Direct Stimulation of the \( \lambda p\alpha Q \) Promoter by the Transcription Effector Guanosine-3',5'-(-bis)pyrophosphate in a Defined in Vitro System*

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The bacterial response to nutritional deprivation, called the stringent response, results in the introduction of the specific nucleotide guanosine-3',5'-(-bis) pyrophosphate (ppGpp). This nucleotide interacts with RNA polymerase and alters its action so that transcription from certain promoters is inhibited, whereas transcription from others seems to be activated. The exact mechanism of transcriprional stimulation by ppGpp in vivo remains unknown. A passive control model has been proposed according to which transcription inhibition during the stringent response at several very active promoters, like those for rRNA and tRNA genes, makes more free RNA polymerase (RNAP) molecules available for transcription at promoters with weak binding affinities for RNAP, thus leading to their passive activation. Among promoters whose transcription is activated by ppGpp in vivo is the histidine operon promoter (hisGp). However, in vitro it is only possible to demonstrate this effect in a coupled transcription-translation system. Here we demonstrate, using another in vivo ppGpp-stimulated promoter, the phage \( \lambda p\alpha Q \) promoter, that activation by ppGpp in a defined in vitro system is direct. A systematic study of ppGpp effects on the stimulation of \( \alpha Q \) revealed that, as in the case of promoters inhibited by this nucleotide, ppGpp decreases the half-life of \( \alpha Q \) open complexes. Our results also indicate that the equilibrium binding affinity of RNA polymerase to \( \alpha Q \) seems not to be affected in the presence of ppGpp. Our data indicate that the mechanism underlying ppGpp stimulation of \( \alpha Q \) is due to an increased rate of productive open complex formation.

When exposed to nutritional deprivation, bacteria respond quickly and efficiently to changing environmental conditions. This is accomplished in part by shutting down those physiological processes whose persistence is not necessarily energetically favorable for survival, such as translation. To shut down protein synthesis, stable RNA synthesis (i.e., rRNA and tRNA) is inhibited. Such action is mediated by a small transcriptional effector molecule, guanosine-3',5'-(bis)pyrophosphate (ppGpp),1 and this general response to starvation and environmental stress is called the stringent response (for review, see Ref. 1). ppGpp interacts with RNA polymerase, probably at an interface between the \( \beta \) and \( \beta' \) subunits (2, 3), and alters its action so that transcription from certain promoters, such as those for stable RNAs, is inhibited, whereas other promoters seem to be activated or unaffected.

Not surprisingly, many promoters stimulated by ppGpp directly transcribe the amino acid biosynthesis operons. Among them is the histidine operon promoter of Salmonella typhimurium, hisG, which has been studied extensively (4–7).

To explain the ppGpp-dependent activation mechanism, a general passive control model has been proposed (8) wherein transcription inhibition at very strong promoters, like those for rRNA and tRNA synthesis, results in making more free RNA polymerase molecules available for transcription at promoters with a low affinity for this enzyme, thus leading to their passive activation. In contrast to the passive model, it was shown by Choy (7) that, in a mixed template in vitro transcription system containing both positively and negatively controlled promoters, transcriptional inhibition and stimulation mediated by ppGpp are independent of each other, thus supporting a direct and active mode of positive transcription control by ppGpp. Still, the conclusion of Choy was based on studies employing an S-30 cell extract and, hence, whether ppGpp interacts directly with RNA polymerase to stimulate transcription or whether another factor participates in this process remained unknown.

In this study we investigated in vitro the phage \( \lambda p\alpha Q \) promoter, a promoter previously uncharacterized for ppGpp stimulation in vitro, but which was previously shown to be stimulated by ppGpp in vivo (9). We found that, in a defined in vitro system, transcription initiating from \( \alpha Q \) is increased 50–60% in the presence of ppGpp in single-round transcription experiments. A systematic study of \( \alpha Q \) stimulation by ppGpp in vitro revealed that, as in the case of promoters inhibited by this nucleotide, ppGpp decreases the half-life of the open complexes. In addition, our results indicate that promoter binding of the RNA polymerase to \( \alpha Q \) is not affected by ppGpp. Rather, our data suggests that ppGpp increases the rate of formation of competent open transcription complexes. Our results clearly demonstrate that effect of ppGpp on \( \alpha Q \) is direct, and, thus, this promoter’s activation in vivo cannot be attributed solely to passive control and does not necessarily require any additional cellular factor.

