SUPPORTING ONLINE MATERIAL

Materials and Methods

Strains and plasmids. We used *E. coli* strain MG1665 and streptomycin-pseudo-dependent strain CH184 (W3110 derivative zhd-126::Tn10 ~*rpsL*(SmP)) kindly provided by Dr. Diarmaid Hughes (Uppsala University) (*1*).

The plasmid pUV12 was previously described (*2*). The fusion *E.coli* gene *rplB-tufA* was constructed in one reading frame by PCR using a pair of overlapped oligos partly complementary to the 3'-end of *rplB* and 5'-end of *tufA* and a pair of specific oligos complementary to the proximal part of *rplB* and distal part of *tufA*. The amplified fragment *rplB-tufA* harboring RBS of *rplB* was cloned into the *Bam*HI site of pUV12. The plasmid pUV12-SD was derived from pUV12 by inserting a 34 bp DNA fragment containing a synthetic RBS from the pET15 vector into the *Bam*HI site. The *Saccharomyces cerevisiae* gene *srb4* was amplified from yeast genomic DNA with specific oligos and cloned into the *Bam*HI site of pUV12-SD just after the RBS.

The plasmid p2EC is equivalent to pATC10 and was described previously (*3*). The plasmid p1EC was obtained by inserting the trp transcription terminator into the *Bam*HI site in p2EC in such a way that there is a unique *Bam*HI site upstream of the terminator sequence. The plasmid p^{RBS}1EC was derived from p1EC by inserting a 37 bp spacer as well as the strong T7 g10 RBS into a *Bam*HI site maintaining the reading frame intact (no stop codons between the ATG and lac operator region).

Measurement of the transcription elongation rate *in vivo*. RNA preparation and dot blot analysis was done essentially as described in (*2*), except that *E. coli* MG1665 or CH184 overexpressing the *lac* repressor from a placK plasmid that harbors the $lacI^q$ gene and LB media were used. Cells harboring pUV12 were grown to an optical density of OD_{600}~0.4. The two plasmids were maintained within the cell by double selection in ampicillin (100 μg/ml) and kanamycin (20 μg/ml). Transcription was induced at time 0 with 1mM isopropyl-β-D-thiogalactoside (IPTG). At 20 s intervals, 0.5 ml samples were withdrawn into precooled (-30°C) plastic tubes containing 0.2 ml stop solution (60% ethanol, 2% phenol, 10mM EDTA, pH8.0) to prevent transcription. RNA was isolated and hybridized as described in (*4*). Only those portions of plots where the hybridization appears as a linear function of time were taken into account.

Although the absolute levels of radioactivity in the dots varied to some extent between experiments, the transcription times were very reproducible.

Determination of translation elongation rate *in vivo*. All measurements were made essentially as described in (*5, 6*). *E. coli* strains MG1655 or CH184 were grown in LB to OD_{600}~0.4. Expression from *lacZ* gene was induced by addition of 1mM IPTG at time zero. Subsequently, samples of 0.2 ml were removed at 15 s intervals and immediately mixed with 0.3 ml ice-cold chloramphenicol solution (1 mg/ml in H_2O). The samples were then processed as described in (*7*) with suitable dilution. After the addition of Na_2CO_3, and centrifugation, the formation of o-nitrophenol was determined by measuring the optical density at 420 nm of each sample. Values were corrected by subtracting the average absorbance of duplicate samples taken immediately after the addition of IPTG and by normalizing cell densities and incubation times. The data were analyzed using a square-root plot (*8*). The number of amino acid residues in the β-galactosidase monomer is 1023. This number is divided by the time required to make the first functional monomers of the enzyme, after addition of IPTG. The results are a measure of the average elongation rate of the ribosomes translating *lac* mRNA.

***In situ* DNA footprinting and RNA analyses**

In situ DNA probing with CAA and subsequent analyses of modifications by primer extension with Klenow fragment of DNA polymerase I were carried out as previously described (*9-11*). *E.coli* MG1655 cells harboring the pLacK and the p^{RBS}1EC (or p2EC or p1EC) plasmids were grown to an OD_{600}~0.4 in 10 ml of M9 medium supplemented with 0.4% glucose, 4 mg/ml casamino acids, 100 μg/ml ampicillin and 20 μg/ml kanamycin, and then induced with 1mM IPTG (if required) for 10 min. For *in situ* modifications, CAA was added to 4% final concentration and the incubation was continued for 5 min. Cells were immediately collected and the plasmid DNA extracted. After denaturation of the templates, 5' labeled primer that anneals downstream of the roadblock site was added and hybridization was allowed to proceed. After primer extension with the Klenow fragment of DNA polymerase I, extension products were analyzed on an 8% sequencing gel in parallel with a dideoxy sequencing ladder.

Quantitative analyses of the *cat* and *bla* transcripts were carried out simultaneously with two 5'-labeled primers that anneal downstream of the roadblock site for the *cat* gene and at the proximal part of *bla* gene. Twenty micrograms of total RNA were used in analysis by primer extension with reverse transcriptase as previously reported (*10*).

In vitro transcription

His^6-RNAP (~2 pmol) was mixed with a 2-fold molar excess of DNA in 20 μl of TB50 (10 mM $MgCl_2$, 40 mM Tris-HCl, pH 7.9, 50 mM KCl) for 5 min at 37° followed by addition of ApUpC (10 μM, Oligos Etc.), GTP and ATP (25 μM) for 10 min. Next, 5 μl TB100-equilibrated Talon Co^{++} affinity bead suspension (Clontech) were added for 5 min at room temperature. 1 μl [α-^{32}P] CTP (3000Ci/mmol; NEN Life Sciences Products) was added for 5 min, followed by washing of the beads in 3x1 ml TB 1000 (as TB 50 but 1000 mM NaCl) and 2x1 ml TB50. The sart-up EC was chased with 1 mM ATP, CTP, UTP and 25 μM GTP for 10 minutes with or without 0.4 μM Rho at 37°C. Reactions were stopped by the addition of 10 μL Stop Buffer [1xTBE; 8M Urea; 20 mM EDTA], samples were phenol/chlorophorm extracted, ethanol precipitated and re-dissolved in 10 μL water plus 10 μL Stop Buffer.

