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ppGpp-Dependent *leuO* Expression in Bacteria under Stress

Ming Fang,* Arundhati Majumder,* Kan-Jen Tsai,† and Hai-Young Wu*,[1]
*Department of Pharmacology, School of Medicine, Wayne State University, Detroit, Michigan 48201; and
†School of Medical Technology, Chung Shan Medical and Dental College, Taichung, Taiwan

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Despite the known potential transcription regulatory role of *leuO* gene product, LeuO, the condition when *leuO* expresses during bacterial growth cycle remains unclear. Mechanistically, *leuO* expression was shown to be part of promoter relay mechanism, however, the factor(s) responsible for the regulation of *leuO* expression is not known. Combining Northern and Western results, we demonstrate in the present communication that *leuO* expression is normally low and enhanced when bacteria are in transition from exponential growth to stationary phase. The stationary phase-associated *leuO* expression is ppGpp dependent and *rpoS* (σ^s factor) independent. © 2000 Academic Press

Key Words: **LeuO; ppGpp; DNA supercoiling; transcriptional regulation; DNA topoisomerase.**

leuO is located at the 1.8 min position and the 2.9 min position of the genetic linkage maps of *E. coli* and *S. typhimurium,* respectively (1, 2). The putative LeuO protein was categorized as a LysR family transcription regulator. Like other LysR proteins, there is a helix-turn-helix DNA binding motif located in the N-terminus of LeuO (3). Its potential involvement in the regulation of various genes was suggested in studies where *leuO* was either overexpressed or underexpressed (4–6). However, exact condition when *leuO* expresses during the bacterial normal growth cycle is unclear. *leuO* mRNA was previously detected when the leucine biosynthesis defected (due to *leu-500* mutant) strain was growing with difficulty in a leucine free medium (7) suggesting that growth stress may be responsible for the regulation of *leuO* expression.

The nucleotide sequences for the *E. coli* and *S. typhimurium* promoter relay regions have been deposited in the GenBank database under NCBI Accession Nos. AF 106955 and AF 106956, respectively.

[1] To whom correspondence should be addressed. Department of Pharmacology, Wayne State University, School of Medicine, 540 E. Canfield Ave., Detroit, Michigan 48201. Fax: (313) 577-6739. E-mail: haiwu@med.wayne.edu.

To reveal how and when *leuO* expression is induced in normal bacterial physiology, we monitored *leuO* expression in both a prototype *S. typhimurium* LT2 strain, CV468, and a prototype *E. coli* K-12 strain, W3110. Northern data indicated that *leuO* expression remains low until cells are entering stationary phase. Detailed immunoblotting analysis confirms that *leuO* expression was enhanced during transition from exponential growth to stationary phase. Codetection of *ilvIH* mRNA under this growth condition indicated that *ilvIH* transcription activity-mediated promoter relay may be responsible for the expression of *leuO in vivo.* This finding is consistent with the previous data obtained using a plasmid model system (8).

Despite the association with stationary phase, *leuO* expression is not dependent on stationary phase-specific *rpoS* (σ^s factor). The *leuO* expression is, however, very sensitive to the cellular ppGpp level, the upstream stress signal of *rpoS* expression. Based on this finding, a role of *leuO* expression in cellular ppGpp level-controlled stress responses is discussed.

MATERIALS AND METHODS

Plasmid constructs. The construction of pWU804LZ has been previously described (7). The map of the reporter plasmid is illustrated in Fig. 1.

Bacterial strains and growth medium. The detailed genotypes of the *S. typhimurium* and *E. coli* strains used in this study are shown in Table 1.

Cells were grown aerobically either at 32°C or 37°C in a synthetic (chemically defined) medium base, SSA, which is a minimal salt buffer [K_2HPO_4 (Sigma) 10.5; KH_2PO_4 (Sigma), 4.5; $(NH_4)_2SO_4$ (Sigma), 1.0; sodium citrate dihydrate (Sigma), 0.97 (each component is in grams per liter of deionized water)] supplemented with 4 μg/ml thiamin, 0.2% glucose, 20 μg/ml guanine, 40 μg/ml adenine, 50 μg/ml $MgSO_4$. When necessary, 14 amino acids (20 μg/ml each of L-histidine, L-lysine, L-tyrosine, L-tryptophan, L-threonine, L-cysteine, L-methionine, L-serine, L-alanine, L-aspartic acid and 40 μg/ml each of L-phenylalanine, L-glutamic acid, L-proline, and L-arginine) were added to form the 14 a. a. SSA medium. Rich medium, Luria Broth, was used in some experiments as indicated. Ampicillin (50 μg/ml) was added to culture as needed.