**Experimental Procedures**

DNA Template Preparation—The \( \lambda p\alpha Q \) promoter region (−103 to +39 bp) was generated by PCR using wild-type \( \lambda \) DNA as a template and the primers 5'-GGCAATCTGCTGATCTGCTG-3' (\( \lambda p\alpha QQ3 \)) and 5'-CACAAGGGTTATCGGAAATACC-3' (\( \lambda p\alpha QQ1 \)). Following treatment with T4 polynucleotide kinase, the DNA fragment was inserted into pHG86 (10), which had been digested previously with Smal. The resu
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In vitro Transcription Assay—In vitro transcription assays were performed in the total reaction volume of 20 μl at 37 °C using 10 nm template and 60 nm RNAP in a transcription buffer containing 50 mM Tris-HCl (pH 8), 10 mM MgCl2, 10 mM β-mercaptoethanol, 10 μg/ml bovine serum albumin, 20 mM KCl, 100 μM ATP, 100 μM GTP, 100 μM UTP, and 10 μM CTP (10 μCi reaction [α-32P]CTP) and either 250 μM GDP or 250 μM ppGpp. RNAP was pre-incubated with GDP or ppGpp for 7 min at room temperature prior to the addition of KCl, followed by a 2-min incubation at 37 °C, and the reaction was started by mixing RNA polymerase with template DNA and NTP substrates. Transcription was terminated after 10 min by the addition of an equal volume of stop and loading solution (95% formamide, 20 mM EDTA, 0.05% bromphenol blue, and 0.05% xylene cyanol). Samples were analyzed on a 7 M urea, 8% polyacrylamide sequencing gel and quantified by phosphorimaging on a Bio-Rad molecular imaging system. In experiments in which CII protein was used (37 nm final concentration), 50 nm KCl was employed.

Open Complex Stability and Promoter Escape Assays—RNAP (60 nm) and a DNA template (10 nm) were incubated with 250 μM GDP or ppGpp at room temperature for 7 min in the transcription buffer, followed by 30 min of incubation at 37 °C with 20 mM KCl. After the addition of heparin (100 μg/ml final), aliquots (10 μl) were removed and added to 10 μl of an NTP mix containing 100 μM ATP, 100 μM GTP, 100 μM UTP, 10 μM CTP (10 μCi reaction [α-32P]CTP), and 100 μg/ml heparin in transcription buffer. Reactions were chased for 7 min, stopped by adding an equal volume of loading buffer, and analyzed as described above. For the promoter escape assays, after 30 min of incubation at 37 °C with 20 mM KCl the NTPs mix was added simultaneously with heparin (100 μg/ml final) to the whole reaction mixture, and, at indicated times, samples (5 μl) were removed and terminated immediately by the addition of stop buffer.

Electrophoretic Mobility Shift Assay—RNAP (0–180 nm) was pre-incubated with 250 μM GDP or ppGpp for 7 min at room temperature in the transcription buffer, followed by the addition of KCl (20 mM final), incubation at 37 °C for 2 min, and the addition of an α-32P-label DNA template (1 μM). After 5 min of incubation at 37 °C in the presence of a paQ template, poly[dI-dC], was added (100 μg/ml final), and the incubation was continued for another 5 min. Then, native loading buffer (30% glycerol, 0.05% bromphenol blue, and 0.05% xylene cyanol) was added (one-sixth of the reaction volume), and the samples were loaded immediately on a running 3.5% polyacrylamide native gel and resolved using 20 mM Tris-HCl (pH 8), 10 mM MgCl2, and 250 mM NaCl. The samples were run and analyzed as described previously (11, 12).

