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Environmental Sensing and Signal Transduction Pathways Regulating Morphopathogenic Determinants of *Candida albicans*

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INTRODUCTION

Opportunistic fungal pathogens, such as *Candida albicans*, are found in the normal gastrointestinal flora and the oral mucosa of most healthy humans. However, in immunocompromised patients, bloodstream infections often cause death, despite the use of antifungal therapies (152). The underlying molecular mechanisms for survival inside the human body and adaptation to various environments are probably distinct but overlapping. Dietary factors, such as an excess of or deficiency in certain nutrients, may alter the endogenous microbial flora. Mechanical factors, such as trauma or occlusive injury, can also alter the microenvironment, deplete the system of "friendly bacteria," and enable the pathogenic fungus to take over. Immunocompromised or immunosuppressed persons, including AIDS patients, neonates, and transplant recipients, are also particularly susceptible to fungal infections.

The most common systemic fungal infection is candidiasis, which accounts for well over half of these invasive mycoses. A single species, *C. albicans*, causes the majority of these infections. Its success stems in part from its capacity to live as a benign commensal in a variety of body locations, most notably the oral cavity, genitalia, and gastrointestinal tract (272). *C. albicans* expresses various traits critical for existence on mucosal surfaces, where a constant but dynamic interplay occurs between innate and acquired host defense mechanisms. The pathogenic *Candida* species also establish well-developed biofilms, which occur easily on various implants and are resistant to antifungal agents (76). The nature of disease resulting from tissue invasion by this organism is complex and depends on a variety of physical and physiological conditions in the host and on specific *C. albicans* traits. The capacity of *C. albicans* to rapidly acquire resistance to antifungal drugs, such as amphotericin B, flucytosine, and a series of azoles, means that continued development of new antifungals remains an important focus for clinicians and pharmaceutical companies.

An important feature of *C. albicans*, relevant to its pathogenesis, is its ability to switch between different morphological forms. *C. albicans* can grow in a single-celled, budding yeast form (blastospore) or in a filamentous form (including both pseudohyphae and true hyphae) (31). A crucial component of this versatility is the ability to survive as a commensal in several anatomically distinct sites, each with its own specific set of environmental pressures. Thus, *C. albicans* must be able to adapt its growth to a range of physiological extremes. To achieve adaptability, the fungus has evolved sophisticated mechanisms of sensing and responding to environmental cues by activating developmental switches that result in coordinated changes in cell physiology, morphology, and adherence. Progress in understanding many aspects of the biology of *C. albicans* has been hindered by the inability to carry out simple, large-scale genetic screens because of the diploid nature of this organism. A major breakthrough in assessing the contribution of specific genes to morphogenesis and virulence occurred with the development of transformation protocols and methods of deleting both alleles of a gene sequentially. Also, whole-genome microarray analysis has now become an important tool for probing signal transduction pathways during morphogenesis in *C. albicans* (102).

Additional interest in the molecular mechanisms of *C. albicans* morphopathogenic determinants originated from the necessity of identifying new drug targets due to increased drug resistance in clinical isolates. There is hope that recently developed techniques of manipulating *C. albicans* and the sequencing of its whole genome will lead to a thorough understanding of its virulence and biology, thus offering the possibility of a knowledge-based approach to the development of novel antifungal agents. A major strategy for determining virulence genes as molecular targets for antifungal drugs and vaccines is to identify a specific biochemical or structural target unique to *C. albicans* (or to fungi in general) in an attempt to specifically and selectively disrupt them and determine their effects on virulence.

In this review, we focus on recent advances in the environmental sensing and signal transduction pathways that mediate the morphogenesis and pathogenesis of *C. albicans*. Where possible, we compare the pathways of *C. albicans* with the analogous pathways/genes in *Saccharomyces cerevisiae*.

DIMORPHISM, AN IMPORTANT VIRULENCE FACTOR

The terms "dimorphism" and "dimorphic fungus" (i.e., existing in two morphological forms) are commonly accepted in reference to *C. albicans*. Strictly speaking, however, this fungus has the ability to adopt a spectrum of morphologies; thus, *C. albicans* can be considered a "polymorphic" or "pleomorphic" organism (71, 289). The production of germ tubes results in conversion to a filamentous growth phase or hypha, also called the mycelial form. The formation of pseudohyphae occurs by polarized cell division when yeast cells growing by budding have elongated without detaching from adjacent cells. Under certain nonoptimal growth conditions, *C. albicans* can undergo the formation of chlamydospores, which are round, retractile spores with a thick cell wall. These morphological transitions often represent a response of the fungus to changing environmental conditions and may permit adaptation to a different biological niche. The transition from a commensal to pathogenic lifestyle may also involve changes in environmental conditions and dispersion within the human host. Although progress has been achieved in recent years, the molecular mechanisms governing these morphogenetic conversions are still not fully understood, partly due to the difficulty of genetic manipulations with *C. albicans* (164), an issue we address briefly below.

GENETIC MANIPULATION WITH *C. ALBICANS*

Sequencing of the genome of *C. albicans* has recently been completed. *C. albicans* has a diploid genome consisting of eight pairs of chromosomes that can be separated by pulsed-field gel

electrophoresis. With a size of ~16 Mb, the haploid genome is slightly larger than that of the model yeast, *S. cerevisiae*. More information on the *C. albicans* genome can be found at www.candidagenome.org (68). Many genes are conserved between *S. cerevisiae* and *C. albicans*, and it is based on this similarity that the mechanisms of many biological processes in *C. albicans* have been discovered. As highlighted throughout this review, in many cases, although the specific components of relevant signaling pathways are conserved, molecular mechanisms and environmental signals have often diverged, most likely because of the coevolution of *C. albicans* and its human host.

C. albicans poses special problems for scientists interested in studying gene function because it is diploid and because CUG is translated into a serine instead of a leucine (164). To analyze the function of a gene, one must disrupt each of the two alleles by transformation. Although methods of transformation (spheroplast-polyethylene glycol, lithium acetate, and electroporation) are patterned after those used with *S. cerevisiae*, the transformation efficiency is poor. For the study of gene function in *C. albicans*, a number of disruption protocols and selectable markers are commonly utilized (300). The most widely used marker is the *URA3* gene, which encodes orotidine-5′-phosphate decarboxylase and confers uracil prototrophy. However, this marker must be used with caution; *URA3* expression levels can affect virulence and are susceptible to chromosome position effects, thereby complicating the analysis of strains constructed with *URA3* as a selectable marker (62, 261, 281, 292). To avoid the use of the *URA3* marker, various other auxotrophic markers, as well as dominant markers, have been developed and are currently used (206, 224, 241, 263). To study the functions of essential genes, the *C. albicans MET3* promoter, which is regulated by the level of methionine and/or cysteine in the medium, or the tetracycline on/off system can be used. These systems allow conditional expression so that the consequences of depletion of a gene product may be investigated (57, 231, 254). In order to study the expression of certain genes at either the RNA level or the protein level, a number of *C. albicans*-optimized reporter constructs, or tags, have been generated (32). The development of these molecular tools has greatly accelerated the elucidation of morphogenesis and pathogenesis in *C. albicans*.

ENVIRONMENTAL SENSING PATHWAYS REQUIRED FOR MORPHOGENESIS AND PATHOGENESIS

All organisms, from bacteria and yeast to higher eukaryotes, respond to changes that occur in the environment. In *C. albicans*, the yeast-to-hypha transition is triggered by various environmental cues, such as serum, *N*-acetylglucosamine (GlcNAc), neutral pH, high temperature, starvation, CO_2, and adherence. In recent years, receptors/sensors that may mediate environmental responses have been identified and partially characterized. Many in vivo and in vitro experiments point to a prominent role for amino acids as nitrogen sources and as ligands for membrane receptors involved in the regulation of cellular morphology and virulence (summarized in Fig. 1 and Table 1). In the following sections, we highlight work aimed at identifying receptors, ligands, and signaling pathways involved in the sensing of environmental signals in *C. albicans*.

Amino Acid Availability Regulates Morphogenesis of *C. albicans*

Amino acid sensing by Csy1. Serum- and amino acid-based media are known to induce filamentous growth in *C. albicans*. Although the mechanism by which amino acids induce filamentation is not well established, several genes have been implicated in the amino acid response. In *S. cerevisiae*, an amino acid-sensing complex (SPS sensor for Ssy1, Ptr3, and Ssy5) that is required for amino acid transporter expression through the induction of proteolytic cleavage of two latent transcription factors has been described (4, 94). Recently a similar sensing complex in *C. albicans* was described (45). Furthermore, it was shown that the amino acid sensor Csy1 (the homolog of the yeast protein Ssy1) plays an important role in filamentation. Loss of Csy1 results in a lack of amino acid-mediated activation of amino acid transport and a lack of induction of transcription of specific amino acid permease genes. Csy1 mutants also show altered colony morphology and hyphal formation in serum- and amino acid-based solid media but not in media that do not contain amino acids. As in *S. cerevisiae*, Csy1 appears to discharge its critical role in amino acid transport and filamentation by promoting proteolytic cleavage of two latent transcription factors, Stp1 and Stp2. Truncated Stp2 migrates to the nucleus and induces the expression of genes required for amino acid uptake, and truncated Stp1 induces genes required for the degradation of extracellular proteins (*SAP2*) and for uptake of peptides (*OPT1*) (198). The Ljungdahl group also discovered that Csh3, the functional homolog of *S. cerevisiae* Shr3, which is involved in endoplasmic reticulum exit after packaging, is essential for the proper uptake and sensing of extracellular amino acids and that a null mutant is unable to switch morphology in response to inducing amino acids (197).

Amino acid sensing (and transport) by the general amino acid permease Gap1. In *S. cerevisiae*, the Gap1 protein functions not only as an amino acid transporter but also as an amino acid sensor for rapid activation of a fermentable growth medium-induced signaling pathway that controls protein kinase A (PKA) targets (83, 140). Receptors with dual roles as sensors have been dubbed "transceptors" (124). Our group has discovered that the *C. albicans* Gap1 transporter, which is a functional homolog of the budding yeast gene, may also be a transceptor important for initiating signal transduction pathways resulting in morphogenesis and virulence. *GAP1* was isolated in a screen to identify and characterize genes that could be involved in the regulation of morphogenesis and virulence induced by GlcNAc, an important hyphal induction regulator of *C. albicans* (35) (see below). A null mutant of *GAP1* has a defect in filamentation under nitrogen starvation conditions, as well as upon the addition of GlcNAc (35). Addition of serum, however, still resulted in the induction of filamentation in the *gap1* mutant, suggesting that Gap1 is not required for serum-induced hyphal formation. As in *S. cerevisiae*, the expression of *GAP1* is strongly repressed in medium containing ammonium as a good nitrogen source. How exactly amino acid transport (and/or sensing) results in hyphal formation remains to be determined. One interesting link is the fact that GlcNAc induces *GAP1* expression at the yeast-to-germ-tube transition. This induction depends on Cph1, the transcrip-

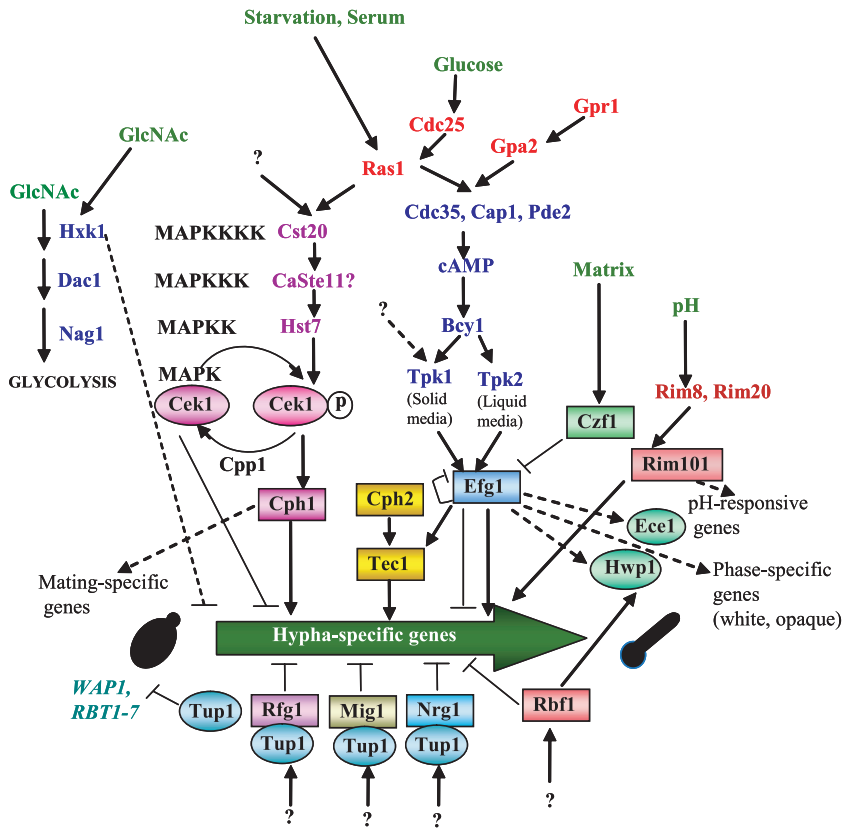

FIG. 1. Regulation of dimorphism in *C. albicans* by multiple signaling pathways. The Cph1-mediated MAPK pathway and the Efg1-mediated cAMP pathway are well-characterized signaling pathways in dimorphic regulation. In *C. albicans*, Ras1 is an important regulator of hyphal development and likely functions upstream of both pathways. In the cAMP-PKA pathway, two catalytic subunits or isoforms of PKA, Tpk1 and Tpk2, have differential effects on hyphal morphogenesis under different hypha-inducing conditions. The MAPK cascade includes Cst20 (PAK), Hst7 (MAPKK), Cek1 (MAPK), and the downstream transcription factor Cph1, which is a homolog of the *S. cerevisiae* transcription factor Ste12. Transcription of the hyphal regulator *TEC1* is regulated by Efg1 and Cph2. Rim101 or Czf1 may function through Efg1 or act in parallel with Efg1. Tup1 is the negative regulator of the hyphal transition. Tup1, recruited by Rfg1, Nrg1, or Mig1, and Rbf1 are also implicated in dimorphic transitions. GlcNAc-inducible hexokinase, Hxk1, plays a negative role in hyphal development under certain conditions. Cell wall proteins (*HWP1* and *ECE1*, etc., which are involved in adherence) are also regulated by Efg1. Transcription factors are shown in rectangular boxes.

tion factor that is the downstream target of the mitogen-activated protein kinase (MAPK) pathway (see below). This may indicate that Gap1-mediated filamentation is regulated by this pathway.

Amino acid sensing by Gpr1. In *S. cerevisiae*, ScGpr1 is a G protein-coupled receptor that functions upstream of the cyclic AMP (cAMP)-PKA pathway and promotes a rapid cAMP increase after the addition of glucose or sucrose (156). In contrast, *C. albicans* Gpr1 is not required for the glucose-induced increase in cAMP (193) but seems to directly or indirectly sense methionine (193). Addition of methionine to wild-type cells results in a rapid internalization of Gpr1, reminiscent of ligand-induced internalization, a typical feature of GPCR systems in higher eukaryotic cells. Gpr1 is also required for the methionine-induced yeast-to-hypha transition (193). Further research is required to determine the mechanism by which Gpr1 activates the PKA pathway and what the role of methionine is in this mechanism. Interestingly, a role for methionine in morphogenesis is not limited to *C. albicans*. Recently, Heitman and colleagues discovered that the addition of methionine results in the internalization of a G protein-coupled receptor (Gpr4) in *Cryptococcus neoformans* and that a *gpr4* null mutant is still responsive to glucose (323). We discuss the Gpr1 receptor in more detail in "cAMP-PKA Pathway," below.