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TABLE 1

Bacterial Strains Used in this Study

Strain	Genotype	Ref.
S. typhimurium		
CV468	*ara9, gal-205*	27
E. coli		
W3110	F⁻ *mcrA mcrB IN(rrnD-rrnE)1* λ	
MC4100	F⁻ *araD139* Δ*(argF-lac)U169 rpsL150* (Str^r) *relA1 flbB5301 deoC1 pstF25 rbsR*	4
CU285	*rpoS*⁻, MC4100	15
CV1444	*ara thi* Δ*(pro-lac)(*λ*243)*	28
CV1457	*ara thi* Δ*(pro-lac)(*λ*243)* Δ*relA25::Kan* Δ*spoT207::cam*	28

β-galactosidase assay. The β-galactosidase activity was measured by hydrolysis of *o*-nitrophenyl β-D-galactoside (ONPG) to produce *o*-nitrophenol (ONP) in permeabilized cells as described by Miller (9). The β-galactosidase specific activity value (nmol of ONP per min per plasmid) is a result of the calculation according to cell number and plasmid copy number at each time point since both numbers increase along with cell growth. The cell number was determined via viable cell counts. The plasmid copy number was determined using southern hybridization. The probe hybridized with *leuABCD* coding region was used to detect the chromosomal DNA. A similar sized probe but hybridized only with plasmid vector sequence was used to detect the plasmid DNA. The radioactive counts of the above two hybridized bands were used for calculating the plasmid copy number in each testing condition. The *lac*⁻ genetic background of CV468 allows the β-galactosidase assay for detecting the expression of plasmid-borne *lacZ* reporter without interference from the *lacZ* activity from host cells.

Northern analysis. The total RNA isolation and Northern analysis were carried out as previously described (7, 8). A 279 bp AccI-AccI DNA fragment isolated from *S. typhimurium ilvI* coding region (position +1550 to +1820 of *ilvI*) (10) was labeled using nick translation and used to detect *ilvIH* mRNA. A synthesized 28mer (5′-CGGAAAACATAAAGACGCTGACAGAGAC-3′) consisting of the DNA sequence downstream of the *S. typhimurium leuO* transcription initiation site was end-labeled by T4 kinase and used to detect *leuO* mRNA.

Western blot analysis. The Western blot analysis was performed as previously described (7). LeuO-specific antiserum raised by injecting the purified over-expressed *S. typhimurium* his-tagged LeuO to a rabbit was used to detect LeuO protein. The purified IgG (1.4 mg/ml) from the antiserum was used at a dilution factor of 1:5000. This antibody somehow detects *S. typhimurium* LeuO more specifically (less background bands as seen in Fig. 3) than *E. coli* LeuO (more background bands as seen in Figs. 4–6).

E. coli DNA topoisomerase I (TopA) antibody was obtained from Drs. Rolf Menzel and Haiyan Qi. The rabbit antibody raised against *E. coli* TopA is equally efficient in immunologically detecting TopA from both *E. coli* and *S. typhimurium* as previously demonstrated (7). 1:5000 diluted TopA antiserum was used in the immunoblotting experiment.

RESULTS AND DISCUSSION

leuO Expression Is Enhanced When S. typhimurium Cells Are Entering Stationary Phase

The reporter plasmid, pWU804LZ (Fig. 1) in which the *lacZ* reporter was transcriptionally fused with the *leu-500* promoter, has previously been used to monitor the promoter relay whereby *ilvIH* transcription activity activates the intermediate *leuO* gene and subsequently activates the *leu-500* promoter (7). Since both the translation product and the transcription activity of the *leuO* gene are simultaneously required for *leu-500* activation (8), *leu-500* promoter activity-mediated expression of the *lacZ* reporter is expected to reliably reflect the expression of *leuO*. This reporter plasmid system was therefore used initially for monitoring the plasmid-borne *leuO* expression indirectly via *leu-500* promoter activity during the entire growth phase of a wild-type *S. typhimurium* LT2 strain, CV468, growing in the 14 a. a. SSA medium (please see Materials and

