

(p)ppGpp Inhibits Polynucleotide Phosphorylase from *Streptomyces* but Not from *Escherichia coli* and Increases the Stability of Bulk mRNA in *Streptomyces coelicolor*[∇]

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ppGpp regulates gene expression in a variety of bacteria and in plants. We proposed previously that ppGpp or its precursor, pppGpp [referred to collectively as (p)ppGpp], or both might regulate the activity of the enzyme polynucleotide phosphorylase in *Streptomyces* species. We have examined the effects of (p)ppGpp on the polymerization and phosphorolysis activities of PNPase from *Streptomyces coelicolor*, *Streptomyces antibioticus*, and *Escherichia coli*. We have shown that (p)ppGpp inhibits the activities of both *Streptomyces* PNPases but not the *E. coli* enzyme. The inhibition kinetics for polymerization using the *Streptomyces* enzymes are of the mixed noncompetitive type, suggesting that (p)ppGpp binds to a region other than the active site of the enzyme. pppGpp also inhibited the phosphorolysis of a model RNA substrate derived from the *rpsO-pnp* operon of *S. coelicolor*. We have shown further that the chemical stability of mRNA increases during the stationary phase in *S. coelicolor* and that induction of a plasmid-borne copy of *relA* in a *relA*-null mutant increases the chemical stability of bulk mRNA as well. We speculate that the observed inhibition *in vitro* may reflect a role of ppGpp in the regulation of antibiotic production *in vivo*.

The alarmone, ppGpp (GDP; 3'-diphosphate) regulates gene expression in bacteria. In the classical stringent response to amino acid starvation in *Escherichia coli*, ppGpp is synthesized on idling ribosomes by the product of the *relA* gene, a (p)ppGpp synthetase (6). ppGpp then inhibits the synthesis of ribosomal and transfer RNAs, decreasing the levels of ribosome and tRNA synthesis when rates of protein synthesis decrease (reviewed in references 3, 6, 31, and 35). ppGpp can also activate transcription in *E. coli*. *E. coli* mutants lacking ppGpp are auxotrophic for a number of amino acids, and ppGpp has been shown to activate transcription of the operons for those amino acids (3, 6, 31, 35). Recent studies indicate that ppGpp interacts with RNA polymerase in concert with the effector protein DksA, and this interaction results in promoter-specific inhibition of transcription, in the case of stable RNA promoters, or the activation of transcription in the case of the promoters for amino acid biosynthesis (11, 12, 18). It has also been argued that ppGpp regulates growth rate in *E. coli* (2, 19), although there is other evidence suggesting that it is not essential to this process (15). Microarray studies indicate that the expression of several hundred genes is affected by changes in ppGpp levels in *E. coli* (13). In other systems, ppGpp is involved in sporulation, stress survival, and virulence (1, 14, 17).

The stringent response also occurs in the soil-dwelling actinomycete, *Streptomyces* (36). *Streptomyces* species contain homologs of *relA* (8, 22, 28), and ppGpp levels increase in response to amino acid starvation in *Streptomyces*, as in *E. coli* (36). Of particular interest is the observation that ppGpp serves as both a negative and a positive regulator of antibiotic production in *Streptomyces* species. Thus, *relA*-null mutants of

Streptomyces clavuligerus overproduce clavulanic acid and cephalomycin C, indicating negative regulation by ppGpp (16). In contrast, ppGpp has been shown to be required for antibiotic production in *Streptomyces griseus* (streptomycin [30]), *Streptomyces coelicolor* (actinorhodin and calcium-dependent antibiotic [7, 21]), and *Streptomyces antibioticus* (actinomycin [22]). In the case of *S. coelicolor*, there is strong evidence indicating that ppGpp activates the transcription of genes that regulate antibiotic production. Thus, a microarray analysis demonstrated that genes for production of the calcium-dependent antibiotic (CDA) and actinorhodin were induced after ppGpp synthesis (20). Induction of the CDA and actinorhodin clusters was accompanied by an increase in transcription of the pathway-specific regulators for those clusters, *cdaR* and *actII-ORF4*, respectively (20). It is noteworthy that induction of the genes for undecylprodigiosin, another of the antibiotics produced by *S. coelicolor*, was not observed in that study.

