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Starvation-Induced Expression of Retron-Ec107 and the Role of ppGpp in Multicopy Single-Stranded DNA Production

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Multicopy single-stranded DNA is found as a small single-stranded RNA-DNA complex in certain wild-type strains of *Escherichia coli* as well as in other gram-negative bacteria. Using the promoter region of the previously characterized retron-Ec107 from *E. coli* ECOR70, I constructed a chromosomally located *lacZ* operon fusion. Examination of expression from the P_{Ec107} promoter showed that activity increased sharply when cells entered stationary phase in rich medium or when they were starved for phosphate. The nucleotide guanosine-3′,5′-bispyrophosphate was found to be a positive regulator of retron-Ec107 expression. Its presence is required for starvation-induced transcription of retron-Ec107 and multicopy single-stranded DNA production. It was also found that expression from the retron promoter is independent of the sigma factor σ^S.

Multicopy single-stranded DNA (msDNA) is a small (65 to 163 bases) single DNA strand covalently bound to an RNA strand through a 2′-5′ phosphodiester linkage (Fig. 1A) (for reviews, see references 12, 14, and 19). Synthesis of msDNA has been proposed to occur through the action of bacterial reverse transcriptase (RT). The gene for RT is typically found just downstream of the region in which msDNA is encoded (Fig. 1B). Primer extension analysis and S1 nuclease mapping indicate that this operon, termed retron (43), is expressed as a single transcript (4, 10, 26). This transcript undergoes folding to produce a secondary structure which is accessible to the retron RT. The bacterial RT then synthesizes a cDNA by using the RNA transcript as a template. This early form of msDNA is further modified by RNase H concomitantly during synthesis of the DNA strand. It has recently been shown that the RT is also responsible for the formation of the 2′-5′ phosphodiester bond between an internal G residue of the RNA and the cDNA (38). msDNA has been found to exist as a complex with one or more proteins; these have yet to be identified, although one may be the RT itself (21, 45).

msDNA is found in a variety of prokaryotes, including *Myxococcus xanthus*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Salmonella* sp., *Nannocystis* sp., *Rhizobium* sp., and *Bradyrhizobium* sp. (34). While the msDNA and RT are well conserved in all *M. xanthus* strains examined (18), they vary greatly in *E. coli*. Only 15% of wild-type *E. coli* strains produce msDNA (11), and these also vary in the size and primary sequence of the msDNA and RT. There is one retron, though, retron-Ec107, which is found in 75% of those *E. coli* strains which produce msDNA and is well conserved in nucleotide sequence and location on the genome (10, 16).

I have examined the production of msDNA-Ec107 under a variety of conditions and have found that a higher level of msDNA is present when cells are starved for nutrients. A chromosomally located *lacZ* operon fused to the promoter region of retron-Ec107 shows a sharp increase in transcription as cells enter the stationary phase of growth. Expression of this operon fusion was found to be independent of σ^S, the sigma factor encoded by the *rpoS* gene and found to be responsible for the expression of many starvation-induced genes (23, 24, 28, 42). A global regulator which is found at higher levels under conditions of nutrient deprivation is guanosine 3′,5′-bispyrophosphate (ppGpp) (for a review, see reference 2). This nucleotide is synthesized under conditions of amino acid starvation by the product of the *relA* gene and under conditions of carbon starvation and general energy deprivation by the product of the *spoT* gene (9, 29, 46). SpoT is also responsible for the degradation of ppGpp to GDP (17). Utilizing null deletions of *relA* and *spoT* and inducible expression of a truncated ppGpp-producing *relA* gene on a plasmid, I show that ppGpp is necessary for the positive regulation of retron-Ec107 expression.

MATERIALS AND METHODS

Bacterial strains and vectors. A. Svitil and J. Zyskind provided pALS13 and pALS14, which carry truncated versions of the *relA* gene (41); M. Cashel sent the K-12 strain CF1693, which carries the *relA*::Kn and *spoT*::Cm null mutations (46); the *lacZ* fusion plasmids pRS414 and pRS415 (39) were kindly sent by R. W. Simons; the suicide vector pCVD442 (5) and strains SY327λ*pir* and SM10λ*pir* were provided by M. Donnenberg; G. Huisman and R. Kolter sent the pDEB2 (*rpoS*$^+$) and pDEB21K (*rpoS*::Kn) plasmids as well as the *rpoS*::Kn strain ZK1000 (1). P1 and P7 transduction of the *relA*, *spoT*, and *rpoS* mutations to the wild-type strain was performed as described by Miller (30). The omega plasmids pHP45Ω and pHP45Ω-Tc (6, 33) carry resistance markers which are flanked on both ends by transcription terminators and stop codons in all six reading frames. The wild-type *E. coli* strain ECOR70 (32, 37), which carries the chromosomally located retron-Ec107 (10), was used for the construction of the operon fusions. The derivative HA4 was constructed from ECOR70 by disruption of the *lacZ* gene (see below). HA4109 is a derivative of HA4 which carries the chromosomal P_{Ec107}-*lacZ* operon fusion (details below). In all of the following experiments, HA4109 is referred to as wild type in Results and figures to distinguish it from derivatives of HA4109.

