenced in *Neurospora* (400), the cold-inducible Ssbp, and Ssz1p. Whereas *S. cerevisiae* has multiple Ssap and Ssbp proteins, *Neurospora* has only one of each. Ssap is required for protein translocation into mitochondria and the ER (191), and each of the three cytosolic Hsp70s contributes to mRNA translation (350, 356). *Neurospora* also has organelle-localized Hsp70s that assist in protein translocation

TABLE 49. Known and predicted *N. crassa* proteins associated with circadian clocks and photobiology

Protein class	*Neurospora* protein	Function	NCU no.	BLAST match				
				Best overall	*S. cerevisiae*	*S. pombe*	Animal[a]	Plant[b]
Clock-associated genes	FRQ	*N. crassa* central clock component	02265.1	*Sordaria fimicola*; e 0.0	None	None	None	None
	WC-1	*N. crassa* blue-light photoreceptor, central oscillator component, 2 PAS domains, 1 LOV domain, GATA zinc finger domain	02356.1	*Podospora anserina*; e 0.0	Ymr1369; e-06	SPCC1902.01; 5e-05	*Drosophila*; e-14	NP851210.1; 4e-18
	WC-2	Forms complexes with WC-1, central oscillator component and blue-light signaling, 1 PAS domain, GATA zinc finger domain	00902.1	*Fusarium solani*; e-138	Ymr136W; 6e-08	None	None	AC026875.23; 3e-07
	CKA	*N. crassa* casein kinase II catalytic subunit involved in FRQ	03124.1	*Candida albicans*; e-148	YorR061W CKA2; e-106	SPAC23C11 CKII; e-130	P28020; e-105	AF370308; e-112
	CKB1	*N. crassa* casein kinase II regulatory subunit involved in FRQ phosphorylation	05485.1	*S. pombe*; 4e-61	Yg109W CKB1; e-53	SPBC2G5.02C CKII; 4e-61	P28021; 3e-46	AF239816; 4e-41
	CKB2	*N. crassa* casein kinase II regulatory subunit involved in FRQ phosphorylation	02754.1	*S. pombe*; 2e-77	Y1r039C RIC1; 8e-4	SPAC1851.04e; 2e-77	XP317865.1; 2e-60	NP181996.1; 1e-58
	HHP1	*N. crassa* casein kinase I involved in FRQ phosphorylation	00685.1	*S. pombe*; e-150	NP015120.1; e-125	NP595760.1; e-150	BAB0347 3.1; e-135	BAB92346.1; e-130
	CAMK	*N. crassa* protein kinase involved in FRQ phosphorylation	09123.1	*Aspergillus nidulans*; 1e-165	Yoi016C CMK2; e-99	SPACUNK12.02c; 4e-67	NP065130.1; 2e-69	NP192383.1; 8e-49
	SHAGGY/GSK-3 homologue	*Drosophila* protein kinase Shaggy involved in phosphorylation and nuclear movement of the central oscillator component TIM	04185.1	*Colletotrichum gloeosporioides*; e-157	Ymi139W Rim11; e-104	SPAC1687.15; e-148	NP571465; e-130	AF43225.1; e-119
	FWD-1	E3 ubiquitin ligase involed in targeting FRQ for proteasomal turnover	04540.1	*Homo sapiens*; e-47	None	SPAC30.05; e-40	*Homo sapiens*; e-47	None
Other light-sensing genes	VVD	*N. crassa* blue-light sensing, PAS/LOV domain	03967.1	*Aspergillus nidulans*; 2e-41	None	None	None	BAA84780.1; 3e-19
	PHY-1	Putative phytochrome red/far-red-light sensing	04834.1	*Pseudomonas putida*; 8e-61	Ylu206W; 3e-09	SPAC27E2.09; 2e-27	None	P55004; 4e-33
	PHY-2	Putative phytochrome red/far-red-light sensing	05790.1	*Agrobacterium tumefaciens*; 2e-33	Yi1147C SLN1; 8e-9	SPAC1834.08; 2e-14	None	P55004; 2e-21
	CRY homolog	Putative blue-light sensing, central oscillator component in mammals, light entrainment of the clock in *Drosophila*	00582.1	*Trichodesmium erythraeum*; 4e-69	Yoi386w PHR1; 4e-26	None	None	NP58461.2; 2e-49
	VELVET homologue	Red- and blue-light sensing in *Aspergillus*	01731.1	*Aspergillus nidulans*; 5e-40	None	None	None	None
	NOP-1	*Neurospora* homologue of bacteriorhodopsin	10055.1	*Leptosphaeria maculans* opsin; 2e-75	Yro2p; e-08	SPCC31H12.02c; 9e-07	None	None
	ORP-1	*Neurospora* opsin-related protein, lacks conserved lysine residue	01735.1	*Coriolus versicolor* Hsp30; 5e-31	Yro2p; 8e-23	SPCC31H12.02c; 8e-12	None	None
Other PAS proteins	Hypothetical protein	4 PAS domains, sensory box histidine kinase/response regulator	03164.1	*Shewanella oneidensis*; 6e-31	Yi1147C SLN1; 2e-10	SPAC27E2.09; 1e-20	None	None
	Predicted protein	3 PAS domains	06390.1	*Caenorhabditis elegans*; e1.4	None	None	None	None
PAS/PAC domain proteins	Hypothetical protein	PAS/PAC domain 1e-88, sensory transduction histidine kinase	02057.1	*Magnetococcus* sp. strain MC-1; 4e-75	Yi1147C SLN1; 4e-18	SPAC183 4.08; 6e-57	None	None
	Hypothetical protein	PAS/PAC domain 3e-59, two-component hybrid sensor and regulator	00939.1	*Anabaena* sp. strain PCC 7120; 4e-23	Yi1147C SLN1; 6e-11	SPAC27E2.09; 2e-25	None	None
	Hypothetical protein	PAS/PAC domain 3e-63, putative two-component histidine kinase Fos-1	07221.1	*Aspergillus fumigatus*; e-161	Yi1147C SLN1; 7e-17	SPAC27E2.09; 3e-36	None	None
	Hypothetical protein	PAS/PAC domain 3e-88, related to two-component histidine kinase *chk-1*	01833.1	*Glomerella cingulata*; 0.0	Ylu206W; e-10	SPAC1834.08; 8e-79	None	None
	Hypothetical protein	PAS/PAC domain 5e-42, probable serine/threonine protein kinase, related to plant NPH-1 protein	07268.1	*Caulobacter crescentus*; 2e-07	None	None	None	T013535; e-07

[a] *Caenorhabditis elegans*, *Rattus norvegicus*, *Xenopus laevis*, *Drosophila melanogaster*, *Anopheles gambiae*, *Mus musculus*, or *Homo sapiens*.
[b] *Arabidopsis thaliana*, *Zea mays*, *Ipomoea nil*, or *Oryza sativa*.

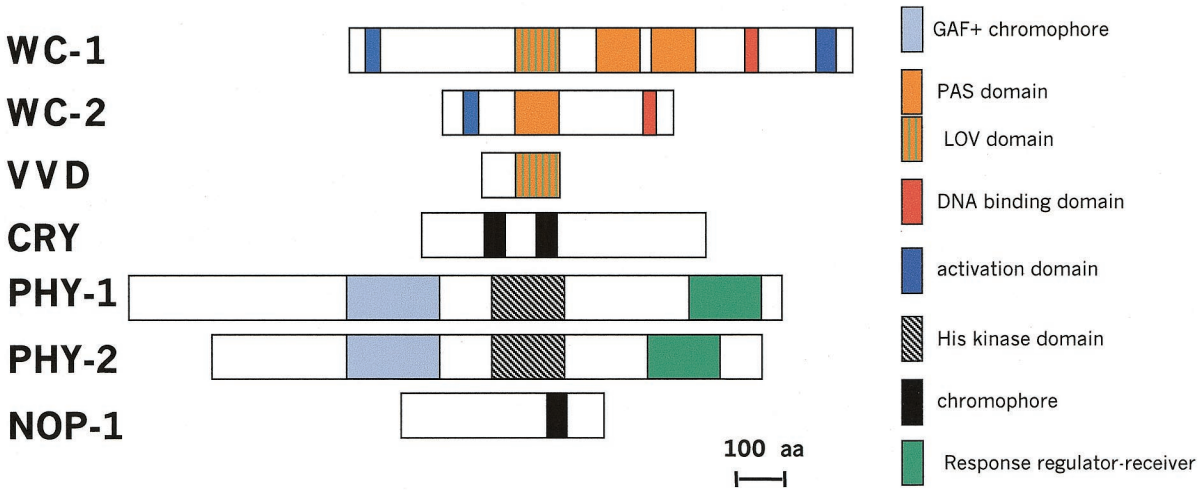

FIG. 16. Real and potential *Neurospora* photoreceptors. The approximate sizes and locations of pertinent protein functional domains are shown for this series of proteins having known or plausible roles in photobiology. WC-1 and WC-2 work together as the White Collar Complex they are known to comprise a photoreceptor that appears to be the circadian photoreceptor and a major blue light photoreceptor in *Neurospora*. VVD is also a blue light photoreceptor that is responsible for modulating the WCC and contributing to photoadaptation. NOP-1 binds retinal and undergoes a photocycle, but the associated photobiology has not been elucidated (see also Fig. 12). Likewise, CRY, PHY-1, and PHY-2 all show strong sequence homology to known photoreceptors from other organisms (Table 49; Fig. 11) but do not yet have any demonstrated role in photobiology. aa, amino acids.

and protein folding within the organelle: Ssc1 in mitochondria (763) and Kar2 (663) and Lhs1 (681) in the ER. *Neurospora* has five other Hsp70-related proteins that do not have known counterparts in fungi and plants but whose closest homologues are a group of related proteins in vertebrates. The mitochondrial Ssq1p of *S. cerevisiae* (763) is absent from *Neurospora*.

Hsp110 of mammals is a distant relative of Hsp70 that is strongly induced by heat shock (463). Its homologue in *Neurospora*, HSP88, was identified by its ability to bind to the major small Hsp (sHsp [see below]) of *Neurospora* (619), HSP30, an affinity also described for HSP110 and the mammalian sHsp (844), suggesting that these two proteins may be cochaperones. The homologous Sse proteins of *S. cerevisiae* are reported to interact physically and functionally with Hsp90 (480).

Hsp90 is a major, conserved heat shock-induced protein that has important functions in signal transduction during normal growth by directly regulating protein kinases and transcription factors. Hsp90 was shown to negatively regulate cell cycle progression in *S. pombe* by its interaction with the Wee1 tyrosine kinase (15). cDNA for the single Hsp90 gene of *Neurospora* was previously sequenced (666), and, like *S. cerevisiae, Neurospora* lacks Grp94, the ER-localized Hsp90 of animal cells. Hsp90 has an amino-terminal ATPase domain to which the inhibitor geldanamycin binds, thereby blocking Hsp90 homodimerization and binding of the p23 cochaperone (630). p23, which couples ATPase activity with substrate release by Hsp90 (890), is present in *Neurospora* but is poorly conserved across species. *Neurospora* also has a homologue of Aha1, which binds to the middle region of Hsp90 and enhances its ATPase activity (486). Assembly of Hsp70 and Hsp90 into a superchaperone folding complex is mediated by the cochaperone Hop/Sti1, which is an adaptor protein that interacts with both chaperones through separate tetratricopeptide repeat (TPR) domains (2). Sti1 is strongly conserved in *Neurospora*. A less strongly conserved homologue of Sti1, Cns1, which binds to Hsp90 (201), is also present in the *Neurospora* genome.

Protein folding by mitochondrial GroEL/Hsp60 has been carefully analyzed. It has been shown that the *Neurospora* HSP60, like other Hsp60s, assembles into two stacked rings of seven monomers each (358). Unfolded proteins bind at hydrophobic apical domains of the Hsp60 structure, refold in the more hydrophilic interior, and are released as ATP is hydrolyzed (865). Its cochaperone GroES/Hsp10 (488), present in *Neurospora*, is moderately conserved across species.

Although a Clp-related Hsp was initially ignored, due to its absence from *D. melanogaster*, where the heat shock response was first characterized, subsequent experiments with *S. cerevisiae* showed that Clp/Hsp104p is important for the acquisition of induced thermotolerance (675). Hsp104p is an ATP-dependent chaperone that, when assembled into hexamers, possesses the unique ability to disentangle protein aggregates after they have formed rather than merely preventing their formation (601). Vertebrates do have a moderately conserved counterpart to Hsp104p (609), and it is strongly conserved in *Neurospora*, as is the mitochondrially localized Clp, Hsp78, which helps turn over unassembled mitochondrial proteins in *S. cerevisiae* (468).

A relatively nonconserved but ubiquitous group of Hsps share a conserved region with α-crystallin of the vertebrate eye lens (363). These small Hsps (sHsps) assemble into large oligomeric particles that function as non-ATP-dependent chaperones and are thought to be the first line of defense against stress (212). The sHsps are nonessential proteins that are induced chiefly in response to stress and during development. There are three sHsps in the *Neurospora* genome, of which the most highly conserved, HSP30, has been characterized (621). RIP mutagenesis indicated that HSP30 helps *Neurospora* survive extended exposure to high temperature coupled with carbohydrate deprivation (620), conditions that would promote

