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Protein Synthesis Is Required for Rapid Degradation of Tubulin mRNA and Other Deflagellation-Induced RNAs in *Chlamydomonas reinhardi*

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After flagellar detachment in *Chlamydomonas reinhardi*, there is a rapid synthesis and accumulation of mRNAs for tubulin and other flagellar proteins. Maximum levels of these mRNAs (flagellar RNAs) are reached within 1 h after deflagellation, after which they are rapidly degraded to their predeflagellation levels. The degradation of alpha- and beta-tubulin RNAs was shown to be due to the shortening of their half-lives after accumulation (Baker et al., J. Cell Biol. 99:2074–2081, 1984). Deflagellation in the presence of protein synthesis inhibitors results in the accumulation of tubulin and other flagellar mRNAs by kinetics similar to those of controls. However, unlike controls, in which the accumulated mRNAs are rapidly degraded, these mRNAs are stabilized in cycloheximide. The stabilization by cycloheximide is specific for the flagellar mRNAs accumulated after deflagellation, since there is no change in the levels of flagellar mRNAs in nondeflagellated (uninduced) cells in the presence of cycloheximide. The kinetics of flagellar mRNA synthesis after deflagellation are shown to be the same in cycloheximide-treated and control cells by in vivo labeling and in vitro nuclear runoff experiments. These results show that protein synthesis is not required for the induced synthesis of flagellar mRNAs, and that all necessary transcriptional control factors are present in the cell before deflagellation, but that protein synthesis is required for the accelerated degradation of the accumulated flagellar mRNAs. Since cycloheximide prevents the induced synthesis and accumulation of flagellar proteins, it is possible that the product(s) of protein synthesis required for the accelerated decay of these mRNAs is a flagellar protein(s). The possibility that one or more flagellar proteins autoregulate the stability of the flagellar mRNAs is discussed.

The cellular response to flagellar detachment in *Chlamydomonas reinhardi* includes a rapid and coordinate induction of the mRNAs for tubulin and other flagellar proteins (25, 27, 37, 38). The accumulation of alpha- and beta-tubulin RNAs is accomplished by both a transient increase in transcription rate and an apparent stabilization of the tubulin transcripts during flagellar regeneration (3, 21). Maximum levels of mRNAs for tubulin and other flagellar proteins accumulate within 1 h, after which these RNAs are rapidly degraded (25, 27, 37, 38). The half-life of tubulin mRNA during this deinduction is only about 20 min, or 2 to 3 times shorter than in nonregenerating, vegetative cells (3). These data suggest that a specific decay mechanism operates during deinduction which accelerates the return of the induced mRNAs to basal levels.

In this report, we show that protein synthesis is not required for the activation of transcription and accumulation of tubulin and other induced mRNAs, but is necessary for the accelerated degradation of these RNAs after induction.

MATERIALS AND METHODS

Cell growth and preparation. *Chlamydomonas reinhardi* vegetative cells, strain 21gr, were grown in medium I of Sager and Granick (35) to approximately 10^6 cells per ml. For experiments, cells were concentrated to 1.5×10^7 cells per ml in spent medium and stirred under illumination for at least 1.5 h before use. For the in vivo labeling study with $^{32}P_i$, cells were grown in a low-phosphate variant of medium I as described previously (3). For nuclear isolations, a cell wall-less *C. reinhardi* strain, CW92 (11), was used. 21gr cells were deflagellated by mechanical shear (34), and CW92 cells were deflagellated by pH shock (43). Cells were stirred under fluorescent illumination during experiments. Flagellar lengths were measured by phase-contrast microscopy. Cycloheximide (Sigma Chemical Co., St. Louis, Mo.) was dissolved in water at 1 mg/ml shortly before use and diluted 100-fold into cell cultures for a final concentration of 10 μg/ml. In one study, anisomycin (Sigma) at a final concentration of 0.4 μM was used to inhibit protein synthesis.

Nucleic acid isolation for RNA abundance determinations. For each sample preparation, 2×10^8 cells were removed from cultures, pelleted and immediately lysed in 0.3 M NaCl–5 mM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid–50 mM Tris hydrochloride (pH 8.0)–2% sodium dodecyl sulfate at a concentration of 10^8 cells per ml. Lysates were quick frozen in liquid nitrogen. Frozen lysates were thawed at 65°C for ca. 20 s in the presence of 120 μg of proteinase K per ml and incubated at room temperature for an additional 5 min before extraction with phenol-chloroform as described previously (3). Total nucleic acid was ethanol precipitated twice and used for RNA dot hybridizations.

