Repression and activation of transcription by Gal and Lac repressors: involvement of alpha subunit of RNA polymerase

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Gal or Lac repressor binding to an upstream DNA segment, in the absence of DNA looping, represses the P1 promoter located on the same face and activates the P2 promoter situated on the opposite face of the DNA helix in the gal operon. Both inhibition and stimulation of transcription requires the physical presence of the C-terminal domain of the alpha subunit of RNA polymerase although the latter is not required for transcription itself. We propose that Gal and Lac repressors inhibit or stimulate transcription initiation by disabling or stimulating RNA polymerase activity at a post-binding step by directly or indirectly altering the C-terminal alpha domain to an unfavorable state at P1 or a more favorable state at P2, respectively.
Keywords: Escherichia coli/gal operon/gene regulation

Introduction

As gene expression begins with transcription catalyzed by RNA polymerase, regulation of gene expression also starts at the level of transcription initiation. Specific regulation of transcription initiation is mediated most often by the DNA sequence-specific regulatory proteins. Such proteins bind to DNA elements cognate to the promoters. For transcription activation, the prevailing model suggests a requirement of a protein–protein contact between activator protein and RNA polymerase bound to adjacent or distant sites on a DNA (Kustu et al., 1991; Ebright, 1993; Ishihama, 1993) and an intact DNA duplex between the two binding sites (Ryu et al., 1994). The traditional model of transcription repression, nevertheless, involves a competition between repressor and RNA polymerase for DNA binding to overlapping sites. In other words, repressor protein binds at or near the promoter to sterically hinder RNA polymerase access to the promoter.

In the galactose (gal) operon of Escherichia coli, Gal repressor (GalR) represses transcription initiation from two overlapping promoters, P1 and P2, by interacting with two operator loci, O_E and O_I, and forming a loop of

the intervening promoter DNA segment (Fritz et al., 1983; Irani et al., 1983; Majumdar and Adhya, 1984). Lac repressor (LacI) can also repress the gal promoters, provided the two gal operators are replaced by lac operators (Haber and Adhya, 1988; Mandal et al., 1990; Choy and Adhya, 1992). O_E and O_I are separated by 114 bp and thus make a loop of 11 helical turns. Neither sliding the promoters within the loop nor increasing the size of the loop up to 30 helix turns affects such repression (Choy et al., 1995). The evidence suggests that inflexibility of the DNA in the loop resists open complex formation by RNA polymerase. While the exact mechanism by which DNA inflexibility brings about repression of two promoters located on the opposite face of the DNA remains to be further investigated, we report here how the repressors play a dual role in gal in the absence of looping, i.e. inhibiting transcription from P1 and stimulating from P2. Both repression and activation require the participation of the C-domain of the alpha subunit of RNA polymerase.

Results

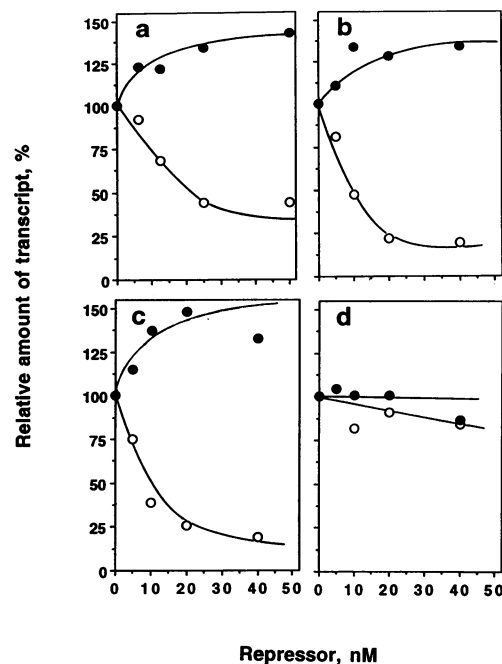### Selective repression of P1 and activation of P2

The two promoters, P1 and P2, in gal are 5 bp apart and thus located on the opposite faces of the DNA helix (Musso et al., 1977). The two operators, O_E and O_I, contain a palindromic DNA sequence and bind to a dimeric form of repressor through a helix-turn-helix motif in each subunit (Majumdar and Adhya, 1987, 1989; Majumdar et al., 1987; Brenowitz et al., 1990). Complete repression of P1 and P2 requires looping of the promoter region formed because of the tetrameric state of the operator-bound dimers (Haber and Adhya, 1988; Adhya, 1989). Since only LacI, and not GalR, forms a tetramer in vitro, looping mediated repression of P1 and P2 in gal has only been shown with LacI in a purified system (Choy and Adhya, 1992). Simultaneous repression of P1 and P2 by GalR, however, has been observed in crude extracts, suggesting that an additional factor is required for tetramerization of GalR (Kuhnke et al., 1986). In the absence of tetramer formation, occupation of the operators by dimeric repressors prevents the DNA looping mediated repression. Under these conditions, P1 activity is inhibited while P2 activity is stimulated (Choy and Adhya, 1992; Goodrich and McClure, 1992).