RESULTS

Activation of the paQ Promoter in a Defined in Vitro System—To elucidate ppGpp-mediated transcriptional activation mechanism in vivo, during the stringent response many attempts to investigate this phenomenon in vitro have been made. However, these attempts were never successful in a defined transcription system, and only when using a coupled transcription-translation system for the hisG promoter was such activation observed in vitro (7). Here, we decided to investigate in vitro a promoter that was shown previously, by the use of promoter-lacZ fusions, to be activated by ppGpp in vivo, namely paQ (9).

It was long thought that in vitro transcription initiating from paQ absolutely requires the presence of the CII activator protein (14, 15). It was suggested that, in the absence of this protein, RNA polymerase is capable of only a weak promoter interaction, leading perhaps to the formation of a closed complex, but not an active open complex. However, we find that the CII requirement holds true only when using a template containing the λR, promoter in addition to paQ (data not shown). This was the case in earlier work on this promoter in which a fragment of the phage λ DNA containing both promoters λcII and paQ was investigated (14, 15). Presumably, because λR is a strong promoter (16), most of the RNA polymerase molecules bind preferentially at this site instead of to paQ. When we employed a PCR template constructed to contain paQ but exclude λR, transcription was observed in the absence of CII. Thus, we investigated the ppGpp-mediated transcriptional activation in vitro, which was previously reported in vivo (9), both in the presence and absence of the CII protein.

The in vitro transcription reactions were carried out in a standard transcription buffer containing the DNA template at 10 nm concentration, RNA polymerase (60 nm), and nucleotides (100 μM) (see “Experimental Procedures” for details). We found that, in this defined in vitro system, transcription initiating from paQ is enhanced by 25–60% in the presence of ppGpp when compared with experiments in which GDP was used at the same concentrations as a control (Fig. 1). These experiments were performed in the absence of CII and employed multiple round reactions. We conclude that ppGpp-mediated activation of paQ is direct and promoter-specific. The degree of activation was salt-dependent (Fig. 1). Because the greatest degree of activation observed was achieved at 20 mM KCl, we used this salt concentration in all subsequent experiments.

The level of ppGpp-mediated activation of the paQ promoter was dose-dependent; however, above 50 μM ppGpp the transcription level appears saturated (Fig. 2). In all subsequent experiments, a saturating level of 250 μM ppGpp or GDP (in control experiments) was used. In addition, we were concerned that the addition of ppGpp might be leading to an indirect effect of adding contaminating ATP and/or GTP (present as 10% contaminants in our ppGpp preparation, data not shown), which would account for the observed stimulatory effects, especially considering that paQ initiates with ATP. However, we titrated ATP in the presence of ppGpp or GDP and chose a concentration of 100 μM ATP for all subsequent experiments,
indicated concentrations prior to mixing with the for 7 min at room temperature, followed by the addition of KCl to the present are obtained from three independent experiments with S.D. presented are obtained from three independent experiments with S.D. of <7%.

because this concentration was saturating for paQ transcription and was unaffected by the addition of contaminating ATP within the ppGpp preparation (data not shown).

When similar experiments were performed in the presence of the CII protein, transcription initiating from paQ seemed to be slightly inhibited rather than activated (Fig. 2). Here, the inhibition was also dose-dependent, reaching ~25% inhibition at the highest ppGpp concentration (750 μM) investigated. However, because the aim of this work was to elucidate the mechanism of the activation of the paQ promoter by ppGpp, subsequent experiments were performed in the absence of CII, where ppGpp-dependent transcription activation could be observed.