Ammonium sensing by Mep2. Ammonium transport in *C. albicans* is mediated by Mep1 and Mep2 (33). Recently it was shown that Mep2, but not Mep1, also functions as a receptor for the induction of filamentous growth under nitrogen starvation conditions, placing Mep2 in the transceptor family of proteins (33, 124). More detailed analysis has shown that, as with the budding yeast protein ScGap1, the C terminus of Mep2 is required for proper sensing but not for transport (33). Signaling by Mep2 functions through both the MAPK and the cAMP-PKA pathways and is Ras1 dependent (see below for more information on the Ras pathway). Under high ammonium conditions, when expression of Mep2 is repressed, filamentation is blocked. A role for Mep2 in polarized morphogenesis is supported by work with both budding yeast and *Ustilago maydis*. In *S. cerevisiae*, ScMep2 is required for pseudohyphal induction (186). The Mep2 homolog of *U. maydis* (Ump1) is able to complement an *S. cerevisiae mep1 mep2 mep3* strain both for ammonium transport and for pseudohyphal induction (269). Interestingly, mutation of a putative PKA phosphorylation site in either Mep2 or Ump1 specifically

TABLE 1. Major morphopathogenic determinants in *C. albicans*

C. albicans protein	*S. cerevisiae* homolog[a]	Protein function	Reference(s)
Gpa2	Gpa2	α subunit of G protein	193, 249
Gpr1	Gpr1	G protein receptor	193, 249
Ras1	Ras2	GTPase	92
Cdc25	Cdc25	Ras-GEF	112, 193
Cdc35/Cyr1	Cyr1	Adenylate cyclase	139, 243
Srv2	Cap1	Adenylate cyclase-associated protein	13
Pde2	Pde2	High-affinity phosphodiesterase	16, 143
Flo8	Flo8	Transcription factor	56
Tpk1/Tpk2	Tpk1/Tpk2	Catalytic subunit of cAMP-dependent PKA	37, 66, 276
Bcy1	Bcy1	Regulatory subunit of cAMP-dependent PKA	58
Cst20	Ste20	PAK	153, 171
Hst7	Ste7	MAPKK	171, 266
Cek1	Fus3/Kss1	MAPK	69
Cpp1	Cpp1	MAPK phosphatase	114, 256
Tec1	Tec1	Transcription factor of TEA/ATTS family	257
Cph1	Ste12	Transcription factor	181, 195
Efg1	Sok2/Phd1	Transcription factor of HLH family	36, 175, 285, 294
Cph2	Hms1	Transcription factor of HLH family	169, 170
Fkh2	Fkh2	Forkhead transcription factor	29
Mcm1	Mcm1	Transcription factor of MADS box family	245
Cdc42	Cdc42	Rho G protein	119, 305
Cln2	Cln2	G_1 cyclin	185
Tup1	Tup1	Transcriptional repressor	41, 210
Nrg1	Nrg1	Transcription factor with zinc finger domain	43, 210, 211
Mig1	Mig1	Transcription factor with zinc finger domain	210
Rfg1	Rox1	DNA-binding partner of Tup1	146, 148
Rbf1	Rbf1	RPG box-binding factor	137, 262
Rap1	Rap1	Transcriptional silencer	34
Rad6	Rad6	Transcription-repressing factor	176
Hog1	Hog1	MAPK for oxidative stress	2, 3, 227
Ssn6	Ssn6	Forms complex with Tup1	136
Sln1	Sln1	Histidine kinases	212
Hxk1	No known homolog	GlcNAc kinase	162, 267, 326
Hwp1	No known homolog	Hyphal wall protein	262, 283, 303
Int1	Int1	Surface glycoprotein	7, 25, 98
Als1	Als1	Adhesin factor	95, 329
Sap1 to Sap9	No known homolog	Aspartyl proteinases	134, 165, 255
Plb1	Plb1	Phospholipase	173, 208
Gcn4	Gcn4	General amino acid control	302
Gcn2	Gcn2	General amino acid control	301
Gap1	Gap1	General amino acid permease	35
Csy1	Csy1	Amino acid sensor	45
Rim20	PalA*	Proteolysis of Rim101	72, 73
Rim8	PalF*	Proteolysis of Rim101	72, 73
Rim101	PacC*	Zinc finger transcription factor	72, 73, 87, 166
Czf1	No known homolog	Putative zinc finger transcription factor	111
Hgc1	Cln21	G_1 cyclin-related protein	332
Sho1	Sho1	Adaptor protein	244
Crz1	Crz1	Calcineurin-regulated transcription factor	250
Mkc1	Mkc1	Mitogen-activated protein kinase	80
Rsr1	Rsr1	Ras-like GTPase	118
Bud2	Bud2	GTPase-activating protein	118
Cln3	Cln3	G_1 cyclin	59
Clb2, Clb4	Clb2, Clb4	B-type cyclin	28
Erg1	Erg1	Epoxidase	232
Mep1, Mep2	Mep1, Mep2	Ammonium permease	33
Cdc4	Cdc4	Ubiquitin ligase	8
Pmt4	Pmt4	Mannosyltransferase	236
Hsl1	Hsl1	Ser/Thr protein kinase	304
Pld1	Pld1	Phospholipase D1	82
Ecm33	Ecm33	GPI-anchored protein	199

[a] *, *A. niger* protein.

blocked pseudohyphal induction, whereas transport capacity was not affected. This result suggests that phosphorylation by PKA is required for Mep2 to be able to exert its effect on pseudohyphal induction.

Role of Gcn4 in nitrogen-regulated morphogenesis in *C. albicans*. Nitrogen availability has profound significance in fungal biology. In response to nitrogen limitation, fungi initiate morphological changes, sexual and asexual sporulation, and

expression of virulence determinants (200). For instance, haploid MATα cells of *C. neoformans*, which typically grow as yeast, develop hyphae and fruiting bodies in nitrogen-deficient media; these processes are inhibited by ammonia (318). In *S. cerevisiae*, starvation for a single amino acid stimulates the expression of genes for all amino acid biosynthetic pathways in a phenomenon termed general amino acid control (GCN response) (122). Nitrogen catabolite repression, the regulation of gene expression in relation to nitrogen availability, has been demonstrated in numerous fungi (200). Though somewhat a misnomer (192), this global control mechanism prevents expression of the many genes required to utilize various secondary nitrogen sources when an adequate supply of preferred nitrogen sources is available, typically ammonium or glutamine (200). Nitrogen regulation is controlled by global positive (Gln3 and Gat1) and negative (Dal80 and Deh1) transcription factors as well as by pathway-specific transcription factors.

Tripathi et al. reported that *C. albicans* can respond to amino acid starvation in at least two ways: by stimulating cellular morphogenesis and by a GCN-like response (302). Both responses are dependent on Gcn4, a functional homolog of the *S. cerevisiae* transcription factor ScGcn4. Hence, Gcn4 plays a central role in coordinating morphogenetic and metabolic responses to amino acid starvation in *C. albicans* (302). In *S. cerevisiae*, ScGcn4 is mainly regulated at the translational level by the eIF-2α kinase ScGcn2 (122). In *C. albicans*, however, Gcn4 appears to be regulated mainly at the transcriptional level, with only a small contribution for Gcn2 (301). These differences between *C. albicans* and *S. cerevisiae* fit with the fact that ScGcn4 has not been implicated in pseudohyphal development (101), and transcript profiling of the GCN response has not revealed any obvious link with pseudohyphal development in *S. cerevisiae* (214). These results suggest a divergence in the cellular roles of Gcn4 between *S. cerevisiae* and *C. albicans* and underscore the importance of amino acids in the regulation of morphogenesis in *C. albicans*. In budding yeast, Gcn4 is targeted for degradation by the Cdc4 ubiquitin ligase complex, and deletion of *CDC4* in *C. albicans* results in constitutive hyphal growth (8). This observation suggests that Cdc4-mediated protein degradation may be involved in the regulation of the dimorphic switch. Possible key targets of Cdc4 include Sol1, a homolog of ScSic1, a cyclin-dependent kinase inhibitor, and transcription factor Tec1 (63, 257), which are all targets of ScCdc4. So far, however, none of the candidates alone account for the hyperfilamentation phenotype of the *cdc4* mutant.

Signal Transduction, Quorum Sensing, and the MAPK Cascade Module

In eukaryotic cells, MAPK cascades are key elements in mediating the transduction of many signals generated at the surface to the nucleus. As in *S. cerevisiae*, a MAPK pathway is involved in filamentation in *C. albicans*. The cascade consists of the kinases Cst20 (homologous to the p21-activated kinase [PAK] kinase Ste20), Hst7 (homologous to the MAPK kinase [MAPKK] Ste7), and Cek1 (homologous to the Fus3 and Kss1 MAPKs) (65, 69, 153, 171, 266, 317) (Fig. 1). The *C. albicans* MAPKK kinase (MAPKKK) Ste11 has not been characterized in detail, although its homolog in *C. glabrata* functionally complements an *S. cerevisiae* Ste11 mutant (51). The transcription factor Ste12 functions downstream of the pheromone-responsive MAPK cascade in budding yeast through a heptamer sequence, TGAAACA, called the pheromone response element (PRE). Genes involved in mating are regulated through the PRE by the Ste12 transcription factor and the regulatory proteins Dig1 and Dig2. By contrast, filamentation gene expression depends on Ste12 acting in concert with a filamentation-specific factor, Tec1, which binds cooperatively with Ste12 to the filamentation response element (64). The *C. albicans* homolog of Ste12 is called Cph1; expression of *CPH1* in *S. cerevisiae* can complement both the mating defect in haploids and pseudohyphal formation in diploid cells (181, 195). Null mutations in any of the genes in the MAPK cascade (Cst20, Hst7, or Cek1) or the transcription factor Cph1 confer a hyphal defect on solid medium in response to many inducing conditions; however, all of these mutants filament normally in response to serum (69, 153, 171). Interestingly, although a *cek1* MAPK mutant strain forms morphologically normal filaments in response to serum, it has a minor growth defect on serum-containing medium (69). The *cek1* mutant strain also has a virulence defect that may be attributable to this growth defect (69). These results indicate that the Cek1 MAPK may function in more than one pathway or that deletion of the gene causes aberrant cross-talk between distinct MAPK cascades, similar to the altered signaling reported for MAPK mutants of *S. cerevisiae*. Recently, clear evidence of an interaction between a second MAPK module—the HOG1 pathway—and the CEK1 pathway has been obtained. Cek1 is activated in certain HOG pathway mutants, apparently to compensate for their defects in cell wall architecture (244). Under conditions that require active growth, Sho1, a sensor protein that links oxidative stress to morphogenesis, is essential for the activation of the Cek1 MAPK (244). Recently, a review of the interactions between the different MAPK pathways in *C. albicans* was published (205).

Other elements of the Cek1 pathway have small but varied effects on virulence. *cst20* mutant strains have a modest virulence defect in a mouse model of systemic candidiasis (171). However, *hst7* and *cph1* mutant strains are able to cause lethal infection in mice at rates comparable to those of wild-type strains (171, 182). In addition to these components, a MAPK phosphatase, Cpp1, that regulates filamentous growth in *C. albicans* has been identified (69, 256). Disruption of both alleles of the *CPP1* gene derepresses hyphal production and results in a hyperfilamentous phenotype. This hyperfilamentation is suppressed by deletion of the MAPK Cek1 (69), indicating that Cpp1 functions primarily in this MAPK pathway. *cpp1* mutant strains are also reduced for virulence in both systemic and localized models of candidiasis (69, 114).

Activation of the MAPK pathway can occur through the Cdc42 small GTPase. Cdc42 binds with high affinity to the Cst20 kinase, as well as to a second PAK kinase, Cla4 (172, 287). Cdc42 and its exchange factor, Cdc24, are both required for hyphal growth (22, 305, 306). Certain point mutants of Cdc42 do not affect its mitotic functions but strongly affect morphogenesis upon serum stimulation. Consistent with this phenotype, the expression of hypha-specific genes is also reduced or transient in these mutants (23, 306). Addition of serum results in a transient increase in *CDC24* expression, and

this increased expression depends on Tec1, the second transcription factor (the homolog of ScTec1) downstream of the Cek1 MAPK module (23). Cdc24 is then recruited to the tip of the hypha. The regulatory relationship between Tec1 and Cdc24 suggests a positive feedback loop that contributes to the level of active Cdc42 at the tip of the germ tube.

Inputs into the Cek1 MAPK pathway may also occur by quorum sensing. Quorum-sensing molecules allow bacteria to monitor their growth and to control cell density-dependent phenomena. Recently, similar regulatory molecules, tyrosol and farnesol, were identified in *C. albicans*, and they are involved in morphogenesis. Studies of the morphological transition from a filamentous to a budding yeast form in *C. albicans* revealed excretion of an autoregulatory substance, 3,7,11-trimethyl-2,6,10-dodecatrienoate, or farnesol, into the medium. Farnesol inhibits filamentous growth and may be involved in developmental signaling (226). Whereas farnesol is a compound that prevents hyphal formation (it is produced during high-density growth), tyrosol stimulates the growth of *Candida* cells and, under the proper conditions, hyphal formation (61, 125). Recent work shows that farnesol may function through the Cek1 MAPK pathway to inhibit morphogenesis, as the addition of farnesol represses the expression of *CPH1* and *HST7* (253). How farnesol is sensed and how the signal is transmitted to this MAPK pathway remains to be determined. Interestingly, *C. albicans* mutants lacking the histidine kinase Chk1 (see below) are refractory to the inhibitory effect of farnesol both in cell suspension and during the formation of a biofilm (158). This indicates a role of two-component signal transduction proteins in quorum sensing and as upstream components of the Cek1 MAPK pathway.

cAMP-PKA Pathway

The cAMP-PKA pathway plays a very important role in filamentation in *S. cerevisiae*, *C. albicans*, and other fungi (177). Nitrogen starvation in *S. cerevisiae* results in the formation of elongated buds termed pseudohyphae, which is dependent on activation of the cAMP pathway (110, 157, 177). In *C. albicans*, an increase in cAMP levels accompanies the yeast-to-hypha transition, and inhibition of the cAMP phosphodiesterase induces this transition (247). Previous reports of cAMP levels during the yeast-to-hypha transition (13, 85, 193, 247) are difficult to compare because of differences in strains and experimental conditions. Nonetheless, it is clear is that the cAMP signal is less pronounced in *C. albicans* than in *S. cerevisiae*. Our understanding of the *C. albicans* cAMP-PKA pathway is based on considerable work with budding yeast, which we briefly review below. We then discuss the different components of the cAMP pathway in *C. albicans* and their roles in morphogenesis in more detail.

Upstream components of the cAMP-PKA pathway. In *S. cerevisiae*, the cAMP-PKA pathway is activated by a G protein-coupled receptor system consisting of the G protein-coupled receptor ScGpr1 and the Gα protein ScGpa2. ScGpr1 was identified in two-hybrid screens with the Gα protein ScGpa2 as bait (156, 324) and in a screen for mutants deficient in glucose-induced loss of heat resistance, a property controlled by the cAMP-PKA pathway (156). Apart from the receptor, ScGpa2 also interacts with ScGbp1/ScKrh2 and ScGbp2/ScKrh1, two proteins that appear to act as Gβ-mimicking subunits, based on structural resemblance with classical Gβ proteins (24, 117). Recently, two different molecular mechanisms by which these kelch repeat proteins function were described (116, 233). It seems that activated ScGpa2 relieves the inhibition imposed by the kelch repeat proteins on PKA, thereby bypassing adenylate cyclase for direct regulation of PKA. It is also clear that both ScKrh1 and ScKrh2 may bind different components of the pathway, thereby functioning as scaffolding proteins. Another interaction partner of ScGpa2 is ScGpg1, which has predicted structural properties typical of a Gγ-like subunit, although ScGpg1 lacks apparent target sequences for posttranslational modifications, which are typical of Gγ subunits (117). The activity of ScGpa2 is also controlled by the RGS protein ScRgs2 (309). Hence, a G protein-coupled receptor (GPCR) system composed of ScGpr1, ScGpa2, and ScRgs2 has been proposed to act as a glucose-sensing system for control of the cAMP pathway (296, 310). Recently we obtained evidence that ScGpr1 is a sensor for sucrose and glucose and that mannose acts as an antagonist (174). This GPCR system is required for pseudohyphal and invasive growth induction, and Sc*GPR1* or Sc*GPA2* mutants, deficient in this morphogenesis, can be suppressed by the addition of cAMP (187, 188, 293).