Bacterial growth and media. *E. coli* was grown either in Luria-Bertani (LB) medium (27) with the appropriate concentration of antibiotics or in morpholinepropanesulfonic acid (MOPS) minimal medium (31) in a 37°C shaking water bath. In addition to other nutrients, MOPS medium contains 1.32 mM phosphate, 9.52 mM ammonia, and 20 mM carbon. MOPS medium which was limited for one of these three macronutrients has the normal amount of other nutrients but has 0.043 mM phosphate, 0.55 mM ammonia, or 1.1 mM glucose for low-P, low-N, or low-G MOPS medium, respectively. The MOPS salts solution used to wash cells consists of 0.523 mM $MgCl_2$, 0.276 mM K_2SO_4, 0.01 mM $FeSO_4$, 5×10^{-4} mM $CaCl_2$, 50 mM NaCl, 40 mM MOPS (pH 7.4), and 4 mM Tricine (pH 7.4). Antibiotics were used in the following concentrations: 100 μg/ml for spectinomycin, 25 μg/ml for streptomycin, 100 μg/ml for ampicillin, 12.5 μg/ml for tetracycline, 50 μg/ml for kanamycin, and 30 μg/ml for chloramphenicol.

Genetic techniques. The spectinomycin/streptomycin resistance (Spc/Sm) cassette of pHP45Ω (33) was removed with *Bam*HI and ligated into the *Bam*HI site of pUC9 (44). *Pvu*II and *Sma*I were used to remove the fragment containing the Spc/Sm cassette and the upstream region of the pUC9 multiple cloning site which includes the *lacZ* promoter region and *lacI*. This fragment was inserted into the *Sma*I site of the suicide vector pCVD442 (5). The 2.1-kb tetracycline resistance

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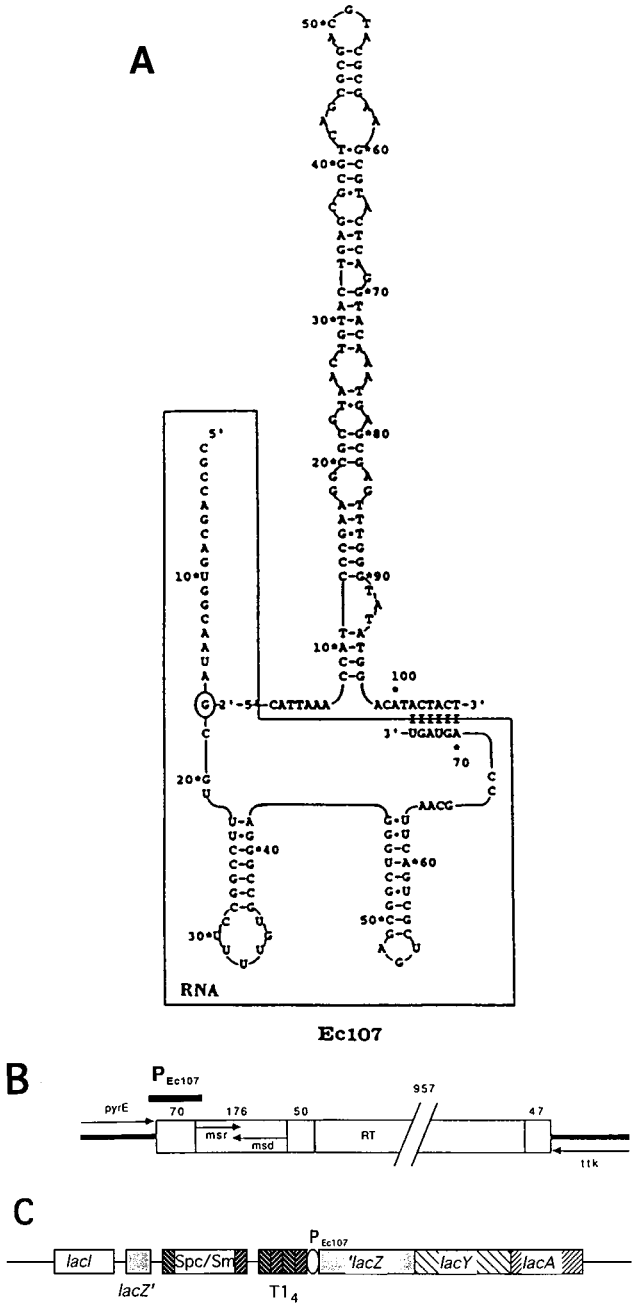