Effect of ppGpp on Stability of Open Complexes at the paQ Promoter—Barker et al., (17) demonstrated that ppGpp decreases the stability of the open complexes of all promoters tested and furthermore hypothesized that ppGpp inhibits only those promoters that form intrinsically short-lived complexes. Our previous report concerning the ppGpp-sensitive promoter λpR (18) was in contrast with the hypothesis of Barker et al. (17), i.e. λpR forms very stable open-complexes and yet is inhibited by ppGpp. In our previous study, we found that although ppGpp destabilizes open complexes formed at the ppGpp-inhibited promoter λpR to a minor extent, overall these complexes remain very stable, even in the presence of ppGpp, and that this effect on open complex stability was not responsible for the observed transcription inhibition of λpR by ppGpp (18). Here, we investigated what the effects would be in the case of a ppGpp-activated promoter. RNA polymerase-promoter complexes were pre-formed on paQ DNA templates (by incubation under conditions specified under “Experimental Procedures”) in the presence of excess enzyme pretreated with either ppGpp or GDP (control experiments). This was followed by the addition of heparin to prevent re-association of RNA polymerase with the template DNA. The reactions were subsequently chased with nucleotides at different times to determine the relative level of promoter occupancy by measuring the level of the paQ-specific transcripts produced. We found that, as in the case of other promoters, ppGpp destabilizes open complexes formed at paQ. We estimated that the average lifetime of the paQ open complex was ~30 min in control experiments, whereas in the presence of ppGpp it was decreased to ~8 min (Fig. 3). This result is also indicative that the model proposed by Barker et al. (17) needs modification, because here we encounter a paradox wherein a ppGpp-activated promoter’s open complexes are more destabilized by ppGpp (~3.75-fold) than by a ppGpp-inhibited promoter, λpR (lifetime decreased from 1 h to 30 min (18)).

It should be noted here that, in the presence of CII, the lifetime of the open complexes was estimated to be ~15 min in control experiments and ~10 min in the presence of ppGpp (data not shown). This suggests that the mechanism of paQ activation by CII does not rely on open complex stabilization, because CII itself decreases the lifetime of such complexes (30 min in the absence of CII and 15 min in its presence).

Effect of ppGpp on paQ in Single Round Transcription Assays—We then investigated whether ppGpp had an effect on paQ transcription under single round conditions. RNA polymerase-paQ complexes were pre-formed at 37°C, and single-round transcription assays were performed by the simultane-
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Effect of ppGpp on Binding of the RNA Polymerase to paQ promoter escape from paQ in the presence of ppGpp. The rate of paQ promoter escape was measured in the presence of 250 μM ppGpp (filled symbols) or 250 μM GDP (open symbols). RNApolymerase (60 nM) and pretreated with ppGpp or GDP was added to paQ DNA template (10 nM) and pre-incubated for 30 min at 37 °C to allow for open complex formation prior to the addition of NTP substrates and heparin (100 μg/ml). RNA samples withdrawn at the indicated times were processed and analyzed as described in the Fig. 1 legend. The results presented are obtained from three independent experiments; S.D. was <7%.

To test the hypothesis that ppGpp enhances the rate of open complex formation at paQ, we used KMN04 footprinting assays in which we investigated the time necessary to form open complexes in the presence of ppGpp or GDP. DNA polymerase was pre-treated with ppGpp or GDP at room temperature in transcription buffer, followed by the addition of KCl and incubation at 37 °C for 2 min. Then, template DNA was added, and, at indicated times, samples were withdrawn and analyzed as described under “Experimental Procedures.” This was done in the presence and absence of the CII activator protein.

The results obtained are shown in Fig. 5. Two distinct transcripts in the early time points are observed as a doublet (denoted by the asterisk in Fig. 5 in the panel labeled -CII), likely representing two distinct initiation sites that are being produced from two distinct open complexes (RPOC) that utilize different transcription start sites; however, at later times, all of the transcription seems to be initiated from a single site, which is consistent with the partitioning of the closed complex into one of two RPOC complexes through a common intermediate complex, RPOCII. In the presence of CII activator protein, all transcription, even at the earliest time point, is initiated from a single start site (Fig. 5, +CII, also denoted by an asterisk) that corresponds to the upper transcript band in the absence of CII. The two transcripts observed (Fig. 5, -CII) here likely arise from the paQ promoter, because one of them (the upper band in Fig. 5, -CII) appears to be 20-fold enhanced when CII is added, and CII binding sites are located directly adjacent to paQ. In addition, point mutations in the core promoter sequences of paQ abolish the production of both transcripts (data not shown). It is apparent from these results (Fig. 5) that the two transcripts, arising presumably from two distinct open complexes (RPOC), are not present in equal amounts and are produced only in the absence of CII. The upper band in the doublet appears to be more abundant compared with the lower band; for example, compare the band intensities in the doublet at 15 min in the +GDP control in Fig. 5 with the +ppGpp at the 15-min time point. With increasing time the lower band diminishes, and the upper band becomes predominant. In the presence of ppGpp this partitioning appears to favor the RPOCII- producing the upper band; for example, compare the doublet at -5 min in the presence of ppGpp with the doublet at 15 min in the presence of GDP. Perhaps this reflects the presence of an intermediate complex leading to the formation of multiple transcriptionally active open complexes and, thus, suggests that ppGpp affects the partitioning and conversion of the closed complex into a specific type of open complex on paQ.

