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Coupling of DNA Replication to Growth Rate in *Escherichia coli*: a Possible Role for Guanosine Tetraphosphate

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Two promoters for the *Escherichia coli* operon that contains the four genes *dnaA*, *dnaN*, *recF*, and *gyrB* were found to be growth rate regulated and under stringent control. Transcript abundance relative to total RNA increased with the growth rate. Changes in transcription from the *dnaAp1* and *dnaAp2* promoters that were induced by amino acid starvation and chloramphenicol and were *relA* dependent were correlated with the stringent response. The abundance of these transcripts per total RNA also decreased in *spoT* mutants as the severity of the mutation increased (guanosine 5′-diphosphate 3′-diphosphate [ppGpp] basal levels increased). Because expression of these promoters appears to be inhibited by ppGpp, it is proposed that one mechanism for coupling DNA replication to the growth rate of bacteria is through ppGpp synthesis at the ribosome.

The overall rate of DNA replication in *Escherichia coli* is determined by the initiation frequency at the origin of replication, *oriC* (12), which in turn appears to be determined by the intracellular concentration of DnaA protein (26). By controlling the level of DnaA protein synthesis concomitant with monitoring of the time of initiation in the cell cycle by flow cytometry, Løbner-Olesen et al. (26) have shown that when the concentration of DnaA protein is artificially increased, initiation events occur earlier in the cell cycle. DnaA protein is the product of the *dnaA* gene, the first gene in the *dnaA* operon. The genes in the *dnaA* operon, *dnaA*, *dnaN*, *recF*, and *gyrB*, encode proteins that participate in synthesis, repair, and structural maintenance of the chromosome. The DnaA protein is required for initiation of replication from *oriC*, as shown by genetic analyses and by the dependence on DnaA protein of *oriC* cell-free replication systems (for reviews, see references 49a and 56). The *dnaN* gene encodes the β subunit of the DNA polymerase III holoenzyme, a subunit that affects processivity and possibly recycling (30). The third gene in the operon, *recF*, codes for a protein involved in recombinational repair (27), and the last gene, *gyrB*, encodes the β subunit of DNA gyrase, an enzyme required for maintenance of chromosomal superhelical density (2).

Promoter identification studies and deletion analysis of the *dnaA* operon suggest that the genes in this operon are expressed in a coordinate and independent manner. Of the eight promoters in this operon (see Fig. 1), only *dnaAp1* and *dnaAp2* provide transcripts that encode DnaA protein, the initiator protein of *E. coli* chromosomal replication. Because all other promoters are within genes and because the very short intergenic sequences do not appear to contain transcription terminators, it is likely that some of the transcripts that originate from *dnaAp1* and *dnaAp2* encode all four proteins. Regulation of these two proximal promoters, therefore, would coordinately affect expression of all four genes.

We have previously demonstrated that the amount of DnaA protein per total protein increases with increasing growth rate (11), and in this study, we found that transcripts from the *dnaAp1* and *dnaAp2* promoters per total RNA also increased with the growth rate. Because the growth rate dependence of DnaA protein synthesis resembles that of rRNA synthesis and because rRNA genes are under both growth rate and stringent controls (14, 15, 43, 44), we examined whether DnaA protein expression might also be under stringent control. The stringent response is induced in bacteria by amino acid starvation and depends on the presence of the *relA* gene product, (p)ppGpp synthetase I (10). This enzyme is an ATP:GTP pyrophosphoryltransferase that synthesizes the phosphorylated nucleotides guanosine 5′-triphosphate 3′-diphosphate (pppGpp) and guanosine 5′-diphosphate 3′-diphosphate (ppGpp). The purified enzyme is active only when bound to ribosomes which contain bound mRNA and codon-specified uncharged tRNA bound at the acceptor (A) site (18, 33). The model that has emerged is that charged/uncharged tRNA ratios, which change in response to shifts in nutrient availability and amino acid starvation, are sensed by cells because uncharged tRNA stimulates the activity of (p)ppGpp synthetase I. This results in changes in pppGpp and ppGpp concentrations that affect expression of many promoters (10), including the promoter for *mioC* (38, 39), which enhances the activity of *oriC* on plasmids (25). The intracellular concentration of ppGpp is inversely correlated with the activity of the rRNA operon promoters and with the growth rate (6, 45), as well as being directly correlated with the expression of the *his* and *lac* promoters (49). To explain these correlations, an RNA polymerase partitioning model has been proposed that suggests that the enzyme exists either bound to ppGpp or unbound and that the two forms of RNA polymerase differ in their promoter affinities (6, 51, 52).

