A Proximal Promoter Element Required for Positive Transcriptional Control by Guanosine Tetraphosphate and DksA Protein during the Stringent Response*\(^{[3]}\)

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Background: ppGpp/DksA regulate genes both positively and negatively during stringency.

Results: Positive control by ppGpp/DksA is linked to RNA polymerase-promoter saturation kinetics dependent on the promoter’s discriminator.

Conclusion: Promoters harboring discriminators that make them easily saturated with RNA polymerase are controlled positively by ppGpp/DksA.

Significance: The discriminator is a cis-acting element dictating the response of the promoter to alterations in ppGpp/DksA and RNA polymerase availability.

The alarmone guanosine tetraphosphate (ppGpp) acts as both a positive and a negative regulator of gene expression in the presence of DksA, but the underlying mechanisms of this differential control are unclear. Here, using uspA hybrid promoters, we show that an AT-rich discriminator region is crucial for positive control by ppGpp/DksA. The AT-rich discriminator makes the RNA polymerase-promoter complex extremely stable and therefore easily saturated with RNA polymerase. A more efficient transcription is achieved when the RNA polymerase-promoter complex is destabilized with ppGpp/DksA. We found that exchanging the AT-rich discriminator of uspA with the GC-rich -P1 discriminator made the uspA promoter negatively regulated by ppGpp/DksA both in vivo and in vitro. In addition, the GC-rich discriminator destabilized the RNA polymerase-promoter complex, and the effect of ppGpp/DksA on the kinetic properties of the promoter was reversed. We propose that the transcription initiation rate from promoters with GC-rich discriminators, in contrast to the uspA-promoter, is not limited by the stability of the open complex. The findings are discussed in view of models for both direct and indirect effects of ppGpp/DksA on transcriptional trade-offs.

Cells of Escherichia coli rapidly inhibit ribosome biosynthesis during transition from exponential growth to stasis \((1,2)\), a response encompassing a swift reduction in rRNA and tRNA biosynthesis \((3)\). This response, called the stringent response of stable RNA synthesis, was first observed during amino acid starvation but is now known to be elicited by a large variety of conditions limiting cellular reproduction \((3)\). The effector molecules of the stringent response are the nucleotides guanosine tetraphosphate and pentaphosphate (collectively referred to as ppGpp)\(^4\) \((4)\) acting together with the small protein DksA \((5,6)\). DksA levels are essentially constant in \(E. \text{coli}\) cells during growth and stasis \((7)\), whereas ppGpp is drastically elevated by conditions causing growth arrest \((4,8,9)\). Two proteins are responsible for ppGpp synthesis as follows: RelA, which is activated during amino acid starvation, and SpoT, activated during other types of starvation and stresses \((3,10–12)\). In contrast to classical transcription factors that bind at or near promoters, DksA and ppGpp regulate transcription by interacting with RNA polymerase (RNAP) without contacting DNA. Specifically, it has been shown in crystals of the \(E. \text{coli}\) RNAP holoenzyme that ppGpp binds in the interface between the \(\beta^{\prime}\) and \(\omega\) subunits of RNAP \((13–15)\), whereas DksA has been structurally positioned in the RNAP secondary channel, \(\geq 30\ \AA\) from the ppGpp-binding site \((16,17)\). The mechanism by which ppGpp and DksA affect transcription is not entirely clear, but they are suggested to repress transcriptional output by affecting different kinetic steps on the pathway to open complex formation \((18)\).

ppGpp/DksA also act as positive effectors of gene expression, and a large number of genes important for maintenance and stress resistance require ppGpp/DksA for their induction during stationary phase and starvation \((19–21)\). Thus, upon growth arrest the rapid increase in ppGpp concentration results in a robust redirection of transcription from proliferation-related genes (e.g., those encoding rRNA, tRNA, and ribosomal proteins) to maintenance-related genes, such as the universal stress proteins, \(uspA\) and \(C-G\) genes \((22–24)\), amino acid biosynthetic genes \((6)\), and genes requiring alternative \(\sigma\) factors for their transcription \((25–27)\). In fact, cells unable to make ppGpp almost completely fail to respond to starvation, as evi

