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Activation of prokaryotic transcription through arbitrary protein–protein contacts

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Many transcriptional activators in prokaryotes are known to bind near a promoter and contact RNA polymerase[1–5], but it is not clear whether a protein–protein contact between an activator and RNA polymerase is enough to activate gene transcription. Here we show that contact between a DNA-bound protein and a heterologous protein domain fused to RNA polymerase can elicit transcriptional activation; moreover, the strength of this engineered protein–protein interaction determines the amount of gene activation. Our results indicate that an arbitrary interaction between a DNA-bound protein and RNA polymerase can activate transcription. We also find that when the DNA-bound 'activator' makes contact with two different components of the polymerase, the effect of these two interactions on transcription is synergistic.

We replaced the carboxy-terminal domain (CTD) of the α-subunit of RNA polymerase (RNAP) (Fig. 1), which is the natural target for many transcriptional activators[1–3], with a heterologous protein domain that does not ordinarily mediate transcriptional activation. To do this, we took advantage of the well defined properties of the CTD of the bacteriophage λ cI protein (λcI).

λcI is a two-domain protein that binds DNA as a dimer, and pairs of dimers bind cooperatively to adjacent operator sites (Fig. 2a)[5]. The amino-terminal domain (λcI-NTD) contacts the DNA and can interact with the σ subunit of RNAP, stimulating transcription when λcI is bound at promoter λP_{RM} (refs 6, 7). The λcI-CTD mediates both dimer formation and the dimer–dimer interaction that results in cooperativity (reviewed in ref. 8) (Fig. 2a). λcI mutants specifically defective for transcriptional activation bear amino-acid substitutions in the λcI-NTD[9], and λcI mutants specifically defective for cooperative binding to DNA bear amino-acid substitutions in the λcI-CTD (ref. 10 and refs therein).

We reasoned that if we replaced the α-CTD with the λcI-CTD, the resulting α-cI chimaera would display a dimeric target that could be contacted by an appropriately positioned λcI dimer (Fig. 2b). We wanted to investigate whether the same protein–protein interaction that ordinarily mediates the cooperative binding of pairs of λcI dimers to the DNA would mediate transcriptional activation when the λcI-CTD is tethered to the α-NTD.

We constructed a derivative of the *lac* promoter termed *plac* O_R2-62, bearing a single λ operator (O_R2) centred 62 base pairs (bp) upstream of the transcription startpoint (at −62) (Fig. 2b) and introduced it in single copy into the chromosome of *Escherichia coli* strain MC1000 F′ *lacI*^{q} to create strain KS1. As expected, λcI alone does not activate transcription from *plac* O_R2-62 (Fig. 3a), because when bound this far away from the promoter it cannot contact the σ-subunit of RNAP. However, λcI stimulated transcription in the presence of the α-cI chimaera approximately 10-fold, as measured by β-galactosidase assay (Fig. 3a). This stimulation was not dependent on the natural activating region located in the λcI-NTD (data not shown). In the absence of the α-cI chimaera, λcI repressed transcription slightly. Furthermore, in the absence of λcI, expression of the α-cI chimaera had no significant effect on transcription from *plac* O_R2-62. Primer extension analysis confirmed that λcI stimulated the production of correctly initiated transcripts (Fig. 3c).

