is better described by the equation:

$$\text{Rate}_{obs} = k_2[\mathbf{1}]/(1+[H^+]/K_2)$$

$$\frac{K(1+[H^+]/K_1)}{(1+[H^+]/K_2)} \cdot [\mathbf{1}]$$

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_1, that is, the ionization constant of the essential lysine:

$$\text{Rate}_{obs} = \frac{(k_2/K)[\mathbf{1}]}{(1+[H^+]/K_1)}$$

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

14. P. Y. Bruice, *J. Am. Chem. Soc.* **112**, 7361 (1990).
15. P. A. Frey, F. C. Kokesh, F. H. Westheimer, *ibid.* **93**, 7266 (1971); F. C. Kokesh and F. H. Westheimer, *ibid.*, p. 7270; L. A. Highbarger, J. A. Gerlt, G. L. Kenyon, *Biochemistry* **35**, 41 (1996).
16. J. K. Lee and K. N. Houk, *Science* **276**, 942 (1997); S. Daopin, D. E. Anderson, W. A. Baase, F. W. Dahlquist, B. W. Matthews, *Biochemistry* **30**, 11521 (1991).
17. X-ray crystallographic programs and methods: (i) HKL (DENZO and SCALEPACK): Z. Otwinowski and W. Minor, *Methods Enzymol.* **276**, 307 (1997); (ii) MERLOT: P. M. D. Fitzgerald, *J. Appl. Crystallogr.* **21**, 273 (1988); (iii) AMORE: J. Navaza, *Acta Crystallogr.* **A50**, 157 (1994); (iv) Program O: T. A. Jones, J.-Y. Zou, S. W. Cowan, M. Kjeldgaard, *ibid.* **A47**, 110 (1991); (v) X-PLOR: A. T. Brünger, J. Kuriyan, M. Karplus, *Science* **235**, 458 (1987); A. T. Brünger, X-PLOR, *Version 3.1: A System for X-ray and NMR* (Yale Univ. Press, New Haven, CT, 1992); (vi) SHELXL: G. M. Sheldrick, SHELXL-96, Program for crystal structure refinement, Göttingen, 1996 (VII). Procheck: R. A. Laskowski, M. W. MacArthur, S. D. Moss, J. M. Thornton, *J. Appl. Crystallogr.* **26**, 283 (1983).
18. L. W. Guddat, L. Shan, J. M. Anchin, D. S. Linthium, A. B. Edmundson, *J. Mol. Biol.* **236**, 247 (1994).
19. R. L. Stanfield, T. M. Fieser, R. A. Lerner, I. A. Wilson, *Science* **248**, 712 (1990).
20. J. A. Arevalo, E. A. Stura, M. J. Taussig, I. A. Wilson, *J. Mol. Biol.* **231**, 103 (1993).
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 partially occupied. However, the density gave no clear indication at this resolution as to its identity.
22. J. S. Richardson and D. C. Richardson, in *Prediction of Protein Structure and The Principles of Protein Conformation*, G. D. Fasman, Ed. (Plenum, New York, 1990), p. 1.
23. I. A. Wilson and R. L. Stanfield, *Curr. Opin. Struct. Biol.* **4**, 857 (1994).
24. The depth of the binding pocket was estimated from measurement of the following: Lys^{H93}Nζ-Thr^{L92}Cα 11.1 Å, Lys^{H93}Nζ-His^{L93}Cα 11.6 Å, Lys^{H93}Nζ-Tyr^{H97}Cα 12 Å.
25. D. R. Davies, E. A. Padlan, S. Sheriff, *Annu. Rev. Biochem.* **59**, 439 (1990); L. C. Hsieh-Wilson, P. G. Schultz, R. C. Stevens, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 5363 (1996).
26. E. A. Kabat *et al.*, Eds, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, ed. 5, 1991).
27. G. W. Zhou, J. Guo, W. Huang, R. J. Fletterick, T. S. Scanlan, *Science* **265**, 1059 (1994).
28. R. L. Brady *et al.*, *J. Mol. Biol.* **227**, 253 (1992).
29. J. Hine and F. A. Via, *J. Am. Chem. Soc.* **94**, 190 (1972); C. Hansch and E. Coats, *J. Pharmacol. Sci.* **59**, 731 (1970); J. Fastrez and A. R. Fersht, *Biochemistry* **12**, 1067 (1973); A. Leo, C. Hansch, D. Elkins, *Chem. Rev.* **71**, 525 (1971).
30. W. P. Jencks, in *Catalysis in Chemistry and Enzymology*, A. Meister, Ed. (Dover, Mineola, NY, 1975).
31. J. R. Knowles, *Nature* **350**, 121 (1991).
32. G. J. Wedemayer, P. A. Patten, L. H. Wang, P. G. Schultz, R. C. Stevens, *Science* **276**, 1665 (1997).
33. G. Hester *et al.*, *FEBS Lett.* **292**, 237 (1991).
34. J. L. Reymond and Y. Chen, *J. Org. Chem.* **60**, 6970 (1995).

