MOLECULAR AND CELLULAR BIOLOGY, Nov. 1985, p. 2959–2966
0270-7306/85/112959-08$02.00/0
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Vol. 5, No. 11

Mechanism for Differential Sensitivity of the Chromosome and Growth Cycles of Mammalian Cells to the Rate of Protein Synthesis

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Received 28 May 1985/Accepted 2 August 1985

It has been documented widely that when the generation times of eucaryotic cells are lengthened by slowing the rate of protein synthesis, the duration of the chromosome cycle (S, G2, and M phases) remains relatively invariant. Paradoxically, when the growth of exponentially growing cultures of CHO cells is partially inhibited with inhibitors of protein synthesis, the immediate effect is a proportionate reduction in the rate of total protein, histone protein, and DNA synthesis. However, on further investigation it was found that over the next 2 h the rates of histone protein and DNA synthesis recover, in some cases completely to the uninhibited rate, while the synthesis rates of other proteins do not recover. We called this process chromosome cycle compensation. The amount of compensation seen in CHO cell cultures can account quantitatively for the relative invariance in the length of the chromosome cycle (S, G2, and M phases) reported for these cells. The mechanism for this compensation involves a specific increase in the levels of histone mRNAs. An invariant chromosome cycle coupled with a lengthening growth cycle must result in a disproportionate lengthening of the G1 phase. Thus, these results suggest that chromosome cycle invariance may be due more to specific cellular compensation mechanisms rather than to the more usual interpretation involving a rate-limiting step for cell cycle progression in the G1 phase.

When inhibitors of protein synthesis are added to cultures of exponentially growing cells, their doubling time lengthens approximately in proportion to the decreased rate of protein synthesis. With moderate inhibition of protein synthesis, almost all of the lengthening occurs in the G1 interval while the S, G2, and M intervals, named collectively by Mitchison (17) as the DNA division or chromosome cycle, are relatively unaffected (14, 15, 19, 20, 21). Rossow et al. (21) studied a Swiss 3T3 cell line which grew with a doubling time of about 16 h, 4 h in the G1 phase and 12 h in the chromosome cycle (S, G2, and M phases). When the rate of protein synthesis in this culture was inhibited to about 35% of the control rate by the addition of cycloheximide, the doubling time increased to about 63 h with a greatly increased G1 phase of about 47 h but only a slightly increased chromosome cycle of 16 h. Liskay et al. (14) have studied the Chinese hamster cell line V79-8 which had no measurable G1 interval; the chromosome cycle of 9.2 h accounted for the doubling time. When the rate of protein synthesis in these cultures was inhibited to 60% that of the control, their doubling times increased to 14.2 h with a G1 phase of 4.2 h but only a slightly increased chromosome cycle of 10.2 h. A relative invariance for the chromosome cycle has also been reported for yeasts (11) and other fungi (16). These results are commonly interpreted in terms of a step just before the S phase, usually in the G1 phase, which is rate limiting for cell cycle progression and which is particularly sensitive to the rate of protein synthesis or other environmental influences, while by unknown mechanisms the chromosome cycle (S, G2, and M phases) is much less limited by these influences (4, 15, 28).

On the other hand it has been shown that inhibition of protein synthesis immediately leads to an almost proportional inhibition of DNA synthesis (8). Considering that most histone and DNA syntheses are known to be coupled, these two sets of results presented a paradox. The rate of DNA synthesis was apparently inhibited in proportion to the inhibition of protein synthesis but the rate of progression through S phase apparently was not.

To attempt to resolve this paradox, we examined the effect of the rate of protein synthesis on the rate of histone protein and DNA synthesis in CHO cells. We found that immediately after the inhibition of protein synthesis, both histone protein and DNA syntheses were inhibited proportionately. However, within a few hours the rates of synthesis of S-phase histones and DNA increased relative to the rates of synthesis of nonhistone proteins. Thus, S-phase cells sense the rate of protein synthesis but compensate the rate of chromatin replication for this inhibition. By analyzing histone mRNA levels, we concluded that a major and, perhaps, the sole compensation mechanism was an elevation in the level of histone mRNA in response to the decreased rate of protein synthesis.

MATERIALS AND METHODS

CHO cells were grown in Hams HF-10 medium supplemented with 10% fetal calf serum, penicillin, and streptomycin. Cells were treated with hydroxyurea or cycloheximide dissolved in phosphate-buffered saline; the solutions were filter sterilized. Cell numbers were analyzed on a Coulter Counter (Coulter Electronics, Inc., Hialeah, Fla.). Growth rates were calculated from 4 days of exponential growth. Cultures were labeled with [^{14}C]lysine (ICN Pharmaceuticals Inc., Irvine, Calif.), at a final concentration of 1 μCi/ml in lysine-free HF-10 medium with 10% fetal calf serum. Cultures were labeled with [^{3}H]thymidine in complete media or in lysine-free medium if duplicate cultures were labeled with [^{14}C]lysine.

Histones were extracted from whole cells with 0.5 N HCl, and the extracts were analyzed by two-dimensional gel electrophoresis as described previously (33). For quantitation of the radioactivity in various proteins, the protein

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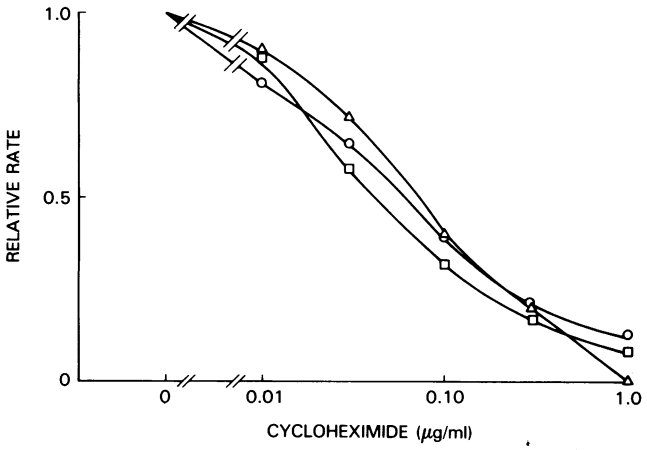