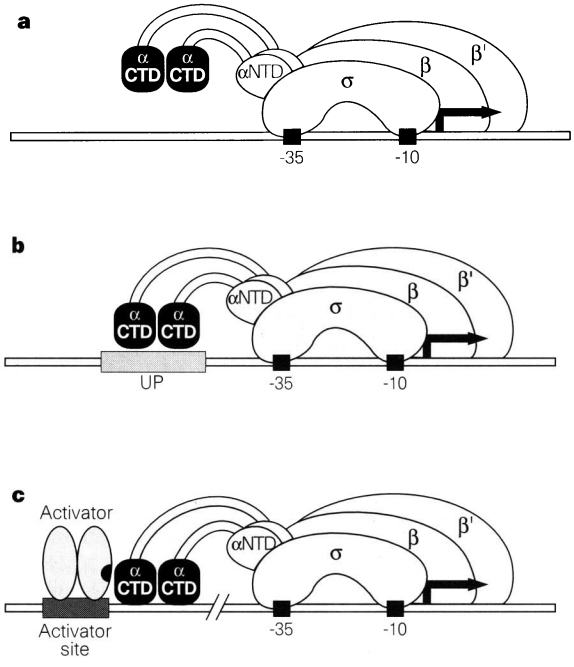

Figure 1 Function of the α-CTD in transcriptional activation. **a**, Basal transcription from a promoter that does not have an associated upstream element or transcriptional activator binding site. RNAP in *E. coli* consists of an enzymatic core composed of subunits α, β and β′ in the stoichiometry $\alpha_2\beta\beta'$, and one of several alternative σ factors responsible for specific promoter recognition. **b**, Stimulated transcription from a promoter bearing an associated upstream element (UP) involves specific protein–DNA interaction between the α-CTD and the UP element. The α-CTD, which is tethered to α-NTD by a flexible linker region, can interact with the DNA UP element that is found upstream of the −35 region of certain particularly strong promoters[30]. **c**, Stimulated transcription from a promoter bearing an associated activator binding site involves specific protein–protein interaction between the α-CTD and the DNA-bound activator.

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The hypothesis that λcI is activating transcription from *plac* O_R2-62 by contacting the λcI-CTD fused to the α-subunit of RNAP predicts that a λcI mutant unable to participate in the dimer–dimer interaction responsible for cooperativity would be unable to activate transcription in our artificial system. To test this prediction, we used a λcI mutant (λcI-D197G) that is unable to bind cooperatively to both adjacent and separated operator sites, but is indistinguishable from wild-type λcI in its ability to dimerize and bind DNA[10]. Unlike wild-type λcI, this mutant failed to activate transcription from *plac* O_R2-62 in the presence of the α-cI chimaera (Fig. 3b).

We then compared λcI-D197G with two λcI mutants with specific but less severe cooperativity defects[10]. Mutant N148D is less defective for cooperativity than mutant R196M, which is in turn less defective than D197G. Mutants N148D and R196M stimulated transcription from *plac* O_R2-62 more weakly than wild-type λcI but more efficiently than λcI-D197G; mutant R196M was the more defective of the two (Fig. 3b). Thus the strength of the interaction mediated by the λcI-CTD correlated with the magnitude of the observed activation.

We have shown that λcI bound at −62 can activate transcription by using its CTD to contact the λcI-CTD fused to the α-subunit of RNAP. It has been previously shown that λcI can also activate transcription when bound at −42 by using its NTD to contact the σ-subunit of RNAP[5–7]. We therefore wanted to see whether a single λcI dimer bound at −42 (close to the promoter) could contact both the α-cI chimaera and the σ-subunit simultaneously, thereby activating transcription more efficiently than λcI does ordinarily. To test this possibility, we used a P_{RM}–*lacZ* fusion bearing a single λcI binding site (O_R2) located at its natural stimulatory position centred at −42. This λP_{RM}–*lacZ* fusion was introduced into *E. coli* strain MC1000 F′*lacI*^{q} on the low-copy plasmid vector pP_{RM}Δ−50 (ref. 11).

Whereas λcI-stimulated transcription from P_{RM} resulted in ~130 units of activity in cells lacking the α-cI chimaera, it resulted in ~1,600 units in cells containing the α-cI chimaera, as measured by β-galactosidase assay (Fig. 4a). Primer extension analysis confirmed that λcI stimulated the production of correctly initiated transcripts in both cases (Fig. 4c).

To show that the high level of λcI-stimulated transcription observed in the presence of the α-cI chimaera depends on both activating surfaces of λcI (the natural one located in its NTD and the artificial one located in its CTD), we made use of two λcI mutants. One is specifically defective for activation through contact with the σ-subunit (λcI-E34A)[9], and the other is specifically defective for activation through contact with the α-cI chimaera (λcI-D197G) (see above). Both mutants stimulated transcription much more weakly than wild-type λcI (less than 10-fold), and, as expected, mutant E34A only stimulated transcription in the presence of the α-cI chimaera (Fig. 4a). We also verified that a double mutant bearing both substitutions failed to stimulate transcription. As when λcI is bound further upstream, the strength of the interaction mediated by the λcI-CTD correlated with the magnitude of the observed activation (Fig. 4b). We also demonstrated that substitution D197G, which abolishes λcI cooperativity, has the same effect when it is present in λcI, in the λcI moiety of the α-cI chimaera, or in both (Fig. 4b).