Genes similar to Sc*GPA2* and Sc*GPR1* have been identified in *C. albicans*, but their precise functions remain unclear. Genetic evidence suggests that *GPA2* may function upstream of both the Cek1 MAPK pathway (249) and the cAMP-PKA pathway (193, 204). Deletion of *GPA2* or *GPR1* produces defects in hyphal formation and morphogenesis in *C. albicans*, which are reversed by exogenous addition of (db)cAMP or by overexpression of downstream components in the pathway. As expected, epistasis analysis revealed that Gpa2 (the Gα protein) acts downstream of Gpr1 (the receptor) in the same signaling pathway, and a two-hybrid assay indicated that the carboxy terminus of Gpr1 interacts with Gpa2. Moreover, expression levels of *HWP1* and *ECE1*, which are cAMP-dependent hypha-specific genes, are reduced in both mutants (204). Interestingly, the morphogenesis defect is present only when cells are cultured on solid media. A possible link between Gpa2 and the CEK1 MAPK pathway was recently solidified by the discovery that Gpa2 is involved in mating. Thus, Gpa2 seems to integrate the nutrient-sensing pathway with the pheromone response MAPK pathway, providing an explanation for why the function of the latter pathway strongly depends on nutritional conditions (27).

Conflicting data regarding the possible ligand for Gpr1 exist. Miwa et al. (204) showed that, as in *S. cerevisiae*, Gpr1 is required for the glucose-induced cAMP signal, but we found that the *gpr1* strain showed the same glucose-induced increase in cAMP as did a wild-type strain (193). However, deletion of either *CDC25* or *RAS1* affected the glucose- and serum-induced cAMP signal, consistent with mediation of the glucose-induced cAMP increase in *C. albicans* by the Cdc25-Ras1 branch of the glucose response pathway and probably not via the Gpr1-Gpa2 branch (193). Addition of serum results in a rapid response, similar to that seen with glucose, supporting a recent finding that glucose is the main factor in serum affecting *Candida* morphogenesis (135). Two important gaps remain in our understanding of the upstream components of the cAMP-PKA pathway in *C. albicans*: (i) the identity of the receptor

activating the Cdc25 guanine nucleotide exchange protein and (ii) the nature of the ligand for Gpr1. No data from budding yeast or *C. albicans* regarding the Cdc25 receptor are available, but, as mentioned above, preliminary investigations aimed at identifying the possible ligand for Gpr1 seem to point to amino acids, specifically methionine, although glucose or other sugars cannot be excluded (193, 194).

The concentration of glucose in the medium is a very important parameter for the hyphal response. Most research groups use normal yeast extract-peptone-dextrose or synthetic complete medium containing 2% glucose. Under these conditions, a wild-type strain displays smooth colonies. However, in the presence of more physiologically relevant concentrations (around 0.1% glucose in the blood), and when there is methionine present in the medium, a wild-type strain rapidly starts to form hyphae. This methionine-dependent, low glucose concentration-induced hyphal production is completely absent in the *gpr1* mutant (194), as well as in an *hgt12* mutant (189). Hgt12 is homologous to *S. cerevisiae* Snf3, which is the high-affinity glucose sensor required for the expression of glucose transporter genes. The pathway that is activated by Hgt12 in *C. albicans* remains to be investigated, but the Hgt12 receptor is required for hyphal development during macrophage infection. Hgt12 does not have the long C-terminal tail that is typical of the yeast Snf3 and Rgt2 glucose sensors. Recently, conflicting data regarding the role of Hgt12 as a sensor have been presented. Brown and colleagues constructed independent Hgt12 mutants and were unable to reproduce the growth or filamentation abnormalities. They showed that Hgt4, another homolog of the yeast Snf3 glucose sensor that does contain a long C-terminal tail, might be the sensor regulating the expression of Hgt12, which, according to these authors, is a normal glucose transporter (48).

Ras1, the master hyphal regulator. Mutants of the single Ras homolog, Ras1, in *C. albicans* are viable but have a severe defect in hyphal growth in response to serum and other inducing conditions (92). In addition, while a dominant-negative Ras1 mutation [Ras1(A16)] causes a defect in filamentation, a dominant-active Ras1 mutation [Ras1(V13)] enhances the formation of hyphae (92). As mentioned above, the morphogenesis defect of a *ras1* mutant can be suppressed by exogenous cAMP, and *ras1* mutants are defective in cAMP induction in response to glucose and serum stimulation. Both phenotypes are shared with mutants lacking the guanine nucleotide exchange factor Cdc25 (193), suggesting that the Cdc25-Ras1 pathway functions upstream of the cAMP-PKA pathway. This is supported by a recent study in which a clear interaction between Ras1 and adenylate cyclase, Cyr1, is demonstrated (90). However, as with the situation in *S. cerevisiae*, Ras1 also appears to function upstream of the Cek1 MAPK pathway (Fig. 1), as the morphogenesis defect of a *ras1* mutant can also be suppressed by overexpressing components of the filament-inducing MAPK cascade. Other evidence for a role in both pathways is that *ras1* mutant strains exhibit a filamentation defect that is similar to that of the *efg1 cph1* mutant strain (see below).

Cyr1, Srv2, and Pde2. *C. albicans* has a single gene homologous to the *S. cerevisiae* adenylate cyclase gene (*CYR1*/*CDC35*). Like Ras1, the cyclase is not essential for growth in *C. albicans* but is required for hyphal development (243). Also as in budding yeast, genes encoding an adenylate cyclase-associated protein (*SRV2*) and two phosphodiesterases (*PDE1* and *PDE2*) are present in the *C. albicans* genome. The Srv2 protein regulates adenylate cyclase activity and is required for wild-type germ tube formation and for virulence in a mouse model of systemic infection; it is the homolog of the *S. cerevisiae* Cap1 protein. Like other mutants in the pathway, *srv2* mutants have defects in morphogenesis, but they can be rescued by the addition of cAMP (13). Interestingly, the basal level of cAMP is higher in the *srv2* mutant than in the wild type (61.8 ± 6.5 versus 45.3 ± 4.6 pmol/mg protein), indicating a possible negative-feedback inhibition.

To characterize more fully the effects of hyperactivation of the cAMP pathway, two groups disrupted the *PDE2* gene, which encodes the high-affinity phosphodiesterase (PDEase) (16, 143). An advantage of studying cAMP hyperactivation by disrupting *PDE2* rather than overexpressing *TPK1* and *TPK2*, which encode the catalytic subunits of PKA, is that the *pde2* mutant displays phenotypes that result solely from an increase in the cAMP level, thereby preempting any unpredictable effects of inequitable overexpression of one cAMP signaling component relative to the others. Sc*PDE1* and Sc*PDE2* encode the low- and high-affinity cAMP PDEases, respectively, in *S. cerevisiae* (190, 221, 252). Budding yeast Sc*pde1* and Sc*pde2* mutants exhibit sensitivity to heat shock and nutrient starvation (190), and Sc*pde2* mutants also have cell wall-related phenotypes, such as lysis upon hypo-osmotic shock, suggesting a role for ScPde2 (and/or cAMP) in the maintenance of cell wall integrity in *S. cerevisiae* (299). Although *C. albicans* also has a low-affinity PDEase encoded by *PDE1* (127), so far only *PDE2* has been studied in detail. As with the situation in budding yeast, *pde2* mutants show cell wall- and membrane-related phenotypes, such as increased sensitivity to membrane-perturbing agents such as sodium dodecyl sulfate or antifungals such as amphotericin B and a strong reduction in the thickness of the cell wall, caused mainly by alterations in the ergosterol and glucan composition (144). *PDE2* mutants are also sensitive to nutrient deprivation and defective in entry into stationary phase and are avirulent in a mouse model of systemic candidiasis (16). Under conditions in which wild-type strains form smooth colonies composed of budding yeasts on agar media, the homozygous *pde2* mutant displayed a wrinkled colony phenotype. The wrinkled colonies exhibited mixtures of elongated yeasts and filamentous forms (pseudohyphae and true hyphae). Germ tube formation by the homozygous *pde2* mutant was accelerated in liquid media compared to wild-type strains. Thus, it is clear that Pde2 is required to regulate the level of cAMP in the cells and that disruption of this enzyme results in a constitutive activation of the PKA pathway.

Adenylate cyclase and CO_2 sensing. *C. albicans* is present in various parts of the body where the CO_2 concentration is more than 150-fold higher (5%) than in atmospheric air (0.033%). In other words, cells growing on the skin face much lower CO_2 concentrations than do cells present in the intestine or blood. The presence of 5% CO_2 strongly induces pseudohyphal development and invasion of the underlying agar (151), a response that requires the catalytic domain of adenylate cyclase (Cyr1/Cdc35) but not Ras1. In many cases, cellular effects observed with CO_2 can be mediated via its hydrated form, bicarbonate. *C. albicans NCE103* encodes a carbonic anhy-

drase that greatly accelerates bicarbonate formation. Nce103 is required for *C. albicans* cells to grow in air but not under high CO_2 concentrations (>0.5%). Nce103 is also required for tissue damage under atmospheric conditions but not under high CO_2 conditions. Whether there is indeed a specific CO_2 sensor protein on the cell membrane (e.g., through aquaporin water channels as in plant and mammalian cells) remains to be established. Klengel et al. have already excluded *C. albicans* Aqy1 as a possible candidate (151). A similar CO_2-sensing pathway in *Cryptococcus neoformans* has also been described (14), and a review of this topic was recently published (15).

PKA. Growth and cellular differentiation of eukaryotic cells depends to a large extent on the activity of cAMP-dependent protein kinases (PKA). PKAs are structurally conserved, consisting of two catalytic subunits that are inactivated by the binding of a homodimer of regulatory subunits. External cues elevate intracellular levels of cAMP, whose binding to the regulatory subunits liberates and thereby activates the catalytic subunits. A comparison of PKA subunit isoforms in *C. albicans* and *S. cerevisiae* reveals several significant differences. (i) In *S. cerevisiae*, PKA is encoded by three paralogues (Sc*TPK1* to Sc*TPK3*) (298), whereas only two paralogues (*TPK1* and *TPK2*) are present in the genome of *C. albicans* (66). (ii) In *C. albicans*, both isoforms have a positive, stimulatory function on the formation of true hyphae, whereas in *S. cerevisiae*, ScTpk2 has a positive function and ScTpk1 and ScTpk3 have negative functions in pseudohyphal development (229).

Although both *C. albicans* Tpk isoforms act positively to regulate hyphal formation, they have various phenotypic effects. For example, *tpk1* mutants are defective in hyphal formation on solid media but are less affected in liquid media (276) (Fig. 1). In contrast, hyphal formation in *tpk2* mutants is partially affected on solid media but is blocked in liquid medium. Because Tpk1 and Tpk2 mainly differ in their N-terminal domains (80 to 90 amino acids), hybrid Tpk proteins, with exchanged N-terminal domains, were tested by Bockmuhl et al. (37). Tests of hybrid proteins suggested that the catalytic domain mediates Tpk protein specificities in filamentation, whereas agar invasion is mediated by the N-terminal domain of Tpk2.

The regulatory subunits of PKA in *S. cerevisiae* and *C. albicans* are encoded by Sc*BCY1* and *BCY1*, respectively (58, 298). Cantore's group examined the morphogenetic behavior of *C. albicans* yeast cells lacking the PKA regulatory subunit and generated a *bcy1 tpk2* double mutant strain, because a homozygous *bcy1* mutant in a wild-type genetic background could not be obtained. In the *bcy1 tpk2* mutant, PKA activity (due to the presence of the *TPK1* gene) was cAMP independent, indicating that the cells harbored an unregulated phosphotransferase activity. This mutant has constitutive protein kinase activity and displays a defective germinative phenotype in GlcNAc and in serum-containing media. In addition, a Tpk1-green fluorescent protein (GFP) fusion is dispersed throughout the cell in the *bcy1 tpk2* double mutant, while it is normally predominantly nuclear in wild-type cells. These genetic studies, together with biochemical evidence, suggest that *C. albicans* Bcy1 may tether the PKA catalytic subunit to the nucleus and thereby perform a pivotal role in regulating the enzymatic activity and availability of PKA in response to growth phase-related nutritional requirements (58).

Efg1 and Efh1, Major Transcriptional Regulators

The *C. albicans EFG1* and *EFH1* genes encode basic helix-loop-helix (bHLH) transcription factors that are members of the APSES family of fungus-specific regulators and involved in morphogenetic processes (81). This class includes StuAp of *Aspergillus nidulans* and Asm1 in *Neurospora crassa*, as well as the Phd1 and Sok2 proteins of *S. cerevisiae*. StuA is involved in the formation of conidiophores, which are important for asexual reproduction in *A. nidulans* (202, 264), while Asm1 of *N. crassa* has a role in ascospore maturation (6). Phd1 enhances and Sok2 suppresses pseudohyphal formation in *S. cerevisiae* (109, 314). In *C. albicans*, Efg1 plays a central role in morphogenesis. Below, we first discuss Efg1 as a downstream component of the PKA pathway. Next, we discuss target genes induced or repressed by Efg1, followed by a more general discussion of the genetic network regulating the expression of yeast- or hypha-specific genes. Finally, we discuss the role of Efg1 in morphogenesis under embedded conditions.

Efg1 is a downstream component in the cAMP-PKA pathway. Several lines of evidence suggest that Efg1 functions downstream of PKA. Overexpression of *TPK2* is unable to suppress the mutant phenotype of *efg1*, whereas overexpression of *EFG1* can bypass the filamentation defect in a *tpk*2 mutant (276). The suppression activity of Efg1 depends on a threonine residue at position 206, a potential phosphorylation site for PKA (36). Induction of morphogenesis after the addition of serum, a process known to be regulated by the PKA pathway, requires Efg1. When *EFG1* transcript levels were examined in transformants overexpressing *TPK1* or *TPK2*, a reduction (about fourfold) of *EFG1* mRNA was observed (294). This finding suggests that Efg1 is down-regulated by its immediate upstream regulator, PKA, but other observations point to autoregulation of *EFG1* gene expression. Overexpression of *EFG1* increases the levels of a smaller 2.2-kb transcript produced by the locus, while the levels of the endogenous 3.2-kb major *EFG1* transcript decline dramatically (294) (Fig. 2). Serial deletion analysis of the promoter region revealed that the TATA box region is required for *EFG1* autoregulation. Binding of Efg1 to the *EFG1* transcriptional initiation region by chromatin immunoprecipitation was also shown. In summary, it appears that the levels and/or activities of Efg1, Tpk1, and Tpk2 are able to down-regulate the major *EFG1* promoter. In addition, Efh1, the homolog of Efg1, also affects Efg1 down-regulation, indicating that at least four proteins can contribute to *EFG1* expression. Negative autoregulation and PKA-mediated down-regulation are probably mediated though the Sin3-Rpd3 (279)-containing histone deacetylase complex (Fig. 2).