35. We thank W. Gilbert and A. Eschenmoser for helpful discussions; the Stanford Synchrotron Radiation Laboratory for data collection time; the staff of beamline 7-1 for their support; G. Fieser, R. Fuller, D. Kubitz, and R. Stefanko for technical assistance; M. Pique for production of figures; and the Alexander von Humboldt Foundation, Germany, for a Feodor Lynen Fellowship (T.H.). Supported by NIH CA27489 (R.A.L., I.A.W., C.F.B.). Coordinates and structure factors for the Fab' 33F12 have been deposited with Brookhaven Data Bank, with PDB ID code 1axt for the coordinate entry.

14 July 1997; accepted 9 November 1997

Transcription Regulation by Initiating NTP Concentration: rRNA Synthesis in Bacteria

Tamas Gaal, Michael S. Bartlett, Wilma Ross, Charles L. Turnbough Jr., Richard L. Gourse*

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 (*rrn* P1) 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 *rrn* P1 promoter] are needed to bind to and stabilize the open complex. ATP and GTP concentrations, and therefore *rrn* P1 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 and to conserve biosynthetic energy at lower growth rates. The relation between growth rate and ribosome synthesis rate, 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 *rrn* 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 transcription by *rrn* P1 promoters. Positive effectors include (i) a promoter upstream (UP) element that increases *rrn* 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 *rrn* P2 and prevent premature transcription termination (*12*). In addition, a negative effector, ppGpp, inhibits transcription from both *rrn* P1 and *rrn* 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 *rrnB* P1 promoter, using promoter or gene mutations to systematically eliminate specific input signals. Transcription from a "minimal" (core)

T. Gaal, M. S. Bartlett, W. Ross, and R. L. Gourse are in the Department of Bacteriology, University of Wisconsin, 1550 Linden Drive, Madison, WI 53706, USA. C. L. Turnbough Jr. is in the Department of Microbiology, University of Alabama, Birmingham, AL 35294, USA.

*To whom correspondence should be addressed. E-mail: rgourse@bact.wisc.edu

rrnB P1 promoter (lacking *rrnB* sequences upstream of −41 with respect to the transcription start site, +1) (Fig. 1B) was growth rate–dependent even in strains unable to make ppGpp (*16*), which implied that some other mechanism—independent of FIS, the UP element, antitermination factors, or ppGpp—is responsible for growth rate–dependent regulation. However, there was no evidence for the binding of potential regulatory proteins (other than RNAP) to the *rrnB* P1 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 *rrnB* 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 *rrnB* 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 *rrnB* P1 varied with solution conditions, increasing with increasing salt concentration or on linear (rather than supercoiled) templates. However, the ATP concentration needed for maximal transcription from *rrnB* 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 *rrnB* P1 activity (*19*).

Six of the seven *E. coli rrn* P1 promoters begin transcription with ATP, but the *rrnD* P1 transcript starts with GTP (Fig. 1B). Maximal transcription of *rrnD* 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 *rrnB* 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 *rrnB* 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, *rrnB* 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_c) with the promoter (P), isomerizes to form an "open" complex (RP_o) in which the double-stranded DNA in the vicinity of the transcription start site is melted, and ultimately binds the initiat-

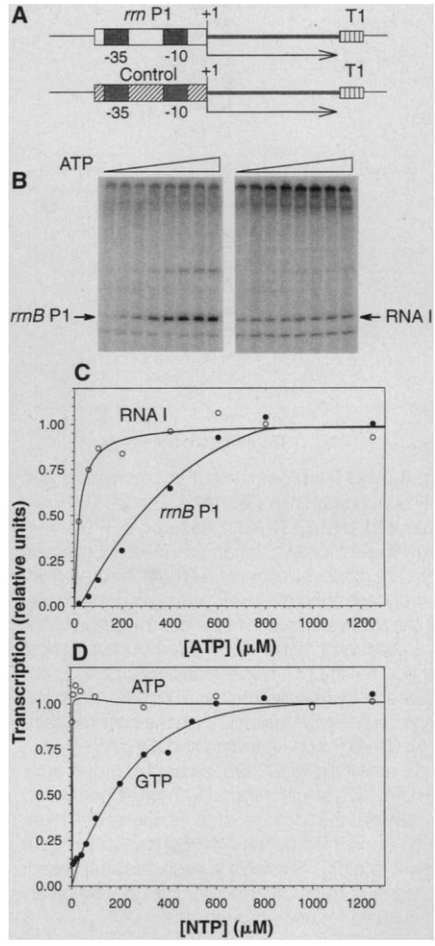

Fig. 2. Effect of ATP or GTP concentration on transcription of *rrn* P1 promoters in vitro. (**A**) Plasmid DNA templates containing different promoters made the same 170-nucleotide (nt) transcript terminated at *rrnB* T1 (*18*). (**B**) Transcription was performed at increasing ATP concentrations with constant GTP, UTP, and CTP (*18*), using either an *rrnB* 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 (*rrnB* P1, ●; cloned RNA I promoter, ○) at each ATP concentration are expressed as a fraction of the plateau value [1.00 (*18*)]. (**D**) Transcription from *rrnD* P1 (*18, 19*) in the presence of varying GTP and constant ATP, UTP, and CTP (●), or with varying ATP and constant GTP, UTP, and CTP (○). 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%.

