is better described by the equation:

\[
\text{Rate}_{\text{obs}} = \frac{k_1[H][L]{/}(1+K_1[H]+K_2)}{1+K_1[H]+K_2}
\]

This equation is simplified by choosing conditions wherein Michaelis-Menten kinetics are not followed and the reaction between antibody and diketone are second order. Under these conditions, the value for [1] in the denominator becomes negligible and the equation reduces to a description of a simple titration curve that reflects K, that is, the ionization constant of the essential lysine:

\[
\text{Rate}_{\text{obs}} = \frac{k_1}{1+K_1[H]}
\]

These kinetic arguments follow from D. E. Schmidt Jr. and F. H. Westheimer [Biochemistry 10, 1249 (1971)].

21. At 1.5σ, the density showed the complete residue; and density appeared close to the Lys H93, which could not be refined as a water molecule and might be a cryoprotectant or precipitant molecule that is

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**Transcription Regulation by Initiating NTP Concentration: rRNA Synthesis in Bacteria**

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The sequence of a promoter determines not only the efficiency with which it forms a complex with RNA polymerase, but also the concentration of nucleoside triphosphate (NTP) required for initiating transcription. *Escherichia coli* ribosomal RNA (rRNA) promoters require high initiating NTP concentrations for efficient transcription because they form unusually short-lived complexes with RNA polymerase; high initiating NTP concentrations [adenosine or guanosine triphosphate (ATP or GTP), depending on the rRNA promoter] are needed to bind to and stabilize the open complex. ATP and GTP concentrations, and therefore rRNA promoter activity, increase with growth rate. Because ribosomal RNA transcription determines the rate of ribosome synthesis, the control of ribosomal RNA transcription by NTP concentration provides a molecular explanation for the growth rate–dependent control and homeostatic regulation of ribosome synthesis.

Protein synthesis is the dominant activity of the bacterial cell (1). Ribosome synthesis rates increase approximately with the square of the growth rate to increase protein synthesis at higher growth rates to conserve biosynthetic energy at lower growth rates. The relationship between growth rate and ribosome syntheses rates, referred to as growth rate–dependent control, was described almost 40 years ago and has been the subject of intensive investigation ever since (2, 3). Models have been proposed to explain the phenomenon, but the molecular mechanism or mechanisms responsible have not been determined (4).

Ribosomal RNA (rRNA) transcription is the rate-limiting step in ribosome synthesis, because ribosomal protein synthesis rates are regulated by feedback mechanisms sensitive to the rRNA concentration (5). In each of the seven rRNA operons in *E. coli*, rRNA is transcribed from two promoters, P1 and P2 (Fig. 1A). Most rRNA transcription at moderate to high growth rates originates from the P1 promoters, whose activities increase with growth rate and are thus responsible for regulation (6). Multiple systems affect rRNA promoters. Positive effectors include (i) a promoter upstream (UP) element that increases rRNA P1 activity by binding the α subunit of RNA polymerase (RNAP) (7–9); (ii) a transcription factor, FIS, that binds to sites upstream of the UP element and interacts directly with RNAP (10, 11); and (iii) antitermination factors that bind to the boxA region in the precursor RNA downstream of rRNA P2 and prevent premature transcription termination (12). In addition, a negative effector, ppGpp, inhibits transcription from both rRNA P1 and P2 during amino acid starvation, a phenomenon referred to as the stringent response (13–15). Overlapping mechanisms influencing rRNA transcription have complicated efforts to identify the major system (or systems) contributing to growth rate–dependent control.

Previously, we evaluated the contributions of the above mechanisms to growth rate–dependent control of the rRNA P1 promoter, using promoter or gene mutations to systematically eliminate specific input signals. Transcription from a "minimal" (core)
was no evidence for the binding of po
erate–dependent regulation. However, there
of FIS, the UP element, antitermination that some other mechanism—independent
of RNAP to the rrn B core promoter region (17). Therefore, we considered the possibility that the concentration of nucleoside triphosphates (NTPs), the substrates of RNAP, might serve as a signal that differentiates rrn P1 from other promoters in a manner that changes with growth rate.

