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# Role of Protein Synthesis in Decay and Accumulation of mRNA during Spore Germination in the Cellular Slime Mold *Dictyostelium discoideum*

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**Spore germination in *Dictyostelium discoideum* is a particularly suitable model for studying the regulation of gene expression, since developmentally regulated changes in both protein and mRNA synthesis occur during the transition from dormant spore to amoeba. The previous isolation of three cDNA clones specific for mRNA developmentally regulated during spore germination allowed for the quantitation of the specific mRNAs during this process. The three mRNAs specific to clones pLK109, pLK229, and pRK270 have half-lives much shorter (minutes) than those of constitutive mRNAs (hours). Using spore germination as a model, we studied the roles of ribosome-mRNA interactions and protein synthesis in mRNA degradation by using antibiotics that inhibit specific reactions in protein biosynthesis. Cycloheximide inhibits the elongation step of protein synthesis. Polysomes accumulate in inhibited cells because ribosomes do not terminate normally and new ribosomes enter the polysome, eventually saturating the mRNA. Pactamycin inhibits initiation, and consequently polysomes break down in the presence of this drug. Under this condition, the mRNA is essentially free of ribosomes. pLK109, pLK229, and pRK270 mRNAs were stabilized in the presence of cycloheximide, but pactamycin had no effect on their normal decay. Since it seems likely that stability of mRNA reflects the availability of sites for inactivation by nucleases, it follows that in the presence of cycloheximide, these sites are protected, presumably by occupancy by ribosomes. No ribosomes are bound to mRNA in the presence of pactamycin, and therefore mRNA degrades at about the normal rate. The data further indicate that a labile protein is probably not involved in mRNA decay or stabilization, since protein synthesis is inhibited equally by both antibiotics. We conclude that it may be important to use more than one type of protein synthesis inhibitor to evaluate whether protein synthesis is required for mRNA decay. The effect of protein synthesis inhibition on mRNA synthesis and accumulation was also studied. mRNA synthesis continues in the presence of the inhibitors, albeit at a diminished rate relative to that of the uninhibited control.**

The concentration of a given protein in a cell is determined by the amount of its mRNA present and its efficiency of translation, while the steady-state level of the mRNA is a function of its rates of synthesis and decay. Individual eucaryotic mRNAs have characteristic rates of decay: some are very stable (hours), others decay rapidly (1 h or less), while some have variable rates of decay under different physiological conditions (1, 3, 6, 7, 13, 14, 16, 19, 20, 22, 24, 30). It is not clear why classes of mRNAs with different stabilities exist, since little work has been performed to determine the factors responsible for mRNA stability. Recent studies have implicated specific sequences in the unique 5′ leader region governing the stability of bacteriophage and human H3 histone mRNA (12, 23), but whether the 5′ region is involved in the decay of all mRNAs is not known. Another investigation (21) also points to the importance of the 3′ portion of the histone H4 gene in the posttranscriptional regulation of gene expression.

The study of mRNA decay during development in a wide variety of organisms has been prompted by the finding that particular proteins are synthesized only during certain stages of the developmental pathway, suggesting that their respective mRNAs have short half-lives. This implies that differential mRNA stability may be one way of regulating not only the amount of a specific protein that is made but also the time during development that it is synthesized. In fact, this kind of regulation takes place during certain stages of development in the slime mold *Dictyostelium discoideum* (8, 18, 22), during induction of various proteins by prolactin (13) and estradiol (6, 16), in histone synthesis specifically during the process of DNA replication (14), in the later stages of myogenesis (1) and erythropoeiesis (3, 7), possibly in c-*myc* gene expression (7, 20), and in the differential expression of photosynthesis genes in *Rhodopseudomonas capsulata* (3). However, the specific mechanisms involved in eucaryotic mRNA decay remain to be elucidated.

