A Complex Control System for Transcriptional Activation from the sid Promoter of Bacteriophage P4

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The sid gene promoter (P_{sid}), which controls expression of the late genes from satellite phage P4, is activated by a unique class of small DNA-binding proteins. The activators from both satellite and helper phages stimulate transcription from P_{sid}. These activators bind to sites centered at position −55 in all the helper and satellite phage late promoters. P4 P_{sid} is unique in that it has an additional activator binding site centered at position −18 (site II). We have constructed a mutant of site II that no longer binds activators. Transcription under the control of satellite phage activators is increased by the site II mutation. In contrast, helper phage activators do not show this increase in transcription from P_{sid} mutated at site II. Competition gel shift analysis reveals that the P4 satellite phage activator, Delta, binds eightfold better to site II than to site I. The products of the sid transcription unit are needed only when a helper phage is present; thus, the satellite phage activators repress transcription until the helper is present to supply a nonrepressing activator.

Satellite (helper-dependent) phages P4 and ΔR73 require head, tail, and lysis genes from a helper phage of the P2 family in order to produce progeny (10, 23, 26). Both satellite phages are temperate, and each has a chromosomally integrated prophage state (1, 10, 23, 26). P4 can also establish a plasmid state, and clear-plaque-type mutants establish this state at a higher frequency than does the wild type (4, 6, 20). When Escherichia coli that is lysogenic for integrated P4 is infected with helper phage, the helper grows well and there is little production of P4 (24). If the infecting helper phage is blocked from replication by a mutation in its own genome or in that of the host, then P4 progeny are produced efficiently (24). P4 is also produced efficiently when P2 infects a bacterium carrying the P4 plasmid (25). When P4 infects a bacterium that is lysogenic for a helper phage, P4 grows well and little P2 is produced. When P4 and a helper phage coinfect a nonlysogenic strain, both phage types are produced, although P4 is produced in greater quantity (5, 23). In order to make use of the helper late genes, the satellite phages carry a gene for derepression of P2 prophage (18), as well as a gene for activation of helper phage late gene promoters (for a review, see reference 17).

P4 and ΔR73 encode transcriptional activators for the expression of the late genes of their helper phages, as well as for the expression of their own late genes. The activators of the satellite and helper phages are small proteins that contain the motif CysX2-CysX2-CysX2-CysX2-Cys and contain one atom of zinc (14, 15, 21). They bind to the consensus sequence TGTX23-ACA (26). The genes for the satellite phage activators lie within a late transcription unit that contains the gene for capsid size determination, sid, followed by the activator gene, s, and the capsid stabilization gene, psu. The promoter for this transcription unit is called P_{sid}. Transcription from this promoter could not be detected from the phage, nor could it be detected in P4-infected, nonlysogenic cells before 40 min. During P4 infection of a P2-lysogenic strain, transcription is detected at 30 min at a high level (3). In contrast to helper phage late promoters, P_{sid} is activated better by helper phage activators than by satellite phage activators, as measured in a two-plasmid system (13). Footprint analysis of helper and satellite phage activators on helper and satellite phage late promoters revealed activator binding sites centered about coordinate −55 from the start of transcription (site I). In addition, P_{sid} has an activator-binding site centered at position −18 (site II), and the products of the sid satellite phage activators appear to have higher affinities for this site than for site I (12, 13). Regulators that bind near −55 usually function to activate transcription (7). This principle holds for P_{sid}, because mutations of the conserved residues in site I abolish promoter activity (27). Regulators that bind near −18 usually function to repress transcription (2). Thus, we suspected that mutation of site II of P_{sid} would abolish a repressive effect. Previous analysis of P_{sid} showed that mutating the first A residue of the ACA of site II (Fig. 1) reduces promoter activity 100-fold (27). This is not surprising, since this nucleotide, at −11, is part of the −10 region that is characteristic of E. coli sigm70 promoters. Since previous work did not specifically target the TGT of site II for mutagenesis, we analyzed these nucleotides and report here their contributions to the activity and specificity of P_{sid}.

Materials and Methods

Bacteria, phages, and plasmid strains. Bacteria, phages, and plasmid strains are described in Table 1.