We have investigated the mechanism by which repressor dimers selectively repress P1 and activate P2 in an in vitro transcription system consisting of a minicircle DNA template which contained only the two gal promoters followed by a transcription terminator to generate two 120 and 125 nt long transcripts respectively (Choy and Adhya, 1993). cAMP and its receptor protein, acting as a complex, also modulate P1 and P2 (Musso et al., 1977; Choy and Adhya, 1993) but have not been studied here. We have
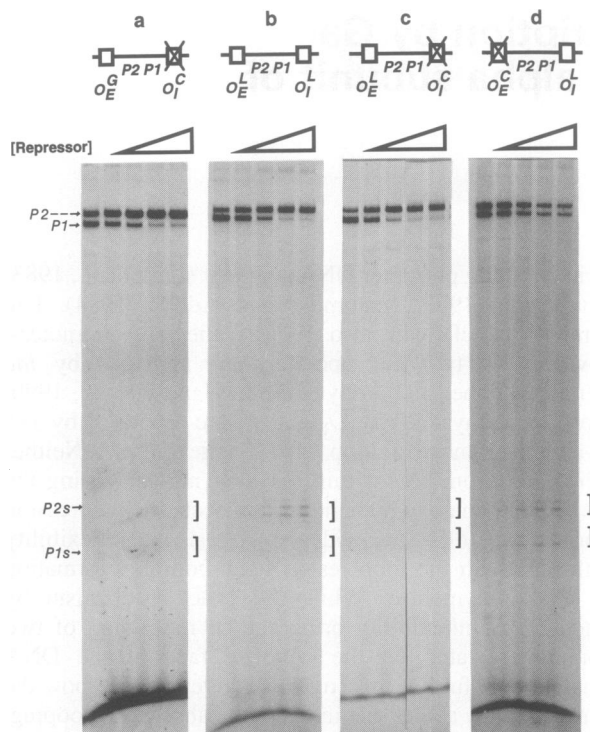
Fig. 1. GalR and LacI[adi] (referred to in the text and the figures as LacI) were titrated using *gal* DNA templates. Reconstituted wild-type RNA polymerase was used for these assays. The transcription products were resolved in denaturing 8% polyacrylamide gel (see Materials and methods). The full-length *P1* and *P2* RNAs at the top are labeled as such; prematurely terminated *P1* and *P2* small RNAs are shown in brackets with labels, *P1s* and *P2s*. (**a**) GalR on O_E^G-O_I^C (O_I^L) DNA (pSA532); (**b**) LacI on O_E^L-O_I^L DNA (pSA510); (**c**) LacI on O_E^L-O_I^C (O_I^G) DNA (pSA512); (**d**) LacI on O_E^C (O_E^G)-O_I^L DNA (pSA532).

Fig. 2. Both full-length and prematurely terminated RNAs were quantified with a beta-scanner (PhosphorImager, Molecular Dynamics, CA) and the percent (%) of RNA (sum of full-length and, if any, terminated RNA) made in the presence of different repressor concentrations relative to the RNA made in the absence of repressor was plotted as a function of repressor concentration. ○, *P1*; ●, *P2*. (**a**) GalR on O_E^G-O_I^C DNA; (**b**) LacI on O_E^L-O_I^L DNA; (**c**) LacI on O_E^L-O_I^C; (**d**) LacI on O(C,E)-O(L,*I*) DNA.

used GalR or a mutant LacI (LacI[adi], referred to as LacI in the remainder of this article). The LacI mutant, like wild-type GalR, does not tetramerize but binds to *lac* operators as dimers with normal efficiencies (Lehming *et al.*, 1988; Brenowitz *et al.*, 1991). The *gal* DNA templates contained either *gal* or *lac* sequences at the two operator loci (O_E^G-O_I^G or O_E^L-O_I^L) for assessing the effect of either GalR or LacI on *P1* and *P2*. Figure 1 shows the results of varying the concentrations of GalR and LacI on RNA synthesis from O_E^G-O_I^G and O_E^L-O_I^L DNA templates, respectively, as analyzed by gel electrophoresis of the products. In the absence of repressor, the 120 and 125 nt long full-length transcripts from *P1* and *P2* respectively were made from both templates by RNA polymerase alone. By the addition of increasing amounts of GalR, synthesis of the full-length RNA from *P1* decreased and that from *P2* increased (panel a). As observed before in this system, the *P2* promoter makes a large amount of aborted RNA oligomers (Choy and Adhya, 1993). The presence of GalR also increased the synthesis of the aborted RNA (data not shown). For LacI, while the levels of full-length *P1* RNA decreased with increasing amounts of repressor, as for GalR, the amount of full-length *P2* RNA increased slightly (panel b). Concomitantly, however, we observed the synthesis of short RNAs ranging from 17 to 26 nt in length. As shown below, these short transcripts were made because of 'road-block' termination of RNA chain elongation by binding of LacI to O_I