Requirement for high concentrations of the initiating NTP for efficient rrn P1 transcription in vitro and in vivo. We used in vitro transcription to test whether varying the concentrations of NTPs, singly or in combination, would affect transcription from rrn P1 promoters differently than from control promoters. Control (RNA I or lacUV5) or rrn B P1 promoters were fused to the plasmid vector at position +1 such that each promoter made a transcript of identical sequence (Fig. 2A) (18, 19). When the concentration of adenosine triphosphate (ATP, the initiating NTP for each promoter) was varied and the concentrations of guanosine, uridine, and cytidine triphosphate (GTP, UTP, and CTP) were kept constant, maximal transcription from rrn B P1 required about 10 times as much ATP as did transcription from control promoters (Fig. 2, B and C) (19). The absolute concentration of ATP required for maximal transcription from rrn B P1 varied with solution conditions, decreasing with increasing salt concentration or on linear (rather than supercoiled) templates. However, the ATP concentration needed for maximal transcription from rrn P1 was greater than for control promoters under all solution conditions (20). Varying the amounts of the other NTPs, individually or together, had no selective effect on rrn B P1 activity (19).

Fig. 1. (A) The rrn B promoter region. Transcripts from promoters P1 and P2 are represented by arrows. DNA regions corresponding to −10 and −35 hexamers (the core promoter), UP element, FIS binding sites, and the BoxA antitermination region are indicated. (B) P1 core promoter sequences (−41 to +6) from the rrn B and rrn D operons. The −10 and −35 hexamers and the transcription start sites are underlined.

Six of the seven E. coli rrn P1 promoters begin transcription with ATP, but the rrn D P1 transcript starts with GTP (Fig. 1B). Maximal transcription of rrn D P1 in vitro was not selectively affected by varying ATP, UTP, or CTP concentrations, but was highly sensitive to GTP concentration (Fig. 2D) (18, 19). Moreover, substitution of G for A at position +1 of rrn B P1 also resulted in a requirement for high GTP, rather than ATP, concentrations (19). Thus, the concentration of the initiating NTP, rather than ATP concentration per se, affects the transcription efficiency of rrn P1 promoters in vitro.

To address whether variation in NTP concentration could account for rrn P1 regulation in vivo, we cultured cells in media supporting different growth rates and analyzed them for NTP content by reversed-phase ion pair high-pressure liquid chromatography (HPLC) (21). ATP and GTP concentrations increased by a factor of about 4 when growth rate increased by a factor of 2 (Fig. 3A) (22), correlating with the increase in rrn P1 promoter activity observed from an rrn B P1 promoter fused to lacZ in the same cells (Fig. 3B).

This correlation suggested but did not prove that the increase in purine nucleotide concentrations with growth rate is responsible for regulation of rrn P1 transcription in vivo, because NTP concentrations could be saturating even at low growth rates (22). Therefore, we uncoupled purine NTP concentrations from growth rate by partially starving cells for pyrimidines, which reduces UTP and CTP concentrations (and growth rate) but increases the amounts of ATP and GTP (23–25). Under these conditions, rrn B P1 transcription increased with the ATP concentration rather than with the growth rate (Fig. 3, C and D). This observation indicated that the concentrations of purine NTPs, rather than the growth rate per se, regulate rrn P1 promoter activity in vivo.

Stabilization of rrn P1 open complexes by the initiating NTP. During transcription initiation, RNAP (R) forms a binary “closed” complex (RP) with the promoter (P), isomerizes to form an “open” complex (RP_1) in which the double-stranded DNA in the vicinity of the transcription start site is melted, and ultimately binds the initiat-

Fig. 2. Effect of ATP or GTP concentration on transcription of rrn P1 promoters in vitro. (A) Plasmid DNA templates containing different promoters made the same 170-nucleotide (nt) transcript terminated at rrn B T1 (18). (B) Transcription was performed at increasing ATP concentrations with constant GTP, UTP, and CTP (18), using either an rrn B P1 promoter template (left panel) or a control promoter template (RNA I, right panel). The 170-nt transcripts are indicated. The 108-nt RNA I transcript, originating from the plasmid’s native RNA I promoter, is visible below the experimental transcript (7, 18). (C) The relative amounts of transcript from Fig. 2B (rrn B P1), cloned RNA I promoter, or at each ATP concentration are expressed as a fraction of the plateau value [1.00 (18)]. (D) Transcription from rrn D P1 (18, 19) in the presence of varying GTP and constant ATP, UTP, and CTP (18), or with varying ATP and constant GTP, UTP, and CTP (18). Data from representative experiments are shown in (B) to (D); each experiment was performed at least three times, and differences in the apparent K_m for ATP or GTP were <5%.