FIG. 1. Structures of msDNA-Ec107, retron-Ec107, and the chromosomal P_{Ec107}-*lacZ* operon fusion. (A) Predicted secondary structure of msDNA-Ec107, including the proposed 3′ RNA-DNA hydrogen binding region. The 2′-5′ branched G residue is circled, and the RNA moiety is boxed (10). (B) Basic structure of the chromosomally located retron-Ec107 of ECOR70. The *msd*, *msr*, and RT regions are indicated (10). The Ec107 promoter contains putative −35 (TGCGA) and −10 (TATCGT) regions and is responsible for expression of the entire retron (10). A heavy overline indicates the promoter region which was used for construction of the *lacZ* operon fusion. (C) Chromosomal *lacZ* operon fusion of HA4109. Transcription terminators are indicated by small dark boxes, and the retron promoter is shown as an oval. The Spc/Sm cassette has three transcription terminators at each end as well as stop codons in all six reading frames. The entire region between *lacZ'* and *'lacZ* was introduced into the chromosome. The figure is not drawn to scale.

cassette from pHP45Ω-Tc (6) was isolated by using *Bam*HI and blunt ended with Klenow fragment. This cassette was then inserted into the *Sma*I site of pRS414 (39). The 4.5-kb *Ssp*I fragment from this construct, which includes the $T1_4$ transcription terminators followed by the tetracycline resistance gene and the downstream region of *lacZ*, was ligated into the *Sma*I site of the suicide vector construct. I isolated the construct which had the upstream region of *lacZ* and the downstream region of *lacZ* in the same orientation such that the *lacZ* gene is disrupted and separated by the Spc/Sm cassette, $T1_4$, and the tetracycline resistance cassette. This construct (pETE4-Tc) was recombined into the *lac* operon of ECOR70 by conjugation of SM10λ*pir* with ECOR70 to create HA4. This recombination was confirmed by Southern blot analysis.

PCR was used to isolate the promoter region of retron-Ec107 and to add an *Eco*RI site to one end of the PCR fragment. This fragment was digested using *Msp*I to cleave an internal site approximately 25 bp downstream of the +1 transcription start site of retron-Ec107. This fragment was blunt ended and digested with *Eco*RI. The *Eco*RI-blunt-end fragment was ligated into the *Eco*RI-*Sma*I site of the operon fusion vector pRS415. This insert includes the retron-Ec107 promoter region from bases 87 to 195. *Ssp*I was used to remove the 2.4-kb fragment containing $T1_4$ and P_{Ec107}-*lacZ*, which was then ligated into pCVD442 to create pETE109 and subsequently recombined into HA4 to produce HA4109 (Fig. 1C). Standard cloning techniques were performed as described by Maniatis et al. (27). *E. coli* CL83 (25) was used for cloning and propagation of plasmids, except in the case of pCVD442 derivatives, in which case SY327λ*pir* was used.

msDNA detection and quantification. Total cellular RNA was isolated from cell samples by the method of Chomczynski and Sacchi (3). msDNA was labeled by treating total RNA with avian myeloblastosis virus RT as described previously (20) except that [α-^{32}P]dATP was used instead of [α-^{32}P]dCTP. In this manner, the DNA moiety of msDNA serves as a primer for RT to extend along the RNA template by utilization of the 3′ DNA-RNA hybrid ends (Fig. 1A) while incorporating [α-^{32}P]dATP into the DNA chain. Samples were run on 4% acrylamide–8 M urea denaturing gels and exposed to X-ray film. Bands were quantified by use of a model 300A Molecular Dynamics Computing Densitometer.

β-Galactosidase assays. At indicated time points, samples of culture were removed for β-galactosidase assays. β-Galactosidase assays were done as described by Miller (30) except that cells were opened by using 15 μl of 0.1% sodium dodecyl sulfate plus 15 μl of chloroform instead of toluene. Data are expressed in β-galactosidase (LacZ) units as a function of optical density at 600 nm (OD_{600}).

RESULTS

Construction of the retron-*lacZ* chromosomal fusion. Plasmid pETE4-Tc was transferred from strain SM10λ*pir* to ECOR70 via conjugation. This plasmid recombined into the native *lac* operon just downstream of the *lacZ* promoter. A series of T1 transcription terminators as well as the Spc/Sm cassette ensured the blocking of any promoter activity from P_{lac}. This prediction was confirmed by the induction of the *lacZ* insertion strain (HA4) with isopropylthiogalactopyranoside (IPTG), which showed the absence of any β-galactosidase activity (data not shown). The Ec107 operon fusion construct was transferred to HA4 and recombined into the chromosome to produce HA4109 (Fig. 1C). I decided to insert the operon fusion into the native *lacZ* because (i) I could delete native *lacZ* gene activity while utilizing the wild-type *lac* operon system and (ii) I wanted to be able to measure transcription expression as well as msDNA levels simultaneously.