(Deuschle *et al.*, 1986; Sellitti *et al.*, 1987). The more prominent set of 22–26 nt RNA are thought to originate from *P2*, whereas the less prominent 17–21 nt size set arise from *P1*. The use of minicircle DNA containing only the *gal* promoter allowed the assignment of the origin of these transcripts to be observed in a single gel without further analysis. Since the actual DNA template used for the GalR experiment shown in Figure 1a was O_E^G-O_I^C, the prematurely terminated transcripts were not observed in Figure 1a but were obtained with O_E^G-O_I^G template (data not shown). Quantification of the radioactive transcripts by direct scanning of the gel is shown graphically in Figure 2. Note that in such quantifications, where applicable, the sum of full-length and prematurely terminated RNA of *P1* or *P2* origin is plotted as a function of repressor concentration. GalR repressed *P1* by ~80% and stimulated *P2* ~50% at 50 nM concentrations (Figure 2a). The pattern of repression by LacI was virtually identical to that of GalR (Figure 2b). In the experiments described below, the behavior patterns of the two repressors were indistinguishable, although the results with only one may have been shown.

To investigate the role of individual operator loci in the dual regulatory behavior of GalR and LacI, i.e. repression of *P1* and activation of *P2*, DNA templates carrying a mutant operator, O_E^C or O_I^C were analyzed. Under these conditions, repressor would bind to one operator and would not be able to form the DNA loop. The results clearly demonstrated that, under conditions of non-looping, occupation of O_E alone by repressor is entirely responsible for *P1* repression and *P2* activation. Repressor dimer binding to O_I is irrelevant to regulation of initiation, but
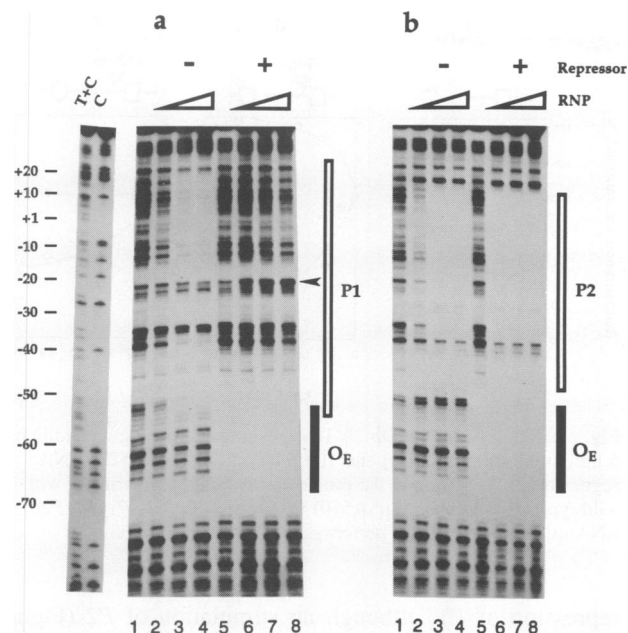
Fig. 3. RNA polymerase titration in the absence (lanes 1–4) or presence of repressor (lanes 5–8) bound to O_E as probed with DNaseI. (a) Results with *gal* DNA carrying a T or G transversion at –19, which specifically inactivates *P2* but leaves *P1* intact (*P1* DNA); (b) Results with the *gal* DNA carrying a G to A transition at –14 which specifically inactivates *P1* but leaves *P2* intact (*P2* DNA) (Bingham *et al.*, 1986). The labeled DNA fragments (20 pM) were mixed with proteins (20 nM repressor when present) in ice and incubated at 37°C for 10 min. After the addition of heparin (0.05 mg/ml) and DNaseI (0.5 µg/ml), the reaction mixture was incubated for an additional 5 min at 37°C. Finally the digestion products were separated on a 6% sequencing gel and visualized by autoradiography. The first two lanes show T+C and C Maxam–Gilbert sequencing reactions. RNA polymerase concentrations used were 0, 50, 200 and 800 nM.

causes partial termination at this locus. For example, LacI at 40 nM showed normal repression of *P1* RNA and stimulation of *P2* RNA in an O_E^L-O_I^C template (Figures 1c and 2c). Note that the short transcripts were not made in the absence of a functional O_I. On the contrary, LacI reduced both the level of *P1* and *P2* full-length RNAs similarly by ~50% in the O_E^C-O_I^L DNA (Figure 1d). The reduction of each species of full-length RNA was compensated for by a corresponding increase in prematurely terminated RNAs originating from *P1* and *P2*. When both full-length and terminated transcripts were quantified and represented graphically together, repression by LacI was almost undetectable at 40 nM for the O_E^C-O_I^L template (Figure 2d).