Roles of Efg1 and Efh1 in morphogenesis in *C. albicans*. Overexpression of Efg1 or Efh1 induces pseudohyphae and opaque-white switching, a specific morphogenetic transition (81, 182, 285). An *efg1* mutant strain has a moderate but not complete defect in hyphal growth in response to many environmental conditions (182). Addition of serum or GlcNAc as an inducer in liquid or on solid medium completely blocks hyphal formation in *efg1* null mutants. In contrast, under microaerophilic or embedded conditions, hyphal formation is unaffected—and may be stimulated—in homozygous *efg1* mutants (111). These results indicate that, depending on environ-

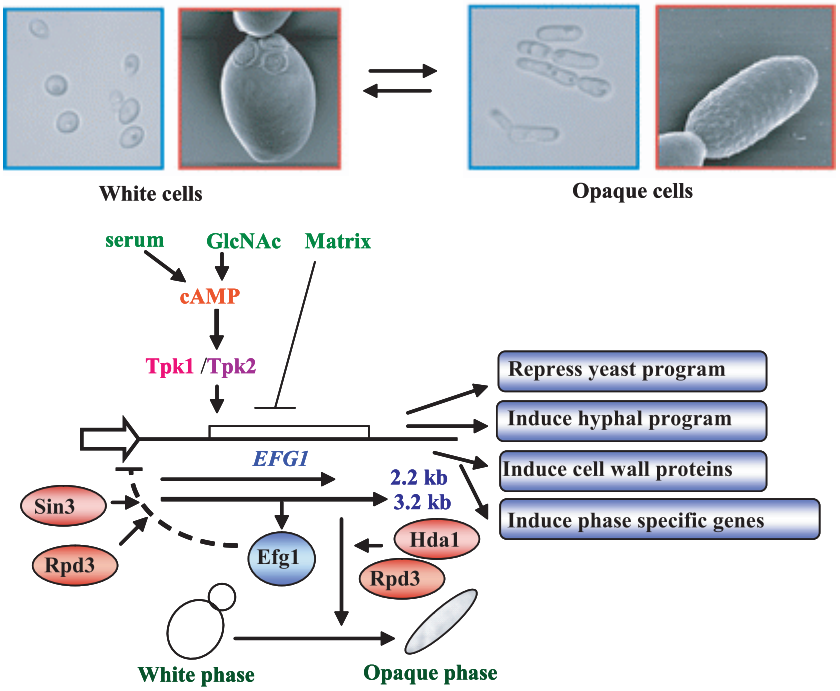

FIG. 2. (Top) White-opaque switching in *C. albicans*. Shown are scanning electron micrographs at a magnification of ×1,000 and bright-field images of white and opaque cells. (Reprinted, with permission, from the *Annual Review of Microbiology* [26], volume 59, © 2005 by Annual Reviews.) (Bottom) Model of morphogenetic regulation by Efg1. Under hypha-inducing conditions (e.g., serum, GlcNAc), Efg1 is induced and activated; under microaerophilic conditions, however, it is repressed. The activated Efg1 (by PKA isoforms Tpk1 and Tpk2) initiates hyphal formation by inducing genes involved in hyphal formation and/or repressing genes directing the yeast form. Efg1 also induces the cell wall proteins (*HWP1*, *HWP2*, and *RBE1*) that are involved in adherence. The phase-specific genes (at the white-to-opaque-phase transition period) are also induced by Efg1. In parallel, Efg1, in conjunction with the Sin3-Rpd3 deacetylase complex, silences chromatin and thereby down-regulates *EFG1* promoter activity (294). The 3.2-kb major transcript of *EFG1* is expressed in the white phase, and the less-abundant 2.2-kb transcript is expressed in opaque cells. The Hda1-Rpd3 deacetylase complex regulates the white-to-opaque-phase transition as well as *EFG1* down-regulation (279).

mental cues (and depending on the genetic background), Efg1 may act as a transcriptional activator and repressor for different subsets of genes, whose balanced activities are essential for yeast, pseudohyphal, and hyphal morphogenesis of *C. albicans*. An *efg1 cph1* double mutant strain has an extreme filamentous growth defect, with no detectable filamentation under almost any conditions tested, including the presence of serum (182). In addition, while the *efg1* mutant strain has a minor reduction in virulence and the *cph1* mutant has little or no defect, an *efg1 cph1* double mutant strain is essentially avirulent in a mouse model of systemic infection (182). Recently, it was shown that this double mutant is still able to cause infections in the kidneys of infected mice. So, although this mutant is avirulent, local proliferation of *C. albicans* cells can occur in certain tissues (60). Thus, Cph1 and Efg1 define elements of two separate pathways that together are essential for both filamentation and virulence in *C. albicans*.

Efg1 and embedded growth. As we have just described, *efg1* mutants have a drastic block in true hyphal formation under most standard induction conditions, but considerable filamentation occurs in certain environments (46, 276). A limited supply of oxygen, as occurs under a coverslip during induction of chlamydospores, allows wild-type cells to form filaments, which is enhanced in *efg1* mutants (275). Similarly, growth of wild-type colonies embedded in agar stimulates filamentation, which still occurs in homozygous *efg1 cph1* mutants (111, 242). Thus, an Efg1-independent pathway of filamentation that is operative under microaerophilic/embedded conditions appears to exist in *C. albicans*. In wild-type strains, the filaments produced under microaerophilic conditions are mostly pseudohyphae; however, they are mostly true hyphae in *efg1* mutants (276). Interestingly, the alternative filamentation pathway is not only independent of Efg1 but may be repressed by it to a certain degree. The enhanced filamentation in *efg1* mutants does not depend on the Cph1 MAPK, because a homozygous *efg1 cph1* strain is as hyperfilamentous as the homozygous *efg1* mutant (276). It is possible that agar embedding generates microaerophilic conditions, which activate the same Efg1-independent pathway of morphogenesis under both conditions. The putative transcription factor Czf1 is probably an important element of the alternative pathway of filamentation in *C. albicans* (47). The central portion of Czf1 contains four clusters of glutamine residues, and the C terminus contains a cysteine-rich region similar to zinc finger elements. Overexpression of *CZF1* stimulates filamentous growth but only under embedded conditions and in certain media lacking glucose. The expression of Czf1 is strongly up-regulated in a *vps34* mutant; as a result, a *vps34* mutant is derepressed in hyphal formation under microaerophilic conditions (150). Vps34 is phosphatidylinositol-3-kinase, which influences vesicular intracellular transport, filamentous growth, and virulence. The exact role of Vps34 in Czf1-mediated morphogenesis is not yet clear. Homozygous

czf1 null mutants filament normally under standard induction conditions, but they are defective in hyphal development when embedded in agar. This defective phenotype occurs only during embedding in certain media, such as complex medium containing sucrose or galactose as carbon sources at 25°C, but not at 37°C, or in media containing strong inducers, including serum and GlcNAc. These characteristics suggest that factors other than Czf1 contribute to filamentation under embedded conditions. The defective phenotype of a *czf1* mutant is exacerbated by the presence of a *cph1* mutation, which by itself shows defects in the types of media used for monitoring the *czf1* phenotype. Thus, although the *cph1* mutant phenotype does not appear to be specific for embedded conditions, it worsens the filamentation defects caused by the *czf1* mutation. Hyperfilamentation of *efg1* single and *efg1 cph* double mutants suggests that Efg1 is a negative modulator of the Czf1 pathway under microaerophilic/embedded conditions. However, recent data show that this hyperfilamentation phenotype of *efg1* mutants may be caused by a Czf1-independent pathway, as *efg1* mutants do not express *CZF1*. Chromatin immunoprecipitation analysis has further indicated that Efg1 and Czf1 interact with the promoter of *CZF1*. This indicates that, like *EFG1*, *CZF1* is autoregulated (312).

Other aspects of *C. albicans* morphogenesis are also dependent on Efg1. Chlamydospore formation requires Efg1 protein in PKA-dependent regulation, as T206 is required (275). Also, recent studies revealed the involvement of *EFG1* in normal biofilm formation (237), as well as in normal filamentation under oxygen limitation conditions (260), which are probably similar to the microaerophilic conditions. Under these conditions, Efg1 seems to function as a repressor of filamentation. In contrast to *efg1*, an *efh1* mutant does not give any clear phenotype except in an *efg1* mutant background. In this case, an *efh1* null mutant is hyperfilamentous under embedded or hypoxic conditions, indicating the cooperation of Efg1 and Efh1 in suppression of an alternative morphogenetic signaling pathway (81).

Efg1 and phenotype switching. As noted briefly above, one form of phenotypic switching is the white-opaque transition, first described by Soll and colleagues in 1987 (268). *C. albicans* colonies of some strains (e.g., WO-1) can switch from the normal size, shape, and white color with high frequency (274) to larger, flatter, and gray colonies. White cells are similar in shape, size, and budding pattern to cells of common laboratory strains. Opaque cells, however, are bean shaped and exhibit three times the volume and twice the mass of white cells (Fig. 2) (268). Transcription of *EFG1* is regulated in a unique fashion during the white-opaque transition (280); the more-abundant 3.2-kb transcription is expressed in the white phase, while the less-abundant 2.2-kb transcription is detected in the opaque phase (Fig. 2). Experiments using deletion or overexpression of *EFG1* show that Efg1 functions downstream of the switch event in the regulation of a subset of white-phase-specific genes involved in the generation of the round white cell shape (278). Furthermore, a detailed promoter analysis suggests that the upstream region of *EFG1* contains overlapping promoters for the expression of white-phase-specific and opaque-phase-specific transcripts (280). Overlapping promoters are also seen for the α and β mRNAs of the *EFG1* homolog StuA in *A. nidulans* (320). It should be noted that *EFG1* is not the only phase-regulated gene expressed as an alternative-molecular-weight transcript during the white-opaque transition in *C. albicans*. The deacetylase *HOS3* is also transcribed as a 2.5-kb transcript in the white phase and as a less-abundant, lower-molecular-weight 2.3-kb transcript in the opaque phase (279). Recently, a key transcription factor required to establish and maintain the opaque growth phase was described by different groups. Like Efg1, this transcription factor, Wor1 (Tos9), binds to its own regulatory region, thereby activating its own expression. It is believed that high levels of Wor1 regulate the epigenetic inheritance of the opaque phase of growth (60, 131, 277).

Efg1 and cell wall dynamics. Recently, several genes encoding cell wall components were shown to be regulated by Efg1, implying cell wall regulation in *C. albicans* morphogenesis. These genes include the cell wall mannoprotein Hwp1 (42, 262), the glycosylphosphatidylinositol (GPI)-anchored cell wall protein *HYR1* (17), and *ALS1*, which encodes a cell surface glycoprotein (95). In order to analyze cell wall dynamics and the regulatory function of Cph1 and Efg1 in the transcriptional control of cell wall genes in a systematic manner, Sohn et al. used a DNA microarray to assay transcriptional profiles from wild-type cells and *cph1*, *efg1*, and *cph1 efg1* double-mutant strains cultured under various yeast- or hypha-inducing conditions (271). Overall, their data demonstrate that Efg1 plays a major role in the regulation of cell wall genes analyzed under both yeast- and hypha-inducing conditions, while Cph1 plays a minor role. During induction of filamentation, many hypha-specific genes that are important for adhesion and virulence are expressed in wild-type cells (in an Efg1-dependent manner). Among those genes are the hyphal wall proteins *HWP1* and *HWP2*. Concomitant with the up-regulation of hyphal wall proteins, the expression of yeast-specific genes such as *YWP1* is down-regulated during the yeast-to-hypha transition. Thus, the reduced virulence of the *efg1* mutant likely reflects both a lack of expression of hypha-specific genes and a change in the overall organization of the cell wall (155, 182). Microarray analysis has further demonstrated that apart from yeast- or hypha-specific genes, Efg1 is also important for the expression of metabolism genes, inducing glycolytic genes and repressing genes essential for oxidative metabolism (81). Finally, Erg3, which is involved in drug resistance, is also down-regulated by Efg1, and *efg1* mutants are more resistant to antifungal agents (183).

Convergent Regulation of Cph1 and Efg1: Involvement of Tec1 and Cph2

Tec1, a member of the TEA/ATTS family of transcription factors, has been shown to regulate hyphal development and virulence in *C. albicans* (257). TEA/ATTS family members AbaA in *A. nidulans* and ScTec1 in *S. cerevisiae* are involved in the regulation of conidiophore formation and pseudohyphal growth, respectively (5, 106). As mentioned before, in *S. cerevisiae* ScTec1 and ScSte12 form a transcription factor complex to specifically activate genes involved in pseudohyphal growth (64, 191). Such complexes have not yet been identified in *C. albicans*. In *C. albicans*, *TEC1* transcription is not regulated by the ScSte12 homolog Cph1 (171) but by Cph2 and Efg1. Cph2, an myc family bHLH protein, regulates hyphal development in

C. albicans (169). *cph2* mutant strains are impaired in hyphal development and in the induction of hypha-specific genes in liquid Lee's medium, and Cph2 is necessary for the transcriptional induction of *TEC1* (Fig. 1). Cph2 binds directly to two sterol regulatory element 1-like elements upstream of *TEC1*. Furthermore, the ectopic expression of *TEC1* suppresses the defect of *cph2* in hyphal development. The function of Cph2 in hyphal transcription is therefore mediated, in part, through Tec1. *tec1* mutants exhibit suppressed filamentation in liquid serum-containing media. *EFG1* overexpression does not suppress the morphological defect of the *tec1* mutant, whereas *TEC1* overexpression has a partial phenotype in the *efg1* mutant (257). These results, coupled with the fact that *efg1* strains have a more severe defect in hyphal development than do *tec1* strains, suggest that Tec1 is downstream of Efg1. In summary, then, it is clear that Tec1 is downstream of both Cph2 and Efg1.

The study of the *C. albicans* yeast-to-hypha transition has been informed by studies performed with budding yeast. Very recently, the group of Snyder performed a detailed analysis of the transcription factor network involved in the yeast-to-pseudohypha transition in *S. cerevisiae*. Predicted binding sites for two transcription factor-encoding genes, Sc*PHD1* and Sc*MGA*, appear upstream of most genes involved in pseudohyphal growth, including Flo8 (38). Overexpression of either Sc*PHD1* or Sc*MGA1* induces pseudohyphal growth, even under noninducing conditions. This indicates that ScPhd1 and ScMga1 are the master regulators in this system. ScPhd1 is the homolog of *C. albicans* Efg1, and ScMga1 is homologous to orf19.3969, which has not yet been characterized in *C. albicans* (44). A common theme for regulation of gene expression during pseudohyphal development is the cooperative binding of transcription factors (probably in preformed complexes, as described above) to target promoters. Cooperative binding between ScTec1 and ScSte12 is well established (64, 191), and ScFlo8 and ScMss1 cooperate to activate Sc*FLO11* or Sc*STA1* expression (149, 307). Other cooperating pairs include ScMga1 and ScFlo8 (38) and the *C. albicans* APSES proteins Efg1 and Efh1 (81). Recently the *C. albicans FLO8* gene was characterized (56). As in *S. cerevisiae*, Flo8 is required for hyphal development and for hypha-specific gene expression. Cooperative binding of two different transcription factors may also occur in *C. albicans*, as Flo8 and Efg1 interact with each other and Flo8 controls a subset of the target genes of Efg1 (56).

Other Positive Regulators: Cdc5, G_1 Cyclins, Int1, Mcm1, and Fkh2

Several other genes in *C. albicans* may contribute to morphogenesis, but information is limited or not well focused on pathogenicity relationships. These include genes encoding the polo-like kinase Cdc5, three major G_1 cyclins, and MADS box and forkhead transcription factor families, among others (Table 1). *C. albicans* cells lacking the polo-like kinase *CDC5* are blocked early in nuclear division. Cell cycle arrest is characterized by the formation of hypha-like filaments under yeast growth conditions. The filaments resemble serum-induced hyphae, and filament formation is independent of Cph1 or Efg1 but requires Cyr1/Cdc35 (11). Microarray experiments confirmed the resemblance of cell cycle-arrested filaments to serum-induced hyphae, as in both cases several targets of hypha-signaling pathways were expressed (10).