An early attempt to identify the *relA* gene in *S. antibioticus* identified another gene instead, namely, the gene for the 3'-5'-exoribonuclease, polynucleotide phosphorylase (PNPase) (23). This situation occurred because *S. antibioticus* PNPase also possesses pppGpp synthetase activity (24). pppGpp (GTP; 3'-diphosphate) is the precursor of ppGpp, and it was initially thought that PNPase might represent an alternative enzyme for ppGpp synthesis in *Streptomyces*. More recent studies indicate that PNPase does not modulate ppGpp levels *in vivo*, and we speculated that the pppGpp synthetase activity might reflect possible regulation of PNPase activity by (p)ppGpp (5). PNPase plays an important role in RNA degradation in *E. coli* and *Streptomyces* and serves both as an exonuclease and as an RNA 3'-polyribonucleotide polymerase [the functional analog of poly(A) polymerase] in both species. Mohanty and Kushner have shown that PNPase is responsible for the G, C, and U residues that occur at low frequency in the poly(A) tails of *E.*

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coli RNAs (29). In *Streptomyces*, the data suggest that PNPase is the sole RNA 3'-polyribonucleotide polymerase present and that it is responsible for the synthesis of the heteropolymeric tails associated with RNAs in those organisms (4, 34). Thus, both the degradative activity (phosphorolysis) and the synthetic activity (polymerization) of PNPase may be important in *Streptomyces*. We thus endeavored to examine the possible role of (p)ppGpp in the regulation of PNPase activity and show here that ppGpp effectively inhibits both the polymerization and the phosphorolysis activities of the enzyme. We also provide evidence that ppGpp increases the chemical half-lives of bulk mRNA in *S. coelicolor*.

MATERIALS AND METHODS

Preparation of (p)ppGpp, substrates, and PNPases. pppGpp and ppGpp were synthesized *in vitro* from GTP and ATP by using crude extracts of *E. coli* strain CF3120, which expresses an inducible form of *relA*, generously supplied by Michael Cashel. (p)ppGpp was purified from reaction mixtures by ion-exchange chromatography on Q Sepharose Fast Flow in the presence of 7 M urea. For some experiments, ppGpp was obtained from Trilink Biotechnologies. [³H]poly(A) was synthesized from [³H]ADP as described previously (25). Untagged polynucleotide phosphorylases from *S. antibioticus*, *S. coelicolor*, and *E. coli* were expressed and purified as described previously (25). The 5601 transcript, derived from the *rpsO-pnp* operon of *S. coelicolor*, was synthesized by using [³²P]CTP as described previously (9).

Conditions for PNPase reactions. Polymerization reactions were performed as described previously (25) in 15- μ l reaction mixtures, incubated for 15 min at 37°C. Mixtures contained 5 mM ADP, 0.45 μ Ci of [³H]ADP (Perkin-Elmer, 33.9 Ci/mmol), and ca. 0.28 μ M PNPase. Kinetic assays for polymerization were performed using a stock solution of 125 mM [³H]ADP with a specific activity of 6 μ Ci/ μ mol, which was diluted to prepare substrate to obtain initial velocities. Preliminary assays (not shown) showed that product formation proceeded linearly at the protein concentrations and for the incubation times used in the experiments reported here. Phosphorolysis reactions were performed as described previously (25) in 15- μ l reaction mixtures, incubated for 10 min at 37°C. Mixtures contained ca. 30,000 cpm of [³H]poly(A), prepared as described previously (25); 5 mM potassium phosphate; and ca. 14 nM PNPase. Phosphorolysis of the 5601 transcript was performed as described previously (9) in 15- μ l reaction mixtures containing 0.43 μ M 5601 RNA (ca. 30,000 cpm), 5 mM potassium phosphate and 28 nM PNPase, which were incubated for 10 min at 37°C. Some reactions contained 1 mM ppGpp. Reactions were stopped by adding Sequencing Stop Solution (Promega) containing formamide, and samples were heated for 5 min at 75°C. Phosphorolysis products were separated on 7 M urea-5% polyacrylamide gels and visualized by autoradiography.