### RNA polymerase binding to promoters in the presence of repressor

The mechanism of selective repression of *P1* and activation of *P2* by repressor bound to O_E was investigated by DNaseI protection experiments. To distinguish between the RNA polymerase protection patterns of the two *gal* promoters, mutant *gal* DNA of the $P1^+P2^-$ or $P1^-P2^+$ genotype caused by single base substitutions were used (Bingham *et al.*, 1986). The DNA fragments contained –76 to +38 of the *gal* sequence in which O_I was absent to assess the effect of repressor bound to O_E only. The binding and protection reactions were carried out in

the presence of heparin to remove non-specific RNA polymerase binding. Figure 3 shows the results of RNA polymerase titration with *P1* (panel a) and *P2* (panel b) DNA. In the absence of repressor, distinguishable patterns of DNaseI protection of RNA polymerase were observed for *P1* and *P2* DNA (lanes 1–4 in both panels). RNA polymerase bound to *P1* protected segment approximately from +24 to –55, while that bound to *P2* protected the region from +10 to –49. The results are in agreement with those obtained previously (Lavigne *et al.*, 1992). These protections represent heparin-resistant open complexes formed at *P1* and *P2*, respectively. Repressor bound to O_E shows protection of the sequence between –50 and –70 (lane 5 in both panels). RNA polymerase in the presence of repressor showed a different pattern of DNaseI protection at the *P1* promoter. DNaseI sensitivity decreased for most of bands between +20 and –17, and increased very noticeably at –23 (shown by an arrow) (lanes 6–8, panel a). Thus, in the presence of repressor, RNA polymerase clearly forms a complex that is heparin-resistant but different from the open complex formed in the absence of repressor.

The DNaseI protection patterns of RNA polymerase on *P2* DNA were qualitatively the same in the absence and presence of repressor (Figure 3, lanes 2–4 versus lanes 6–8 in panel b). Quantitatively, there was increased open complex formation at *P2* in the presence of repressor. Total protection of the promoter region required 200 nM RNA polymerase in the absence of repressor but only 50 nM RNA polymerase in the presence of repressor. Thus, RNA polymerase formed open complexes at the *P2* promoter more readily in the presence of O_E bound repressor in the absence of the *P1* promoter.

Topography of gal DNA–protein complexes

It is interesting that repressor bound to the upstream O_E locus (at position –60.5) represses transcription initiation from *P1* located farther (at +1) and not from *P2* located closer (at –5). To better understand the spatial relationship between repressor bound to O_E and RNA polymerase bound to *P1*, we constructed a projection diagram of the repressor and RNA polymerase on B-DNA (Figure 4a). Methylation protection and ethylation interference assays have shown that *E.coli* RNA polymerase, when bound to a promoter, covers mostly one face of the DNA helix upstream of the –10 region (Simpson, 1980; Siebenlist *et al.*, 1980). Since the *P1* and *P2* promoters are separated by half of one DNA helical turn, assuming 10.5 bp per turn, RNA polymerase, when bound to these two promoters (not simultaneously), must occupy the opposite face of the helix. The projection pattern of RNA polymerase at *P1* is considered in Figure 4a. The contacts made by GalR at O_E obtained by DNase protection, dimethyl sulfate protection and ethylation interference assays (Busby *et al.*, 1987; Majumdar and Adhya, 1987, 1989; Attey *et al.*, 1994) showed that the repressor occupying O_E (as well as O_I) is on the same face of DNA as RNA polymerase at *P1* (Figure 4a). Since the repressor does not prevent RNA polymerase from binding to the *P1* promoter, as shown above, we conjectured that the act of repression of *P1* is related to the co-occupancy of the repressor at O_E and RNA polymerase at *P1* on the same side of DNA (see also Figure 5a). We tested this idea using a DNA
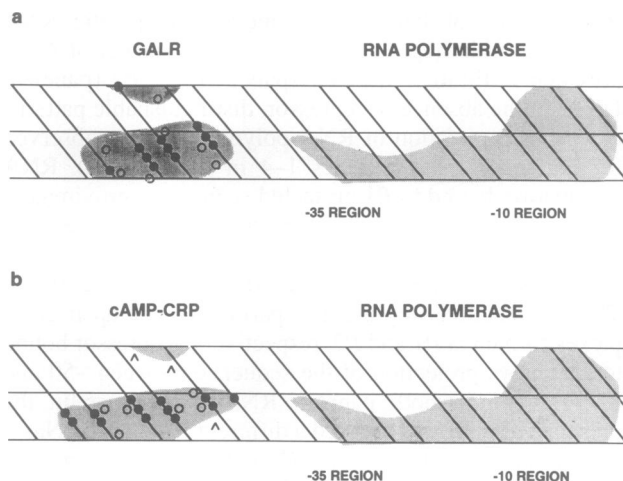
Fig. 4. Projection patterns of (a) GalR and RNA polymerase and (b) cAMP•CRP and RNA polymerase footprints on *gal* and *lac* DNA, respectively. Footprinting results were adapted from Simpson (1980), Siebenlist *et al.* (1980), Majumdar and Adhya (1987, 1989), Busby *et al.* (1987) and Attey *et al.* (1994) to generate the projection patterns. ●, ethylated phosphates that interfere with protein binding; ○, guanine bases that are protected by protein binding from dimethyl sulfate attack; ∧, purine bases that are hypersensitive to dimethyl sulfate attack. For simplicity the details of protection and interference points are not shown for RNA polymerase.
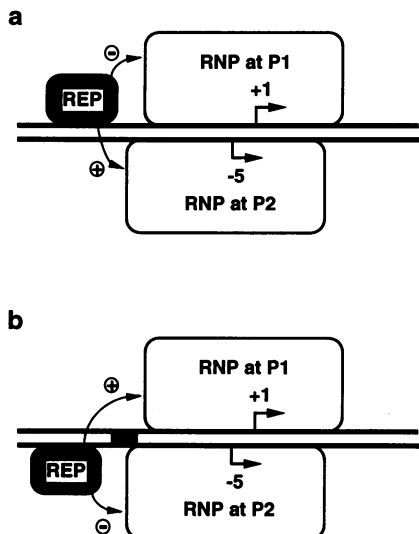
Fig. 5. A cartoon of topography of repressor–DNA–RNA polymerase (at *P1* or *P2* promoter) complex for (a) wild-type DNA and (b) DNA with a 5 bp insertion (shaded) between O_E and the promoter segment. Details are given in the text.