FIG. 1. Effect of cycloheximide on the rates of protein and DNA synthesis and multiplication of CHO cells. Cultures were preincubated with the indicated concentration of cycloheximide for 0.5 h and then labeled in the presence of the same concentration of cycloheximide for an additional 0.5 h either with 1 μCi of [^{14}C]lysine per ml or 1 μCi of [^{3}H]thymidine per ml in complete Hams HF-10 medium. (Hams HF-10 medium contains 3 μM thymidine). The rates of protein and DNA synthesis were determined as [^{14}C]lysine or [^{3}H]thymidine incorporated into trichloroacetic acid-precipitable material. The growth rate was determined from daily cell counts during 4 days of exponential growth in the indicated concentration of cycloheximide. In 1.0 μg of cycloheximide per ml, no increase in cell number was obtained; this growth rate was set to zero. Symbols: □, rate of protein synthesis; ○, rate of DNA synthesis; △, rate of cell multiplication.

islands were excised from the dried and fluorographed gels, digested overnight in scintillation vials with H_2O_2-NH_3 (95 parts of 30% H_2O_2 and 5 parts of concentrated NH_4OH). Aquassure (10 ml; New England Nuclear Corp., Boston, Mass.) was added for scintillation counting.

[^{3}H]thymidine incorporation was determined by the method of Schmidt and Thannhauser (23) as modified by Wu and Wilt (35) and was normalized per unit of optical density at 260 nm.

RNA levels were determined by the cytoplasmic dot blot method of White and Bancroft (32). The dot blots were hybridized with a nick-translated (Amersham kit with [^{32}P]dCTP) pBR322 plasmid containing a mouse H4 gene (gift from A. Seiler-Tuyns; 25) to determine histone H4 mRNA levels. A nick-translated pBR322 plasmid containing a chicken β-actin gene (gift from B. Paterson; 7) was used as the control. For quantitation, the dots were cut out and put in scintillation vials. The filter material was dissolved in 1 ml of methylcellosolve; 10 ml of Aquassure was added for scintillation counting.

For analysis of cell cycle distributions, CHO cells were seeded in T-150 culture flasks and grown for 48 h. Growth was exponential with a doubling time of 15 h after an initial lag of 12 h. At 48 h, the media were changed to include various concentrations of cycloheximide. Cell numbers were maintained below 2×10^6 per T-150 flask to minimize changes to the cell cycle distribution because of crowding (36). After 24 h, cells were prepared for flow cytometry analysis with chromomycin A3 (10). Analysis was performed on a Becton-Dickinson fluoresence-activated cell sorter (FACS) analyzer.

RESULTS

For exponentially growing CHO cells, the rates of DNA synthesis and cell doubling seem to be closely linked to the rate of protein synthesis (Fig. 1). At 0.03 μg of cycloheximide per ml, the rate of cell doubling was decreased to 70 to 75% of the uninhibited value (Fig. 1). These values agree with others reported in the literature. Calculated from the data of Liskay et al. (14), the rate of cell doubling of another Chinese hamster cell line (V79-8) was decreased to 65% of the control in 0.05 μg of cycloheximide per ml. A Swiss 3T3 cell line studied by Rossow et al. (21) doubled at approximately 75% of the uninhibited rate when grown in 0.03 μg of cycloheximide per ml. Thus, there does not seem to be great difference in the ability of cycloheximide to inhibit the multiplication of these different strains of mammalian cells.

High concentrations of cycloheximide which prevent cell multiplication are known to lead to elevations in the level of histone mRNA (3, 13, 22, 27, 30, 31; R. S. Wu, E. Sariban, and W. M. Bonner, J. Cell Biol., 99:134a, 1984). Sariban et al. (22) have shown that this increased amount of histone mRNA was translated, albeit very inefficiently, due to the concomitant inhibition of protein synthesis. We asked whether concentrations of cycloheximide which still permitted exponential growth could lead to increased histone mRNA levels. At 0.3 μg/ml, cycloheximide still permitted cell doubling at 20% of the uninhibited rate (Fig. 1). This concentration of cycloheximide did lead to increased histone H4 mRNA levels (Fig. 2). After a lag of half an hour, there was a rapid rise in the H4 mRNA level over the first hour followed by a slower rise, while the level of another mRNA, β-actin, did not change during the time period of this experiment. This result shows that concentrations of cycloheximide low enough to allow exponential growth of CHO cells led to relative increases in the level of histone mRNA.

Results obtained by Sariban et al. (22) have indicated that increased and decreased histone mRNA levels affect the synthesis pattern of histones; thus, we studied in some detail the effect of 0.3 μg of cycloheximide per ml on the synthesis of protein and DNA (Fig. 3). When 0.3 μg of cycloheximide per ml was added to exponentially growing cultures of CHO cells, the synthesis of proteins, histones, and nonhistones was inhibited to 20% of the control level (Fig. 3A versus 3B). However, after 4 h in cycloheximide, the synthesis of most but not all histone isoprotein species had increased relative

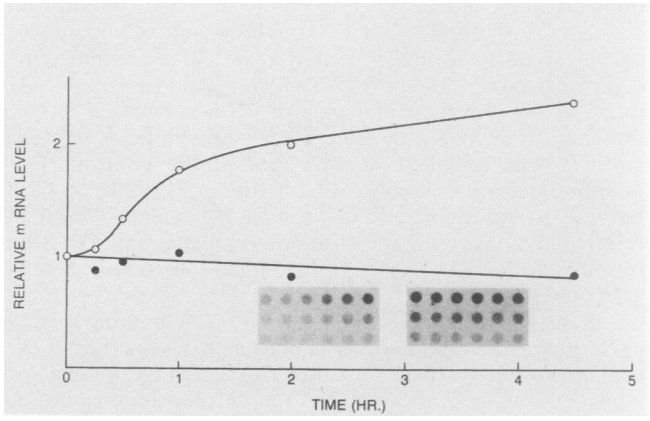

FIG. 2. Elevation of histone H4 mRNA levels during growth of CHO cells in 0.3 μg of cycloheximide per ml. Exponentially growing cultures of CHO cells were treated with cycloheximide for the indicated times. Cytoplasmic dot blots were prepared from these cultures and hybridized as described in the text. Symbols: ○, histone H4 mRNA level, fluorograph of dot blots in lower left; ●, β-actin mRNA level, fluorograph of dot blots in lower right. In dot blots, top, middle, and bottom rows correspond to 4×10^5, 2×10^5, and 1×10^5 cells per dot, respectively.