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\(^{4}\) The abbreviations used are: ppGpp, guanosine tetraphosphate; RNAP, RNA polymerase; qPCR, quantitative PCR; Er\(^{\alpha}\), RNA polymerase saturated with \(\sigma^{\alpha}\); \(N_{dsd}\), negative discriminator; \(P_{dsd}\), positive discriminator.
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denced by the proteome of starved ΔrelA ΔspoT double mutants being almost identical to that of exponentially growing cells (28). In other words, the transcriptional trade-off between proliferation and maintenance-related activities normally seen in cells of E. coli is abolished in cells lacking ppGpp.

How ppGpp/DksA regulates the trade-off between proliferation and maintenance, mechanistically, is not fully understood. Promoters that are negatively regulated by ppGpp/DksA, such as rRNA promoters controlling the expression of rRNA, form extremely unstable complexes with RNAP. These unstable complexes are further destabilized by ppGpp/DksA (5, 29, 30). Factors contributing to the short half-life of RNAP-rRNA promoter complexes include suboptimal discriminator sequences (the sequence preceding the transcriptional start point downstream of the −10 region (31)), suboptimal −35 elements, and suboptimal spacing between the −10 and −35 hexamers (32, 33). The destabilization of RNAP-rRNA promoter complexes by ppGpp/DksA observed in vitro might explain why ppGpp/DksA negatively affects transcriptional output from rRNA promoters also in vivo (18, 34). However, all RNAP-promoter complexes studied in vitro, including promoters positively regulated by ppGpp/DksA, are destabilized by the addition of ppGpp/DksA (5, 6, 35, 36). Thus, it has been difficult to explain positive control of transcription by ppGpp/DksA through changes in RNAP-promoter complex stability. Instead, it has been suggested that ppGpp/DksA fail to inhibit the output from promoters that make long-lived complexes because RNAP escapes to the elongation cycle before promoter occupancy declines (18). Alternatively, it was proposed that the positive effect of ppGpp/DksA on promoters induced during the stringent response is indirect and due to a presumed increase in free RNAP polymerase levels resulting from RNAP falling off promoters controlling the expression of rRNA (37). The argument of indirect control is based also on the premise that promoters positively regulated by ppGpp/DksA have an intrinsically low affinity for RNAP and require high concentration of RNAP for transcription (29, 37, 38). Whether RNAP concentrations actually increase during a stringent response and whether promoters positively regulated by ppGpp/DksA have, in general, a low affinity for RNAP is not known. Measurements have shown that the levels of free RNAP are elevated rather than reduced in cells lacking ppGpp (22), and calculations suggest that the free RNAP concentration rises with increasing growth rates, i.e. with decreasing levels of ppGpp (39). Such results are difficult to reconcile with the notion that elevated ppGpp levels would cause an increase in the availability of free RNAP.

The promoters of the usp genes, encoding the universal stress proteins UspA, -C, -D, -E, -F, and -G, are σ70-dependent and strongly regulated by ppGpp in a positive manner (40, 41). In this work, using hybrid uspA promoters, we found that the 5-bp AT-rich discriminator region immediately downstream from the Puspa −10 element is required for positive control by ppGpp/DksA and that this region renders the promoter easily saturated by stabilizing the RNAP-promoter complex. Based on the saturation kinetics data presented, we suggest that the defining character of a promoter positively regulated by ppGpp/DksA is its relatively poor ability to clear out RNAP, ppGpp/DksA, by destabilizing the RNAP-promoter complex, allows RNAP to escape and embark on elongation. In contrast, we propose that the rate of transcription from promoters containing GC-rich discriminators downstream from their −10 element is not limited by the promoter interaction. Thus, a further destabilization by ppGpp/DksA will only have a negative effect on the transcriptional output of such promoters. The data are discussed in view of models for how RNAP availability affects transcriptional trade-offs.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains and Growth Conditions**—Bacterial strains are listed in supplemental Table SI. All promoter-lacZ constructs in this work were incorporated into bacteriophage λRS45 and integrated in the Escherichia coli chromosomal λ att site as described previously (42). Transformation of the E. coli overproduction plasmids (43) and transductions of the ΔdksA, ΔrelA, and ΔspoT alleles were introduced into the different strains by standard methods. Cultures were grown in Erlenmeyer flasks in M9 defined medium supplemented with a limiting concentration of glucose (0.08%), thiamine (10 µM), and all the amino acids in excess (44) at 37 °C. When necessary, antibiotics were used in the following concentrations: carbenicillin 50 µg ml⁻¹, chloramphenicol 30 µg ml⁻¹, and kanamycin 50 µg ml⁻¹.