template carrying a 5 bp insertion between the *gal* promoters and O_E (now at position –65.5). Such an insertion would change the angular orientation of O_E with respect to the promoters and place the repressor bound to O_E on the same face of DNA as RNA polymerase at *P2* and opposite to that at *P1* (Figure 5b). The results clearly showed a complete switch; compared with the wild-type control (Figure 6a), LacI at 40 nM decreased transcription from *P2* and increased that from *P1* on this DNA template (Figure 6b). Furthermore, insertion of a 10 bp DNA segment, which is equivalent to a full DNA helical turn and thus is expected to restore the original angular orientation of O_E with respect to the promoters, showed
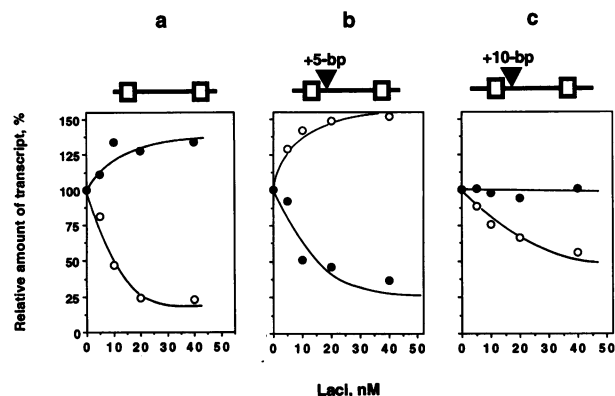

Fig. 6. Effect of LacI on O_E^L-O_I^+ DNA templates carrying an additional 5 bp [(b) plasmid pSA521] and 10 bp [(c) plasmid pSA528] DNA segments between O_E and the promoters. A control experiment with wild-type DNA template (pSA510) is shown in (a). ○, *P1*; ●, *P2*. RNA quantifications were performed as described in Figure 2.

repression of *P1*, although no stimulation of *P2* (Figure 6c). These results gave strong credence to the model that the face of RNA polymerase binding with respect to the repressor at O_E is a critical determinant of differential behavior of repressor toward the two promoters.

