Supplementary Material:

Strains and strain construction

Strains and plasmids are listed in Table S1. Promoter-*lacZ* fusions were constructed using pDG3661, a modified version of integration vector pDG1661 (Guerout-Fleury *et al*, 1996). Since *lacZ* and *amyE* are in opposing orientations in these constructs, transcription terminators were inserted between the two genes (one from the *rrnO* operon was inserted just downstream of *lacZ* and one from the *rrnB* operon was inserted just downstream of *amyE* front) to prevent potential readthrough into either gene (for details see GenBank accession number AY618310). Promoter fragments were inserted using *EcoRI* and *HindIII* restriction sites. The *rrnB* P1, *rrnB* P2, and P*veg* reporters were constructed with the sequence TCT adjacent to position +1 (the transcription start site) to avoid placing an A (from the *HindIII* site) adjacent to +1. (This insertion actually makes the initial transcribed sequence match the natural *rrnB* P1 initial transcribed sequence.) The *rrnB* promoters were 2 to 3-fold more active than the *veg* promoter in LB, consistent with previous reports that P*veg* is quite strong (Le Grice *et al*, 1986), and the *hag* promoter appeared at least 5-fold weaker than P*veg* (data not shown). The *rrnO* P1 and P2 reporter constructs used in experiments other than Fig. 1 contained *rrnO* sequences to position +10 (rather than +1) followed by the *Hind*III site and *lacZ*. All constructs were verified by DNA sequencing.

We emphasize that the transcription activities reported here are relative to transcription from other promoters synthesizing the same RNA sequence. This

eliminates the possibility that the promoter-specific differences in activity reported could derive from changes in mRNA half-life rather than from changes in synthesis rate. In theory, the half-live of this RNA might vary in response to a nutritional change under investigation, either exaggerating or reducing the magnitude of the observed response in a systematic fashion. For this reason, we emphasize not the magnitude of the response but the promoter specificity of the response to changes in growth conditions. In practice, differences in RNA lifetime were not observed under the conditions tested (log phase or at different times during outgrowth from stationary phase; see below).

Promoter-*lacZ* fusions were integrated into the *amyE* locus of the *B. subtilis* chromosome by transforming appropriate strains with appropriate plasmids (Dubnau & Davidoff-Abelson, 1971). Double crossover recombinants at *amyE* were selected for resistance to chloramphenicol (5 μg/ml) and sensitivity to spectinomycin (100 μg/ml), erythromycin (1 μg/ml) and lincomycin (25 μg/ml), as described (Guerout-Fleury *et al*, 1996). Δ*relA* strains were created by transforming appropriate strains containing the desired promoter-*lacZ* fusions with chromosomal DNA isolated from TW30 (Wendrich & Marahiel, 1997) and selecting for resistance to erythromycin and lincomycin.

Media and growth conditions

Cells were grown either in LB or in a MOPS buffered defined medium: 50 mM MOPS (pH 7.0), 1mM $(NH_4)_2SO_4$, 0.5 mM KH_2PO_4, 2 mM $MgCl_2$, 2 mM $CaCl_2$, 50 μM $MnCl_2$, 5 μM $FeCl_3$, 10 mg/L thiamine, amino acids (50 μg/ml) as

indicated, and glucose at the concentrations indicated in the figure legends. The "wild-type" *B. subtilis* strains used are auxotrophic for trp and phe. The Δ*relA* strains required ile, leu, val, and met for growth in addition to trp and phe (Gropp *et al*, 1994; Wendrich & Marahiel 1997). All experiments with *B. subtilis* were conducted at 37°C. The *E. coli* cells for the control experiment shown in Fig. 2D were grown at 30°C, since they contain a λ prophage controlled by a temperature-sensitive λ repressor. Serine hydroxamate (SHX), α-methyl glucoside (methyl α-D-glucopyranoside), and antibiotics were from Sigma, and decoyinine was from Biomol.

Primer extension

Primer extension was performed with M-MLV reverse transcriptase as recommended by the manufacturer (Promega) with 1-10 μl purified RNA. The ^{32}P 5'-labeled primer (3540/BgalR: 5' CAGTAACTTCCACAGTAGTTCACCAC-3') hybridized 89 nt downstream from the junction of the promoter fragment used for creation of the *lacZ* fusion. Samples were electrophoresed on 7M urea 5.5 % or 9% polyacylamide gels, quantified by phosphorimaging (ImageQuant Software, Molecular Dynamics), and normalized to cell number (OD_{600}) and the RM band.