**Plasmid Construction**—All promoter-lacZ transcriptional fusions were cloned into pTL61-T prior to integration into the E. coli chromosomal λ att site. Mutations within the uspA promoter were performed using Phusion® site-directed mutagenesis kit (Finzymes). The UP-PupspA promoter was constructed such that the −60 to −39 part of the rrnB P1 promoter UP element (45) was fused into the same position upstream of the uspA promoter. A PCR-amplified portion of the uspA promoter region was subcloned into EcoRI and BamHI sites of the in vitro transcription plasmid pTE103 (46) using In-Fusion™ Dry-Down PCR cloning kit (Clontech) creating pBG100-102. The pBG103 plasmid was constructed by GenScript USA Inc. pRLG597 was a kind gift from the Gourse laboratory (47). All plasmids are listed in supplemental Table SII.

**β-Galactosidase Activity Assays**—Relative β-galactosidase levels were determined as described previously (48) with some modifications (49). The β-galactosidase activity is expressed as shown in Equation 1,

\[
\frac{1000 \cdot A_{\text{reaction}}}{A_{420} \cdot \text{reaction time (min)} \cdot \text{sample volume (ml)}}
\]

(Eq. 1)

**DksA Purification**—His-tagged DksA was overexpressed and purified according to Åberg et al. (36), with the exception that the cells were disrupted using a French press.

**RNA Extraction and Quantitative PCR (qPCR)**—Wild type, ΔdksA, and the ΔrelA ΔspoT strains containing promoter-lacZ fusions were grown at 37 °C in M9 defined medium (see above). Samples for RNA extraction were taken in transition to stationary phase (A420 ~ 2.5–3.5). Total RNA was isolated from cultures with RNAsafe protect bacteria mini kit from Qiagen according to the manufacturer’s instructions with subsequent
E. coli moter derivatives were 347 and 220 nucleotides, respectively. The transcript generated from the
uspA promoter upon entry of wild type (Wt), ΔrelA ΔspoT (ppGpp0), and ΔdksA cells into stationary phase. Average induction ratios (stationary phase promoter activity/exponential growth promoter activity) are shown, and error bars represent S.E. (n = 6).

For single-round in vitro transcription used in open complex stability assays, 11 nM ErΔ70 with 200 μM ppGpp and/or 5 μM DksA (or appropriate buffer) was preincubated for 7 min. To determine functional open complexes, preincubated mixtures were added to 1 nM supercoiled plasmid templates and incubated for an additional 10 min to allow open complex formation. Heparin (0.1 mg ml⁻¹) or DNA (100 nM of 197-bp double-stranded consensus promoter DNA (see 5, 51)) was added as competitor, and 8-μl aliquots were removed at the indicated times to tubes containing NTPs (concentrations as above). For the zero time point, the competitor was added as a mixture with NTPs. Reactions were stopped after 15 min with 6× stop/loading buffer, and transcripts were analyzed as described above.