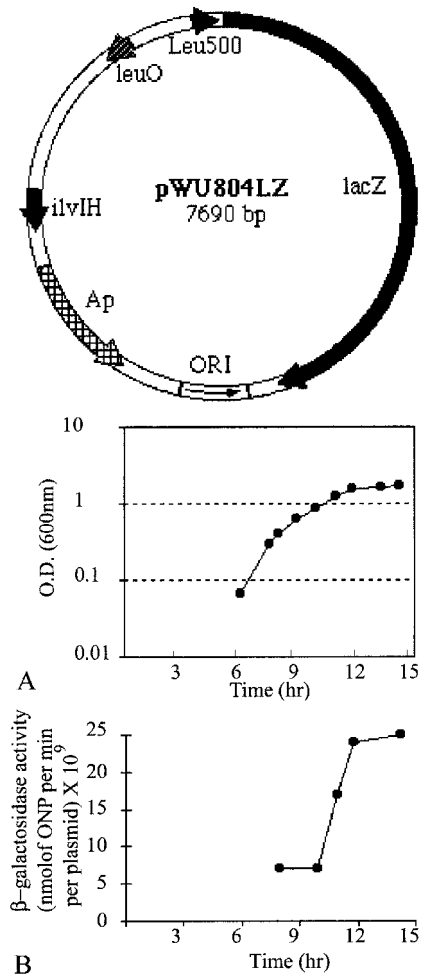

FIG. 1. The indirect detection of plasmid-borne *leuO* expression in *S. typhimurium* when cells are entering stationary phase. pWU804LZ-harboring CV468 was grown in 14 a. a. SSA medium. During cell growth, both the OD_{600} (A) and β-galactosidase activity (B) were measured and plotted against time postinoculation. *leuO* expression was indirectly detected via the activity of the plasmid-borne *leu-500* promoter based on the fact that the coupling of *leuO* promoter activity and its gene product is required for *leu-500* activation. The β-galactosidase activity has been normalized against plasmid copy number so that the activity unit is nmol of ONP per minute per plasmid.

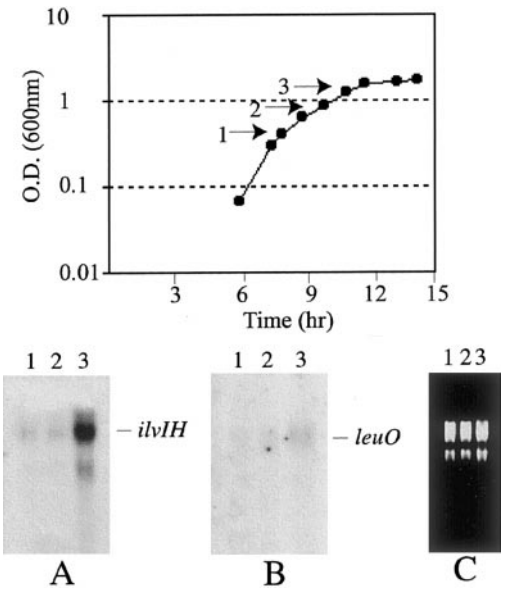

FIG. 2. The expression of chromosomal *ilvIH* and *leuO* in *S. typhimurium* when cells are entering stationary phase. CV468 cells were grown in 14 a. a. SSA and harvested at OD_{600} of 0.4 (1), 0.8 (2) and 1.35 (3) for the isolation of total RNA. The *ilvIH* and *leuO* mRNA were detected by Northern analyses and shown in A and B, respectively. The loading of equal amount total RNA was verified by the ethidium bromide stained gel (C).

Methods for the detailed formula). 14 a. a. SSA medium is exactly the same as SSA(-leu) medium described in our previous studies (7, 8, 11, 12). The same growth condition is used for ensuring a direct comparison between the current and previous data. The β-galactosidase activity was shown to remain low during the early- and mid-log phases, it started to increase as cell density exceeded certain level in late-log phase (at O.D. 600 nm = 0.8), and reached a maximal level when the cells entered the stationary phase (at O.D. 600 nm = 1.5) (Figs. 1A and 1B). This result suggests that the plasmid-borne *ilvIH* and *leuO* may be induced and subsequently activate the *leu-500* promoter in the reporter plasmid as cells are entering stationary phase (during the period between O.D. 600 nm readings 0.8 and 1.5).

We, therefore, directly monitored *ilvIH* and *leuO* mRNA in CV468 using Northern analysis. Both *ilvIH* and *leuO* mRNA were barely detectable in the early- or mid-log phases (lanes 1 and 2 in Figs. 2A and 2B) but significantly increased when cells are entering stationary phase (lane 3 in Figs. 2A and 2B). It appears that at their chromosomal locations, expressions of *ilvIH* and *leuO* are low until cells exceed certain cell density. Together with the previous data showing expression of *ilvIH* and *leuO* in CH601, a leucine biosynthesis defected *S. typhimurium* strain, at the end of prolonged lag phase in a leucine free minimal medium (7), the coupled expression of both genes at the transition stage suggests that *ilvIH*-initiated promoter relay mechanism may indeed be responsible for *leuO* expression under some difficult growth conditions (stresses) including entering stationary phase.