The results with these λcI mutants demonstrate that for an activator capable of contacting two components of RNAP, the effect of these two contacts on transcription can be synergistic. Specifically, the amount of transcription observed in the presence of wild-type λcI and the α-cI chimaera (~1,600 units) is much greater than the sum of that observed in the presence of the two single mutants (80 and 140 units). Because the fractional occupancy of the λcI binding site is ~85% under the conditions of the experiment shown in Fig. 4 (results not shown), this increased activation cannot be explained by an increase in the fractional occupancy of the λcI binding site. Whereas the λcI-CTD-mediated contact presumably

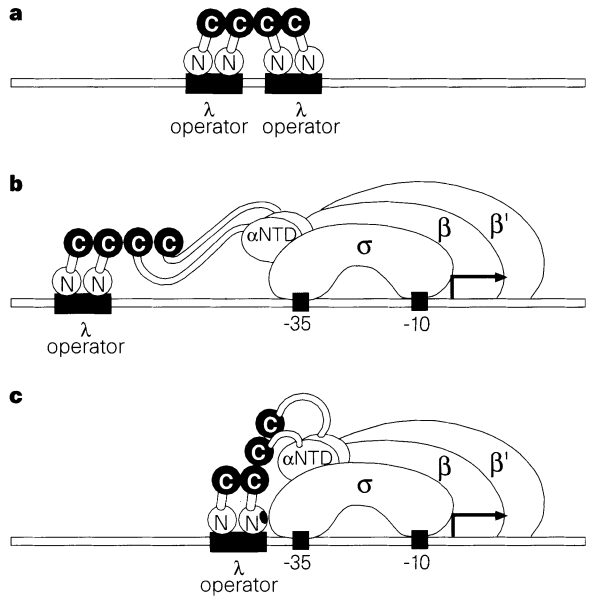

Figure 2 a, Interaction of adjacently bound λcI dimers. Shown are two λcI dimers (with each monomer depicted as a dumbbell to illustrate its domain structure) cooperatively bound to two adjacent operators (black boxes). The interaction between the two dimers, which is responsible for the cooperativity, is mediated by the CTD. **b**, Replacement of RNAP α-CTD by the λcI-CTD permits interaction with the CTD of a DNA-bound λcI dimer. The artificial promoter derivative *plac* O_R2-62 is shown: this bears the λ operator O_R2 centred 62 bp upstream of the transcriptional start site of the *lac* promoter. **c**, An appropriately positioned λcI dimer can make two contacts with an RNAP molecule whose α-CTD has been replaced by the λcI-CTD. The artificial promoter derivative P_{RM}Δ-50 is shown.

elicits activation simply by recruiting RNAP to the promoter, the λcI-NTD-mediated contact has been shown to promote the formation of transcriptionally active 'open complexes'[12]. Thus, in our artificial system, a single λcI dimer is evidently able to exert both of these effects at once.

This artificial form of activation is like that of the *E. coli* cAMP receptor protein (CRP) at class II CRP-dependent promoters. When bound at a site centred near position −42, CRP makes two functional contacts with RNAP, using one activating region to contact the α-CTD and a second activating region to contact a target site in the α-NTD[13].

Our results with two different promoters indicate that a protein domain can mediate transcriptional activation when tethered to the α-NTD simply by providing a surface that can be contacted by a DNA-bound protein. This activation presumably results from a strengthened association of RNAP with the promoter. Our findings are consistent with the proposal that natural activators that interact with the α-CTD function by recruiting RNAP to the promoter, but they indicate that a direct protein–DNA interaction involving the α-CTD (Fig. 1c) is not in principle essential for this domain to function as an activation target[3,4].

Our results suggest that a system analogous to the yeast two-hybrid system for detecting protein–protein interactions[14] could now be used in *E. coli*. That is, our artificial system could in principle be used to detect any protein–protein interaction between a protein domain fused to the α-NTD and a second domain fused to a suitable DNA-binding domain. As the equilibrium dissociation constant for the interaction of λcI dimers in solution is $\sim 10^{-6}$ M, and cooperative binding to DNA probably involves this same interaction[15], any protein–protein interaction of comparable (or greater) strength could be expected to mediate transcriptional