Increase in transcription from the retron promoter P_{Ec107} upon entry into stationary phase. Strain HA4109 was inoculated from an overnight culture into LB, 20 mM glucose MOPS plus 0.1% Casamino Acids, 20 mM glucose MOPS, or 20 mM glycerol MOPS medium. In LB medium and the two glucose MOPS media, there is a small increase in activity in late exponential phase (Fig. 2), but only LB and MOPS supplemented with Casamino Acids show a sharp increase in transcription as cells enter the stationary phase of growth. As the 20 mM glucose MOPS culture enters stationary phase, there is only a slight increase compared with growth in richer media. Expression from cells grown in glycerol never decreased to the low basal levels seen in the other media and may have a higher basal level because of its slow growth rate. The growth rates of HA4109 in these media were 2.0 doublings per h in LB, 1.43 doublings per h in 20 mM glucose MOPS plus 0.1% Casamino Acids, 1.18 doublings per h in 20 mM glucose MOPS, and 0.77 doubling per h in 20 mM glycerol MOPS.

Starvation for carbon, nitrogen, or phosphorous. To determine whether the retron fusion was induced upon starvation

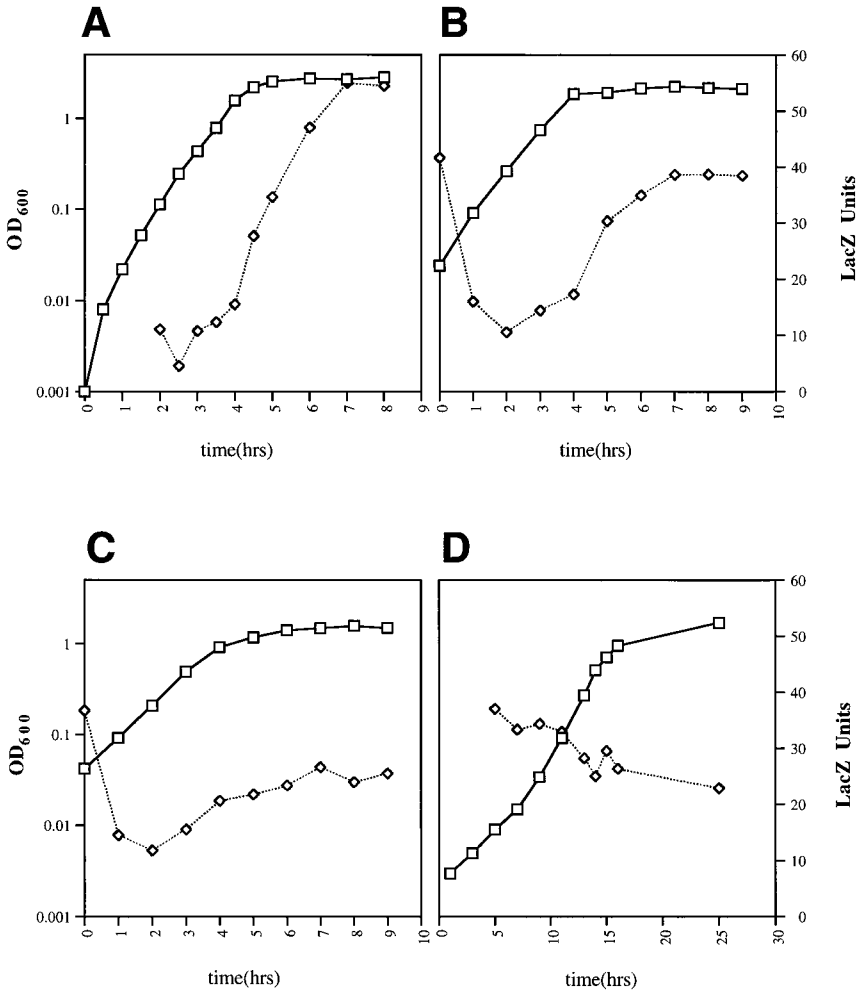


FIG. 2. Expression in β-galactosidase (LacZ) units from the retron-Ec107 promoter in various media. An overnight culture of HA4109 grown in 20 mM glucose MOPS medium was diluted 1:1,000 into fresh LB (A), 20 mM glucose MOPS plus 0.1% Casamino Acids (B), 20 mM glucose MOPS (C), or 20 mM glycerol MOPS (D) medium. Samples were removed at the indicated time points and assayed for β-galactosidase activity. OD_{600} is indicated by squares, and LacZ units are indicated by diamonds.

for different macronutrients, HA4109 was grown in MOPS medium which was limited for either glucose, ammonia, or phosphate. All three cultures had a higher level of induction as they entered the starvation phase of growth (Fig. 3A), but the level of expression varied. Phosphate starvation resulted in the highest level of expression compared with glucose and ammonia limitation. This expression may require a slowing of growth which occurs as cells enter the stationary phase or the presence of a secondary metabolite as opposed to just the absence of a required nutrient. To examine if the absence of one of these nutrients was enough to induce expression, HA4109 was grown in 20 mM glucose MOPS until mid-exponential phase. The cells were then collected, washed, and equally distributed into MOPS medium which was devoid of carbon, nitrogen, or phosphate. As shown in Fig. 3B, the carbon-starved and nitrogen-starved cultures showed a very mild increase in expression, whereas the phosphorus-starved culture showed a very sharp increase in expression upon transfer from nutrient-sufficient medium. This may be due to starvation for phosphate, or it may also be due to the continued slow rate of growth by the phosphorus-starved culture. This induction upon phosphate starvation is independent of the *pho* operon (data not shown).