Measurement of bulk mRNA half-lives. *S. coelicolor* M600 (a *relA*⁺ parental strain), M653 (a *relA* strain containing the *relA* gene under the control of the inducible *tipA* promoter) (21), and M570 (a *relA* strain) (8) were generously provided by Andrew Hesketh of the John Innes Centre, Norwich, England. M570 containing pIJ8600 was constructed in our laboratory and was designated JSE571. pIJ8600 is the cloning vector used for overexpression of *relA* in M653 (21).

All *Streptomyces* strains were grown in minimal medium (26) containing carboxymethyl cellulose rather than polyethylene glycol as a dispersant. To measure mRNA half-lives liquid cultures were grown at 30°C for the appropriate lengths of time when 15 ml was removed to a 50-ml screw cap tube. [³H]uridine (37.5 μ Ci [DuPont NEN], 31.9 Ci/mmol) was added, and the samples were incubated for 10 min at 30°C. Actinomycin D (Sigma) was then added to these samples to a final concentration of 75 μ g/ml. Incubation was continued with the removal of 1-ml samples to 0.2 ml of 50% trichloroacetic acid (TCA) at 0, 5, 10, 15, 20 and 60 min, after actinomycin addition. TCA samples were collected as described previously, and the cpm in mRNA were calculated by subtracting the cpm values obtained at 60 min, representing stable RNAs, from the values obtained at the earlier time points (5). Half-lives were determined by regression analysis of the decay data. *S. coelicolor* M600 and M570 were grown to A_{450} values of 0.4 to 0.5 (ca. 7 h postinoculation) for the measurement of half-lives during exponential phase and to A_{450} values of ≥ 1.2 (26 h postinoculation) to measure half-lives in stationary phase. Strains M653 and JSE571 were grown to A_{450} values of ≥ 1.2 (26 h postinoculation) when half-lives were measured as described above. Thio-strepton was then added to separate 15-ml samples of each culture to a final

concentration of 25 μ g/ml, and the samples were incubated for 60 min at 30°C to induce ppGpp production in M653 (21). The half-lives were then measured as described above.

The half-lives of bulk mRNA were also measured in two *E. coli* strains, CF1648 (*relA*⁺) and CF1652 (Δ *relA::kan*), generously donated by Michael Cashel (38). The strains are isogenic apart from the *relA* mutation. The strains were grown in 10 to 12 ml of M9 medium (33) containing 0.2% each of glucose and Casamino Acids. Exponential-phase measurements were performed at A_{600} values of 0.2 to 0.3, and stationary-phase measurements were performed at A_{600} values of ~ 1.0 . To measure half-lives, 25 μ Ci of [³H]uridine was added to 10-ml cultures, and the samples were incubated for 2 min at 37°C. Rifampin and nalidixic acid (Sigma) were then added to these cultures to final concentrations of 500 and 20 μ g/ml, respectively, and the cultures were incubated for two additional minutes. Incubation was continued with the removal of duplicate 0.4-ml samples to 0.1 ml of 50% TCA at 0, 2, 4, 6, 8, and 30 min after rifampin addition. The half-lives were measured as described for the *Streptomyces* assays with the 30-min cpm values representing stable RNAs.

RESULTS AND DISCUSSION

Examination of the effects of (p)ppGpp on the polymerization reactions catalyzed by PNPase. The exact intracellular concentrations of ppGpp during normal growth have not been determined in *Streptomyces*; however, Riesenberg et al. attempted to correlate intracellular concentrations of ppGpp with ppGpp levels presented as pmol/mg of dry weight. These authors estimated that an intracellular ppGpp concentration of 0.5 mM corresponded to ca. 1,500 pmol/mg of dry weight (32). ppGpp levels of 200 to 500 pmol/mg of mycelial dry weight have been measured in *S. antibioticus*, *S. coelicolor*, and other streptomycetes (7, 20, 22, 30), corresponding to intracellular concentrations of ca. 0.07 to 0.17 mM, based on the estimates of Riesenberg et al. Local concentrations within the mycelium might well be higher. In *E. coli*, it is known that millimolar concentrations of ppGpp accumulate during the stringent response and that (p)ppGpp concentrations of 0.1 to 0.5 mM inhibit exopolyphosphatase from *E. coli* (27). Thus, we utilized concentrations up to 1 mM in the studies reported here. The effects of (p)ppGpp on polymerization of [³H]ADP are shown in Fig. 1. It can be seen that ppGpp effectively inhibited the polymerization reactions catalyzed by the streptomycete enzymes but did not inhibit the *E. coli* enzyme. ppGpp at 1 mM inhibited polymerization by the *Streptomyces* enzymes ca. 90%. pppGpp also inhibited the streptomycete PNPases but to a lesser extent than did ppGpp. We consistently observed a slight stimulation of the *E. coli* PNPase by pppGpp as shown in Fig. 1. The mechanism of this stimulation is not currently known. No inhibition of polymerization by either compound was observed at the concentrations tested.