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ing nucleotide: \( R + P \rightarrow R_P \rightarrow R_P \rightarrow R_P_{\text{NTP}} \) (26). At most characterized promoters, \( R_P \) is relatively stable, with a half-life of 30 min to several hours under typical conditions in vitro (27). However, open complexes at \( rrrnP1 \) promoters are exceptionally unstable (28–30), with half-lives generally one to two orders of magnitude shorter than those at more typical promoters under comparable solution conditions. At \( rrrnP1 \) promoters, initiating NTP concentration dependence and open complex stability are strongly affected by salt concentration and template conformation in vitro (20, 28–30), which suggests that the requirement for high NTP concentrations might be related to complex instability.

Direct evidence for a role of NTPs in stabilizing \( rrrnP1 \) promoter–RNAP complexes was obtained by measuring the half-life of complexes in the presence and absence of the initiating NTP (Fig. 4) (31, 32). At \( rrrnB \) \( P1 \), which initiates with ATP, 2 mM ATP increased the half-life of the complex by a factor of about 3 (Fig. 4A), whereas GTP had little or no effect. At \( rrrnD \) \( P1 \), which initiates with GTP, 2 mM GTP (but not ATP) increased the half-life, again by a factor of about 3 (Fig. 4B). These data suggest that the initiating NTP concentration influences the fraction of \( rrrnP1 \) promoters present as open complexes, thereby affecting the amount of transcription.

Mutations that alter growth rate–dependent regulation were identified in the \( rrrnB \) \( P1 \) promoter (16) and in \( rpoB \) and \( rpoC \), encoding the \( \beta \) and \( \beta' \) subunits of RNAP (33). The properties of complexes formed with the mutant promoter or the mutant RNAPs confirmed the importance of NTP concentration and open complex stability for \( rrrnP1 \) regulation in vivo. A single base substitution at position –1 in \( rrrnB \) \( P1 \) (\( rrrnB \) \( P1 \) C-1T) that resulted in high transcription at all growth rates (Fig. 5A) (16) drastically altered the ATP concentration dependence of the promoter: Maximal transcription of the mutant promoter required about one-tenth the amount of ATP required for maximal transcription of the wild-type promoter in vitro (Fig. 5B) (34). Furthermore, the complex containing the mutant promoter was about 5.5 times as

![Fig. 3. \( rrrnB \) \( P1 \) promoter activity correlates with ATP concentration in vivo. (A) ATP and GTP were measured by HPLC from cultures of RLG3492 with different growth rates (doublings per hour) (21). The concentration of ATP (●) differed from the concentration of GTP (○) at each growth rate, but the relative increase in concentration between the lowest and highest growth rates was almost identical for the two nucleotides. (B) \( rrrnB \) \( P1 \) promoter activity ([\( \beta \)-galactosidase units] from an \( rrrnB \) \( P1 \) promoter–\( lacZ \) fusion (27)) in the cultures used in (A). (C) ATP concentration and (D) \( rrrnB \) \( P1 \) promoter activity in RLG3493, a car::Tn10 derivative of RLG3492, at different growth rates (25). Symbols in each panel represent averages of three different samples of two different cultures for each growth rate.

![Fig. 4. Stabilization of \( rrrnP1 \) promoter–RNAP complexes by initiating NTPs in vitro. Complexes of \( rrrnB \) \( P1 \) (A) or \( rrrnD \) \( P1 \) (B) were formed with RNAP in the presence of ATP (●) or GTP (○), or in the absence of NTPs (control; ○). Symbols represent the fraction of complexes remaining at times after heparin addition (32). A representative experiment is shown, but differences between observed half-lives in the presence or absence of the initiating NTPs were highly reproducible (≤10% error).