Supplemental Figures and Legends

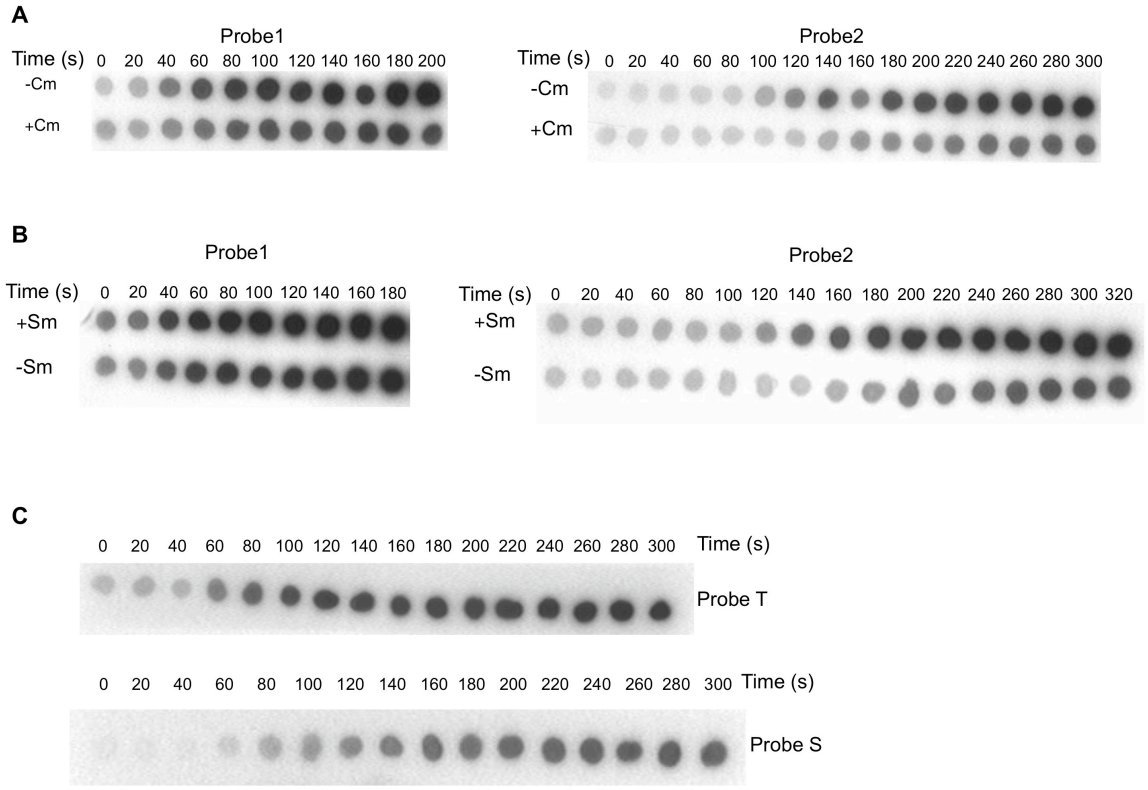

Fig. S1. **Dot blots used to generate the induction curves of Fig. 1B (A), Fig. 1C (B) and Fig. 1D (C).**

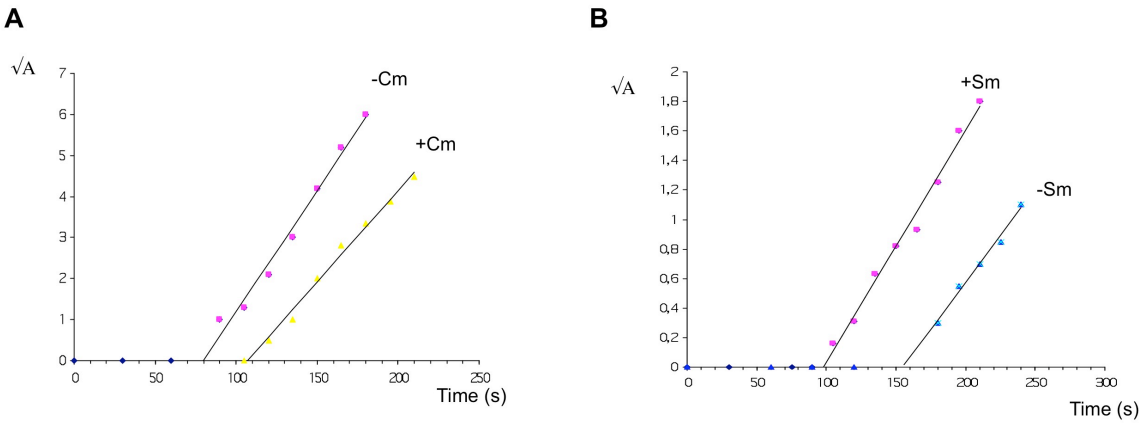

Fig. S2. **The effect of Cm (A) and Sm (B) on the translation rate of MG1655 and SmP strains, respectively**. Representative plots demonstrate the induction kinetics of β-galactosidase: absorbance values plotted against time. Extrapolation to an absorbance of zero gives the time required for synthesis of the first β-galactosidase monomers. The averaged calculated rates from three independent experiments are shown in Table 1.