LeuO Level Increases during Transition from Exponential Growth to Stationary Phase in S. typhimurium

Northern data indicated that *leuO* expression was triggered in the late-log phase. The reporter plasmid study also show that the *leuO* expression indirectly mediated β-galactosidase activity that remains high in the entire stationary phase once the expression of *leuO* is triggered in late-log phase (Fig. 1). However, whether the *leuO* expression is transient or constant remains unclear since the turnover rate of the reporter gene product, β-galactosidase, may be very different from that of *leuO* gene product, LeuO. The constant high level of β-galactosidase activity in the stationary phase may be misleading since β-galactosidase is a very stable protein (13). More importantly, detailed time course experiment is required for unraveling the exact starting point of LeuO increase since the Northern data demonstrated only an approximate condition when *leuO* expresses. To address these two questions, we monitored LeuO protein level using Western blot at various time points during cell growth (Fig. 3). The immunoblot results show that the normally barely detectable LeuO protein was increased at O.D. 600 nm = 1.2 (lane 3 in Fig. 3B) which is approximately the same time point when *leuO* mRNA was detected in Fig. 2B (lane 3) and LeuO protein level maximized 1 h later at O.D. 600 nm = 1.5 (lane 4 in Fig. 3B). Both Western and Northern results consistently supported that *leuO* expression was increased during transition from exponential growth to stationary phase when cell density exceeded certain point in late-log phase (O.D. 600 nm = 1.2).

As an internal control, the same membrane was blotted with *E. coli* TopA antibody (Fig. 3C). The result indicated that TopA level remains constant throughout the entire growth of CV468 and again demonstrated that *leuO* expression is decisively controlled by the *ilvIH* transcription activity-mediated promoter relay rather than the cellular TopA level. The same conclusion has been drawn previously using another *S. typhimurium* strain, CH601 (7).

leuO Expression during Transition from Exponential Growth to Stationary Phase in E. coli

According to the DNA sequence alignment between *E. coli* and *S. typhimurium*, there is about 78% sequence homology in the 1.9 kb chromosomal region involving the relay of *ilvIH*, *leuO* and *leuABCD* promoters (the sequences can be obtained from NCBI Genbank; Accession No. AF 106955 and AF 106956). Based

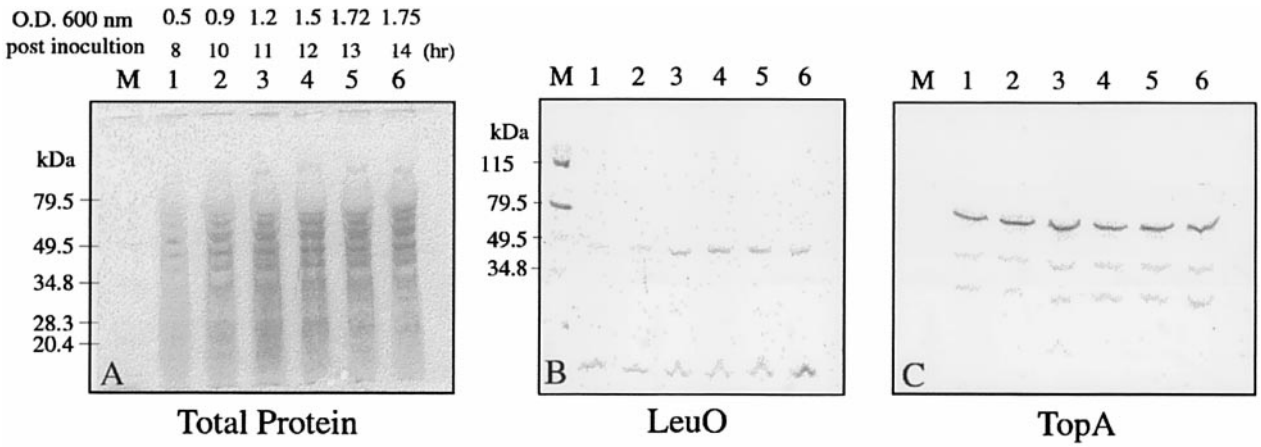

FIG. 3. *S. typhimurium* LeuO level increases during transition from exponential growth to stationary phase and slightly decreases in stationary phase. Aliquots of CV468 culture in 14 a. a. SSA medium were harvested and prepared for immunoblotting at various time points during the cell growth. Both the OD_{600} readings and the elapsed incubation time point of each sample are indicated above each lane in the coomassie blue stained gel (panel A). The immunoblotting results using the NC membrane which contains the total proteins electrotransferred from the gel (A) for detecting LeuO and TopA are shown in B and C, respectively. The theoretical molecular mass of *S. typhimurium* LeuO is 37.4 kDa.