Construction of pBJ86 to pBJ89. The expression plasmids pBJ86 (ΔR73 Delta), pBJ87 (Delta), pBJ88 (Ogr), and pBJ89 (Pag) were made as follows. The lacZ-bearing plasmid pR931 (8) was cleaved with BamHI; the cohesive ends were filled in by using Klenow fragment, and the product was cleaved with Eco57I. Activator gene fragments were prepared from pBJ17 (11) (ΔR73 Delta), pBJ47 (12) (ΔR73 Delta), pBJ49 (11) (Ogr), and pBJ72 (11) (Pag) by cleavage with HindIII, followed by filling in with Klenow fragment and cleavage with Eco57I. The appropriate fragments were purified by gel electrophoresis and ligated.

Construction of psid93. In order to construct a promoter fusion of P_{sid} to lacZ, pB93 and pR9414 were digested with EcoRI and BamHI and the 293-bp fragment from pB93, carrying P_{sid}, was ligated to the large fragment of pR9414.

Mutagenesis of site II of P_{sid}. The first three nucleotides of site II from P_{sid} were mutated to the complementary sequence. This mutation was accomplished by replacing the TGT at −26 to −24 with ACA on a primer and amplifying pB93 by PCR (Fig. 1). The mutagenic primer was 5'-TCATGTGTGACCAG TGTG-3', which corresponds to P4 coordinates 9525 to 9543 (the ACA in boldface is the altered sequence). The primer for the opposite strand was the 20-mer T3 promoter from New England Biolabs. The PCR product was ligated and cleaved with EcoNI and BamHI. The fragment containing the mutant promoter was isolated and ligated to the large EcoNI-to-BamHI fragment of pB93.
The PCR product was ligated in case some circular templates had been copied completely. If the circular templates had been copied, then ligation would make the product more stable after subsequent cleavage with restriction enzymes. The ligation step may not be necessary for success. The presence of the ACA mutation and the absence of other mutations were ascertained by sequence analysis.

Construction of bacteria with \( P_{s45} \) and mutant \( P_{s45} \) fused to \( lacZ \) in the host chromosome. The wild-type and mutant \( P_{s45} \) promoters were introduced into \( \lambda \) phage carrying \( lacZYA \) (\( \lambda RS45 \)) by recombination in vivo, as described by Simons et al. (22), with psid93 as the source of \( P_{s45} \) and psid as the source of mutant \( P_{s45} \). These phages were called \( \lambda RS45sid \) and \( \lambda RS45sidmut \), respectively.