lacZ

ATG ACC ATG ATT ACG GAT TCA CTG GCC GTC GTT TTA CAA CGT CGT GAC TGG GAA AAC CCT GGC GTT ACC CAA
CTT AAT CGC CTT GCA GCA CAT CCC CCT TTC GCC AGC TGG CGT AAT AGC GAA GAG GCC CGC ACC GAT CGC CCT
TCC CAA CAG TTG CGC AGC CTG AAT GGC GAA TGG CGC TTT GCC TGG TTT CCG GCA CCA GAA GCG GTG CCG GAA
AGC TGG CTG GAG TGC GAT CTT CCT GAG GCC GAT ACT GTC GTC GTC CCC TCA AAC TGG CAG ATG CAC GGT TAC
GAT GCG CCC ATC TAC ACC AAC GTA ACC TAT CCC ATT ACG GTC AAT CCG CCG TTT GTT CCC ACG GAG AAT CCG
ACG GGT TGT TAC TCG CTC ACA TTT AAT GTT GAT GAA AGC TGG CTA CAG GAA GGC CAG ACG CGA ATT ATT TTT GAT
GGC GTT AAC TCG GCG TTT CAT CTG TGG TGC AAC GGG CGC TGG GTC GGT TAC GGC CAG GAC AGT CGT TTG CCG
TCT GAA TTT GAC CTG AGC GCA TTT TTA CGC GCC GGA GAA AAC CGC CTC GCG GTG ATG GTG CTG CGT TGG AGT
GAC GGC AGT TAT CTG GAA GAT CAG GAT ATG TGG CGG ATG AGC GGC ATT TTC CGT GAC GTC TCG TTG CTG CAT
AAA CCG ACT ACA CAA ATC AGC GAT TTC CAT GTT GCC ACT CGC TTT AAT GAT GAT TTC AGC CGC GCT GTA CTG GAG
GCT GAA GTT CAG ATG TGC GGC GAG TTG CGT GAC TAC CTA CGG GTA ACA GTT TCT TTA TGG CAG GGT GAA ACG
CAG GTC GCC AGC GGC ACC GCG CCT TTC GGC GGT GAA ATT ATC GAT GAG CGT GGT GGT TAT GCC GAT CGC GTC
ACA CTA CGT CTG AAC GTC GAA AAC CCG AAA CTG TGG AGC GCC GAA ATC CCG AAT CTC TAT CGT GCG GTG GTT
GAA CTG CAC ACC GCC GAC GGC ACG CTG ATT GAA GCA GAA GCC TGC GAT GTC GGT TTC CGC GAG GTG CGG ATT
GAA AAT GGT CTG CTG CTG CTG AAC GGC AAG CCG TTG CTG ATT CGA GGC GTT AAC CGT CAC GAG CAT CAT CCT
CTG CAT GGT CAG GTC ATG GAT GAG CAG ACG ATG GTG CAG GAT ATC CTG CTG ATG AAG CAG AAC AAC TTT AAC
GCC GTG CGC TGT TCG CAT TAT CCG AAC CAT CCG CTG TGG TAC ACG CTG TGC GAC CGC TAC GGC CTG TAT GTG
GTG GAT GAA GCC AAT ATT GAA ACC CAC GGC ATG GTG CCA ATG AAT CGT CTG ACC GAT GAT CCG CGC TGG CTA
CCG GCG ATG AGC GAA CGC GTA ACG CGA ATG GTG CAG CGC GAT CGT AAT CAC CCG AGT GTG ATC ATC TGG TCG
CTG GGG AAT GAA TCA GGC CAC GGC GCT AAT CAC GAC GCG CTG TAT CGC TGG ATC AAA TCT GTC GAT CCT TCC
CGC CCG GTG CAG TAT GAA GGC GGC GGA GCC GAC ACC ACG GCC ACC GAT ATT ATT TGC CCG ATG TAC GCG CGC
GTG GAT GAA GAC CAG CCC TTC CCG GCT GTG CCG AAA TGG TCC ATC AAA AAA TGG CTT TCG CTA CCT GGA GAG
ACG CGC CCG CTG ATC CTT TGC GAA TAC GCC CAC GCG ATG GGT AAC AGT CTT GGC GGT TTC GCT AAA TAC TGG
CAG GCG TTT CGT CAG TAT CCC CGT TTA CAG GGC GGC TTC GTC TGG GAC TGG GTG GAT CAG TCG CTG ATT AAA
TAT GAT GAA AAC GGC AAC CCG TGG TCG GCT TAC GGC GGT GAT TTT GGC GAT ACG CCG AAC GAT CGC CAG TTC
TGT ATG AAC GGT CTG GTC TTT GCC GAC CGC ACG CCG CAT CCA GCG CTG ACG GAA GCA AAA CAC CAG CAG CAG
TTT TTC CAG TTC CGT TTA TCC GGG CAA ACC ATC GAA GTG ACC AGC GAA TAC CTG TTC CGT CAT AGC GAT AAC
GAG CTC CTG CAC TGG ATG GTG CGC CTG GAT GGT AAG CCG CTG CAA AGC GGT GAA GTG CCT CTG GAT GTC GCT
CCA CAA GGT AAA CAG TTG ATT GAA CTG CCT GAA CTA CCG CAG CCG GAG AGC GCC GGG CAA CTC TGG CTC ACA
GTA CGC GTA GTG CAA CCG AAC GCG ACC GCA TGG TCA GAA GCC GGG CAC ATC AGC GCC TGG CAG CAG TGG CGT
CTG GCG GAA AAC CTC AGT GTG ACG CTC CCC GCC GCG TCC CAC GCC ATC CCG CAT CTG ACC ACC AGC GAA ATG

GAT TTT TGC ATC GAG CTG GGT AAT AAG CGT TGG CAA TTT AAC CGC CAG TCA GGC TTT CTT TCA CAG ATG TGG ATT GGC GAT AAA AAA CAA CTG CTG ACG CCG CTG CGC GAT CAG TTC ACC CGT GCA CCG CTG GAT AAC GAC ATT GGC GTA AGT GAA GCG ACC CGC ATT GAC CCT AAC GCC TGG GTC GAA CGC TGG AAG GCG GCG GGC CAT TAC CAG GCC GAA GCA GCG TTG TTG CAG TGC ACG GCA GAT ACA CTT GCT GAT GCG GTG CTG ATT ACG ACC GCT CAC GCG TGG CAG CAT CAG GGG AAA ACC TTA TTT ATC AGC CGG AAA ACC TAC CGG ATT GAT GGT AGT GGT CAA ATG GCG ATT ACC GTT GAT GTT GAA GTG GCG AGC GAT ACA CCG CAT CCG GCG CGG ATT GGC CTG AAC TGC CAG CTG GCG CAG GTA GCA GAG CGG GTA AAC TGG CTC GGA TTA GGG CCG CAA GAA AAC TAT CCC GAC CGC CTT ACT GCC GCC TGT TTT GAC CGC TGG GAT CTG CCA TTG TCA GAC ATG TAT ACC CCG TAC GTC TTC CCG AGC GAA AAC GGT CTG CGC TGC GGG ACG CGC GAA TTG AAT TAT GGC CCA CAC CAG TGG CGC GGC GAC TTC CAG TTC AAC ATC AGC CGC TAC AGT CAA CAG CAA CTG ATG GAA ACC AGC CAT CGC CAT CTG CTG CAC GCG GAA GAA GGC ACA TGG CTG AAT ATC GAC GGT TTC CAT ATG GGG ATT GGT GGC GAC GAC TCC TGG AGC CCG TCA GTA TCG GCG GAA TTC CAG CTG AGC GCC GGT CGC TAC CAT TAC CAG TTG GTC TGG TGT CAA AAA TAA