In vivo labeling. $^{32}P_i$ labeling of RNA was performed as described in detail previously (3). Briefly, samples of cells were labeled with 80 μCi of $^{32}PO_4$ (Amersham Corp., Arlington Heights, Ill.) per ml for 5-min pulses during regeneration. At the end of each labeling period, cells were pelleted through ice-cold medium I and lysed, and the lysate was quick frozen in liquid nitrogen. Nucleic acid was prepared from thawed lysates as described above, and RNA was purified by centrifugation through a CsCl cushion (16).

Nuclear isolation and in vitro transcription. *C. reinhardi*

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CW92 cells were treated continuously with 10 μg of cycloheximide per ml in medium I beginning at 20 min before deflagellation. Nuclei were isolated from control and cycloheximide-treated cells exactly as described previously (21) before deflagellation and at 40, 80, and 150 min after deflagellation and frozen at −80°C. Isolated nuclei were assayed for transcriptional activity as described previously (21), except that labeled ribonucleotides in the reaction were [α-^{32}P]GTP and [α-^{32}P]CTP instead of [α-^{32}P]UTP. Transcription reactions were stopped after 45 min; total nucleic acids were extracted three times with phenol-chloroform and once with chloroform and precipitated with 70% ethanol. The precipitates were suspended in 10 mM Tris hydrochloride–1 mM sodium EDTA (pH 7.5) and chromatographed through a 0.7-ml column of P-60 (Bio-Rad Laboratories, Richmond, Calif.). The total nucleic acid content of the samples was determined by measuring absorbance at 260 nm.

Hybridizations. RNA dot filters were prepared as described previously (37), with the following exception: 4 μg of total nucleic acid, rather than purified RNA, was applied per dot. In comparative hybridizations, we found that accumulation curves were the same whether total nucleic acid or CsCl-purified RNA was dotted (data not shown). Nondenatured DNA will not bind to nitrocellulose filters. It appears that any DNA inadvertently denatured and bound in these preparations makes no significant contribution to the hybridizable sequences on the filter. Nick translation of plasmid DNA with [^{32}P]dCTP or [^{32}P]dGTP was performed as described previously (37). Plasmid DNAs contained cDNA sequences for alpha-tubulin (pcf4-2), beta-tubulin (pcf8-31), two nontubulin-induced sequences (pcf3-21 and pcf6-110), and a constitutively expressed sequence (pcf2-40) inserted into the vector pBR322 (37). Prehybridization, hybridization, and filter washes were carried out as previously described (37).

Plasmid DNA dot filters were prepared as described previously (21). For hybridization with in vivo-labeled RNA, 4 μg of each plasmid DNA per dot was applied in duplicate, and 200 μg of ^{32}P-RNA were added to each bag in a total volume of 2.5 ml of hybridization buffer. For hybridization with in vitro-synthesized RNA, 4 μg of each plasmid per dot was applied in triplicate to nitrocellulose, and 12 μg of total nucleic acid was added to each bag in a total volume of 1 ml. Prehybridization, hybridization, and washing were performed as described previously (21).

RESULTS

Tubulin RNA levels are altered by inhibition of protein synthesis. Regeneration of full-length flagella in *C. reinhardi* is dependent upon protein synthesis. In the absence of protein synthesis, two approximately half-length, functional flagella are assembled from a premade, flagellar protein precursor pool (34). Figure 1A shows flagellar regeneration kinetics in cells in which protein synthesis was inhibited by cycloheximide. The dosage used (10 μg/ml) inhibits protein synthesis by >95% within 5 min in *C. reinhardi* (34). The uppermost curve shows flagellar regeneration in control cells (no cycloheximide). Full-length flagella (ca. 12 μm) are assembled within 90 min. The remaining three curves, in descending order, show flagellar regeneration in cells which were continuously exposed to cycloheximide from 30 and 2 min after deflagellation and 20 min before deflagellation. The final length of the regenerated flagella is clearly dependent on the amount of new protein synthesis permitted after flagellar detachment. If these partial-length flagella are amputated, no flagellar regeneration occurs (34). Thus, regeneration in the presence of cycloheximide effectively depletes the flagellar precursor pool (at least of some essential component) which is usually replenished by induced flagellar protein synthesis.

Tubulin mRNA levels were analyzed in these cells. RNA samples were isolated at time points throughout regeneration, dotted onto nitrocellulose filters, and hybridized with nick-translated probes containing alpha-and beta-tubulin cDNA sequences. Autoradiograms of these filters are shown in Fig. 1B. Hybridization was quantified by scintillation counting of excised dots, and Fig. 1C shows the combined data from two separate determinations of beta-tubulin RNA levels. Beta-tubulin RNA in control, deflagellated cells accumulates and decays with characteristic kinetics (3, 27, 37, 38); the peak level is reached at 40 min, and the RNA decays nearly back to basal levels within 2 h. When cells are exposed to cycloheximide from 20 min before deflagellation, beta-tubulin RNA accumulates at about the same rate as in the control cells, but the levels remain elevated, at least for the 2-h duration of these studies. In addition, the maximum level of RNA attained is slightly higher in cycloheximide-treated cells. A similar accumulation pattern results when cycloheximide is added at 2 min after deflagellation (data not shown). However, when cycloheximide is added to regenerating cells at 30 min after deflagellation, an intermediate pattern is observed. Beta-tubulin RNA accumulates at the same rate, but a significant reduction in its abundance occurs by 2 h. This difference was consistent in each of the two experiments combined here.