Cyclin-dependent protein kinases (Cdks) regulate major cell cycle transitions in eukaryotic cells. In *S. cerevisiae*, three G_1 cyclins, ScCln1, ScCln2, and ScCln3, activate the Cdc28 Cdk to promote G_1-phase progression (30). *C. albicans* contains homologs of the *S. cerevisiae* G_1 cyclins, including Ccn1 (= ScCln1), Hgc1, and Cln3 (www.candidagenome.org). Deletion of *CCN1* results in the inability to maintain hyphal growth under certain conditions (185), while deletion of *HGC1* prevents hyphal growth under all hypha-inducing conditions (332). Ccn1 and Hgc1 are not essential for progression through the cell cycle (they have a slow-growth phenotype), suggesting that these G_1 cyclin homologs in *C. albicans* have evolved important roles in hyphal morphogenesis as opposed to cell cycle progression. The induction of three known hypha-specific genes, *HYR1*, *ECE1*, and *HWP1*, in *ccn1* mutant cells in response to serum is slightly reduced (about 50%) in comparison to that in wild-type cells. On the other hand, in liquid amino acid-based Lee's medium, the *ccn1* strain shows a profound defect in the transcriptional activation of all three hypha-specific genes. Thus, Ccn1 may coordinately regulate hyphal development with signal transduction pathways in response to various environmental cues (185). Cln3, however, is essential for the *C. albicans* yeast cell cycle. When unbudded cells are depleted for Cln3, they increase in size but do not bud (12, 59). Eventually, however, these enlarged cells spontaneously form true hyphae. Cln3 seems to be less important for the hyphal cell cycle, although morphological abnormalities are observed in Cln3-depleted cells when they are treated with hypha-inducing signals such as serum (59). Apart from the G_1 cyclins, *C. albicans* also contains two B-type cyclins, Clb2 and Clb4, that are important for morphogenesis but affect polarized growth negatively (28).

Other kinases have also been investigated for their role in pseudohyphal development. The Nim1 kinases, Gin4 and Hsl1, are important for the formation of septin structures, and both *gin4* and *hsl1* mutants form pseudohyphae constitutively (319). In *S. cerevisiae*, ScHsl1 regulates the tyrosine phosphorylation of the cyclin-dependent protein kinase Cdc28 by deactivating the protein kinase ScSwe1, which regulates the G_2/M transition (295). Interestingly, in *C. albicans*, the Hsl1-Swe1-Cdc28 pathway appears to be important for cell elongation of both the yeast and hyphal forms and for virulence (304). Gin4-depleted pseudohyphae are unable to form hyphae when challenged with serum, but this can be overcome by ectopic expression of Gin4 from the *MET3* promoter. Thus, Gin4 plays an important role in regulating the developmental switch from pseudohyphae to hyphae (Fig. 3) (319).

Another morphogenesis factor, Int1, was originally cloned by virtue of its limited homology to vertebrate leukocyte integrins (98). Like *cph1* mutants, *C. albicans int1* strains have a reduced ability to form hyphae in response to most hypha-inducing conditions but form apparently normal hyphae in the presence of serum (100). The C terminus of *Int1* has homology to *S. cerevisiae* Bud4, which is localized to the septin rings at the mother-bud neck (7). *C. albicans INT1* induces filamentous growth in *S. cerevisiae*, and this property has been used to explore the cytoskeleton components required for *INT1*-induced filamentous growth. Sla2, a cytoskeleton protein, may

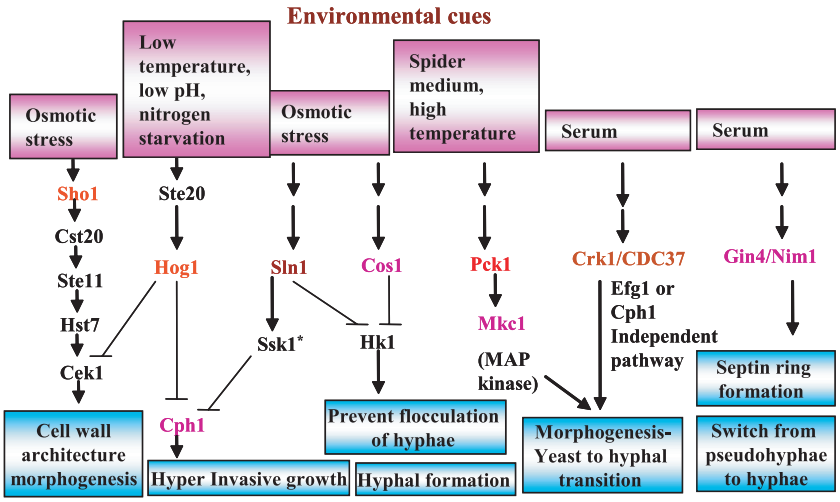

FIG. 3. Multiple environmental conditions activate the kinase cascade, resulting in coordination of the stress response with morphogenesis. Arrows indicate activation; lines with bars indicate inhibition. See the text for details.

interact with Int1 to mediate morphogenesis by modulating the actin skeleton (7). In *C. albicans*, Int1 is important in axial budding pattern and colocalizes with the Cdc3 septin in a ring at the mother-bud neck of yeast and pseudohyphal cells. Under conditions that induce hyphae, both Cdc3 and Int1 localize to a ring distal to the junction of the mother cell and germ tube. Thus, placement of the Int1/septin ring with respect to the mother-daughter cell junction distinguishes yeast/pseudohyphal growth from hyphal growth (99).

Finally, several cell cycle-regulated transcription factors, such as the MADS box and forkhead transcription factors, are also required for morphogenesis. The *C. albicans* homolog of the MADS box transcription factor *MCM1* was identified in a screen for genes that could activate pFLO11::*lacZ* expression in *S. cerevisiae* (245). Either overexpression or repression of *MCM1* induces hyphae in *C. albicans*, indicating that correct timing of expression is important for normal morphogenesis.

In *S. cerevisiae*, two forkhead transcription factors, Sc*FKH1* and Sc*FKH2*, regulate the expression of genes whose transcription peaks in early M phase (163, 333), including mitotic cyclins. In addition, Sc*fkh1* Sc*fkh2* mutants display constitutive pseudohyphal growth. *C. albicans* has only one forkhead homolog, *FKH2*. Cells lacking this gene also form constitutive pseudohyphae under all yeast and hyphal growth conditions tested. *fkh2* mutants exhibit reduced expression of hypha-specific mRNAs in the presence of serum, while under yeast growth conditions expression of several genes encoding proteins likely to be important for cell wall separation is reduced. Together, these results imply that Fkh2 is required for morphogenesis of true hyphae as well as of yeast cells. Cph1 and Efg1 are not required for pseudohyphal morphology of *fkh2* mutants, implying that Fkh2 acts in a parallel or downstream pathway. Cells lacking Fkh2 do not damage human epithelial and endothelial cells in vitro, suggesting that Fkh2 contributes to *C. albicans* virulence (29).

Transcriptional Repressor Tup1

As highlighted throughout this review, activation of signal transduction pathways causes transcription factors to induce global expression programs that direct cells among distinct developmental pathways. The Tup1 transcription factor is required to keep cells in the yeast form in the presence of glucose and other noninducing conditions. In *S. cerevisiae*, the ScTup1 protein regulates about 60 genes involved in glucose regulation, oxygen stress response, and DNA damage (79). The *C. albicans* Tup1 homolog (41) contains seven conserved WD40 repeats at the C terminus, which likely promote interaction with DNA-binding proteins (DBPs), and an N-terminal domain that promotes interaction with the Ssn6 corepressor, as in budding yeast (147, 154). A homozygous *C. albicans tup1* mutant grows in filamentous form in all media tested; filaments on most media have characteristics of pseudohyphae, but in some media true hyphae appear. Pseudohyphae of a *tup1* mutant, unlike pseudohyphae produced by *EFG1* overexpression (285), cannot be induced to form germ tubes or true hyphae by the addition of serum (41). Tup1 has activities besides repression of filamentation, because *tup1* mutants exhibit pleiotropic phenotypes, including a failure to grow at 42°C and misshapen cell walls. In epistasis experiments, most of the filamentation phenotype induced by the *tup1* mutation is abolished by the presence of an *efg1* mutation, while a *cph1* mutation has very little effect. These and other genetic results indicate that Efg1 is the main contributor to the *tup1* hyphal phenotype. Genes repressed by Tup1 have been identified recently, of which some are expressed in a filament-specific manner (42). Braun et al. used subtractive hybridization to identify six genes termed "repressed by *TUP1*" (RBT), whose expression is regulated by Tup1 (40). One of the genes (*HWP1*) has previously been characterized, and a seventh *TUP1*-repressed gene (*WAP1*) was recovered due to its high similarity to *RBT5* (Fig. 1). These genes all encode secreted or cell surface proteins, and four out of the seven (*HWP1*, *RBT1*, *RBT5*, and *WAP1*) encode putatively GPI-modified cell wall proteins. The remaining three,

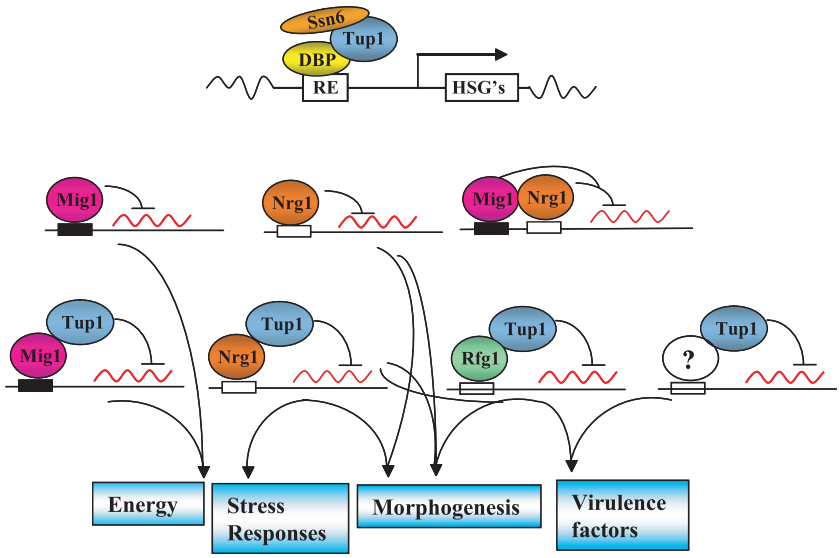

FIG. 4. (Top) Ssn6 is not directly required for Tup1-mediated repression of hypha-specific genes (HSG's), and Tup1 may interact directly with the DBP. The DBP-responsive element (RE) may affect Ssn6 dependency. (Bottom) Model summarizing the transcriptional repression mediated by Tup1, Nrg1, Mig1, and Rfg1 in *C. albicans*.

RBT2, *RBT4*, and *RBT7*, encode, respectively, an apparent ferric reductase, a plant pathogen-related protein (PR-1), and a putative secreted RNase T2.

In *S. cerevisiae*, ScTup1 forms a transcriptional corepressor complex in concert with ScSsn6 and regulates a diverse set of genes controlled by mating type, glucose, oxygen, and DNA damage (270). Although neither ScTup1 nor ScSsn6 itself has any DNA-binding activity, each is recruited to a specific promoter through interaction with distinct upstream DBPs, such as ScMig1, ScRox1, ScCtr1, and ScNrg1 (270). *C. albicans* Ssn6 encodes a putative global transcriptional corepressor (136). Its expression level declines significantly in response to strong hyphal inducers, such as serum. Overexpression of Ssn6 causes increased filamentation and decreased virulence (136). Mutants lacking Ssn6 display a stubby pseudohyphal growth pattern, derepressed expression of filament-specific genes in response to increased temperature, and failure to develop true hyphae. Such morphological defects are not rescued by overexpression of *TUP1*, *CPH1*, or *EFG1*. Recent data indicate that Ssn6 is not essential for repression of hypha-specific genes, at least under a few conditions (Fig. 4), and *ssn6* mutants form filaments in response to serum in the absence of Cph1 and Efg1. These results suggest that filamentation of *ssn6* mutants is not dependent on the MAPK or cAMP protein kinase pathways (104).

Tup1 repression with Nrg1, Mig1, and Rfg1. The DBP Nrg1 contains a zinc finger domain that is conserved in transcriptional regulators from fungi to humans. It is most closely related to ScNrg1, which represses transcription in a Tup1-dependent fashion. *nrg1* mutant cells are predominantly filamentous under non-filament-inducing conditions and show attenuated virulence. Nrg1 represses several filament-specific genes, such as *ECE1* and *HWP1*. Most of these genes contain an Nrg1 response element in their promoters. These genes constitute a subset of those under Tup1 control, providing further evidence that Nrg1 acts by recruiting Tup1 to target genes (43, 104, 210, 211).

In *S. cerevisiae*, another transcriptional repressor, ScMig1, targets the ScTup1-ScSsn6 complex to the promoters of glucose-repressed genes to repress their transcription. Murad et al. (210) provided new insights into the regulatory functions of Tup1, Nrg1, and Mig1 in *C. albicans*. Nrg1 and Tup1 regulate a set of genes different from those regulated by Mig1 and Tup1. This is consistent with the idea that Mig1 and Nrg1 target the Tup1 repressor to a specific subset of *C. albicans* genes (210) (Fig. 4). However, Mig1 and Nrg1 repress other *C. albicans* genes in a Tup1-independent fashion (210, 211). An HMG protein, Rfg1, homolog of *S. cerevisiae* ScRox1, is also an important DBP that recruits Tup1 to the promoters of hyphal growth genes. Recently, Kadosh and Johnson identified 61 hypha-specific genes that are induced in response to growth in serum at 37°C by using whole-genome microarray analysis, and they showed that approximately half of the genes are under the negative control of the Rfg1, Nrg1, and/or Tup1 transcriptional repressors (145).

Other Negative Regulators: Rap1, Rbf1, and Rad6

In *S. cerevisiae*, the ScRap1 protein acts as both a transcriptional silencer and a structural protein at telomeres by binding to a sequence designated the RPG box (84). The *C. albicans* Rap1 homolog is not essential for survival but is required to repress pseudohypha formation under conditions favoring yeast growth (34). A second *C. albicans* protein, Rbf1, is not homologous to Rap1 but binds to the RPG box of *S. cerevisiae* (137). Rbf1 contains two glutamine-rich regions embedded within a region with weak similarity to bHLH domains, which binds to RPG sequences. Homozygous *rbf1* null mutants are constitutively filamentous; the filaments formed are characteristic of pseudohyphae rather than true hyphae (137). Virulence of the *rbf1* mutant in the mouse model of systemic infection

was significantly attenuated (137). Recently, by screening for sequences that mediate Rbf1-dependent transcriptional regulation, target genes were identified in the heterologous host *S. cerevisiae*. Among the genes identified as Rbf1 targets was the white-phase-specific gene *WH11* (273); the level of *WH11* transcripts is reduced in homozygous *rbf1* mutants compared to wild-type cells (137). Another repressing factor is the Rad6 protein, which, besides contributing to UV protection, represses hyphal growth under inducing conditions by an unknown pathway; its deficiency under noninducing conditions generates a pseudohyphal phenotype (176). All of these repressors have clear homologs in *S. cerevisiae*, and this may be the reason that they seem to be more involved in the repression of pseudohyphae than of true hyphae. In general, it also becomes clear that the regulation of morphogenesis is tightly controlled by a whole group of activators and repressors.