To examine this phenomenon further, we performed a kinetic analysis by varying the concentration of ADP in reaction mixtures containing either *S. antibioticus* or *S. coelicolor* PNPases. The results of this analysis are shown in Fig. 2 and Table 1. Figure 2 shows Eadie-Hofstee plots of the kinetic data, and it can be seen that both the maximum velocity and the K_m values of the reaction were affected by 0.5 and 1 mM ppGpp, indicating that ppGpp is a mixed or true noncompetitive inhibitor of the polymerization reaction. This conclusion is confirmed by the data of Table 1, which show a change in K_m and k_{cat} in the presence of ppGpp. ppGpp affects k_{cat} to a greater degree than it does K_m and, interestingly, the K_m for ADP decreased somewhat at 1 mM ppGpp in the case of *S. antibioticus* PNPase and at both ppGpp concentrations in the case of

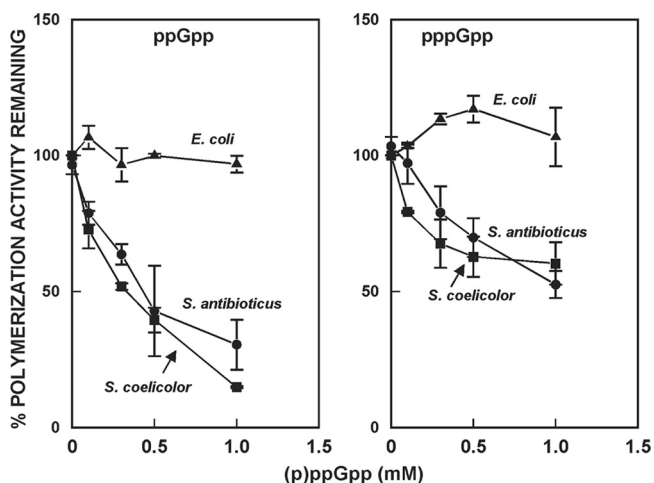


FIG. 1. Inhibition of the polymerization activity of PNPase by (p)ppGpp. The data in the figure show the percentage of the polymerization activity observed in the absence of (p)ppGpp that remained when the reactions were performed in the presence of the indicated concentrations of (p)ppGpp and are the averages of duplicates from two separate experiments \pm the standard error of the mean (SEM).

the *S. coelicolor* enzyme. k_{cat}/K_m decreased by \sim 8-fold in the case of *S. antibioticus* PNPase and $>$ 5-fold in the case of the *S. coelicolor* enzyme.

For comparison with the results described above, we also measured the kinetic constants for polymerization using *E. coli* PNPase. The results of this analysis are also shown in Table 1. Under our assay conditions, the k_{cat} value for *E. coli* polymerase was greater than that observed for either of the *Streptomyces* enzymes, but the K_m value was somewhat higher as well. As expected, 0.5 mM ppGpp had essentially no effect on the kinetic constants for the *E. coli* enzyme.

Effects of (p)ppGpp on phosphorolysis. We examined the effects of (p)ppGpp on phosphorolysis using [3 H]poly(A), synthesized as described previously (25). The results of the analysis are shown in Fig. 3. In this figure, the 100% value is the level of phosphorolysis observed in the absence of ppGpp and lower percentages reflect the inhibition of phosphorolysis by ppGpp. Thus, for example, the phosphorolysis activity of the *S. antibioticus* PNPase measured in the presence of 0.5 mM ppGpp was only ca. 30% of that observed in the absence of ppGpp. Again, the activities of the *Streptomyces* enzymes were inhibited by ppGpp; 1 mM ppGpp essentially abolished phosphorolysis of the poly(A) substrate. pppGpp was much less effective than ppGpp with the *Streptomyces* enzymes and did not substantially affect the activity of *E. coli* PNPase. ppGpp produced only about a 10% inhibition of the *E. coli* enzyme, even at a concentration of 1 mM. To obtain additional information on the effects of ppGpp on the phosphorolysis reactions, we examined the phosphorolysis of a model RNA substrate, the 5601 transcript, derived from the *rpsO-pnp* operon of *S. coelicolor* and used by us in other studies (9). The effects of 1 mM ppGpp on phosphorolysis of the substrate by the three PNPases are shown in the autoradiogram presented in Fig. 4. Again, ppGpp had no effect on the phosphorolysis of this substrate catalyzed by the *E. coli* PNPase (lanes 6 and 7). All of the products normally observed upon phosphorolysis of