We previously showed that it was possible to assess, for bacteria, the role protein synthesis plays in the degradation of particular mRNAs by using inhibitors specific for individual reactions in protein synthesis (5). This approach enabled us to determine the effects of ribosome-mRNA interactions on mRNA degradation and to localize sites on the mRNA which appear to be particularly important for decay. In a prior study, we determined the half-lives of constitutive mRNAs and those developmentally regulated during spore germination (18). More recently, we used spore germination in *D. discoideum* as a model for revealing the role of protein synthesis in eucaryotic mRNA decay. The results presented here demonstrate that inhibition of protein synthesis per se does not regulate decay of spore-specific developmentally

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regulated mRNAs. What is important is the interaction of the mRNA with the ribosomes.

## MATERIALS AND METHODS

**Organism.** The isolation, storage, and activation of wild-type *D. discoideum* B spores have been described previously (10).

**Isolation of RNA.** Total RNA was isolated from activated spores by using glass beads to break the spores or cells instead of grinding them in solid $CO_2$ as previously described (11). Approximately $1.5 \times 10^9$ to $2 \times 10^9$ spores or germinating spores were harvested at the times indicated in each experiment and rapidly frozen in a dry ice-ethanol bath. Nitric acid-washed glass beads (4 g; diameter, 0.45 to 0.5 mm; B. Braun Co.) were chilled on ice in a 15-ml glass screw-cap tube. The spore pellets were thawed, 2 ml of HMK (50 mM HEPES [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; pH 7.5], 40 mM magnesium acetate, 20 mM KCl) containing 40 μl of 10% sodium dodecyl sulfate was added to each sample, and the cells were transferred to the tube containing the glass beads. HMK-saturated phenol (2 ml) and 4 M sodium acetate (0.2 ml; pH 7.5) were added. The tubes were shaken on a Vortex mixer (The Vortex Manufacturing Co.) at full speed twice for 30 s each time. The contents were transferred to 15-ml Corex (Corning) centrifuge tubes, and 2 ml of $CHCl_3$ was added. The tubes were again agitated on a Vortex mixer and subsequently centrifuged for 10 min at 10,000 × *g* and 4°C to effect separation of the phases. The aqueous phases were collected, and 1 ml of phenol and 1 ml of $CHCl_3$ were added to each. The phenol phases were reextracted with 1 ml of HMK. The aqueous phases were combined, and 1.5 ml of phenol and 1.5 ml of $CHCl_3$ were added to each. This step was repeated, and the resultant aqueous phase was extracted an additional time with 3 ml of $CHCl_3$. Ethanol (2.5 volumes, 95%) was added to each aqueous phase, and the RNA was precipitated overnight at −20°C. The RNA was collected by centrifugation at 10,000 × *g* for 25 min. The pellets were washed once with 5 ml of ethanol (80%) at −20°C and lyophilized. Each pellet was finally suspended in 250 μl of sterile glass-distilled $H_2O$. Agitation by vortexing with glass beads has proven to be a rapid and essentially quantitative procedure for breaking the spores, which are extremely resistant to breakage by conventional methods. In addition, the presence of phenol during the breakage must minimize degradation of RNA, since we have never observed significant degradation of RNA during this procedure.

**Isolation of plasmid DNA and cloned insert.** The purification of plasmid DNA and the isolation of the *D. discoideum* cDNA inserts were carried out as described previosly (18).

**Labeling of DNA probe.** Isolated insert was nick translated to a specific activity of about $10^7$ cpm/μg with [α-$^{32}$P]dCTP and a nick-translation kit according to the specifications of the manufacturer.

**Northern blot and RNA dot-blot analyses.** Northern blot and RNA dot-blot analyses were performed as described previously (18). The hybrids formed were quantitated by scintillation counting of the bands or dots excised from the nitrocellulose filter. The amount of labeled insert hybridized was proportional to the concentration of RNA in the range tested.