Methods that can change the concentration of ppGpp up to 500-fold and that were used in this study to determine whether promoters *dnaAp1* and *dnaAp2* of the *dnaA* operon are stringently regulated include (i) amino acid starvation, which greatly stimulates ppGpp synthesis in a *relA*$^+$-carrying strain and reduces ppGpp synthesis in a *relA* mutant, and (ii) addition of chloramphenicol, which leads to a reduction in the rate of synthesis of ppGpp (10). The intracellular amounts of RNAs originating from these two promoters were affected by these conditions such that we conclude that both promoters are stringently controlled but *dnaAp2* is more strongly affected by the stringent response than is *dnaAp1*. Our results with *spoT* mutants deficient in ppGpp pyrophosphorylase activity suggest that an increased intra-

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cellular concentration of ppGpp leads to a net decrease in DnaA protein concentration.

MATERIALS AND METHODS

Bacterial strains and plasmids. The *E. coli* strains used in this study included EMG2 F^+ λ^+, CF1014 F^- *thi-1 argE3 proA2 thr-1 leuB6 mtl-1 xyl-5 ara-14 galK2 lacY1 rspL31 supE44 cdd gyrA*, CF1018 (CF1014 *relA1 spoT*$^+$), CF947 (CF1014 *relA*$^+$ *spoT201*), CF948 (CF1014 *relA1 spoT202*), and CF949 (CF1014 *relA1 spoT203*). The strains were kindly provided by B. Bachmann and M. Cashel. *spoT* alleles *spoT202* and *spoT203* cannot be recovered in *relA*$^+$ cells, presumably because the higher ppGpp level would prevent growth (45).

Plasmid pAC17 (see Fig. 1) was constructed for preparation of the *dnaA* complementary-strand RNA probe. The 817-base-pair *Eco*RI-*Alu*I fragment from plasmid pRB100 (9) that contains the two promoters of the *dnaA* gene was cloned into the *Eco*RI and *Sma*I sites of plasmid vector pBluescript KS(+) (Stratagene).

Media and growth conditions. Unless otherwise indicated, cultures were grown at 37°C in Luria broth containing 0.2% glucose. The antibiotic ampicillin or chloramphenicol was added at 50 μg/ml for plasmid selection when necessary. The growth conditions used to study growth rate regulation were previously described (11). The cells from which RNA was isolated and used in the experiment described in Fig. 2 are the same as those used by Chiaramello and Zyskind (11) to examine the amounts of DnaA protein present in cells at different growth rates. For the experiments described in Fig. 3 and 4, the cultures were grown at 37°C in M9 minimal medium containing 0.4% glucose and supplemented with only required amino acids (40 μg/ml). An overnight culture was diluted to an optical density at 450 nm of 0.025 in the same medium. When cell densities reached an optical density at 450 nm of 0.3, a sample of the culture was removed (zero time) before any inhibitor was added. Amino acid starvation was accomplished by addition of valine at 500 μg/ml (see Fig. 3) to starve the cells for isoleucine (23) or by addition of serine hydroxamate to a concentration of 8.4 mM (see Fig. 4) to provoke serinyl-tRNA limitation (44). Chloramphenicol was added at 150 μg/ml (see Fig. 3) to block protein synthesis. For each treatment, culture samples were taken 15 and 45 min thereafter. The cells were then chilled and centrifuged, and total RNA was extracted. The *spoT* mutants (see Fig. 5) were grown at 37°C in M9 minimal medium containing 0.4% glucose and supplemented with only required amino acids (40 μg/ml), and the cultures were allowed to reach an optical density at 450 nm of 0.4 before extraction of total RNA.