rplB

ATG GCA GTT GTT AAA TGT AAA CCG ACA TCT CCG GGT CGT CGC CAC GTA GTT AAA GTG GTT AAC CCT GAG CTG CAC AAG GGC AAA CCT TTT GCT CCG TTG CTG GAA AAA AAC AGC AAA TCC GGT GGT CGT AAC AAC AAT GGC CGT ATC ACC ACT CGT CAT ATC GGT GGT GGC CAC AAG CAG GCT TAC CGT ATT GTT GAC TTC AAA CGC AAC AAA GAC GGT ATC CCG GCA GTT GTT GAA CGT CTT GAG TAC GAT CCG AAC CGT TCC GCG AAC ATC GCG CTG GTT CTG TAC AAA GAC GGT GAA CGC CGT TAC ATC CTG GCC CCT AAA GGC CTG AAA GCT GGC GAC CAG ATT CAG TCT GGC GTT GAT GCT GCA ATC AAA CCA GGT AAC ACC CTG CCG ATG CGC AAC ATC CCG GTT GGT TCT ACT GTT CAT AAC GTA GAA ATG AAA CCA GGT AAA GGC GGT CAG CTG GCA CGT TCC GCT GGT ACT TAC GTT CAG ATC GTT GCT CGT GAT GGT GCT TAT GTC ACC CTG CGT CTG CGT TCT GGT GAA ATG CGT AAA GTA GAA GCA GAC TGC CGT GCA ACT CTG GGC GAA GTT GGC AAT GCT GAG CAT ATG CTG CGC GTT CTG GGT AAA GCA GGT GCT GCA CGC TGG CGT GGT GTT CGT CCG ACC GTT CGC GGT ACC GCG ATG AAC CCG GTA GAC CAC CCA CAT GGT GGT GGT GAA GGT CGT AAC TTT GGT AAG CAC CCG GTA ACT CCG TGG GGC GTT CAG ACC AAA GGT AAG AAG ACC CGC AGC AAC AAG CGT ACT GAT AAA TTC ATC GTA CGT CGC CGT AGC AAA TAA

tufA

GTG TCT AAA GAA AAA TTT GAA CGT ACA AAA CCG CAC GTT AAC GTT GGT ACT ATC GGC CAC GTT GAC CAC GGT AAA ACT ACT CTG ACC GCT GCA ATC ACC ACC GTA CTG GCT AAA ACC TAC GGC GGT GCT GCT CGT GCA TTC GAC CAG ATC GAT AAC GCG CCG GAA GAA AAA GCT CGT GGT ATC ACC ATC AAC ACT TCT CAC GTT GAA TAC GAC ACC CCG ACC CGT CAC TAC GCA CAC GTA GAC TGC CCG GGG CAC GCC GAC TAT GTT AAA AAC ATG ATC ACC GGT GCT GCT CAG ATG GAC GGC GCG ATC CTG GTA GTT GCT GCG ACT GAC GGC CCG ATG CCG CAG ACT CGT GAG CAC ATC CTG CTG GGT CGT CAG GTA GGC GTT CCG TAC ATC ATC GTG TTC CTG AAC AAA TGC GAC ATG GTT GAT GAC GAA GAG CTG CTG GAA CTG GTT GAA ATG GAA GTT CGT GAA CTT CTG TCT CAG TAC GAC TTC CCG GGC GAC GAC ACT CCG ATC GTT CGT GGT TCT GCT CTG AAA GCG CTG GAA GGC GAC GCA GAG TGG GAA GCG AAA ATC CTG GAA CTG GCT GGC TTC CTG GAT TCT TAT ATT CCG GAA CCA GAG CGT GCG ATT GAC AAG CCG TTC CTG CTG CCG ATC GAA GAC GTA TTC TCC ATC TCC GGT CGT GGT ACC GTT GTT ACC GGT CGT GTA GAA CGC GGT ATC ATC AAA GTT GGT GAA GAA GTT GAA ATC GTT GGT ATC AAA GAG ACT CAG AAG TCT ACC TGT ACT GGC GTT GAA ATG TTC CGC AAA CTG CTG GAC GAA GGC CGT GCT GGT GAG AAC GTA GGT GTT CTG CTG CGT GGT ATC AAA CGT GAA GAA ATC GAA CGT GGT CAG GTA CTG GCT AAG CCG GGC ACC ATC AAG CCG CAC ACC AAG TTC GAA TCT GAA GTG TAC ATT CTG TCC AAA GAT GAA GGC GGC CGT CAT ACT CCG TTC TTC AAA GGC TAC CGT CCG CAG TTC TAC TTC CGT ACT ACT GAC GTG ACT GGT ACC ATC GAA CTG CCG GAA GGC GTA GAG ATG GTA ATG CCG GGC GAC AAC ATC AAA ATG GTT GTT ACC CTG ATC CAC CCG ATC GCG ATG GAC GAC GGT CTG CGT TTC GCA ATC CGT GAA GGC GGC CGT ACC GTT GGC GCG GGC GTT GTT GCT AAA GTT CTG GGC TAA