Cycloheximide was also added to nondeflagellated cells, and the relative beta-tubulin RNA levels were determined at a number of times after addition. The data (Fig. 1C) demonstrate that cycloheximide causes no significant change in the level of tubulin RNA in nondeflagellated cells for up to 3 h of exposure.

Effect of cycloheximide on other mRNAs. The response to deflagellation in *C. reinhardi* involves the accumulation of a large number of mRNAs, in addition to the tubulin RNAs (25, 37). The flagellum is composed of more than 150 different proteins (31), and many of these other (induced) RNAs code for flagellar structural proteins (25). Some undoubtedly code for proteins other than flagellar components, such as a subset of heat shock proteins which are induced by deflagellation (May and Rosenbaum, unpublished results) and possible regulatory (nonstructural) proteins. All of the mRNAs induced by deflagellation will be referred to here as flagellar mRNAs. The accumulation curves for these other, nontubulin flagellar mRNAs, like the tubulin RNAs, show a rapid and coordinate induction and a rapid return to basal levels. However, there are characteristic differences in the timing and magnitude of peak RNA levels attained as well as how rapidly levels return to baseline (25, 37).

Figure 1D shows data for one of these RNAs, 3-21. cDNA clone pcf3-21 codes for an mRNA of ca. 2,400 bases which is strongly induced by deflagellation (37). Peak accumulation of this RNA always occurs shortly before the tubulin RNAs reach their maxima, and its decay is somewhat faster (37; unpublished data). The effect of protein synthesis inhibition on the levels of 3-21 RNA is qualitatively similar to the effect on tubulin RNA. The initial rate of accumulation of 3-21 RNA is the same in control and drug-treated cells. When cycloheximide is added to the cells 20 min before deflagellation, there is a delayed return to basal levels. When cycloheximide is added to cells at 30 min after deflagellation, the RNA levels during the return are intermediate. Although the differences between control and experimental curves are