Repressor–RNA polymerase contact

As mentioned above, one of the requirements of transcription activation, for example, by cAMP and its receptor–protein complex, cAMP•CRP, at the *lac* promoter in *E.coli* is a contact between DNA-bound cAMP•CRP and RNA polymerase. Such a protein–protein contact occurs through specific amino acid residues in a surface loop of CRP and C-terminal domain of the alpha subunit(s) of the RNA polymerase (Ebright, 1993; Ishihama, 1993). The chemical protection and interference results of cAMP•CRP binding to the *lac* promoter showed that the face of cAMP•CRP occupancy at position –61.5 is on the same face of the DNA helix as RNA polymerase (Simpson, 1980; Siebenlist *et al.*, 1980), a topography which is remarkably similar to that of repressor at O_E and RNA polymerase at *P1* on *gal* DNA (Figure 4a and b). The center of the GalR binding site (–60.5) at *gal* is located only 1 bp upstream of the center of the cAMP•CRP binding site (–61.5) at *lac*. We therefore argued that the selective repression of *P1* by the O_E-bound repressor may also involve the C-terminal domain of alpha. The C-terminal 80 amino acids of the alpha subunit is an independently folded domain, which is dispensable for the RNA polymerase function at many promoters (Ishihama, 1993; Blatter *et al.*, 1994). Homogeneous RNA polymerase holoenzyme reconstituted *in vitro* using alpha subunits lacking various segments of the C-terminal domain were also fully functional at the *gal* promoters (Igarashi and Ishihama, 1991). We tested whether the C-terminus of alpha is required for repression of *P1* by studying the effect of repressor on *gal* transcription with RNA polymerase composed of truncated alpha subunits. The following results were obtained with GalR dimer with O_E^G-O_I^C DNA template (Figures 7 and 8a) and LacI dimer with O_E^L-O_I^C DNA template (Figure 8b). The templates containing the O_I^C allele were chosen so as to eliminate the partial transcription termination effects of repressors at O_I. With RNA polymerase reconstituted from
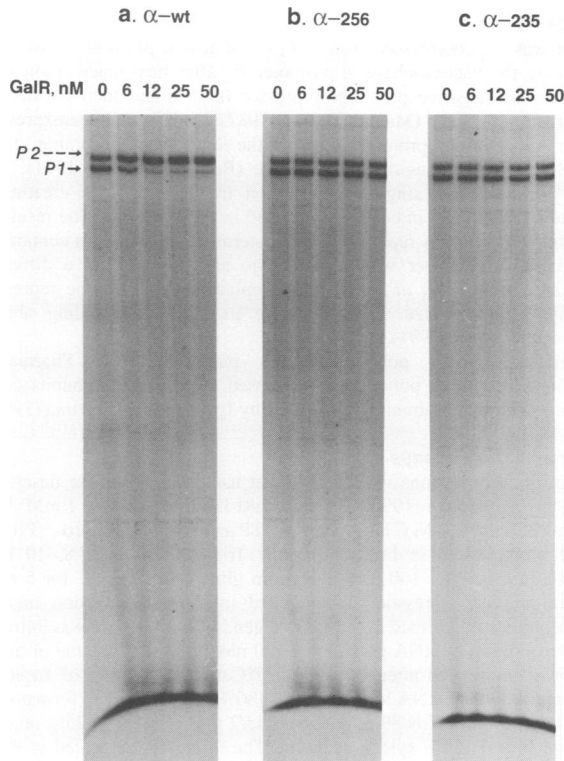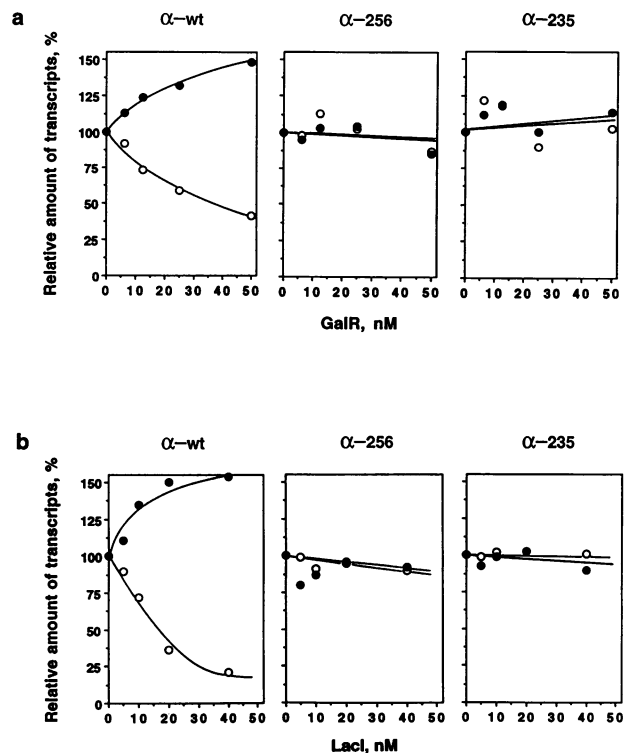
Fig. 7. GalR was titrated using O_E^G-O_I^G DNA (pSA509) with reconstituted RNA polymerases as described in Figure 1. (a) RNA polymerase with intact alpha; (b) with alpha with 256 amino acids; (c) with 235 amino acids.