**TABLE 1. Bacterial strains, phages, and plasmids**

<table>
<thead>
<tr>
<th><strong>E. coli C. derivatives, phage, or plasmid</strong></th>
<th>Characteristic(s)</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E. coli C. derivatives</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-2420</td>
<td>F(^{+}); prototrophic ( \Delta(argF-lac)U169 )</td>
<td>12</td>
</tr>
<tr>
<td>C-2420(( \text{asidwt} ))</td>
<td>C-2420 harboring an integrated ( \lambda ) phage that carries the wild-type ( sid ) promoter fused to ( lacZYA )</td>
<td>This work</td>
</tr>
<tr>
<td>C-2420(( \text{asidmut} ))</td>
<td>Same as for C-2420(( \text{asidwt} )), except that the ( sid ) promoter is mutated in site II</td>
<td>This work</td>
</tr>
<tr>
<td><strong>Phages</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P4</td>
<td>Wild-type P4 phage</td>
<td>23</td>
</tr>
<tr>
<td>P4 ( \text{sid mut} )</td>
<td>P4 mutated at site II of ( P_{s45} )</td>
<td>This work</td>
</tr>
<tr>
<td>P4 ( \text{vir1} )</td>
<td>Does not lysogenize</td>
<td>23</td>
</tr>
<tr>
<td>P4 ( \text{vir1 bins1} )</td>
<td>Does not lysogenize; transactivation deficient</td>
<td>9</td>
</tr>
<tr>
<td>( \lambda RS45 )</td>
<td>( \lambda ) phage carrying parts of ( blu ) and ( lacZ ), as well as all of ( lacY ) and ( lacA )</td>
<td>22</td>
</tr>
<tr>
<td>( \lambda RS45sid )</td>
<td>( \lambda RS45 ) carrying ( P_{s45} ) fused to ( lacZYA )</td>
<td>This work</td>
</tr>
<tr>
<td>( \lambda RS45sidmut )</td>
<td>( \lambda RS45 ) carrying mutant ( P_{s45} ) fused to ( lacZYA )</td>
<td>This work</td>
</tr>
<tr>
<td><strong>Plasmids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pB( \Delta93 )</td>
<td>ColE1 origin; Ap(^{+}); the ( sid ) promoter carries a deletion that removes DNA upstream of ( -93 ), and the promoter functions like the wild type</td>
<td>11</td>
</tr>
<tr>
<td>pBJ17f</td>
<td>ColE1 origin; Ap(^{+}); bears DNA that expresses ( \delta R73 ) Delta</td>
<td>13</td>
</tr>
<tr>
<td>pBJ47</td>
<td>ColE1 origin; Ap(^{+}); bears DNA that expresses P4 Delta</td>
<td>12</td>
</tr>
<tr>
<td>pB172</td>
<td>ColE1 origin; Ap(^{+}); bears DNA that expresses Ogr</td>
<td>13</td>
</tr>
<tr>
<td>pBJ186</td>
<td>p15A origin; Kn(^{+}) ( \delta R73 ) &amp; provides controlled expression of ( \delta R73 ) Delta</td>
<td>This work</td>
</tr>
<tr>
<td>pBJ157</td>
<td>p15A origin; Kn(^{+}) ( lacP ) P4 &amp; provides controlled expression of P4 Delta</td>
<td>This work</td>
</tr>
<tr>
<td>pBJ158</td>
<td>p15A origin; Kn(^{+}) ( lacP ) ogr &amp; provides controlled expression of Ogr</td>
<td>This work</td>
</tr>
<tr>
<td>pBJ159</td>
<td>p15A origin; Kn(^{+}) ( lacP ) pag &amp; provides controlled expression of Pag</td>
<td>This work</td>
</tr>
<tr>
<td>pcr1-1</td>
<td>( P_{s45} ) site II mutant made from pB( \Delta93 )</td>
<td>This work</td>
</tr>
<tr>
<td>pCW4</td>
<td>pUC19 carrying the large P4 BamHI fragment (nt 4264–10659)</td>
<td>This work</td>
</tr>
<tr>
<td>pRS414</td>
<td>ColE1 origin; Ap(^{+}); lacks a promoter and Shine-Dalgarno sequence for ( lacZYA )</td>
<td>22</td>
</tr>
<tr>
<td>psid93</td>
<td>ColE1 origin; Ap(^{+}); wild-type ( P_{s45} ) fused to lacZYA</td>
<td>This work</td>
</tr>
<tr>
<td>psidcr</td>
<td>ColE1 origin; Ap(^{+}); ( P_{s45} ) mutated in site II fused to lacZYA</td>
<td>This work</td>
</tr>
</tbody>
</table>
Gel shift analysis of wild-type and mutant promoters with Delta. Gel shift analyses were conducted as described by Julien and Calendar (12), except that they were conducted with P4 Delta that had the MBP fusion cleaved from it. The P25 fragment (nt 93 to +200) was made from pB49 or pC1-3 by digestion with EcolI and BamHI. The binding reaction was performed in 15 μl containing 25 mM Tris- HCl (pH 8.0), 14% glycerol, 133 mM NaCl, 20 μM EDTA, 1 mM β-mercaptoethanol, and 0.133 μg of poly(dI-dC) per μl. Approximately 50 fmol of 32P-labeled P25 fragment and 12 pmol of Delta or Pag were used in each binding reaction mixture.

Factor Xa cleavage. The cleavage of MBP from Delta was carried out according to the suggestions of the manufacturer (New England Biolabs) except that the cleavage buffer was 20 mM Tris- HCl (pH 8.0)–250 mM NaCl–2 mM CaCl2–10% glycerol. One microgram of Factor Xa was added to buffer with 25 μg of MBP-Delta, and the reaction mixture was incubated at room temperature for 5 h. The cleavage reaction was more than 80% complete as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

DNAase I footprint analysis. Increasing amounts of Delta were footprinted on sid wild-type and mutant promoters as described in the work of Julien and Calendar (12). This procedure used the Delta-MBP-Delta mixture; titration was begun at 4 pmol, and then the amount of protein used in each subsequent reaction mixture was doubled. A constant amount (100 fmol) of 32P-labeled P25 was used in each 20-μl reaction mixture. The P25 fragments were generated as described above for the gel shift.