TABLE 50. Heat shock and stress proteins

Protein family	Name/feature	NCU no.	BLAST match				
			Best overall	*S. cerevisiae*	*S. pombe*	Animal[a]	Plant[b]
Hsp70	Hsp70	09602.1	*Paracoccidiodes brasiliensis*; 0.00	0.00	0.00	0.00	0.00
	Grp78/Kar2:ER	03982.1	*Aspergillus amawori*; 0.00	0.00	0.00	0.00	0.00
	Ssb	02075.1	*Aspergillus nidulans*; 0.00	0.00	0.00	e-177	e-175
	Ssc1:Mt	08693.1	*S. cerevisiae*; 0.00	0.00	e-178	e-166	e-145
	Ssz1	00692.1	*S. cerevisiae*; e-104	e-104	e-102	8e-54	3e-49
	Lhs1:ER	09485.1	*Anopheles gambiae*; 4e-73	2e-43	3e-65	1e-64	1e-52
	KIAA0417	00573.1	*Mus musculus*; 7e-26	1e-05	2e-06	7e-26	4e-05
	KIAA0417	01499.1	*Homo sapiens*; 1e-23	1e-06	2e-04	1e-23	2e-04
	Hspa12b	04396.1	*H. sapiens*; 5e-21	5e-06	4e-04	5e-21	7e-04
	KIAA0417	09471.1	*H. sapiens*; 6e-10			6e-10	
	KIAA0417	03288.1	*M. musculus*; 7e-05			7e-05	
Hsp110	HSP88	05269.1	*S. pombe*; 0.00	e-168	0.00	e-136	e-132
Hsp90	Hsp90	04142.1	*Podospora anserina*; 0.00	0.00	0.00	0.00	
Hsp90 associated	p23/Wos2	01792.1	*S. pombe*; 8e-20	2e-16	8e-20	2e-11	1e-05
	Aha1	04087.1	*S. pombe*; 2e-71	3e-51	2e-71	6e-37	2e-19
	Hop/Sti1	00714.1	*S. pombe*; e-138	e-129	e-138	3e-95	2e-91
	Cns1	06340.1	*S. pombe*; 5e-26	7e-25	5e-26	1e-16	2e-20
Hsp60	Hsp60:Mt	01589.1	*Coriolus immitis*; 0.00	0.00	0.00	e-162	e-173
Hsp10	Hsp10:Mt	04334.1	*S. pombe*; 4e-28	6e-27	4e-28	2e-17	1e-23
Clp	Hsp104	00104.1	*P. brasiliensis*; 0.00	0.00	0.00	8e-46	0.00
	Hsp78:Mt	02630.1	*Leptosphaeria maculans*; 0.00	0.00	5e-38	0.00	
sHsp	HSP30	09364.1	*A. nidulans*; 7e-27	0.69	2e-05		4e-04
	[mito]	07232.1	*A. nidulans*; 8e-23	1.3	6e-04		2e-04
		09420.1	*A. nidulans*; 8e-17		0.20		0.41
Hsp40	Ydj1	07414.1	*S. pombe*; e-107	2e-98	3e-107	2e-85	9e-70
	Ydj1	00465.1	*S. pombe*; 93-67	3e-49	9e-67	3e-56	2e-49
	Mdj1:Mt	05196.1	*S. pombe*; 7e-79	3e-55	7e-79	8e-47	1e-60
	Zuo1	03009.1	*S. pombe*; 3e-81	3e-60	3e-81	2e-41	1e-26
	Scj1:ER	CAD7098 8.1	*S. pombe*; 1e-68	4e-60	1e-68	2e-53	3e-59
	Sis1	03732.1	*S. pombe*; 4e-61	3e-31	4e-61	2e-44	3e-56
	Sec63:ER	00169.1	*S. pombe*; 4e-61	1e-41	4e-61	3e-44	2e-27
	[TPR domains]	00170.1	*S. pombe*; 3e-80	9e-12	3e-80	2e-69	3e-37
	Mandelate racemase	07064.1	*Burkholderia fungorum*; e-150	3e-11	1e-29	e-121	5e-21
		02432.1	*P. anserina*; e-129	9e-43	1e-20	2e-40	4e-36
	Djp1	06052.1	*P. anserina*; e-169	2e-35	2e-50	1e-18	1e-26
	Hlj1:ER	03335.1	*S. pombe*; 9e-29	4e-19	9e-29	2e-19	9e-13
	[TPR domain]	02424.1	*P. anserina*; e-161	2e-11	5e-16	1e-43	2e-10
		05710.1	*Rhodospirillum rubrum*; 9e-12	2e-07	5e-07	2e-11	4e-11
		05199.1	*Salmonella enterica*; 1e-11	3e-08	8e-11	5e-11	5e-11
		04305.1	*Plasmodium falciparum*; 9e-12	1e-09	3e-08	4e-10	9e-11
		04145.1	*Chlorobium tepidum*; 3e-11	4e-07	9e-08	5e-11	6e-08
		01284.1	*P. falciparum*; 1e-10	3e-09	2e-05	2e-06	5e-08
GrpE	Mge1:Mt	01516.1	*S. cerevisiae*; 2e-41	2e-41	1e-39	3e-25	1e-23
	Fes1	04172.1	*S. cerevisiae*; 4e-17	4e-17	5e-14	0.25	2e-06
	Sls1:ER	00968.1	*Yarrowia lipolytica*; 4e-11	5e-08		4e-06	6.2
Bag-1		01221.1	*S. pombe*; 0.015		0.015	0.081	
Cyclophilin	Cyp40/Cpr6	03853.1	*M. musculus*; 1e-81	4e-76	1e-72	1e-81	2e-71
	CypC/Cyp1	00578.1	*Aspergillus niger*; 5e-58	9e-28	2e-51	8e-53	2e-51
	Cyp20/Cpr1:Mt/Cyt	00726.1	*Fusarium sporotrichioides*; 4e-70	3e-47	2e-48	9e-47	6e-44
	CypB:ER	01200.1	*Aspergillus niger*; 1e-65	2e-48	2e-60	2e-57	5e-50
	[U-snRNP-assoc]	02614.1	*Echinococcus multilocularis*; 4e-50	3e-43	8e-47	6e-49	6e-45
	KIAA0073 [WD40] [nucleus]	09819.1	*H. sapiens*; e-153	8e-27	e-134	e-153	e-142
	[U-Box]	00181.1	*Drosophila melanogaster*; 2e-83	4e-16	2e-30	2e-83	5e-39
	[nucleus]	08514.1	*Arabidopsis thaliana*; 2e-47	3e-15	2e-44	5e-45	2e-47
	Ppil4 [RNA-Bind] [nucleus]	07179.1	*S. pombe*; 6e-99	1e-10	6e-99	2e-85	1e-82
FKBP	FKBP13:Mt/Cyt	04140.1	*P. anserina*; 2e-29	4e-23	6e-24	3e-26	4e-26
	FKBP22:ER	02455.1	*P. anserina*; 1e-36	2e-11	2e-07	2e-16	1e-12
	Fpr4:Nucleus	03241.1	*S. cerevisiae*; 6e-36	6e-36	4e-17	1e-26	3e-20
	Fpr1	04371.1	*S. pombe*; 4e-17	7e-13	4e-17	8e-13	1e-11
Hsp30	ORP-1	01735.1	*Coriolus versicolor*; 5e-31	7e-23	8e-12		5.1
	NOP-1	10055.1	*L. maculans*; 2e-75	1e-08	9e-07		
HSF	HSF	08512.1	*H. sapiens*; 2e-22	7e-16	1e-19	2e-22	3e-15
		08480.1	*S. cerevisiae*; 3e-04	3e-04	0.018	0.029	0.006

[a] *Caenorhabditis elegans*, *Drosophila melanogaster*, *Mus musculus*, or *Homo sapiens*.
[b] *Arabidopsis thaliana* or *Oryza sativa*.

oxidative stress. The *hsp30* RIP mutants were defective in glucose-phosphorylating activity and in mitochondrial protein import (617, 618). The next most highly conserved sHsp of *Neurospora*, which, like HSP30, is induced by heat shock, has the properties of an imported mitochondrial protein with a targeting presequence (MitoProt [152]) that was, unfortunately, omitted by automated gene prediction programs. There are also sHsps of plants and *D. melanogaster* with mitochondrial targeting presequences that are imported into mitochondria (539). Conditions under which the least highly conserved sHsp of *Neurospora* is expressed are not known. Despite their sequence divergence, these three sHsps of *Neurospora* are most similar to one another in the N-terminal domain that is predicted to form an amphipathic helix (HelixWheel, www.site.uottawa.ca/~turcotte/resources/HelixWheel/ [M. Turcotte, 1996]), a predicted structure that is also conserved.

The largest class of Hsps in *Neurospora* is the DnaJ/Hsp40 proteins, which number 18 in the genome; 16 Hsp40s have been identified in the *S. cerevisiae* genome (871). Despite the similar number of Hsp40 proteins in *Neurospora* and *S. cerevisiae*, many of them are unrelated to one another. The Hsp40 class is defined minimally by containing a "J domain" through which the proteins interact with Hsp70. Hsp40s function as independent chaperones, as well as being cochaperones for Hsp70s, whose ATPase activity they enhance (23). Several Hsp40s have a domain structure throughout that resembles that of the prokaryotic DnaJ, including a central cysteine-rich zinc finger domain and a characteristic C-terminal domain, as well as the N-terminal J domain (871). There are four of these Hsp40s in *Neurospora* that correspond most closely to the *S. cerevisiae* proteins Ydj1p (two), Scj1p, and Mdj1p. Like Ydj1p, the *Neurospora* Ydj1p homologues terminate in a CAAX motif that signals isoprenylation and that is required for Ydj1p association with nuclear membranes (126). Scj1p and Mdj1p are located in the ER lumen and mitochondrial matrix, respectively, where they cooperate with organellar Hsp70s in protein folding (665, 736). *Neurospora* also has a homologue of Sis1p, an essential cytosolic protein in *S. cerevisiae*, which has the conserved J and carboxyl domains (871).

The other conserved Hsp40s of *Neurospora* have only the J domain. These include homologues of ER membrane-localized Sec63, which interacts with luminal Hsp70 in protein import (671); Zuo1, which binds Ssb Hsp70 on ribosomes (872); and cytosolic Djp1, which is required for protein import into peroxisomes (335). *Neurospora* has a less strongly conserved counterpart of the ER membrane-localized Hlj1p (316). Other Hsp40-related proteins that contain the J domain do not have obvious counterparts in *S. cerevisiae*. Two of these proteins contain TPR domains, and one is in the mandelate racemase enzyme family. Of the unidentified Hsp40s of *Neurospora*, two show moderate similarity and five show little similarity to hypothetical proteins of other species. Hsp40s of *S. cerevisiae* that are absent from the *Neurospora* genome include microsomal Jem1p and mitochondrial Mdj2p and Jac1p, which interacts with the minor mitochondrial Hsp70 Ssq1p (836). Some of the unique Hsp40s of *Neurospora* may interact with Hsp70 proteins for which no orthologue is evident in other organisms.

Prokaryotic GrpE is a nucleotide exchange factor for DnaK, and in *S. cerevisiae* mitochondria the GrpE homologue, Mge1p, forms a complex with mitochondrial Hsp70/Ssc1p and Hsp40/Mdj1p (93). This complex is essential for the import of proteins into mitochondria and for their folding. *Neurospora* has a conserved homologue of Mge1p; however, much less strongly conserved in *Neurospora* and other species is an ER-localized protein, Sls1p, which was shown in *S. cerevisiae* to act as a nucleotide exchange factor for Kar2p, the ER Hsp70 (391). *Neurospora* also has a counterpart to *S. cerevisiae* Fes1p, a cytosolic homologue of Sls1p that interacts with Ssa1p. Fes1p is associated with polysomes, and it facilitates translation together with Ssa1p and Ydj1p (390). Bag-1 is another type of protein shown to be an Hsp70 cochaperone in mammalian cells and to act as its nucleotide exchange factor (344). Bag-domain proteins are poorly conserved overall, but they were identified in *Neurospora*, *S. cerevisiae*, and *S. pombe* by the presence of conserved residues essential for Hsp70 interaction (746, 774). The *S. cerevisiae* Bag-domain protein Snl1p, which bears little sequence similarity to the *N. crassa* protein, was shown to function as a cochaperone of Hsp70 (746).

Immunophilins are *cis-trans* peptidyl-prolyl isomerases that are expressed at higher levels during heat shock and that assist in protein folding; they are present in the cytosol and in organelles. In *Neurospora*, one gene encodes both the cytosolic and the mitochondrial cyclophilins, which bind cyclosporin A (801), and, similarly, one gene encodes the cytosolic and the mitochondrial FK506 binding proteins, FKBPs (802). Like other organisms, *Neurospora* also has a cyclophilin and an FKBP that localize to the ER (745), as well as a nuclear FKBP. Three cyclophilins of *Neurospora* are predicted to be nuclear (PredictNLS [165]); one contains an RNA binding domain, one contains a WD40 repeat, while the third contains a U-box (ring finger) domain. In addition to acting as independent chaperones, a subset of immunophilins contain a TPR domain through which they bind to Hsp90 and Hsp70 as part of the superchaperone complex (582). *Neurospora* has one TPR-containing 40-kDa cyclophilin compared with two in *S. cerevisiae*: Cpr6p and Cpr7p, which bind to Hsp90 (205). Similar to *S. cerevisiae*, none of the four *Neurospora* FKBPs has a TPR domain comparable to that of mammalian FKBP51 and FKBP52 that would indicate involvement with the Hsp90/Hsp70 superchaperone complex.

Neurospora has two seven-transmembrane helix proteins that are homologous to the plasma membrane-localized Hsp30p of *S. cerevisiae*. *S. cerevisiae* Hsp30p is a non-α-crystallin-related protein that protects the membrane ATPase from high-temperature stress (592). The more strongly conserved homologue in *Neurospora* (ORP-1; see also Tables 44 and 49) may be functionally similar to Hsp30p. The less strongly conserved homologue is an opsin (NOP-1), which has been characterized and shown to bind retinal (see also "Major signal transduction pathways" above) (Tables 44 and 49) (78).

The heat shock transcription factor (HSF) is central to the mechanisms used by cells to dramatically increase the transcription of Hsp genes. HSF becomes activated by the accumulation of unfolded proteins, resulting from heat shock or other stresses (803), which diverts the Hsp70 and Hsp90 chaperones from their inhibitory binding to HSF (352). Plants have multiple HSFs (21 for *A. thaliana* [571]), while vertebrates have four and *S. cerevisiae* has only one (780). *Neurospora* has two HSFs, one of that is moderately conserved and one that is divergent.

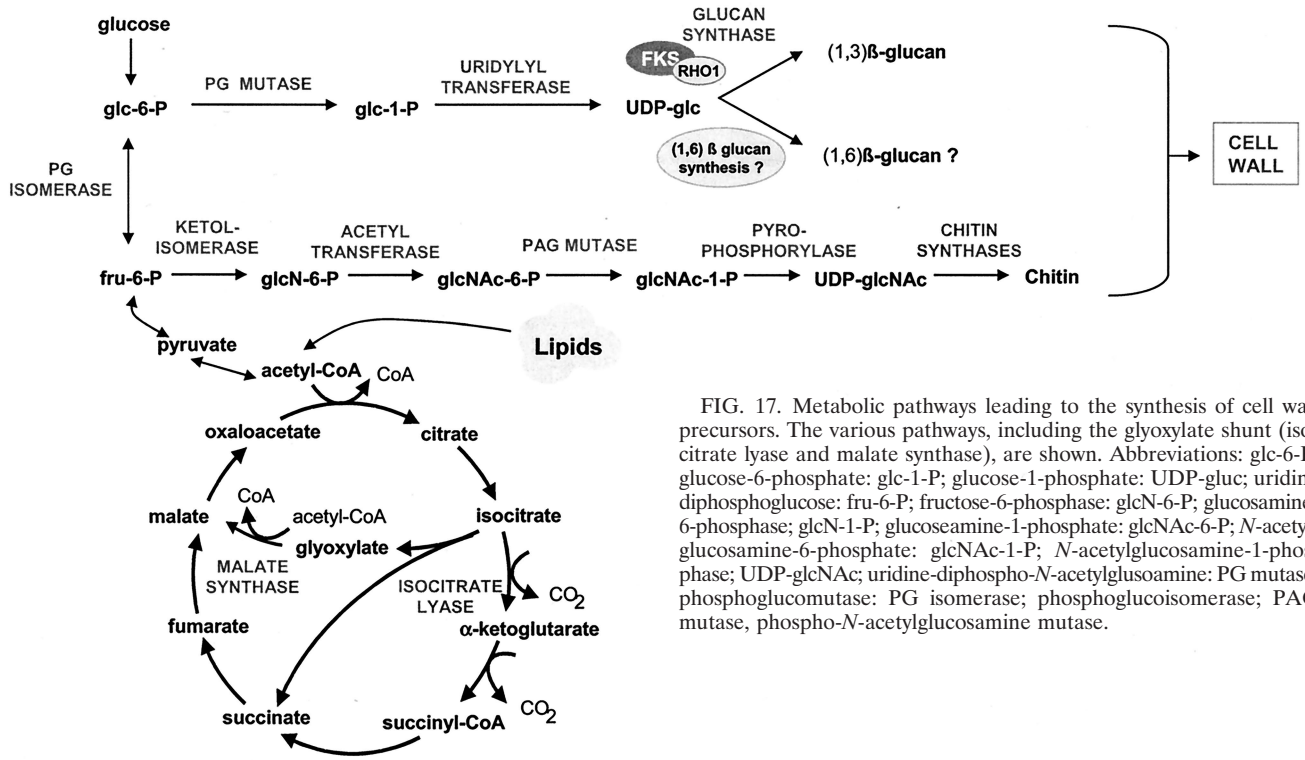

FIG. 17. Metabolic pathways leading to the synthesis of cell wall precursors. The various pathways, including the glyoxylate shunt (isocitrate lyase and malate synthase), are shown. Abbreviations: glc-6-P; glucose-6-phosphate: glc-1-P; glucose-1-phosphate: UDP-gluc; uridine diphosphoglucose: fru-6-P; fructose-6-phosphase: glcN-6-P; glucosamine-6-phosphase; glcN-1-P; glucoseamine-1-phosphate: glcNAc-6-P; *N*-acetylglucosamine-6-phosphate: glcNAc-1-P; *N*-acetylglucosamine-1-phosphase; UDP-glcNAc; uridine-diphospho-*N*-acetylglusoamine: PG mutase; phosphoglucomutase: PG isomerase; phosphoglucoisomerase; PAG mutase, phospho-*N*-acetylglucosamine mutase.

The assembly of HSF into homotrimers is mediated by its coiled-coil domains (748). However, most stress-induced HSFs are constrained from trimerization, until activated, by intramolecular coiled-coil formation (852). The HSF of *S. cerevisiae*, on the other hand, forms trimers and binds to DNA in the absence of stress (748), presumably due to a lack of strong intramolecular interactions. The conserved HSF of *Neurospora*, like the stress-induced HSF1 of mice, has three regions with high propensity for coiled-coil formation (490), suggesting that the *Neurospora* HSF would require activation for homotrimer formation. The more divergent HSF of *Neurospora* possesses an HSF-like DNA binding domain, but it has no predicted coiled-coils.