Protein purification

B. subtilis RNAP, histidine-tagged on the β' subunit (Qi & Hulett, 1998), was purified from strain MH5636 as described (Anthony *et al*, 2000). The σ^A and δ subunits of RNAP were overproduced from plasmids pCD2 and pFL31,

respectively, generous gifts from J. Helmann (Cornell Univ.), and purified as described (Juang & Helmann, 1994; Lopez de Saro *et al*, 1999). Core RNAP, σ^A, and δ were reconstituted in storage buffer (50 mM Tris-HCl pH 8.0, 0.1 M NaCl, 3 mM 2-mercaptoethanol, 50% glycerol) for 30 min at 30°C. Titration experiments were carried out to ensure saturation of core RNAP with σ^A and δ. Typically, σ^A and δ were used at 8 and 10-fold molar excess to core RNAP, respectively. *E. coli* RNAP was purified as described (Burgess & Jendrisak, 1975) and was a generous gift from R. Landick (Univ. of Wisconsin).

mRNA half-life determination

Half-life was measured by standard procedures: rifampicin (final concentration 200 μg/ml), which prevents RNA synthesis beyond the first few nucleotides, was added to cultures at OD_{600} ~ 0.3 in the same medium used in Fig. 4A, and total RNA was extracted at various times after rifampicin addition. The fraction of mRNA remaining was determined by primer extension.

Determination of NTP and ppGpp concentrations

Wild-type and Δ*relA* cells (RLG7554 and RLG7580, respectively) were grown in the same medium used for primer extension assays plus ^{32}P KH_2PO_4 (20 μCi/ml). Samples (100 μl) were pipetted into 100 μl 11.5 M formic acid, vortexed, left on ice for 20 min, and stored overnight at -80°C (see also Paul *et al*, 2004b; Schneider *et al*, 2003; Wendrich & Marahiel, 1997). After microcentrifugation (5 min, 4°C) to remove cell debris, 10 μl was examined by

TLC (BakerFlex) in 0.85 M (for ATP and GTP) or 1.5 M KH_2PO_4 (for ppGpp) (pH 3.4), and quantified by phosphorimaging. The identities of ATP, GTP, and ppGpp were verified by comparison with commercial preparations of the nucleotides run alongside and visualized by UV shadowing (Schneider *et al*, 2003).

Supplemental References

Aiyar SE, Gourse RL, Ross W (1998) Upstream A-tracts increase bacterial promoter activity through interactions with the RNA polymerase alpha subunit. *Proc Natl Acad Sci USA* **95:** 14652-14657

Anthony LC, Artsimovitch I, Svetlov V, Landick R, Burgess RR (2000) Rapid purification of His(6)-tagged *Bacillus subtilis* core RNA polymerase. *Protein Expr Purif* **19:** 350-354

Burgess RR, Jendrisak JJ (1975) A procedure for the rapid, large-scale purification of *Escherichia coli* DNA-dependent RNA polymerase involving Polymin P precipitation and DNA-cellulose chromatography. *Biochemistry* **14:** 4634-4638

Dubnau D, Davidoff-Abelson R (1971) Fate of transforming DNA following uptake by competent *Bacillus subtilis*. I. Formation and properties of the donor-recipient complex. *J Mol Biol* **56:** 209-221

Estrem ST, Ross W, Gaal T, Chen ZW, Niu W, Ebright RH, Gourse RL (1999) Bacterial promoter architecture: subsite structure of UP elements and

interactions with the carboxy-terminal domain of the RNA polymerase alpha subunit. *Genes Dev* **13:** 2134-2147

Guerout-Fleury AM, Frandsen N, Stragier P (1996) Plasmids for ectopic integration in *Bacillus subtilis*. *Gene* **180:** 57-61

Gaal T, Barkei J, Dickson RR, deBoer HA, deHaseth PL, Alavi H, Gourse RL (1989) Saturation mutagenesis of an *Escherichia coli* rRNA promoter and initial characterization of promoter variants. *J Bacteriol* **171:** 4852-4861

Gropp M, Eizenman E, Glaser G, Samarrai W, Rudner R (1994) A *relA*$^{(S)}$ suppressor mutant allele of *Bacillus subtilis* which maps to *relA* and responds only to carbon limitation. *Gene* **140:** 91-96

Juang YL, Helmann JD (1994) A promoter melting region in the primary sigma factor of *Bacillus subtilis*. Identification of functionally important aromatic amino acids. *J Mol Biol* **235:** 1470-1488

Le Grice SF, Shih CC, Whipple F, Sonenshein AL (1986) Separation and analysis of the RNA polymerase binding sites of a complex *Bacillus subtilis* promoter. *Mol Gen Genet* **204**: 229-236.

Lopez de Saro FJ, Yoshikawa N, Helmann JD (1999) Expression, abundance, and RNA polymerase binding properties of the delta factor of *Bacillus subtilis*. *J Biol Chem* **274:** 15953-15958

Qi Y, Hulett FM (1998) PhoP-P and RNA polymerase sigma A holoenzyme are sufficient for transcription of Pho regulon promoters in *Bacillus subtilis*: PhoP-P activator sites within the coding region stimulate transcription *in vitro*. *Mol Microbiol* **28:** 1187-1197

Ross W, Thompson JF, Newlands JT, Gourse RL (1990) *E. coli* Fis protein activates ribosomal RNA transcription *in vitro* and *in vivo*. *EMBO J* **9:** 3733-3742

Schneider DA, Gaal T, Gourse RL (2002) NTP-sensing by rRNA promoters in *Escherichia coli* is direct. *Proc Natl Acad Sci USA* **99:** 8602-8607