ppGpp is a positive regulator of retron-Ec107 expression. The guanine nucleotide ppGpp is a global regulatory signal involved in the regulation of many *E. coli* genes (2). The cellular level of ppGpp has been shown to increase under conditions of nutrient deprivation via synthesis involving the *relA* and *spoT* gene products. RelA is involved in synthesis of ppGpp through a ribosome-binding manner during conditions of amino acid starvation. SpoT synthesizes ppGpp independently of ribosome binding during carbon- and energy-limiting conditions (7). To determine whether ppGpp could induce the retron fusion, I used a truncated *relA* gene (*relA′*) which produces a metabolically unstable RelA protein that is *relC* independent but can produce wild-type levels of ppGpp (36, 41). This truncated *relA′* is carried on plasmid pALS13 and is under control of the IPTG-inducible P_{tac} promoter. Plasmid pALS14, which was used as a control, is truncated further and does not produce ppGpp. Derivatives of strain HA4109, carrying either pALS13 or pALS14, were grown in LB medium until mid-exponential phase, when IPTG was added to the cultures to induce *relA′* expression. With the strain carrying plasmid pALS13, there is a decrease in the growth rate of the culture followed by a sharp increase in transcription from the retron

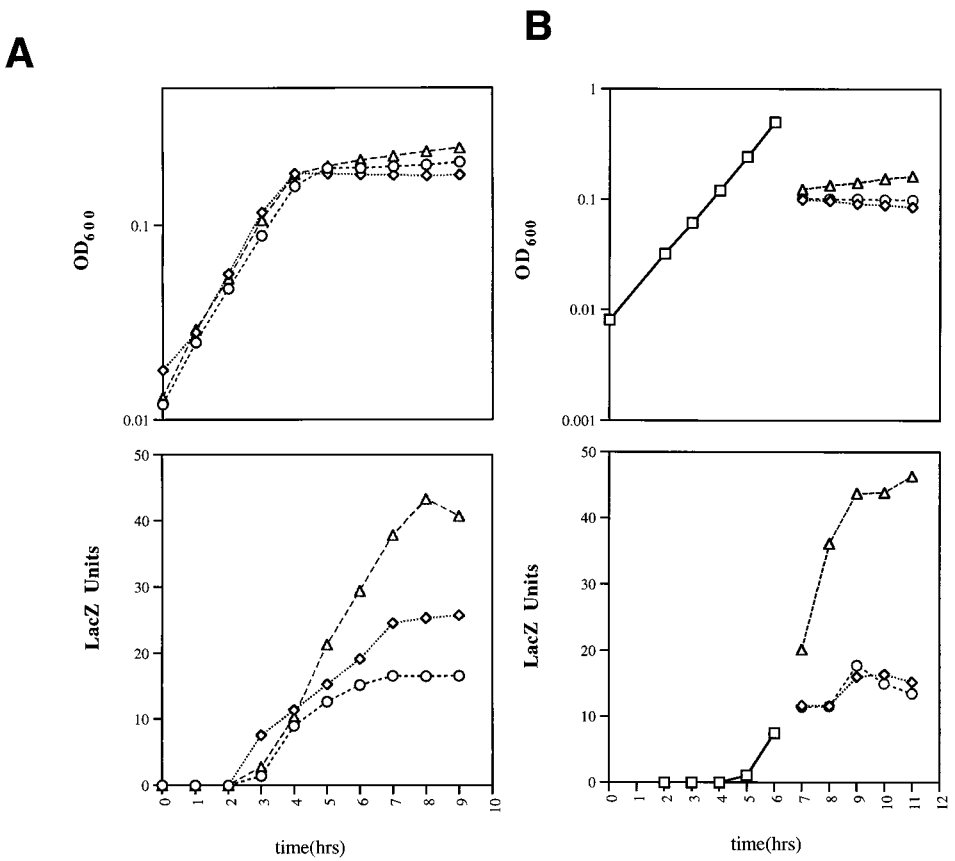
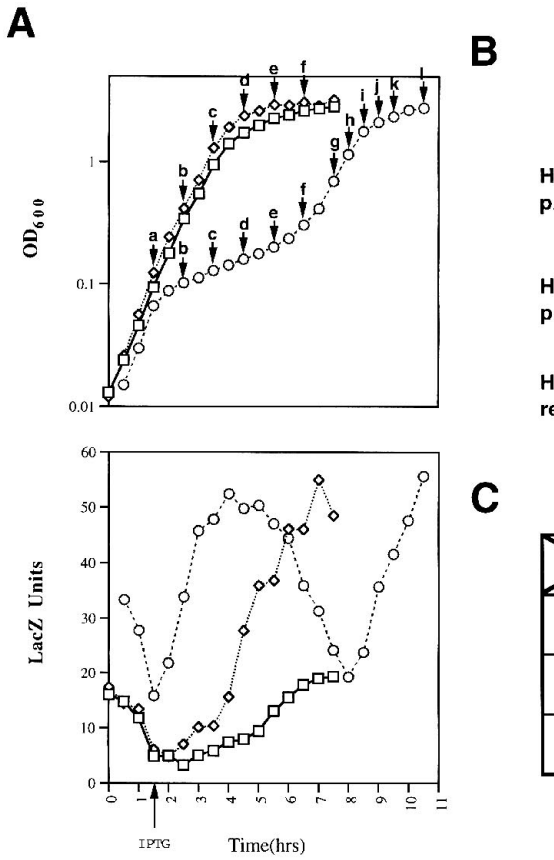