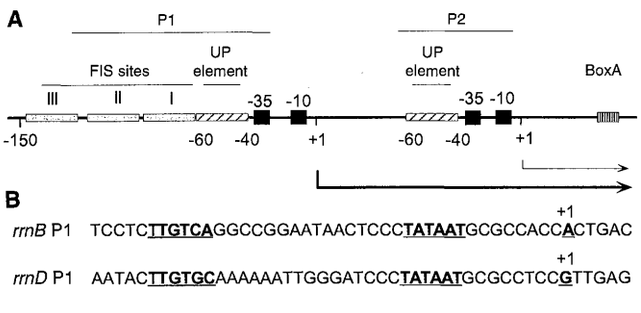

Fig. 1. (**A**) The *rrnB* promoter region. Transcripts from promoters P1 and P2 are represented by arrows. DNA regions corresponding to −10 and −35 hexamers (the core promoter), UP elements, FIS binding sites, and the BoxA antitermination region are indicated. (**B**) P1 core promoter sequences (−41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

ing nucleotide: $R + P \rightleftharpoons RP_c \rightleftharpoons RP_o \rightleftharpoons RP_{NTP}$ (*26*). At most characterized promoters, RP_o is relatively stable, with a half-life of 30 min to several hours under typical conditions in vitro (*27*). However, open complexes at *rrn* P1 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 *rrn* P1 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 *rrn* P1 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 *rrnB* 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 *rrnD* 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 *rrn* P1 promoters present as open complexes, thereby affecting the amount of transcription.

Mutations that alter growth rate–dependent regulation were identified in the *rrnB* P1 promoter (*16*) and in *rpoB* and *rpoC*, encoding the β and β′ 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 *rrn* P1 regulation in vivo. A single base substitution at position –1 in *rrnB* P1 (*rrnB* 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

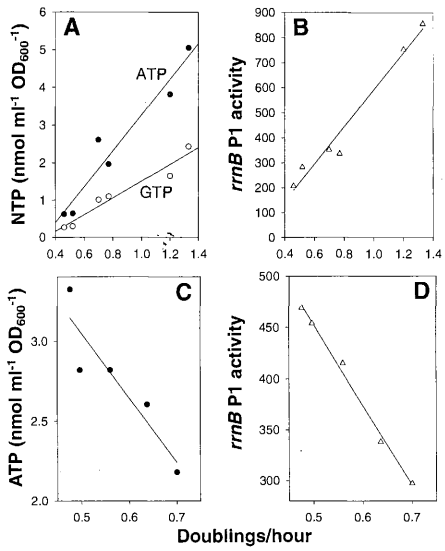

Fig. 3. *rrnB* P1 promoter activity correlates with ATP concentration in vivo. (**A**) ATP and GTP were measured by HPLC from cultures of RLG3492 with different growth rates (doublings per hour) (*21*). The concentration of ATP (●) differed from the concentration of GTP (○) at each growth rate, but the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

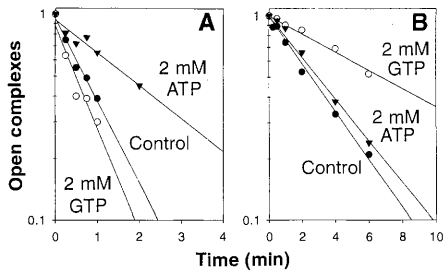

Fig. 4. Stabilization of *rrn* P1 promoter–RNAP complexes by initiating NTPs in vitro. Complexes of *rrnB* P1 (**A**) or *rrnD* P1 (**B**) were formed with RNAP in the presence of ATP (▼) or GTP (○), or in the absence of NTPs (control; ●). Symbols represent the fraction of complexes remaining at times 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).