Fig. 8. Effect of GalR (a) and LacI (b) on $P1$ and $P2$ transcription in the presence of RNA polymerases reconstituted with intact alpha (left panels), with alpha subunits truncated after amino acid 256 (middle panels) and after amino acid 235 (right panels) (Igarashi and Ishihama, 1991). The DNA templates were O_E^G-O_I^C (pSA532 for GalR) and O_E^C-O_I^C for (pSA512 for LacI). RNA quantifications were performed as in Figure 2. O, $P1$; ●, $P2$.

intact alpha, GalR at 50 nM concentration, as expected, inhibited the synthesis of $P1$ RNA 4-fold and stimulated $P2$ RNA >50% (Figure 8a, left panels). Nevertheless, with RNA polymerase reconstituted from α-256 (missing the C-terminal 73 of the 329 amino acids) (middle panels) or α-235 (missing 94 amino acids from the C-end) (right panels), GalR neither repressed $P1$ nor activated $P2$ RNA synthesis. Identical results were obtained with reconstituted RNA polymerases and LacI dimer (Figure 8b). Taken together, these results show that the C-terminal domain of alpha is needed for repression of $P1$ and activation of $P2$ mediated by O_E-bound repressor.

Models of repression and activation by repressor

It has been suggested that the stimulation of $P2$ is an indirect effect of repression of $P1$ because of RNA polymerase partitioning (Goodrich and McClure, 1992). In this model, inhibition of $P1$ occurs by exclusion of RNA polymerase binding by repressor at this promoter, thus making more RNA polymerase available to initiate from $P2$. Our results with mutant *gal* DNA templates ($P1^+P2^-$ and $P1^-P2^+$) strongly suggested that independent mechanisms govern the observed repression of $P1$ and activation of $P2$. DNaseI protection experiments showed an increase in RNA polymerase $P2$ open complex formation in the presence of repressor. Repressor stimulation of $P2$ transcription was the same in wild-type and $P1^-P2^+$ DNA (data not shown). Based on these results and the observation that RNA polymerase reconstituted with truncated alpha subunit was defective in $P2$ activation, we propose that repressor stimulates $P2$ transcription by directly or indirectly changing the C-terminal domain of

the alpha subunit of RNA polymerase. Although the C-terminal domain alpha is not essential for transcription initiation at $P2$ (Igarashi and Ishihama, 1991; Attey *et al.*, 1994), the proposed change in this domain is more favorable to transcription initiation from $P2$.

Repressor inhibited transcription from $P1$ in the $P1^+P2^-$ DNA in the same manner as with wild-type DNA (data not shown). In the presence of repressor, we detected an RNA polymerase–$P1$ complex which is heparin-resistant but qualitatively different from the open complex formed in the absence of repressor. It is important to point out that although DNaseI protection results may suggest a partial overlapping of RNA polymerase (+24 to –55) and repressor (–50 to –70) binding sites, one should note that the pattern of DNaseI digestion does not necessarily provide information on the spatial arrangement of proteins on DNA. The detection of an RNA polymerase–$P1$ complex in the presence of repressor rules out the possibility of repressor inhibiting the binding of RNA polymerase to $P1$ in its entirety. We propose that repressor prevents a step after closed complex formation. Since the repression of $P1$ promoter requires an intact C-terminal domain of the alpha subunit of RNA polymerase and since the C-terminal domain of alpha is not necessary for open complex formation at $P1$ (Igarashi and Ishihama, 1991; Attey *et al.*, 1994), we conclude that the repressor disables RNA polymerase by directly or indirectly altering the C-terminal domain of the alpha subunit to a state which resists a productive open complex formation.

Discussion

While the two *gal* promoters encoded on opposite faces of the DNA helix are repressed by DNA looping, we have demonstrated that repressor bound to the upstream operator, O_E, in the absence of looping, represses one promoter and stimulates the other by participation of the C-terminal domain of the alpha subunit of RNA polymerase. Since the C-terminal domain is not needed for transcription from the *gal* promoters, we envisage that the inhibitory and stimulatory effects are generated by O_E-bound repressor which directly or indirectly alters the alpha domain to an unfavorable state at *P1* and to a favorable state at *P2* for transcription initiation. Our observations about the dual behavior of Gal and Lac repressors have biochemical significance. The inference that both Gal and Lac repressors, which have 60% similarities in amino acid sequence, can inhibit and stimulate transcription initiation by physically modulating a subunit of RNA polymerase after binding, makes the structure–function study of the regulatory proteins interesting. Whether such modulation needs direct physical contact between repressor and alpha remains to be investigated. Several other regulatory proteins, e.g. cAMP•CRP (Kolb *et al.*, 1993), TyrR (Pittard and Davidson, 1991), cI protein of bacteriophage λ (Ptashne, 1992), OxyR (Bolker and Kahmann, 1989; Storz *et al.*, 1990) and MerR (Summers, 1992), are known to have similar dual functions in various promoters. Although the activator role of cAMP•CRP and cI has been shown by both genetic and biochemical experiments (Bushman *et al.*, 1989; Ebright, 1993; Ishihama, 1993; Kuldell and Hochschild, 1994; Li *et al.*, 1994) to occur through contact with RNA polymerase, the repressor role is usually thought to be passive, i.e. inhibiting RNA polymerase binding in its entirety (Lanzer and Bujard, 1988; Brodolin *et al.*, 1993; Schlax *et al.*, 1995), except for MerR for which an action at a post-RNA polymerase binding step has also been suggested (Frantz and O'Halloran, 1990; Heltzel *et al.*, 1990).