![Fig. 5. Effects of promoter or RNAP mutations on growth rate–dependent regulation, NTP concentration dependence, and open complex stability. (A) Activities (in \( \beta \)-galactosidase units) of the \( rrrnB \) \( P1 \) (–46; +1) wild-type (RLG2663) or C-1T mutant (RLG2665) promoters were measured using promoter–\( lacZ \) fusions in cultures grown at different growth rates (16). (B) In vitro transcription of wild-type or C-1T mutant \( rrrnB \) \( P1 \) promoters with wild-type RNAP at different ATP concentrations (34). Promoter activities are expressed as fractions of the plateau values (1.0). (C) Decay of open complexes containing wild-type RNAP or wild-type or C-1T mutant \( rrrnB \) \( P1 \) promoters. Decay curves depict the fraction of open complexes remaining at different times after heparin addition, as described for Fig. 4 (32, 35). (D) Growth rate–dependent regulation was measured as in (A), using strains with wild-type RNAP (RLG3950) or \( rpoC \Delta 215–220 \) mutant RNAP (RLG3951), and an \( rrrnB \) \( P1 \) (–61 to +50) promoter–\( lacZ \) fusion (33). Because the promoter–\( lacZ \) fusions used to monitor transcription activity are different in (A) and (D), the absolute activities should not be compared directly (32). (E) In vitro transcription with wild-type or \( rpoC \Delta 215–220 \) RNAPs and the wild-type promoter at different ATP concentrations (34). (F) Decay of open complexes containing wild-type \( rrrnB \) \( P1 \) promoter (–61 to +50) and wild-type or \( rpoC \Delta 215–220 \) mutant RNAP. The lower salt concentration used in (F) resulted in a slightly slower decay rate for the promoter–wild-type RNAP complex than was observed in (C) (35).
stable as the wild-type complex (Fig. 5C) (34). The simplest interpretation of these results is that the mutation allows high rrnB P1 activity at low growth rates, because the promoter is transcribed efficiently even at low ATP concentrations.

A deletion of amino acids 215 to 220 in the RNA polymerase subunit (rpoCA215–220) resulted in low activity of rrnB P1 promoters lacking FIS sites at all growth rates (Fig. 5D) (33). Relative to wild-type RNA polymerase, the mutant RNA polymerase required 8 to 11 times as much ATP for comparable rrnB P1 transcription in vitro (Fig. 5E), but not for RNA I transcription (33). Moreover, rrnB P1–mutant RNA polymerase complexes were about 8% as stable as wild-type complexes (Fig. 5F) (35). These data suggest that rrn P1 expression in rpoCA215–220 mutant strains is altered because the NTP concentrations present even at the highest growth rates are insufficient to stabilize rrn P1 open complexes.

Model for homeostatic control of ribosome synthesis by NTP sensing. These data support a model (Fig. 6) in which purine NTP pools control the rate of rRNA transcription—and thereby the rate of ribosome synthesis and the amount of translation—by stabilizing rrn P1–RNA polymerase complexes in vivo. Intracellular ATP and GTP concentrations are determined by their rates of synthesis and consumption; synthesis rates are determined by nutritional conditions (which influence the efficiency of fermentation and respiration), and consumption is determined to a large extent by the amount of protein synthesis [ATP for amino acid biosynthesis and tRNA charging; GTP for tRNA binding to the ribosome and ribosome translation (36)]. Transient imbalances between NTP generation and consumption thus create a feedback signal to readjust the RNA synthesis rate to the translation rate and the nutritional state of the cell.

Many previous observations are consistent with the predictions of this model. Conditions that inhibit translation—for example, chloramphenicol treatment or mutations in the translation apparatus, which would be expected to reduce the drain on purine NTP pools—result in overproduction of rRNA (37, 38). Conversely, conditional mutants of the glycolytic enzyme fructose 1,6-diphosphate aldolase have reduced amounts of ATP at the restrictive temperature, which might explain the observed transcription inhibition of rrn P1 promoters (39).

The model also provides a molecular explanation for the feedback control of rRNA synthesis previously observed. Overall rRNA expression remains relatively constant in situations that might be expected to perturb it (3). For example, total rRNA transcription remains roughly the same when cells contain as few as 4 or as many as 21 functional rRNA operons (38, 40), when rrn antitermination is defective because of rts mutations (41), or when rrn P1 transcription activation is defective because of fis or rpoA mutations (7, 10). In addition, transcription from rrn P1 promoters is decreased when cells overproduce rRNA from a PL promoter (42). The adjustments in rrn P1 promoter activity in each of these situations can be attributed to over- or underproduction of translating ribosomes, resulting in changes in ATP and GTP pools.

Mechanism of NTP sensing by rrn P1 promoters. The effect of purine NTP concentration on rrn P1 promoter activity involves stabilization of the RNA polymerase–rrn complex. NTPs are the substrates of transcription; however, the initiating NTP concentration, because they are subject to growth rate–dependent regulation, make unstable open complexes, or share other characteristics with rrnB P1 (38, 45). Initiating NTP concentrations potentially could affect any promoter whose expression is limited by the stability of its open complex with RNA polymerase and is poised such that physiologically relevant NTP concentrations could affect its lifetime. Regulation of rRNA transcription by purine NTP concentration apparently is not limited to bacteria: ATP and GTP pools control mammalian rRNA synthesis as well, although the mechanism responsible is not understood (46).

