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In Vitro Transcription from the Late Promoter of Bacteriophage P4

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The late genes of satellite bacteriophage P4 are cotranscribed from a single promoter which shares little homology with known classes of *Escherichia coli* promoters (E. Dale, G. Christie, and R. Calendar, J. Mol. Biol. 192:793–803, 1986). In a coupled transcription-translation system, the P4 late gene promoter was activated by either the δ protein of P4 or the *ogr* protein of helper phage P2 in the absence of any other phage-encoded factor. δ-dependent transcription was inhibited by antibodies to the σ^{70} subunit of *E. coli* RNA polymerase but was restored by purified σ^{70}, indicating that activation of transcription by the δ protein of P4 is dependent on the σ^{70} holoenzyme.

For successful lytic multiplication, satellite bacteriophage P4 requires the products of the capsid, tail, and lysis genes (late genes) of its temperate helper phage P2 (21; Fig. 1). P4 uses the product of its *sid* gene (also a late gene) to cause assembly of a smaller capsid which can accommodate the P4 genome but not the larger P2 genome (19).

Transcription from the four P2 late promoters in vivo is dependent upon *Escherichia coli* RNA polymerase (15) and the product of the P2 *ogr* gene, a protein of 72 amino acids (3, 6); in addition, it depends upon replication of P2 DNA (22). When P2 DNA does not replicate, transcription of the P2 late genes can be transactivated by the product of the P4 δ gene (22, 23; Fig. 1). The δ protein contains 166 amino acids and is homologous to the P2 *ogr* protein (12, 14; C. Halling and R. Calendar, unpublished data).

The P4 late genes are transcribed from a single promoter, P_{sid} (Fig. 1 and 2), which can be activated in vivo by the P2 *ogr* protein expressed from a plasmid (7). In the absence of the P2 *ogr* protein, the P4 δ protein can activate transcription from P_{sid} (7, 9). Thus, the *ogr* protein of P2 and the δ protein of P4 are each independently capable of activating transcription from the same late gene promoters.

Both P4 and its helper phage P2 are thought to utilize the host RNA polymerase core throughout their development because all stages of transcription by these phages are rifamycin sensitive (15). The *ogr* and δ proteins may interact directly with RNA polymerase. A mutation in the α subunit of RNA polymerase (*rpoA109*) blocks late P2 transcription, and mutations in the *ogr* gene overcome this block (8, 24). The *rpoA109* mutation also blocks δ-dependent activation of P_{sid}, and a mutation in the δ gene (*org4*) suppresses this effect (7; M. Sunshine, personal communication). Because the P2 and P4 late promoters lack obvious −10 and −35 hexamers that would be recognized by the σ^{70} holoenzyme (5, 7), we have considered the possibility that the *ogr* and δ proteins are alternate sigma factors for RNA polymerase. However, the *ogr* and δ proteins are more basic and smaller (M_r, 9,000 and 19,000, respectively) than any known sigma factor, and they do not share the conserved regions common to a number of other known sigma factors (3, 6, 9a, 12, 14).

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MATERIALS AND METHODS

Transcription-translation reactions. Plasmid templates were transcribed and translated in an S-30 extract of *Salmonella typhimurium* SK419 (13) which was prepared by a modification of the method described by Artz and Broach (1, 10, 16). In experiments in which the P2 *ogr* and P4 δ proteins were synthesized in vitro (Table 1), the driver plasmid from which the *ogr* protein was synthesized was pRF5, described by Christie et al. (6), and that from which the δ protein was synthesized, pCH13, was constructed by C. Halling and R. Calendar. The amount of driver plasmid in each reaction mixture is indicated. The reporter plasmid was pSidZ (7.5 μg per reaction; Fig. 2) or the control plasmid pRS229 (also called pNK736; 5.0 μg per reaction), in which *lacZ* transcription is under control of the *lac*UV5 promoter (20). After 60 to 90 min of incubation, the entire 50-μl sample was assayed for β-galactosidase activity by addition of the colorimetric substrate *o*-nitrophenyl-β-D-galactopyranoside. The amount of *o*-nitrophenol (ONP) product was determined as described by Miller (17).

In experiments in which the P4 δ protein was made by induction (Table 2), crude extracts containing the P4 δ protein were prepared from *E. coli* M5219 *lacZ* λ *c*Its (pCH13) (δ-overproducing plasmid of Halling and Calendar) by disrupting cells in a French press and using the supernatant of centrifugation at 30,000 × *g*. Strain M5219 carries an amber mutation in *lacZ* and the defective, temperature-inducible prophage λ *c*I857ΔH1 *bio252* (18). Cells were grown at 30°C (uninduced) and then shifted to 40°C for 3 h (induced for δ protein). Extracts were added in the amounts indicated to the *Salmonella* coupled transcription-translation system described in the legend to Table 1. In the first 11 reactions, an extract from induced cells was used. For reactions 12 to 14, which were run in a separate experiment, the extract was from induced or uninduced cells, as indicated. The P_{sid} and *lac*UV5 promoters were carried on reporter plasmids pSidZ (Fig. 2) and pRS229 (20) respectively. The amounts of the templates were pSidZ, 7.5 μg and pRS229, 5 μg. Incubation time and measurement of β-galactosidase activity were as shown in Table 1.