**Preparation of polysomes from germinating spores.** The spore suspensions were incubated at 23°C with vigorous shaking. At the time indicated below, each portion was poured over crushed ice, and the germinating spores were rapidly sedimented and frozen. The suspensions were monitored microscopically for percent germination. The frozen spore pellets were suspended in 1 ml of buffer containing 50 mM HEPES (pH 7.5), 40 mM $MgCl_2$, 25 mM KCl, 5% sucrose, 4% Cemusol NP12 (Sfos), and 1 g of glass beads (0.45 to 0.50 mm diameter). The suspension was mixed on a Vortex mixer, at the highest speed, twice for 20 s each time at 1-min intervals and was kept on ice between the two mixing steps. Control experiments with amoebae which were broken only by detergent or by the above method showed that the method involving the use of beads to break cells results in excellent polysome preparations. The lysate was centrifuged at 5,000 × *g* for 3 min, and the supernatant (optical density at 260 nm, approximately 30) was applied to a 30-ml, 15 to 30% sucrose density gradient, made up in the above buffer without Cemusol. The gradients were centrifuged in a Spinco L265-B for 3.5 h at 22,500 rpm and analyzed with an ISCO density gradient fractionator.

**Materials.** Nogalamycin and pactamycin were the generous gifts of Paul F. Wiley, The Upjohn Co., Kalamazoo, Mich. *Pst*I, nitrocellulose sheets for Northern transfers, and the nick-translation kit were obtained from Bethesda Research Laboratories, Gaithersburg, Md. [α-$^{32}$P]dCTP (ca. 400 Ci/mmol) was obtained from Amersham Corp., Arlington Heights, Ill. Nitrocellulose sheets for RNA dot blotting, Elutip-d columns, and the micro-sample filtration manifold were purchased from Schleicher and Schuell, Inc., Keene, N.H.

## RESULTS

**Effect of protein synthesis inhibition on mRNA half-lives.** Three previously isolated cDNA clones specific for sequences developmentally regulated during spore germination were used as probes for their respective mRNAs. mRNAs complementary to clones pKL109 and pLK229 are expressed in spores and germinating spores, but their levels decrease during hours 2 and 3 of germination. pRK270 mRNA is present in very low amounts in dormant spores but is found predominantly in 1- and 1.5-h germinating spores and to a lesser extent in 2- and 3-h germinating spores (Fig. 1) (17). A typical Northern blot depicting the presence of these mRNAs during normal germination is shown in Fig. 1. The figure shows that all three mRNAs can be analyzed in

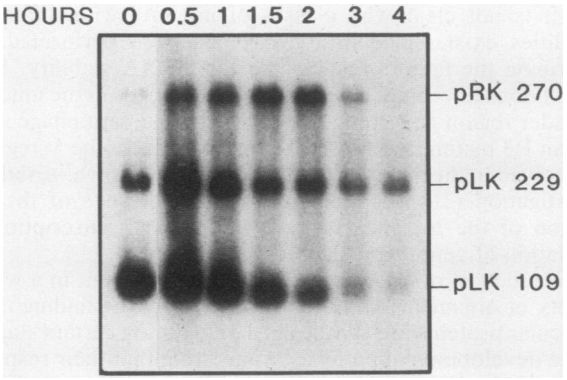


FIG. 1. Northern blot analysis of the time course of expression of developmentally regulated mRNAs during spore germination. The procedures for isolation, size fractionation, and hybridization of the mRNAs are given in Materials and Methods. The sizes of the mRNAs are as follows (in kilobases): pRK270, 2.7; pLK229, 1; pLK109, 0.5 at 0 h and 0.4 thereafter.

one experiment, although not all experiments were performed with the three probes simultaneously.

The effect of inhibiting protein synthesis on the decay of these mRNAs was determined. In germinating spores, inhibition of protein synthesis requires high concentrations of antibiotic to achieve greater than 90% inhibition. Cycloheximide (400 μg/ml) and pactamycin (300 μg/ml) were effective inhibitors, specifically stopping protein synthesis immediately after addition at any time during the germination sequence (data not shown). Puromycin was also tested but did not completely inhibit protein synthesis, even at concentrations as high as 750 μg/ml.