It should be noted here that, in the presence of the CII protein, no intermediate complexes were observed and that the “final” single transcript was observed to be present in the first 20 s of the experiment. This observation suggests further that paQ stimulation by CII results from a quick conversion to this single initiation complex and, thus, implies that the effect of ppGpp in the absence of CII is to increase the rate of formation of this final complex; in other words, ppGpp mimics the presence of CII at paQ.
one permanganate footprinting assay are shown in Fig. 6. It is clear that, in the presence of ppGpp, open complexes are formed more rapidly when compared to the control reaction with GDP (Fig. 6C). The detection of the first open complexes is already apparent in the first 20 s of the experiment in both cases; in the presence of ppGpp ~60% more of such complexes were formed, but on the average (three experiments), stimulation was ~20–30%. As a control for sample-to-sample variation, quantification of footprint reactivities was normalized to nonspecific reactivities (present ± RNAS) observed at a position far removed from the promoter. Such difference persists until the 10th minute of the experiment, where the number of the open complexes reaches more or less the same level and no differences are detectable (Fig. 6C). These results support the hypothesis that ppGpp affects the rate of formation of productive open complexes at this promoter.

**DISCUSSION**

Although the stringent response is a global regulatory process, and it has been known for over 30 years that the stimulation of certain promoters occurs in amino acid starved-cells (1), the mechanism of this stimulation has remained obscure. A current model of how this mechanism operates is the passive regulation model (8, 17). Never before has activation by ppGpp been demonstrated in a well defined in vitro transcription system. Here, we investigated transcriptional stimulation of the bacteriophage *paQ* promoter. Our results indicate that such activation is direct and, at least in the case of this promoter, no additional effectors are necessary. Thus, the model of passive control may need further modification. Clearly, direct stimulation of promoter activity by ppGpp is possible.

It has been implied by Barker et al. (17) that ppGpp destabilizes the open complexes formed at all promoters; however, only those promoters forming intrinsically unstable or “short lived” open complexes are inhibited. We demonstrated, however, that although open complexes formed at *paQ* are destabilized by ppGpp, transcription is actually stimulated and not inhibited by this promoter. Although the proposed model of Barker et al. (17) may hold true for the *rrr* promoters that form extremely unstable open complexes, the generalization of this model to the behavior of all promoters needs to be re-evaluated in the light of the behavior of *paQ*, which can be directly stimulated by ppGpp.