β-Galactosidase synthesis dependent upon induction of activators from plasmids. C-2420 (sidmut) and C-2420 (sidmut) were transformed with either pBJ86, pBJ87, pBJ88, or pBJ89 (producing Δr73 Delta, P4 Delta, Ogr, or Pag, respectively). Each strain was inoculated into 15 ml of Luria-Bertani broth with 30 μg of kanamycin per ml and the cultures were grown to a density of 0.6 OD in an A600 between 0.150 and 0.200. IPTG (1 mM; isopropyl-β-thiogalactopyranoside) was added to each culture, and after one more hour of growth, the cultures were placed on ice and samples were removed and assayed for β-galactosidase activity in quadruplicate according to the method of Miller (15).

β-Galactosidase synthesis dependent upon P4 infection. C-2420 (sidmut) and C-2420 (sidmut) were grown in 15 ml of Luria-Bertani broth at 37°C. When the A600 reached 0.2, P4, P4 vir, or P4 vir banl was added at a multiplicity of infection of 10. Cultures were returned to 37°C, and samples were taken at 0, 20, 40, 60, and 120 min postinfection and plated on ice. Each sample was then assayed for β-galactosidase activity in quadruplicate (19).

Competition gel shifts. To conduct titrating gel shifts of Delta or Pag on the two different sites, from P4Δ, we used 32P-labeled oligonucleotides. For site I the oligonucleotides used were 5′-AGTAGATGAGTCTCCGTGTCAGGGCGTGCACATCTGAAATG-3′ (oligonucleotide 1) and 5′-CATTCGAGATGTCGCAACCTTGAGACAGAGCTCATCCTTGTAG-3′ (oligonucleotide 2), and for site II the oligonucleotides used were 5′-GGCTGTGTGTGGTCGCCGCGTGATGTCGATTCTTCTAAATGTACACCGGACACAAACGACAGCGC-3′ (oligonucleotide 3) and 5′-TAAAAGAAAATGTTGAGCTGTTACACCCGGACACAAACGACAGCGC-3′ (oligonucleotide 4). The consensus nucleotide sequences are underlined.

Oligonucleotides 1 and 3 were end labeled with [γ-32P]ATP and T4 polynucleotide kinase, according to the suggestions of the manufacturer (New England Biolabs). Site I oligonucleotides were then annealed with 300 ng of the labeled oligonucleotide 1 and 500 ng of oligonucleotide 2, contained in 250 mM NaCl–100 mM Tris-HCl (pH 7.6)–100 mM MgCl2–5 mM dithiothreitol. The reaction mixture was incubated at 93°C for 3 min and then allowed to slowly cool to room temperature. Site II oligonucleotides were treated in the same way. To make unlabeled specific competitor DNA, unlabeled oligonucleotides were annealed. Using 1.13 nM (approximately 30,000 cpm) site I or site II and a constant amount of protein (93 μM for MBP-Delta plus 120 μM for MBP-Pag plus Pag), we titrated unlabeled site I. The nucleic acids were added before the protein. Using the same amount of substrate (1.33 nM), we also titrated unlabeled site II. The concentrations of specific competitor (cold DNA) used started at 4% and went as high as 256% (competitor/substrate). The buffer and nonspecific competitor used were as described above.

To measure the effect of the competitor, we measured the amount of DNA in the shifted band by phosphorimagery analysis. That amount was then expressed as the fraction of the amount of DNA that was shifted when no specific competitor was used. The adjusted amounts of shifted DNA were then graphed against the amount of specific competitor used.

RESULTS

Activator binding properties of mutated site II. To investigate the role of the binding site (site II) centered at −18 of P4Δ, we used site-directed mutagenesis to change nt −24 to −26 from TATG(T/G) to ACA (Fig. 1). Sequence analysis confirmed the presence of the desired mutation and the absence of other mutations.