The effect of these inhibitors on polysome stability was also investigated to ascertain that they inhibit protein synthesis in *D. discoideum* in the same way as reported previously (29). A 60-ml portion of *D. discoideum* B spores at $10^8$/ml was activated and allowed to germinate for 1 h. At this time, 75% of the spores were swollen and 25% remained spores. Then the suspension of germinating spores was divided into three 20-ml portions. Nothing was added to one portion (control; Fig. 2A), to the second was added 400 μg of cycloheximide per ml (Fig. 2B), and to the third was added 300 μg of pactamycin per ml (Fig. 2C). The suspensions were incubated with vigorous shaking for 1 h, and polysomes were prepared as described in Materials and Methods. At 2 h, the control suspension contained 8% amoebae, 78% swollen spores, and 14% spores; the pactamycin- and cycloheximide-treated spores contained approximately 90% swollen spores and 10% spores. Inhibition of protein synthesis by cycloheximide resulted in polysome stabilization (Fig. 2B), while addition of pactamycin led to polysome disaggregation (Fig. 2C). These findings support the conclusion that, as expected, cycloheximide inhibits elongation and pactamycin inhibits initiation.

We previously showed that the intercalating antibiotic nogalamycin was an effective inhibitor of the synthesis of all species of RNA but had little effect on protein synthesis (9) and could be used to determine the half-lives of the mRNAs described above (18). In the following experiment, activated spores were allowed to germinate for 1 h, and then the spore suspension was divided into three parts. Cycloheximide (400 μg/ml) was added to the first portion, pactamycin (300 μg/ml) was added to the second, and the third served as an uninhibited control. At 1 h, spores were swelling (10); 5 to 10 min later, nogalamycin (300 μg/ml) was added to inhibit further RNA synthesis. Samples were removed at the times indicated in Fig. 3, and RNA was isolated as described in Materials and Methods. The relative amount of each specific mRNA was determined by Northern blot analysis (Fig. 1) as described in Materials and Methods or by dot blots. The amount of mRNA present immediately before the addition of nogalamycin was considered 100%.

The results of the blots are depicted graphically in Fig. 3. The lines on the following decay curves represent the decay of the respective mRNA in the control, uninhibited sample. We have also chosen to draw the curves on linear graphs. The bars are the standard error of the mean for each point. pLK229 and pRK270 mRNA were shown previously (18) to have half-lives of approximately 25 and 40 min, respectively. It is clear that in the presence of cycloheximide, all the mRNAs are much more stable than in its absence or in the presence of pactamycin. The half-lives in the presence of pactamycin are approximately the same as those observed in the control samples. pKL109 mRNA was previously shown to have a biphasic decay, one component apparently decaying in minutes and the other component decaying in hours

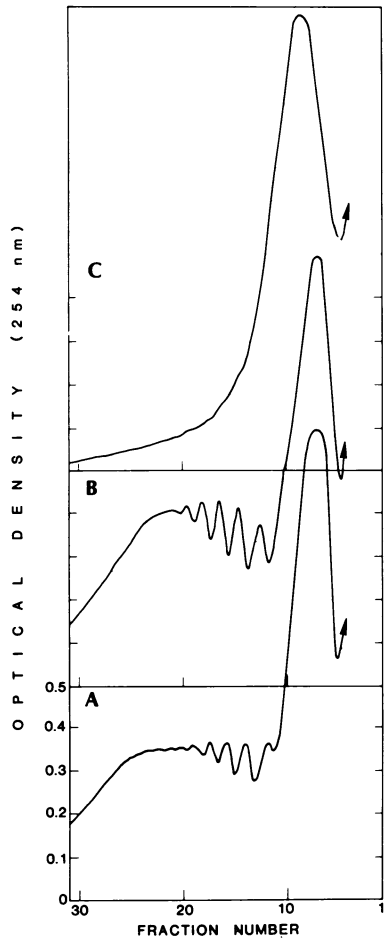


FIG. 2. Effect of inhibitors on polysome distribution during spore germination. Polysomes were prepared as described in Materials and Methods. (A) Control (no addition); (B) 400 μg of cycloheximide per ml was added; (C) 300 μg of pactamycin per ml was added.

(18). This mRNA was stable in the presence of cycloheximide but apparently decayed in the presence of pactamycin with the essentially normal biphasic kinetics observed in the untreated control (Fig. 3).

The effect of inhibition of protein synthesis in the decay of a stable mRNA was also investigated. The same experiment as done to obtain Fig. 3 was performed, except that pLK125 (18) was used as a probe. pLK125-specific mRNA has a half-life of about 230 min with first-order decay kinetics. The half-life is relatively unaffected in the presence of cycloheximide (Fig. 4). However, this mRNA is much more labile in the presence of pactamycin than in its absence, with a half-life of about 60 min.