Our studies reveal that binding of the RNA polymerase to *paQ* seems not to be affected by the presence of ppGpp. Rather, our data seems to indicate that ppGpp affects the rate of formation of productive open transcription complexes (Fig. 6). We suggest the presence of an intermediate closed complex, RPCC1 which partitions into one of two transcriptionally active open complexes; the presence of an intermediate complex between closed and open complexes was originally proposed by Buc and McClure to explain kinetic properties of the *lacUV5* promoter (20). Here we propose the existence of such an intermediate closed complex at 37 °C, whereas the intermediate complex observed by Buc and McClure was only observed at lower temperatures because of the entropic requirement for conversion of the intermediate closed complex to the open complex on *placUV5* (20). This intermediate closed complex is apparent on *paQ*, but only in the absence of the CII activator; in the presence of CII no intermediate is formed, as evidenced by the immediate formation of a single RNA species (Fig. 5), and in the presence of ppGpp this intermediate is still formed but is more readily partitioned into an open complex that has a higher efficacy for RNA synthesis (Figs. 5 and 6). The presence of a similar intermediate closed complex between closed and open complexes was recently observed in vivo on *lac* promoter variants fused to AraC activation sites (21), and, similar to the observations made here, the appearance of intermediate promoter complexes was abrogated by the action of the AraC activator protein, which led to direct conversion of closed into open promoter complexes, as is apparent here when the CII activator is included (Fig. 5, +CII). Thus, ppGpp appears to affect the isomerization rate of the conversion of the closed complex into the open complex most likely by stabilizing an intermediate closed complex (RPCC1), which partitions into one of two open complexes, RP1C1 or RP2C1 (Fig. 7); the partitioning into RP1C1 is favored in the presence of ppGpp and leads directly to the formation of a stable, highly active initial transcribing complex ITCC1, whereas in the absence of ppGpp the intermediate complex is not stabilized and, thus, more is converted into an inactive closed promoter complex. Thus, the main effect of ppGpp at this promoter is to lower the rate of the back reaction from the open to the closed promoter complex as outlined in Fig. 7, allowing more of the intermediate closed complex to accumulate, which, in turn, leads to a higher probability of forming highly productive open complex 1.

It should be mentioned that these results, together with our previous results (18), are consistent with our overall hypothesis that the incoming infecting phage assesses the levels of ppGpp as a means of monitoring the physiological state of the host at the moment of infection and responds appropriately. The *paQ*-derived transcript is an antisense RNA that inhibits expression of the Q gene, coding for an anti-terminator protein that is indispensable for expression of late phage genes (including cell lysis genes) during lytic development (14, 15). Thus, *λpr* inhibition, investigated in vivo (9, 19, 21) and in vitro (18), and *λpaQ* activation, both mediated by high intracellular levels of...
ppGpp, would contribute to the lysogeny pathway in phage development. Internally consistent with this hypothesis, also, is the CII-independent, ppGpp-mediated stimulation of \(^{/}\text{paQ}\) that we observed here, because the \(^{/}\text{CII}\) gene is the first gene in the \(^{/}\text{paQ}\) transcription unit, and its expression would be inhibited because of ppGpp-inhibitory regulation (18, 19), obligating all “downstream” genetic regulatory events to be CII-independent.

The modest level of activation observed (50–60%) here for the \(^{/}\text{paQ}\) promoter is significantly less than the estimated 20-fold activation observed on the \(^{/}\text{hispG}\) promoter in vitro using an 30 S cell extract (5). The reason for this modest versus robust activation by ppGpp when comparing \(^{/}\text{paQ}\) expression to that of \(^{/}\text{his}\) may lie in the physiological role played by ppGpp in these two cases. In the case of \(^{/}\text{his}\) activation by ppGpp, histidine biosynthesis must be induced to a level sufficient to provide for histidine prototrophy. In the case of \(^{/}\text{his}\) induction during an amino acid starvation, protein synthesis is simultaneously restricted severely because of the accumulation of deacylated tRNAs, and, likewise, the translation of \(^{/}\text{his}\) mRNAs is severely hampered under amino acid starvation conditions. Thus, a dramatic induction of \(^{/}\text{hispG}\) promoter activity may be necessary to achieve a sufficient level of \(^{/}\text{his}\) mRNAs for adequate translation and production of the histidine biosynthetic enzymes. In the case of \(^{/}\text{paQ}\) promoter, the produced transcript acts as an antisense RNA; thus, translation of \(^{/}\text{paQ}\) RNA is not required for its action. Therefore, a much lower level of induction may be necessary for effective \(^{/}\text{paQ}\) transcript levels to be achieved to give a particular physiological outcome, in this case, the implementation of the lysogeny over the lytic
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pathway. In addition, the lysis versus lysogeny decision is governed by such a tightly regulated genetic program that only small difference in the genetic output of any particular gene involved in this regulation may be sufficient to tip the balance in favor of one or the other outcome; thus, the 50% activation of paQ by ppGpp may represent such a case.