infB

ATG ACA GAT GTA ACG ATT AAA ACG CTG GCC GCA GAG CGA CAG ACC TCC GTG GAA CGC CTG GTA CAG CAA TTT GCT GAT GCA GGT ATC CGG AAG TCT GCT GAC GAC TCT GTG TCT GCA CAA GAG AAA CAG ACT TTG ATT GAC CAC CTG AAT CAG AAA AAT TCA GGC CCG GAC AAA TTG ACG CTG CAA CGT AAA ACA CGC AGC ACC CTT AAC ATT CCT GGT ACC GGT GGA AAA AGC AAA TCG GTA CAA ATC GAA GTC CGC AAG AAA CGC ACC TTT GTG AAA CGC GAT CCG CAA GAG GCT GAA CGC CTT GCA GCG GAA GAG CAA GCG CAG CGT GAA GCG GAA GAG CAA GCC CGT CGT GAG GCA GAA GAA TCG GCT AAA CGC GAG GCG CAA CAA AAA GCT GAA CGT GAG GCC GCA GAA CAA GCT AAG CGT GAA GCT GCT GAA CAA GCG AAA CGT GAA GCT GCG GAA AAA GAC AAA GTG AGC AAT CAA CAA GAC GAT ATG ACT AAA AAC GCC CAG GCT GAA AAA GCC CGC CGT GAG CAG GAA GCT GCA GAG CTC AAG CGT AAA GCT GAA GAA GAA GCG CGT CGT AAA CTC GAA GAA GAA GCA CGT CGC GTT GCT GAA GAA GCA CGT CGT ATG GCG GAA GAA AAC AAA TGG ACT GAT AAC GCG GAA CCG ACT GAA GAT TCC AGC GAT TAT CAC GTC ACT ACT TCT CAA CAT GCT CGC CAG GCA GAA GAC GAA AGC GAT CGT GAA GTC GAA GGC GGC CGT GGC CGT GGT CGT AAC GCG AAA GCA GCG CGT CCG AAG AAA GGC AAC AAA CAC GCT GAA TCA AAA GCT GAT CGT GAA GAA GCA CGC GCA GCA GTA CGT GGC GGT AAA GGC GGA AAA CGT AAA GGT TCT TCG CTG CAG CAA GGC TTC CAG AAG CCT GCT CAG GCC GTT AAC CGT GAC GTT GTG ATC GGC GAA ACT ATC ACC GTT GGC GAA CTG GCG AAC AAG ATG GCG GTT AAA GGC TCT CAG GTC ATC AAA GCG ATG ATG AAA CTG GGC GCA ATG GCA ACC ATC AAC CAG GTT ATC GAT CAG GAA ACC GCA CAG CTG GTT GCT GAA GAG ATG GGC CAT AAA GTT ATC CTG CGT CGT GAA AAC GAG CTG GAA GAG GCG GTA ATG AGC GAC CGT GAC ACG GGT GCT GCG GCT GAA CCG CGC GCG CCG GTT GTG ACC ATC ATG GGT CAC GTT GAC CAC GGT AAA ACC TCT CTG CTG GAC TAC ATT CGT TCA ACG AAA GTG GCC TCT GGC GAA GCG GGC GGC ATT ACC CAG CAC ATT GGT GCA TAC CAC GTT GAA ACT GAA AAC GGC ATG ATC ACC TTC CTG GAC ACC CCG GGG CAC GCC GCG TTT ACT TCA ATG CGT GCT

CGT GGT GCG CAG GCA ACG GAC ATC GTA GTC CTG GTT GTT GCT GCC GAC GAC GGT GTG ATG CCG CAG ACC ATC
GAA GCA ATC CAG CAC GCG AAA GCG GCG CAG GTA CCG GTG GTG GTT GCA GTG AAC AAG ATC GAT AAA CCA GAA
GCT GAT CCG GAT CGC GTT AAG AAC GAA CTC TCC CAG TAC GGC ATC CTG CCG GAA GAG TGG GGC GGT GAA AGC
CAG TTC GTA CAC GTA TCT GCG AAA GCG GGT ACC GGT ATC GAT GAA CTG CTG GAC GCT ATC CTG CTG CAG GCG
GAA GTT CTG GAG CTG AAA GCG GTA CGT AAA GGT ATG GCG AGC GGT GCG GTT ATC GAA TCC TTC CTC GAT AAA
GGT CGT GGT CCG GTT GCT ACC GTT CTG GTA CGT GAA GGT ACT CTG CAC AAG GGC GAT ATC GTT CTG TGT GGC
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TCC GTC ATC TAT AAC CTG ATT GAC GAA GTG AAA GCG GCG ATG AGC GGT ATG CTG TCT CCG GAA CTG AAA CAG
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ATT GCT TAA

srb4

ATG ACA ACG GAA GAT CCA GAT TCA AAT CAC TTA AGT TCC GAA ACT GGC ATT AAA TTG GCA TTG GAC CCG AAC TTA
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GCA GGA AAA GCA GAT ACT AGT ATT CGA CTA GAA GGT GAT GAG TTA GAG AAT AAA ACT AAG AAA GAC AAT GAT
AAG AAC TTA AAA TTT TTG AAG AAT AAA GAT TCT CTA GTC AGT AAT CCA CAC GAA ATT TAT GGC TCC ATG CCG TTG
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CAA AAT GAG ATT AAG CAG CTT GGT AGT GAT AGT AGT GAC GGT CAT AAC AGC GAG AAG AAG GAC ACT GAT GGC
GCT GAT GAG AAT GTA CAA ATT GGA GAA GAT TTC ATG GAA GTG GAT TAT GAA GAT AAA GAT AAT CCA GTG GAT TCA
CGA AAT GAA ACA GAC CAC AAA ACG AAT GAA AAT GGC GAG ACC GAT GAT AAT ATT GAA ACG GTA ATG ACA CAG
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ACG AAA GAT ATC AGA AAG CAA ATC CAA CTT TTG AAA AAG ATC ATT TTT GAA AAA GAA CTG ATG TAC CAA ATA AAG
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GAA AAA TTT GAA ATC GAG TTG TTG TCC CTT GAC GAT GAC TCC ATT GTC AAT CAT GAA CAA GAC TTA CCA AAA ATC
AAC GAC AAG AGA GCA AAT TTA ATG CTT GTT ATG TTG AGA CTA TTA TTA GTC GTT ATA TTC AAG AAA ACA TTA CGA
TCG AGA ATA AGC TCA CCC CAC GGA CTG ATC AAT TTG AAT GTT GAC GAT GAT ATC TTA ATA ATA CGT CCC ATT CTT
GGT AAA GTT CGG TTT GCT AAT TAC AAA CTG TTA CTA AAA AAA ATC ATA AAG GAT TAC GTG CTC GAT ATA GTT CCT
GGC TCA AGT ATA ACA GAA ACG GAA GTT GAG AGA GAA CAA CCT CAA GAA AAT AAA AAC ATT GAT GAT GAA AAT ATA
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CCA TTA ACT GAG CAC AAA AGC CCT AAT CTA AGT TTA ATG CTC GAA AGT CCT AAC TAT TGT AAC GCA CTC ATT CAC
ATC AAG TTT TCA GCT GGT ACG GAA GCC AAC GCA GTG TCC TTT GAC ACA ACA TTT TCT GAT TTT AAA GAA GTA GAG
GAC TTC CTA CAT TTT ATT GTC GCT GAG TAC ATC CAG CAA AAG AAG GTG TAA