on the similar gene positioning and the similar presence of upstream AT-rich sequences, it is not surprising to find that *leuO* expression in *E. coli* is also enhanced in late-log phase. A prototype *E. coli* K12 strain, W3110, was used here for monitoring *leuO* expression by Western analysis (Fig. 4). For a direct comparison, the previously used medium, 14 a. a. SSA medium, and incubation temperature (32°C) was used again in this initial study. *S. typhimurium* LeuO-specific antibody was used for detecting *E. coli* LeuO. More background bands were observed in all *E. coli* Western results. Western blots using preimmune serum (data not shown) was used for unambiguously distinguishing the LeuO band from the background bands. Western data shown that LeuO level increased slightly in late-log phase at O.D. 600 nm = 1.5 (Fig. 4, lane 6) and the level maximized when cells entered stationary phase (Fig. 4, lanes 8 and 9). As seen in *S. typhimurium*, expression of *leuO* in *E. coli* similarily remains low until cells are in transition from exponential growth to stationary phase (Fig. 4). The establishment of LeuO expression condition in *E. coli* is important for investigating further the cause of stationary phase-associated *leuO* induction since more *E. coli* mutants than *S. typhimurium* mutants are available for testing. Similar stationary phase-associated LeuO expression pattern was detected when cells were grown in rich medium, Luria Broth, at 37°C (data not shown).

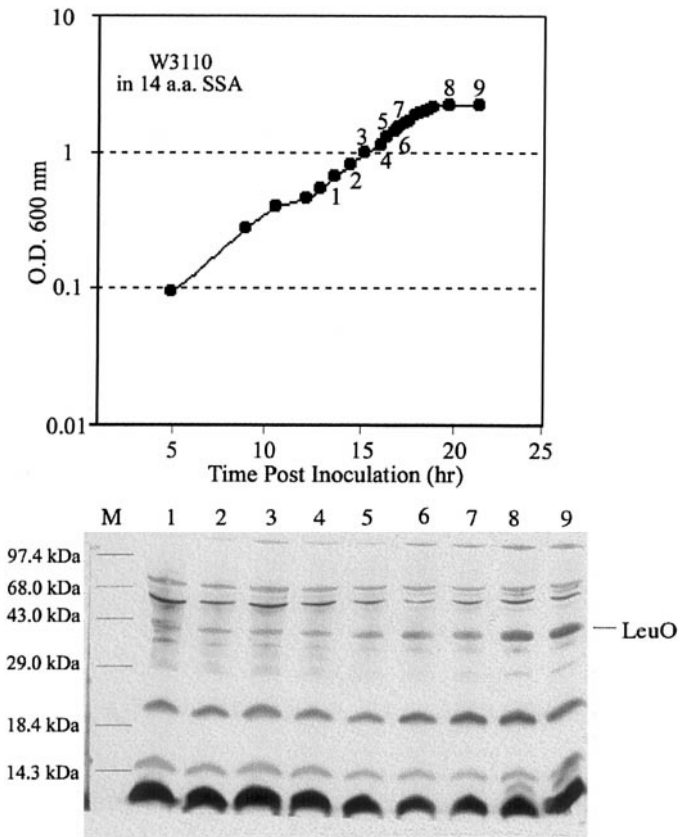

FIG. 4. Late-log/stationary phase-specific *leuO* expression in *E. coli*. *E. coli* K-12 strain, W3110, was grown in 14 a. a. SSA medium at 32°C with aeration. Aliquots of culture were collected at nine time points marked with filled circles and Arabic numerals in the growth curve. Time points 1 and 2 are in early log phase; time points 3, 4, and 5 are in mid-log phase; time points 6 and 7 are in late log phase; while time points 8 and 9 are in stationary phase. The lane numbers of the Western blotting result correspond to the above time point assignment. *E. coli* LeuO with a theoretical molecular mass of 39.9 kDa is marked.

leuO Expression Is Unrelated to rpoS Controlled Stationary Phase Regulatory Cascade

What triggers *leuO* expression in bacteria? The stationary phase-association of *leuO* expression has prompted us to consider that *leuO* expression may be part of *rpoS* regulatory cascade during the stationary