We expected that Delta would no longer bind to site II with this alteration. To demonstrate this, we conducted gel shift experiments. Previous experiments (12, 13) employed a mixture of Delta and MBP fused to Delta. For the gel shift experiments reported here, we cleaved MBP-Delta in this mixture using Factor Xa. Although initial attempts to cleave MBP from Delta had produced inactive aggregates of Delta (12), we were able to overcome this problem by using 250 mM NaCl–10% glycerol. As shown in Fig. 2, when cleaved Delta was incubated with the wild-type promoter, there were two shifted bands (lane 2), but when cleaved Delta was incubated with the mutant promoter, there was only one (lane 5). These results indicate that Delta cannot bind to mutated site II. A DNase I footprint analysis of both wild-type and mutant promoters was conducted with a mixture of Delta and MBP-Delta. No protection of mutant site II was observed (Fig. 3). One helper phage activator, Pag from phage PSP3, was also tested by gel shift analysis for binding to mutated site II (Fig. 2, lanes 3 and 6). The preparation was a mixture of MBP-Pag and Pag, cleaved with Factor Xa. As seen for Delta, Pag appears to bind to the shifted band by phosphorimagery analysis. That amount was then expressed as the fraction of the amount of DNA that was shifted when no specific competitor was used. The adjusted amounts of shifted DNA were then graphed against the amount of specific competitor used.

FIG. 2. Gel shift analysis of wild-type and mutant sid promoters (12). Lanes 1 to 3 contain wild-type DNA (50 fmol, labeled with 32P), and lanes 4 to 6 contain mutant DNA. Lanes 1 and 4 have no protein added, whereas lanes 2 and 5 contain 12 pmol of Delta and lanes 3 and 6 contain 12 pmol of Pag. The Delta and Pag preparations were prepared as mixtures of MBP-activator plus activator (14) which had been cleaved with Factor Xa (New England Biolabs).

FIG. 3. DNase I footprint analysis with Delta and wild-type or mutant P4Δ. The first eight lanes contain wild-type P4Δ, and the second eight lanes contain the mutated P4Δ. Lanes 1 and 9 contain no protein. Lanes 2 and 10 contain 4 pmol of the wild-type-MBP-Delta mixture, and each succeeding lane contains twice as much protein as the preceding lane.
to only one site on P\textsuperscript{sid} when site II is mutated. DNase I footprint analysis with MBP-Pag and Pag showed that Pag does not bind to mutant site II (data not shown).

In vivo activities of wild-type and mutant P\textsuperscript{sid} in the presence of helper and satellite phage activators. Since our site II mutant promoter did not bind activators, we were in a position to test whether binding of site II by activators affects regulation of transcription from P\textsuperscript{sid}. In order to control the promoter copy number, we placed the wild-type and mutant P\textsuperscript{sid} promoters, fused to lacZ, in the E. coli chromosome. Wild-type and mutant P\textsuperscript{sid} were crossed into \( \lambda \) phage, and lysogenic strains were constructed as described in Materials and Methods. The lysogenic strains are called C-2420(\( \lambda \)sid\textsuperscript{mut}) and C-2420 (\( \lambda \)sid\textsuperscript{mut}). In order to test activation of transcription from these promoters, we introduced plasmids that express P4 Delta, \( \delta R73 \) Delta, PSP3 Pag, or P2 Ogr under control of lacI. Induction of these activators with IPTG showed that both Delta and Pag could bind to this altered site II in vitro. We also used wild-type P4 as a source for Delta and determined the difference in levels of activation between the two promoters. In this case, basal transcription of the wild-type promoter was reduced and the mutant promoter was 30-fold more active than the wild type (Fig. 5C). These results corroborate the data from the Delta overexpression experiment (Fig. 4) and also indicate a true biological function for the repression at site II on P\textsuperscript{sid}. We attempted to perform a similar analysis using infection with the P2 wild type, but such infection did not cause any increase in the synthesis of \( \beta \)-galactosidase. P2 inhibits the growth of infected E. coli, whereas P4 does not, so P2 may prevent the expression of chromosomal genes.

**Competition gel shift analysis.** We hypothesized that the difference between the levels of activation of P\textsuperscript{sid} by Delta and Pag was due to their respective affinities for site II. We suspected that Delta represses transcription of P\textsuperscript{sid} because it binds better to site II than to the activating site (site I). To test this model, we determined the relative affinities of Pag and Delta for the two different sites in P\textsuperscript{sid}. We conducted gel mobility shift assays using \( ^{32} \)P-labeled site I and site II and the Deltas repress transcription by binding to site II. In contrast, both of the helper phage activators, Ogr and Pag, gave similar levels of transcriptional activation for wild-type and mutant promoters.