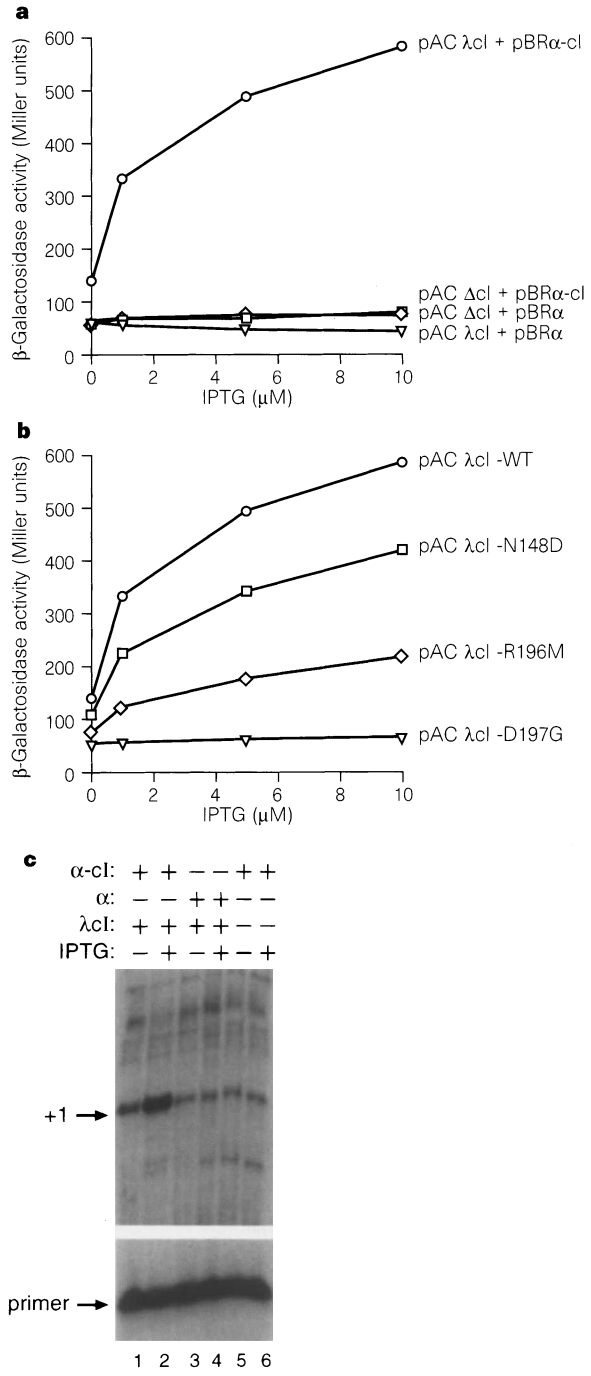

Figure 3 a, Effect of λcI on transcription *in vivo* from *plac* O_R2-62 in the presence of α-cI fusion protein. KS1 cells harbouring the indicated plasmids were assayed for β-galactosidase as described[28]. pACYC-derived plasmids either encoded λcI (pACλcI) or did not (pACΔcI); pBR322-derived plasmids either encoded the α-cI chimaera (pBRα-cI) or wild-type α (pBRα). **b**, Effects of λcI cooperativity mutants on transcription *in vivo* from *plac* O_R2-62 in the presence of the α-cI chimaera. KS1 cells containing plasmid-encoded α-cI chimaera (pBRα-cI) together with plasmids expressing either wild-type λcI or the indicated cooperativity mutant derivative were assayed for β-galactosidase activity. The abilities of the mutants to bind cooperatively to adjacent and non-adjacent operators have been measured previously[10]: λcI wild type > λcI-N148D > λcI-R196M > λcI-D197G. **c**, Primer extension analysis of transcripts produced from *plac* O_R2-62 in the presence of λcI and the α-cI chimaera. Total RNA was isolated from KS1 cells harbouring plasmids encoding the indicated proteins, and primer extension analysis was done by using a primer complementary to the *lacZ* transcript produced by the *plac* O_R2-62 promoter. Primer extension products produced by correctly initiated *plac* O_R2-62 transcripts are indicated by +1. Excess unincorporated primer is shown in the lower panel.