RESULTS

Changing the Promoter Strength of uspA Does Not Affect Positive Regulation by ppGpp and DksA—In previous studies (22, 52), we have analyzed a uspA promoter spanning 227 bp upstream and 163 bp downstream from the transcriptional start site. Here, we constructed a uspA promoter harboring only the four core elements as follows: the −10 region, the −35 region, the spacer region, and the sequence immediately downstream from the −10 element (Fig. 1A). The regulation of this promoter was found to be identical to the wild type promoter and positively controlled by ppGpp and DksA in vivo (Fig. 1, B and C). We conclude that any cis-acting regulatory elements responding to ppGpp and/or DksA must be present within this core promoter region.
The activation of positively regulated promoters by ppGpp (in the presence of DksA) upon the stringent response has been proposed to be indirect and due to an increased concentration of free RNAP holoenzyme (Eσ70) released from stable RNA promoters (37, 38). Furthermore, it is suggested that promoters activated during the stringent response have low affinity for Eσ70 and cannot be activated until sufficient levels of free Eσ70 are available. This model predicts that improving the strength of the uspA promoter with, for example, the consensus sequence for σ70 binding or by fusing an UP-element upstream from the −35 region (Fig. 2A) should lessen the requirement for ppGpp/DksA and reduce the induction ratio during stringency. However, the uspA hybrids constructed harboring consensus −10/−35 sequences or UP elements showed enhanced basal expression in exponential phase but were still dependent on ppGpp and DksA for their induction (Fig. 2, B and C). Reducing the strength of the promoter, e.g. by altering the spacer length from 17 to 18 bp, is in the affinity model predicted to make the promoter even more dependent on ppGpp/DksA (37).

However, this was not the case for the uspA promoter (Fig. 2, B and C). Thus, altering the strength of the uspA promoter did not alleviate or enhance its dependence on ppGpp/DksA or its induction characteristics during entry into the stationary phase.

AT-rich Region Downstream from the −10 Element Is Required for Activation by ppGpp/DksA Both in Vivo and in Vitro—The GC-rich sequence downstream from the −10 element (31), referred to here as a negative discriminator (N_{disc}), is important for negative control by ppGpp/DksA (30, 53, 54). The promoter, PrnB P1, required for rRNA synthesis, harbors such an N_{disc} sequence (Fig. 3A). Less is known about the nucleotide requirements at the corresponding position in promoters positively regulated by ppGpp/DksA. The sequence downstream from the −10 element in the uspA promoter is AT-rich rather than GC-rich (Fig. 3A). We tested if this sequence of PrnB P1 is important for positive regulation by ppGpp/DksA by exchanging this region into an N_{disc} identical to the discriminator of rrrnB P1. Furthermore, we made a hybrid promoter with only the first 5 bp of N_{disc} followed by the wild type uspA sequence to create a mixed discriminator (Fig. 3A, N_{disc}), preserving the length of the authentic P_{disc}. As seen in Fig. 3B, the uspA promoter is induced 4-fold in stationary phase in vivo, but the uspA N_{disc} hybrid totally lost its stationary phase induction as did the uspA mixed discriminator promoter.

To elucidate the effect of ppGpp and DksA on the promoters constructed, we performed multiple-round in vitro transcription assays with super-coiled plasmids harboring the hybrid uspA promoters as templates. In the reconstituted in vitro assay, DksA and ppGpp had a positive synergistic effect on the transcription of PrnB, but this effect was not only abolished but reversed for the uspA harboring the N_{disc} (Fig. 3, C and D). As expected, the expression from rrrnB P1 (containing its authentic N_{disc}) was synergistically affected by ppGpp and DksA in a negative manner (Fig. 3E) (5, 55). The repression of PrnB harboring the N_{disc} seen in vitro was confirmed in vivo by qPCR (Fig. 3F). We conclude that the AT-rich region downstream from the uspA −10 element is necessary for positive control by ppGpp and DksA, and we hereafter call this a positive discriminator (P_{disc}).
this promoter. A further weakening of the interaction between the N\textsubscript{dsc} and the ρ\textsuperscript{70} subunit of RNAP by ppGpp/DksA is anticipated to facilitate negative promoter regulation by destabilizing the RNAP-promoter open complex even more (18, 30). Less is known about the stability of the open complex for promoters harboring a P\textsubscript{dsc} and whether this stability is responsible for positive regulation by ppGpp/DksA. To address this, we measured the decay rates of competitor-resistant RNAP-promoter open complexes for P\textsubscript{uspa-P\textsubscript{dsc}} and compared them with the hybrid variant with an N\textsubscript{dsc}. As shown in Fig. 4A, the P\textsubscript{uspa} promoter has a very stable RNAP-promoter open complex that marginally decayed over the time frame of the experiment. The N\textsubscript{dsc} markedly destabilized this RNAP-promoter complex suggesting that the intrinsic stability of the uspa RNAP-promoter complex is strongly influenced by the discriminator sequence (Fig. 4A). Moreover, the intrinsically unstable rrnB P\textsubscript{1-N\textsubscript{dsc}} RNAP-promoter complex became stabilized when the N\textsubscript{dsc} was swapped for a P\textsubscript{dsc} (Fig. 4B).