GROWTH AND REPRODUCTION

Cell Wall

The fungal cell wall protects the organism from an osmotically and, in the case of pathogens, an immunologically hostile environment. The cell wall not only serves a supportive function but also plays a dynamic role in all aspects of fungal physiology. For filamentous fungi, including *Neurospora*, growth and cell wall assembly occur only at each hyphal apex (119, 324, 656, 668, 799, 851, 863). This is in contrast to yeasts, where extension occurs at bud tips, followed by intercalary growth (reviewed in references 424, 479, and 493). *Neurospora* walls are composed of 7 to 10% chitin (a polymer of *N*-acetylglucosamine [GlcNAc]), 25% (1,3)β-linked glucans, 35% other glucans, and 10% proteins (47, 668). Each carbohydrate polymer is synthesized de novo at hyphal tips. The synthase responsible for production of each polymer is transported in an inactive form to hyphal tips in vesicles that fuse with the apical plasma membrane and then begin extruding each carbohydrate polymer through the membrane (372). The wall is assembled exterior to the membrane by processes not completely understood.

Glucan synthases. Although *S. cerevisiae* cell wall structure and assembly have served as the general models for fungal cell wall assembly, recent results, including the analysis of the *Neurospora* genome, have underscored some fundamental differences. Most notable is the observation that *Neurospora* lacks not only (1,6)β-linked glucans but also all of the enzymatic machinery required for (1,6)β-glucan synthesis encoded in the *S. cerevisiae* genome (see below).

(i) (1,3)β-Glucan synthesis. The synthesis of (1,3)β-linked glucan is catalyzed by an enzyme complex composed of at least two proteins, the (1,3)β-glucan synthase catalytic subunit encoded by a single gene in *Neurospora* (FKS) and a regulatory subunit, Rho1 (Fig. 17; Table 51) (35, 398, 576, 661). In *S. cerevisiae*, there are two and possibly three genes, FKS1, FKS2, (and FKS3), each encoding a catalytic subunit of (1,3)β-glucan synthase (510). *Neurospora* has only one FKS gene, which encodes a protein of 1955 amino acids. Correspondingly, the *Neurospora rho-1* gene encodes a protein of 195 amino acids. The substrate for (1,3)β-glucan synthase activity is UDP-glucose, which is synthesized from glucose-6-phosphate using two enzymes, phosphoglucomutase and a uridylyl transferase (Fig. 17; Table 51).

There is evidence that in situ, β(1,3)-linked glucans branch through (1,6)β-linkages from the main (1,3)β-glucan chain (243). In addition, it is likely that (1,3)β-glucans are cross-linked to chitin (243). Unfortunately, the enzymes responsible

TABLE 51. Glucan synthases

Enzyme	EC no.	NCU no.	BLAST match				
			Best overall	*S. cerevisiae*	*S. pombe*	Animal	Plant
(1,3)β-Glucan synthase	2.4.1.34	06871.1	*C. immitis* FKS/0.0	FKS2; 0.0	NP 588501; 0.0	*M. musculus* NP_080570/1e-60	*A. thaliana* NP_196804/2e-88
GTPase Rho		08683.1	*S. pombe* RHO2/5e-81	RHO2; 2.5e-62	RHO2; 2e-81	*M. musculus* Arha2/1e-28	
GTPase Rho		01484.1	*A. fumigatus* Rho1/1e-32	RHO1; 1.6e-30	RHO1; 2e-32	*X. laevis* AAH43876/1e-160	*B. napus* CAB60109/1e-155
Phosphoglucomutase	5.4.2.2	10058.1	*A. oryzae* PgmA/0.0	PGM2; 2.1e-184	NP_596153; 0.0	*M. musculus* AAH26626/1e-138	*M. acuminata* MWUGPA/1e-128
UTP-glucose-1-phosphate uridylyltransferase	2.7.7.9	02797.1	*S. pombe* NP_588132/0.0	UGP1; 3.4e-170	NP_588132; 0.0	*A. gambiae* EAA08752/0.0	*A. thaliana* NP_177278/0.0
Killer toxin resistance protein 5		02349.1	*P. anserina* CAD60785/0.0	KRE5; 1.1e-42	GPT1; 0.0	*M. musculus* Ges1/1e-109	*A. thaliana* NP_176916/1e-106
Mannosyl-oligosaccharide glucosidase	3.2.1.106	03657.1	*S. pombe* NP_594106/0.0	CWH41; 2.2e-88	NP_594106; 0.0	*M. musculus* BAC27099/0.0	*A. thaliana* NP_201189/0.0
Glucan 1,4-α-glucosidase homologue	3.2.1.-	04203.1	*S. pombe* NP_593490/0.0	ROT2; 3.6e-195	NP_593490; 0.0	*R. norvegicus* SI/1e-87	*P. pinaster* XYL1/1e-142
Glucan 1,4-α-glucosidase homologue	3.2.1.-	04674.1	*A. nidulans* AgdB/0.0	ROT2; 5.9e-68	NP_593996; 1e-163	*H. sapiens* CANX/9e-86	*H. tuberosus* CAA84491/1e-80
Calnexin precursor	None	09265.1	*A. niger* CkxA/1e-168	CNE1; 2.2e-40	CAL1; 1e-135		
Killer toxin resistance protein 1?	None	05229.1		WSC4; 1.8e-10	NP_588031; 3e-14	*M. musculus* Gfpt1/0.0	*A. thaliana* NP_189051/0.0
Ketol isomerase	2.6.1.16	07366.1	*S. castellii* GFA1/0.0	GFA1; 3.2e-236	NP_596011; 0.0	*C. elegans* Gna-1/6e-24	*A. thaliana* NP_197081/1e-24
Glucosamine-phosphate-*N*-acetyltransferase	2.3.1.4	01902.1	*C. albicans* GNA1/1e-35	GNA1; 7.2e-26	GNA1; 2e-20	*D. melanogaster* NP_648588/1e-120	*A. thaliana* BAB9465/1e-102
Phosphoacetylglucosamine mutase	5.4.2.3	07458.1	*C. albicans* AGM1/1e-126	PCM1; 7.2e-81	NP_592933; 1e-116	*D. rerio* AAH44137/1e-118	*A. thaliana* NP_181047/7e-97
UDP-*N*-acetylglucosamine pyrophosphorylase	2.7.7.23	02109.1	*C. albicans* UAP1/1e-134	UAP1; 5.2e-110	NP_596832; 7e-91	*M. cephalus* Pgi-1/1e-173	
Phosphoglucose isomerase	5.3.1.9	07281.1	*A. oryzae* PgiA/0.0	PGI1; 1.4e-196	PGI1; 0.0		
Isocitrate lyase	4.1.3.1	04230.1	*M. grisea* Icl1/0.0	ICL1; 1.2e-165	NP_595067; 1e-90	*C. elegans* Gei-7/1e-150	*I. batatas* ICL/1e-166
Malate synthase	4.1.3.2	10007.1	*A. nidulans* ACUE/0.0	MLS2; 6.7e-181			*G. hirsutum* CAA36546/1e-143

for branch synthesis or for chitin-glucan cross-linking have not been identified with certainty in any fungal organism.

(ii) (1,6)β-Glucan synthesis. (1,6)β-Linked glucans play a pivotal role in cell wall assembly in yeast, where they interconnect all other wall components into a lattice (424, 479). Genetic analysis of mutants defective in (1,6)β-glucan synthesis has revealed the involvement of a number of gene products that are localized throughout the secretory pathway and at the cell surface (94, 110, 536, 720, 721, 739). In sharp contrast, (1,6)β-linked glucans are not found in a number of filamentous fungi, including *Neurospora* and *A. fumigatus* (243). In addition, analysis of the *Neurospora* genome has indicated the presence of homologues of only 4 of the 10 genes involved in (1,6)β-glucan synthesis. For example, all of the *S. cerevisiae* proteins that function in the ER, Cwh41p, Rot2p, Kre5p, and Cne1p, are present in *Neurospora*. Kre11p, which appears to be involved in the transport of nascent (1,6)β-glucans from the ER to the Golgi, is present only in *S. cerevisiae* and not in *Neurospora* (or in *A. fumigatus*). The Golgi genes, *KRE6* and *SKN1*, are present in *S. cerevisiae, A. nidulans*, and *A. fumigatus* but not *Neurospora*. None of yeast cell surface genes, *KRE9*, *KNH1*, and *KRE1* are present in *Neurospora*.

These results indicate that cell wall synthesis may be fundamentally different in filamentous fungi and *S. cerevisiae* and that caution should be exercised when using budding yeast as a model for cell wall assembly in filamentous fungi.

Chitin substrate synthesis—the Leloir pathway. The substrate for each chitin synthase isozyme is UDP-N-acetylglucosamine (UDP-GlcNAc), which is synthesized from fructose-6-phosphate by the Leloir pathway (Fig. 17; Table 51) (466, 529). The first pathway-specific enzyme is a ketol-isomerase that synthesizes glucosamine-6-phosphate (GlcN-6-P) and glutamate from glutamine and fructose-6-phosphate. The *Neurospora* enzyme has been studied in detail and is feedback inhibited by the end product of the pathway, UDP-GlcNAc (221). The second pathway-specific enzyme (the acetyltransferase) forms *N*-acetylglucosamine-6-phosphate and CoA from GlcN-6-P and acetyl-CoA. The third enzyme, the mutase, forms *N*-acetylglucosamine-1-phosphate (GlcNAc-1-P) from GlcNAc-6-P. The final enzyme, the pyrophosphorylase, catalyzes the formation of UDP-GlcNAc and PP_i from UTP and GlcNAc-1-P. *Neurospora* has only a single gene for each enzyme of the pathway, similar to the situation in budding yeast and other filamentous fungi.

Cell wall precursors. An overview of the metabolic machinery to synthesize the precursors for the cell wall polymer synthases is shown in Fig. 17. As indicated, the substrates for (1,3)β-glucan and chitin synthase are synthesized from hexose-phosphate precursors. Interestingly, fungi, including *Neurospora*, grow on a number of suboptimal carbon sources, including lipids. Under these conditions, *Neurospora* utilizes the glyoxylate shunt (428) to form the sugar substrates required for carbohydrate wall assembly. The enzymes of the shunt—isocitrate lyase and malate synthase—are similar to those from *S. cerevisiae* and are present in single copy in *Neurospora*. The genes encoding each of these enzymes are presented in Table 51.

Chitin synthases. In their pioneering work, Galzer and Brown (284) utilized *Neurospora* when first describing cell-free chitin synthase activity. *Neurospora* has since been the organ-

TABLE 52. *Neurospora* chitin synthase components

Enzyme	NCU no.	BLAST match				
		Best overall	*S. cerevisiae*	*S. pombe*	Animal[a]	Plant[b]
Chitin-UDP acetylglucosaminyl transferase	03611.1	*Emericella nidulans* ChsB/0.00	e-151	e-160	e-22; *D. melanogaster*	No hits
Chitin-UDP acetylglucosaminyl transferase	05239.1	*E. nidulans* ChsA/0.00	0.00	0.00	5e-17; *C. elegans*	No hits
Chitin-UDP acetylglucosaminyl transferase	04251.1	*Exophiala dermatitidis* CHS2/0.00	0.00	0.00	5e-15; *C. elegans*	No hits
Chitin-UDP acetylglucosaminyl transferase	09324.1	*Magnaporthe grisea* CHS4/0.00	0.00	e-13	5e-16; *D. melanogaster*	No hits
Chitin-UDP acetylglucosaminyl transferase	04350.1	*Ustilago maydis* CHS6/0.00	e-125	5e-10	4e-20; *D. melanogaster*	e-08[c]
Chitin-UDP acetylglucosaminyl transferase	04352.1[d]	*U. maydis* CHS6/0.00	e-122	6e-11	2e-21; *D. melanogaster*/ *C. elegans*	3e-04
Chitin-UDP acetylglucosaminyl transferase	05268.1	*A. fumigatus* CHSD/0.0	2e-13	e-05	e-10; *C. elegans*	No hits
Chitin synthase associated	07435.1	*S. pombe* CHS5/e-64	2e-63	e-64	2e-10; *C. elegans*	No hits
Chitin synthase associated	04511.1	*S. pombe* Hypo./e-140	2e-65[e] (Bud7p)	e-140	No hits	No hits
Chitin synthase associated	05720.1	*S. cerevisiae* chs7p/e-72	e-72	No hits	No hits	No hits

[a] *Caenorhabditis elegans*, *Drosophila melanogaster*, *Mus musculus*, or *Homo sapiens*.
[b] *Arabidopsis thaliana*.
[c] Based on the myosin component of the protein.
[d] Based on manual annotation, this predicted polypeptide spans contig 3.225 nucleotides 69201 to 72540 and encodes a 1,112-amino-acid polypeptide (in contrast to the 143-amino-acid prediction in the *Neurospora* database).
[e] Even though chs6p was used to identify NCU04511.1, when the latter was used as a query on the yeast database, bud7p was the closest match.

ism of choice in many of the significant phases of progress made in the research of chitin synthesis, including the description of chitosomes and subsequent localization of chitin synthase in vesicular organelles (735), the cloning of a first chitin synthase from a filamentous fungus (878), and the use of partial chitin synthase gene sequences as a phylogenetic tool (127).

As has been determined for a variety of fungi, *Neurospora* has multiple chitin synthase-encoding genes, some of which have been functionally analyzed (74–76, 878). Based on the analyses performed to date, it appears that there is at least some redundancy in chitin synthase-encoding genes in *Neurospora*. Such redundancies have also been demonstrated in other filamentous fungi. Based on the full *Neurospora* genome sequence, there are three additional chitin synthase-encoding genes (other than the four mentioned above), including NCU04350.1, which has an apparent myosin motor-like domain (Table 52) (263). This suggests that at least in some instances there may be a direct association between a chitin synthase and cytoskeletal elements. Similarly, seven chitin synthases have been identified in *A. fumigatus*.

In *S. cerevisiae*, several other genes have been associated with chitin biosynthesis (although their products probably do not catalyze the reaction involving the polymerization of the UDP-GlcNAc substrate into chitin). Interestingly, the *S. cerevisiae* Sbe2p and Sbe22p proteins (677), which have been implicated in trafficking of chitin synthases (and perhaps other components of the cell wall biosynthetic machinery), do not have apparent homologues in *Neurospora*. However, Chs5p, Chs6p, and Chs7p, most probably involved in the budding yeast Chs3p transport and/or activation (129, 678, 797, 901), have apparent homologues in *Neurospora* (Table 52). Both the similarities and the differences between the *S. cerevisiae* and the *Neurospora* chitin synthase-related machinery are intriguing. Similar catalytic components are present in both (even though there are more such components in the filamentous fungus, perhaps due to its morphological complexity). In contrast, a more pronounced divergence is apparent in at least one of the additional components involved in regulation of chitin synthase activity. This, again, could be linked with the significant differences in the morphology of the different species (which may require different modes of regulation). Another possibility is that these components play a secondary role in *S. cerevisiae* chitin synthesis, even though they have been associated with the cell wall biosynthetic process. The notion that these proteins are involved in other functions is supported by experimental evidence (678). Interestingly, higher eukaryotes that produce chitin (e.g., *C. elegans* and *D. melanogaster*) appear to contain fewer members of the chitin synthase gene family (Table 52). Whether chitin biosynthesis in these (and perhaps similar) organisms has been streamlined or whether additional, as yet unidentified, components have altered the way in which chitin production is regulated is yet to be determined.

As expected, the fact that plants and mammals lack chitin is also evident from the absence of structural chitin synthase homologues. The unique composition of the fungal cell wall has been a favored target in the continuous search for antifungal compounds (279, 293, 325). Even though direct and indirect inhibitors of chitin synthesis have been identified, their use as commercial antifungals has been limited; this may be due to the combination of their pharmacological properties and the potential difficulties in inhibiting a process that can apparently be performed by several enzymes in a compensatory manner. However, the recent commercial introduction of glucan synthase inhibitors is a clear indication that cell wall components are valid and potentially rewarding targets for the development of antifungal compounds (see also "Relationship to animal and plant pathogens" below).