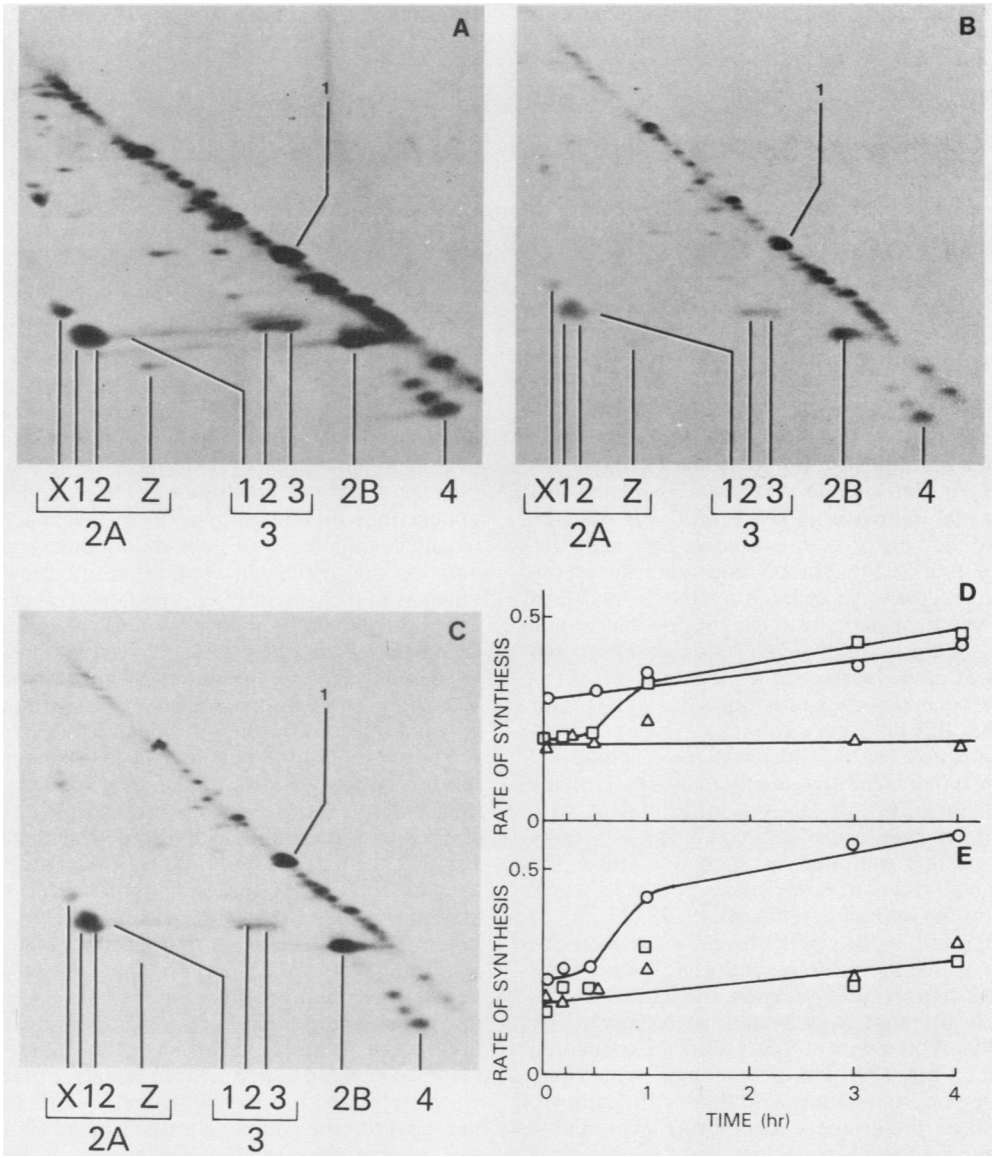

FIG. 3. Recovery of histone and DNA synthesis during growth of CHO cells in cycloheximide. Exponentially growing CHO cells were incubated with 0.3 μg of cycloheximide per ml for the indicated times and then labeled for 15 min with 1 μCi of [^{14}C]lysine per ml in lysine-free Hams HF-10 medium containing 10% fetal calf serum and 0.3 μg of cycloheximide per ml. Cell proteins were extracted and analyzed as described in the text. Duplicate cultures were labeled under the same conditions with 1 μCi of [^{3}H]thymidine per ml in Hams HF-10 medium (containing 3 μM thymidine). These samples were processed as described in the text. (A through C) Fluorographs of two-dimensional gels of cellular histones and other proteins. (A) Uninhibited control (for setting of the 100% values). (B) One minute after the addition of cycloheximide. (C) Four hours after the addition of cycloheximide. (D and E) Quantitative analysis of particular protein islands from gels shown in panels A, B, and C, as well as other gels not shown. (D) symbols: ○, [^{3}H]thymidine incorporation per optical density unit at 260 nm; □, average of all the core histone islands marked in panel A (not including H1, noted by 1 in panels A through C; each of the four core histones behaved in a manner similar to this average; △, Average of six nonhistone proteins form the diagonal and off the diagonal. (E) Symbols: ○, H2A.1/H2A.2; △, H2A.X; □, H2A.Z.

to that of the nonhistone proteins (Fig. 3B versus 3C). When a group of six nonhistone proteins, as well as the histone proteins from the gels shown in Fig. 3A, B, and C and from other gels at intermediate time points (data not shown), were analyzed, the rate of synthesis of the nonhistone proteins was found not to change significantly over the course of the experiment (Fig. 3D). In contrast, the rate of total histone synthesis increased in a biphasic manner; after a 0.5 h lag, it increased rapidly over the next 0.5 h and then more slowly over the next 3 h (Fig. 3D). At 7 h, the rate of histone synthesis had decreased slightly (data not shown), presumably because more cells were completing the S phase than were entering it. The kinetics of the induction of histone protein synthesis were almost identical to the kinetics of the increase in histone mRNA levels shown in Fig. 2.