Materials and methods

Plasmids

Construction and functional elements of plasmids used to generate supercoiled 'minicircles' *in vivo* have been described previously (Choy and Adhya, 1993). Briefly, the parental plasmid carried a multiple cloning site into which the *gal* promoter segment was inserted. The promoter–terminator region was located on the plasmid between the λ phage attachment site, *attP*, and the corresponding bacterial site, *attB*. DNA minicircles carrying the *gal* promoter followed by the transcription terminator were generated by site-specific recombination between the *attP* and *attB* sites *in vivo* in a host (SA1751) that provided the λ integrase and the host integrase factor, IHF. The minicircles were extracted and purified by gel electrophoresis.

pSA508, the parental plasmid, contained no promoter at the multiple cloning site (Choy and Adhya, 1993). pSA509 contained a 288 bp segment of *gal* promoter (−197 to +91) cloned between *Eco*RI and *Pst*I sites of pSA508. pSA510 is identical to pSA509 except that the *gal* operators (O_E^G and O_I^G) were replaced with the consensus *lac* operator sequence (O_E^L and O_I^L). pSA512 and pSA532 were identical to pSA510 except that they contain the *gal* operator sequence at the internal operator (O_I^G) and external operator (O_E^G), respectively. pSA521 contains a 5 bp insertion at position −50.5 in pSA510. pSA532 contains a 10 bp insertion at position −50.5 in pSA510.

DNA fragments used for DNaseI footprinting experiments were from the plasmids pSA541 and pSA542, in which *gal* sequences from −76 to +91 were cloned between *Eco*RI and *Pst*I sites of pSA508 (see legend of Figure 3 for details).

Proteins

GalR was hyperexpressed from a P_L–*galR* fusion plasmid (pAM2) by inducing the bacteriophage λ promoter P_L after heat inactivation of a temperature-sensitive prophage repressor (*cI854*) and then purified as described previously (Majumdar *et al.*, 1987). LacI^adi was hyperexpressed from a constitutive promoter fused to the *lacI*^adi gene (Lehming *et al.*, 1988) and purified as described before (Brenowitz *et al.*, 1991). The *lacI*^adi gene has a single-base deletion in the *lacI* gene creating a frameshift mutation in codon 330 of 360 in the *lacI* gene. The resultant mutant repressor has replaced the 31 C-terminal amino acids containing a mini-leucine zipper with a 16 amino acid segment of a different sequence (Alberti *et al.*, 1991). The replacement makes the repressor unable to tetramerize while retaining its full DNA-binding ability (Brenowitz *et al.*, 1991).

Wild-type RNA polymerase was purchased from Pharmacia. Reconstituted RNA polymerase composed of wild-type subunits or of C-truncated alpha subunits was as used by Igarashi and Ishihama (1991).

Transcription assays

Transcription reactions were carried out using the procedure described by Choy and Adhya (1993). Briefly, 2 nM DNA template, 0.1 mM ATP, 0.1 mM GTP, 0.1 mM CTP, 0.01 mM UTP and 10–20 μCi of [α-^{32}P]UTP were preincubated in buffer (20 mM Tris–acetate, pH 7.8, 10 mM magnesium acetate, 100 mM potassium glutamate) at 37°C for 5 min. When present, repressor was included in the preincubation mix at concentrations described in the figure legends. Transcription was initiated by the addition of RNA polymerase (20 nM) in a total volume of 50 μl and was terminated after 10 min at 37°C by the addition of an equal volume (50 μl) of RNA loading buffer [80% (v/v) deionized formamide/ 1× TBE (89 mM Tris/89 mM boric acid/2 mM EDTA)/0.025% bromophenol blue/0.025% xylene cyanole]. The mixture was heated at 90°C for 2 min and electrophoresed in 8 M urea/8% polyacrylamide sequencing gels (40 cm×0.4 mm). The RNA transcripts were quantitated as determined by c.p.m. with an Ambis β-scanner.

DNaseI experiments

The 195 bp DNA fragments of *Xba*I (located 81 bp upstream of the *Eco*RI site) to *Bst*EII (+38 of *gal* sequence) from pSA541 and pSA542 were labeled at the *Xba*I site with Klenow (Brenowitz and Snear, 1989), purified from 4% polyacrylamide gel and used for DNaseI protection experiments. The experiments were conducted essentially as described by Brenowitz and Snear (1989).

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