Fig. S3. Rare codons distribution in the tested genes. tRNA species for 8 triplets (CUA, UCC, ACA, CCU, CCC, UCA, CCA, AGG) are extremely scarce in E.coli (*12*). In addition, two moderately used codons (UUA and GUC) are also limited by their tRNA concentrations. In total, these 10 rare codons are expected to produce the slowest translation rates (*13*).

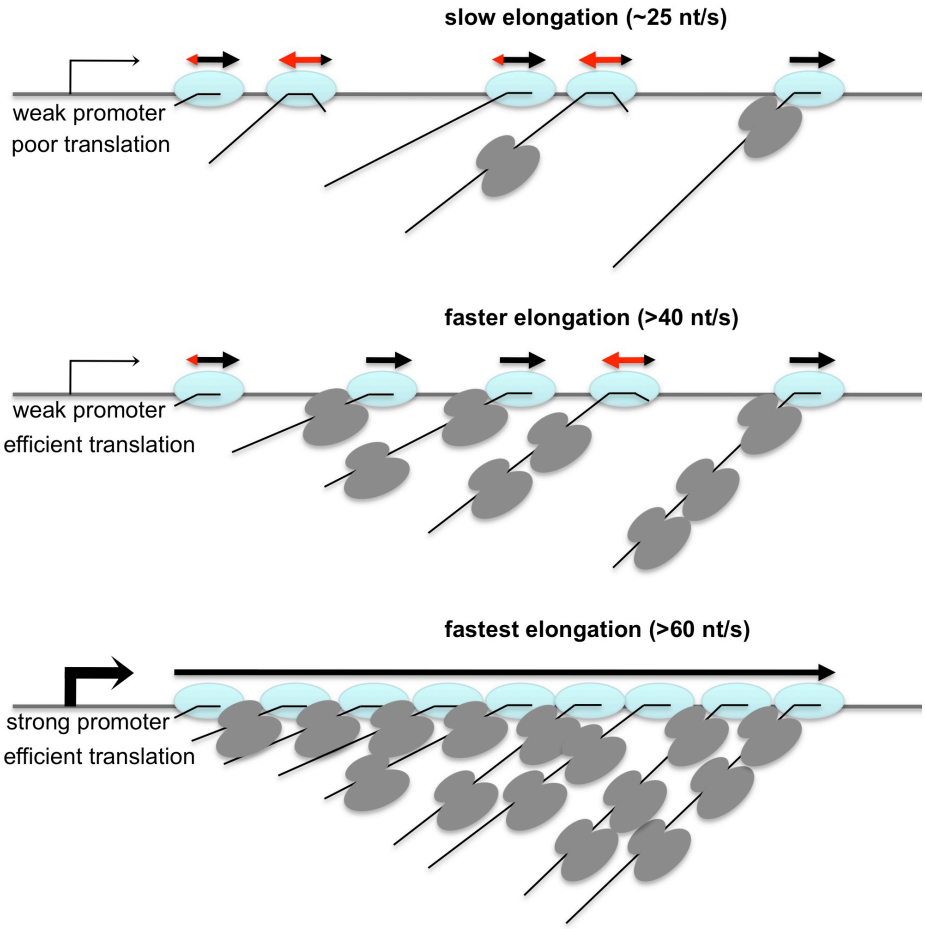

Fig. S4. Macromolecular trafficking and cooperation in controlling bacterial gene expression. Schematics summarize the results of the present study, which together with our previous study (*4*), establish a relationship between promoter strength, translation and transcription elongation rates. Trailing ECs and ribosomes are capable of "pushing" the leading ECs forward by physically suppressing RNAP backtracking (indicated by red arrows). Thus, the more RNAP molecules engaged in transcription of a gene, and the higher the speed of its translation, the faster its overall transcription elongation rate. Since elongation is usually the rate-limiting step of the transcription cycle (it takes minutes to complete a transcript), such a direct coordination between initiation, elongation and translation ensures precise adjustment of the transcriptional yield to translational needs. The cooperation mechanism explains why the transcription elongation rate matches the translation elongation rate so precisely (Table 1), and how transcription elongation depends on codon usage (Table 2), growth phase (fig. S5), growth rate (fig. S6) (*2*), and promoter strength (*4*).