**Accumulation of mRNA during inhibition of protein synthesis.** The relationship between protein and mRNA synthesis was also explored. Dormant spores were activated, and the protein synthesis inhibitor was added immediately after activated spores were suspended in germination buffer. Nogalamycin was not present for these experiments. Samples were taken at the indicated times, and the specific mRNA content at each time was estimated by Northern blot analysis. The decision to add antibiotic immediately after spore activation and not later was made because the maximum amount of synthesis of the three mRNAs studied

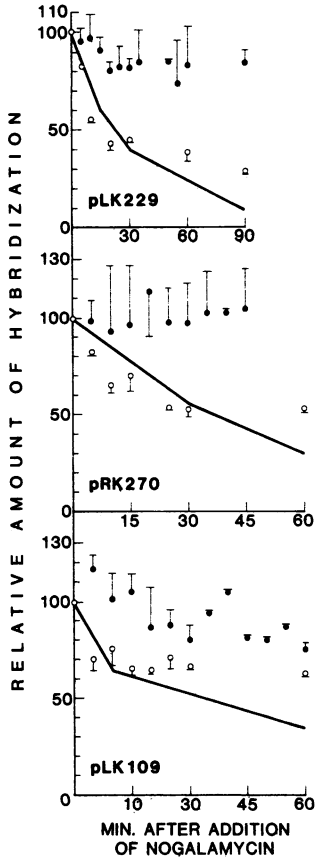


FIG. 3. Effect of inhibition of protein synthesis on the decay of pLK109, pLK229, and pRK270 mRNA. Decay was measured as described in Results. The circles are the actual datum points: ○, pactamycin-treated; ●, cycloheximide-treated. Bars represent the standard error of the mean for each point, and the solid line is the control decay curve which was previously reported (18). For pLK229, the cycloheximide experiment was performed three times and the pactamycin experiment was performed twice. For pRK270, each point represents four to seven separate determinations. For pLK109, the cycloheximide experiment was performed four times and the pactamycin experiment was performed twice.

occurred from 0 h (after activation) to approximately 1.5 h during normal germination. Synthesis either stopped or was greatly diminished later in germination (17, 18).

The Northern blot of an experiment involving the use of pactamycin to inhibit protein synthesis is presented in Fig. 5. As in the previous experiments, the hybridized bands were cut out of the nitrocellulose filter and counted. The subsequent data in Fig. 6 are graphic representations of Northern blot experiments with each of the inhibitors. The amount of radioactivity in each mRNA band is given relative to the zero time, which was arbitrarily set at unity. All other times are relative to this value. In these figures, the open bars indicate the levels of the specific mRNAs in uninhibited spores. As we showed previously (17), pLK109- and pLK229-specific mRNAs are present in spores, and their concentrations increase two- to threefold 0.5 to 1 h during germination and finally decrease rapidly thereafter. pRK270 mRNA is present in very low concentrations in spores, and its concentration increases seven- to eightfold at 1 to 1.5 h, and its level also decreases rapidly thereafter. All three mRNAs have half-lives of less than 1 h (18).

In contrast to what occurred during normal germination,

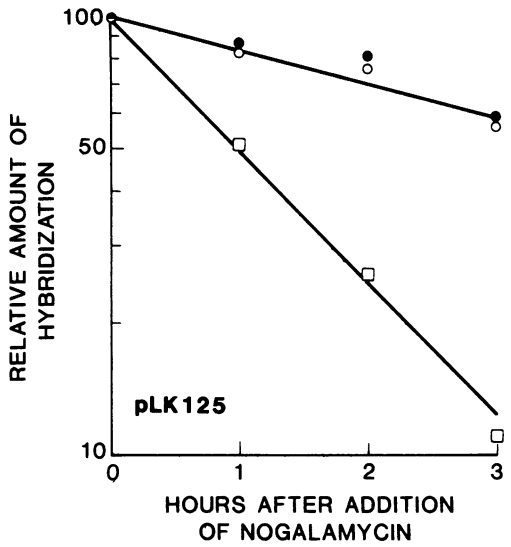


FIG. 4. Effect of inhibition of protein synthesis on the decay of pLK125 mRNA. The experiment was performed as described in the legend to Fig. 3, except that samples were taken at 1, 2, and 3 h after addition of drugs. Symbols: ○, control (no drug treatment); ●, cycloheximide-treated; □, pactamycin-treated.