Although the rate of synthesis of most histone isoprotein species recovered after the inhibition of protein synthesis, the rate of synthesis of several species, in particular the H2A isoprotein species H2A.X and H2A.Z, did not (Fig. 3E). We have shown previously (33) that the synthesis of H2A.X and H2A.Z are not linked to the synthesis of DNA; thus, CHO cell cultures synchronized in the G1 phase or treated with

inhibitors of DNA synthesis had very low rates of synthesis for H2A.1 and H2A.2 but almost normal rates for H2A.X and H2A.Z. Sariban et al. (22) have shown that when chromatin replication is inhibited by treating CHO cells with growth-arresting concentrations of cycloheximide, the synthesis of the H2A.1 and H2A.2 increases relative to that of H2A.X and H2A.Z. Figure 3E shows a similar result when growth-permitting concentrations of cycloheximide are used. The rates of synthesis of H2A.X and H2A.Z do not seem to change significantly with time, while those of H2A.1 and H2A.2 do increase with time. The H2A isoprotein synthesis ratio (H2A.1 + H2A.2)/(H2A.X + H2A.Z) approximately doubles over the course of the 4 h, an increase very similar to the proportionate increase in histone mRNA levels (Fig. 2).

In this experiment DNA synthesis was also measured in duplicate cultures (Fig. 3D). The rate of DNA synthesis was immediately inhibited almost but not quite to the same extent as was the rate of histone synthesis. Evidence that this immediate partial inhibition of DNA synthesis is mediated through an effect on protein synthesis has been discussed by Sariban et al. (22). Total histone synthesis recovered after 1 h to the same level as DNA synthesis, and then the two levels of synthesis rose more slowly together. Such initial differences in the extent of inhibition of DNA and histone synthesis at growth-arresting concentrations of cycloheximide have been reported previously (1, 8, 24). Our results indicate that this difference can also exist at growth-permitting concentrations of cycloheximide (see below).

It has been shown that treatment of exponentially growing cell cultures with inhibitors of DNA synthesis leads to a decrease in histone mRNA levels (3, 5, 9, 12, 26). If protein synthesis, then, is also inhibited in such a culture, the histone mRNA level rises, in some cases reaching levels above that found in the uninhibited control (9, 22, 27, 31). If these larger changes in histone mRNA levels are reflected in a larger recovery of histone protein synthesis, the relationship between these two would be further substantiated.

Hydroxyurea at 0.1 mM was added to exponentially growing CHO cells. This concentration allows exponential growth at a reduced rate (29; data not shown), with DNA synthesis rate being approximately 25% that of the control. Histones synthesized under these conditions exhibited a pattern between the G1- and S-phase patterns (33), which is consistent with the partial inhibition of DNA synthesis and the lowered level of S-phase histone mRNA (Fig. 4A). Immediately after the addition of 0.3 μg of cycloheximide per ml, the rates of synthesis of all the proteins visible on these two-dimensional gels (Fig. 4A versus 4B) were inhibited to a similar extent. During the next 4 h, the rates of synthesis of most histone proteins increased substantially (Fig. 4B versus 4C), while the rates of synthesis of the nonhistone proteins and H2A.X and H2A.Z did not increase significantly. Figure 4D shows that the rate of synthesis of H2A.1 and H2A.2 increased sixfold, from 12% to 77% of the control value (Fig. 4A). These results substantiate the relationship between the increase in histone mRNA concentrations and the recovery of histone synthesis. In addition, a comparison of the experiments shown in Fig. 3 and 4 demonstrates that direct limitations on the rate of DNA synthesis can increase the extent of recovery of S-phase histone synthesis when protein synthesis is inhibited.

The selective recovery in the synthesis of the major structural components of chromatin raises the question as to whether other components required for completion of the chromosome cycle, such as elements of the mitotic apparatus, might have similar controls. The gels used in Fig. 3 and 4, as well as sodium dodecyl sulfate-acetic acid-urea-hexadecyl trimethyl ammonium bromide two-dimensional gels (data not shown), do not show any other proteins which have synthetic rates that react similarly. These gels do show a variety of nonhistone proteins from both the nucleus and cytoplasm; however, these proteins are still only a small fraction of the total proteins in the cell. The combination of the protocols used in Fig. 3 and 4 with a more general protein separation method (18) could reveal whether the synthesis of any other protein components are controlled in the same manner as that of S-phase histones.

We investigated the recovery of DNA synthesis throughout the growth range, and checked the validity of [^{3}H]thymidine incorporation as a measure of DNA synthesis. Figure 5 shows the temporal recovery of [^{3}H]thymidine incorporation after the addition of various concentrations of cycloheximide. Hams HF-10 medium contains 3 μM thymidine as a normal constituent. These conditions flood the endogenous thymidine pool (6) so that when [^{3}H]thymidine is added, the precursor pool should have a constant specific activity, and tritium incorporation into DNA should reflect changes in the rate of DNA synthesis rather than changes in the endogenous thymidine pool. To check this assumption, two sets of cultures were also treated with 1 μM fluorodeoxyuridine to inhibit endogenous thymidine synthesis. Figure 5 shows that the recovery of tritium incorporation was not affected by the presence of fluorodeoxyuridine.