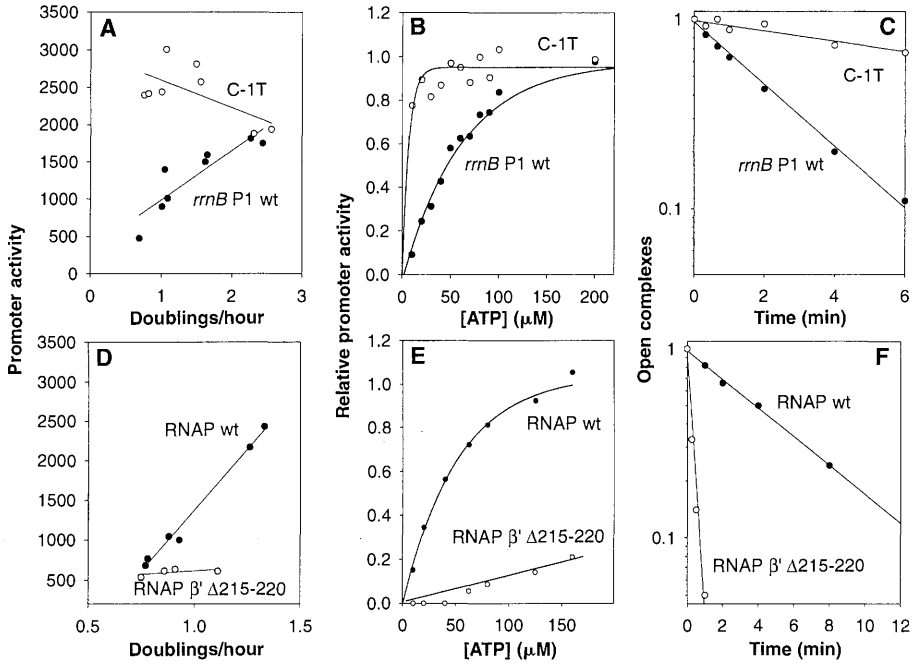

Fig. 5. Effects of promoter or RNAP mutations on growth rate–dependent regulation, NTP concentration dependence, and open complex stability. (**A**) Activities (in β-galactosidase units) of the *rrnB* P1 (–46, +1) wild type (RLG2663) or C-1T mutant (RLG2665) promoters were measured using promoter-*lacZ* fusions in cultures grown at different growth rates (*16*). (**B**) In vitro transcription of wild-type or C-1T mutant *rrnB* 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 *rrnB 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 *rpoCΔ215–220* mutant RNAP (RLG3951), and an *rrnB* 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 (*8*). (**E**) In vitro transcription with wild-type or *rpoCΔ215–220* RNAPs and the wild-type promoter at different ATP concentrations (*34*). (**F**) Decay of open complexes containing wild-type *rrnB* P1 promoter (–61 to +50) and wild-type or *rpoCΔ215–220* 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 RNAP β′ subunit (*rpoCΔ215–220*) resulted in low activity of *rrnB* P1 promoters lacking FIS sites at all growth rates (Fig. 5D) (*33*). Relative to wild-type RNAP, the mutant RNAP 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 RNAP complexes were about 8% as stable as wild-type complexes (Fig. 5F) (*35*). These data suggest that *rrn* P1 expression in *rpoCΔ215–220* 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–RNAP 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 translocation (*36*)]. Transient imbalances between NTP generation and consumption thus create a feedback signal to readjust the rRNA 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 *nus* 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 λP_L 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 RNAP–*rrn* P1 complex. NTPs are the substrates of transcription, but we emphasize that the initiating NTP affects the *rrn* P1 promoter complex before catalysis occurs. The initiating NTP most likely functions as a ligand that binds to the open complex, presumably at the active site, leading to an increase in the observed half-life of the complex and a greater chance for productive transcription before the complex dissociates (*31*). The higher the NTP concentration, the greater the fraction of promoters in open complexes with RNAP, and the higher the extent of transcription.

The promoter sequences responsible for the instability of the *rrn* P1 open complex are not understood. The determinants are likely to be complex, involving multiple aspects of promoter architecture. The sequence just upstream of the transcription start site is a likely determinant, because the C → T change at position –1 (*43*) increased the stability of the *rrnB* P1 open complex (Fig. 5C). It is striking that all seven *rrn* P1 promoters initiate transcription at the unusual distance of 9 base pairs (bp) from the –10 hexamer and contain the least preferred NTP for initiation, CTP, at the preferred positions 7 and 8 bp from the –10 hexamer (*3, 44*). However, the C-1T mutation increased stability without altering the transcription start site (*13*), indicating that start site position alone apparently is insufficient to account for this instability.

Other promoters (including the other *rrn* P1 promoters and many tRNA promoters) may also be controlled by variation in 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 RNAP 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 RNAP holoenzymes in *Bacillus subtilis* (*49*).

Overlap in rRNA regulation mechanisms. The NTP-sensing mechanism need

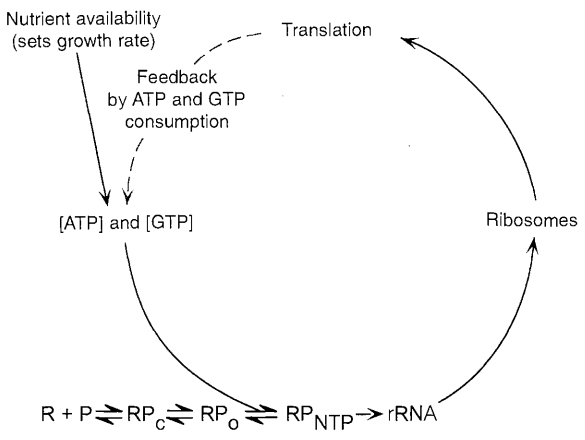

Fig. 6. Model for homeostatic regulation of rRNA transcription and ribosome synthesis by the initiating NTP concentration. ATP and GTP, whose concentrations vary with growth rate (nutrient availability), regulate rRNA transcription by stabilizing RNAP (R)–*rrn* P1 promoter (P) open complexes (RP_o). rRNA transcription determines the rate of ribosome synthesis and therefore the amount of translation. ATP and GTP are consumed during the process of translation, resulting in a feedback signal affecting *rrn* P1 transcription. Initiating NTP pools reflect the balance between protein synthesis rates and nutritional conditions.