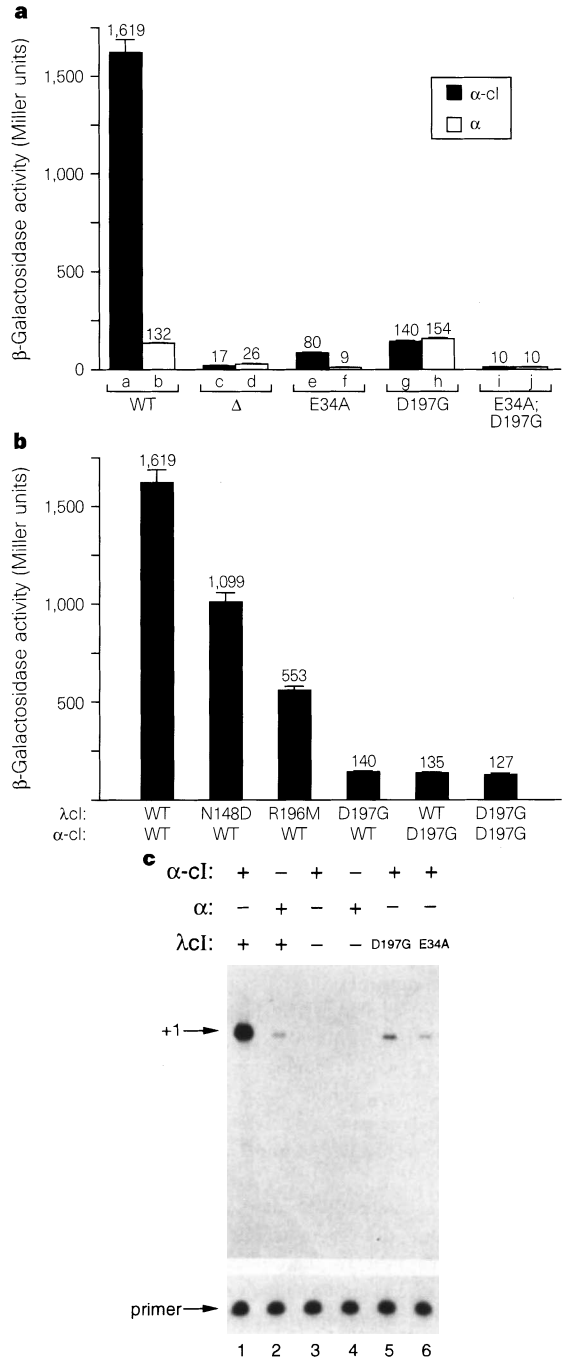

Figure 4 a, Effects of wild-type and mutant λcI proteins on transcription *in vivo* from P_{RM}Δ-50 in the presence of α-cI chimaera or wild-type α. MC1000 F′*lacI*^{q} cells harbouring the reporter plasmid pP_{RM}Δ-50 and containing the indicated plasmid-encoded proteins were assayed for β-galactosidase. pACYC-derived plasmids directed expression of wild-type λcI (WT), no λcI (Δ), or the indicated λcI mutant, whereas pBR322-derived plasmids directed expression of either the α-cI chimaera (a, c, e, g, i) or wild-type α (b, d, f, h, j). **b**, Effects of λcI cooperativity mutants on transcription *in vivo* from P_{RM}Δ-50 in the presence of α-cI chimaera. MC1000 F′*lacI*^{q} cells carrying the reporter plasmid pP_{RM}Δ-50 together with a pACYC-derived plasmid directing expression of either wild-type λcI (WT) or the indicated λcI cooperativity mutant, and a pBR322-derived plasmid directing expression of either the α-cI fusion protein (WT) or the D197G mutant derivative were assayed for β-galactosidase. **c**, Primer extension analysis of transcripts produced from P_{RM}Δ-50 in the presence of both the α-cI chimaera and wild-type or mutant λcI protein. Total RNA was isolated from MC1000 F′*lacI*^{q} cells harbouring the reporter plasmid pP_{RM}Δ-50 together with plasmids encoding the proteins indicated. Primer extension analysis was done using the primer described previously[11]. Primer extension products produced by correctly initiated P_{RM} transcripts are indicated by +1. Excess unincorporated primer is shown in the lower panel.

activation. We have shown that two interacting protein domains from yeast which interact more strongly than the λcI-CTDs mediate greater transcriptional activation when fused respectively to the α-NTD and to λcI (S.L.D., J.K.J. and A.H., manuscript in preparation). We do not know the interaction strength that would give maximal activation: depending on the particular promoter, and if the interaction between the DNA-binding protein and its recognition site were especially tight, a sufficiently strong protein–protein interaction might impede promoter clearance, resulting in repression[16] rather than activation.