Nucleotide concentrations affect the expression of many operons by mechanisms different from that reported here for rrn P1 promoters. For example, changes in amounts of pyrimidine NTPs can alter expression of pyrimidine biosynthesis and salvage operons by affecting start site selection, reiterative transcription, transcription elongation, transcription attenuation, and translation initiation efficiency (24, 44, 47). Adenine nucleotides modulate transcription by phosphorylation or dephosphorylation of components of transcription complexes (48). Adenine nucleotides have also been proposed to affect anti–σ factor function and thereby control transcription by at least two RNA holoenzymes in Bacillus subtilis (49).

Overlap in rRNA regulation mechanisms. The NTP-sensing mechanism need
not account for all aspects of rRNA regulation. Ribosomal RNA promotors integrate multiple input signals: RNAP $\alpha$ and $\sigma$ subunit interactions with promoter DNA, FIS, ppGpp, antitermination factors, and rrn P2 all contribute to rRNA synthesis in vivo (3), and regulatory mechanisms affecting rRNA expression may partially overlap. For example, deletion of the fis gene does not decrease overall rRNA transcription or alter growth rate–dependent regulation, because there are compensating increases in rrn P1 core promoter activity, presumably through feedback signaling involving the NTP-sensing mechanism described above (10). Conversely, RRNP mutations (such as $\tau$ppGpp)$\Delta 215$–220; Fig. 5) that decrease rrn P1 core promoter activity are compensated for, in part, by FIS: FIS activates the $\beta'$ mutant RNAP more than wild-type RNAP and restores growth rate–dependent regulation (33, 50), because FIS concentrations vary with nutritional conditions (17, 51).

Overlapping regulatory mechanisms appear to be an intrinsic feature of rRNA synthesis, perhaps because of the central role played by rRNA transcription in cell physiology. Additional systems may also contribute to rRNA regulation, either independently or by influencing the NTP-sensing or FIS-dependent activation mechanisms. In fact, any condition that alters rrn P1 promoter–RNAP stability could potentially play a role in rRNA regulation (52). For example, the mediator of stringent control, ppGpp, which is produced in large amounts after amino acid starvation, inhibits transcription by decreasing the half-life of the rrn P1 open complex (19, 50, 53), perhaps making the complex unable to be “rescued” by normal intracellular NTP concentrations. ppGpp is dispensable for transcription from the $\delta 9$ promoter (–60 to +1), but even the low concentrations of ppGpp $\ldots$ 16, 59
dissociate at the same position in the vector as the rrn P1 or lacUV5 promoters or at the RNA I promoter’s natural position in the plasmid (Fig. 2B). In Fig. 2B, the plasmid template containing the rrn P1 promoter (–60 to +10) inserted into pRLG770 (pRLG3426) yielded identical results. Identical results were obtained with plasmids pRLG3286 (ppGpp (–60 to +1) (19)). Reactions contained 5 mM 1.25 mM GTP, 200 mM UTP, 10 mM ATP, 10 mM CTP, and 5 M Ci of [32P]CTP, or 5 M to 1.25 mM ATP, 200 mM UTP, 200 mM ATP, 200 mM CTP, and 5 M Ci of [32P]CTP.


20. The concentration of ATP required for half-maximal transcription of rrn P1 (apparent $K_{m}$) on a supercoiled template ranged from about 5 mM at 30 mM KC1 to more than 1500 mM at 200 mM KC1. On a linear template, the apparent $K_{m}$ for ATP was about 500 mM at 100 mM KC1.

21. NTPs were measured in PGL3492, a derivative of VH1000 with a lambda prohagone containing an rrn B P1 (–46 to +1) promoter–lacZ fusion (16). VH1000 is a lac–lacZ–pyrE– derivative of MG1655 (J. B. Bact.

15. M. Cashel, D. G. Frankel, C. E. Squires and R. L. Gourse, J. Bacteriol. 175, 1421 (1993). Cells were grown in C medium [M. Aper and B. Ames, J. Bacteriol. 133, 149 (1978) containing 0.4 mM MgSO4 and 0.4% sucrose, glycerol, or glucose in the absence or presence of all NTPs (200 mM/ml).