RESULTS AND DISCUSSION

We designed a coupled transcription-translation assay to detect transcription from the P4 late promoter, P_{sid}. For this

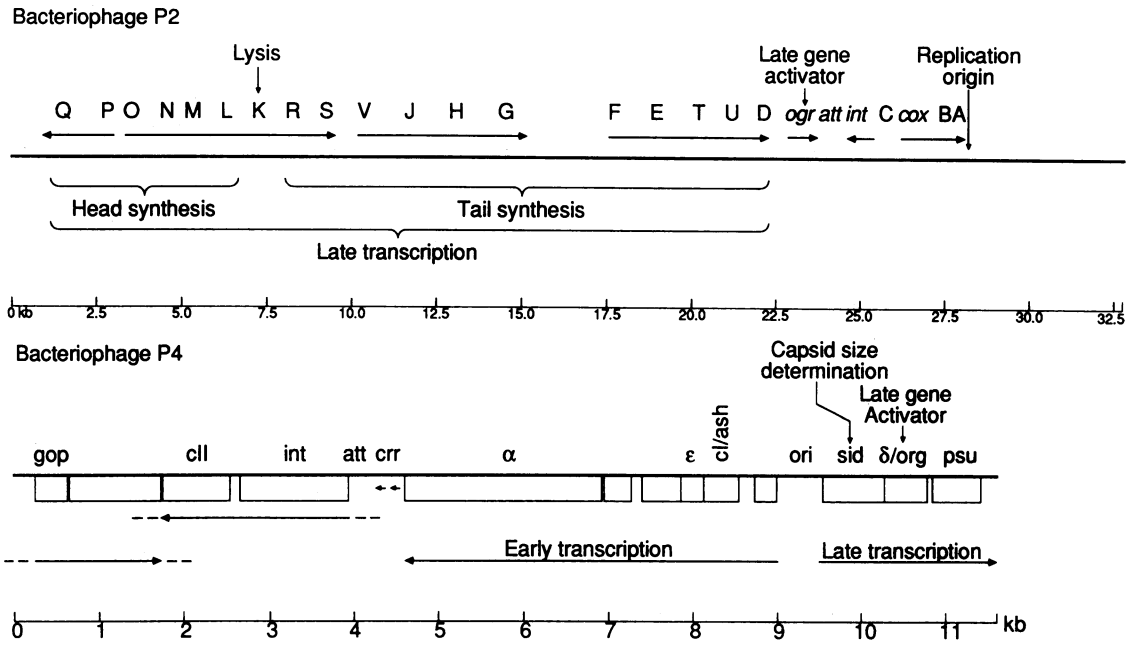

FIG. 1. Genetic maps of phages P2 and P4, with transcription units depicted by horizontal arrows. The P2 genome is 33.3 kilobases (kb), and the P4 genome is 11.6 kb (2).

purpose P_{sid} was inserted into the *lacZ* expression vector pMC1403 (4). The resulting reporter plasmid, pSidZ, carries an in-frame fusion of the 5′ portion of the *sid* gene to *lacZ* (Fig. 2) to permit measurement of β-galactosidase activity as a sensitive indicator of expression from P_{sid}. The P2 *ogr* gene and the P4 δ gene were cloned into driver plasmids such that their respective gene products could be overproduced (6; Halling and Calendar, unpublished data). To test for activation of transcription from P_{sid} by the *ogr* and δ gene products in vitro, we programmed an S-30 extract with the appropriate driver plasmid and the reporter plasmid pSidZ (Table 1). In the absence of any phage-encoded factors, expression from P_{sid} was not detected (Table 1, reaction 3). The product of the *ogr* or δ gene, transcribed and translated in situ, could activate transcription from P_{sid} (reactions 4 to 7). At the concentrations of driver plasmids that gave maximal activity (reactions 5 and 7), the level of β-galactosidase production from P_{sid} was half of that obtained from the strong promoter *lac*UV5, which was carried on the control reporter plasmid pRS229 (20). These results demonstrate that the *ogr* and δ proteins can activate transcription from P_{sid} in the absence of other phage-encoded factors.