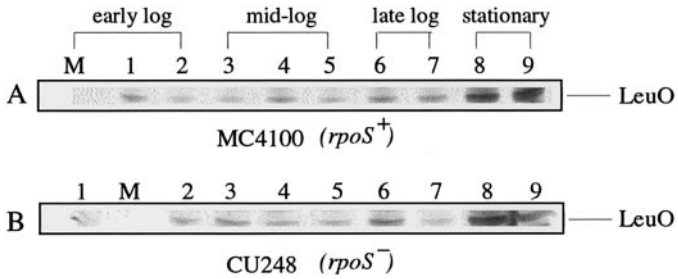

FIG. 5. LeuO expression is independent of *rpoS* genetic background. MC4100, *rpoS*$^+$ strain and CU285, MC4100 *rpoS*$^-$ were grown in LB and harvested at time points in various growth phases as indicated.

phase (14). Notably, a recent study has shown that *leuO* expression causes the reduction of σ^s, the *rpoS* gene product, at cold temperature via affecting the translational expression of *rpoS* by the repression of the synthesis of the small *dsrA* RNA (4). In order to know whether *leuO* expression is dependent or independent on *rpoS* regulatory cascade, we monitored the LeuO protein level during the growth of an isogenic *rpoS*$^+$ and *rpoS*$^-$ pair in LB. CU285, a *rpoS*$^-$ MC4100, was derived from MC4100, a *rpoS*$^+$ strain (15, 16). Results showed that LeuO level increase started in late-log phase (lane 6 in both Figs. 5A and 5B) and maximized in stationary phase (lanes 8 and 9 in both Figs. 5A and 5B). The pattern of *leuO* expression remains the same in both strains (compare Figs. 5A and 5B) suggesting that *leuO* expression is independent of the *rpoS* regulatory cascade.

ppGpp Is Required for leuO Expression

Besides the association with the stationary phase, *leuO* expression was also previously linked to a stress growth condition when a *leu-500* mutant, CH601, grew in a leucine free medium (7). Cellular ppGpp level is known to be elevated upon the decrease of growth rate when cells are under such a growth stress and when entering stationary phase (17). The same study (17) demonstrated that cellular ppGpp level determines *lrp* expression. And *lrp* gene product, Leucine-responsive regulatory protein (Lrp) is known to be the positive regulator required for activating *ilvIH* transcription activity (18) which is crucial for initiating a promoter relay mechanism (7). Since *leuO* expression is part of the promoter relay mechanism, it is possible that ppGpp is the signal responsible for the observed stationary phase-associated *leuO* expression. To test this possibility, Western analyses were used to monitor LeuO expression in a pair of ppGpp$^+$ and ppGpp$^-$ isogenic strains. A typical late-log/stationary phase associated *leuO* expression was detected in the ppGpp$^+$ strain but not in the ppGpp$^-$ strain (Fig. 6). More strikingly, the background low level leuO expression was abolished in the ppGpp$^-$ strain. This result strongly suggested ppGpp is the stress signal responsible for the observed induction of *leuO* expression when cells are at transition from exponential growth to stationary phase.

Our data links *leuO* expression to late-log/stationary phase during bacterial growth cycle. Despite that ppGpp is the stress signal for the stationary phase-specific *rpoS* stress response (4, 14, 19). Strikingly, *leuO* expression is dependent solely on the stress signal, ppGpp. Obviously two related but independent stress responses must have occurred under such a growth condition. Together, the best explanation for the present data is that *leuO* expression is induced indirectly via the increase of cellular Lrp level in response to ppGpp level increase caused by the slow growth rate when cells are entering stationary phase. *ilvIH* transcription activity-initiated promoter relay