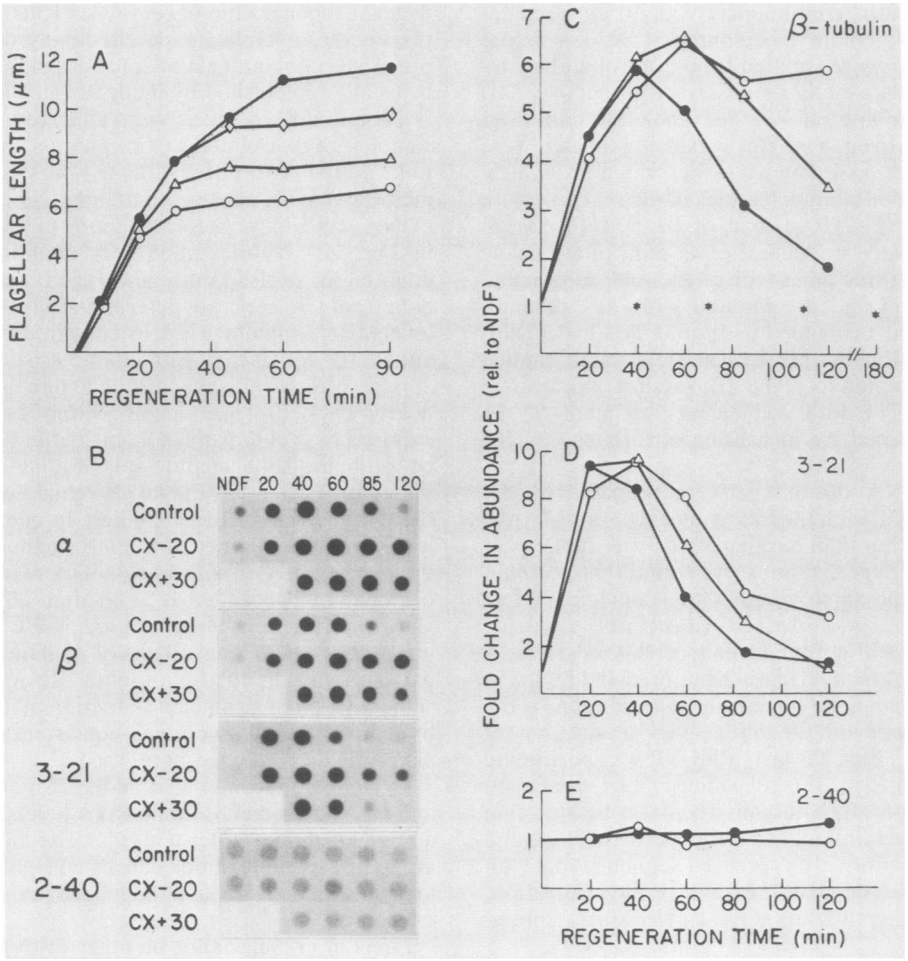

FIG. 1. Effect of inhibition of protein synthesis of flagellar regeneration and specific RNA levels. Cells were deflagellated and allowed to regenerate flagella in the presence or absence of cycloheximide (CX). One cell culture received no cycloheximide; others were exposed continuously to 10 μg of cycloheximide per ml from 30 or 2 min after deflagellation or 20 min before deflagellation. Cycloheximide was also added to nondeflagellated cells, which has no effect on flagellar length. RNA was prepared from time points during regeneration, and the relative abundance of several specific RNAs was determined by RNA dot hybridizations to excess nick-translated plasmid DNAs, as described in Materials and Methods. Hybridization data represent the averaged results of two separate experiments. A, Flagellar regeneration curves from one experiment. Symbols: (●) no cycloheximide, (◇) cycloheximide at +30 min, (△) cycloheximide at +2 min, (○) cycloheximide at −20 min. B, Sample autoradiograms of hybridized RNA dots from one experiment. Equal amounts (4 μg) of sample were dotted in triplicate; only a single row is shown. Hybridized dots were excised and counted. Triplicate values were averaged, and the background (counts per minute bound to dots containing no RNA) was subtracted. In panels C through E, the averaged data from two experiments are plotted as the fold change in RNA abundance over the level determined for nondeflagellated cells (NDF, equal to 1). Symbols: (●) cycloheximide, (△) cycloheximide at +30 min, (○) cycloheximide at −20 min, (*) cycloheximide added to nondeflagellated cells. RNA: C, beta tubulin RNA; D, 3-21 RNA (nontubulin, flagellar RNA); E, 2-40 RNA (constitutive RNA).

small, both independent experiments gave virtually identical results. The effect of cycloheximide on 3-21 RNA is, though, much less dramatic than the effect on tubulin RNA levels.

Many RNAs in *C. reinhardi* do not change in abundance after deflagellation, and these have been defined as constitutive RNAs (37). Figure 1E shows relative levels of one constitutive RNA, 2-40, in the presence and absence of cycloheximide. Unlike the effect on the flagellar RNAs described above, there is little or no change in the abundance of this RNA during regeneration caused by cycloheximide. Only the data for regenerating cells treated with cycloheximide at −20 min are shown; levels also remain roughly constant when the drug is added at 30 min after deflagellation and in nondeflagellated cells exposed to the drug for 3 h.

Flagellar RNA levels remain elevated for over 3 h in cycloheximide. An additional study was done to determine how long tubulin RNA levels remain elevated in cycloheximide. For this study, cycloheximide was added at 20 min before deflagellation, and RNA samples were prepared over the course of 3.5 h. The data for alpha- and beta-tubulin RNAs are shown in Fig. 2A and B. The effect of cycloheximide on tubulin RNA levels is quite dramatic—at 3 h after deflagellation, tubulin RNA is still present at a concentration equal to, or greater than, the maximum level attained in control cells. It appears that levels begin to drop by 3 h. The decline in levels at this time has been observed in other experiments (data not shown). It would be of questionable value, however, to carry out the measurements much longer. After 4 h in cycloheximide, the cells begin to show signs of distress, such as impaired motility.

Figure 2C shows the levels of 3-21 flagellar RNA in these cells, and Fig. 2D shows levels of another induced flagellar RNA, 6-110. (cDNA clone pcf6-110 hybridizes to an 820-nucleotide RNA of unknown coding capacity [37].) The