not account for all aspects of rRNA regulation. Ribosomal RNA promoters integrate multiple input signals: RNAP α and σ 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, RNAP mutations (such as *rpoCΔ215–220*; Fig. 5) that decrease *rrn* P1 core promoter activity are compensated for, in part, by FIS: FIS activates the β′ 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, 30, 53*), perhaps making the complex unable to be "rescued" by normal intracellular NTP concentrations. ppGpp is dispensable for growth rate–dependent control (*16, 54*), but even the low concentrations of ppGpp that are present during steady-state growth could conceivably supplement the NTP-sensing mechanism.

Models for the control of rRNA synthesis involving substrate limitation were considered, and discarded, previously (*55, 56*). In particular, after an upshift it was found that both NTP pools and rRNA transcription ultimately reached higher steady-state levels, but NTP concentrations dropped transiently while stable RNA synthesis increased almost immediately (*56*). These data were interpreted to mean that NTP concentrations do not correlate with rRNA transcription rates. The apparent conflict with the NTP-sensing model proposed here might reflect the contribution of additional mechanisms to regulation during growth transitions. Consistent with this hypothesis, *rrn* P2–derived transcripts are responsible for most rRNA transcription immediately after an upshift, and P1-derived transcripts become dominant only after about 30 min (*57*). Because transcription from the *rrnB* P2 promoter, which initiates with several C residues (*58*), is insensitive to reduced CTP concentrations in vitro (*19*), *rrn* P2 promoter activity could account for the reported transient inverse correlation between NTP concentrations and rRNA synthesis during an upshift. It is also possible that the increase in rRNA transcription that occurs immediately after upshift could result, in part, from loss of inhibition by ppGpp, because ppGpp concentrations drop quickly after shifts before attaining new steady-state levels (*59*).

In summary, the sequence of a promoter determines the concentration of the initiating NTP required for maximal transcription efficiency. At *rrn* P1 promoters, unstable open complexes serve as sensors of the concentration of the initiating NTP (ATP or GTP). Purine NTP concentrations reflect the nutritional state as well as the translational activity of the cell, and they satisfy the role of a feedback effector of rRNA transcription. NTP sensing thus provides a molecular explanation for the growth rate–dependent regulation that is observed even in the absence of all other systems known to affect rRNA transcription.