Other MAPK Pathways Involved in Morphogenesis

The HOG MAPK pathway: response to oxidative stress. The recent annotation of the *C. albicans* genome sequence revealed four putative osmosensor proteins: Chk1, Nik1 (Cos1), Sln1, and Ssu81 (Sho1). As for other signaling pathways, the organization of the osmosensing pathways in *C. albicans* has been informed by studies with budding yeast. In *S. cerevisiae*, the ScHog1 MAPK pathway is involved in osmotic stress signaling. The pathway starts with the activation of cell membrane-bound receptor proteins (ScSho1, ScMsb2, and ScSln1) that act to sense external osmolarity, feeds through the HOG MAPK pathway, and ends with the synthesis of osmoprotectants such as glycerol (70, 123). The ScSln1 branch is required to induce expression of several genes in response to very high solute levels and operates over a broader range of osmolarities than does the ScSho1/ScMsb2 branch. Under osmotic stress, ScSln1 promotes phosphorylation of a downstream target protein, ScYpd1, which continuously transfers a phosphate group to the response regulator protein ScSsk1. This pathway activates two partially redundant MAPKKKs, ScSsk2 and ScSsk22. The ScSho1/ScMsb2 branch requires ScCdc42, ScSte20, and ScSte50 to activate the MAPKKK ScSte11. Any of the three MAPKKKs is able to activate ScPbs2, which then phosphorylates ScHog1. Phosphorylation of ScHog1 by the MAPKK ScPbs2 results in its nuclear localization and causes regulation of gene expression through several transcription factors—ScHot1, ScSko1, and ScSmp1—and probably also through ScMsn1, ScMns2, and ScMsn4 (105, 228, 240, 248, 315).

Although most components of these pathways are conserved in *C. albicans*, it seems that, instead of osmolarity, the sensors are activated by oxidative stress. Mechanisms of defense against oxidative stress are especially relevant for many pathogenic fungi, such as *C. albicans*, since the neutrophil-macrophage system controls infection (308) through an oxidative killing mechanism. The Hog1 MAPK mediates an adaptive response to both osmotic (as in yeast) and oxidative stress in *C. albicans*. Hog1 also participates in two distinct morphogenetic processes, either as a repressor (yeast-to-hypha transition) or as an inducer (chlamydospore formation) (3). The HOG pathway appears to be a major virulence determinant: the pathway represses the serum-induced yeast-to-hypha transition in *C. albicans* and also represses filamentous growth under other partially inducing conditions, such as low temperature, low pH, or nitrogen starvation (86) (Fig. 3). Hog-mediated repression of the yeast-to-hypha switch is independent of the Efg1 and Cph1 transcription factors. A triple deletion mutant, *efg1 cph1 hog1*, is able to form filaments under subinducing conditions, similar to a *hog1* mutant. In contrast, the function of Hog1 in chlamydospore formation is dependent on the *CEK1* MAPK pathway (86). The exact mechanism by which Hog1 represses hypha formation is still unclear but may involve activation of the transcriptional repressor Rbf1.

The upstream sensors of the Hog1 MAPK pathway, Ssu81 (Sho1) and Sln1, have clear roles in oxidative stress tolerance and morphogenesis. Although *sho1* mutants are sensitive to oxidative stress, the signal appears to travel mainly through the other branch of the pathway via the Sln1-Ssk1 components (244). Sho1 is essential for the activation of the Cek1 MAPK under conditions that require active cell growth and/or cell wall remodeling, and *cek1* mutants are defective in morphogenesis on different hypha-inducing media. So it seems that in *C. albicans*, Sho1 links oxidative stress, cell wall biogenesis, and morphogenesis. The *SLN1* gene encodes a two-component histidine kinase containing a sensor and regulator domain. In vitro autophosphorylation activity has been shown for the Sln1 protein (325). In *S. cerevisiae*, activation of Sln1 occurs at normal osmolarity and leads to phosphorylation (and thereby inactivation) of the Ssk1 regulator. Although the *C. albicans* Sln1 and Ssk1 proteins are the direct homologs of the *S. cerevisiae* Sln1 and Ssk1 proteins, they are not essential in sensing hyperosmolarity. However, hyphal development of *sln1* and *ssk1* null mutants is blocked on starvation-type media and is severely impaired on serum agar, while filamentation is normal in all liquid media (54, 212, 325). Interestingly, while growth of the *ssk1* mutant under nitrogen starvation conditions does not allow formation of hyphae, invasive growth is stimulated significantly compared to the wild-type strains (54). Apart from Sln1 and Sho1, two other two-component histidine kinases have been described, Nik1 (Cos1) and Chk1 (54, 325). The Cos1 protein is a homolog of the *Neurospora crassa* Nik1 histidine kinase, which regulates hyphal growth and protection against osmotic stress. In *C. albicans*, null mutants lacking *NIK1* or C*HK1* have no defect in osmoprotection but are significantly defective in hyphal formation on solid media (starvation-type or medium containing serum) but not in liquid media (1, 325). In addition, the Chk1 histidine kinase is needed to prevent flocculation of hyphae (53). Interestingly, deletions of *SLN1* or *NIK1* alleles in a *chk1* mutant restored filamentation and virulence, suggesting that Sln1 and Nik1 act upstream of Chk1 via a negative regulator (Fig. 3) (325). Expression of *CHK1* is down-regulated in an *sln1* mutant but is not changed in a *nik1* mutant, under most conditions tested (179). Thus, histidine kinase pathways, including Sln1 and Nik1, are possibly down-regulated during hyphal development on agar surfaces and within agar.

Role of the PKC pathway in *C. albicans* morphology. In *S. cerevisiae*, the protein kinase C (PKC)-MAPK "cell integrity pathway" is involved in responses to a wide variety of stresses, including nutritional (311) and osmotic (103) stresses. These environmental signals are sensed by a family of sensor proteins: ScMtl1, ScWsc1, ScWsc2, ScWsc3, and ScMid2. The cell surface sensors transmit the signal to ScRom2, a guanine nu-

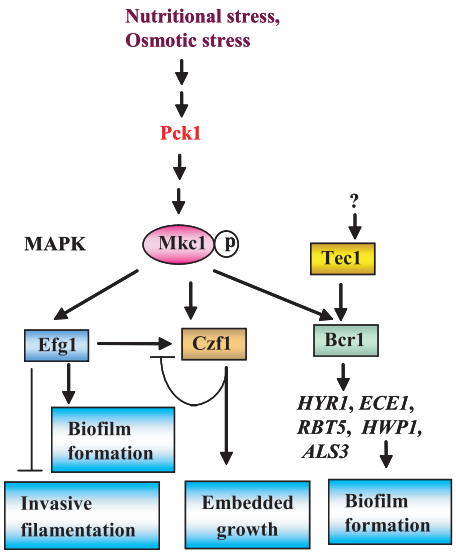

FIG. 5. Downstream targets of the PKC pathway. Under environmental stress conditions, the MAPK Mkc1 is activated in a Pck1-dependent manner. Then, it activates transcription factors Efg1, Czf1, and Bcr1 under different conditions for morphogenesis in *C. albicans*. *CZF1* expression requires Efg1 and is negatively regulated by Czf1. Tec1 regulates the expression of *BCR1*.

cleotide exchange factor protein of the GTP-binding protein ScRho1. ScRho1 then activates the ScPkc1 protein kinase, which in turn activates a MAPK module consisting of ScBck1; two MAPKKs, ScMkk1 and ScMkk2; and the MAPK ScSlt2 (Mpk1). ScSlt2 then activates downstream transcription factors such as ScRlm1, involved in the activation of cell wall genes, and SBF, a heterodimeric transcription factor required for G_1-specific gene expression (120, 123, 178). Based on these data with budding yeast, mutants in the *C. albicans* PKC pathway homologs have been tested for defects in morphogenesis (Fig. 5). A *pkc1* null strain has a cell lysis defect, which is osmotically remediable; however, normal hyphal morphogenesis occurs in stabilized liquid serum media (230). The downstream target of Pkc1, the MAPK Mkc1 (homolog of yeast Slt2), is also affected in morphogenesis under certain conditions (215, 217). Homozygous *mkc1* mutant cells have cell wall defects and are recovered in lower counts with shorter hyphae in the target tissues of infected mice. These data show that Mkc1 is clearly necessary for virulence in a mouse model of systemic infection (80).

Mkc1 is phosphorylated in the presence of oxidative stress, changes in osmotic pressure, cell wall damage, and a decrease in the growth temperature. The phosphorylation of Mkc1 largely depends on Pkc1, but under certain conditions Hog1 is required for Mkc1 phosphorylation, illustrating the interconnection between different MAPK pathways in *C. albicans*. One possibility is that Hog1 may be required for the expression of genes that are important for the glycosylation of the receptors (Wsc or Mid2 homologs) that activate the Mkc1 pathway (216). Kumamoto has further investigated the role of Mkc1 as a kinase that is specifically activated upon contact with a solid support (160, 161). This is important from a clinical point of view, as biofilm formation on various implants is currently the biggest problem in hospitals. Mkc1 is required for invasive hyphal growth and normal biofilm development (Fig. 5). The transcription factors that are activated (or indirectly induced) by Mkc1 remain to be identified, but possible candidates are Efg1 and Czf1. As described previously, Efg1 is required for biofilm development (237). Czf1 is required for filamentous growth under embedded and semisolid conditions (47), and ectopic expression of the Czf1 gene restores the filamentation defect of the *mkc1* mutant. Another potential downstream target of Mkc1 is the zinc finger transcription factor Bcr1. Bcr1 is required for biofilm formation but not for hyphal growth. The expression of *BCR1* depends on the hyphal regulator Tec1, and the downstream effectors of Bcr1 are genes encoding cell surface proteins and adhesins (Fig. 5) (223).

GlcNAc Catabolic Pathway of *C. albicans*

GlcNAc is one of the known in vitro inducers of the yeast-to-hypha transition in *C. albicans* and promotes germ tube formation in *C. albicans* within 3 h. The response of *C. albicans* to GlcNAc involves dramatic changes in the enzyme levels of the aminosugar catabolic pathway. The most interesting feature of this pathway is that all of the genes involved in the catabolism of GlcNAc exist in a cluster (162) housing six genes (Fig. 6). Three of the cluster genes encode known enzymes—glucosamine-6-phosphate deaminase (Nag1), GlcNAc-6-phosphate deacetylase (Dac1/Nag2), and GlcNAc kinase (Hxk1/Nag5)—that act sequentially on GlcNAc to generate fructose-6-phosphate, which is fed into the glycolytic pathway (Fig. 6). The notable traits of this metabolic pathway are that it is inducible by GlcNAc and that GlcNAc can be utilized as a sole carbon source. The transcripts for these genes are induced within minutes of each other (162), indicating the activation of a *trans*-acting factor(s) that enables the coordinated expression of the catabolic pathway genes. GlcNAc was unable to induce the other three genes in the cluster, which encode predicted transmembrane proteins (Nag3/Tmp1 and Nag4/Tmp2) and a cytosolic protein with a possible GTP-binding site (Nag6) (258, 326, 327). Disruption of the Nag regulon cluster, including deletion of *DAC1*, *NAG1*, and *HXK1*, results in a mutant that is unable to grow on aminosugars and which is avirulent in a murine model of systemic candidiasis (267). Hyphal formation under GlcNAc-inducible conditions is attenuated in the mutant, but it shows hyperfilamentation and a change in colony morphology under other filament-inducing conditions, such as Spider (where mannitol is used as the carbon source) or SLAD (nitrogen starvation) medium. When the genes (*NAG1*, *DAC1*, and *HXK1*) are disrupted individually, the growth pattern remains the same, i.e., all three mutants show impaired growth in GlcNAc but normal growth in glucose. The hyperfilamentation phenotype on Spider and SLAD media appears attributable to disruption of the *NAG1* gene. Virulence in a murine model of systemic candidiasis is variably affected in the three individual mutants, with the strongest attenuation for the *hxk1* mutant, followed by the *dac1* and *nag1* mutants (267, 326). In summary, these three genes are involved not only in metabolism of GlcNAc but also in morphogenesis and virulence. These processes do not seem to be related to one another. Experiments with immobilized GlcNAc, unavailable to the cells, show that GlcNAc utilization by the cells is not required for germ tube induction (290). This clearly points toward sensing of

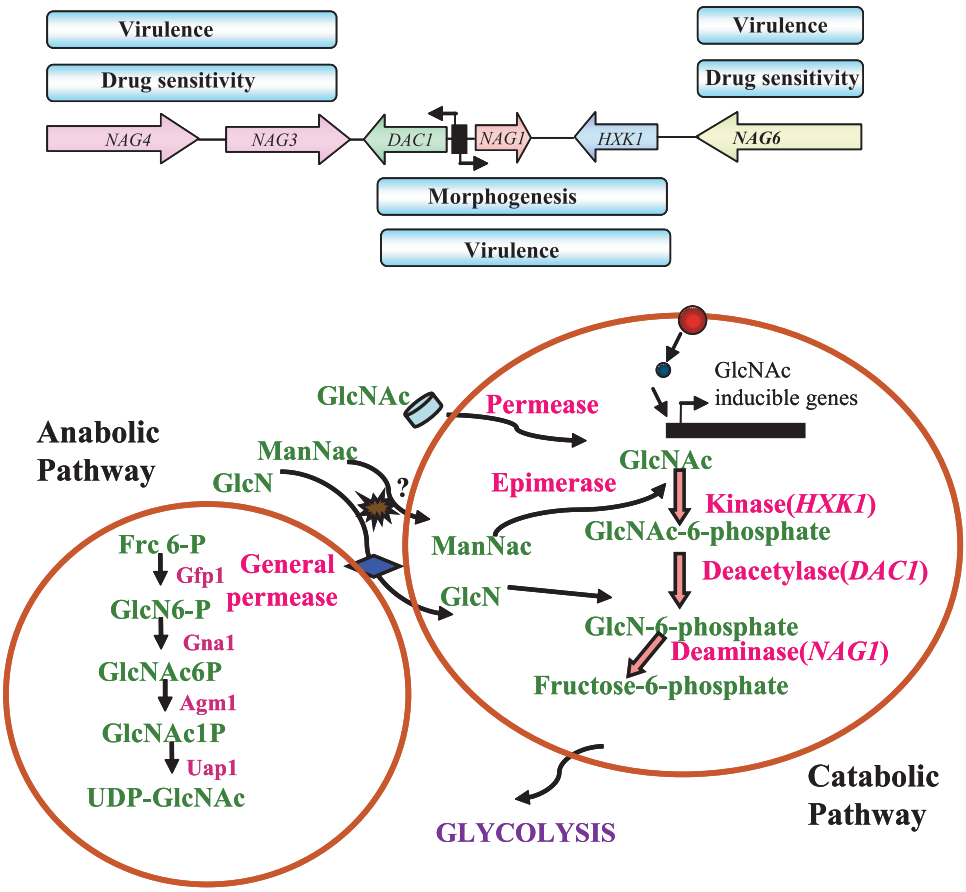

FIG. 6. (Top) Chromosomal organization of the *NAG* gene cluster in *C. albicans*. The organization of the cluster of genes on chromosome 6 and their direction of transcription are illustrated. *NAG1* and *DAC1* are transcribed in opposite directions from a bidirectional promoter. (Bottom) Metabolism of GlcNAc in *C. albicans*. GlcNAc is synthesized by *C. albicans* from fructose-6-phosphate by sequential action of glutamine: Frc 6-P amidotransferase (Gfp1), GlcN6-P acetyltransferase (Gna1), GlcNAc6P mutase (Agm1), UDP-GlcNAc pyrophosphorylase (Uap1). On the other hand, GlcNAc permease, GlcNAc kinase, GlcNAc-6-phosphate deacetylase, and GlcN-6-phosphate deaminase act sequentially on GlcNAc to generate the fructose-6-phosphate that is fed into the glycolytic pathway. *N*-Acetylmannosamine epimerase converts *N*-acetylmannosamine, which is imported in the cell by an unknown permease (?), into GlcNAc. Glucosamine can directly enter the cell via a general sugar permease and is converted to glucosamine-6-phosphate by the action of a GlcN-kinase.

GlcNAc at the cell surface, but which receptor is involved is not yet understood.