Another major difference between ppGpp induction of the his operon and λpaQ is the fact that his promoter induction requires the addition of an S30 cell extract for the ppGpp effects to be observed, whereas paQ seems to respond to ppGpp in the absence of an S30 extract. The reason for this difference may be to be observed, whereas ppGpp effects may reflect the nature of the paQ genetic program. That is to say, as it still responds to the availability of host factors for processes such as replication and, in this case, the physiological state of the host as gauged by the level of ppGpp. That is to say, once the phage has "assessed" the physiological state of the host via ppGpp levels, it then implements its response independently of additional host factors and, thus, direct ppGpp-mediated paQ stimulation may simply reflect the nature of the phage to operate as an autonomous genetic element.

Clearly, the phage employs host regulatory systems specifically for its development, and a direct stimulation of transcription from the paQ promoter by ppGpp may be beneficial under certain conditions. Although in this report we demonstrate for the first time that ppGpp can directly activate transcription in the absence of any other factors apart from RNA polymerase, it remains to be elucidated whether such a mechanism is specific only for phage promoter(s) or whether it also operates similarly at certain promoters present on the bacterial chromosome.

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REFERENCES
1. Cashel, M., Gentry, D. R., Hernandez, V. J., and Vinella, D. (1996) in Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology (Neidhart, F. C., Curtiss, R., III, Ingraham, J. L., Lin, E. C. C., Low, K. B., Magasanik, B., Reznikoff, W. S., Riley, M., Shaehchter, M., and Umbarger, H. E., eds) Vol. 2, pp. 1458–1496, American Society for Microbiology, Washington, D. C.
2. Chattopadhyay, D., Pujita, N., and Ishihama, A. (1998) Genes Cells 3, 279–287
3. Touloukhonov, I. I., Shulgina, I., and Hernandez, V. J. (2001) J. Biol. Chem. 276, 5991–5999
4. Stephens, J. C., Arzt, S. W., and Ames, B. N. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 4389–4393
5. Riggs, D., Mueller, R. D., Kwan, H. S., and Arzt, W. S. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 9333–9337
6. Choy, H. (1997) Biochim. Biophys. Acta 1353, 61–68
7. Choy, H. E. (2000) J. Biol. Chem. 275, 6783–6789
8. Zhou, Y. N., and Jin, D. J. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 2908–2913
9. Slominska, M., Neubauer, P., and Wegrzyn, G. (1999) Virology 262, 431–441
10. Giladi, H., Igarashi, K., Ishihama, A., and Oppenheim, A. B. (1992) J. Mol. Biol. 227, 985–990
11. Cashel, M. (1974) Anal. Biochem. 57, 100–107
12. Cashel, M., and Kalbacher, B. (1970) J. Biol. Chem. 254, 2309–2318
13. Shetland, Y., Shifrin, A., Ziv, T., Teff, D., Kobay, S., Kohler, O., and Oppenheim, A. B. (2000) J. Bacteriol. 182, 3111–3116
14. Hoopes, B., and McClure, W. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 3134–3138
15. He, Y. N., and Rosenberg, M. (1986) J. Biol. Chem. 261, 11838–11843
16. Kainz, M., and Roberts, J. W. (1995) J. Biol. Chem. 270, 808–814
17. Barker, M. M., Gaal, T., and Gourse, R. L. (2001) J. Mol. Biol. 305, 689–702
18. Petrynska, K., Wegrzyn, G., and Hernandez, V. J. (2002) J. Biol. Chem. 277, 45785–45791
19. Vogel, U., and Jensen, K. F. (1994) J. Biol. Chem. 269, 16236–16241
20. Buc, H., and McClure W. R. (1985) Biochemistry 24, 2712–2723
21. Lozinski, T., Ellinger, T., and Bujard, H. (2001) Nucleic Acids Res. 29, 3873–3881
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