The use of expression plasmids is likely to yield concentrations of activators that are higher than those found during phage infection. Thus, we also used P4 infection to measure activation of P\textsuperscript{sid} in C-2420(\( \lambda \)sid\textsuperscript{mut}) and C-2420 (\( \lambda \)sid\textsuperscript{mut}). Figure 5A shows that infection by P4 vir\textsuperscript{II} phage causes the chromosomal mutant P\textsuperscript{sid} to be activated 15-fold better than wild-type P\textsuperscript{sid}. When infection was carried out with a P4 Delta null mutant, \( \beta \)-galactosidase synthesis was not increased over the level seen in uninfected cells (Fig. 5B), confirming that Delta is responsible for the observed activation. We also used wild-type P4 as a source for Delta and determined the difference in levels of activation between the two promoters. In this case, basal transcription of the wild-type promoter was reduced and the mutant promoter was 30-fold more active than the wild type (Fig. 5C). These results corroborate the data from the Delta overexpression experiment (Fig. 4) and also indicate a true biological function for the repression at site II on P\textsuperscript{sid}. We attempted to perform a similar analysis using infection with the P2 wild type, but such infection did not cause any increase in the synthesis of \( \beta \)-galactosidase. P2 inhibits the growth of infected E. coli, whereas P4 does not, so P2 may prevent the expression of chromosomal genes.

**Effect of our site II mutation on phage growth.** In order to test the effect of our site II mutation on the P4 life cycle, we passaged our P\textsuperscript{sid} site II mutation into wild-type P4 phage. This mutant phage (P4 \( \delta R73 \) mut) made tiny plaques, and we were unable to grow a high-titer stock. Thus, repression at site II by Delta appears to be important for proper regulation of gene expression, even during P4 lytic growth in the presence of a helper prophage.

**Discussion**

We have studied the role of an activator binding site II in the sid promoter. We mutated three nucleotides in the upstream end of the consensus sequence and demonstrated that neither the satellite phage activator, P4 Delta, nor the helper phage activator, Pag, could bind to this altered site II in vitro. We also studied the expression of wild-type and mutant promoters after fusing them to lacZ and inserting them into the host chromosome. Wild-type P\textsuperscript{sid} was activated by P4 Delta, \( \delta R73 \) Delta, and two helper phage activators, Ogr and Pag, supplied from plasmids. The levels of activation were all within the same range, although Pag worked better than Ogr or either of the Deltas. The mutant P\textsuperscript{sid} was activated approximately 10-fold better by the P4 and \( \delta R73 \) Deltas than by Ogr or Pag. When
Delta was supplied by P4 infection, it also worked much better on the mutant P\textsubscript{sid} than on the wild-type promoter. Thus, the binding of Delta to site II appears to repress transcriptional activation by Delta. In contrast, the absence of site II does not greatly affect activation by Ogr or Pag. Repression of transcription by Delta at site II helps to keep P\textsubscript{sid} from being overexpressed in the absence of helper phage, which prevents wasteful expression of the P4 capsid synthesis proteins Sid and Psu (whose genes are transcribed from P\textsubscript{sid}) and of Delta. None of these gene products are needed until helper phage infects the same cell.

We have also shown that Delta binds eightfold better to site II than to site I but that Pag binds equally well to both. This finding suggests that at least part of the difference in levels of transcriptional activation is due to the relative affinities of the activators for the two sites. An additional reason for the differences in specificities of activators for P\textsubscript{sid} might be the nature of the protein-DNA complexes formed at site II. Delta bound to site II might contact and inhibit RNA polymerase, while Pag and Ogr bound to site II might contact and inhibit RNA polymerase, while Pag and Ogr bound to site II might have no contact with the transcribing enzyme.

When P4 produces progeny in the presence of a helper phage, transcription from P\textsubscript{sid} occurs sooner and reaches a higher level (3). This effect is due to the helper phage activator, which causes transcription of P\textsubscript{sid} directly. However, the \(\delta\) gene is in the transcription unit controlled by P\textsubscript{sid}, so helper phage activator also causes more expression of satellite phage activator. The contributions of these two activators to increased P\textsubscript{sid} expression have not been assessed rigorously. One possibility is that the concentration of helper phage activator is much higher than the concentration of satellite phage activator. This higher concentration of helper phage activator might cause site II to be occupied mostly by this activator, which does not repress transcription. Satellite phage activators appear to bind much more tightly to site II than do helper phage activators, so the helper phage activator would have to be present in much greater concentration than satellite phage activator in order to overcome repression. Helper phage activator might cause increased expression of P\textsubscript{sid} from replicating, circular P4 DNA. Helper phage activators are, in fact, more efficient than satellite phage activators on plasmid-borne P\textsubscript{sid} (13). This efficiency of helper phage activators on P\textsubscript{sid} is not apparent when the promoter is in the host chromosome (Fig. 4). The high
The level of \( P_{sid} \) expression during P4 lytic infection might also be due to binding of helper phage activator to one binding site and binding of satellite phage activator to the other site. High-level \( P_{sid} \) expression might also be due to formation of heteromultimers between satellite and helper phage activators. Such hypothetical heteromultimers might bind site I and activate transcription unusually well, and they might repress transcription poorly at site II.