The capacity to metabolize GlcNAc is present in various bacteria, such as *Escherichia coli* (313), *Klebsiella pneumoniae*, and *Vibrio* species (328), but, surprisingly, is absent in *S. cerevisiae*. The main reason for this seems to be the absence of genes homologous to *NAG1*, *DAC1*, and *HXK1*. A notable exception is *S. cerevisiae* strain 3059, a clinical isolate, which grew well on both glucose- and GlcNAc-containing media (265). How this GlcNAc is metabolized in this particular strain is not yet known. The functions of the other genes in the Nag regulon are not yet clear. *NAG3* and *NAG4* have more than 80% homology to each other and have significant sequence homology to *S. cerevisiae* Ypr156C and Ygr138C (both transporters of spermine) (162, 327). *NAG3* and *NAG4* are not required for GlcNAc uptake, as there was no difference between the growth of a *nag3 nag4* mutant in GlcNAc-containing medium and the growth of the wild-type strain (258). The *nag3 nag4* mutant became sensitive to cycloheximide, 4-nitroquinoline 1-oxide, and 1,10-phenanthroline. The above observations indicate that though *NAG3* and *NAG4* are present in the same gene cluster as the catabolic pathway genes, they are not indispensable for GlcNAc metabolism or GlcNAc-mediated signaling (258). *NAG6* encodes a protein involved in drug sensitivity that has no human or murine homolog (327). This, together with the fact that Nag6 is required for virulence, makes it an interesting target for antifungals.

β-*N*-Acetylglucosaminidase and virulence. β-*N*-Acetylglucosaminidase is a chitobiase that likely acts together with chitinase to release GlcNAc from chitin by hydrolysis (20). This process could have two biological effects: first, it may provide an appropriate carbon source to the fungus; second, it may facilitate adhesion of *Candida* cells to host tissues by bringing about conformational changes in the cell wall (55). The enzyme is reported to be induced by GlcNAc and repressed by other sugars, such as glucose (94-fold lower), fructose, galactose, and mannose (220). Thus, GlcNAc not only regulates its own catabolic pathway but also regulates the availability of GlcNAc to the cell. β-*N*-Acetylglucosaminidase is secreted and deposited into various parts of the cell wall of both the yeast

and hyphal forms. A mutant deficient in β-*N*-acetylglucosaminidase exhibits attenuated virulence in a mouse model of candidiasis (141), although it shows normal growth. The *HEX1* gene encodes β-*N*-acetylglucosaminidase and produces a transcript that is 200 nucleotides longer when isolated from GlcNAc-grown cells than from glucose-grown cells, implying that alternate transcription termination sites are used depending on the growth conditions (55). Temperature and pH changes also affect the level of *HEX1* transcripts and β-*N*-acetylglucosaminidase activity.

pH Regulation in *C. albicans*

C. albicans causes infections in a broad range of host niches, which show significant differences in ambient pH. For example, the mouse systemic pH is 7.3, whereas the pH in the rat vagina is 4.5. The ability of *Candida* to react appropriately to (among other environmental variables) rather different pH environments is crucial for its pathogenicity. The environmental pH acts as a manipulator for many physiological functions, including morphogenesis (93). Under optimal (37°C) temperature conditions, filamentation is favored by ambient pH values close to neutral and is considerably reduced at pH values lower than 6. In contrast, the yeast form predominates almost exclusively at pH 4 (50).

Genes involved in pH regulation. The pathway controlling pH-responsive gene expression has been most extensively dissected for the ascomycete *Aspergillus nidulans* (234). Central to the pH response is the pH-dependent activation of the zinc finger transcription factor encoded by *pacC* (297). PacC is synthesized in an inactive form, which is activated at alkaline pH by proteolytic removal of the carboxy terminus (203). Proteolysis is dependent on six genes: *palA*, *palB*, *palC*, *palF*, *palH*, and *palI* (297). The activated form of PacC induces the expression of alkaline pH-expressed genes and represses acidic pH-expressed genes (297). This regulatory pathway is apparently conserved, as various elements have been identified in *Yarrowia lipolytica* and *S. cerevisiae*. The *Y. lipolytica RIM101* gene product is activated by carboxy-terminal truncation and is required for alkaline pH-dependent expression of *XPR2*-encoded protease (168). In *S. cerevisiae*, the *PacC* homolog, Sc*RIM101*, was initially identified as controlling meiosis and haploid invasiveness (180, 286). ScRIM101 and the yeast homolog of PalB, ScCpl1, have been implicated in a pH-dependent growth response of yeast (97). Genes homologous to *palA* and *palI* are also present in *S. cerevisiae* (77, 218). Saporito-Irwin et al. used differential screening techniques to isolate genes with a pH-dependent expression profile and identified *PHR1*, a prototypical alkaline pH-expressed gene encoding a protein of 548 residues, carrying a glycosylphosphatidylinositol lipid anchor, which is required for apical cell growth and morphogenesis (251). *PHR1* is homologous to Sc*GAS1*, which encodes a GPI-anchored protein of the *S. cerevisiae* cell surface (225). *PHR1* is strongly expressed under conditions of alkaline pH but not at any pH below 5.5 (251). A second pH-regulated gene, designated *PHR2* (207), is expressed at an ambient pH below 5.5 and also plays a role in morphogenesis (251). Deletion of either one or both genes has a strong effect on virulence. Deletion of *PHR1* results in pH-conditional defects in growth, morphogenesis, and virulence, evident at neutral to alkaline pHs. Conversely, a *phr2* null mutant exhibits similar pH-conditional defects in growth and morphogenesis that manifest at acidic rather than alkaline pH values. Engineered expression of *PHR1* at acidic pH in a *phr2* mutant strain and *PHR2* at alkaline pH in a *phr1* mutant strain complements the defects in the opposing mutant. A strain lacking both *PHR1* and *PHR2* shows pH-independent constitutive growth and morphological defects (108, 207). When such strains were tested for pathogenicity in various niches of the host, the virulence phenotype paralleled the pH dependence of the in vitro phenotypes. The *phr1* mutant was avirulent in a mouse model of systemic infection but uncompromised in its ability to cause vaginal infection in rats. The virulence phenotype of a *phr2* mutant was the inverse. Heterozygous mutants exhibited partial reductions in their pathogenic potential, suggesting a gene dosage effect (75). Another pH-regulated gene, *PRA1*, a homolog of surface antigens of *Aspergillus* species, with maximal expression at neutral pH, has an important role in host-pathogen interactions during candidal infection (259).

To investigate the pH-dependent regulation of *PHR1* and *PHR2* in *C. albicans*, Fonzi et al. isolated and characterized *PRR1* (pH response regulator), which is the *C. albicans* homolog of *palF* (now renamed *RIM8*). Expression of *RIM8* is pH dependent, and a mutant lacking *RIM8* is defective in pH-dependent regulation of gene expression. *PHR1* is no longer induced at alkaline pH, and *PHR2* is no longer repressed in both alkaline and acidic pH. Thus, Rim8 is a component of the pH response pathway in *C. albicans*. A *rim8* mutant exhibited no morphological abnormalities at either pH; however, it cannot form hyphae on serum-containing medium (235). The ability to form hyphae on serum is not restored by forced expression of *PHR1*, indicating that additional *RIM8*-dependent genes are required for hyphal development. As discussed above, *HWP1* is an Efg1 target gene required for normal filamentation, but it also appears to be downstream of Rim8. Expression of *HWP1* is induced in the absence of *RIM8*, indicating that the pH response pathway is not required for the expression of *EFG1*-dependent genes. This suggests that the pH response pathway is distinct from the *EFG1*-dependent signaling pathway (235). In this regard it might be noted that *EFG1* lies downstream of *TPK2*, which encodes a cAMP-dependent protein kinase (276). *Rim101* of *S. cerevisiae* contains a functionally significant recognition site for phosphorylation by cAMP-dependent protein kinases (286). Although this site is not conserved in the *C. albicans* homolog, two potential phosphorylation sites are present, and this could provide a regulatory connection between Tpk2, Rim101, and Efg1.

Rim101-dependent and -independent pathways. Fonzi's group characterized the full-length homolog of *pacC*, which they designated *PRR2* (for "pH response regulator," now renamed *RIM101*). *C. albicans RIM101* encodes a protein of 604 amino acids that shares two structural features with other members of the Rim101 family. First, the zinc finger domain of the Rim101 family is highly conserved; *C. albicans* Rim101 has 57% identity and 87% similarity to the *S. cerevisiae* homolog in this region. Second, *C. albicans* Rim101 has an 84-amino-acid C-terminal region with 32% D/E residues, which is conserved in other members of this family (in *S. cerevisiae*, *Y. lipolytica*, and *A. nidulans*).

As with its homologs in other species (see above), *C. albi-*

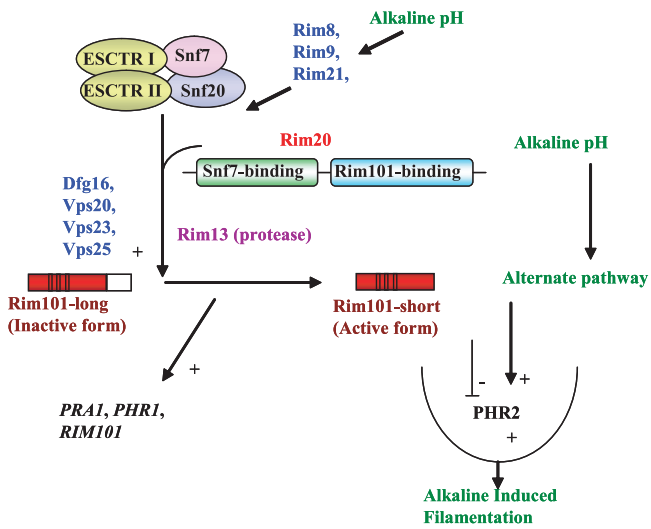

FIG. 7. Alkaline responses in Rim101 regulation. Alkaline pH stimulates Rim101 activity through increased expression and proteolytic activation. The ESCRT-I, ESCRT-II, and Snf7-Vps20 complexes are required for Rim101 activation. The Rim13p-dependent C-terminal proteolytic processing event also depends on Rim20, Rim8, and other transmembrane proteins. Full-length Rim101-long does not have a known activity. Processed Rim101-short is required for the alkaline response, which includes activation of alkaline pH-induced genes, repression of alkaline pH-repressed genes, and filamentation. Since Rim101 is an alkaline pH-induced gene, its expression may depend on autoregulation by Rim101-short. Alkaline pH also stimulates a RIM101-independent pathway. This pathway activates *PHR2* expression and stimulates filamentation in conjugation with Rim101.

cans Rim101 activity is controlled by proteolytic processing. Under acidic conditions, Rim101 exists primarily in a full-length "long" form (180). Under alkaline conditions, a carboxyl-terminal portion is cleaved to yield the active "short" form. Proteolysis is controlled by pH through the action of a number of gene products, including Rim20, Rim8, Rim13, and Rim9 (78, 180) (Fig. 7). Together with two other pH-regulatory genes, *RIM8* and *RIM20*, Rim101 acts to induce expression of *PRA1*, *PHR1*, and *RIM101* itself under alkaline conditions (238). In the absence of *RIM101*, alkaline and acidic pH-expressed genes, such as *PHR2*, are no longer repressed (239). The same phenotype is observed in the *rim8* mutant (235). PacC homologues from three filamentous fungi bind the consensus sequence 5′-GCCARG-3′ in vitro (251, 288, 297), and a similar DNA sequence (5′-NCCAAG-3′) is recognized by Rim101 of *C. albicans*.

Unlike *Aspergillus* and *Yarrowia* spp., *C. albicans* remains pH responsive in the absence of the *RIM101* pathway. For example, a dominant *RIM101-405* allele, which complements the filamentation defect of the *rim101* null mutant, promotes filamentation very weakly at acidic pH. Thus, the uncoupling of Rim101 processing from the upstream regulators does not completely bypass the control of filamentation by external pH. In addition, *PHR2* becomes an alkaline pH-induced gene in cells that lack *RIM101* pathway functions. Thus, both morphological and gene expression responses suggest the existence of a *RIM101*-independent pH response pathway (Fig. 7). This pathway has two roles: to stimulate *PHR2* expression at alkaline pH and to act in conjunction with Rim101 to activate filamentation (72). Davis et al. also found that the RIM101 pathway is necessary in vivo for pathogenesis. Using the mouse model of hematogenously disseminated systemic candidiasis, they showed that both *rim101* and *rim8* mutants have significant reductions in virulence. The virulence defect of the *rim8* mutant is not due solely to a failure to process Rim101, since the *rim8* mutant has a more pronounced virulence defect than does a *rim101* mutant. These observations suggest that both processed and unprocessed Rim101 may have a function during infection. Thus, it seems also that unprocessed Rim101 has a functional role in target gene regulation (73).

Participation of endocytic machinery in pH signaling. Rim20 is a Bro1-domain protein; these are proteins that normally participate in endosome metabolism, but in *C. albicans* Rim20 is required for alkaline pH-stimulated cleavage of Rim101 (321). Rim101 cleavage removes a C-terminal PEST-like sequence allowing the N-terminal zinc finger domain to regulate transcription (167, 180). Rim20 is thought to function as an adaptor (similar to the yeast Bro1 gene), binding Snf7, to bring the protease Rim13 in close proximity with Rim101, thus promoting Rim101 cleavage and activation (Fig. 7) (322). Snf7 is a component of machinery designated ESCRT (for "endosomal sorting complexes required for transport"), a group of three protein complexes that sort endocytic cargo into multivesicular bodies (9, 159). A connection between ESCRT machinery and Rim101 signaling was predicted based on two-hybrid assay-based interactions between Rim20 and Snf7 (138). Also, mutants with defects in the ESCRT subunits fail to produce cleaved Rim101 (67, 322). A GFP-Rim20 fusion is located in the cytoplasm under acidic conditions; under alkaline conditions, it is located to punctate foci (39). All known proteins required for Rim101 cleavage, i.e., the arrestin homolog Rim8; the transmembrane proteins Rim9 and Rim21; and Dfg16, Snf7, Vps20, Vps23, and Vps25, are required for the appearance of Rim20-GFP to punctuate foci. Dfg16 and Rim21 are seven-transmembrane domain-containing proteins and therefore may constitute the sensors for external pH (21, 234). Sensing of external pH may actually be triggered by the Rim8-directed endocytosis of these two receptor proteins (21). Evidence for this hypothesis comes again from work performed with *Aspergillus*. The Rim8 homolog PalF behaves as a pH-responsive arrestin: alkaline growth conditions induce its phosphorylation and ubiquitination. This is the first example of an upstream component of the Rim101 pathway that is directly controlled by pH (121).

DOWNSTREAM TARGETS OF THE DIFFERENT ENVIRONMENTAL SENSING PATHWAYS INVOLVED IN MORPHOGENESIS

Adhesins

The virulence factors expressed or required by *Candida* species, and in particular by *C. albicans*, vary depending on the type of the infection (i.e., mucosal or systemic), the site and stage of infection, and the nature of the host response (89). One of the important virulence attributes is adhesion. Most studies focus on the role of two well-characterized adhesins (Hwp1 and the *ALS* family) in the morphogenesis, pathogenicity, and phenotype switching of *C. albicans* (291).