We found that ppGpp and DksA alone had very little effect on the stability of the open complex of P\textsubscript{uspa-P\textsubscript{dsc}} but that ppGpp and DksA synergistically destabilize the promoter (Fig. 4C). The open complex stability of the P\textsubscript{uspa-N\textsubscript{dsc}} was difficult to access because of the massive destabilizing effects obtained by DksA, which has previously been shown for the rrnB P\textsubscript{1-N\textsubscript{dsc}} and some r-protein promoters containing the GC-rich sequence downstream from the −10 element (56). Although the addition of ppGpp had a modest negative effect on the stability of the open complex, no transcript from the promoter could be detected when DksA was added alone or together with ppGpp (Fig. 4D). These experiments show that the P\textsubscript{dsc} of the uspa promoter contributes greatly to the stability of the RNAP-promoter complex and that ppGpp and DksA destabilize this complex regardless of whether the promoter harbors a P\textsubscript{dsc} or an N\textsubscript{dsc}.

**Opposite Effects of ppGpp/DksA on the Kinetic Properties of a Promoter with Different Discriminators**—Because ppGpp/DksA destabilize the RNAP-promoter open complex at promoters both negatively and positively affected by ppGpp/DksA, we wondered whether ppGpp/DksA instead might affect the saturation kinetics differentially at N\textsubscript{dsc} and P\textsubscript{dsc} promoters. We tested this in a multiple-round *in vitro* transcription assay with increasing concentrations of RNAP, with or without ppGpp.
and DksA, and the $V_{\text{max}}/K_m$ values were determined to reveal the promoters’ ability to compete for RNAP. For P_{uspA}-P_{dsc}, $V_{\text{max}}/K_m$ values increased almost 2-fold with the addition of ppGpp and DksA (Fig. 5, A and C). This increase is also seen as an increase in $V_{\text{max}}$. By definition, $V_{\text{max}}$ is not limited by promoter binding but indicates the rate at which transcripts are produced when the promoter is constantly occupied with an RNA polymerase. We therefore suggest that an increased $V_{\text{max}}$ indicates an increased overall promoter clearance rate or rather an increased rate in one or more rate-limiting steps on the path leading from a promoter-bound RNA polymerase to promoter clearance. The reverse saturation kinetics was observed for the P_{uspA}-N_{dsc}; without any factors, this promoter was extremely difficult to saturate with RNAP, and the $V_{\text{max}}/K_m$ value decreased 2-fold by the addition of ppGpp and DksA (Fig. 5, B and C). Thus, ppGpp/DksA has the exact opposite effect on the kinetic properties of the P_{dsc} and N_{dsc} promoters, which is consistent with the in vivo behavior of these promoters (Fig. 5D).

In addition to the direct negative effect by DksA and ppGpp on transcription initiation (5, 29, 30, 34), promoters regulated by ppGpp/DksA have been shown also to be regulated “passively” by alterations in RNAP availability (22, 28, 39). Specifically, elevated levels of free RNAP increase relative transcription from rnrB P1 while decreasing transcription from P_{uspA} (22). Decreasing RNAP gave the exact opposite effect (28). Because the uspA-N_{dsc} promoter displayed a higher capacity than the authentic P_{uspA}-P_{dsc} to utilize high levels of RNAP in vitro, we tested whether the P_{uspA}-N_{dsc} promoter responded differently to elevated levels of RNAP in exponential phase also in vivo. This was indeed the case; a 2-fold overproduction of RNAP induced relative expression from the P_{uspA}-Ndsc promoter in exponential growing cells, similar to P_{rrnB}P1, although the P_{uspA}-P_{dsc} was repressed in relative terms as described previously (Fig. 5E) (22).