when protein synthesis was inhibited by either cycloheximide or pactamycin, the mRNA levels remained high. It is noteworthy that three new transcripts, denoted X, Y, and Z, were observed to accumulate when protein synthesis was inhibited (Fig. 5). This figure presents an experiment with pactamycin, although the results were the same with cycloheximide. In a separate experiment in which the Northern blot was hybridized individually with the probes, it was shown that the three new bands hybridized solely to pLK229 cDNA (data not presented). We have evidence that these new transcripts represent other species of pLK229 mRNA normally present in too low a concentration to be detected by the method we used to perform the Northern blot hybridization (data not shown). It would seem that inhibiting

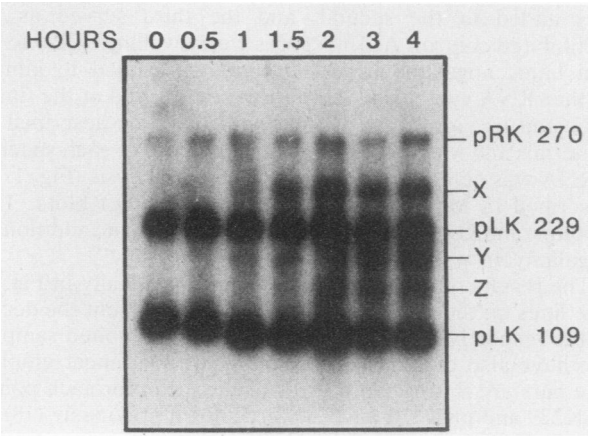


FIG. 5. Northern blot analysis of the effect of inhibition of protein synthesis on the expression of developmentally regulated mRNAs during spore germination. Pactamycin was added to the activated spores immediately after their suspension in germination buffer. Samples were taken at the indicated intervals, and the mRNAs were size fractionated and identified as described in Materials and Methods. Nogalamycin was not present for this experiment.

protein synthesis either stimulates their synthesis or stabilizes the mRNAs and allows their accumulation and detection.

## DISCUSSION

It is possible to determine the role of ribosome-mRNA interactions in mRNA decay by the use of antibiotics that inhibit specific steps in the protein synthesis pathway (5). Cycloheximide inhibits the elongation step of protein synthesis (29). Polysomes accumulate in inhibited cells because ribosomes do not terminate normally and new ribosomes enter the polysome. Pactamycin inhibits initiation, and consequently, polysomes break down in the presence of this drug (29). Under these conditions, the mRNA is essentially free of ribosomes, except for a 40S ribosome in the initiation site. The effect of these inhibitors on the decay of three *D. discoideum* developmentally regulated mRNAs was studied.