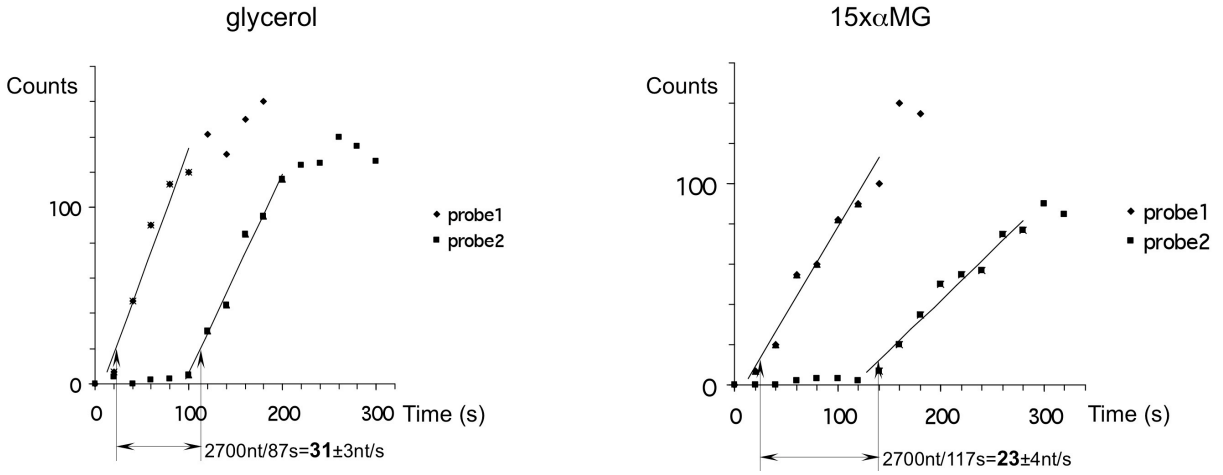

Fig. S5. Effect of carbon source on transcription elongation rate. Panels display representative induction curves used to calculate the elongation rate. Rates were calculated as in Fig. 1A. "Glycerol" – cells were grown in M9 minimal media supplemented with glycerol (0.5%) and Casamino Acids (0.2%); "αMG" – cells were grown in glucose minimal media in the presence of α-methyl-glucoside at the 15x to glucose ratio.

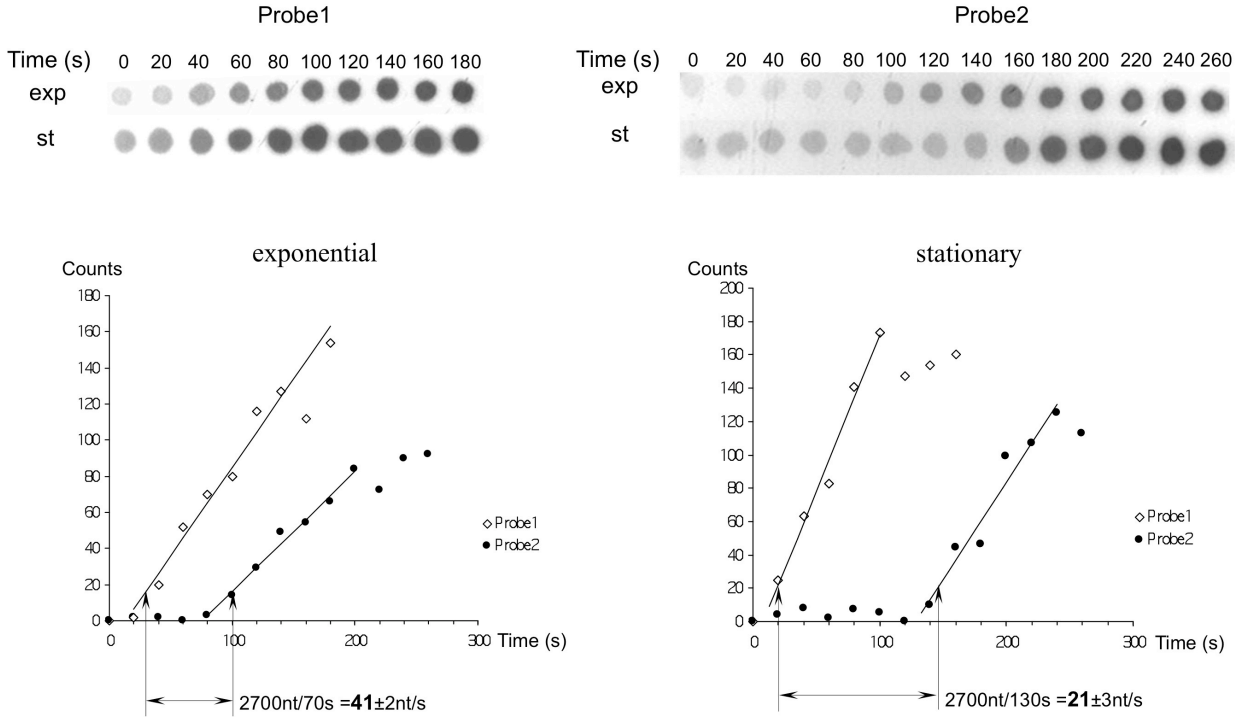

Fig. S6. **Transcription elongation rate depends on growth phase.** Representative dot blots of the early (1) and late (2) probes from the early exponential (OD_{600}~0.4) and stationary (OD_{600}~2.5) phase of growth of MG1655 were used to generate the induction curves shown. Rates were calculated as in Fig. 1A.