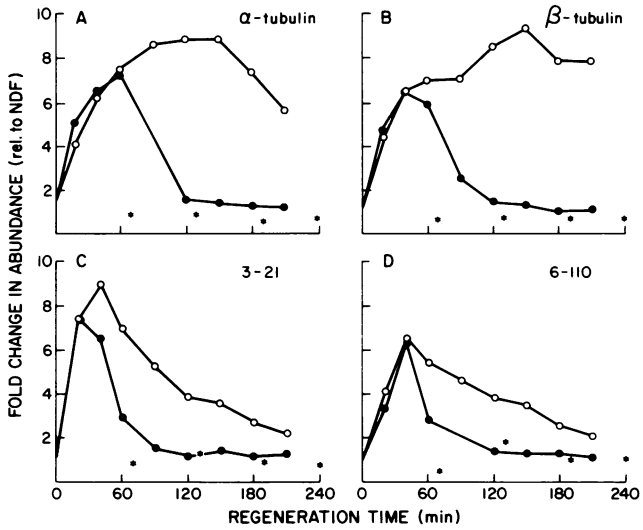

FIG. 2. Effect of cycloheximide on several induced flagellar RNAs. Data were obtained as described in the Fig. 1 legend and are presented as the fold change in RNA abundance over nondeflagellated cell levels. Symbols: (●) no cycloheximide, (○) 10 μg of cycloheximide per ml added 20 min before deflagellation, (*) cycloheximide added to nondeflagellated cells. RNAs: A, alpha tubulin RNA; B, beta tubulin RNA; C, 3-21 RNA; D, 6-110 RNA. The range of counts per minute for each filter were as follows: α-tubulin (696 to 6,111 cpm), β-tubulin (462 to 3,547 cpm), 3-21 (98 to 881 cpm), and 6-110 (75 to 485 cpm).

effect of cycloheximide on this flagellar mRNA is quite similar to the effect on 3-21 RNA. The flagellar RNAs in cycloheximide-treated cells accumulate very much like those in control cells, but the subsequent return to basal levels is much slower and is not completed by 3.5 h. Again, the effect of protein synthesis inhibition on the abundance of these RNAs is not as great as the effect on the tubulin RNAs.

The levels of the constitutive RNA 2-40 were also determined (data not shown). This RNA remained at approximately constant levels for 4 h in control and drug-treated cells.

Effect of cycloheximide on flagellar mRNA levels is specific. It is known that inhibitors of peptide elongation, such as cycloheximide, may have a general, though not dramatic (ca. twofold), mRNA-stabilizing effect due to polysome freezing (40). The most direct way to demonstrate that this general effect is not responsible for the elevated flagellar RNA levels observed in cycloheximide would be to use protein synthesis inhibitors with different modes of action, such as puromycin or pactamycin. Unfortunately, these drugs will not inhibit protein synthesis in *C. reinhardi* presumably because the cells are impermeable to them. We do know that the elevated flagellar RNA levels are not due to a side effect of cycloheximide unrelated to protein synthesis inhibition, since identical results are obtained with anisomycin, another elongation inhibitor (data not shown).

Two observations weaken the possibility that a nonspecific stabilizing effect of polysome freezing could account for the results described above. First, there is no increase in tubulin RNA levels in nondeflagellated cells exposed to cycloheximide (Fig. 1C, 2A, and 2B). The half-life of beta-tubulin RNA in vegetative *C. reinhardi* cells is ca. 45 min (3). If the effective half-life of the RNA were, for example, doubled by a general stabilizing effect, a significant increase in levels (1.5-fold) would be evident within 90 min; this is not seen. Furthermore, to explain the elevated tubulin RNA levels in cycloheximide-treated, regenerating cells, a stabilization much greater than twofold would have to be invoked. Secondly, if the effect of cycloheximide on tubulin RNA levels were due simply to polysome freezing, then the tubulin RNA present in the cytoplasm when cells receive cycloheximide at 30 min after deflagellation should be as effectively stabilized as the tubulin RNA in the cells which received cycloheximide before deflagellation. This is clearly not the case, since about half of the tubulin RNA in cells treated with cycloheximide at 30 min has decayed by 2 h (Fig. 1C). This argument can also be applied in the case of 3-21 RNA (Fig. 1D). We conclude that the elevated levels of flagellar mRNA observed in regenerating cells exposed to cycloheximide are caused by the absence (or reduced levels) of a product of protein synthesis. Since cycloheximide prevents the replenishment of the flagellar precursor pool, it is possible that the required product(s) could be tubulin itself or other flagellar proteins or both.

Effect of cycloheximide on transcription of flagellar RNAs. Evidence was presented previously (3) which indicated that the loss of tubulin RNA sequences after induction is due to an accelerated decay mechanism. One obvious interpretation of the effect of cycloheximide on tubulin RNA levels is that protein synthesis is required for the activation of this decay mechanism. It is also possible that cycloheximide exerts its effect at the level of transcription. In particular, an increase in the duration of the transcriptional burst which follows deflagellation (3) would result in accumulation curves much like those shown in Fig. 1C, 2A, and 2B. Both in vivo and in vitro measurements of transcription were made to determine whether the rates of synthesis of tubulin RNA and other flagellar RNAs during regeneration are altered by inhibition of protein synthesis.