REFERENCES AND NOTES

1. F. C. Neidhardt, in Escherichia coli *and* Salmonella typhimurium*: Cellular and Molecular Biology*, F. C. Neidhardt *et al.*, Eds. (American Society for Microbiology, Washington, DC, ed. 2, 1996), pp. xi–xii.
2. M. Schaechter, O. Maaloe, N. O. Kjeldgaard, *J. Gen. Microbiol.* **19**, 592 (1958); O. Maaloe and N. O. Kjeldgaard, *Control of Macromolecular Synthesis: A Study of DNA, RNA, and Protein Synthesis in Bacteria* (Benjamin, New York, 1966).
3. R. L. Gourse, T. Gaal, M. S. Bartlett, J. A. Appleman, W. Ross, *Annu. Rev. Microbiol.* **50**, 645 (1996); C. Condon, C. Squires, C. L. Squires, *Microbiol. Rev.* **59**, 623 (1995); J. Keener and M. Nomura, in (*1*), pp. 1417–1431.
4. Most models for rRNA regulation have focused on proposed direct or indirect effects of ppGpp [A. A. Travers, *Nature* **263**, 641 (1976); J. Ryals, R. Little, H. Bremer, *J. Bacteriol.* **151**, 1261 (1982); K. F. Jensen and S. Pedersen, *Microbiol. Rev.* **54**, 89 (1990); A. G. Marr, *ibid.* **55**, 316 (1991)].
5. M. Nomura, R. L. Gourse, G. Baughman, *Annu. Rev. Biochem.* **53**, 75 (1984); J. M. Zengel and L. Lindahl, *Prog. Nucleic Acid Res. Mol. Biol.* **47**, 331 (1994).
6. R. L. Gourse, H. A. deBoer, M. Nomura, *Cell* **44**, 197 (1986).
7. W. Ross *et al.*, *Science* **262**, 1407 (1993).
8. L. Rao *et al.*, *J. Mol. Biol.* **235**, 1421 (1994).
9. E. E. Blatter, W. Ross, H. Tang, R. L. Gourse, R. H. Ebright, *Cell* **78**, 889 (1994); T. Gaal *et al.*, *Genes Dev.* **10**, 16 (1996).
10. W. Ross, J. F. Thompson, J. T. Newlands, R. L. Gourse, *EMBO J.* **9**, 3733 (1990).
11. A. J. Bokal IV, W. Ross, T. Gaal, R. C. Johnson, R. L. Gourse, *ibid.* **16**, 154 (1997).
12. C. L. Squires, J. Greenblatt, J. Li, C. Condon, C. Squires, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 970 (1993).
13. C. A. Josaitis, T. Gaal, R. L. Gourse, *ibid.* **92**, 1117 (1995).
14. R. Gafny, S. Cohen, N. Nachaliel, G. Glaser, *J. Mol. Biol.* **243**, 152 (1994).
15. M. Cashel, D. R. Gentry, V. J. Hernandez, D. Vinella, in (*1*), pp. 1458–1496.
16. M. S. Bartlett and R. L. Gourse, *J. Bacteriol.* **176**, 5560 (1994).
17. J. A. Appleman, W. Ross, J. Salomon, R. L. Gourse, in preparation.
18. Multiple-round transcription was performed at 22°C essentially as described (*7*). Supercoiled plasmid templates were derivatives of pRLG770 (*10*), with either the *rrnB* P1 promoter (−46 to +1, where +1 is the transcription start site; pRLG2294), RNA I promoter (−61 to +1; pRLG3265), or *lacUV5* promoter (−46 to +1; pRLG3422), inserted 170 bp upstream of the *rrnB* T1 terminator. In Fig. 2, B and C, 25-μl reaction volumes contained 0.2 nM supercoiled DNA template, 40 mM tris-acetate (pH 7.9), 10 mM $MgCl_2$, bovine serum albumin (BSA) at 0.1 mg/ml, 170 mM KCl, 1 mM dithiothreitol (DTT), 5 μM to 1.25 mM ATP, 10 μM CTP, 200 μM GTP, 200 μM UTP (Pharmacia, HPLC pure), and 5 μCi of [α-^{32}P]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 concentration, whether it was located 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. 2D, the plasmid template contained the *rrnD* P1 promoter (−60 to +10) inserted into pRLG770 (pRLG3426). Identical results were obtained with plasmid pRLG3266 [*rrnD* P1 (−60 to +1) (*19*)]. Reactions contained 5 μM to 1.25 mM GTP, 200 μM ATP, 200 μM UTP, 10 μM CTP, and 5 μCi of [α-^{32}P]CTP, or 5 μM to 1.25 mM ATP, 200 μM GTP, 200 μM UTP, 10 μM CTP, and 5 μCi of [α-^{32}P]CTP.
19. T. Gaal and R. L. Gourse, unpublished data.
20. The concentration of ATP required for half-maximal transcription of *rrnB* P1 (apparent K_{NTP}) on a supercoiled template ranged from about 5 μM at 30 mM KCl to more than 1500 μM at 200 mM KCl. On a linear template, the apparent K_{NTP} for ATP was about 500 μM at 100 mM KCl.
21. NTPs were measured in RLG3492, a derivative of VH1000 with a lambda prophage containing an *rrnB* P1 (−46 to +1) promoter-*lacZ* fusion (*16*). VH1000 is a *lacI*$^-$ *lacZ*$^-$ *pyrE*$^+$ derivative of MG1655 [B. J. Bachmann, in (*1*), ed. 1, 1987, pp. 1190–1219; K. F. Jensen, *J. Bacteriol.* **175**, 3401 (1993)]. Cells were grown in C medium [M. Alper and B. Ames, *J. Bacteriol.* **133**, 149 (1978)] containing 0.4 mM $MgSO_4$ and 0.4% succinate, glycerol, or glucose in the absence or presence of all 20 amino acids (20 μg/ml). Samples were withdrawn after three or four generations of steady-state growth ($OD_{600} \approx 0.5$) and treated with formaldehyde, and NTPs were extracted from unwashed cells with KOH [R. Little and H. Bremer, *Anal. Biochem.* **126**, 381 (1983)] and measured by HPLC (C-18 Alltech nucleoside/nucleotide column) as recommended by the manufacturer. β-Galactosidase activities were measured as described (*54*) from the same cultures.
22. Earlier studies also indicated that ATP concentration increased with growth rate [F. C. Neidhardt and D. G. Fraenkel, *Cold Spring Harbor Symp. Quant. Biol.* **26**, 63 (1961); J. S. Franzen and S. B. Binkley, *J. Biol. Chem.* **236**, 515 (1961); R. C. Smith and O. Maaloe, *Biochim. Biophys. Acta* **86**, 229 (1964); A. S. Bagnara and L. R. Finch, *Eur. J. Biochem.* **36**, 422 (1973)]. ATP and GTP concentrations in vivo were estimated to be about 3.0 mM and 1 mM, respectively [J. Neuhard and P. Nygaard, in (*1*), ed. 1, 1987, pp. 445–473], although the actual free, unbound ATP and GTP concentrations in cells are unknown. The NTP concentrations required in vitro (*20*) likely underestimate those needed for *rrn* P1 transcription in vivo, where the degree of supercoiling is lower and the ionic conditions are higher. The K_{NTP} for initiating NTPs reported previously in vitro for other promoters is much higher than for elongating NTPs, but is still lower than the apparent K_{NTP} reported here for *rrn* P1 promoters [W. R. McClure, C. L. Cech, D. E.