Hyphal Morphogenesis

Determining and maintaining cell shape is a fundamental prerequisite for proper development of any organism. The defining characteristic of filamentous fungi is the development of hyphae, tip-growing cellular elements that undergo regular branching, exhibit high developmental versatility, and respond to a myriad of signals during their invasive and exploratory growth within the natural environment. Hyphal compartments are frequently multinucleate, and movement of organelles between compartments is facilitated by the existence of incom-

TABLE 53. Classification of proteins important for cell polarity development[a]

S. cerevisiae GO[b] term	Orthologues found in *Neurospora* (homology over >60% of protein length)	Domains conserved (e<-10)	No matches found
Establishment of polarity	ABP1^{++}, ACT1^{++}, BCK1*, BEM1°, BNI1^{++}, BUD6^{++}, CDC3**, CDC10**, CDC11**, CDC12**, CDC24°, CDC42°, CKA1*, CKA2*, CKB1*, CKB2*, CLA4*, EXO70°°, EXO84°°, MLC1, PWP2, RGA1°, RGA2°, RHO1°, RHO2°, RHO3°, RHO4°, ROM1°, ROM2°, SEC3°°, SEC5°°, SEC6°°, SEC8°°, SEC10°°, SEC15°°, MYO2^{++}, TPM1^{++}, TPM2^{++}	BEM2°, BEM3°, BOI1, BOI2, CDC43, MSB1, SHS1**, SPA2°,	BEM4°, GIC1°, GIC2°, PEA2°, LAS1, MSB2, SPH1°, SLG1°, ZDS1°, ZDS2°
Exocyst	EXO70°°, EXO84°°, SEC3°°, SEC5°°, SEC6°°, SEC8°°, SEC10°°, SEC15°°		
Polarisome	BNI1^{++}, BUD6^{++}	SPA2°	PEA2°, SPH1°
Bud site selection	AXL1, BUD6, BUD7, BUD23, BUD31, BUD32, RSR1°, STE20*	BUD10, BUD2°, BUD4, BUD5°, BUD13, BUD14, BUD20, RAX1, RAX2	BUD3, BUD8, BUD9, BUD16, BUD17, BUD19, BUD22, BUD25, BUD26, BUD27, BUD28, BUD29, BUD30, HKR1, YOR300W
Axial budding	AXL1, CDC3**, CDC10**, CDC11**, CDC12**, ERV14°°, MYO1^{++}, PFY1^{++}, RSR1°	BUD10, BNR1^{++}, BUD2°, BUD4, BUD5°, ELM1*, GIN4°, KCC4°, PAN1^{++}	BUD3, GIC1°, GIC2°
Invasive growth	CDC24°, CDC42°, RGA1°, RGA2°	BEM3°, DIA3, DIA4, RIM20, SPT3^{+}, STE12^{+}	DFG16, DIA1, DIA2, DIG1^{+}, DIG2^{+}, FLO8^{+}, MUC1^{+}, NRG1^{+}, NRG2^{+}, RIM8, RIM21, RXT2
Pseudohyphal growth	BCY1*, BMH1, BMH2, CDC24°, CDC42°, CDC55*, DFG5, GPA2*, KSS1*, MEP2, PGU1, PLC1*, RAS2°, RGA1°, RGA2°, SHO1*, STE7*, STE11*, STE20*, TPK1*, TPK2*, TPK3*	BEM3°, BUD5°, DFG10, DIA3, DIA4, ELM1*, FKH1^{+}, FKH2^{+}, PHD1^{+}, SOK2^{+}, SPA2°, STE12^{+}	ASH1^{+}, BUD8, DIA1, ECM23, FLO8^{+}, GPR1*, HMS1^{+}, HMS2^{+}, MSS11^{+}, MUC1^{+}, PAM1, TEC1^{+}, SPH1°, SRO9°

[a] *, signaling components; **, septation machinery; °, Rho- and Ras-type GTPases modules and interacting proteins; °°, secretory pathway; $^{+}$, transcriptional regulators; $^{++}$, actin cytoskeleton.
[b] GO, gene ontology.

plete cross walls. Over the last few years, evidence has accumulated that, similar to cells of higher eukaryotes, the microtubule cytoskeleton provides the structural basis for the long-distance vectorial transport of secretory vesicles toward the growing hyphal tip (464, 701). Secretory vesicles then fuse with the tip to deliver membrane and materials required for continuous cell wall synthesis. This is thought to be coordinated by the *Spitzenkörper*, a fungal-specific organelle assemblage which is localized in the hyphal apex and serves as a vesicle supply center (49, 283). Through its microtubule-dependent movement and positioning within the hyphal apex, this organelle complex determines the shape and growth directionality of the hypha (48, 656). New hyphal tips are generated in subapical regions by branching, which requires some ill-defined signal(s) to establish the site of the new bud emergence and to regulate the spacing of branch points along the hypha.

Generation of hyphal polarity. On a molecular level, the best-described example of polar growth is the budding yeast *S. cerevisiae*. Many genes required for cellular development have been identified and arranged into functional hirarchies in this organism (203, 632, 633). This interaction map can be used as a starting point for the analysis of similarities and differences that control the different morphologies of filamentous fungi and unicellular yeasts. In *S. cerevisiae*, polarized growth is mediated by a series of steps, including the action of cortical landmark proteins, Rho- and Ras-type GTPases, that polarize the actin cytoskeleton and direct the motor-driven transport of secretory vesicles and cell wall components to the site of growth (566). One key component for the establishment of cortical landmarks that determine the future bud site is the small GTPase Rsr1p, which acts in combination with information from the previous division site. Additional factors, such as the septins, several other Bud proteins, and the exocyst component Sec3p, act in combination with the DNA content of the cell and the available growth conditions to further specify an axial or bipolar budding pattern (45, 235). Following bud site establishment, Cdc42p-dependent organization of the actin cytoskeleton and the recruitment of the budding machinery occur, finally leading to actin-dependent targeted secretion and cell wall formation at the site of polarization (summarized in reference 236).

(i) Proteins important for cell polarity development. To determine the conservation of components that constitute the morphogenetic network leading to polarized growth in *S. cerevisiae*, all proteins that are associated with polarity-related gene ontology (GO) terms at the Saccharomyces Genome Database were compared with the *Neurospora* genome (Table 53). Most proteins that represent the core machinery of cellular morphogenesis, such as components of cAMP and MAPK signaling pathways, Ras- and Rho-type GTPases, proteins that are necessary for the coordinated polarization and organization of the actin cytoskeleton and the secretory pathway, and proteins that constitute the septation machinery, are present in the *Neurospora* genome. Little is known about their function in *Neuropora* or other filamentous fungi, and experiments are needed to clarify their role in filamentous growth. However, in addition to this highly conserved core machinery that governs cellular polarity, other genes that modulate the different morphologies of *S. cerevisiae* (e.g., establishment of different budding patterns or pseudohyphal/invasive growth) are either ab-

sent from or highly diverged in *Neurospora*. In contrast to the true hyphae of filamentous fungi, the pseudohyphae produced by yeast under nitrogen starvation conditions are the result of unipolar budding that leads to chains of uninucleate elongated cells with no apparent communication between cellular compartments (540). Although the two signaling pathways that regulate pseudohyphal differentiation and invasive growth in *S. cerevisiae*—the MAPK and cAMP modules—are conserved among yeast and filamentous fungi, the key transcription factors Flo8p, Muc1p, and Tec1p, as well as other transcriptional regulators that are implicated in the switch from budding to pseudohyphal growth (Dig1p, Dig2p, Hms1p, Hms2p, Nrg1p, Nrg2p, and Mss11p), are not detectable in the *Neurospora* genome. Thus, different sets of transcription factors appear to regulate true hyphal versus pseudohyphal development.

In its budding mode, *S. cerevisiae* can exist in different forms, each with a specific cell morphology and cell division pattern. The typical yeast cell is ellipsoid, and haploid cells bud in an axial pattern with the bud formed next to the preceding site of cytokinesis. However, in diploid yeast cells, daughter cells bud 180° from their birth site in a bipolar manner. Interestingly, most BUD proteins that are specifically involved in the generation of these different budding patterns are not conserved in *Neurospora* (Table 53), suggesting that novel (and to date unknown) mechanisms have been developed in filamentous fungi to define the sites of the new hyphal tip emergence and to regulate spacing of branch points along the hypha. Also of interest is that the genes encoding several proteins that bind to Rho-type GTPases in budding yeast (*BEM4*, *GIC1*, *GIC2*, *NIP100*, and *ZDS2*) or act as downstream effectors of Rho proteins (*PEA1*, *SLG1*, *SPH1*, *WSC1*, *WSC2*, and *WSC3*) either are not found in the *Neurospora* genome or are highly diverged.

(ii) Rho-type GTPases as key regulators of polarity. Rho-type GTPases are molecular switches that cycle between an active (GTP bound) and an inactive form (GDP bound). Transition between these two forms is achieved through GTPase-activating proteins (GAPs), leading to the inactive form, and GDP-GTP exchange factors (GEFs), that activate the small G protein. Originally, Rho proteins were described as key regulators of the actin cytoskeleton, but now it has been shown that they influence an amazing variety of cellular processes that are crucial for coordinated morphogenesis (for reviews, see references 224 and 820). Therefore, a comparison of the *Neurospora* set of these master regulators of polarity with their budding yeast orthologues is required to elucidate similarities and differences that may contribute to the different morphologies in the two organisms.

A phylogenetic analysis shows that *Neurospora* Rho proteins and available fungal sequences of Rho1, Rho2, Rho3, and CDC42 fall into distinct subgroups (Fig. 18; Table 54), and experimental data from *S. cerevisiae* and other filamentous fungi suggest that there is at least a central set of conserved functions for these proteins. *S. cerevisiae* Rho1p regulates the organization of the actin cytoskeleton and is also required to maintain cell wall stability. This is achieved through two independent mechanisms. Rho1p activates cell wall synthesis through the activation of the MAPK pathway, which monitors cell wall integrity, and it is also directly required to stimulate glucan synthase activity, which catalyzes the synthesis of the main structural component of the yeast cell wall. Similarly,

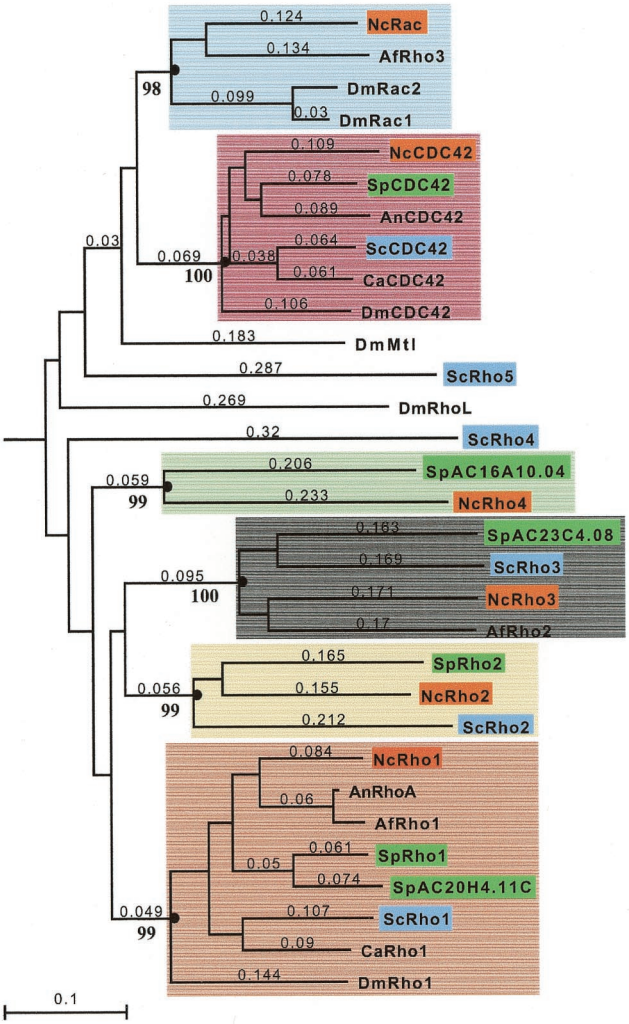

FIG. 18. Phylogenetic tree of *Neurospora* Rho proteins. Rho proteins were manually annotated and analyzed using Clustal W. The numbers adjacent to nodes indicate the percentages of 1,000 additional bootstrap trials in which the indicated protein groups were found.

Rho1 was also found to be part of the glucan synthase complex that localizes to zones of active growth at the hyphal apex in *A. fumigatus* (57). A role for cell wall integrity was also suggested for Rho1 of *Ashbya gossipii*, since deletion mutants show reduced filamentous growth and a high rate of lysis (848). Deletion of the *A. gossipii* gene encoding Bem2 (which was suggested to act as a Rho1-specific GAP in this fungus) resulted in aberrant switching from isotropic to polar growth during germination and maintenance of polarity during hyphal elongation (849). Areas of lost polarity showed a uniform actin distribution, suggesting that hyperactivity of Rho1 may result in depolarization of the actin cytoskeleton. Rho3p is important for coordinated polarization of the actin cytoskeleton and the secretory apparatus in *S. cerevisiae. Trichoderma reseei rho3* can complement the corresponding yeast mutant, and overexpression of this protein can also suppress several late-acting secretory pathway mutations, suggesting conserved functions (829). Surprisingly, deletion of *T. reseei rho3* shows no morphological defects, while deletion of *RHO3* is lethal in yeast. This example

TABLE 54. Rho GTPase modules in *Neurospora*

Neurospora protein	Orthologue(s)[a]		*S. cerevisiae* domains conserved	Proposed role in filamentous fungi	Interacting Rho protein in *S. cerevisiae*
	S. cerevisiae	*S. pombe*			
Rho proteins					
NCU01484.1/Rho1	Rho1p	Rho1; SpAC20H4.11C		Part of β(1,3)-glucan synthase complex, regulation of actin organization and cell wall integrity	
NCU08683.1/Rho2	Rho2p	Rho2			
NCU0600.1/Rho3	Rho3p	SpAC23C4.08		Interaction with secretory pathway, maintenance of polarity	
NCU03407.1/Rho4	Rho4p	SpAC16A10.04			
NCU02160.1/Rac				Actin organization	
NCU06454.1/CDC42	Cdc42p	Cdc42		Essential for establishment and maintenance of polar growth	
NCU03346.1					
Regulators of Rho proteins					
RhoGAP proteins					
NCU02689.1	Lrg1p				Cdc42p, Rho1p, Rho2p
NCU02524.1			Bem2/3		Cdc42p, Rho1p
NCU00553.1	Rgd1p				Rho3p, Rho4p
NCU00196.1			Bag7/Sac7		Rho1p, Rho2p
NCU07688.1	Rga1p/2p				Cdc42p, Rho1p
NCU09537.1	Rgd2p				Cdc42p, Rho5p
NCU02915.1					
NCU07622.1					
RhoGEF proteins					
NCU06067.1	Cdc24p				Cdc42p
NCU00668.1	Rom1p/2p				Rho1p
NCU02131.1	Tus1p				Rho1p
NCU06579.1					
NCU02764.1					

[a] Domain structure conserved and homology over >60% of protein length.

clearly illustrates that although it can be assumed that core functions of the Rho GTPases are conserved between yeast and filamentous fungi, a detailed analysis is necessary to clarify their exact roles during hyphal morphogenesis.

For the CDC42 module, several mutants are available in filamentous fungi. In *A. gossipii*, loss of *CDC42* as well as *CDC24* (acting as a GEF for Cdc42p) leads to apolar growth of the spore and inability to initiate polarized growth during germination (848). Experiments with dominant-negative and dominant-active mutants in *Penicillium marneffei* indicate that CDC42 is essential to maintain polarity during hyphal elongation in addition to establishing polarity during germination (101). No functional data are available for Rho2 and Rho4 in *Neurospora* or other filamentous fungi. In *S. cerevisiae*, these proteins appear to function in a partially redundant manner with Rho1p and Rho3p, respectively. While members of the Rho2 group are evolutionarily conserved, the available sequences constituting the Rho4 class are quite strongly diverged (Fig. 18), making it difficult to predict potential functions. Similarly, *S. cerevisiae* Rho5p is an orphan protein that cannot be correlated to any other available fungal Rho protein.

In addition to these five Rho proteins (Rho1 to Rho4 and CDC42), the *Neurospora* genome contains a clear ortholog of Rac that is conserved in filamentous fungi and higher eukaryotes but is missing from all sequenced yeasts (*S. cerevisiae*, *S. pombe*, and *C. albicans*). Interestingly, this type of Rho protein is especially important for the growth and development of polarity in neuronal cells and may play a similar role in the filamentous fungi. Although the suggestion that the hyphal cell is a simpler version of a neuron may seem far-fetched, several examples from the area of motor proteins and organelle transport support this view (343, 421, 538, 700, 831). Interestingly, several potential Rac homologs are present in the dimorphic fungus *Yarrowia lipoytica* (357). The deletion of one Rac protein revealed that it is not an essential gene. Its loss does not impair actin organization in *Y. lipoytica* cells but does prevent the switch to filamentous growth, indicating an important function during hyphal morphogenesis. On the other hand, in *P. marneffei*, Rac colocalizes with actin at the tips of vegetative hyphal cells and at septa (102). Loss of Rac in this organism results in growth defects in both vegetative hyphal and conidiophore cell types, such that cells become depolarized and the actin cytoskeleton is severely disrupted. These data suggest that Rac proteins can play a crucial role in actin-dependent polarized growth and division. Finally, the *Neurospora* genome contains an unusual protein with a domain that has weak homology to Rho in filopodia (NCU03346.1). Orthologues of this unusual protein are also found in the genome sequences of *M. grisea* and *A. fumigatus*, implying that this protein has a function that is unique for filamentous fungi; however, no functional data are currently available.