Role of Hwp1 in morphogenesis and pathogenicity. Proadhesive and proinvasive factors of *C. albicans* contribute to disease by mediating the penetration of host tissues. Filamentous forms, particularly true hyphae, embed themselves within the superficial, keratinized layer of stratified squamous epithelium and grow by apical extension (283). The true hyphae are extensions of germ tubes that emerge from yeasts. To explore the role of surface proteins in tissue invasion, the function of *HWP1*, a developmentally regulated gene in germ tubes and true hyphae, was studied (283). *HWP1* encodes an outer mannoprotein, with a cell surface-exposed NH_2-terminal domain and COOH-terminal features conferring covalent integration into cell wall β-glucan. It may belong to a unique subset of GPI-anchored proteins characterized by the presence of a conserved structural motif, suggesting that it imparts a general property, e.g., interaction with specific surface proteins or wall polysaccharides. The surface localization of Hwp1 is compatible with diverse functions, from cell wall assembly to cell signaling. The evidence for the common occurrence of both membrane- and cell wall-anchored forms of cell wall-localized proteins comes from studies using fusion proteins consisting of α-galactosidase fused to C-terminal sequences from GPI-anchored cell wall proteins (196). Staab et al. showed that a 325-kDa GPI-anchored membrane species of Hwp1 is the precursor of a 301-kDa intermediate, which becomes covalently attached to β-glucan in the cell wall. This intermediate form may be released from membranes by phosphatidylinositol-specific phospholipase C, and it is found in the soluble fraction in cytoplasmic extracts and in small amounts in culture supernatants (282). *HWP1* has no known *S. cerevisiae* homolog.

HWP1 was originally isolated as a hypha- and germ tube-specific gene from a differential screen (283, 291). *HWP1* is conditionally required for hyphal formation: the ability to form hyphae on solid media is severely reduced in an *HWP1* heterozygous mutant and eliminated in the null mutant. In the presence of serum, colonies of the null mutant produce peripheral hyphae but at reduced levels compared to the wild type. All mutants of *HWP1* maintain the ability to invade agar directly beneath the colony and to form germ tubes in liquid serum-containing cultures. An *hwp1* null mutant has reduced virulence in a hematogenously disseminated murine model (262, 283), germinates less readily in the kidneys of infected mice, and causes less endothelial cell damage (303). When tested in vitro, the *hwp1* mutants germinate normally in liquid media containing 10% serum but exhibit reduced hyphal development when grown on serum-containing agar (262). In the murine kidney, the organisms are almost certainly exposed to serum constituents. The finding that the null mutants germinated very poorly in vivo suggests that the signal transduction pathway(s) that induces germination on solid media may also regulate germination in the murine kidney. The transcriptional and functional regulations of Hwp1 are summarized in Fig. 8. Staab et al. reported that the Hwp1 protein might act as an adhesin by serving as a substrate for host cell transglutaminases (283). More recently they have shown that Hwp1 participates in cross-links with proteins on the mammalian mucosa (292). In a model system of oroesophageal candidiasis, which is caused by the combined action of fungal virulence factors and host inflammatory responses when protective immunity is absent (18), the wild-type strain resulted in extensive alterations

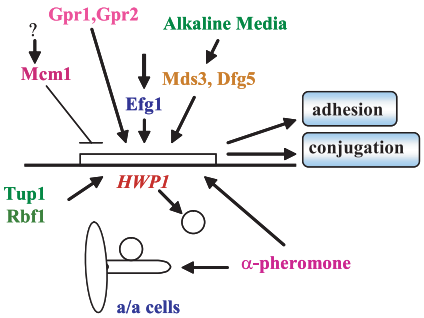

FIG. 8. Transcriptional and functional regulation of *HWP1*. Transcription of *HWP1* is regulated by Efg1. Interestingly, the hyphal gene repressors Tup1 and Rbf1 are positive regulators of Hwp1, whereas the MADS box transcription factor Mcm1 (245) negatively regulates *HWP1* transcription. The pH regulators Mds3 and Dfg5 are required for expression of *HWP1* in alkaline medium (74). *HWP1* is expressed in conjugation tubes of **a**/**a** cells by α pheromone of opposite-mating-type cells (184). The cell surface protein Hwp1 is involved in adherence and plays a role in mating.

of the lingual and esophageal mucosa, whereas mice treated with the *hwp1* mutant were not affected. Thus, *HWP1* is a promising target for the development of antifungal drugs for treatment of oroesophageal candidiasis.

***ALS* family and adhesion.** The *ALS* gene family of *C. albicans* encodes large cell surface glycoproteins with a three-domain structure that are implicated in the process of adhesion to host surfaces (126, 128). The *ALS* (for "agglutinin-like sequence") family includes eight genes, *ALS1* to *ALS7* and *ALS9*. Recent work showed that *ALS3* and *ALS8*, which were presumed to represent separate loci, are the same gene, eliminating *ALS8* from the family nomenclature (331). In *C. albicans*, *ALS* genes are found on three different chromosomes: chromosomes 3 (*ALS6* and *ALS7*), R (*ALS3*), and 6 (*ALS1*, *ALS2*, *ALS4*, *ALS5*) (96, 128). Each Als protein has a relatively conserved N-terminal domain, a central domain consisting of tandem copies of a highly conserved 108-bp unit of a repeated motif, and a serine-threonine-rich C-terminal domain that is relatively variable across the family. Within a given *ALS* gene, the size of tandemly repeated regions varies considerably between alleles due to differences in the numbers of copies of the 108-bp sequence. The different *ALS* family members are differentially regulated and exhibit variability in size and expression. Additionally, examination of various *C. albicans* isolates showed that certain strains lack *ALS* genes that are present in others (128, 129). Finally, allelic sequence polymorphisms can be found outside of repeated regions. These sequence differences are more pronounced within the 3′ end of the gene, which encodes the heavily glycosylated portion of the mature protein (128). Als1 was the first cell surface protein identified that functions as a downstream effector of filamentation in *C. albicans*. Overexpression of *ALS1* causes extensive flocculation and the formation of large aggregates of cells, a phenotype similar to that seen for *S. cerevisiae* Flo11. Both proteins function as effectors of filamentation and mediate adherence and flocculation (96, 115). Als1 functions as an effector of the cAMP-PKA pathway via Efg1 but does not appear to be an effector of the MAPK pathway (95). Als1 appears to be essential for virulence in hematogenously disseminated infection in

a murine model (52). Recently, a function for Als3 in biofilm formation was found by performing genome-wide expression analysis of the *bcr1* mutant, which is affected in biofilm formation. Als3 expression is strongly down-regulated in a *bcr1* mutant (223). *ALS3* overexpression rescues the *bcr1* mutant biofilm defect in vivo, arguing for an important role for Als3 under these in vivo conditions (222, 330).

Extracellular Hydrolytic Enzymes

In susceptible hosts, *C. albicans* enters the bloodstream and causes deep-seated infection in target organs. Because the organisms must cross the endothelial cells of the blood vessels to enter these organs, the interaction between *C. albicans* and vascular endothelial cells is likely to be a critical step in the initiation of a disseminated infection. One factor that contributes to this process of virulence is hydrolytic enzyme production. The three most significant extracellular hydrolytic enzymes produced by *C. albicans* are the secreted aspartyl proteinases (Sap), phospholipase B enzymes, and lipases. All 10 *SAP* genes of *C. albicans* encode preproenzymes approximately 60 amino acids longer than the mature enzymes, which are processed when transported via the secretory pathway (213).

***SAP* gene expression in the yeast-to-hypha transition.** The hypha-deficient *efg1* mutant is, in contrast to the *cph1* mutant, not able to invade or damage tissue (see above). However, whether this could be explained solely by a block in dimorphism was not clear. Hube's group investigated the expression of the 10 *SAP* genes under hypha-inducing conditions (5% serum, different pH values, and different temperatures) in different genetic backgrounds (wild type, *efg1* mutant, and *cph1* mutant) (133). Expression of *SAP4* to *SAP6* was strongly reduced in *efg1* mutants in serum-containing medium, which is a strong environmental signal for hyphal growth of wild-type cells (316). However, expression of all three genes was enhanced in both *efg1* and *cph1* mutants when hyphal formation was induced via a pH and temperature shift protocol, suggesting that the transcription factors Efg1 and Cph1 can be either positive or negative regulators of *SAP4* to *SAP6* expression. Since *efg1* mutants do not produce hyphal cells under these conditions, the expression of *SAP4* to *SAP6* is not strictly linked to hyphal morphology but is regulated by another factor that regulates hyphal formation. Expression of Sap antigen during systemic infections has long been recognized in antigen-antibody studies (246). Staib et al. demonstrated high-level expression of *SAP5* in all phases of infection with wild-type cells and a late induction of *SAP2* and *SAP6* using in vivo expression technology. With Sap-specific antibodies, Sap1 to Sap3 proteinases were detected on both yeast and hyphal wild-type cells, while the Sap4- to Sap6-specific antigen was identified mostly on penetrating hyphal cells, conferring hypha-specific expression of Sap4 and Sap6. The transcription factors Cph1 and Efg1 are involved in the expression of *SAP5*, as the avirulent *cph1 efg1* mutant does not express this gene (284). Although expression of *SAP2* was high in all in vivo samples investigated and large amounts of Sap1 to Sap3 antigen were found on all types of wild-type *Candida* cells, the level of expression did not correlate with the importance of the corresponding gene for invasion. Only mutants lacking *SAP6* had strongly reduced abilities to invade and damage parenchymal organs, despite the fact that hyphal production was normal and all other proteinase genes were still expressed (91). It can be concluded that the reduced virulence of hypha-deficient mutants is not only due to the inability to form hyphae but also due to modified expression of *SAP* genes normally associated with hyphal morphology.

Phospholipase B. Phospholipases are important pathogenicity determinants in *C. albicans*. They play a significant role in damaging cell membranes and invading host cells. High phospholipase production is correlated with increased adherence and a higher mortality rate in animal models (201). Various phospholipases reported from *C. albicans* include phospholipases A, B, C, and D (219). Phospholipase A and lysophospholipase activities are found in the cell wall of yeast cells and hyphae. Enzyme activity is higher in the walls of older yeast cells than of younger cells and is more prominent at the tip of growing hyphae (113). A *C. albicans* strain deleted for the phospholipase B gene *PLB1* has significantly attenuated virulence (107, 173). Although deletion of *PLB1* does not produce any detectable effects on *Candida* adherence to human endothelial or epithelial cells, the ability of the *plb1* null mutants to penetrate host cells is dramatically reduced (209). Thus, phospholipase B may well contribute to the pathogenicity of *C. albicans* by abetting the fungus in damaging and traversing host cell membranes, processes which likely increase the rapidity of disseminated infection (173). Expression of *PLB1* is regulated by nutritional supplementation, environmental factors, and the growth phase of the *C. albicans* cells, as well as by physiological conditions. The differential expression of *PLB1* in response to environmental factors may be correlated to host-specific components available to *C. albicans* (208). These data prove that phospholipase B is essential for *Candida* virulence and pave the way for studies directed at determining the mechanism(s) through which phospholipases modulate virulence in this organism. The phospholipase D1 has been reported to be important for morphological transition under certain conditions (132).

FUTURE CHALLENGES

The mechanism of the yeast-to-hypha transition and identification of potential virulence attributes of *C. albicans* is a critical problem that requires further investigation for the development and improvement of targeted antifungal therapy. In the past, progress in *C. albicans* research was slow due to the lack of effective molecular tools, such as stable plasmid expression systems, limited mating ability, and the absence of known sporulation. The recent annotation of the *C. albicans* genome (68), together with the development of a whole range of molecular tools (e.g., codon-optimized reporter constructs), has greatly accelerated the pace of genetic research with this organism. In this review, we have highlighted studies that reveal the dynamics of cellular signaling during the yeast-to-hypha transition in *C. albicans*. Morphogenesis in *C. albicans* is regulated by a limited number of transcription factors, such as Efg1, Cph1, Rim101, Tup1, Bcr1, Czf1, Tec1, Nrg1, and Rbf1. These transcription factors are induced and/or activated by a whole array of environmental signals through a number of signaling pathways. Many of these transcription factors have

common downstream targets, which are the real triggers of morphogenesis. In the classical view, we see environmental sensing as a system in which a membrane protein is the upstream component of a signal transduction pathway. Recent studies have shown that this is not always the case. The sensing of environmental CO_2 concentrations seems to occur directly at the level of carbonic anhydrase and adenylate cyclase and does not seem to involve a membrane receptor. Similar systems may exist for the sensing of ammonia or other gases or some of the small quorum-sensing molecules.

Several important questions remain unanswered. (i) How do the different signals leading to morphogenesis and virulence affect the transcription of specific subsets of genes? What are the transcription factors that mediate the responses? The major transcription factor Efg1 is a key regulator of the yeast and hyphal program, but it is not required, or may be even inhibitory, for the later phase of filament formation. These observations suggest that additional regulators are involved in the control of morphogenesis and that there is a strong interplay between different signal transduction pathways. Alternatively, more feedback loops of gene regulation, such as for *EFG1* and *WOR1*, will probably be discovered. A future challenge will be to identify all transcription factors involved in morphogenesis and how they interact with each other or how they are interdependent. (ii) How are morphogenesis and virulence regulated in vivo? Mutants that are either nonfilamentous (e.g., *cph1 efg1* mutants) or hyperfilamentous (e.g., *pde2* or *hxk1* mutants) in vitro have strongly reduced virulence. Thus, it seems that a balance in filamentation is critical at the time of invasion and establishment of virulence. (iii) A third important issue that needs to be clarified is how the aminosugar GlcNAc induces cellular morphogenesis. Aminosugar utilization is an attribute of pathogenic yeast, and knockout of this catabolic pathway abolishes virulence. (iv) Finally, although several membrane receptors have been described, our knowledge of ligands remains limited. In this area of research, comparison with *S. cerevisiae* may be of limited use, as *C. albicans* has coevolved with its host. For instance, whereas in *S. cerevisiae* we have clearly shown that glucose and sucrose are the ligands of the G protein-coupled receptor Gpr1, the ligand of the homologous receptor in *C. albicans* remains to be identified. In addition, we will have to look into specific host components as potential ligands for certain receptors.

FINAL OUTLOOK

C. albicans is a suitable organism for studying dimorphism and pathogenicity. Major challenges still remain in determining how cells sense different sets of specific environmental signals and how the various signaling cascades process and integrate these signals. The phrase "many components but few connections" can characterize the current knowledge of the mechanisms of yeast-to-hypha transitions in *C. albicans*. Therefore, efforts should be directed toward analyses of functional linkages in addition to an expansion of the list of components affecting morphogenesis and virulence. A human-curated assembly of the *C. albicans* genome sequence has been released (www.candidagenome.org/) (44, 142). Bruno and Mitchell describe different strategies for overcoming the limitation of *C. albicans* gene functional analysis (49), and Noble and Johnson describe the use of new "lab" strains (avoiding the use of the *URA3* marker) and "high-throughput" gene deletion strategies (224). Comparative genome analysis provides important clues about the evolution of the species and its mechanisms of pathogenesis (142). Comparative analysis of genetic and biochemical pathways in *S. cerevisiae* and *C. albicans* has also been extremely fruitful in revealing pathways regulating virulence in *C. albicans*. With the current speed of identification of genomes of other pathogens (e.g., *C. glabrata* and *C. dubliniensis*), comparative genomics will be an important tool in the future to identify broad-spectrum antifungal targets and to understand fungal pathogenesis. The ability of *C. albicans* to pass through the diverse body tract requires niche-specific gene expression (130). Recently, tools for monitoring the expression of specific genes in different locations of the host have become available (19). The availability of new functional genomic technologies, including DNA microarrays for high-throughput gene expression profiling and knock-out mutant collections, should enable functional characterization of novel genes. As has been achieved for *S. cerevisiae*, the *C. albicans* community should put together a consortium to generate a deletion strain collection, as well as conditional mutant collections for essential genes. While technologies for rapid gene deletion construction exist (88, 224), such collections should be constructed with the use of dominant and reusable markers in order to avoid marker problems and for the generation of multiple deletions. At the signaling level, new strategies for identifying nonlinear complexities in signal transduction, such as cross-talks and feedback loops, must be developed. As a result, our view and appreciation of *Candida* biology and pathogenesis should undergo something just short of a revolution in the very near future.

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