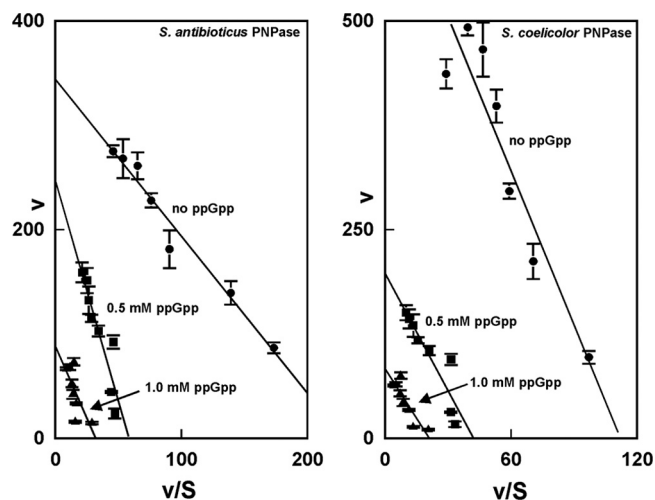


FIG. 2. Eadie-Hofstee plots of kinetic data for *S. antibioticus* and *S. coelicolor* PNPases in the polymerization reaction. Kinetic assays were performed as described previously (25) and in Materials and Methods. Velocities are expressed as $\mu\text{mol min}^{-1}$, and substrate concentrations are indicated in mM. The data in the figure are averages of two sets of duplicates \pm the SEM. The kinetic parameters were calculated from the regression statistics corresponding to the slopes and intercepts of the plots.

the 5601 transcript were observed in the presence or absence of ppGpp. In contrast, 1 mM ppGpp completely abolished the production of RP1, RP3, and RP4 by PNPases from *S. coelicolor* (lanes 2 and 3) and *S. antibioticus* (lanes 4 and 5). These results indicate that ppGpp inhibits the phosphorolysis of not only a model substrate, poly(A), but also a substrate corresponding to a naturally occurring transcript in *S. coelicolor*.

Effects of ppGpp on the stability of bulk mRNA in *S. coelicolor*. How might the observation of ppGpp inhibition of PNPase reflect a regulatory role for ppGpp and PNPase in *Streptomyces*? As indicated above, ppGpp positively regulates antibiotic production in several *Streptomyces* species, including the paradigm for streptomycete studies, *S. coelicolor* (7, 20, 22, 30). ppGpp levels rise just prior to the onset of antibiotic production in these organisms and, although the levels decline subsequently, they do not fall to zero. ppGpp levels are maintained at ca. 25% of peak levels for hours to days following the initial rise in concentration (22, 36). Again, as indicated above, PNPase functions as both an exonuclease and as an RNA 3'-polynucleotide polymerase in *Streptomyces* (4, 34). Both functions appear to be important in *Streptomyces* since: (i) *Streptomyces* spp. do not contain an RNase R homolog (39), so PNPase is the major if not the only 3'-5'-exonuclease present in *Streptomyces*; (ii) *Streptomyces* spp. do not contain a dedicated poly(A) polymerase, so PNPase must synthesize the 3' tails that facilitate the degradation of streptomycete RNAs (4, 34); and (iii) PNPase is an essential enzyme in *Streptomyces* (4). Thus, PNPase almost certainly functions as a critical determinant of transcript stability in *Streptomyces*. As demonstrated here, ppGpp inhibits PNPase activity in *Streptomyces*. The inhibition of both the polymerization and the phosphorolysis activities of PNPase would be expected to increase transcript stability. It is conceivable, therefore, that ppGpp, which persists at significant levels in *Streptomyces* after antibiotic synthe-