A major complication in determining the functions of small G proteins is that the number of GAPs and GEFs that have been uncovered in the sequencing projects far outnumber the GTPases that they regulate (e.g., the *D. melanogaster* genome contains only six Rho proteins but about 20 GEFs and more

than 20 GAPs [5]). This is also true for *Neurospora*, where the GAP and GEF repertoires are both expanded relative to budding yeast (especially if *S. cerevisiae* paralogues are not considered in the comparison [Table 54]). The task for the future will be to elucidate the regulatory networks for all the Rho proteins and their regulators and to determine how the different pathways interact to generate a well-shaped hypha.

Cytoskeleton and motor proteins. Mechanochemical enzymes responsible for intracellular cytoskeletal transport can be grouped into three superfamilies: the microtubule-based kinesins and dyneins and the actin-associated myosins (341, 342, 413). Based on the sequence similarity of their ATP-hydrolyzing motor domains, several families or classes within each superfamily can be defined that often correlate with conserved cellular functions. The genome of the unicellular yeast *S. cerevisiae* encodes six kinesins, one cytoplasmic dynein, and five myosins. In contrast, mammalian genomes encode more than 50 distinct kinesins, four functional classes of dyneins, and over 40 distinct myosins (31, 623). These numbers suggest that as eukaryotic cells increase in volume and morphological complexity, there is a corresponding increase in the complexity of cytoskeletal transport systems. This is borne out in our analysis of *Neurospora*, with an elaboration of certain cytoskeletal components reflecting the more complex morphology of *Neurospora* than of *S. cerevisiae* (Table 55).

(i) Structural components. The main structural components of the cytoskeleton are actin filaments, microtubules, and intermediate filaments. The *Neurospora* genome did not offer any major surprises at the level of the structural components of the cytoskeleton. All *S. cerevisiae* genes coding for the actin and microtubule cytoskeleton as well as actin binding and microtubule-associated proteins are strongly conserved in *Neurospora* (204, 434, 688). Ten actin-related proteins (Arps) have been found in *S. cerevisiae* (684), and *Neurospora* encodes all but Arp7p and Arp9p, which are involved in chromatin remodeling (120). The Arp2p-Arp3p complex and formins participate in two independent actin nucleation pathways (634). The components of the Arp2p-Arp3p complex are well conserved in *Neurospora;* however, *Neurospora* encodes only a single formin, while *S. cerevisiae* and *S. pombe* contain two and three, respectively. In addition to proteins required for actin and microtubule formation and function, an ortholog of *S. cerevisiae* Mdm1p was identified in the *Neurospora* genome. Mdm1p is important for mitochondrial and nuclear distribution in *S. cerevisiae* (512) and shows sequence similarity to vimentin and keratin, suggesting that an intermediate filament system is a universal component of eukaryotic cells.

(ii) Kinesins. The extremely high growth rate (>1 μm/s) and highly polar form of *Neurospora* requires that a large amount of material for cell wall synthesis, as well as various organelles, be transported toward hyphal tips. Intracellular transport in budding yeast is exclusively myosin dependent. In contrast, filamentous fungi, similar to higher eukaryotes, utilize a combination of actin- and microtubule-based systems.

Examination of the *Neurospora* genome indicates that it encodes 10 distinct kinesins, of which 4 are likely to be involved in cytoplasmic transport (Table 55). Conventional kinesin purified from animal sources is the founding member of the kinesin superfamily and is involved in a wide spectrum of cytoplasmic transport processes (401, 831). A relative of animal conventional kinesin, Nkin, was first identified and characterized in *Neurospora* (756), but additional work has shown that members of this family are present in all filamentous fungi examined to date (464, 648, 755, 862). Nkin was proposed to be involved in the transport of secretory vesicles toward the growing tip in *Neurospora* (701, 702), but the analysis of orthologs in other filamentous fungi suggested that in addition to polarized secretion, conventional kinesin might be necessary for the organization of vacuoles and could affect microtubule dynamics (648, 757). These results argue for multiple functions of a single motor protein in filamentous fungi, similar to what has been described for animal kinesins. Mutational analysis of Nkin has identified regions of cargo association and regulation of the ATPase activity that are conserved between fungal and animal kinesins (421, 700). Interestingly, fungal conventional kinesins lack copurifying light chains (422), which, in addition to the C terminus of the motor protein itself, were proposed to function in cargo attachment and motor activation in animals (830), suggesting that this fungal motor could serve as a simplified model to study motor-cargo interaction and its regulation.

Additional *Neurospora* kinesins implicated in organelle transport include two of the unc104 family and one related to KIF21A. Both families are involved in the transport of a variety of cytoplasmic cargoes in metazoan systems (84, 502). Interestingly, members of the KIF21 group are restricted to filamentous fungi and higher eukaryotes and not present in unicellular yeasts, suggesting a potential role in long-range transport processes. KIF21 orthologs were also found in *U. maydis* and the thermophilic fungus *Thermomyces lanuginosus* (670, 846), suggesting that this family may be widely used for vesicular transport in filamentous fungi.

In *S. cerevisiae*, five kinesin motors are required to build up counteracting forces to organize the mitotic and meiotic spindle. The C-terminal motor Kar3p, the functionally redundant BimC family members Kip1p and Cin8p, and the proteins Kip2p and Kip3p, which have at least partly overlapping functions, function together with cytoplasmic dynein for spindle assembly and chromosome segregation (summarized in reference 338). In contrast to both budding and fission yeast, *Neurospora* is more streamlined and has a minimal set of only one mitotic kinesin per subfamily with no apparent overlap in function. The orthologues of NCU04581.1 and NCU00927.1 have been studied in *A. nidulans* (KlpA and BimC, respectively [573]), and the results suggest the existence of conserved functions between yeast, filamentous fungi, and higher eukaryotes for these proteins (for a detailed analysis of mitotic motors in filamentous fungi, see reference 12).

In addition to these minimal components, the *Neurospora* genome contains two other kinesins that are known to be involved in mitosis in metazoan cells (NCU05180.1 and NCU05028.1 [6 and 265]) are not found in the *S. cerevisiae* genome. A possible function for these motor proteins during fungal mitosis remains to be determined, but their existence suggests mechanistic similarities in spindle formation and function between animals and filamentous fungi that are not shared with unicellular yeasts. This is also reflected by the absence (Spc25p, Spc29p, Spc34p, Spc42p, and Ndc1p) or high divergence (Bbp1p, Cnm1p, Stu1p, Spc24p, Spc72p, Spc105p, and Spc110p) of components of the *S. cerevisiae* spindle pole body

TABLE 55. Comparison of fungal motor proteins

NCU no.	Orthologue(s)		Family/class	Proposed role(s)
	S. cerevisiae	*S. pombe*		
Kinesins				
09730.1	Smy1p	Klp3 (SPAC1834.07)	Conventional kinesin	Transport of secretory(?) vesicles, nuclear positioning, microtubule dynamics
06733.1	NF[a]	NF	Unc104	Vesicular transport
03715.1	NF	SPAC144.14		
06832.1	NF	NF	Kif21/chromokinesin	Vesicular transport, DNA binding
04581.1	Kar3p	Pkl1/Klp1 (SPAC3A11.14C)	C-terminal	Dyanamics if spindle microtubules, counteracts BimC-like motors
		Klp2 (SPAC664.10)		
06144.1	Kip3p	Klp5 (SPBC2F12.13)	Kip3	Spindle positioning, spindle elongation during anaphase, microtubule disassembly
		Klp6 (SPBC649.01C)		
00927.1	Kip1p, Cin8p	Cut7 (SPAC25G10.07C)	BimC/Eg5	Spindle assembly and centrosome separation during mitosis
05180.1	NF	SPBC15D4.01C (also named SPBC2D10.21C)	Fast evolving/pavarotti	Organization of the mitotic spindle
02626.1	Kip2p	Klp4/Tea2 (SPBC1604.20C)	Kip2	Heterogenous group: Kip2p has mitotic functions (partly overlapping with Kip3p), while Tea2 seems to alter the dynamics of interphase microtubules
05028.1	NF	NF	KID	Chromosome alignment in metaphase
Myosins				
01440.1	Myo2p, Myo4p	Myo5 (SPCC1919.10c)	Class V	Organelle transport
		Myo4 (SPBC2D10.14c)		
02111.1	Myo3p, Myo5p	Myo1 (SPBC146.13c)	Class I	Endo-/exocytosis
00551.1	Myo1p	Myo2 (SPCC645.05c)	Class II	Actin organisation, cytokinesis
		Myo3 (SPAC4A8.05c)		
04350.1	NF	NF	Chitin sythase-myosin fusion protein	Specific for filamentous fungi
Dynein subunits				Nuclear movement, spindle elongation, retrograde vesicle transport
06976.1	Dyn1p	Dhc1	Dynein heavy chain	
09142.1	Pac11p	SPBC646.17	Dynein intermediate chain	
09982.1	NF	NF	Dynein light intermediate chain	
02610.1	Dyn2p	Dlc (SPAC926.07)	Dynein light chain, LC8	
03882.1	NF	Dlc (SPAC1805.08)	Dynein light chain, Tctex-1	
09095.1	NF	NF	Dynein light chain, LC7	
Dynactin subunits				Dynein-cargo interaction, nuclear movement, spindle elongation, retrograde vesicle transport
03483.1	NIP100p	NF	Dynactin p150^{Glued}	
00257.1	NF	NF	Dynactin p62	
03563.1	NF	NF	Dynactin Arp11	
08375.1	NF	NF	Dynactin p50/dynamitin	
04247.1	Arp1p	Actin-like protein (SPBC1347.12)	Dynactin Arp1	
04043.1	NF	NF	Dynactin p27	
07196.1	NF	NF	Dynactin p25	
Not defined	NF	NF	Dynactin p24	
Lis-1 complex				Dynein regulation, nuclear movement, spindle elongation, retrograde vesicle transport
04534.1	Pac1p	NF	LIS1	
04312.1	Pac1p	NF	LIS1	
08566.1	NF	NF	NUDE/RO11	

[a] NF, not found by conventional BLAST searches.

compared to other organisms. Especially interesting is one kinesin (NCU05180.1) that shows only weak sequence conservation in the normally well-conserved motor domain. The *D. melanogaster* orthologue of this kinesin ("pavarotti") was originally identified in a screen for fast-evolving proteins by cross-hybridization with *D. virilis* and *D. yakuba* cDNA libraries (687) and subsequently shown to act in mitosis. It seems that their fast-evolving nature and not their sequence similarity is a key feature of this subfamily. NCU02626.1 is part of a heterogenous subfamily of kinesins that is defined by a centrally located motor domain; however, sequence conservation in this subfamily is restricted to the motor domain. The *S. cerevisiae* member of this subfamily, Kip2p, functions in spindle assembly and nuclear positioning (530), while the corresponding *S.*

pombe tea2 mutants display altered microtubule dynamics during interphase (112). *U. maydis kin1* mutants have no discernible phenotype (464).

(iii) Myosins. *Neurospora* has only four myosins, with a single member in each of the three classes found in *S. cerevisiae* (Table 55). Analysis of the *A. nidulans* protein corresponding to the *Neurospora* class I myosin, MyoA, suggests that it functions in endocytosis and secretion (513, 578, 870). Work with both yeasts and several vertebrate systems suggests that the other two *Neurospora* myosins (NCU00551.1, class II; NCU01440.1, class V) are probably involved in cytokinesis and organelle transport, respectively (111, 718, 856); however, experimental data are lacking for *Neurospora* or other filamentous fungi. In addition, *Neurospora* encodes an unconventional myosin domain linked to a class V chitin synthase domain. This unusual myosin has also been identified in other filamentous fungi and appears to function in cell wall synthesis and maintenance of cell wall integrity (263, 599). Mutational analysis has indicated that both the myosin and chitin synthase domains are required for correct cellular function (349).

(iv) Dynein. Cytoplasmic dynein is the most complex of the motor proteins operating in the cytoplasm (341). Purified mammalian cytoplasmic dynein consists of a heavy chain (>4,000 residues), an intermediate chain, a light intermediate chain, and three distinct light chains (347, 416). An additional multisubunit complex, known as dynactin, is required for all known dynein functions and consists of at least distinct 10 subunits (Table 55) (345). In mammals, cytoplasmic dynein is required for numerous intracellular transport processes; however, in *S. cerevisiae*, dynein function is restricted to ensuring proper nuclear movement and distribution between mother and daughter cells during cell division. Consistent with this restricted role, *S. cerevisiae* dynein and dynactin subunits are highly diverged relative to those of higher eukaryotes. In contrast, cytoplasmic dynein in *Neurospora* is required for retrograde transport of membranous organelles, as well as for nuclear movement (702). Examination of the *Neurospora* genome shows that dynein/dynactin subunits of *Neurospora* are more similar to those of metazoans than to those of *S. cerevisiae*. Some of the dynein/dynactin subunits present in filamentous fungi and metazoans (DLIC, the "roadblock" DLC, $p150^{Glued}$, dynamitin/p50, p62, p27, and p25) are not detectable in either *S. cerevisiae* or *S. pombe* (Table 55). Two of the dynactin subunits, p24/p22 of the shoulder/sidearm subcomplex and Arp11 of the Arp1 pointed-end complex (211, 693), have undergone significant change in all organisms, with clear matches seen only between closely related organisms. Haploinsufficiency of LIS1, a dynein regulator, results in a defect in neuronal migration and subsequent brain development (818). Interestingly, *Neurospora* appears to possess two LIS1 proteins, while other unicellular and filamentous fungi and metazoans appear to have only one. The significance of this duplication is not known.

Cyclin/CDK Machinery

Oscillations in the activity of cyclin-dependent kinases (Cdk) drive the eukaryotic cell cycle (549, 561). These enzymes are complexes of catalytic (Cdk) and regulatory (cyclin) subunits. In most cases it is cyclin abundance that oscillates in the cell cycle. In *S. cerevisiae*, the cell cycle alternates between two states, depending on whether mitotic B-type cyclin/Cdk activity is high (in the S, G_2, and M phases) or low (in G_1). The G_1 cyclins are thought to elevate mitotic cyclin/Cdk activity by promoting the degradation of mitotic cyclin/Cdk inhibitors, whereas the anaphase-promoting complex reduces kinase activity by degrading mitotic cyclins, thereby completing the cycle (549, 561). In many eukaryotes the proteins and pathways involved in cell cycle transitions are highly redundant and have specialized functions. In *S. cerevisiae*, for example, most of the current cyclin genes are derived from gene duplications. Because of RIP (709), we anticipated that *Neurospora* should have fewer, presumably less specialized cyclins than the yeasts or higher eukaryotes do.

In both *S. cerevisiae* and *S. pombe*, there is only one major Cdk responsible for cell cycle transitions (*CDC28* and *cdc2*, respectively), while in humans there are five Cdks with cell cycle roles (550). There are additional Cdks that do not play major roles in cell cycle progression; some of these proteins are involved in phosphate metabolism, transcription, or less well defined processes. *Neurospora*, like *S. cerevisiae*, has a Cdk family that includes a Cdc2-like Cdk (*cdc-2*), a Pho85p-like Cdk, and Cdks likely to be involved in transcription (NCU07172.1, NCU06685.1, and NCU03659.1). It also has a Cdk (NCU07880.1) with a PITSLRE motif, which is absent from *S. cerevisiae* but present in *S. pombe*. We conclude that *Neurospora* has just one Cdk (*cdc-2*) that is primarily responsible for cell cycle progression.