**DISCUSSION**

In this work, we used P_{uspA} as a model to determine which part of the promoter region is required for positive control by ppGpp/DksA and ppGpp. We hypothesized that exchanging core elements of the uspA promoter with core promoter domains or elements from an rrr promoter could potentially switch the behavior of the promoter to becoming negatively regulated by ppGpp/DksA. Indeed, we found that the 5-bp AT-rich discriminator region (P_{dsc}, AAGGA) immedi-
ately downstream from the −10 element is critical for positive control by ppGpp/DksA and that swapping this region to the 8-bp discriminator of rrnB (Ndsc, GCGCCACC) is sufficient to switch the uspA promoter into being negatively regulated by ppGpp/DksA. In addition, exchanging the Pdsc to the Ndsc rendered the RNAP-promoter complex unstable, and the promoter became difficult to saturate with RNAP, a hallmark of the rrn promoters. ppGpp/DksA destabilized both the RNAP-uspA-Pdsc promoter complex and the RNAP-uspA-Ndsc promoter complex, but the effect of ppGpp/DksA on the kinetic properties of uspA-Pdsc and uspA-Ndsc was the exact opposite. Specifically, uspA-Pdsc and uspA-Ndsc displayed an increased and decreased $V_{\text{max}}/K_{\text{m}}$ value, respectively, upon addition of ppGpp/DksA. Based on these results, we suggest that the defining character of a promoter positively regulated by ppGpp/DksA is its poor ability to clear itself from RNAP and that ppGpp/DksA, by destabilizing the RNAP-promoter complex, somewhat alleviates this “problem” and allows RNAP to escape and embark on elongation. In contrast, promoters containing an Ndsc are already clearing at a close to maximal rate, and a further destabilization by ppGpp/DksA, by allosterically modifying the interaction between RNAP and the Ndsc (34), will negatively affect the transcriptional output of such promoters. This view differs from a previous model (18), which suggests that ppGpp/DksA fail to inhibit the output from promoters that make long-lived complexes because RNAP escapes to the elongation cycle before promoter occupancy declines (18). This notion could potentially explain why

![FIGURE 5. Pdsc and Ndsc elements affect the saturation properties of a promoter in an opposite manner. A, multiple-round in vitro transcription with increasing concentrations of RNA polymerase (0.5, 1.5, 3.75, 7.5, 15, 44, 75, and 150 nM of E70) from the PusA-Pdsc promoter with 5 μM DksA and 200 μM ppGpp (closed circles) or with no addition of factors (open circles). Data curve was fitted to the Michaelis-Menten model. Representative data are shown. B, multiple-round in vitro transcription as in A from the PusA-Ndsc promoter. C, kinetic parameters calculated from three independent experiments of A and B fitted to the Michaelis-Menten model using Origin (Origin Lab), mean ± S.E.D, relative promoter activity upon entry of cells into stationary phase assayed by qPCR. Samples were taken in early stationary phase, in cells harboring promoter lacZ fusions, PusA-Pdsc (open bar), PusA-Ndsc (light gray bar), and PrnB P1-Ndsc (closed bar). Data presented are the log average promoter activity ($\beta$-galactosidase transcript) in wild type cells over the expression in the corresponding ppGpp0 cells with error bars representing S.E. (n ≥3). E, average promoter activity in exponential growing cells with 2-fold overproduction of E70. Promoters tested were PusA-Pdsc (open bar), PusA-Ndsc (light gray bar), and PrnB P1-Ndsc (closed bar). Data presented are the log average promoter activity upon E70 overproduction (expression in cells with overproduced E70 (+1 mM isopropyl 1-thio-β-β-galactopyranoside added) over the expression in cells without overproduction (no isopropyl 1-thio-β-β-galactopyranoside added)) with error bars representing S.E. (n ≥10).]
ppGpp/DksA fail to inhibit transcription from P\text{\textsubscript{dsc}} promoters in contrast to N\text{\textsubscript{dsc}} promoters but cannot account for the positive effect of ppGpp/DksA on the \textit{in vitro} transcription of the former promoters.