Isolation and purification of total cellular RNA and plasmid DNA. Preparation of total cellular RNA and plasmid DNA was performed essentially as described previously (11).

Enzymes and chemicals. Restriction enzymes were purchased from Stratagene or Boehringer Mannheim Biochemicals, and T4 DNA ligase was from Bethesda Research Laboratories, Inc. Reaction conditions suggested by the manufacturer were used. We obtained an RNA transcription kit from Stratagene, RNase T1 and RNase A from Boehringer Mannheim, and placental RNase inhibitor from Promega Biotec. Rifampin, chloramphenicol, and serine hydroxamate were purchased from Sigma Chemical Co.

RNase protection analysis. After linearization of pAC17 with *Nco*I, the *dnaA* antisense RNA probe (654 nucleotides) was transcribed by using T3 RNA polymerase and [α-^{32}P]UTP (400 Ci/mmol; Amersham Corp.) as previously described (31). The riboprobe was treated with RNase-free DNase (Stratagene) for 10 min at 37°C and then precipitated with ethanol after the addition of 100 μg of carrier RNA per ml (Bethesda Research Laboratories) with ethanol. The probe was dissolved in 100 μl of hybridization buffer (80% formamide, 40 mM PIPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid] [pH 6.4], 0.4 M NaCl, 1 mM EDTA) and stored at −20°C. In each experiment, 30 or 50 μg of total cellular RNA was dissolved in 30 μl of hybridization buffer. At least a 50-fold excess of the probe was added to each hybridization reaction and heated to 85°C for 5 min. The annealing reaction was performed at 50°C for 18 h. The RNA mixture was diluted with 350 μl of RNase digestion buffer (10 mM Tris hydrochloride [pH 7.5], 5 mM EDTA, 300 mM NaCl) containing 40 μg of RNase A per ml (20 U/ml) and 2 μg of RNase T1 per ml (700 U/ml) and incubated at 37°C for 45 min (31). Proteinase K (20 U/ml) treatment of the protected fragments was performed at 37°C for 15 min in 10% sodium dodecyl sulfate. After phenol-chloroform-isoamyl alcohol (25:24:1) extraction, the RNA duplex was concentrated by ethanol precipitation after addition of carrier RNA (100 μg/ml). The RNA samples were suspended in 10 μl of loading buffer (95% formamide, 0.5× TBE [28], 0.1% xylene cyanol) and heated for 3 min at 85°C. Half of the sample was fractionated on a 6% polyacrylamide–8 M urea–1× TBE gel. The sizes of the RNase-resistant fragments were determined by using pBR322 digested with *Msp*I for size markers. The pBR322 *Msp*I fragments were labeled by the fill-in reaction of the DNA polymerase I large fragment in [α-^{32}P]dCTP and [α-^{32}P]dGTP (29). The gel was exposed to AGFA-Curix RP2 film with Du Pont Cronex Lightning-Plus screens at −70°C for different periods of time. Autoradiograms from low exposure times were scanned with an LKB Ultrascan XL laser densitometer, and the area of peaks was integrated with the LKB Gelscan XL software package. The density signal of each band was calculated from the area of the peak and the width of the band.

RESULTS

The *dnaA* promoters are growth rate regulated. DnaA protein is growth rate regulated in that the ratio of DnaA protein to total protein displayed a fivefold increase as the growth rate increased from 0.55 to 2.00 doublings per h (11). We asked whether the growth rate dependence of DnaA protein expression seen at the protein level reflects control at the transcriptional level, as has been found for rRNA operon promoters (13, 14, 43, 44). The strategy used was based on direct measurement of the two cellular transcripts arising from the *dnaA* promoter region by RNase protection of a riboprobe (31). To determine whether the two *dnaA* promoter activities changed in a manner characteristic of growth rate control of stable RNA, total cellular RNA was extracted from cells grown under different growth conditions to achieve a range of growth rates from 0.55 to 2.00 doublings per h; in fact, the cultures used in the immunoblot analysis of DnaA protein performed by Chiaramello and Zyskind (11) were also used here. A specific amount of RNA (30 μg) was hybridized to the *dnaA* antisense RNA probe (Fig. 1) under conditions of probe excess, followed by digestion with single-strand-specific RNases T1 and A. The remaining RNA was then fractionated on a denaturing polyacrylamide gel (Fig. 2). The *dnaA* transcripts produced two bands, as expected (9), one approximately 215 nucleotides long (*dnaAp2*) and another approximately 300 nucleotides