Neurospora has three cyclin genes (Table 56) that are likely to be involved in cell cycle control. By comparison, *S. cerevisiae* and *S. pombe* have nine and five cyclins, respectively, involved in cell cycle progression, while humans have four classes of cell cycle cyclins (cyclins A, B, D, and E), with more than one member in each class (550). NCU02114.1 (*cln-1*) is likely to be a G_1 cyclin because the *S. cerevisiae* (*CLN1* to *CLN3*) and *S. pombe* (*puc1*) G_1 cyclins are more closely related to it than to the other two *Neurospora* cyclins (Table 56). In fact, *CLN1* and *CLN3* show no significant similarity to any other *Neurospora* cyclin (Table 56). Both NCU02758.1 (*clb-1*) and NCU01242.1 (*clb-3*) are B-type cyclins. CLB-1 contains the mitotic destruction motif typical of mitotic cyclins (652). It is related to G_2/M cyclins from *S. cerevisiae* (*CLB1*/*CLB2*) and *S. pombe* (*cdc13*) (Table 56) and the NIME B-type cyclin from *A. nidulans* (574). CLB-3 may play an earlier role—perhaps in S phase—because it is more similar to *CLB3*/*CLB4* from *S. cerevisiae* and *cig1* from *S. pombe* (Table 56). It appears that *A. fumigatus* also contains these three cyclins—all closely related to the *Neurospora* proteins (data not shown). We conclude that the cyclin/Cdk complexes in *Neurospora* are very streamlined compared to the better-studied model organisms. However, the *Neurospora* life cycle is far more complex than that of *S. cerevisiae* and *S. pombe*. How *Neurospora* manages to achieve more complex developmental programs with apparently less diversity in protein complexes is a question worth addressing in future cell cycle studies.

Even with a limited set of cyclins, however, most of the key components of the cell cycle machinery are present in *Neurospora*. Thus, *Neurospora* conforms to the view that the machinery and the "wiring" of processes that regulate cell division are conserved among eukaryotes. An apparent lack of Cdk inhib-

TABLE 56. The cyclin family of *Neurospora*[a]

Neurospora locus vs *S. cerevisiae* genome		*S. cerevisiae* locus vs *Neurospora* genome		*Neurospora* locus vs *S. pombe* genome		*S. pombe* locus vs *Neurospora* genome	
NCU02114.1	*CLB3* (1e-27)	*CLN1*	NCU02114.1 (1e-16)	NCU02114.1	*cdc13* (4e-32)	*puc1*	NCU02114.1 (1e-30)
	CLB4 (5e-26)		NCU01242.1 (>e-5)		*puc1* (1e-31)		NCU01242.1 (8e-18)
	CLB6 (1e-23)		NCU02758.1 (>e-5)		*cig1* (2e-30)		NCU02758 (5e-16)
	CLN3 (2e-22)	*CLN2*	NCU02114.1 (1e-14)		*cig2* (3e-26)	*cig1*	NCU01242.1 (8e-76)
	CLB2 (3e-22)		NCU02758.1 (1e-08)		*rem1* (9e-18)		NCU02758 (4e-66)
	CLB5 (6e-20)		NCU01242.1 (>e-5)	NCU01242.1	*cdc13* (4e-74)		NCU02114.1 (7e-25)
	CLB1 (1e-19)	*CLN3*	NCU02114.1 (9e-15)		*cig1* (4e-72)	*cig2*	NCU02758 (2e-79)
	CLN1 (3e-14)		NCU01242.1 (>e-5)		*cig2* (1e-67)		NCU01242.1 (1e-65)
	CLN2 (2e-12)		NCU02758.1 (>e-5)		*rem1* (3e-47)		NCU02114.1 (8e-28)
NCU01242.1	*CLB3* (7e-78)	*CLB1*	NCU02758.1 (4e-72)		*puc1* (4e-17)	*rem1*	NCU01242.1 (2e-48)
	CLB4 (6e-75)		NCU01242.1 (2e-53)	NCU02758.1	*cdc13* (2e-94)		NCU02758 (1e-41)
	CLB2 (5e-57)		NCU02114.1 (3e-19)		*cig2* (2e-78)		NCU02114.1 (7e-19)
	CLB1 (5e-53)	*CLB2*	NCU02758.1 (2e-85)		*cig1* (4e-64)	*cdc13*	NCU02758 (1e-101)
	CLB6 (5e-48)		NCU01242.1 (2e-57)		*rem1* (2e-41)		NCU01242.1 (9e-74)
	CLB5 (3e-47)		NCU02114.1 (3e-22)		*puc1* (3e-15)		NCU02114.1 (2e-30)
	CLN3 (5e-07)	*CLB3*	NCU01242.1 (3e-82)				
	CLN2 (>e-5)		NCU02758.1 (7e-67)				
	CLN1 (>e-5)		NCU02114.1 (2e-26)				
NCU02758.1	*CLB2* (4e-81)	*CLB4*	NCU01242.1 (8e-80)				
	CLB1 (1e-74)		NCU02758.1 (1e-65)				
	CLB3 (6e-67)		NCU02114.1 (3e-25)				
	CLB4 (2e-62)	*CLB5*	NCU02758.1 (3e-51)				
	CLB6 (3e-58)		NCU01242.1 (3e-48)				
	CLB5 (4e-52)		NCU02114.1 (1e-19)				
	CLN2 (2e-07)	*CLB6*	NCU02758.1 (1e-59)				
	CLN3 (2e-05)		NCU01242.1 (1e-48)				
	CLN1 (>e-5)		NCU02114.1 (5e-23)				

[a] Sequences were compared using BLAST, and the corresponding *P* value in each case is indicated in parentheses. The databases used in the analysis are as follows: *S. cerevisiae*, http://www.yeastgenome.org/; *N. crassa*, http://www-genome.wi.mit.edu/annotation/fungi/neurospora/; *S. pombe*, http://www.sanger.ac.uk/Projects/S_pombe/.

itors from BLAST searches is not surprising, since these proteins have very little, if any, sequence similarity. NCU07565.1, however, has weak similarity to budding yeast *FAR1*. Three *Neurospora* proteins with predicted ankyrin repeats (NCU07934.1, NCU02967.1, and NCU01098.1) all display weak similarity to human p16/INK4, an ankyrin repeat protein. Ankyrin repeats are important for Cdk inhibition, but not all ankyrin repeat proteins are Cdk inhibitors (53).

Finally, in addition to cyclin binding, Cdks need to be phosphorylated on a conserved threonine residue to be activated. In *S. cerevisiae*, Cak1p catalyzes this phosphorylation, while mammals use a different Cdk for this activation (550). *Neurospora* probably uses the same mechanism found in *S. cerevisiae* for Cdk activation, since it contains a Cak1p-like predicted protein (NCU04426.1).

Asexual and Sexual Sporulation

Spore formation is a common mechanism among fungi for reproduction, dispersal, and survival under harsh conditions (7, 184). For most pathogenic fungi, spores are the major source of infection of their hosts. Many filamentous fungi reproduce both sexually and asexually. The asexual cycle is mitotic, while the sexual cycle involves mating and meiosis.

Macroconidiation. In the filamentous ascomycetes, asexual reproduction involves the production of macroconidia at the tips of specialized hyphae called conidiophores (7, 751). Macroconidiation begins with the differentiation of aerial hyphae that grow perpendicular to the surface mycelium. Following a period of apical aerial growth, the aerial hyphae switch to a budding mode of growth that results in the formation of proconidial chains within the conidiophore. As the conidia reach maturity, conidial separation takes place. Free conidia are released and are dispersed primarily by air currents. The macroconidia germinate rapidly, allowing efficient reproduction of the fungus. Macroconidiation can be induced by environmental signals including heat shock, desiccation, and carbon or nitrogen starvation (808). Furthermore, the endogenous circadian clock regulates the timing of conidiation in *Neurospora* (484). Asexual development in the filamentous ascomycetes is considered to be a relatively simple process and is not essential for viability. Thus, these organisms serve as excellent models for uncovering the genes involved in, and the mechanisms of, asexual spore differentiation. Using both forward and reverse genetic techniques, key components of the macroconidiation pathway have been identified in *Neurospora* and *A. nidulans*.

The *A. nidulans* FadA and FluG proteins are considered to be upstream regulators of conidiation, since they affect the expression levels of a key regulator of development, *brlA* (7). FluG produces an extracellular factor that signals conidiophore development, while FadA is a heterotrimeric G-protein α subunit which, when activated, blocks sporulation. Homologues of both FluG and FadA are present in the *Neurospora* genome (NCU04264.1 and GNA-1, respectively), and mutation of *gna-1* leads to multiple developmental phenotypes, including defects in macroconidiation (369). In contrast, there appears to be significantly less conservation, between these two species, of key components that act downstream of FadA and FluG (see below).

In *A. nidulans*, the FlbC, FlbD, BrlA, AbaA, and WetA proteins are required for the normal production of conidiospores and StuA and MedA are developmental modifiers that are necessary for the normal spatial organization of the conidiophore (7). There are no *Neurospora* homologues of BrlA or AbaA. There is a hypothetical *Neurospora* protein (NCU06975.1) with weak homology to the C terminus of WetA and a hypothetical protein (NCU03043.1) with similarity to FlbC, but the identity is limited primarily to the zinc finger domain. A homologue of the MedA transcription factor is present in *Neurospora* (NCU07617.1) and in *M. grisea* (ACR1). ACR1 is a stage-specific negative regulator of conidiation in *Magnaporthe* (454). A putative *Neurospora* homolog of *flbD, rca-1*, was shown to complement the *A. nidulans flbD* mutation. Surprisingly, mutation of *rca-1* has no effect on conidiation in *Neurospora* (726). Similarly, a *stuA* homologue, *asm-1*, is required for sexual development but not macroconidiation in *Neurospora* (25). In *Neurospora*, the ACON-2, ACON-3, FL, and FLD proteins are required for macroconidiation (752). Of these, the only corresponding gene that has been cloned is *fl* (40). No homologues of FL are found in other ascomycetes, including *A. nidulans* and *M. grisea*. The closest match to FL in the database is to a hypothetical *Neurospora* protein (NCU09205.1).

The *con* genes are a set of cloned *Neurospora* loci that are preferentially expressed during conidiation but are not essential for development (69). In most cases, searches using the *con* gene sequences yielded few clues to their cellular functions. However, the closest match to CON-7 is another hypothetical *Neurospora* protein (NCU07846.1). NCUO7846.1 has similarity to a putative transcriptional regulator from *M. grisea* (AAB69694.1), suggesting that CON-7 and the related *Neurospora* protein may be involved in transcriptional regulation. EAS (CCG-2) is a fungal hydrophobin that is induced during macroconidiation and coats the mature condiospore, rendering the spores hydrophobic and air dispersible (62, 455). Searches using EAS identified a putative second *Neurospora* hydrophobin (NCU08457.1). This finding is surprising, since deletion of *eas* (*ccg-2*) results in wet, clumpy spores that lack the hydrophobic rodlet layer (62), and suggests either a different role for the NCU08457.1 hydrophobin or a requirement for EAS (CCG-2) for its expression and activity.

In summary, the apparent lack of conservation of key regulators of conidiation in *A. nidulans* and *Neurospora* suggests the possibility that macroconidiation has evolved independently in these two organisms. In contrast, there appears to be significant conservation among the upstream signaling components, perhaps reflecting the similar ways in which these fungi respond to the same environmental signals to initiate macroconidial development.

Meiosis and the sexual cycle. In fungi, meiosis is intimately associated with sporulation (436). The sexual development of filamentous ascomycetes is characterized by the formation of a fertilized fruiting body containing asci, which, in turn, enclose the progeny spores. In contrast, yeasts form an ascus directly from a single diploid cell without the involvement of a fruiting body. Numerous genes have been identified that are involved in meiosis and ascus development in *Neurospora* and closely related filamentous ascomycetes (456, 638; see also "Meiotic recombination" above).

In *Neurospora*, a single isolate can produce both female and male reproductive structures, but sexual reproduction can take place only between strains of opposite mating type, *matA* and *mat***a** (reviewed in references 436 and 638). The formation of female reproductive structures (protoperithecia) is induced by nitrogen limitation. These structures produce specialized hyphae (trichogynes) that exhibit chemotropic growth toward male cells (usually conidia or hyphae) of the opposite mating type in an apparent pheromone response pathway (81, 87, 414). Contact between the trichogyne and the male cell leads to entry of the male nucleus into the trichogyne and its subsequent transport to the ascogonium cell of the protoperithecium (reviewed in references 169, 184, and 638). The male and female nuclei do not fuse immediately after fertilization but instead undergo a series of mitotic divisions to produce an ascogenous hyphal mass. Later, nuclei of opposite mating types pair and undergo simultaneous mitotic divisions at the tips of ascogenous hyphae to yield distinct cell types, including the binucleate cell in which karyogamy (nuclear fusion) takes place. Each resulting diploid cell immediately enters meiosis, followed by a postmeiotic mitosis, to yield an ascus containing eight ascospores.

S. cerevisiae has been extensively used for the analysis of meiosis and sporulation. To identify genes that are regulated during meiosis and conserved across yeast species, the *S. cerevisiae* and *S. pombe* meiotic transcriptomes were compared (507, 686). These analyses identified a group of approximately 75 similarly regulated meiotic genes, including components of the anaphase-promoting complex and genes involved in recombination, sister chromatid cohesion, and synapsis (686). From these analyses, a list of 74 yeast genes that are essential for meiosis and sporulation, but not for mitotic growth, was compiled and the sequences were compared to the fly, worm, mouse, and human genomes (www.biozentrum.unibas.ch/personal/primig/gamates). A comparison of several of these core meiotic gene products to the *Neurospora* sequence database revealed several interesting features (Table 57).

Of the three known transcription factors specifically involved in meiotic gene transcription in *S. cerevisiae* (Abf1p, Ume6p, and Ndt80p [147, 268, 333]), only Ndt80p, a meiosis-specific transcription factor that induces genes at the end of prophase, appears to be conserved in *Neurospora*. Transcription of Ndt80p is itself dependent on Ime1p, which activates the expression of early sporulation genes; an ImeIp homologue is also present in *Neurospora*. Ume6p, which interacts with Ime1p and recognizes a conserved *URS1* site in the promoters of many genes that are activated early in meiosis and are associated with chromosome pairing and recombination, is absent from *Neurospora*. Consistent with the lack of Ume6p, several of the products of the genes in *S. cerevisiae* known to be regulated by this factor (854) are not present in *Neurospora* (with the exception of Spo11p). Ime2p is the founding member of a family of protein kinases that are required for effective progression through meiotic development. Ime2p is essential for the induction of meiosis-specific genes and for the activation of meiotic DNA replication in *S. cerevisiae*. Orthologs of Ime2p and Rim15p (required for Ime2p expression) are present in *Neurospora;* however, a regulator of Ime2p expression, Rim4p, is absent.

TABLE 57. Meiosis and sexual sporulation

S. cerevisiae enzyme	Function	*Neurospora* NCU no., gene name	BLAST e value	Best overall BLAST match to *Neurospora*	Homologue/ orthologue in plants and animals?
Meiosis					
Abf1	ARS1 binding protein/transcriptional regulator	None			
Ama1	Activator of meiotic anaphase-promoting complex	1572.1	2e-42	*S. cerevisiae* AMA1 AAK61800	Yes
Cdc16	Subunit of anaphase-promoting complex	1377.1	1e-102	*S. pombe* Cut9 NP593301	Yes
Csm1	Chromosome segregation in meiosis	None			No
Doc1	Component of the anaphase-promoting complex	8731.1	5e-17	*A. thaliana* expressed protein NP565433	Yes
Dmc1	Meiotic recombination	No match		AAB39323	
Hmf1	DNA helicase meiotic crossing over	9793.1	1e-149	*S. cerevisiae* HMF1 NP588310	Yes
Hop1	Homologous chromosome synapsis	None			No
Hop2	Synaptonemal complex component	None			
Isd2	IME2-dependent signaling	None			
Ime2	Serine/threonine kinase; positive regulator of meiosis	1498.1	7e-69	*S. pombe* Ser/Thr protein kinase NP593607	Yes
Ime4	Activates IME1	None			No
Mam1	Monoorientation of sister kinetochores	7984.1	2e-8	*S. pombe* monopolin complex component CAD88639	No
Mei4	Chromosome pairing	None			No
Mei5	Synapsis and meiotic recombination	None			No
Mek1 (Spo13)	Serine/threonine protein kinase	2814.1, 9123.1	2e-35, 2e-34	*H. sapiens* protein kinase CHK2 isoform a NP009125	Yes
Mlh3	Mismatch repair	None			Yes
Mnd1	Meiotic recombination	None			
Mpc54	Meiotic spindle pole body component	0658.1, 9063.1	4e-08, 9e-07	*E. histolytica* myosin heavy chain T18296	Yes
Mre11	Meiotic DNA DSB formation	8730.1	1e-123	*S. cerevisiae* Smc4 NP013187	Yes
Msh4	Meiotic recombination	2230.1, Msh2	1e-40	*S. cerevisiae* homologue of MutS AAA34802	Yes
Msh5	Reciprocal recombination between homologs	9384.1	1e-58	*L. maculans* mismatch repair protein	Yes
Mum2	Premeiotic DNA synthesis	None			
Ndj1	Premeiotic DNA synthesis	None			
Ndt80	Meiosis-specific gene	9915.1	4e-8	*S. cerevisiae* NDT80 P38830	No
Pch2	Pachytene checkpoint	None			No
Rec8	Recombination and sister chromatid cohesion	None			Yes
Rec102	Meiotic DNA DSB formation	None			No
Rec104	Meiotic DNA DSB formation	None			No
Rec107	Meiotic DNA DSB formation	None			No
Rec114	Meiotic DNA DSB formation	None			No
Red1	Synaptonemal complex formation	None			No
Rim4	Regulator of IME2 expression	None			No
Rim11	IME1/transcriptional regulator	4185.1	1e-106	*C. gloeosporioides* protein kinase GSK AAN32716	Yes
Rim15	Required for IME2 expression	7378.1	1e-103	*S. pombe* Cek1p NP588310	Yes
Sae2	Meiotic DNA DSB procesing	None			No
Sae3	Meiotic recombination	None			No
Spo1	Meiotic spindle pole body duplication	3141.1	7e-46	*P. chrysogenum* phospholipase B P39457	Yes
Spo11	Endoribonuclease meiotic DSB formation	1120.1/REC12	2e-10	*C. cinereus* Spo11 AAF26720	Yes
Spo12	Regulates meiosis	None			
Spo19	Meiosis-specific GPI protein	None			No
Spo69	Sister chromatid cohesin component	None			
Sps1	Serine/threonine protein kinase	772.1	3 3-72	*D. discoideum* severin kinase AAC24522	Yes
Ume6	Transcriptional regulator	None			No
Zip1	Synaptonemal complex formation	658.1	8e-24	*E. histolytica* myosin heavy chain T18296	Yes
Zip2	Synaptonemal complex formation	None			No
Zip3	Recombination nodules and synapses	None			No
Sporulation					
Ady2	YaaH family of putative transporters	6043.1	2e-53	*P. anserina* CAD60593	No
Ady3	Mediates assembly of the Don1p containing structure at the leading edge of the prospore membrane via interaction with components of the spindle pole body	None			No
Ady4	Sporulation	None			
Dit1	Spore wall maturation protein	None			
Dtr1	Dityrosine transporter spore wall assembly	1411.1	9e-39	*S. cerevisiae* A (acid, azole) Q (quinidine) resistance NP009599	No
Isc10	Required for spore formation	None			No