To investigate the recovery of histone synthesis throughout the whole growth range, we took advantage of the differential response of the synthesis of the H2A isoprotein species to the inhibition of protein synthesis, as shown in Fig. 3. The rate of H2A.X and H2A.Z synthesis was taken as a measure of the initial and unrecovered rates of protein synthesis, while the rate of H2A.1 and H2A.2 synthesis was taken as a measure of the rate of recovered histone synthesis. The advantage of this procedure is that both values can be obtained from one two-dimensional gel sample, eliminating potential problems of changing amino acid pools and differential losses between samples. Figure 6A shows the result of such an experiment in which cultures of CHO cells were pretreated for 3.5 h with cycloheximide concentrations that covered the complete growth range (Fig. 1); proteins in the cultures were then labeled for 2 h in the same concentrations of cycloheximide. The results show that the rates of synthesis of the S-phase H2A isoprotein species H2A.1 and H2A.2 were considerably higher than were those for the basal H2A isoprotein species H2A.X and H2A.Z. The rate of total histone synthesis (Fig. 6A) was higher than the basal rate but not as high as the S-phase-specific H2A.1/H2A.2 rate because it contained contributions from basal synthesis in S-phase and G1- and G2-phase cells (33). The ratio of the H2A.1/H2A.2 and H2A.X/H2A.Z rates is shown in Fig. 6C. This histone compensation ratio is a measure of the recovery of histone protein synthesis after and during the inhibition of overall protein synthesis.

In the experiment shown in Fig. 5, the recovery of DNA synthesis was measured over a time course of 4.5 h. The rate of DNA synthesis measured in cells after 4.5 h of cycloheximide treatment was higher than the initial rate by a similar difference as was found for histone synthesis in Fig. 6A (Fig. 6B). The ratio of the two curves in Fig. 6B, the DNA compensation ratio, is plotted in Fig. 6C. This ratio follows the histone compensation ratio up to 0.03 μg of cycloheximide per ml and then levels off and falls. Also shown in Fig. 6C is the total compensation ratio, which is that ratio of

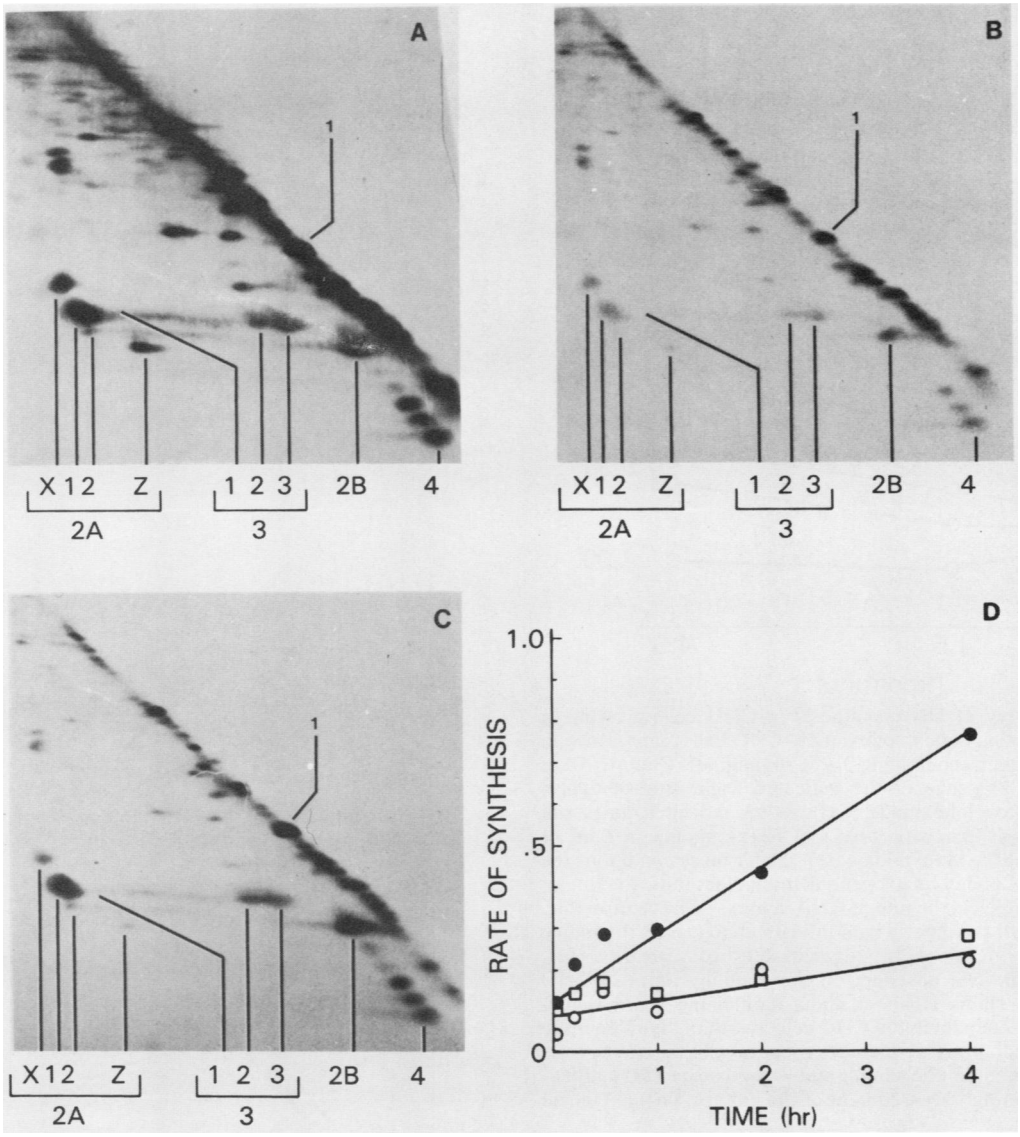

FIG. 4. Recovery of histone synthesis during incubation of hydroxyurea-pretreated CHO cells with cycloheximide. This experiment was carried out in parallel with that shown in Fig. 4, except that the cultures were treated with 0.1 mM hydroxyurea for 1 h before 0.3 μg of cycloheximide per ml was added. (A) Hydroxyurea control. (B) One minute after the addition of cycloheximide. (C) Four hours after the addition of cycloheximide. (D) Quantitative analysis of H2A isoproteins. Symbols: ●, H2A.1/H2A.2; ○, H2A.X; □, H2A.Z.

recovered histone and DNA synthesis that would be necessary to totally restore these rates to their uninhibited values.