We are unsure of the mechanism for repression and activation at \( P_{sid} \). Our current models for repression involve steric hindrance of RNA polymerase, as shown in Fig. 8. For the model in Fig. 8A, there is no Delta present and basal transcription should occur. This situation should occur early in P4 infection before Delta has been produced from \( P_{sid} \). For the model in Fig. 8B, after basal transcription has produced a certain amount of Delta, Delta binds to site II because it has

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**FIG. 6.** Amounts of labeled DNA bound by Delta in the presence of specific competitors. Gel mobility shift experiments were conducted with constant amounts of \( ^{32}P \)-labeled site I or site II and various amounts of unlabeled site I (A) or site II (B). The shifted species was measured with a phosphorimager and then plotted against the ratio of unlabeled oligonucleotide to labeled oligonucleotide.

**FIG. 7.** Amounts of labeled DNA bound by Pag in the presence of specific competitors. The procedures and labels are the same as those used to obtain the results shown in Fig. 6.
the higher affinity of the two sites and represses transcription. This repressed state should be maintained until either a helper phage infects or activator is supplied from another source. For the model in Fig. 8C, an infecting helper phage has supplied either Pag or Ogr, which then binds site I. The activator now bound at site I can recruit RNA polymerase to the promoter, leading to the initiation of transcription, thereby overcoming the repressing effects of Delta bound at site II. This model does not explain why only Delta, of all the factors which bind at site II, represses transcription. This phenomenon may be explained by the observed affinities of Delta and Pag to the two different sites. Delta at low levels will bind preferentially to site II, resulting in repression of transcription. When a certain threshold level of Delta is reached, then the additional Delta binds to site I and activates transcription. Pag, however, has equal affinities for both sites and may therefore either bind to site I, and activate transcription, or bind to site II, and repress transcription, with equal probabilities. Note that in the normal in vivo case, a basal level of Delta would be present and bound at site II, leading to preferential binding of Pag to site I and thereby causing activation of the sid promoter. In all of these cases, binding of a factor, Pag, Ogr, or Delta, to site I leads to the recruitment of RNA polymerase to the promoter region. The transcribing enzyme can now, with the aid of one of the transcriptional activators, replace the repressive factor bound at the initiation site. Steric hindrance, however, may not provide the correct explanation for repression by binding of Delta at site II. Perhaps upon binding to the separate sites, Delta undergoes different conformational changes, becoming appropriate for either activating or repressing transcription. The conformational changes leading to the repression of transcription would be specific to Delta and not the helper phage activators, which do not need this down regulation. Therefore, only Delta would repress transcription when it was bound at site II. Indeed, the concept of alternate DNA-binding sites differentially affecting the conformation of the same bound protein is very intriguing but not uncommon. Many examples of this allosteric effect have been recently reviewed by Lefstin and Yamamoto (16). Experiments aimed at differentiating between these two hypotheses are under way in our laboratory.

To summarize, we have shown that P4 and φR73 Delta repress transcription when site II is present but that Ogr and Pag do not. In addition, we have shown that at least part of that difference is due to differential levels of binding to the two sites in P4sid.

The models in Fig. 8 imply that only P2 Ogr and P4 Delta control the expression of P4sid. We tested this proposition, at the suggestion of Erich Six, by using a bacterial strain that is lysogenic for P2 carrying a deletion of P2 ogr. We infected this strain with P4 and measured transcription from P4sid using primer extension. The removal of P2 Ogr from the cell did not greatly reduce P4sid transcription, and the stimulation of P4sid transcription was very marked, when compared to that observed in a nonlysogenic strain. Thus, we plan to determine which P2 gene, in addition to ogr, causes P4sid transcription.

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