Our findings provide a parallel with recent findings in yeast[17–21]. The discovery[17] that transcriptional activation can be triggered by a fortuitous interaction between a DNA-bound protein lacking a classical activating region and a mutant form of a component of the RNAP II holoenzyme[22,23], suggested a simple recruitment model for gene activation[17]. (Recruitment of the TATA-binding protein to the promoter can also elicit transcriptional activation[18–20].) Analysis of the interaction involving a DNA-binding protein and a component of the RNAP II holoenzyme has established a correlation between its strength as measured *in vitro* and the degree of activation *in vivo*[21]. Further, addition of an acidic activating region to the DNA-binding protein, allowing additional contacts with the transcription machinery, results in a dramatic increase in the activation observed in the presence of the mutant holoenzyme[21]. These findings in yeast and ours in *E. coli* imply that activation in both prokaryotes and eukaryotes can be elicited by a simple protein–protein contact between a DNA-bound activator and an available target surface on the RNAP holoenzyme. □

Methods

Plasmids and strains. pACλcI harbours the wild-type cI gene under the control of the *lacUV5* promoter. Plasmid pKB280 (ref. 24) was cut with *Eco*RI and *Hin*dIII to yield a fragment containing the cI gene; the ends of this fragment, together with those of pACYC184 digested with *Hin*dIII and *Hin*cII, were made flush using Klenow and the two fragments ligated to generate pAC280. pACλcI was constructed by replacing the *Hin*dIII–*Bsty*I fragment of pAC280 with the corresponding *Hin*dIII–*Bam*HI fragment from pLR2 (ref. 10). pACλcI-D197G, pACλcI-R196M, pACλcI-N148D, pACλcI-E34A and pACλcI-E34A; D197G (made by cloning the appropriate restriction fragments from previously described plasmids[10,11] into pACλcI) are identical to pACλcI except that they encode mutant derivatives of λcI with the indicated amino-acid changes. pACΔcI is identical to pA3HΔcI (ref. 10) and does not encode functional λcI.

The plasmid pBRα-cI encodes residues 1–248 of the α-subunit of *E. coli* RNAP fused to residues 132–236 of λcI under the control of tandem *lpp* and *lacUV5* promoters. The hybrid α-cI gene was amplified by the PCR and cloned into *Eco*RI–*Bam*HI-digested pBRα (a derivative of pHTf1α; ref. 25) to create pIBRα-cI. The *Hin*dIII–*Bam*HI fragment from pLR2 (ref. 10) was cloned into pIBRα-cI to make pBRα-cI. pBRα-cI-D197G was similarly constructed using the *HindIII–Bam*HI fragment from pLR2-D197G (ref. 10). PCR-amplified DNA was sequenced to verify that no errors had been introduced, and expression of α-cI fusion protein was confirmed by western blot using a polyclonal λcI antiserum (data not shown).

The *lac* promoter derivative *plac* O_R2-62 was constructed by cleaving the plasmid KJ306 (ref. 26) with *Hin*cII and inserting a 31-bp linker sequence. *plac* O_R2-62 on a plasmid vector was transferred to a lysogenic phage and integrated in single copy into the chromosome of MC1000 F′ *lacI*^{q} by lysogeny[27], creating strain KS1.

Experimental procedures. For the experiments shown in Fig. 3, cells were grown in LB supplemented with carbenicillin (50 μg ml^{-1}), chloramphenicol (25 μg ml^{-1}) and kanamycin (50 μg ml^{-1}) together with IPTG at the concentration indicated. Assays were done at least three times in duplicate on separate occasions, with similar results. Values are the averages from one experiment; duplicate measurements differed by <10%. In the primer extension analysis (Fig. 3c), IPTG (isopropyl-β-D-thiogalactoside) was added to the growth medium to a final concentration of 5 μM where indicated. Conditions for experiments shown in Fig. 4 were as for Fig. 3, except that tetracycline (35 μg ml^{-1}) was added to the growth medium. For Fig. 4, duplicate assays were done on duplicate cultures and data are expressed as the means of the four measurements. Experiments were done at least three times and a typical data set is shown; standard deviations were less than 10% and are indicated by error bars.

Primer extension analysis. RNA was isolated as described[28]; primer labelling and primer extension assays were done essentially according to ref. 29. Transcriptional start sites were identified by electrophoresis of primer extension products alongside dideoxy sequencing reactions done with the same oligonucleotide used in the primer extension reactions (data not shown).

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