In a comparable experiment, the mRNA levels for histone H4 and β-actin were measured; dot blots of this experiment, as well as the quantitative elevation of histone H4 mRNA levels, are shown in Fig. 6C. (β-Actin mRNA levels did not change significantly.) The very close correspondence between the recovery of S-phase histone protein synthesis and the histone mRNA level is striking, suggesting that modulation of S-phase mRNA levels may be the major mechanism of compensation.

The curves in Fig. 6C interrelate in three ways, depending on the extent of inhibition of protein synthesis. Up to cycloheximide concentrations of 0.03 μg/ml, all the curves rise together, indicating that in this range chromatin biosynthesis can completely recover to control rates. This range corresponds to that reported by Liskay et al. (14), in which the chromosome cycle in the Chinese hamster cell line V79-8 was not significantly lengthened.

At cycloheximide concentrations of 0.1 and 0.3 μg/ml, cells still grow exponentially, but the chromosome cycle also begins to lengthen (14). The curves (Fig. 6C) in this range diverge. Neither the histone nor the DNA compensation ratio increases as much as the total compensation ratio, but while the histone compensation ratio still rises gradually, the DNA compensation ratio starts to fall. A possible reason for this can be seen in Fig. 3D; the rate of DNA synthesis initially is not inhibited as much as the rate of protein synthesis, and as the rate of histone synthesis rises, the two rates become much closer after 1 h. The finding that some DNA replication continues even when protein synthesis is almost completely inhibited has been documented widely (1, 8, 24). These results (Fig. 3D) indicate that such a phenomenon may also appear when protein synthesis is only par-

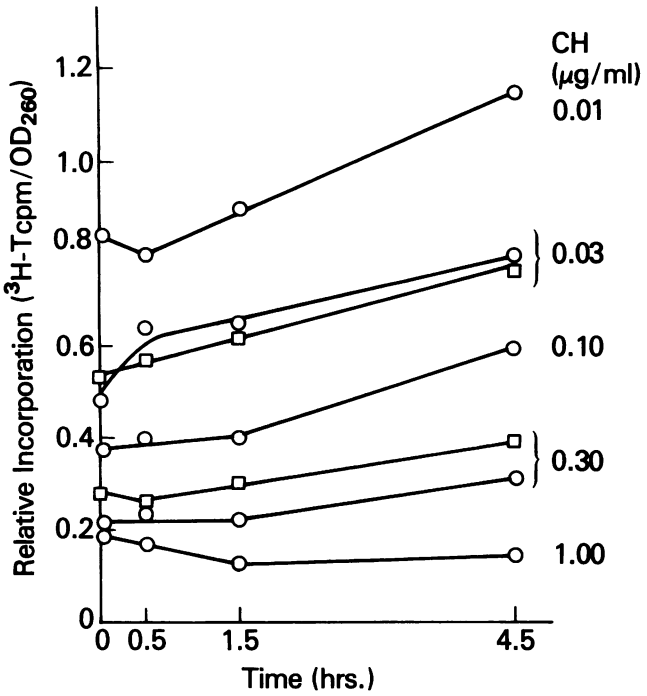

FIG. 5. Recovery of DNA synthesis in CHO cells growing in various concentrations of cycloheximide. CHO cells were grown in Hams HF-10 medium (containing 3 μM thymidine). Cultures were incubated in the presence of the indicated (right side of figure) concentrations of cycloheximide (CH) for the indicated times and then labeled for 30 min with 1 μCi of [^{3}H]thymidine per ml of medium containing 3 μM thymidine (○). Under these conditions the thymidine pool is flooded by exogenous thymidine; thus, the rate of ^{3}H incorporation reflects the rate of DNA synthesis. To confirm this, 1 μM fluorodeoxythymidine, which inhibits endogenous thymidine synthesis, was included in several sets of cultures (□). Fluorodeoxyuridine had no effect on the doubling times of CHO cells in complete Hams HF-10 medium (containing 3 μM thymidine), but it completely inhibited CHO cell growth in thymidine-free Hams HF-10 medium. The incorporation of [^{3}H]thymidine was analyzed by the method of Schmidt and Thannhauser (23) and was normalized to the total DNA content of the culture. OD_{260}, Optical density at 260 nm.

tially inhibited and may increase as protein synthesis is further inhibited.

At cycloheximide concentrations above 1.0 μg/ml, which prevent multiplication of CHO cells, the histone compensation ratio reached a maximum, but the DNA compensation ratio fell below 1; the rate of DNA synthesis decreased rather than increased with time. It may also be worth noting that significantly more histone synthesis may be occurring in high concentrations of cycloheximide (Fig. 6A) than is indicated by the measurement of the inhibition of total protein synthesis.