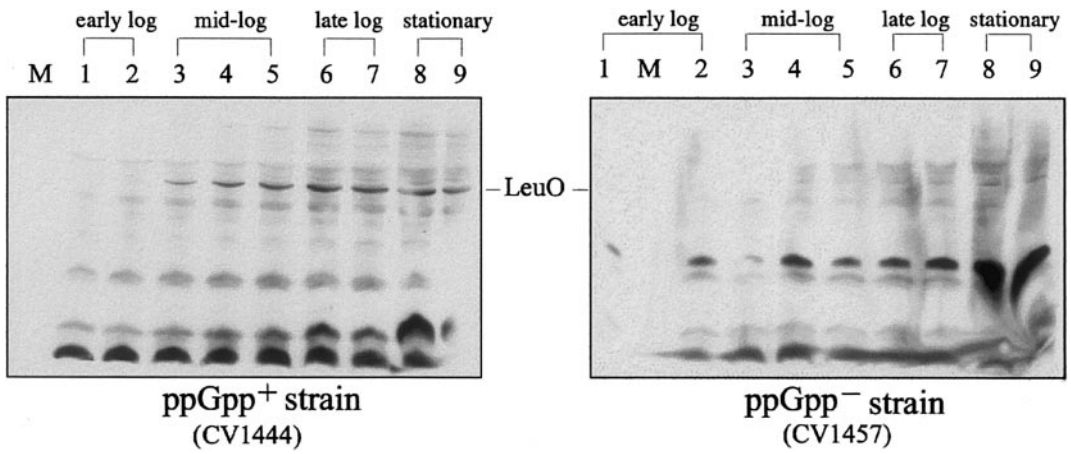

FIG. 6. ppGpp is required for the late-log/stationary phase-associated LeuO expression. A ppGpp wild-type strain, CV1444, and an isogenic ppGpp free strain, CV1457, were grown in LB. Samples were harvested at time points in various growth phases as indicated. Position of the LeuO band is marked.

mechanism causes the *leuO* induction as one of the many stress responses including *rpoS*-dependent stress responses under this growth condition. The link between *leuO* expression and growth stress has provided important clues for the current effort of unraveling the molecular detail how *leuO* expression is regulated.

The conditional *leuO* expression in growth stress-related responses may represent a type of gene expression regulation which is not essential for exponentially growing cells under standard laboratory growth conditions but important for cells to survive in nature. Unlike the fast growing conditions in the laboratory, bacteria in nature often encounter various stresses such as limiting nutrient. As a consequence, bacteria have evolved to survive under the stress conditions by inducing a series of genes for increasing resistance to the stresses (reviewed in 20, 21). Since these types of genes are not essential for the exponentially growing cells, mutations in these genes often lack phenotypes under standard laboratory testing conditions. Due to the cryptic phenotypes in laboratory conditions, the regulation and functions of these genes have been understudied until recently. A number of stress-induced gene regulation pathways have been elucidated, such as expression of *cadBA*, *proU*, *stpA*, etc., under various induced conditions (22–26). The discovery of conditional expression of *leuO* in response to late log/stationary phase-associated stress and other stresses will further broaden our knowledge of the regulation of expression of important genes in nature.