FIG. 3. Expression of P_{Ec107}-*lacZ* during starvation for a single macronutrient. (A) An overnight culture of HA4109 grown in 20 mM glucose MOPS medium was diluted 1:1,000 into MOPS medium which was limited for either phosphate (0.043 mM; △), ammonia (0.55 mM; ◇), or glucose (1.1 mM; ○). The cultures were grown to stationary phase at 37°C, and samples were removed as indicated for β-galactosidase assays. (B) An overnight culture of HA4109 grown in 20 mM glucose MOPS medium was diluted 1:1,000 into fresh 20 mM glucose MOPS medium (□) and was grown until mid-exponential phase. At an OD_{600} of 0.5, the cells were pelleted and washed twice with ice-cold MOPS salts. These cells were resuspended in MOPS salts, and equivalent aliquots were diluted into MOPS medium which contained either no glucose (○), no phosphate (△), or no nitrogen (◇). Samples were taken before and after reinoculation and assayed for β-galactosidase.

promoter (Fig. 4A). After induction, LacZ activity reaches a level which corresponds to that seen at starvation of wild-type bacteria in rich medium. It then decreases as cells resume a high rate of growth, but LacZ expression increases again when the cells enter the stationary phase. The latter parts of the growth and LacZ unit curves with the strain harboring pALS13 resemble the corresponding curves of the control strain carrying pALS14 (Fig. 4A). These results indicate that ppGpp has a positive regulatory effect on expression from the P_{Ec107} retron promoter.

To determine whether ppGpp is required for the starvation-induced expression of the retron, I constructed null mutations at the *relA* and *spoT* alleles of HA4109. Growth of the *relA* strain in LB medium showed an increase in *lacZ* expression and msDNA levels upon entry into stationary phase similar to that of the wild-type strain (data not shown), but there was only a slight increase in those of the *relA spoT* double mutant (Fig. 4A and B). These data indicate that expression upon starvation in this medium is dependent on *spoT* but is *relA* independent. The *spoT* gene cannot be deleted singularly, since this deletion is lethal in $relA^+$ cells (46). msDNA levels were compared by taking culture samples at the indicated time points and extracting total cellular RNA. RNA concentration was measured, and 12 to 14 μg was labeled with [α-^{32}P]dATP. Since msDNA is self-priming (Fig. 1A), the DNA moiety is extended with incorporation of ^{32}P. The samples were run on denaturing acrylamide gels and exposed to film, and the msDNA bands were quantified by densitometry. All of the values obtained were divided by the lowest value to give the relative intensities (Fig. 4C). There is a 3-fold increase in the msDNA level as the *relA spoT* strain enters stationary phase, compared to a 20-fold increase of msDNA seen in the wild type (Fig. 4B and C). Interestingly, an increase in expression from the retron promoter does not lead to an increase in the level of msDNA upon induction of *relA′* in pALS13 (Fig. 4A and B), although the level of msDNA is significantly higher once cells enter stationary phase (Fig. 4B). This higher level of msDNA seen in HA4109/pALS13 compared with HA4109/pALS14 may be due to a stable retron transcript which accumulates in HA4109/pALS13 and is finally utilized to make msDNA once the cells enter stationary phase. These data suggest that ppGpp is required for optimum expression of the retron but other factors particular to the stationary phase of growth are required for msDNA production.

Expression from the retron-Ec107 promoter is independent of the sigma factor σ^S. The sigma factor σ^S, which is encoded by the *rpoS* gene, is responsible for the expression of more than 30 stationary-phase proteins (28). It was found that deletion of *rpoS* causes a decrease in the viability of cells after prolonged periods of starvation as well as greater susceptibility to environmental damage from oxidation, hyperosmosis, and heat stress (28). The *rpoS* gene is positively regulated by ppGpp (8,



	a	b	c	d	e	f	g	h	i	j	k	l
HA4109 pALS13	1.0	1.6	1.1	1.0	1.4	5.7	6.0	2.3	31	64	73	152
HA4109 pALS14	1.1	1.0	1.3	23	27	17						
HA4109 relA spoT	1.3	1.4	1.4	1.6	4.3	3.8						