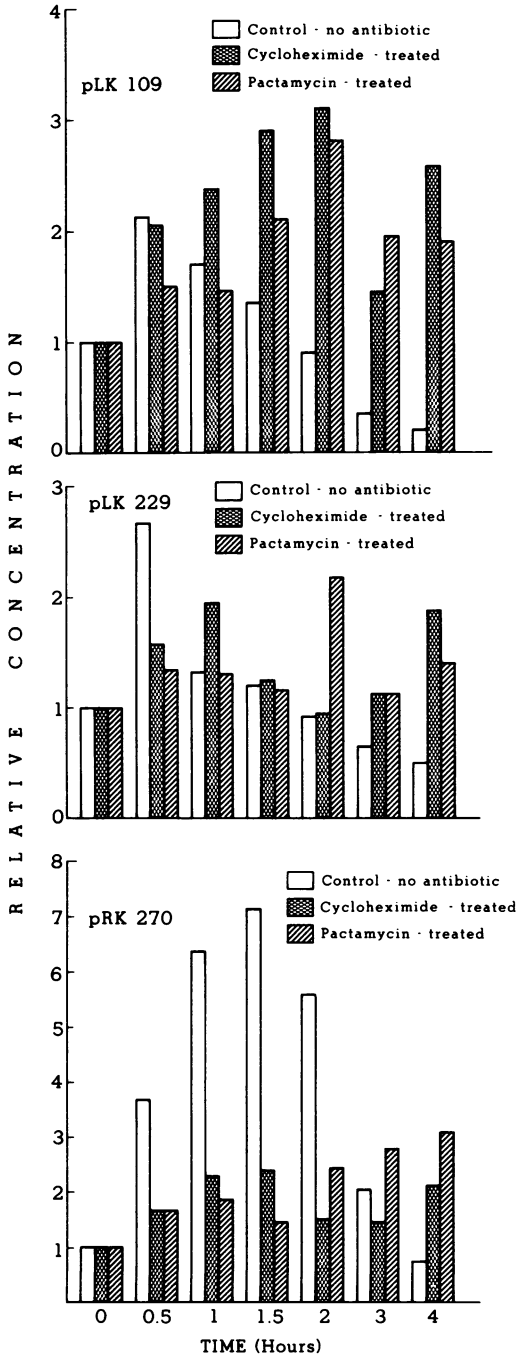


FIG. 6. Effect of inhibition of protein synthesis on the accumulation of mRNA. The experiments were performed as described in the legend to Fig. 5, and the data were obtained as described in the legend to Fig. 1 and 3. The data were normalized to a time zero value of unity. The control values were obtained in experiments similar to that described in Fig. 1.

The results show (Fig. 3) that the developmentally regulated pLK109, pLK229, and pRK270 mRNAs were stabilized in the presence of cycloheximide but that pactamycin had no effect on their normal decay. In cycloheximide-treated cells, the mRNAs are bound to ribosomes. Since the stability of mRNA may reflect the availability of sites for inactivation by nucleases, it follows that in the presence of cycloheximide, these sites are protected. Presumably, the ribosomes bound to the mRNA occupy these nuclease-sensitive sites, rendering the mRNA stable. No ribosomes are bound to mRNA in the presence of pactamycin except at the initiation site, and mRNA degrades at about the normal rate. The results indicate that the occupancy of the initiation site by a 40S subunit is not sufficient to stabilize these mRNAs. If the 5′ portion of the mRNA is important in mRNA stabilization, as has recently been shown for two mRNAs (12, 23), then perhaps an 80S subunit is required to shield this region from nuclease attack. In addition, since mRNA is unstable in the presence of pactamycin, it is clear that mRNA translation is not necessary for its degradation.

Another interesting conclusion derives from the observation that mRNA decay in the presence of pactamycin is the same as in its absence. This can mean that the rate of decay of these mRNAs is not influenced in any way by normal ribosome movement during protein synthesis. It is only when the ribosomes are stalled on the mRNA, as when cycloheximide is present, that ribosomes can protect the mRNA. Obviously, for the normal situation, ribosomes are not in a putative initiation of degradation site long enough to make the mRNA unavailable for degradation.

The data further indicate that a labile protein is probably not involved in mRNA decay or stabilization, since protein synthesis is inhibited equally by both drugs and cycloheximide stabilizes and pactamycin does not. However, we have not ruled out the possibility that pactamycin stabilizes the putative labile protein and thus the protein can be used in mRNA decay in the presence of this drug.

We also determined the decay of a stable mRNA in the presence of the protein synthesis inhibitors. pLK125 mRNA is a constitutive mRNA (present throughout all stages of growth and development) whose half-life is approximately 230 min (18). Surprisingly, this mRNA is destabilized in the presence of pactamycin, (half-life, about 60 min) and is unaffected in the presence of cycloheximide (Fig. 4). This very interesting finding may mean that the polysomes interact with a component of the cell (e.g., cytomatrix) and that disruption of this interaction by pactamycin (or other means) renders the mRNA susceptible to degradation. mRNA sequestered in the polysome would be stable. However, the finding may also simply be interpreted to mean that stabilization of mRNA can occur by polysome shielding. When polysomes are disaggregated, the mRNA is released and is made available to the degradative processes in the cell because sensitive sites on the molecule are no longer protected.