Although the effect of cycloheximide on the cell distribution has been carried out on several cell lines, including a Chinese hamster cell line (14) it has not been shown how the cell cycle distribution of CHO cells changes under these conditions. If the S phase or the chromosome cycle as a whole does not lengthen proportionally as the growth cycle lengthens, then it becomes a smaller fraction of the total growth cycle and the cell cycle distribution will show fewer cells in the S phase when the steady-state distribution is restored (21). Such results, obtained after CHO cells are grown for 24 h in cycloheximide, are shown in Fig. 7A. The

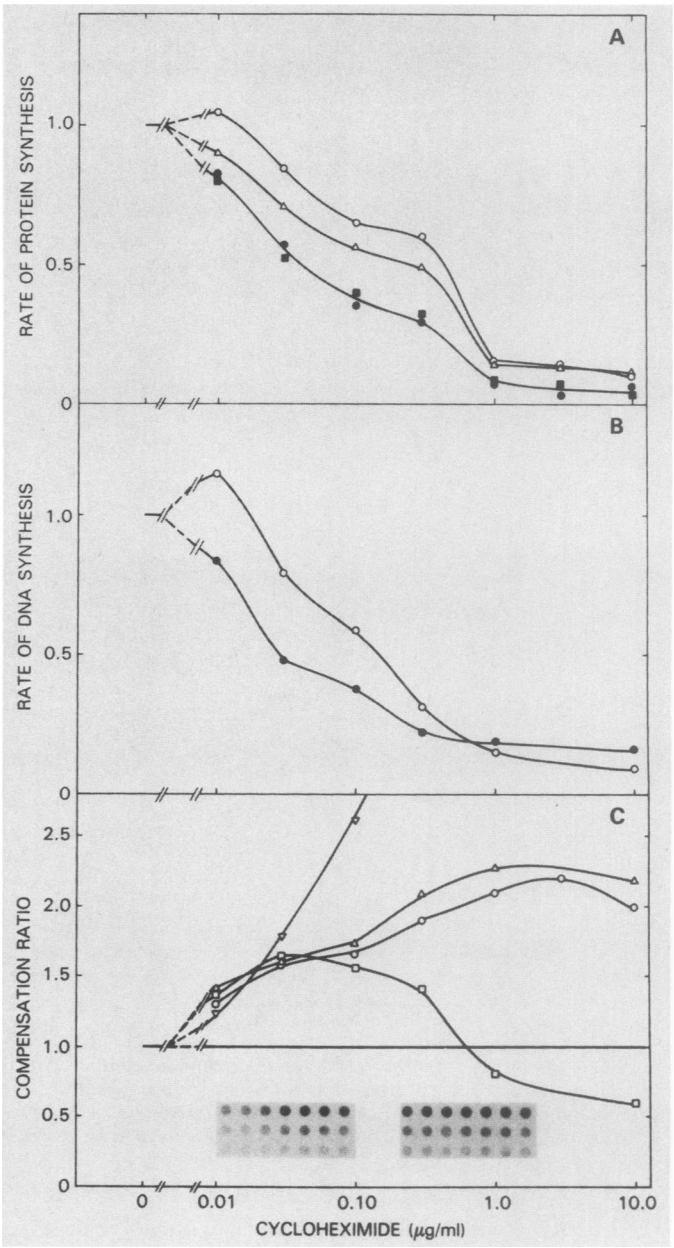

FIG. 6. Recovery of histone and DNA synthesis during growth of CHO cells in various concentrations of cycloheximide. (A) Histone synthesis. Cultures were labeled for 2 h in the presence of cycloheximide after growth in the same concentration of cycloheximide for 3.5 h. Symbols: △, Average of all core histone proteins; ○, H2A.1/H2A.2; ●, H2A.Z; ■, H2A.X. (B) DNA synthesis. Cultures were labeled for 0.5 h, 1 min after the addition of cycloheximide (●). Cultures were labeled for 0.5 h, 4.5 h after the addition of cycloheximide (○). (C) Compensation ratios. Histone compensation ratio. Ratio of H2A.1/H2A.2 to H2A.X/H2A.Z from panel A (○). Histone H4 mRNA level after 2 h of treatment with cycloheximide (△) (see Fig. 2 and the text for details; dot blots were also arranged as described in the legend to Fig. 2). DNA compensation ratio; ratio of the 4.5 h DNA synthesis curve to the 1-min DNA synthesis curve (□). Total compensation curve (the theoretical amount of compensation necessary to restore histone and DNA synthesis to the uninhibited control rate) (▽).

inset shows the cell cycle distribution of the uninhibited cell culture compared with that of the culture grown overnight in 0.3 μg of cycloheximide per ml.

In Fig. 7B the inverse ratio of the fraction of the cell population in mid S phase after growth in cycloheximide for 24 h with the elevation in the level of histone mRNA 4 h after the addition of cycloheximide is compared. The close correspondence of these two curves suggests that the magnitude of the increase in histone mRNA levels in the cultures is sufficient to account for the magnitude of the decrease in the fraction of cells in S phase 24 h later. This mechanism suggests that the increased histone mRNA level in the culture is a transient phenomenon because the histone mRNA level per S-phase cell increases more rapidly than the fraction of S-phase cells decreases. Thus, as the fraction of S-phase cells decreases, the average histone mRNA level, based on the total number of cells in the culture and not just S-phase cells, should return to values close to those found in the control cultures. When the histone mRNA levels in cultures grown in cycloheximide for 24 or 48 h were analyzed, the average histone mRNA levels were found to be similar to the control value (data not presented).

DISCUSSION

Mitchison (17) has suggested that the cell cycle should be considered as two cycles, a growth cycle encompassing cytokinesis to cytokinesis and a DNA division cycle encompassing chromosome synthesis (S), condensation (G2), and segregation (M). We use the term chromosome cycle for the latter.

The results presented here show that CHO cells can compensate for decreased efficiency of protein synthesis by increasing the rates of synthesis of the major chromatin structural protein components, S-phase histones, relative to the synthesis of other proteins. CHO cells seem to be able to completely compensate the rate of chromatin biosynthesis for a 40% decrease in the rate of protein synthesis and to partially compensate the rate of chromatin biosynthesis for up to a 70 to 80% decrease in the rate of protein synthesis.