Schneider DA, Murray HD, Gourse RL (2003) Measuring control of transcription initiation by changing concentrations of nucleotides and their derivatives. Meth Enzymol *370*: 606-617

Wendrich TM, Marahiel MA (1997) Cloning and characterization of a *relA/spoT* homologue from *Bacillus subtilis*. *Mol Microbiol* **26:** 65-79

Table I. Bacterial strains and plasmids

Strain/plasmid	Genotype	Reference
B. subtilis		
MH5636	*rpoC*-His10	(Qi & Hulett, 1998)
MO1099	*trpC2 pheA1 amyE*::mls	(Guerout-Fleury *et al*, 1996)
TW30	*trpC2 lys-3* Δ*relA*::mls	(Wendrich & Marahiel, 1997)
RLG6930	MO1099 *amyE*::Cm *rrnB* P1-P2 (P1 -248, P2 +8)-*lacZ*	this study
RLG6937	MO1099 *amyE*::Cm *rrnO* P2 (-38/+10)-*lacZ*	this study
RLG6943	MO1099 *amyE*::Cm *rrnO* P2 (-77/+50)-*lacZ*	this study
RLG7027	MO1099 *amyE*::Cm *rrnO* P1 (-38/+10)-*lacZ*	this study
RLG7028	MO1099 *amyE*::Cm *rrnO* P1 (-77/+10)-*lacZ*	this study
RLG7029	MO1099 *amyE*::Cm *rrnO* P2 (-77/+10)-*lacZ*	this study
RLG7030	MO1099 *amyE*::Cm *rrnO* P1 (-227/+10)-*lacZ*	this study
RLG7369	MO1099 *amyE*::Cm *rrnO* P1 (-38/+1)-*lacZ*	this study
RLG7370	MO1099 *amyE*::Cm *rrnO* P2 (-38/+1)-*lacZ*	this study
RLG7372	MO1099 *amyE*::Cm *rrnB* P1 (SUB-39/+1)-*lacZ*	this study
RLG7373	MO1099 *amyE*::Cm *rrnB* P1 (-58/+1)-*lacZ*	this study
RLG7374	MO1099 *amyE*::Cm *rrnB* P2 (SUB-38/+1)-*lacZ*	this study
RLG7375	MO1099 *amyE*::Cm *rrnB* P2 (-57/+1)-*lacZ*	this study
RLG7376	MO1099 *amyE*::Cm P*veg* (-38/+1)-*lacZ*	this study
RLG7391	MO1099 *amyE*::Cm P*hag* (-43/+4)-*lacZ*	this study
RLG7392	MO1099 *amyE*::Cm P*hag* (-96/+4)-*lacZ*	this study
RLG7553	MO1099 *amyE*::Cm *rrnB* P2 (-38/+1)-*lacZ*	this study
RLG7554	MO1099 *amyE*::Cm *rrnB* P1 (-39/+1)-*lacZ*	this study
RLG7555	MO1099 *amyE*::Cm P*veg* (-38/-1,+1G)-*lacZ*	this study
RLG7580	MO1099 Δ*relA*::mls *amyE*::Cm *rrnB* P1 (-39/+1)-*lacZ*	this study
RLG7581	MO1099 Δ*relA*::mls *amyE*::Cm P*veg* (-38/-1,+1G)-*lacZ*	this study
RLG7584	MO1099 *amyE*::Cm *rrnB* P1 (-352/+1)-*lacZ*	this study
RLG7585	MO1099 *amyE*::Cm *rrnB* P1 (-39/-1,+1A)-*lacZ*	this study

E. coli

RLG1100	*E. coli rrnB* P1 (-88/+1)-*lacZ*	(Gaal *et al*, 1989)
RLG3074	*E. coli rrnB* P1 (-66/+50)-*lacZ*	(Estrem *et al*, 1999)
RLG3097	*E. coli rrnB* P1 (SUB-39/+50)-lacZ	(Estrem *et al*, 1999)
RLG6921	pCD2 (*B. subtilis sigA*)	(Juang & Helmann, 1994)
RLG7023	pFL31 (*B. subtilis rpoE*)	(Lopez de Saro *et al*, 1999)

plasmid

pRLG770	promoter vector	(Ross *et al*, 1990)
pRLG2230	pRLG770 with *E. coli rrnB* P1 (-41/+50)	(Aiyar *et al*, 1998)
pRLG6214	pRLG770 with *E. coli rrnB* P1 (-66/+50)	(Schneider *et al*, 2002)
pRLG6555	pRLG770 with *E. coli rrnB* P1 (-66/+9)	(Barker & Gourse, 2001)
pRLG7558	pRLG770 with P*veg* (-38/-1, +1G)	this study
pRLG7595	pRLG770 with P*veg* (-38/+1)	this study
pRLG7596	pRLG770 with *B. subtilis rrnB* P1 (-39/+1)	this study
pRLG7597	pRLG770 with *B. subtilis rrnB* P1 (-39/-1/+1A)	this study
pRLG7598	pRLG770 with *B. subtilis rrnB* P1 (-58/+1)	this study
pRLG7599	pRLG770 with *B. subtilis rrnB* P1 (SUB-39/+1)	this study