Transcription in vivo. ^{32}P-RNA prepared from cells pulse-labeled for 5-min intervals in vivo was hybridized to specific plasmid DNAs to determine the relative rates of synthesis of flagellar RNAs during regeneration in the presence and absence of cycloheximide (Fig. 3). As reported previously (3), deflagellation induces a rapid burst of synthesis of alpha- and beta-tubulin RNA which reaches its maximum rate within 15 min, followed by a rapid decline (Fig. 3A and B). In the presence of cycloheximide, the kinetics of tubulin RNA synthesis during regeneration are similar to those of controls, although the return to basal synthesis rate appears to be a little faster. Likewise, the kinetics of synthesis of 3-21 RNA and 6-110 RNA during regeneration are also similar in control and cycloheximide-treated cells (Fig. 3C and D), although the initial rates of synthesis of these flagellar RNAs are somewhat reduced in cycloheximide-treated cells relative to controls. These measurements indicate that there is no substantial change in flagellar RNA synthesis during regeneration as a result of protein synthesis inhibition.

Transcription in vitro. Analysis of tubulin RNA transcripts made in vitro by isolated nuclei are consistent with the measurements made in vivo (Fig. 4). There is no significant difference between control and experimental nuclei in the extent or the duration of the transcriptional induction of the tubulin RNAs. The kinetics of induction in both control and cycloheximide-treated cells are somewhat prolonged in these experiments relative to the others reported (3) (Fig. 3). This is because nuclei were prepared from a cell wall-less strain of *C. reinhardi*, CW92, which exhibits slower flagellar regeneration than the wild type (Keller, unpublished data).

These in vivo and in vitro measurements of transcription

show that the elevated levels of tubulin mRNAs and other flagellar mRNAs which occur in regenerating cells when protein synthesis is inhibited (Fig. 1C and D, Fig. 2) are not due to increased mRNA synthesis. Neither a higher rate of transcription nor a prolongation of the transcriptional burst was observed in drug-treated cells.

Stabilization of flagellar mRNAs by cycloheximide. These data strongly indicate that the induced, flagellar RNAs are more stable in the absence of protein synthesis, and that the accelerated decay mechanism which usually operates after induction requires protein synthesis.

The accumulation curves presented in Fig. 2 can be used to provide a measure of the extent of stabilization conferred by protein synthesis inhibition. Figure 5 shows logarithmic plots of the loss of flagellar mRNAs, 3-21 and 6-110, from their peak accumulated levels. In control regenerating cells, both RNAs decay with half-lives of about 20 min. In the presence of cycloheximide, these RNAs decay with half-lives of ca. 80 min (3-21) and 110 min (6-110), which represent 4-fold and 5.5-fold stabilizations, respectively. These half-life values are actually overestimates of the true half-lives, because continuing RNA synthesis contributes to the RNA levels measured.

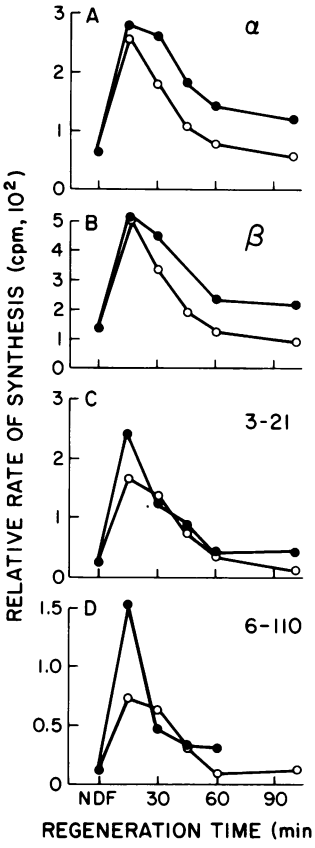

FIG. 3. Relative rates of synthesis of several flagellar RNAs during regeneration. ^{32}P-RNA, labeled in vivo with 5-min pulses of $^{32}P_i$, was hybridized to nitrocellulose filters containing duplicate dots of four specific plasmid DNAs and pBR322 DNA (vector). Hybridization was quantified by scintillation counting of individual dots. Data are presented as the average counts per minute hybridized per dot (minus the counts per minute hybridizing to pBR322 vector DNA). Time points represent the end of each 5-min labeling period. Symbols: (●) no cycloheximide, (○) 10 μg of cycloheximide per ml added 20 min before deflagellation. RNAs: A, alpha-tubulin RNA; B, beta-tubulin RNA; C, 3-21 RNA; D 6-110 RNA.