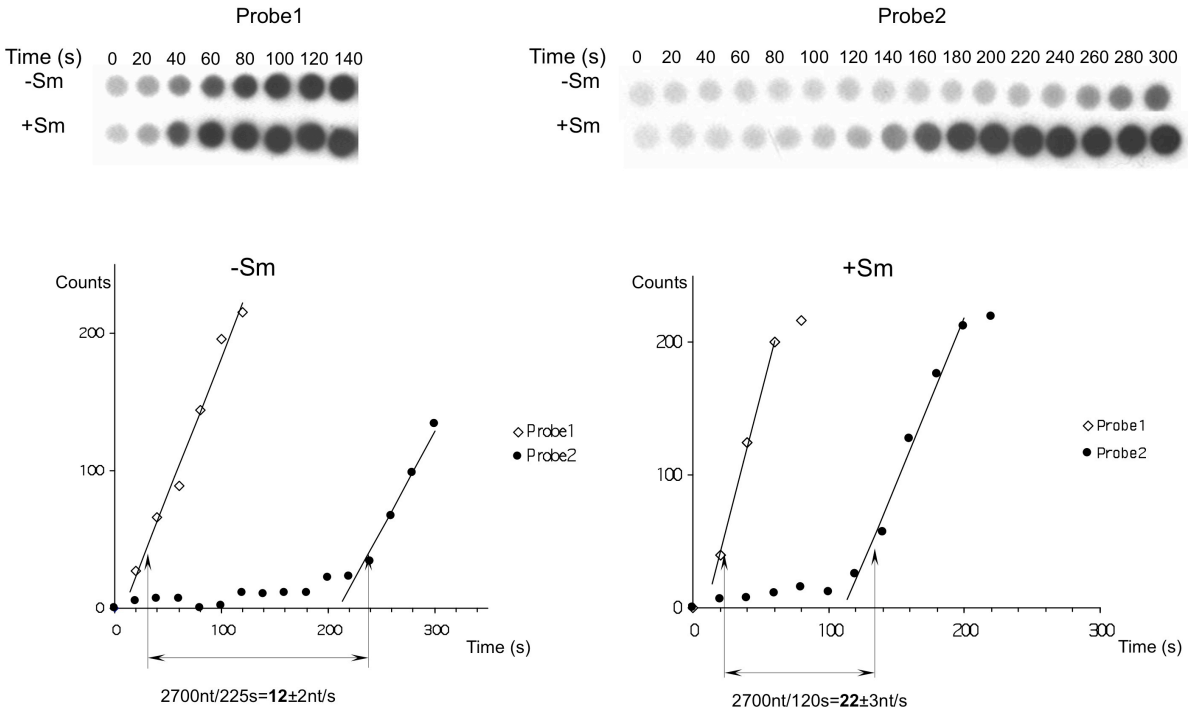

Fig. S7. **Effect of the slow ribosome mutation (*rpsL*[SmP]) and streptomycin (Sm) on transcription elongation rate in the stationary phase.** Representative dot blots of the early (1) and late (2) probes from the stationary (OD_{600}~2.5) phase of growth of the "slow ribosome" strain CH184 were used to generate the induction curves shown. Rates were calculated as in Fig. 1A. Slow transcription rate of CH184, as compared to that of MG1655 (fig. S6), and its acceleration by Sm demonstrate that the transcription elongation rate remains under tight translational control in the stationary phase of growth.

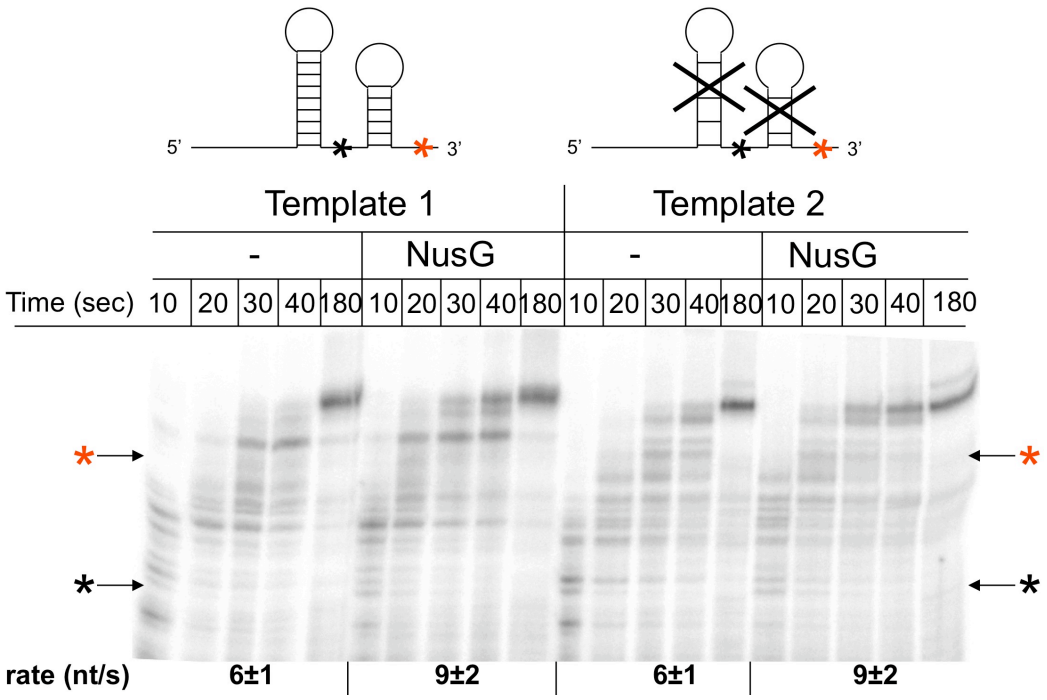

Fig. S8. **Effect of local secondary structures and NusG on the elongation rate in vitro**. Templates 1 and 2 are identical in sequence, except in two strong hairpins that are mutated in template 2 so they cannot fold: hairpins #1 and #2 have 9 and 6 bp perfect GC-rich stems, respectively, and Template 2 carries three mismatches in each of the hairpins stem. Asterisks indicate the location of potential pause sites (7-10 nt downstream of the base of the hairpin stem), which are not detectable under specified chase conditions (10 μM NTPs) in either case. No difference in the overall elongation rates (nt/s) is detected between the two templates, demonstrating that neither hairpin affects the rate of elongation. Moreover, the anti-backtracking factor NusG (0.1 mg/ml) increased the rate of elongation to the same extent on both templates. Thus, backtracking-type pauses, not hairpins, determine the elongation rate. In vivo footprinting and roadblocking experiments (Fig. 3 and 4) demonstrate that the ribosome suppresses RNAP backtracking. We, therefore, conclude that the stimulating effect of a ribosome on transcription elongation occurs predominantly as a result of its anti-backtracking mechanism.

Supplemental References and Notes

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14. Our results imply that the ribosome has to be *immediately* behind the moving RNAP most of the time to control the overall rate of RNAP movement. Indeed, translation initiation (including 30S-mRNA complex formation, 50S binding, and IF3release) occurs in less than 2 seconds at the majority of genes in E.coli (*15, 16*). In this short time window RNAP can only move 20-40 nt away from RBS. Because RNAP elongates much slower in the absence of translation (Table 1), the ribosome should be able to catch up to RNAP within the first 100-200 nt. RBS mutations do not affect significantly the rate of initiation unless ribosome binding is impaired by a strong secondary structure, which is rare (*17*). Therefore, for the majority of E.coli genes there must be only short stretches of naked RNA between the moving RNAP and the first trailing ribosome (the one which determines the rate of transcription) in the beginning of a gene. The minimal distance between the ribosome and RNAP should also prevent Rho from terminating transcription prematurely; Rho requires only about 100 nt of naked RNA (without strong hairpins) to load and terminate transcription (*18*).
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