Continued on following page

TABLE 57—*Continued*

S. cerevisiae enzyme	Function	*Neurospora* NCU no., gene name	BLAST e value	Best overall BLAST match to *Neurospora*	Homologue/ orthologue in plants and animals?
Pfs1	Prospore formation	None			No
Sma1	Spore membrane assembly	None			No
Smk1	MAPK involved in cell wall formation	9842.1	1e-75	*C. lagenarium* MAPK AAL50116	Yes
Spo14	Phospholipase D	3955.1	4e-51	*S. pombe* putative phospholipase D1 NP592986	Yes
Spo16	Early meiotic protein required for efficient spore formation	None			No
Spo20	v-SNARE, spore wall maturation	9243.1	5e-8	*S. cerevisiae* SNAP 25 homologue NP013730	No
Spo22	Meiosis-specific phospholipase A2 homolog	None			
Spo71	Spore wall maturation	None			
Spo73	Sporulation	None			
Spo74	Sporulation	None			
Spo75	Sporulation	8776.1	1e-33	*S. cerevisiae* NP013993	Yes
Spo77	Sporulation	None			
Spr1	Glucan 1,3-β-glucosidase	3914.1	3e-53	*C. immitis* β-glucosidase 6 AAL09830	Yes
Spr3	Spore wall assembly	3795.1	4e-65	*A. nidulans* septin AAK21000	Yes
Sps18	Transcription factor	7734.1	9e-20	*S. cerevisiae* Gcs1p NP010055	Yes
Sps100	Spore wall maturation	None			No
Ssp1	Spore wall maturation	None			No
Ssp2	Spore wall maturation	None			No
Swm1	Spore wall maturation	None			No

Given that the process of meiosis is similar in distantly related organisms, it is surprising to find that very few proteins specific to meiotic chromosome behavior in *S. cerevisiae* appear to be conserved in *Neurospora* (Table 57). When the proteins are shared between *Neurospora* and *S. cerevisiae*, they are in most cases also present in higher eukaryotes. Thus, while several of the regulators of meiosis appear conserved, many of the other proteins are not. Conspicuously absent from *Neurospora* are proteins with similarity to those required in *S. cerevisiae* for premeiotic DNA synthesis, chromosome segregation and pairing, sister chromatid cohesion, and pachytene checkpoint control. However, a cursory comparison of *Neurospora* proteins to proteins in other higher eukaryotes known to be involved in meiosis identified potential *Neurospora* homologues of gene products required for pachytene checkpoint control (*nim-1*, related to the mouse NimA kinase; CHK2, a *C. elegans* checkpoint control protein required for meiosis; and NCU02814.1), chromatid adhesion (human CDCA1 and NCU06568.1), and chromosome segregation and pairing (mouse SMC11 and NCU01323.1). These data point to *Neurospora* as an evolutionary intermediate between yeast and higher organisms with respect to the proteins involved in meiosis and suggest that there are only a limited number of core meiotic genes conserved among eukaryotic organisms.

Less than half of the conserved *S. cerevisiae* sporulation-specific gene products examined are present in the *Neurospora* genome (Table 57). Similar to meiosis-specific proteins, the signaling components required for sporulation appear to be more strongly conserved than the structural proteins. In any case, proteins that do show similarity are likely to be involved in spore or ascus formation in *Neurospora*.

FUNGAL PATHOGENESIS AND HUMAN DISEASE

Relationship to Animal and Plant Pathogens

Ideally, antifungal compounds should target gene products essential for the growth and development of the fungus in the host without affecting the function of the host cells. In addition, drugs that target a large variety of fungi are especially useful; however, many identified fungal virulence factors are pathogen specific. Development of a broad-acting drug involves the identification of gene products which have essential functions in many fungi but which are absent in or otherwise greatly differ from those found in humans. Nonetheless, this does not rule out the development and application of compounds that exhibit antifungal efficacy, even though they target cellular components common to the pathogen and the host (for example, benzimidazoles that inhibit β-tubulin polymerization). Systems which fungi possess and which mammals lack include cell walls and a variety of membranous components. Even though plants possess carbohydrate polymer-based cell walls, there are sufficient structural differences that provide a basis for specific antifungal agents that can be used for plant protection. Existing drugs take advantage of these differences; for example, azoles and polyenes are compounds that inhibit the function of the fungal cell membrane. Inhibitors of β(1,3)-glucan synthesis, the candins, are being used to inhibit fungal cell wall function (237), and the investigation of cell wall synthesis continues to uncover potential antifungal targets (705).

Animal pathogens. Even though *Neurospora* is not a pathogen, it shares numerous properties with related fungal pathogens, and its genome can be used to identify targets that generally differ between humans and fungi. In addition, the genome sequence allows a comparison between nonpathogens and pathogens, which will aid in the identification of putative virulence factors used to adapt to human hosts. One interesting finding from analysis of the genome sequence is that *Neurospora* has several genes similar to those used to synthesize the polysaccharide capsule, a known virulence factor found in the pathogen *Cryptococcus neoformans*, the causative agent of fatal meningoencephalitis in AIDS patients (Table 58) (850). The capsule of *C. neoformans* surrounds the cell wall and mediates the immune response with the host. Multiple effects of the capsule include the ability to inhibit inflammatory cyto-

TABLE 58. Human pathogenesis-associated genes in *Neurospora*

Neurospora gene	Homologue	Organism	Pathogenesis function
NCU06430.1	*CAP10*	*C. neoformans*	Capsule
NCU02336.1			
NCU05123.1			
NCU02119.1			
NCU05916.1	*CAP59*	*C. neoformans*	Capsule
NCU04473.1	*CAP60*	*C. neoformans*	Capsule
nik-1/os-1	*COS1*	*C. albicans*	Unknown
NCU07221.1	*fos-1*	*A. fumigatus*	Unknown

kine production and complement factor and leukocyte migration, all of which contribute to evasion of the host immune system. In addition, the capsule prevents efficient phagocytosis by macrophages and thus leads to persistence of the pathogen in the host. *Neurospora* possesses proteins (Table 58) similar to those encoded by three genes (*CAP10*, *CAP59*, and *CAP60*) that are implicated in capsule formation in *C. neoformans* and are absent from *S. cerevisiae* (98). The finding that *Neurospora* lacks a fourth cap gene (*CAP64*), a glycosyltransferase required for O acetylation (*CAS1*) of the main capsule polysaccharide, and a UDP-glucuronate decarboxylase (*CAS2*), may explain the absence of this structure in *Neurospora* and also suggests noncapsule functions for *CAP10*/*CAP59*/*CAP60* in filamentous fungi. Laccase is also a known virulence factor for *C. neoformans*, and *Neurospora* has several laccase genes (see below).

Emerging antifungal targets are processes regulated by two-component signal transduction systems (see "Environmental sensing" above) (Table 58). HKs found in *C. albicans* (Cos1/CaNik1) and *A. fumigatus* (Fos-1), which have been shown to affect virulence, have homologues in *Neurospora*: NIK-1 and NCU07221.1, respectively (153, 704). Compounds with activity against these types of systems have been described but are not currently in use (492).

The analysis of the *Neurospora* genome has shown that 63% of the predicted proteins lack homologues in *S. cerevisiae*, a species that is commonly used in antifungal screening processes. Unshared components that are possible antifungal targets include a wealth of two-component signal transduction systems, multicomponent cytoskeletal motor complexes, etc. It is likely that additional potential targets for antifungal development are present within the approximately 40% of the putative *Neurospora* ORFs without homologues in other organisms.

Plant pathogens. Members of the fungal kingdom exhibit a broad spectrum of lifestyles, ranging from saprophytic to obligate parasitism. Furthermore, the same fungal species can sometimes exhibit different lifestyles depending on the host range and/or environmental conditions. In some instances, pathogencity and virulence genes were defined as such because when discovered, their only observable role was in determining the pathogenic attributes of the organism studied. In other instances, analysis of many gene products involved in functions that were not immediately or intuitively associated with pathogenicity later resulted in the appreciation that they are required for the pathogenic phase.

Even though *Neurospora* is not known to be intimately associated with living plants, the genome sequence has revealed the presence of genes whose putative products are highly similar to those shown to be strictly associated with pathogenicity in other organisms (Table 59). Genera containing these proteins include *Botrytis* (292), *Colletotrichum* (360), *Magnaporthe* (195), *Nectria* (311), and others (174). All of the listed putative proteins, with the exception of NCU5730.1, which resembles the *N. haematococca* PEP2 gene product (311), have apparent homologues in *M. grisea* and *F. graminearum*. Furthermore, similar proteins (with the exception of NCU07432.1, a tetraspanin-like protein) are also found in the human pathogen *A. fumigatus*, supporting the possibility that the respective gene products are likely to function in ways that are not limited to plant-pathogen interactions.

TABLE 59. *Neurospora* plant pathogenicity-related proteins

Protein	NCU no.	BLAST match				
		Best overall	*S. cerevisiae*	*S. pombe*	Animal[a]	Plant[b]
Nitrogen deprivation	03370.1	*B. cinerea*; 6e-38	None	None	None	None
Probable cutinase percursor[c]	09663.1	*M. tuberculosis* probable cutinase; 9e-6	None	None	None	None
Pathogenicity-related protein	08038.1	*M. grisea* MAS3/ASG1; 3e-41	None	No hits	None	None
N. crassa Histidine kinase (NIK1)	02815.1	*N. crassa* NIK1 and 8 other fungi; 0.0	3e-16	5e-59	2e-7; *H. sapiens*	2e-68
Membrane protein that mediates differentiation in response to inductive substrate cues	02903.1	*M. grisea* PTH11; 3e-11	None	None	None	None
N. crassa PLS1	07432.1	*Colletotrichum lindemuthianum*; 3e-73	None	None	None	None
Pathogenicity-related protein	06170.1	*M. grisea* MAS3/ASG1; e-17	None	None	8e-9; *D. melanogaster*	None
Pathogenicity protein	05521.1	*M. grisea* PATH531; 4e-70	5e-17	6e-13	5e-19; *M. musculus*	e-13
Pathogenicity protein	02604.1	*M. grisea* PTH10; 2e-55	2e-20	2e-35	7e-16; *D. melanogaster*	2e-11
Putative transcription factor	07846.1	*Botrytis cinerea*; 8e-28	None	None	4e-8; *D. melanogaster*	2e-7
PEP2 Pathogenicity cluster	05730.1	*Nectria haematococca*; 7e-40	None	None	None	None
Putative transcription factor	01422.1	*Botrytis cinerea*; 3e-47	e-9	3e-29	6e-21; *M. musculus*	8e-14

[a] *Caenorhabditis elegans*, *Drosophila melanogaster*, *Mus musculus*, or *Homo sapiens*.
[b] *Arabidopsis thaliana*.
[c] Not required for virulence in *F. solani*, but still a potential cutinase in *Neurospora*.

TABLE 60. *Neurospora* proteins exhibiting similarities to human proteins associated with disease

Enzyme	Disease or disorder	NCU no.	BLAST match			
			Best overall	*S. cerevisiae*	*S. pombe*	Human
With homologues in yeast						
10 chloride channel protein	Dent's disease (renal)	06624.1	*S. pombe*/e-124	e-135	e-162	e-124
DNA ligase I polydeoxyribonucleotide synthase [ATP]	Immunodeficiency	09706.10	*S. pombe*, *R. norvegicus* DNL1/7e-61	4e-46	7e-61	3e-60
		06481.1	*S. cerevisiae* Cdc9/0.0	0.0	e-116	e-146
Calcium/calmodulin-dependent protein kinase type I (CAM kinase 1)	Cancer	09212.1	*S. pombe* Srk1/e-133	8e-92	e-133	5e-57
		09123.1	*E. nidulans* KCC1/e-166	e-96	2e-64	7e-69
Probable ATP-dependent permease (multidrug resistance-associated protein 1)	Cancer related	09012.1	*S. cerevisiae* YCFI/0.0	0.0	0.0	0.0
Probable ATP-dependent permease (multidrug resistance-associated protein 2)	Cancer related	08358.1	*S. cerevisiae* YBT1/0.0	0.0	e-146	e-180
Copper-transporting ATPase (copper pump 2)	Wilson's disease	07531.1	*S. cerevisiae* Pca1/0.0	0.0	7e-70	8e-66
		08341.1	*A. thaliana* RAN1/0.0	e-175	0.0	e-170
Carnitine acetyltransferase	CPT2 deficiency/metabolic	08002.1	*S. cerevisiae* Cat2/e-123	e-123	None	6e-86
TSC2	Tuberous sclerosis type 2	4105.1	*S. pombe* SPAC630.13c/e-100	None	e-100	NP_066399.1; 3e-36
COT1 kinase	Myotonic dystrophy	07296.1	*S. cerevisiae* Cbk1/e-151	e-151	e-144	2e-67[a]
Without homologues in yeast						
Tripeptidyl-peptidase I precursor, TPP-I (CLN2)	Ceroid-lipofuscinosis	08656.1	*A. oryzae* aorsin/7e-33	None	None	4e-18
		08418.1	*A. oryzae* tripeptidylpeptidase A/e-145	None	None	5e-59
		04903.1	*C. brassiana* tripeptidylpeptidase precursor/1e-89	None	None	5e-38
Calpain-like thiol protease	Limb girdle MD$^+$ 2A-CAPN3	1151.1	*N. crassa* NCU00251.1/e-120	None	None	5e-38
		0251.1	*N. crassa* NCU01151.1/e-110	None	None	9e-30
		3355.1	*N. crassa* NCU01151.1/8e-98	None	None	8e-37

[a] Also similar to human NDR kinase (e-108).

In addition to the listed gene products, many components known to be involved in both pathogen- and non-pathogen-related functions of fungi are clearly present in *Neurospora*. The potentials for metabolism or efflux of toxic plant metabolites (e.g., P450 cytochrome oxidases and ABC transporters, respectively), biotic sensing and stress signal transduction, and polyketide biosynthesis are just a few examples of cellular machinery capable of performing such functions that are present in *Neurospora*. It is clear that the differences in the gene content of *Neurospora* and *Magnaporthe* will very soon be a focus of investigation. These fungi are considered close relatives but are estimated to have evolved from a common ancestor 50 to 150 Mya ago. Given this evolutionary distance, it is not surprising that they appear to share only about 60% of their genes. The question of whether their common ancestor was a plant pathogen, a saprophyte, or a nonpathogenic symbiont may remain unanswered until additional fungal genome sequences are available. The presence of apparent homologues of genes for secondary metabolism and plant pathogen virulence factors in *Neurospora* suggests that the lineage leading to *Neurospora* is just as likely to have lost its ancestral ability to parasitize plants as the lineage leading to *Magnaporthe* is to have gained parasitism (269).