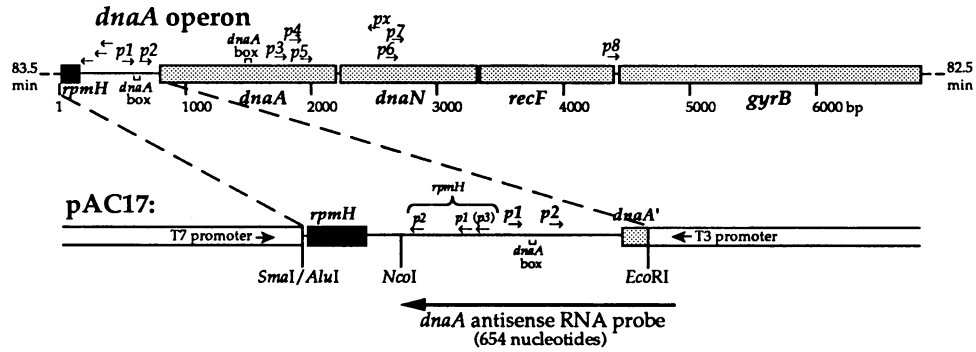


FIG. 1. Section of *E. coli* genetic map containing the *dnaA* operon and a map of pAC17. Nucleotide sequence analysis, gene assignments, and activities and locations of promoters have been described (1–4, 7, 16, 17, 32, 35–37, 41, 42). Base number 1 is at an *Alu*I site and is base 132, as described by Hansen et al. (17). Two 9-mer DnaA boxes are indicated. One lies between *dnaAp1* and *dnaAp2*, where binding of DnaA protein appears to repress the activities of these two promoters (5, 9, 20, 54). The other is within the *dnaA* gene and acts in an orientation-dependent manner to repress transcription (46). The construction of pAC17 is described in Materials and Methods. bp, Base pairs.

long (*dnaAp1*). The two *dnaA* promoters displayed similar, significant reductions in transcriptional activity as the growth rate decreased. The data obtained by densitometric scanning of the autoradiogram (Fig. 2, bottom) showed 70 and 74% decreases in the abundance of transcripts from the *dnaAp1* and *dnaAp2* promoters, respectively. The upstream promoter (*dnaAp1*) was less transcriptionally active than the downstream promoter (*dnaAp2*) at each of the growth rates studied. There was only a slight variation in the [*dnaAp2*]/[*dnaAp1*] ratio between 3.2 and 4.0 as the growth rate was modified, indicating that the promoters were affected equally by growth rate changes. The anomalous increase in transcript abundance seen in glycerol-grown cells (109-min generation time; Fig. 2) was also found in the immunoblot analysis of DnaA protein (11). In fact, the overall response of the *dnaA* promoters to the growth rate was similar to the response of the DnaA protein concentration observed with immunoblot analysis performed on cell extracts (11). Thus, these results indicate that growth rate regulation occurs mainly at the transcriptional level and affects both promoters.