TABLE 1. Kinetic parameters for the polymerization reactions in the presence or absence of ppGpp^a

ppGpp concn (mM)	Mean \pm SEM						k_{cat}/K_m (1 mol ⁻¹ min ⁻¹ , 10 ³)		
	K_m (mM)			k_{cat} (min ⁻¹)			<i>S. antibioticus</i>	<i>S. coelicolor</i>	<i>E. coli</i>
	<i>S. antibioticus</i>	<i>S. coelicolor</i>	<i>E. coli</i>	<i>S. antibioticus</i>	<i>S. coelicolor</i>	<i>E. coli</i>			
0	1.46 \pm 0.12	6.14 \pm 0.43	14.6 \pm 1.2	80.5 \pm 2.9	492 \pm 43.4	731 \pm 224	55.1	80.1	50.1
0.5	4.24 \pm 0.77	4.68 \pm 0.32	12.6 \pm 2.0	59.1 \pm 6.5	141 \pm 14.1	672 \pm 179	13.9	30.1	53.3
1.0	2.78 \pm 0.26	3.96 \pm 0.68	-	21.2 \pm 4.2	59.3 \pm 6.7	-	7.6	15.0	-

^a The K_m and k_{cat} data are averages of two sets of duplicates.

sis is initiated, could increase the stability of transcripts for proteins required for antibiotic production, by inhibiting the activity of PNPase. This strategy, along with the persistence of low levels of protein and RNA synthesis, would ensure that sufficient amounts of the necessary proteins remain available to support antibiotic production.

To test this hypothesis, we have determined the chemical half-lives of bulk mRNA in *S. coelicolor* under several sets of conditions. In the initial set of experiments, *S. coelicolor* M600 and M570 were grown to mid-exponential phase (A_{450} of \sim 0.5, ca. 7 h postinoculation), a portion of the cultures was removed, RNAs were radioactively labeled, and RNA synthesis was then inhibited with actinomycin as described in Materials and Methods. A second portion of the cultures was removed 26 h postinoculation in the stationary phase, when the production of undecylprodigiosin and actinorhodin had begun in M600. The chemical half-lives of the bulk mRNA were determined as described previously (5) and in Materials and Methods. It is apparent from Fig. 5 and Table 2 that the half-life of bulk mRNA increased by a factor of 1.8 in stationary phase in M600, from 3.2 to 5.7 min. No such increase was observed with M570, the *S. coelicolor* *relA*-null mutant. This is exactly the outcome that is predicted if ppGpp inhibits mRNA decay during the stationary phase.

To determine whether ppGpp rather than some other agent

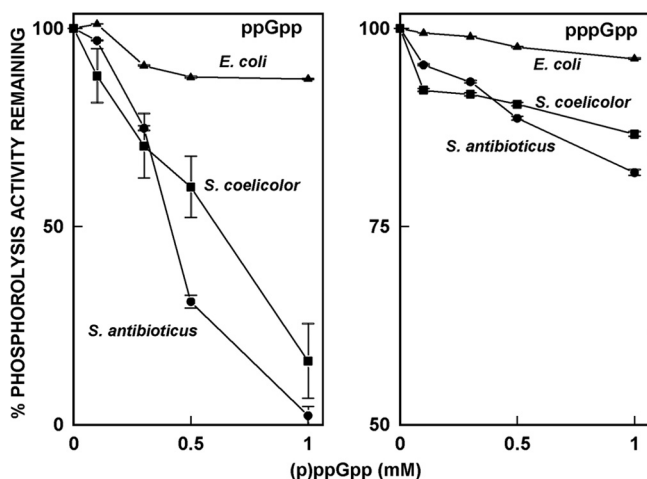


FIG. 3. Inhibition of the phosphorolysis activity of PNPase by (p)ppGpp. In this figure, the 100% value is the level of phosphorolysis observed in the absence of ppGpp for each enzyme, and lower percentages reflect the inhibition of phosphorolysis by ppGpp. The data in the figure are the averages of duplicates from two separate experiments \pm the SEM.