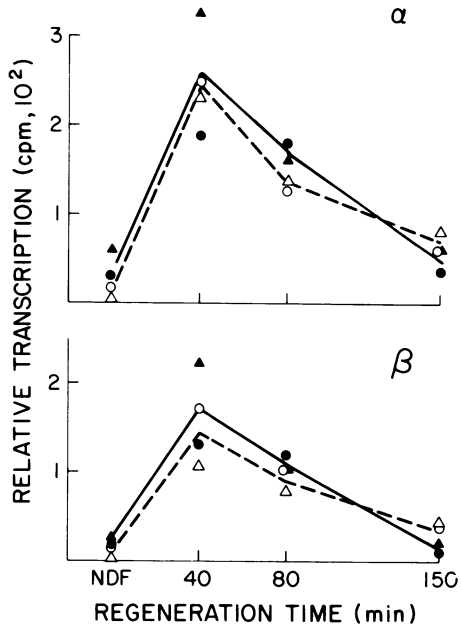

FIG. 4. Effect of cycloheximide on tubulin transcription in isolated nuclei. Nuclei were isolated at 40, 80, and 150 min after deflagellation from cells receiving no cycloheximide treatment and from cells treated with 10 μg of cycloheximide per ml (starting 20 min before deflagellation) and from nondeflagellated cells (NDF). Nuclei were incubated in standard transcription assays, and tubulin RNA synthesized in vitro was detected by hybridization with dots of α- and β-cDNA plasmids. Hybridized dots were excised and counted in a scintillation counter. Each data point represents the average of counts per minute hybridized to triplicate dots of plasmid after subtraction of background hybridization to dots of vector pBR322 without an insert. Data from two transcription assays using nuclei from a single nuclear isolation are plotted; (●, ▲, —) no cycloheximide, (○, △, -----) with cycloheximide. The curves represent the averages of each two data points.

It is not possible to apply this type of analysis to the stabilization of alpha- and beta-tubulin RNAs, since significant decay does not occur during the course of the experiment (Fig. 2A and B). It is quite clear, though, that the tubulin RNAs are even more strongly stabilized than are the other two flagellar mRNAs analyzed.

DISCUSSION

Detachment of the flagella of *C. reinhardi* causes a rapid induction of the mRNAs for tubulin and other flagellar proteins. In this report, we have demonstrated that protein synthesis is not required for this induction. The burst in transcription rate and the initial accumulation of these sequences is very similar in cycloheximide-treated cells and in controls. This means that all (protein) factors required for the activation of flagellar mRNA synthesis are present in the vegetative cell. This finding may not be surprising in view of the speed of the induction. In fact, cycloheximide-treated cells can be deflagellated again 40 min after the first deflagellation and undergo a second round of tubulin RNA accumulation with kinetics similar to the first round (unpublished results). This suggests that transcription factors are either present in excess or that they are reutilized in multiple inductions.

Alpha- and beta-tubulin RNAs decay very rapidly after

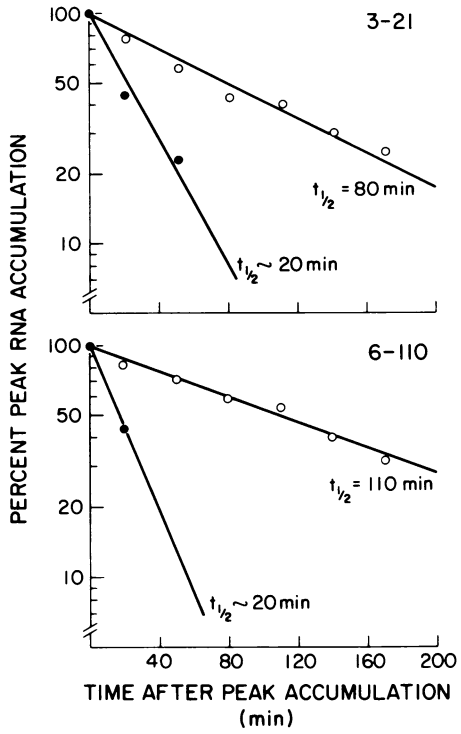

FIG. 5. Half-lives of two flagellar RNAs during deinduction in the presence and absence of cycloheximide. The loss of 3-21 and 6-110 RNAs after their peak in abundance in regenerating cells is plotted as the percentage of peak levels. The data are taken from the accumulation curves shown in Fig. 2C and D. Although there are not enough data points to obtain accurate half-life values for the control (no cycloheximide) decay, the extremely rapid loss (half-life of <20 min) of these sequences in the absence of cycloheximide is clear from data presented in Fig. 1 and 2 as well as other (36; unpublished data) accumulation curves. Symbols: (●) no cycloheximide, (○) cycloheximide.

induction by deflagellation, exhibiting a half-life of less than 25 min (3). Since this decay is more rapid than can be explained by the usual half-lives of these mRNAs in *C. reinhardi* (45 to 55 min), a mechanism must exist which accelerates their degradation after regeneration. Apparent accelerated degradation of tubulin mRNA has been described in other systems also (23, 36). The acceleration in decay rate appears to be specific for induced, flagellar messages, since no decrease in levels of uninduced (constitutive) RNAs is observed (3, 37) (Fig. 1E). However, a transient increase in RNA degradation rate would have a smaller effect on the levels of relatively long-lived RNAs. Thus, a transient increase in decay rates of all mRNAs throughout the cell is not ruled out by these data if all of the constitutive RNAs examined have long half-lives. The data in this report demonstrate that protein synthesis is required for this rapid decay of the tubulin RNAs as well as for at least two other flagellar RNAs.