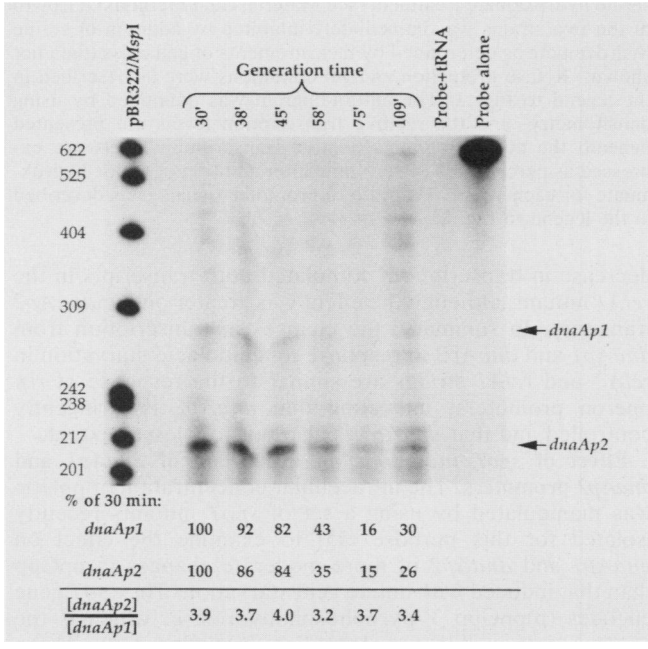


FIG. 2. Growth rate-dependent regulation of *dnaAp1* and *dnaAp2* promoters. The growth rate of strain EMG2 was changed by varying the source and concentration of carbon and energy as described by Chiaramello and Zyskind (11). Total cellular RNA (30 μg) isolated from these cells grown at various growth rates was hybridized with the *dnaA* antisense RNA probe. The samples were treated with RNases T1 and A (see Materials and Methods), and the remaining RNA was analyzed in a 6% polyacrylamide–8 M urea gel. The gel was run until the marker dye reached 20 cm, and the gel was exposed to film for 7 days at −70°C. The two *dnaA* transcripts are indicated by an arrow on the right. The sizes (in nucleotides) of the pBR322 *Msp*I fragments are shown on the left. Beneath the autoradiogram is a summary of the densitometer analysis of the signal from the *dnaAp1* and *dnaAp2* promoters. The numbers are percentages of the signal obtained for a 30-min generation time. The ratio of promoter signals is the ratio of the density signals of the two bands, which is a reflection of the relative concentration of each transcript.

Stringent control of the *dnaA* operon. Transcription of *rrn* operons is both stringently controlled and growth rate regulated, so we examined whether the *dnaAp1* and *dnaAp2* promoters were also stringently controlled. The stringent response, which occurs with amino acid starvation of a *relA*$^+$ strain, is the immediate decrease in rRNA and tRNA synthesis. We induced the stringent response by adding valine to *relA*$^+$ cells to starve them for isoleucine (23). The amounts of *dnaAp1* and *dnaAp2* mRNA in cells treated with valine decreased to 38 and 7%, respectively, after 15 min, with little further change after 45 min (Fig. 3). In contrast, in this same strain, chloramphenicol stimulated the amount of the *dnaAp2* transcript and had no effect on the amount of the *dnaAp1* transcript (Fig. 3). These two treatments also have opposite effects on the rate of stable RNA synthesis (10). Amino acid starvation results in an immediate decrease in stable RNA synthesis and accumulation of ppGpp, whereas addition of chloramphenicol provokes an increase in the rate of rRNA and tRNA with a concomitant decrease in ppGpp intracellular concentration. The *dnaAp2* transcript appears to be stringently controlled because the response of this transcript is similar to that of stable RNA. The *dnaAp1* transcript, however, was less affected by these treatments. The [*dnaAp2*]/[*dnaAp1*] ratio is indicative of differential promoter responses, and in the case of valine treatment, this ratio decreases such that the *dnaAp1* promoter appears as transcriptionally active as the *dnaAp2* promoter. This ratio increases with chloramphenicol treatment, because the