18. Multiple-round transcription was performed at 22°C essentially as described (49). Plasmid templates were derivatives of pRLG770 (10), with either the rrn P1 promoter (–46 to +1, where +1 is the transcription start site) (pRLG2294), RNA I promoter (–61 to +1 (pRLG3565), or lacUV5 promoter (–46 to +1) (pRLG3422), inserted 170 bp upstream of the mtrB terminator. In Fig. 2B and C, 25 µl reaction volumes contained 0.2 mM supercoiled DNA template, 40 mM tris–HCl, pH 7.9, 0.05 mM MgCl2, bovine serum albumin (BSA) at 0.1 mg/ml, 170 mM KC1, 1 mM dlthiostrepton (DTT), 5 µM to 1.25 mM ATP, 10 mM CTP, 200 mM UTP (phosphoril, HPLC pure), and 5 M Ci of [32P]CTP (DuPont). Reactions were initiated by the addition of 4 nM RNAP and terminated after 15 min, as described (10). Samples were subjected to electrophoresis on 5.5% polyacrylamide–7 M urea gels, which were dried, visualized, and then quantified by phosphorimaging (ImageQuant software, Molecular Dynamics). Transcription of the RNA I promoter did not vary with ATP concentrations, perhaps because of the central role played by rRNA transcription in cell physiology. Additional systems may also contribute to rRNA regulation, either independently or by influencing the NTP-sensing or FIS-dependent activation mechanisms. In fact, any condition that alters rrn P1 promoter–RNAP stability could potentially play a role in rRNA regulation (52).

23. Identical results were obtained with plasmids pRLG3286 (ppGpp (–60 to +1) (19)). Reactions contained 5 mM 1.25 mM GTP, 200 mM UTP, 10 mM ATP, 10 mM CTP, and 5 M Ci of [32P]CTP, or 5 M to 1.25 mM ATP, 200 mM UTP, 200 mM UTP, 10 mM CTP, and 5 M Ci of [32P]CTP.

Promoter-RNAP complex stability was measured on plasmids pRLG2294 and pRLG2295 (S4) as described in (32), except that no NTPs were included until after heparin addition. In Fig. 5G, reactions contained 40 mM tris-acetate (pH 7.9), 10 mM MgCl2, BSA (0.1 mg/ml), 1 mM DTT, and 30 mM KCl, with 2 mM ATP or GTP (Pharmacia, HPLC pure) as indicated for maximal transcription. The amount of ATP for maximal transcription initiation, only slightly more ATP than was needed by the RNA I promoter at each solution condition (19).

In Fig. 5G, stability of wild-type and mutant promoter-RNAP complexes were measured on plasmids pRLG2294 and pRLG2295 (S4) as described in (32), except that no NTPs were included until after heparin addition. In Fig. 5F, reactions contained 40 mM tris-acetate (pH 7.9), 10 mM MgCl2, BSA (0.1 mg/ml), 1 mM DTT, 10 mM KCl, 5 mM NTP, and 3 mM plasmid DNA, and the NTP mixture added after heparin addition contained 4 mM ATP, 20 mM GTP and UTP, and 10 mM [α-32P]UTP. Plasmid DNA in Fig. 5F (pRLG598) contained the mP1 (–88 to +50) promoter. The mutant RNAP also made more stable complexes with the RNA promoter, indicating that the mutation does not identify a site of interaction with DNA specific to mP1 promoters (33).


42. The top (nontemplate) strand base is indicated, but either the top or bottom strand could be the crucial determinant.


49. This probably explains why the growth rate regulation–defective mutant RNAPs are not lethal to the cell and reduce the growth rate by only about 25%.


51. Because salt concentration and degree of supercoiling modulate mRNA transcription efficiency in vitro (28, 29), it is possible that changes in the internal ionic environment or in chromosomal supercoiling could contribute to regulation of RNA transcription. However, the media used in our studies to vary growth rates did not systematically alter the ionic environment, and changes in the external ionic environment have much less effect on protein-DNA interactions in vivo than observed in vitro (S. Cayley, B. Lewis, H. Gutman, M. T. Record Jr., J. Mol. Biol. 222, 281 (1991). Similarly, the extent of supercoiling does not correlate in a systematic manner with growth rate (V. L. Balke and J. D. Gralla, J. Bacteriol. 169, 4399 (1987)).


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