There have been many reports describing elevation in levels of specific mRNAs in response to inhibition of protein synthesis. In some cases, the effect has been shown to be due, at least in part, to increased transcription of the gene in the presence of protein synthesis inhibitors (14, 15, 17, 33). These observations suggest that the expression of such genes is regulated by labile protein repressors. In other cases, a specific stabilization of mRNA by protein synthesis inhibition has been shown, e.g., histone mRNA in a number of mammalian culture cell types (12, 39, 41), c-*myc* RNA in some human cell lines (10), and other (32, 42). Two features in common to histone mRNA, c-*myc* mRNA, and tubulin mRNA are (i) their levels are cell cycle regulated, at least in some cell types (2, 19, 20, 22, 36), and (ii) they exhibit particularly short half-lives relative to other mRNAs in their respective cell types (3, 4, 10, 19, 23). Adjustable stability may be a general, effective means of regulating cell-cycle-specific mRNAs.

There are two basic ways in which cycloheximide might be preventing the usual rapid breakdown of flagellar mRNA which follows induction. Protein synthesis may be required (i) for the synthesis or activation of a new, possible specific, RNase, or (ii) for an alteration in the flagellar mRNP population itself, which renders it particularly vulnerable to degradation by extant nucleases.

Although there are a number of documented cases of activation of apparently specific mRNA decay (1, 5, 6, 23, 28), the mechanism(s) for the events is not known. The only well-documented case in eucaryotes of the induction of specific nuclease activity is the activation of the 2-5A-synthetase–RNase L system by double-stranded RNA and interferon (reviewed in reference 26), although it is also possible that the degradation of host mRNA by some animal viruses (29) is mediated by induced RNase activities. In some cases, specific destabilization may involve the association of targeted RNAs with newly synthesized or activated destabilizing proteins (or, alternatively, the dissociation of stabilizing proteins). Hayward and Shapiro have identified a protein whose properties suggest a role in the reversible stabilization-destabilization of vitellogenin mRNA in the presence and absence of estrogen (18). Dreyfuss et al. (13) have documented a striking change in the protein composition of cytoplasmic messenger ribonucleoproteins in the presence of transcription inhibitors. A presynthesized, cytoplasmic protein, not previously associated with mRNA, binds in large quantities to messenger ribonucleoproteins in the presence of the inhibitors, and a role as a stabilization protein is suggested. Analysis of the composition of the induced flagellar messenger ribonucleoproteins during induction and deinduction and in the presence of cycloheximide may reveal differences in the proteins bound.

One possibility is that the product of protein synthesis required for the rapid decay of the flagellar mRNAs is tubulin itself (or any set of induced flagellar proteins). Ben-Ze'ev et al. (4) and Cleveland et al. (8, 9) have presented evidence that tubulin may autoregulate the levels of its own mRNA; this effect appears to be mainly posttranscriptional (7). Autoregulation of mRNA levels by half-life alteration has also been proposed to explain the destabilization of histone RNA produced in excess by extra gene copies in *Saccharomyces cerevisiae* (30). In *C. reinhardi* an early drop in the flagellar precursor pool during regeneration (due to flagellar assembly) and its subsequent replenishment by new protein synthesis (25) correlates well with the stabilization of tubulin transcripts early in regeneration and their subsequent accelerated decay (3). If the stability of tubulin RNA is inversely related to the size of the tubulin pool, then inhibition of new tubulin synthesis during regeneration should result in a stabilization of the tubulin RNAs. In fact, we now know that the flagellar mRNAs stabilized in the presence of cycloheximide can be selectively destabilized, in the continued presence of cycloheximide, by inducing the cells to resorb their flagella, thereby increasing the soluble, cytoplasmic tubulin pool (Baker and Rosenbaum, manuscript in preparation). This finding makes the possibility that the

stability of the flagellar mRNAs is regulated by one, or a number, of their protein products even more attractive.

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ADDENDUM IN PROOF

We would like to note the recent publication of two articles in which the authors demonstrate apparent autoregulation of tubulin synthesis by a cytoplasmic control mechanism which acts to alter tubulin mRNA stability (J. M. Caron, A. L. Jones, L. B. Rall, and M. W. Kirschner, Nature (London) **317**:648–651, 1985; M. F. Pittenger and D. W. Cleveland, J. Cell Biol. **101**:1941–1952, 1985.).

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