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REFERENCES

1. Berlyn, M. K. B., Low, K. B., Rudd, K. E., and Singer, M. (1996) Linkage map of *Escherichia coli* K-12. *In Escherichia coli and Salmonella:* Cellular and Molecular Biology (Neidhardt, F. C., Curtiss, R., III, Ingraham, J. L., Lin, E. C. C., Low, K. B., Magasanik, B., Reznikoff, W. S., Riley, M., Schaechter, M., and Umbarger, H. E., Eds.), ed. 9, Vol. 2, pp. 1715–1902, American Society for Microbiology, Washington, D.C.
2. Sanderson, K. E., Hessel, A., Liu, S.-L., and Rudd, K. E. (1996) The genetic map of *Salmonella typhimurium*. *In Escherichia coli and Salmonella:* Cellular and Molecular Biology (Neidhardt, F. C., Curtiss, R., III, Ingraham, J. L., Lin, E. C. C., Low, K. B., Magasanik, B., Reznikoff, W. S., Riley, M., Schaechter, M., and Umbarger, H. E., Eds.), ed. 8, Vol. 2, American Society for Microbiology, Washington, D.C.
3. Henikoff, S., Haughn, G. W., Calvo, J. M., and Wallace, J. C. (1988) A large family of bacterial activator proteins. *Proc. Natl. Acad. Sci. USA* **85,** 6602–6606.
4. Klauck, E., Böhringer, J., and Hengge-Aronis, R. (1997) The LysR-like regulatory LeuO in *Escherichia coli* is involved in the translational regulation of *rpoS* by affecting the expression of the small regulatory DsrA-RNA. *Mol. Microbiol.* **25,** 559–569.
5. Shi, X., and Bennett, G. N. (1995) Effects of multicopy LeuO on the expression of the acid-inducible lysine decarboxylase gene in *Escherichia coli. J. Bacteriol.* **177,** 810–814.
6. Ueguchi, C., Ohta, T., Seto, C., Suzuki, T., and Mizuno, T. (1998) The *leuO* gene product has a latent ability to relieve silencing in *Escherichia coli. J. Bacteriol.* **180,** 190–193.
7. Fang, M., and Wu, H.-Y. (1998b) Suppression of *leu-500* mutation in *topA*$^+$ *Salmonella typhimurium* strains: The promoter relay at work. *J. Biol. Chem.* **273,** 29929–29934.
8. Fang, M., and Wu, H.-Y. (1998a) A promoter relay mechanism for sequential gene activation. *J. Bacteriol.* **180,** 626–633.
9. Miller, J. H. (1972) Experiments in Molecular Genetics, pp. 352–355, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
10. Squires, C. H., DeFelice, M., Lago, C. T., and Calvo, J. M. (1983) *ilvHI* locus of *Salmonella typhimurium*. *J. Bacteriol.* **154,** 1054–1063.
11. Tan, J., Shu, L., and Wu, H.-Y. (1994) Activation of the *leu-500* promoter by adjacent transcription. *J. Bacteriol.* **176,** 1077–1086.
12. Wu, H.-Y., Tan, J., and Fang, M. (1995) Long-range interaction between two promoters: Activation of the *leu-500* promoter by a distant upstream promoter. *Cell* **82,** 445–451.
13. Kepes, A. (1969) Transcription and translation in the lactose operon of *Escherichia coli* studied by *in vivo* kinetics. *Prog. Biophys. Mol. Biol.* **19,** 199–236.
14. Lange, R., Fischer, D., and Hengge-Aronis, R. (1995) Identification of transcription start sites and the role of ppGpp in the expression of *rpoS*, the structural gene for the δ^s subunit of RNA polymerase in *Escherichia coli. J. Bacteriol.* **177,** 4676–4680.
15. Yamashino, T., Kakeda, M., Ueguchi, C., and Mizuno, T. (1994) An analogue of the DnaJ molecular chaperone whose expression is controlled by sigma s during the stationary phase and phosphate starvation in *Escherichia coli. Mol. Microbiol.* **13,** 475–483.
16. Casadaban, M. J. (1976) Transposition and fusion of the *lac* genes to selected promoters in *Escherichia coli* using bacteriophage lambda and Mu. *J. Mol. Biol.* **104,** 541–555.
17. Landgraf, J. R., Wu, J., and Calvo, J. M. (1996) Effects of nutrition and growth rate on Lrp levels in *Escherichia coli. J. Bacteriol.* **178,** 6930–6936.
18. Platko, J. V., Willins, D. A., and Calvo, J. M. (1990) The *ilvIH* operon of *Escherichia coli* is positively regulated. *J. Bacteriol.* **172,** 4563–4570.
19. Shiba, T., Tsutsumi, K., Yano, H., Ihara, Y., Kameda, A., Tanaka, K., Takahashi, H., Munekata, M., Rao, N. N., and Kornberg, A. (1997) Inorganic polyphosphate and the induction of *rpoS* expression. *Proc. Natl. Acad. Sci. USA* **94,** 11210–11215.
20. Rao, N. N., and Kornberg, A. (1999) Inorganic polyphosphate regulates responses of *Escherichia coli* to nutritional stringencies, environmental stresses and survival in the stationary phase. *Prog. Mol. Subcell. Biol.* **23,** 183–195.
21. Zambrano, M. M., and Kolter, R. (1996) GASPing for life in stationary phase. *Cell* **86,** 181–184.
22. Bhriain, N., Dorman, C. J., and Higgins, C. F. (1989) An overlap between osmotic and anaerobic stress responses: A potential role for DNA supercoiling in the coordinate regulation of gene expression. *Mol. Microbiol.* **3,** 933–942.
23. Free, A., and Dorman, C. J. (1997) The *Escherichia coli stpA* is transiently expressed during growth in rich medium and is induced in minimal medium and by stress conditions. *J. Bacteriol.* **179,** 909–918.

24. Goransson, M., Sorden, B., Nilsson, P., Dagberg, B., Forsman, K., Emanuelsson, K., and Uhlin, B. E. (1990) Transcription silencing and thermoregulation of gene expression in *Escherichia coli. Nature (London)* **344,** 682–685.

25. Higgins, C. F., Dorman, C. J., Stirling, D. A., Waddell, L., Booth, I. R., May, G., and Bremer, E. (1988) A physiological role for DNA supercoiling in the osmotic regulation of gene expression in *S. typhimurium* and *E. coli. Cell* **52,** 569–584.

26. Meng, S.-Y., and Bennett, G. N. (1992) Regulation of the *Escherichia coli cad* operon: Location of a site required for pH induction. *J. Bacteriol.* **174,** 2670–2678.

27. Dubanau, E., and Margolin, P. (1972) Suppression of promoter mutations by the pleiotropic *supX* mutations. *Mol. Gen. Genet.* **117,** 91–112.

28. Svitil, A. L., Cashel, M., and Zyskind, J. W. (1993) Guanosine tetraphosphate inhibits protein synthesis *in vivo.* A possible protective mechanism for starvation stress in *Escherichia coli. J. Biol. Chem.* **268,** 2307–2311.