FIG. 4. Induction of ppGpp synthesis in derivatives of HA4109. HA4109:*relA spoT* (□), HA4109/pALS13 (○), and HA4109/pALS14 (◇) were grown overnight and diluted 1:1,000 into fresh LB medium. At t = 1.5 h, IPTG was added to the HA4109/pALS13 and HA4109/pALS14 cultures to a final concentration of 200 μM. At indicated times, culture samples were removed for β-galactosidase assays (A) and RNA preparations for msDNA labeling (B). Amounts of total RNA per sample were 12.3 μg for HA4109:*relA spoT*, 14.5 μg for HA4109/pALS13, and 14.25 μg for HA4109/pALS14. (C) Relative intensities calculated as described in the text.

22, 24), and so it is possible that the retron promoter is recognized by this sigma factor. The *rpoS* gene was disrupted in HA4109, and β-galactosidase activity was measured during growth in LB medium. There was no decrease in the amount of LacZ units of the *rpoS* mutant compared with that of wild-type (Fig. 5A). Indeed, the *rpoS* strain actually showed a slightly higher level of activity in stationary phase even though the growth pattern of the *rpoS* mutant was identical to that of the wild type. The levels of msDNA in the *rpoS* mutant were also similar to those of the wild-type strain (data not shown). Expression of σ^S on a multicopy plasmid was studied by using pDEB2, which carries the native *rpoS* gene (1). I compared the levels of *lacZ* fusion expression by using the HA4109:*rpoS*::Kn mutant which carried either pDEB2 (*rpoS*$^+$) or the control plasmid pDEB21K (*rpoS*::Kn). Expression from the control HA4109:*rpoS*/pDEB21K was similar to that from the wild type, but that of HA4109:*rpoS*/pDEB2 showed only a very slight increase above basal levels after entry into stationary phase (Fig. 5B). The negative effect on retron expression through overexpression of *rpoS* may be due to expression of an inhibitory element of the retron by σ^S. It may also be due to greater competition for RNA polymerase holoenzyme between σ^S and σ^{70}. I have also transformed the *rpoS* mutant with pALS13 and induced synthesis of ppGpp, which has been shown to be a positive regulator of σ^S expression (8, 22, 24). Both the growth patterns and *lacZ* expression of HA4109:*rpoS*/pALS13 were almost identical to those of HA4109/pALS13 (data not shown); this finding provides further evidence that expression from the retron promoter is independent of σ^S.

DISCUSSION

msDNA and the retron responsible for its synthesis are found in many different gram-negative bacteria. Although its structure and presence are well conserved in *M. xanthus*, msDNA is quite diverse in *E. coli* and is found in only 15% of wild-type strains. Also, its deletion in *M. xanthus* led to no deleterious effects on the normal developmental cycle of this soil bacterium under laboratory conditions (13). The presence of msDNA in only a small percentage of *E. coli* and other bacteria found to produce msDNA also indicates that these strains have no obvious requirement for a retron. Indeed, considering that msDNA is quite complex in its unique structure and its association with protein(s), there is no known function or advantage to making msDNA under standard laboratory conditions. Since bacteria tend to express those genes which are needed during those periods of growth or environmental conditions in which they would be most useful, I thought that it would be interesting to determine whether the retron is expressed in a regulatory manner and under which conditions that expression is warranted. I decided to examine the expression of retron-Ec107 because it is the most abundant retron in *E. coli*, being found in 75% of those *E. coli* strains which produce msDNA. Also, the codon usage of the Ec107 RT is