Human Disease Genes

There are over 200 predicted *Neurospora* proteins exhibiting significant similarity to human gene products that, when altered, have been demonstrated to cause disease (Table 60). The range of proteins associated with human diseases that is represented in the *Neurospora* genome is vast and includes those implicated in diseases of the immune system, metabolic disorders, neurological impairments, and cancer (for a list of examples, see Table 60). Although many of these proteins have counterparts in *S. cerevisiae* and *S. pombe*, some of the features of *Neurospora* (multinucleate, multicellular, and exhibiting distinct and diverse developmental phenotypes) provide an attractive platform for investigating the functions of these genes with the intention of increasing our understanding of the relevant human disease. Furthermore, the observation that in some instances the *Neurospora* proteins have high similarity to proteins in one (but not both) of the yeasts (e.g., TSC2 and carnitine acetyltransferase) may have evolutionary significance. In many cases, *Neurospora* has more than one predicted protein that is associated with the same disorder (e.g., DNA ligase, CAM kinase 1, and copper-transporting ATPase), raising the question of whether both (or any) of the structurally related proteins are relevant to the respective human diseases.

Two human disease genes associated with ceroid lipofusci-

nosis and limb-girdle muscular dystrophies (LGMD) are represented in the *Neurospora* genome but are absent from both *S. cerevisiae* and *S. pombe*. Ceroid lipofuscinosis is an inherited degenerative disease characterized by neuronal cytoplasmic inclusions that stain positively for ceroid and lipofuscin (168). Affected individuals develop retinal degeneration, seizures, myoclonus, ataxia, rigidity, and progressive dementia. Individual genes mutated in six forms of ceroid lipofuscinosis have been identified. The products of these genes fall into two distinct categories comprising either soluble lysosomal enzymes (CLN1 and CLN2) or predicted transmembrane proteins of unknown structure and function (CLN3, CLN5, CLN6, and CLN8). *Neurospora* has three predicted polypeptides that show significant structural similarity to the CLN2 class of proteins. The *CLN2* gene encodes tripeptidyl peptidase 1, which cleaves tripeptides from the N-terminus of small proteins before their degradation by other lysosomal proteases.

The LGMDs are a heterogenous group of genetically determined progressive disorders of skeletal muscle with a primary or predominant involvement of the pelvic or shoulder girdle musculature. At least 15 genes have been identified which, when mutated, can cause LGMD, among them calpain-3, a Ca^{2+}-activated cysteine protease, responsible for LGMD2A (650). The fact that two such proteins (NCU03355.1 and NCU01151.1) are present in *Neurospora* but not in yeasts is in line with the general observation that significantly more Ca^{2+}-signaling proteins are present in *Neurospora* than in *S. cerevisiae* (Table 47) A. Zelter, M. Bencina, B. J. Bowman, O. Yarden, and N. D. Read, unpublished data).

Many of the other gene products known to be associated with LGMD (e.g., dysferlin, telethonin, myotilin, FKRP, and sarcoglycan beta [894]) do not have structural homologues in *Neurospora* or in *S. cerevisiae* and *S. pombe*. The absence of these other LGMD proteins in fungi could be because they are involved in sarcomeric functions, and fungi lack sarcomeres. Should these proteins be involved in additional (nonsarcomeric) cellular activities, it is conceivable that their *Neurospora* functional homologues would not be identified by BLAST-based structural similarity searches.

The observation that six of the unique *Neurospora* gene products associated with human disease are proteases is intriguing. It could be argued that *Neurospora* would be expected to have an abundance of proteases due to its ability to metabolize a very diverse set of nutrients compared to yeasts. However, the six proteases are not predicted to be secreted (Table 26) and are therefore not likely to be involved in extracellular digestion processes. This suggests the existence of fundamental cellular processes involving these proteases that are shared between *Neurospora* and humans but are absent from yeasts; it also suggests that *Neurospora* is an excellent system for the study of these processes.

The observation that *Neurospora* has an abundance of transporters (see "Metabolic processes and transport" above) (269) is also relevant to human disease. For example, there are at least two highly similar copper transporters (NCU07531.1 and NCU08341.1) associated with Wilson's disease, an inherited disorder that causes the body to retain copper and can result in severe brain damage, liver failure, and death. The structural and functional dissection of these two transporters may provide important information concerning copper transport and the metabolic consequences of impaired copper transport in eukaryotes. The finding of two related proteins in *Neurospora* suggests the potential for studying subtleties in the copper uptake process in this organism. Another exciting medically related potential of studying the multitude of transporters in *Neurospora* is the elucidation of drug uptake/resistance processes transporters (e.g., NCU09012.1 and NCU08358.1), which are associated with the success or failure of drug treatments in humans. These, of course, are also of prime interest from the viewpoint of successful application of antifungals (see "Relationship to animal and plant pathogens" above).

Some of the *Neurospora* proteins that have significant homology to human disease gene products are also very similar to additional human proteins. For example, the *Neurospora* COT1 kinase (877) is highly similar to the human myotonic dystrophy kinase but may be even more homologous to the human NDR kinase, which is involved in cell proliferation and tumor development (779). Thus, on the one hand, the similarity of *Neurospora* proteins to specific human counterparts poses an exciting avenue for functional analysis in relation to human diseases. However, in most cases, determining the extent of functional homology of the *Neurospora* (or any given model organism) gene product to the human disease-associated protein awaits analysis.

PERSPECTIVES AND FUTURE DIRECTIONS

This analysis of the *Neurospora* genome is provided as a starting point for further understanding of filamentous fungi and other multicellular eukaryotes and to facilitate progress not only in basic research but also in study of animal and plant pathogenesis, biotechnology, and biodegradation. With just over 10% of the gene complement of 10,000 ORFs analyzed, the investigation has already revealed many unexpected and exciting avenues for future studies. Many predicted *Neurospora* proteins have no homologues in the yeasts *S. cerevisiae* and *S. pombe* but are similar to proteins in animals, plants, and other filamentous fungi. Furthermore, *Neurospora* contains numerous gene products that are found in the two yeasts but for which the *Neurospora* protein is a better match for the corresponding animal or plant protein. These features suggest that *Neurospora* is an excellent model system for studies of numerous aspects of biology.

The related arenas of chromosome structure and gene regulation appear ripe for future productive analysis. For example, *Neurospora* possesses a virtually complete repertoire of vertebrate histone-modifying enzymes, suggesting that this organism may be an excellent system in which to describe the histone code and its role in epigenetic regulation of gene expression and development. The compilation of transcription factors offers clues that will benefit researchers working on a multitude of biological processes that contain a transcriptional regulatory component. Remarkably, only 14 of the 186 annotated transcription factor genes had previously been cloned and characterized. The $Zn(II)_2Cys_6$ fungal binuclear cluster family appears to have several family members that have partners, suggesting their involvement in a combinatorial regulatory process. The C2H2 zinc finger transcription factor family was divided into two groups, one that was most similar to factors from unicellular yeasts and one that was most similar to

proteins from filamentous fungi and animals. This dichotomy could represent a point of divergence in the evolution of promoters. In the future, it will be interesting to determine which genes are controlled by these two groups of factors and to investigate their relative importance to gene regulation in *Neurospora*.

The formation and maintenance of silent chromatin in *Neurospora* appears to be a variation of the HP1/Swi-6-mediated pathway of *S. pombe*. A connection between DNA methylation and the HP1/Swi-6 pathway was first experimentally determined for *Neurospora* and has now also been established in plants and animals. In contrast to *Neurospora*, the formation of silent chromatin in *S. cerevisiae* relies on complexes of Sir proteins, which appear absent from the *Neurospora* genome. While some of the processes are shared between *Neurospora*, fission yeast, plants, and animals, some differences have also been revealed from the genome sequence. It will be interesting to determine exactly how *Neurospora* recognizes and maintains methylated DNA, given the fact that it lacks recognizable homologues of plant and animal proteins involved in methylated DNA binding. In addition, the cytosine DNA methyltransferases of *Neurospora* are distinct from those found in other eukaryotes.

Besides DNA methylation, an additional three distinct genome defense mechanisms have been identified in *Neurospora*. RIP is a process that extensively mutates and methylates duplicated DNA sequences in the haploid genome during the sexual phase of development. RIP is thought to protect the genome from invasion by transposons. Two other mechanisms which rely on RNA-mediated gene silencing have also been discovered: quelling and meiotic silencing. In fact, genome analysis indicates that there are orthologues of the known components of eukaryotic RNA-dependent silencing in *Neurospora* (Argonaute-like translation initiation factors, Dicer-like RNases, etc.). In addition, further analysis of the *Neurospora* proteins indicates that they, like the other fungal proteins, fall into two distinct clades, suggesting that they function in similar but distinct pathways. These predictions can now be tested.

The recombination machinery of *Neurospora* is more like that of other filamentous fungi and mammals than that of *S. cerevisiae;* which has many genes required for recombination that have no identified homologue in the more complex organisms. Even *S. cerevisiae* Spo11p, responsible for initiating recombination by a DSB, has limited homology to the equivalent proteins of higher eukaryotes and requires several accessory proteins that are not needed in other organisms. These data suggest that recombination may be achieved in a variety of ways in different species and emphasize the need for detailed analysis in other tractable species such as *Neurospora*. Additionally, *Neurospora* has genes known to exert a level of recombination control (133, 134) not seen in *S. cerevisiae*, and the question whether such regulation occurs in other eukaryotes might be answered by their cloning.

The genome contains a unique diversity of proteins, both animal-like and plant-like, involved in various areas of metabolism that will make *Neurospora* an attractive eukaryotic system for study. However, functional studies are needed to fully exploit and augment the information from the genome sequence. For example, the identity of the sensors for carbon, nitrogen and sulfur, as well as the subsequent signal transduction pathways that regulate the assimilation of these critical metabolites, cannot be predicted from genome analysis and remain unknown. Similarly, an accurate description of the mitochondrial proteome is needed, since analysis of the genome sequence did not yield a complete inventory of the proteins in this organelle.

Neurospora possesses a wide array of environmental sensory capabilities and promises to be an outstanding model for investigations of signaling. The larger number of HKs and GPCRs in *Neurospora* compared to *S. cerevisiae* and *S. pombe* predicts the existence of novel signaling pathways and/or networking interactions not found in yeasts. The completely unexpected identification of cryptochrome and phytochrome homologues has sparked renewed interest in the photobiology of *Neurospora*, leading investigators to look more closely at the role of light in the development and real-world biology of this organism and of filamentous fungi in general. Homologues of many of the key proteins involved in the release of Ca^{2+} from internal Ca^{2+} stores could not be recognized in *Neurospora*, indicating that there are significant differences between the Ca^{2+}-signaling machinery in filamentous fungi and that in animals and plants. The different intracellular Ca^{2+} release mechanisms in filamentous fungi may provide novel targets for antifungal agents.

The polarized hyphal cell growth of *Neurospora* is reflected in its complement of proteins involved in generating the hyphal structure that in many cases is quite distinct from proteins that carry out similar functions in yeast cells. Analysis of cell wall polymer synthesis genes demonstrated that *Neurospora* lacks the (1,6)β-glucan synthesis pathway found in *S. cerevisiae*. In addition, components of the chitin synthesis machinery are more complex in *Neurospora*. Together, these results confirm the long-held idea that cell wall assembly in yeasts is not a universal model for fungal cell wall assembly.

Although *S. cerevisiae* has polarized modes of growth—production of psuedohyphae—the mechanisms used to generate these structures and the true hyphae in filamentous fungi are very different. The genome analysis bears this out, since key transcription factors necessary for the switch from budding to filamentous growth in yeast are not present in *Neurospora*. In addition, proteins involved in bud site selection are absent from *Neurospora*, suggesting novel processes for the determination of emerging hyphal branches and their spacing. The presence of the small GTPase Rac in the *Neurospora* genome, which is absent from *S. cerevisiae*, may have implications for actin-dependent polarized hyphal cell growth and division. In addition, *Neurospora* has a larger complement of cytoskeletal components than does *S. cerevisiae*. An unusual myosin protein containing a chitin synthase domain has been identified and has been shown to function in cell wall synthesis and integrity in other filamentous fungi.

An intriguing question arises from analysis of the cyclin/CDK machinery. *Neurospora* has one Cdk (CDC-2) that is primarily responsible for cell cycle progression. However, it has only three cyclins, in contrast to the nine in *S. cerevisiae* and three in *S. pombe*. It may be that RIP has limited the set of *Neurospora* cyclins. However, given the more complex life cycle of *Neurospora*, this finding also suggests the possibility of novel mechanisms for regulation of cell cycle progression. In addi-

tion, functional analysis of the cyclin and Cdk genes may reveal the mechanisms leading to the observed asynchrony between the cell cycle and cell division in *Neurospora*.

The differentation of asexual spores, or conidia, has been well characterized in *Neurospora* and *A. nidulans*. The genes involved in these two processes appear to be quite different, suggesting that they have evolved independently. The production of sexual spores, ascospores, requires meiosis. Comparison of genes known to be involved in meiosis in *S. cerevisiae* revealed that only one transcription factor is conserved between the two species (Ndt80p) and that a key transcriptional regulator of meiotic genes in *S. cerevisiae* (Ume1p) is absent from *Neurospora*.

In most cases, the roles of *Neurospora* genes exhibiting structural similarity to human disease genes have yet to be determined. Are the biochemical and cellular functions of these genes similar to those observed in humans? If so, the amenability of *Neurospora* to genetic and physiological manipulations may prove extremely useful in the functional dissection of these proteins (especially in cases when these are not produced by the yeast model systems) with the long-term objective of intervening in the function of these genes or gene products. Are the consequences of mutations in such genes as detrimental in filamentous fungi as they are in higher eukaryotes? The answer to this question not only may provide additional insight into the biology of fungi but also may help to establish new links between developmental complexity and genome evolution.

Finally, as full fungal genome sequences become available, there will be increased interest in and need for comparative analysis at all levels (from locus organization to nucleotide sequence [see also reference 876]). In the future, data analysis rather than data acquisition will be the limiting factor to progress. Appropriate software must be developed to minimize the need for repeated manual (or even program-based) analyses, since many of the requirements can be defined a priori. There are numerous choices to be made regarding how genome data are curated, maintained, and funded, with consideration given to existing models for effectively organizing the data. Addressing these issues in a timely and organized manner represents an important milestone in the quest to maximize and speed the application of fungal genome information to medicine, agriculture, industry, and the environment.

ACKNOWLEDGMENTS

We are indebted to Matthew Springer and David Perkins for the use of previously published figures and legends, and we thank David Jacobson and David Perkins for providing a high-resolution version of Fig. 1. We thank Markus Aebi and Albert Courey for their comments and suggestions concerning the Dolichol pathway and the transcription factor analyses, respectively.

This analysis was supported by National Institutes of Health grant GM48626 and National Science Foundation grant MCB-0296055 (to K. A. Borkovich), National Institutes of Health grant S06 GM53933 (to L. A. Alex), Israel Science Foundation (to O. Yarden), Royal Society grant 066392/Z/01/Z and the Darwin Trust (to N. D. Read), Deutsche Forschungsgemeinschaft grant SPP1111 (to S. Seiler), National Institutes of Health grants GM58529 and NS39546 (to D. Bell-Pedersen), National Science Foundation grant MCB-0235871 (to M. Plamann), National Research Initiative of the USDA Competitive Research Grants Office 2001-35100-10618 (to N. Plesofsky), Deutsche Forschungsgemeinschaft grant Schu698/3 (to G. Mannhaupt and U. Schulte), Natural Sciences and Engineering Research Council 3041 and the Canadian Institutes of Health Research MOP-13170 (to F. E. Nargang), National Institutes of Health grant R37 GM34985 (to J. C. Dunlap), National Science Foundation grant MCB-0084509 (to J. J. Loros), National Institutes of Health grant MH44651 and the Norris Cotton Cancer Center Core Grant at Dartmouth Medical School (to J. C. Dunlap and J. J. Loros), Australian Research Council grant DP0345994 (to D. E. Catcheside), National Institutes of Health grant GM58770 (to R. Aramayo), National Institutes of Health grant GM062377 (to M. Polymenis), and National Institutes of Health grant GM35690 (to E. U. Selker).

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