Johnston, *J. Biol. Chem.* **253**, 8941 (1978); W. C. Nierman and M. J. Chamberlin, *ibid.* **254**, 7921 (1979)].
23. P. Poulsen and K. F. Jensen, *Mol. Gen. Genet.* **208**, 152 (1987).
24. F. Qi and C. L. Turnbough Jr., *J. Mol. Biol.* **254**, 552 (1995).
25. Purine NTP concentrations were increased at low growth rates (Fig. 3C) by growing strain RLG3493 [a *car-403::Tn10* derivative of RLG3492 (*21*)] under conditions of pyrimidine limitation; *car-403::Tn10* inactivates the first enzyme in the pyrimidine biosynthesis pathway (*24*), resulting in pyrimidine auxotrophy. In the presence of uracil (pyrimidine excess), growth and regulation of *rrn* P1 transcription were identical to that of RLG3492 (*19*). Different degrees of pyrimidine limitation were achieved by growing cells with uridine 5′-phosphate (UMP) at selected concentrations of Mg^{2+}, which determined the rate at which UMP could be used as a pyrimidine source and reduced growth rates to different extents. Cells were grown in C medium (*21*) containing 1 mM arginine, 0.4% glucose, 0.25 mM UMP, and 0.2 to 0.8 mM Mg_2SO_4. The *rrnB* P1 promoter–*lacZ* fusion in RLG3493 indicated that *rrn* P1 activity increased under pyrimidine-limiting conditions. However, pyrimidine-limited cells do not overproduce full-length rRNA (*24*) [U. Vogel, S. Pedersen, K. F. Jensen, *J. Bacteriol.* **173**, 1168 (1991)], possibly because of compensating effects on other aspects of the rRNA transcription process.
26. M. T. Record Jr., W. S. Reznikoff, M. L. Craig, K. L. McQuade, P. J. Schlax, in (*1*), pp. 792–820.
27. M. E. Mulligan, J. Brosius, W. R. McClure, *J. Biol. Chem.* **260**, 3529 (1985); M. S. Bartlett, M. M. Barker, R. L. Gourse, unpublished data.
28. R. L. Gourse, *Nucleic Acids Res.* **16**, 9789 (1988).
29. S. Leirmo and R. L. Gourse, *J. Mol. Biol.* **220**, 555 (1991).
30. K. L. Ohlsen and J. D. Gralla, *Mol. Microbiol.* **6**, 2243 (1992); *J. Biol. Chem.* **267**, 19813 (1992); *J. Bacteriol.* **174**, 6071 (1992).
31. Stabilization of promoter open complexes by NTPs has also been reported by others (K. L. McQuade, E. Courtenay, P. E. Schlax, O. V. Tsodikov, M. T. Record Jr., unpublished data; J. Villemain, R. Guajardo, R. Sousa, *J. Mol. Biol.*, **273**, 958 (1997).
32. Promoter-RNAP complex stability was measured as described (*29*). RNAP (20 nM) and plasmid DNA (0.5 nM) were incubated for 20 min at 22°C in 40 mM tris-acetate (pH 7.9), 10 mM $MgCl_2$, BSA (0.1 mg/ml), 1 mM DTT, and 30 mM KCl, with 2 mM ATP or GTP (Pharmacia, HPLC pure) as indicated in Fig. 4, A and B. NaCl was added to achieve comparable monovalent cation concentrations in control reactions lacking NTPs. No evidence for contamination of ATP or GTP with other nucleotides was detected by HPLC analysis. At time 0, heparin (10 μg/ml) was added, followed by removal of 10-μl samples at timed intervals and addition of 200 μM ATP, GTP, CTP, and 10 μM [α-^{32}P]UTP to initiate a single round of transcription. Transcripts were resolved on 5.5% polyacrylamide–7 M urea gels and quantified by phosphorimaging.
33. M. S. Bartlett, T. Gaal, W. Ross, R. L. Gourse, in preparation.
34. Transcription with wild-type or mutant RNAPs in Fig. 5, B and E, was performed as in Fig. 2 (*18*), except that the salt concentration was 100 mM rather than 170 mM KCl. Thus, the ATP concentration required for maximal transcription of wild-type *rrnB* P1 (−46 to +1; plasmid pRLG2294) in Fig. 5, B and E, was lower than required in Fig. 2 [150 to 200 μM ATP (apparent K_{NTP} = 35 μM) at 100 mM KCl, compared with about 1000 μM ATP (apparent K_{NTP} = 360 μM) at 170 mM KCl (*20*)]. Relative to wild-type *rrnB* P1, the C-1T mutant *rrnB* P1 promoter (−46 to +1; plasmid pRLG2295) required about one-tenth 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*).