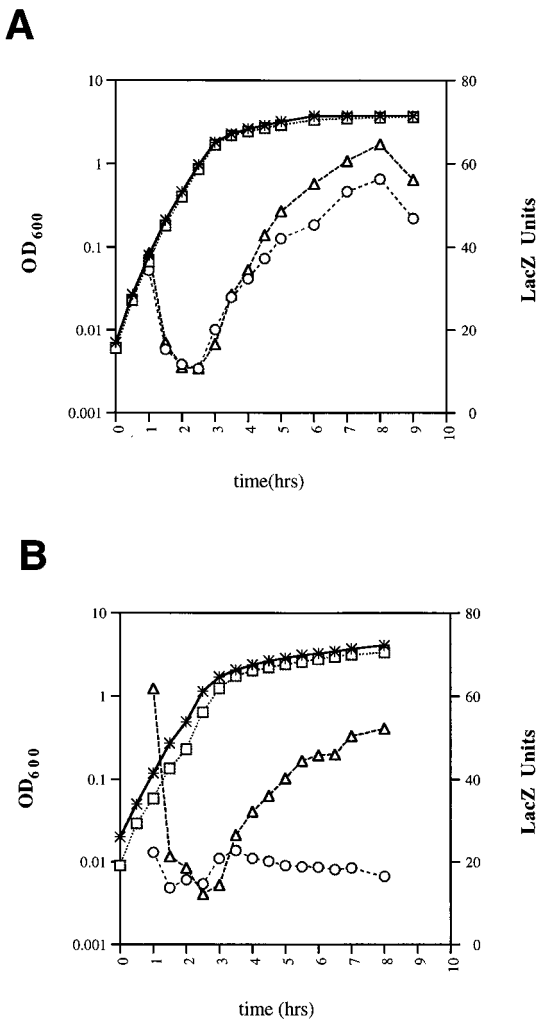


FIG. 5. Effect of *rpoS* deletion and overexpression on P_{Ec107}. Overnight cultures of HA4109 (OD_{600}, *; LacZ, ○) and HA4109:*rpoS* (OD_{600}, □; LacZ, △) (A) and of HA4109:*rpoS* harboring either pDEB2 (OD_{600}, *; LacZ, ○) or pDEB21K (OD_{600}, □; LacZ, △) (B) were diluted 1:1,000 into fresh LB medium and grown at 37°C. The multicopy plasmid pDEB2 carries the *rpoS* gene, and so there is an overexpression of σ^S as HA4109:*rpoS*/pDEB2 enters stationary phase. HA4109:*rpoS* carrying plasmid pDEB21K (*rpoS*::Kn) does not express *rpoS* and was used as a control. At indicated times, samples were removed for β-galactosidase assays.

similar to that of other *E. coli* genes, while most other retron RTs differ in their codon usage from their *E. coli* hosts (10). Retron-Ec107 is also the only characterized retron in *E. coli* which is not associated with phage or phage-like genes and therefore is probably more stable within the chromosome. Through construction of a P_{Ec107}-*lacZ* operon fusion within the chromosomal *lac* allele of the wild-type ECOR70 strain, I obtained the opportunity to examine expression from the retron promoter without altering the normal msDNA production environment within the cell itself. Also, this provided the possibility to examine the levels of msDNA produced under those same conditions. It was found that the retron is more highly expressed under conditions of starvation. This expression is most pronounced when cells enter stationary phase in rich medium or when they are starved for phosphate. This high level of expression is dependent on the presence of ppGpp and can indeed be induced under conditions of nutritional abundance by use of the *relA'* plasmid pALS13, which synthesizes ppGpp when the *relA'* gene is induced by IPTG. However, it is obvious that other factors are involved in the production of msDNA because an increase in retron expression does not necessarily lead to an increase in the msDNA level. There is a requirement for RT in the synthesis of msDNA from the transcript; thus, even though msDNA synthesis is dependent on the presence of this transcript, RT must be present to utilize this transcript as a template. Also, msDNA is found as a complex with proteins, as shown by sucrose gradient and footprinting experiments (21, 45). If these proteins are not present for proper folding and/or protection of msDNA, it may not be properly synthesized or may be subject to degradation by exo- and endonucleases. This would explain how ppGpp could induce expression of the retron while not leading to an increase in the level of msDNA during exponential growth, although it appears that the cells must be in stationary-phase growth for msDNA production to occur. I had thought it possible that the retron is transcribed through use of the sigma factor σ^S, since σ^S is responsible for the expression of more than 30 genes upon entry into stationary phase and is also positively regulated by ppGpp. However, I have shown that expression of retron-Ec107 is independent of σ^S and is in fact slightly higher if *rpoS* is deleted.

It is quite interesting that the retron is under regulatory control. If msDNA were some form of selfish DNA, regulation by the cell would be wasteful, as its high expression under starvation conditions would indicate. Under these conditions, the cell is expressing genes to scavenge micro amounts of nutrients, protect itself from environmental stresses as it lies in this low metabolic state, and prepare itself to switch to a growing state once nutrients become available again. One might argue that this regulation is found under laboratory conditions and may be coincidental, but positive regulation by ppGpp is one which would occur in nature, where many bacteria go through a feast-and-famine type of life cycle and depend on ppGpp as a global regulator of those genes necessary for survival. There are also other factors involved in regulation of the retron. For example, a *cya* deletion which prevents accumulation of cyclic AMP has a negative effect on retron-Ec107 expression (unpublished data). These data indicate that regulation of the retron is more complex than stated here and that more work needs to be done.

Interestingly, Singer and Kaiser (40) have recently shown that ppGpp is responsible for the positive regulation of genes which are expressed as *M. xanthus* enters into the fruiting-body development phase of growth. When *M. xanthus* undergoes starvation at high cell density, the cells undergo the development of fruiting bodies in which a small fraction of the cells differentiate into myxospores (for a recent review, see reference 15). Since retron-Mx162 is well conserved in *M. xanthus* and is probably as old as many of its other genes (12, 35), it would be interesting to see if the *Myxococcus* retron is also positively regulated by ppGpp. Deletion of the Mx162 and Mx65 retrons of *M. xanthus* did not have any obvious effect on the normal cell cycle of this strain (13), but perhaps in the less optimal conditions which nature offers these bacteria, there is some advantage to survival.

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