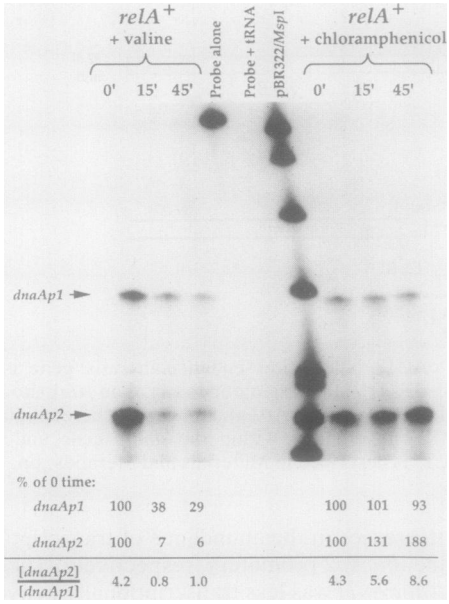


FIG. 3. *dnaA* transcript abundance after isoleucine starvation and chloramphenicol treatment. Total cellular RNA was extracted from *relA*$^+$ strain CF1014 after amino acid starvation (addition of valine at 500 μg/ml) and chloramphenicol treatment as described in Materials and Methods. Growth was immediately inhibited by addition of valine or chloramphenicol as determined by cell mass measurements (data not shown). This RNA (50 μg) was hybridized to a 50-fold excess of the riboprobe covering the *dnaA* promoter region and then treated with RNases A and T1 (see Materials and Methods). Half of the reaction was loaded on a 6% polyacrylamide–8 M urea gel. The gel was run until the marker dye reached 20 cm and was exposed to film for 5 days at −70°C. The RNase-resistant fragments corresponding to the two *dnaA* transcripts are indicated by arrows to the left. Below the gel, results of the densitometer analysis are expressed as percentages of zero time for each type of treatment. The ratio of promoter signals is as described in the legend to Fig. 2.

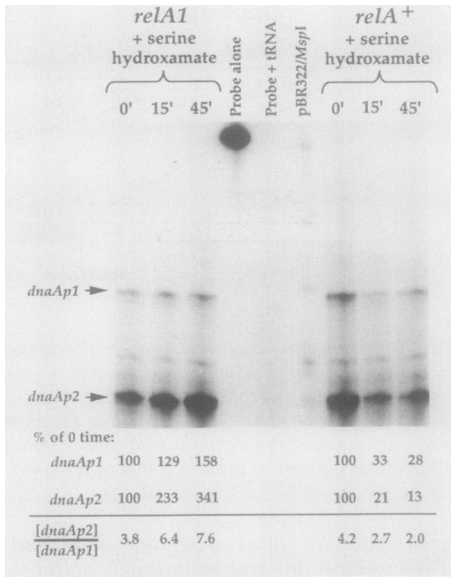
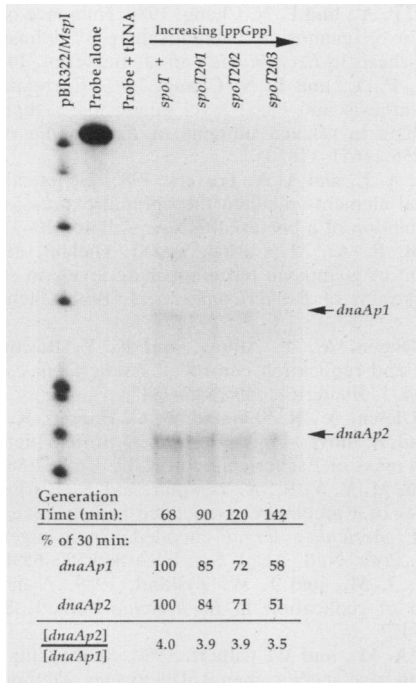


FIG. 4. Stringent control of *dnaA* expression: *relA* dependence of amino acid starvation on *dnaA* transcript abundance. RNase protection analysis was performed on total cellular RNA isolated from *relA1* mutant strain CF1018 and *relA*$^+$ strain CF1014 after serine hydroxamate treatment (see Materials and Methods). Growth of the two strains was immediately inhibited by addition of serine hydroxamate as determined by measurements of cell mass (data not shown). RNase protection and gel conditions were as described in the legend to Fig. 3. The autoradiogram was quantified by using densitometry, and the relative transcription levels are presented beneath the autoradiogram. Relative transcription levels are expressed as percentages of zero time after addition of serine hydroxamate for each strain. The ratio of promoter signals is as described in the legend to Fig. 2.

dnaAp2 transcript is more affected than the *dnaAp1* transcript.