35. In Fig. 5C, stabilities of wild-type and mutant promoter-RNAP complexes were measured on plasmids pRLG2294 and pRLG2295 (*34*) 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 $MgCl_2$, BSA (0.1 mg/ml), 1 mM DTT, 10 mM KCl, 5 nM RNAP, and 3 nM plasmid DNA, and the NTP mixture added after heparin addition contained 4 mM ATP, 200 μM GTP and UTP, and 10 μM [α-^{32}P]CTP. Plasmid DNA in Fig. 5F (pRLG589) contained the *rrnB* P1 (−88 to +50) promoter. The mutant RNAP also made less stable complexes with non-rRNA promoters, indicating that the mutation does not identify a site of interaction with DNA specific to *rrn* P1 promoters (*33*).
36. F. C. Neidhardt, J. L. Ingraham, M. Schaechter, *Physiology of the Bacterial Cell* (Sinauer, Sunderland, MA, 1990).
37. E. Lund and N. O. Kjeldgaard, *Eur. J. Biochem.* **28**, 316 (1972); V. Shen and H. Bremer, *J. Bacteriol.* **130**, 1109 (1977); J. R. Cole, C. L. Olsson, J. W. B. Hershey, M. Grunberg-Manago, M. Nomura, *J. Mol. Biol.* **198**, 383 (1987); M. Yamagishi, H. A. deBoer, M. Nomura, *ibid.*, p. 547.
38. S. Jinks-Robertson, R. L. Gourse, M. Nomura, *Cell* **33**, 865 (1983).
39. A. Bock and F. C. Neidhardt, *J. Bacteriol.* **92**, 470 (1966); M. Singer *et al.*, *ibid.* **173**, 6249 (1991).
40. C. Condon, S. French, C. Squires, C. L. Squires, *EMBO J.* **12**, 4305 (1993).
41. R. A. Sharrock, R. L. Gourse, M. Nomura, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 5275 (1985).
42. R. L. Gourse, Y. Takebe, R. A. Sharrock, M. Nomura, *ibid.*, p. 1069.
43. The top (nontemplate) strand base is indicated, but either the top or bottom strand base could be the crucial determinant.
44. J. Liu and C. L. Turnbough Jr., *J. Bacteriol.* **176**, 2938 (1994).
45. H. Kupper, R. Contreras, H. G. Khorana, A. Landy, in *RNA Polymerase*, R. Losick and M. Chamberlin, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1976), pp. 473–484; K. B. Rowley, R. M. Elford, I. Roberts, W. M. Holmes, *J. Bacteriol.* **175**, 1309 (1993); L. Nilsson and V. Emilsson, *J. Biol. Chem.* **269**, 9460 (1994); W. Ross, J. Salomon, W. M. Holmes, R. L. Gourse, unpublished data.
46. I. Grummt and F. Grummt, *Cell* **7**, 447 (1976).
47. H. R. Wilson, C. D. Archer, J. Liu, C. L. Turnbough Jr., *J. Bacteriol.* **174**, 514 (1992); C. L. Turnbough Jr., K. L. Hicks, J. P. Donahue, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 368 (1983); C. Liu, L. S. Heath, C. L. Turnbough Jr., *Genes Dev.* **8**, 2904 (1994); J. P. Donahue and C. L. Turnbough Jr., *J. Biol. Chem.* **269**, 18185 (1994); A.-H. T. Tu and C. L. Turnbough Jr., *J. Bacteriol.* **179**, 6665 (1997).
48. A. Ninfa, in (*1*), pp. 1246–1262.
49. S. Alper, A. Dufour, D. A. Garsin, L. Duncan, R. Losick, *J. Mol. Biol.* **260**, 165 (1996).
50. 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. C. A. Ball, R. Osuna, K. C. Ferguson, R. C. Johnson, *J. Bacteriol.* **174**, 8043 (1992).
52. Because salt concentration and degree of supercoiling modulate rRNA 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 rRNA 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. Guttman, 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**, 4499 (1987)].
53. J. P. Donahue and C. L. Turnbough Jr., *J. Biol. Chem.* **265**, 19091 (1990).
54. T. Gaal and R. L. Gourse, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 5533 (1990).
55. M. Cashel and J. Gallant, *J. Mol. Biol.* **34**, 317 (1968).
56. C. Beck, J. Ingraham, O. Maaloe, J. Neuhard, *ibid.* **78**, 117 (1973).
57. J. A. Appleman, T. Gaal, R. L. Gourse, unpublished data; P. Sarmientos, S. Contente, G. Chinali, M. Cashel, in *Gene Expression*, D. H. Hamer and M. Rosenberg, Eds. (Liss, New York, 1983), pp. 65–74.
58. H. A. deBoer and M. Nomura, *J. Biol. Chem.* **254**, 5609 (1979); E. Lund and J. E. Dahlberg, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5480 (1979).
59. V. J. Hernandez and H. Bremer, *J. Biol. Chem.* **265**, 11605 (1990).
60. Supported by NIH grants GM37048 (R.L.G.) and GM29466 (C.L.T.). We thank R. Landick for RNAP; V. J. Hernandez for VH1000; T. Record and K. McQuade for sharing ideas and for communicating results; A. Grossman, R. Landick, and N. Shimamoto for helpful discussions; J. Southworth for technical assistance; and A. Appleman, M. Dreyfus, D. Dubnau, K. McQuade, R. Mooney, M. Nomura, R. Schleif, M. Springer, C. Squires, and T. Record for comments on the manuscript.

28 April 1997; accepted 27 October 1997