Many investigators (see, e.g., references 7, 15, and 25)

used cycloheximide (or inhibitors such as emetine or anisomycin which act similarly) to study whether protein synthesis is required for mRNA decay. In general, rather labile mRNAs were studied, and not all mRNAs or all populations of mRNA are stabilized by treatment with protein elongation inhibitors (2). The investigators uniformly found that the mRNA was stabilized, leading them to conclude that a labile protein was involved in mRNA decay, although polysome shielding of mRNA is a well-known effect of the mechanism by which cycloheximide inhibits protein synthesis. The effect of other inhibitors with different modes of action was not studied.

Others who used inhibitors possessing different mechanisms of action found that the results obtained were dependent on the model system studied. Both cycloheximide and pactamycin were found to stabilize histone mRNA when DNA replication is stopped (27) and to stabilize c-*myc* mRNA (20), two mRNAs that have very short half-lives. These investigators correctly indicate a requirement for continuing protein synthesis in the regulation of the decay of these mRNAs. In contrast, the mRNAs of tyrosine aminotransferase (28) and vesicular stomatitis virus (26), although stable in the presence of cycloheximide, were normally unstable in the presence of pactamycin or puromycin. The last two studies and our present findings show that some mRNAs can be stabilized by polysome shielding resulting from inhibitors analogous to cycloheximide. Furthermore, the stabilization is not due to inhibition of protein synthesis per se, or because a labile protein important for decay turns over rapidly and is not replenished under these conditions. Finally, it has also been recently concluded (4) that histone mRNAs in polysomes are better targets for destabilization upon inhibition of DNA synthesis than those released from the polysomes. We therefore consider it important for investigators studying the role of protein synthesis in mRNA decay to evaluate their data in the light of our findings and to use in their studies inhibitors of the different reactions in protein synthesis, e.g., elongation and initiation, which result in different ribosome-mRNA configurations.

The relationship between protein and mRNA synthesis was also studied. In these experiments, the protein synthesis inhibitor was added at the initiation of the germination process, and the levels of the developmentally regulated mRNAs were determined at later times (Fig. 6). In the uninhibited control, the levels of pLK109 and pLK229 mRNAs reached a peak at about 1 h and the level of pRK270 reached a peak at 1.5 h after germination was initiated; the levels decreased later in germination, consistent with previously described data (17, 18). The results indicate that (i) new mRNA was being synthesized during germination, (ii) the synthesis was shut off at about 1 to 1.5 h after germination was initiated, and (iii) the mRNAs degraded rapidly. Since mRNA is stabilized in the presence of cycloheximide, if mRNA were being synthesized at the control rate, then a greater accumulation than that of the control should have been observed. This did not occur. If the rate of synthesis in the presence of pactamycin were equal to the control value, then the level of mRNA reached early in germination should have been equal to that of the control. This also was not seen. It is therefore obvious that in the presence of the inhibitors, some mRNA synthesis occurs, albeit at a diminished rate relative to that of the uninhibited control. Our results are not surprising in view of the voluminous literature showing that mRNA is made in the presence of inhibitors of protein synthesis; although the level of transcription varies depending on the system studied (e.g., 20, 25, 28).

The fact that the final mRNA levels attained (at 3 to 4 h) in the presence of the inhibitors were higher than the initial amount (at 0 h) shows that mRNA synthesis was not shut off under these conditions. The rate of synthesis was obviously higher in the presence of pactamycin than in the presence of cycloheximide, because, to reiterate, decay occurred in the presence of the former drug and not in the presence of the latter (Fig. 3). These results might indicate that in the absence of protein synthesis, a switch necessary for shutting off synthesis of the developmentally regulated mRNAs does not occur. Perhaps this switch requires protein synthesis or is a protein itself. It is noteworthy that inhibition of protein synthesis did not stop the normal processing of pLK109 mRNA (Fig. 5), which is approximately 500 bases in spores and is processed during germination to about 400 bases (17).

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