was likely to be responsible for the observations just described, we examined the effects of induction of the *relA* gene on the stability of bulk mRNA. We utilized *S. coelicolor* M653, containing the *relA* gene under the control of the thiostrepton-inducible *tipA* promoter (21). As a control, M570 containing pIJ8600 (JSE571), the vector utilized to clone the *relA* gene, was used. M653 and JSE571 were grown to stationary phase, and a portion of each culture was treated with [³H]uridine and actinomycin as described in Materials and Methods. A separate portion of each culture was brought to 25 μ g/ml with thiostrepton to induce *relA* expression (21). The half-lives of bulk mRNA were measured, and the results are shown in Fig. 5 and Table 2. The half-life of bulk mRNA prior to induction of *relA* in M653 was ca. 6.6 min, whereas the half-life increased to ca. 11.8 min after induction, again a 1.8-fold increase. Thiostrepton treatment produced no increase in half-life in JSE571, the strain containing the cloning vector only. These data strongly suggest that induction of *relA* in *S. coelicolor* M653 and the subsequent production of ppGpp by RelA leads to an increase in the stability of bulk mRNA. The observation that the half-life of bulk mRNA in exponential phase in M600 was shorter than in any of the strains in which *relA* was disrupted suggests that in addition to its role in ppGpp synthesis, *relA* may be involved in other physiological processes that affect mRNA stability. Effects of *relA* disruption on the growth and morphology of *S. coelicolor* have been reported (7).

We also examined the half-lives of bulk mRNA in two strains of *E. coli* to provide a comparison with the data de-

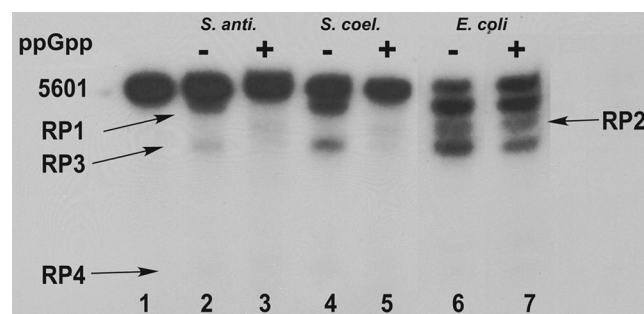


FIG. 4. Gel electrophoresis of PNPase digests of the 5601 transcript. ³²P-labeled 5601 RNA was synthesized as described previously (9). Gels were subjected to autoradiography. Lane 1, undigested 5601 transcript; lanes 2 and 3, digestion with *S. antibioticus* PNPase; lanes 4 and 5, digestion with *S. coelicolor* PNPase; lanes 6 and 7, digestion with *E. coli* PNPase. The reaction mixtures corresponding to lanes 3, 5, and 7 contained 1 mM ppGpp. The product designations at the left and right of the figure relate the bands produced to the 3'-endpoints mapped previously by cDNA cloning and sequencing (9).