The amino acid analog L-serine hydroxamate was used to examine the *relA* dependence of the response to amino acid starvation shown in Fig. 3. This analog rapidly inhibits growth of *E. coli* by inhibiting the activity of seryl-tRNA synthetase (50). Without charged seryl-tRNA, a stringent response is produced in *relA*$^+$ strains, but the opposite effect occurs in *relA* mutants (44). After addition of L-serine hydroxamate, a 36-fold increase in a *relA*$^+$ strain and a 10-fold decrease in a *relA1* mutant strain in the ppGpp basal levels have been observed (49). When the *dnaA* promoters were examined for response to serine hydroxamate in *relA*$^+$- and *relA*-carrying cells (Fig. 4), the abundance of the *dnaAp2* transcript followed the pattern observed for the *rrnBp1* promoter (44) and that expected for stringent control. The effect was less pronounced for the *dnaAp1* transcript, as seen in the effect of serine hydroxamate on the ratio of the two transcript concentrations shown in the bottom of Fig. 4. Serine starvation of the *relA*$^+$ strain caused a decrease in the abundance of both transcripts (Fig. 4), in agreement with that observed after isoleucine starvation (Fig. 3). This decrease in *dnaA* transcript prevalence caused by amino acid starvation is dependent on the *relA* gene product, (p)ppGpp synthetase I. Serine hydroxamate, rather than causing a decrease in transcription, stimulated both transcripts in the *relA1* mutant, although the effect was greater on the *dnaAp2* transcript. In summary, the changes in transcription from *dnaAp1* and *dnaAp2* in response to amino acid starvation in *relA*$^+$ and *relA1* strains are similar to the response of *rrn* operon promoters, indicating that *dnaAp2* is stringently controlled and that *dnaAp1* is also but to a lesser extent.

Effect of *spoT* mutations on expression of *dnaAp1* and *dnaAp2* promoters. The intracellular concentration of ppGpp was manipulated by using a set of *spoT* mutants recently isolated for this purpose (45) to examine the effect on *dnaAp1* and *dnaAp2* of more moderate changes in ppGpp than that induced with amino acid starvation. The *spoT* gene encodes (p)ppGpp 3′-pyrophosphohydrolase, which is the enzyme responsible for degrading (p)ppGpp (19). These mutants, which were selected for the ability of *spoT* mutants to suppress the 3-aminotriazole sensitivity of *relA* strains, each contain increasing amounts of (p)ppGpp. A strict correlation of ppGpp with the growth rate, as well as with the expression of a stringently controlled promoter, was observed in these mutants (45). As the severity of the mutation increased, resulting in increased ppGpp levels, the growth rate decreased (generation time increased) and the activity of the stringently controlled promoter *rrnAp1* decreased. We also observed differences in the growth rates of these mutants (Fig. 5), which are isogenic except for the *spoT* and *relA* alleles, with a range of generation times from 68 min for the *spoT*$^+$ strain to 142 min for the most severely affected *spoT* mutant, *spoT203*. This mutant exhibits an eightfold increase in ppGpp concentration relative to the



Generation Time (min):	68	90	120	142
% of 30 min:				
dnaAp1	100	85	72	58
dnaAp2	100	84	71	51
[*dnaAp2*]/[*dnaAp1*]	4.0	3.9	3.9	3.5

FIG. 5. Effects of different *spoT* mutants on expression of *dnaAp1* and *dnaAp2* promoters. RNA analysis by RNase protection assay was performed on total cellular RNA extracted from strains CF1014, CF947, CF948, and CF949. These RNAs (30 μg) were hybridized to the antisense *dnaA* probe (Fig. 1) and treated with RNases A and T1 as described in Materials and Methods. Half of the samples were loaded on a 6% polyacrylamide–8 M urea gel. The gel was run until the marker dye reached 20 cm and was exposed to film for 3 days at −70°C. The positions of the two *dnaA* transcripts are indicated by arrows. The arrow at the top of the autoradiogram indicates the increase in the intracellular concentration of ppGpp associated with increased severity of the *spoT* mutations, as measured by Sarubbi et al. (45). The gel was quantified by using densitometry, and the relative transcription levels are expressed as percentages of the signal obtained for the wild-type parental strain. The ratio of promoter signals is as described in the legend to Fig. 2.

wild-type strain (45). Figure 5 illustrates the variation in the abundance of *dnaA* transcripts in these mutants. As the severity of the *spoT* mutation increased, the amount of transcription coming from *dnaAp1* and *dnaAp2* was reduced. A similar decrease in DnaA protein was also observed by immunoblot analysis (data not shown). This pattern of expression is similar to what we found when the growth rate was manipulated by the medium composition (Fig. 2) and shows that there is an inverse correlation between the intracellular level of ppGpp and the concentration of *dnaA* transcripts.