With respect to passive regulation by alteration of the availability of free RNAP, the data obtained in this work on the differential saturation characteristics of P\text{\textsubscript{dsc}} and N\text{\textsubscript{dsc}} promoters are in line with a model presented by Jensen and Pedersen (57). This model highlights that promoters of genes whose final products (e.g., ribosomes) are in exceptionally high demand need to exhibit a high maximal clearing capacity. Indeed \textit{rrn} P1 exhibits an exceedingly high maximal velocity of expression and initiates transcription with one of the highest frequencies known (57–59). From this it follows that such promoters require a high RNAP concentration for saturation, and \textit{rrn} promoters are, in this model, predicted to be favored by elevated levels of free RNAP. In line with this model, elevating RNAP levels ectopically has been shown to boost \textit{rrn} expression \textit{in vivo} (22). In contrast, promoters that are saturated already at low concentrations of RNAP, such as \textit{uspA} and the promoter of the amino acid biosynthetic operon \textit{thrABC}, would not be favored by elevated RNAP levels and are, in fact, repressed \textit{in vivo} by an increased availability of RNAP (Fig. 5E) (22). We now link this response of the \textit{uspA} promoter to the nature of the discriminator; the repression of \textit{uspA} upon elevated RNAP levels can be completely reversed by swapping the P\text{\textsubscript{dsc}} to the N\text{\textsubscript{dsc}} (Fig. 5E), which markedly increased the \(V_{\text{max}}\) of the promoter (Fig. 5, A–C). Thus, we believe that the differential clearing capacity of promoters harboring P\text{\textsubscript{dsc}} or N\text{\textsubscript{dsc}} sequences may explain how they respond both to ppGpp/DksA (decreasing the stability of the RNAP-promoter complex) and altered levels of free RNAP.

To what extent the behavior of the \textit{uspA} promoter and its P\text{\textsubscript{dsc}} can be extrapolated to other promoters induced by ppGpp/DksA is presently not known. This class of promoters, including those of amino acid synthesis genes (6) and \textit{fimB} P2 (36), has in common that they form very stable RNAP-promoter open complexes that are destabilized by DksA and/or ppGpp (6, 36). These promoters also harbor AT-rich, rather than GC-rich, regions downstream from their −10 element. However, it has not been determined whether they are saturated at relatively low concentrations of RNAP and whether ppGpp/DksA affects such saturation kinetics. In the case of the \textit{fimB} P2 promoter, DksA enhances the ability of RNAP to bind the promoter \textit{in vitro} while being dispensable for induction \textit{in vivo} (36), suggesting that several mechanisms of DksA-dependent control may act in parallel. In addition, the GreA and GreB proteins, which are structurally similar to DksA and occupy the secondary channel of RNAP (17, 60), can stimulate expression from \textit{fimB} P2 in the absence of DksA (36), indicating that several factors besides DksA and ppGpp can modulate the transcriptional output of this class of promoters. Notably, GreB can compensate for the loss of DksA also in the negative control of the \textit{rrnB} P1 (55).

At present, it is not clear how the P\text{\textsubscript{dsc}} confers such extreme stability of the RNAP-promoter complex. It has recently been shown in a crystal structure of a bacterial RNAP-promoter open complex containing a promoter DNA fragment with a consensus discriminator sequence (GC-rich) that amino acid residues of the \(\sigma\) and the \(\beta\) subunits of RNAP make direct nucleotide contacts with the non-template strand of the discriminator sequence (61). However, some interactions between RNAP and the discriminator were nonspecific with respect to the discriminator nucleotides (61). Thus, it is possible that the AT-rich region of a P\text{\textsubscript{dsc}} displays alternative interaction characteristics that could increase the stability of the RNAP-promoter complex and its sensitivity for positive regulation by ppGpp/DksA. A renewed interest in the discriminator region seems called for as it appears to be a key \textit{cis}-acting element governing the robust and global transcriptional rerouting required for bacterial fitness and survival during transitions from growth- to maintenance-related activities.

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