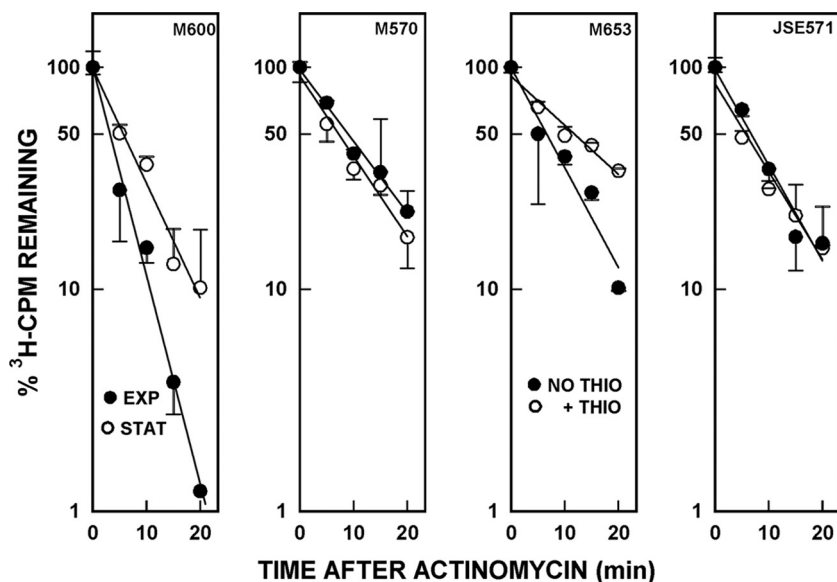


FIG. 5. Decay curves of bulk mRNAs in various *S. coelicolor* strains. Cultures were grown and treated as described in Materials and Methods, and actinomycin D was used to inhibit transcription. Measurements were made during exponential (EXP) and stationary (STAT) phases for M600 and M570 and during stationary phase for M653 and JSE571. Samples of the latter two strains were also treated with thiostrepton to induce expression of the *tipA* promoter. The cpm values obtained at each time point were plotted as a percentage of the cpm present at time zero, set arbitrarily as 100. The data in the figure are the averages of duplicate measurements from two independent experiments \pm the SEM.

scribed above for *S. coelicolor*. As shown in Table 2, the chemical half-lives were about 2 min during the exponential phase of growth in the parental *relA*⁺ strain and in the *relA*-null mutant. The half-life observed in stationary phase for the parental strain was also approximately 2 min, while a slight increase (~1.3-fold) was observed in the *relA*-null mutant. If this increase has physiological significance, it is presumably not due to an effect of ppGpp.

In summary, we have shown that (p)ppGpp inhibits both polymerization and phosphorolysis by *Streptomyces* PNPases but not by *E. coli* PNPase. The observation that ppGpp exhibits

mixed noncompetitive inhibition of polymerization suggests that the inhibitor binds to a region of the *Streptomyces* PNPases other than the active site (10). Analysis of the crystal structure of *S. antibioticus* PNPase suggests a candidate region for ppGpp binding. In particular, helices α 3 and α 4 of the PNPase, along with two of the β strands, form a solvent-filled pocket which does not bind tungstate (37). Thus, these structural elements are potential components of the site for (p)ppGpp binding. It should be possible to identify the binding site using analogs of ppGpp that can be cross-linked to the protein.

Our data also suggest that ppGpp stabilizes bulk mRNA during stationary phase in *S. coelicolor* and in strains in which ppGpp synthesis is ectopically induced. Although we did not measure ppGpp levels in the experiments reported here, we note that no stabilization of mRNA was observed when exponential- and stationary-phase cultures of *S. coelicolor* M570 (*relA*) were compared or when thiostrepton-treated and untreated cultures of JSE571 were compared (Fig. 5 and Table 2). It should be noted further that although the half-life of bulk mRNA was only increased by a factor of 1.8 in the experiments described here, ppGpp might well exert a greater effect on the stability of particular mRNAs. Although it is possible to speculate as to the identities of mRNAs whose stability might be affected by ppGpp levels, the most effective way to examine this question would be to perform a global analysis of mRNA half-lives under various physiological conditions in *S. coelicolor*. Experiments are in progress to examine further the roles of ppGpp and PNPase as determinants of mRNA stability during the stationary phase in this organism.

ACKNOWLEDGMENT

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TABLE 2. Chemical half-lives of bulk mRNA in various *S. coelicolor* and *E. coli* strains

Strain	Conditions or description	Mean half-life (min) \pm SEM ^a
<i>S. coelicolor</i>	M600 Parental strain, exponential phase	3.2 \pm 0.2
	M600 Parental strain, stationary phase	5.7 \pm 0.6
	M570 <i>relA</i> -null mutant, exponential phase	8.9 \pm 0.8
	M570 <i>relA</i> -null mutant, stationary phase	7.2 \pm 0.7
M653	Strain M570 with inducible <i>relA</i> with no thiostrepton	6.6 \pm 0.9
	Strain M570 with inducible <i>relA</i> plus thiostrepton	11.8 \pm 1.6
JSE571	With pIJ8600, no thiostrepton	6.8 \pm 0.8
	With pIJ8600 plus thiostrepton	5.7 \pm 0.9
<i>E. coli</i>	CF1648 <i>relA</i> ⁺ ; exponential phase	2.6 \pm 0.3
	CF1648 <i>relA</i> ⁺ ; stationary phase	2.2 \pm 0.3
	CF1652 <i>relA</i> -null mutant, exponential phase	2.3 \pm 0.2
	CF1652 <i>relA</i> -null mutant, stationary phase	3.0 \pm 0.2

^a Half-lives were calculated from the data shown in Fig. 5 or from similar plots in the case of the *E. coli* measurements.

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