DISCUSSION

The first step in initiation identified is the formation of a complex of 20 to 40 molecules of DnaA-ATP with *oriC*, the origin of replication (8). This step appears to be rate limiting; consequently, the concentration of DnaA protein is thought to determine when in the cell cycle initiation occurs (26, 34). We have recently found that expression of DnaA protein is growth rate regulated in that its cellular concentration is directly proportional to the growth rate (11). Based on these data, a model has been proposed to explain the discrepancy between the function of DnaA protein as the principal controller of initiation of replication and the fact that its concentration is growth rate regulated (28). This model is based on observations by Sekimizu et al. that there are different forms of DnaA protein: the active form, DnaA-ATP, and inactive forms, DnaA-ADP, DnaA bound to acidic phospholipids, and DnaA unbound to a nucleotide or phospholipid (47, 48). We show here that growth rate regulation of DnaA protein is at the level of transcription from the *dnaAp1* and *dnaAp2* promoters. Because all four genes in this operon are downstream of these promoters, the intracellular concentrations of the β subunit of the DNA polymerase III holoenzyme (*dnaN* product), RecF protein (*recF* product), and the β subunit of gyrase (*gyrB* product) are predicted to change in a growth rate-regulated manner as well.

The observed *relA*-dependent changes in the abundance of *dnaAp1* and *dnaAp2* transcripts with amino acid starvation strongly support the interpretation that these two promoters are stringently controlled (Fig. 3 and 4). Also, as the severity (higher ppGpp basal levels) of the *spoT* mutation increased, the levels of *dnaAp1* and *dnaAp2* transcripts decreased, as expected for stringent control (Fig. 5). Travers (53) predicted that the *dnaAp2* promoter was stringently controlled after comparing the sequence of *dnaAp2* to the sequences of stringently regulated promoters. The combined activity of the *dnaAp1* and *dnaAp2* promoters is weak, being 13 and 30% of the activities of the uninduced *tac* and *lacUV5* promoters (35), respectively. This suggests that *dnaAp1* and *dnaAp2* are the weakest promoters subject to stringent control that have been analyzed and is inconsistent with the proposal by Yamagishi et al. (55) that the effect of stringent control is only to inhibit a rate-limiting step for very active transcription.

A strict correlation between ppGpp concentration and rRNA and tRNA synthesis exists for many different conditions, including (i) amino acid starvation and chloramphenicol treatment of *relA*$^+$ and *relA* mutant cells (6, 21, 22), (ii) *relA*$^+$ and *relA* mutant cells grown at different growth rates (6, 24, 40), and (iii) use of *spoT* mutants (45), suggesting that one of the primary mechanisms for controlling the growth rate is via ppGpp. It is intriguing to consider that ppGpp, whose synthesis is coupled to the rate of protein synthesis and whose concentration appears to control the activities of promoters for genes involved in both protein and DNA syntheses, as well as amino acid biosynthesis, is an effector molecule coupling DNA replication to the growth rate. For example, if protein synthesis were to slow down because of decreased nutrient availability, synthesis of ppGpp would increase, leading to inhibition of *dnaA* operon promoters *dnaAp1* and *dnaAp2*. The synthesis rates of DnaA protein and the β subunit of the DNA polymerase III holoenzyme would decrease, leading to loss of the ability to initiate new rounds of replication and possibly to a decreased rate of replication fork movement. The opposite response would occur if protein synthesis were to speed up in response to nutrient enrichment of the environment. In summary, the demonstration that *dnaAp1* and *dnaAp2* are regulated by a growth rate-dependent mechanism and are stringently controlled suggests that one way in which DNA replication is coordinated with the growth rate is via ppGpp synthesis at the ribosome.

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