Liskay et al. (14), studying the growth kinetics of a Chinese hamster cell line, showed that when protein synthesis was inhibited by 40%, there was a 54% increase in the length of the growth cycle (doubling time) but only a 10% increase in the length of the chromosome cycle (S, G2, and M phases). In a calculation analogous to those shown in Fig. 6C, these numbers result in a compensation ratio of 1.4 for the chromosome cycle compared with the growth cycle (1.54/1.10 = 1.4). Figure 6C shows that we were able to obtain maximum compensation ratios of 1.65 for DNA synthesis in CHO cells. Thus, the extent of complete compensation of histone and DNA synthesis measured in this study agrees well with the extent of complete compensation of the chromosome cycle measured by Liskay et al. (14). In these studies on CHO cells, we concentrated on the S phase rather than on the chromosome cycle as a whole. It is possible that proteins required in large amounts for mitosis, such as the tubulins, also may have rates of synthesis compensated for by the efficiency of protein synthesis.

It is important that the length of the growth cycle changes proportionately with the rate of protein synthesis; thus, compensation of chromatin biosynthesis does not in any obvious way allow the cell to grow faster. Since the G1 phase is the portion of the growth cycle remaining after the chromosome cycle is completed and since it is usually a small part of the growth cycle in uninhibited cells, chromosome cycle compensation must, as a direct consequence, result in a disproportionate lengthening of the G1 phase. This disproportionate lengthening may give the appearance of a particular sensitivity of the G1 phase itself to the rate of protein synthesis as well as the appearance of cell cycle arrest. Our results do not suggest that any part of the growth cycle, such as the G1 phase, is particularly sensitive to the efficiency of protein synthesis. In contrast, these results demonstrate that S-phase cells as well as G1-phase cells

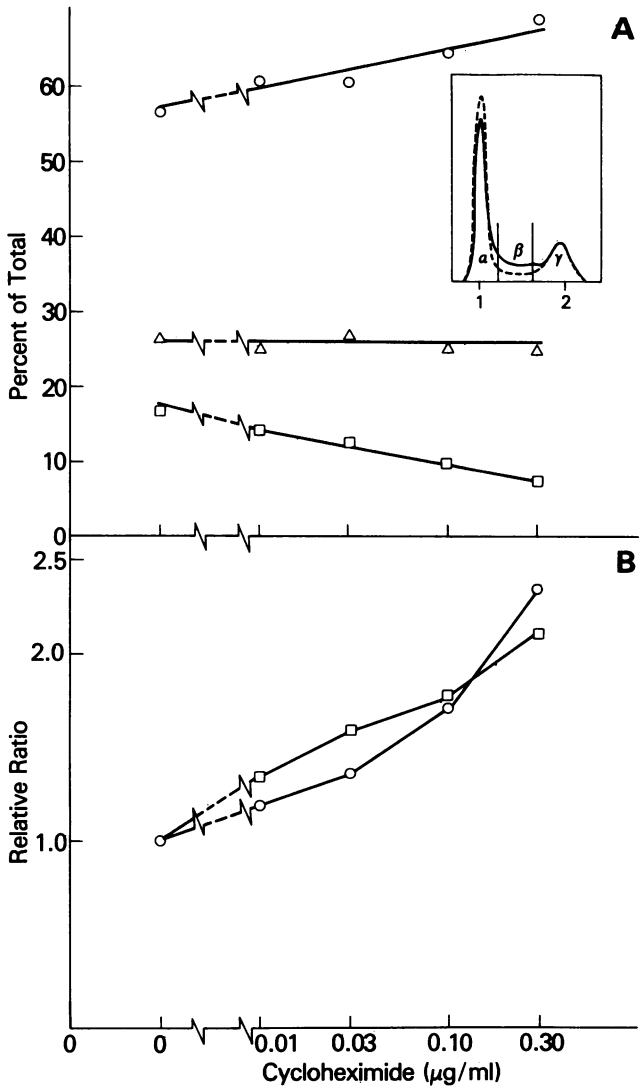

FIG. 7. Cell cycle distributions of CHO cells grown in various concentrations of cycloheximide. Cultures of CHO cells were grown exponentially for 2 days in Hams HF-10 medium and then for 24 h in Hams HF-10 medium containing the indicated concentrations of cycloheximide. Care was taken to prevent cell crowding (less than 20,000 cells per cm^2). Cells were harvested, stained with chromomycin A3, and analyzed in a Becton-Dickinson FACS Analyzer (10,000 cells in each analysis). Two typical distributions are shown in panel A inset (solid line, uninhibited control; dotted line, culture grown for 24 h in 0.3 μg of cycloheximide per ml). (A) Percentage of total cells in each of the three regions indicated in the inset. Symbols: ○, α; □, β; △, γ. (B) Relative ratios. Symbols: ○, Fraction of cells in region β of the cell cycle distributions relative to that of the control (inversed); □, histone H4 mRNA levels relative to that of the control.

sense changes in the efficiency of mRNA translation but that in S-phase cells, the concentration of S-phase histone mRNA is increased to compensate chromosome cycle processes for this decreased efficiency.

The results of this study were obtained with cycling cells and, as such, are directly relevant to proliferating cells grown in nutritionally complete media at low densities. Cells released from nonproliferating G0 states (induced by nutritional or serum deprivation, or contact inhibition) may be a different situation (2); the pattern of histone synthesis differs between the G0 state and G1-phase cells (34). We currently are using these techniques to study these relationships in normal and transformed cells, as well as the effect of these perhaps more physiological methods of limiting cell growth on chromosome cycle compensation.

Our results suggest that the compensating mechanism may be primarily the elevation of histone mRNA levels to completely or partially offset the decrease in the efficiency of protein synthesis. One possible mechanism for the elevation of histone mRNA levels is that the free cytoplasmic histone autoregulates its own synthesis. Such a mechanism was proposed by Butler and Mueller (5) to account for decreases in histone protein synthesis when DNA synthesis was inhibited, but such a model could be applicable here (22). Thus, the differential sensitivity of the growth and chromosome cycles may be explained by a fairly straight forward and plausible chain of etiological relationships. A decrease in the rate of protein synthesis leads to a proportional increase in the length of the growth cycle and a proportional decrease in the concentration of free cytoplasmic histone. By autoregulation, the level of histone mRNA rises, leading to a recovery in histone protein synthesis which in turn leads to recoveries in DNA and chromatin biosynthesis.

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