To measure the activity of δ protein produced in vivo, crude extracts were prepared from cultures of *E. coli* M5219 (18), which carries the δ-overproducing plasmid pCH13 (Halling and Calendar, unpublished data). Extracts prepared from cultures induced for δ expression were able to activate transcription from P_{sid} in vitro (Table 2, reactions 2 to 6 and 12), whereas an extract from an uninduced culture gave little activation (reaction 13). Extracts from induced cultures did not activate transcription from the control promoter *lac*UV5 (reactions 8 to 11), indicating that their effects at P_{sid} were specific. As a further indication of specificity, amounts of crude extract that nonspecifically inhibited expression from the *lac*UV5 promoter (reactions 10 and 11) could still acti-

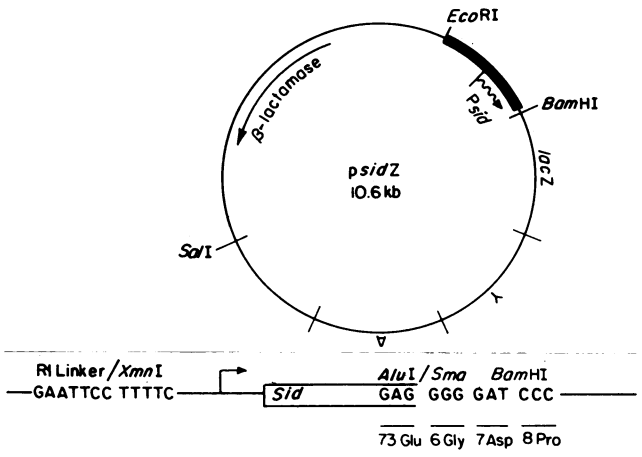

FIG. 2. Plasmid pSidZ. Phasmid P420Δ43 (11) was cleaved with *Xmn*I; *Eco*RI linkers were added, and the DNA was cleaved with *Eco*RI and *Alu*I. The *Eco*RI-modified *Xmn*I-to-*Alu*I fragment containing P_{sid} (7) was isolated and ligated to pMC1403 (4), which had been digested with *Eco*RI and *Sma*I. This construction encodes a fusion protein; the first 73 amino acids of the P4 *sid* protein are fused to amino acid 6 of β-galactosidase. kb, Kilobases.

TABLE 1. Activation of transcription from the P4 late promoter, P_{sid}, by the P2 *ogr* or P4 δ protein synthesized in situ

Reaction	Driver plasmid, amt (μg)	Reporter plasmid	pmol of ONP/min
1	pRF5 (*ogr*), 7.5	None	≤60
2	pCH13 (δ), 7.5	None	≤60
3	None	pSidZ (P_{sid})	≤60
4	pRF5 (*ogr*), 7.5	pSidZ (P_{sid})	1,700
5	pRF5 (*ogr*), 3.0	pSidZ (P_{sid})	18,600
6	pCH13 (δ), 15.0	pSidZ (P_{sid})	11,400
7	pCH13 (δ), 7.5	pSidZ (P_{sid})	19,200
8	None	pRS229 (*lac*UV5)	38,980

TABLE 2. In vitro assay of crude extracts from cells induced for expression of the P4 δ protein

Reaction	μl of extract (temp [°C])	Promoter	pmol of ONP per min
1	0	P_{sid}	200
2	0.19	P_{sid}	1,940
3	0.45	P_{sid}	5,100
4	1.0	P_{sid}	12,500
5	2.5	P_{sid}	13,000
6	5.0	P_{sid}	3,140
7	0	*lac*UV5	63,900
8	0.45	*lac*UV5	64,800
9	1.0	*lac*UV5	60,300
10	2.5	*lac*UV5	27,200
11	5.0	*lac*UV5	2,990
12	0.25 (40)	P_{sid}	5,630
13	0.25 (30)	P_{sid}	270
14	0	P_{sid}	60

vate P_{sid} (reactions 5 and 6). Since the activity of the δ gene product is detectable in crude extracts, we can use the coupled system to monitor its purification.

To determine whether the σ^{70} subunit of RNA polymerase was required for activation of transcription by the δ protein, δ-dependent expression from P_{sid} in the coupled transcription-translation system was challenged with polyclonal antibodies to *E. coli* σ^{70}. When increasing amounts of anti-σ^{70} serum were added to a set of reactions containing δ protein and the pSidZ template, transcription from P_{sid} was inhibited (Table 3). As expected, antibodies to σ^{70} inhibited transcription from the *lac*UV5 promoter but did not inhibit transcription from the *glnA* promoter, which is dependent on the alternate sigma factor encoded by the *ntrA* gene (Table 3; reference 13). These results were consistent with the view that activation of transcription from P_{sid} requires σ^{70} as well as the δ protein. To confirm this view, we demonstrated that a monoclonal antibody to σ^{70} (M. Strickland, N. Thompson, and R. Burgess, Biochemistry, in press) also inhibited transcription from P_{sid} and from the *lac*UV5 promoter (Fig. 3A) and that purified σ^{70} restored transcription from both promoters (Fig. 3B). We conclude that transcription from P_{sid} depends on the σ^{70} holoenzyme, and therefore the δ protein does not appear to be an alternate sigma factor.

How, then, might the δ and *ogr* proteins activate transcription? Deletion of a region 60 base pairs upstream of the start site for the P4 late transcript (E. Dale, Ph.D. thesis, University of California, Berkeley, 1987) or the corresponding region upstream of the start site for a P2 late transcript (N. Grambow, M.S. thesis, Medical College of Virginia, Richmond, 1987) abolishes promoter activity. Because the P4 and all of the P2 late promoter regions are similar around −60 (7), it seems likely that the δ and *ogr* activator proteins bind there. As a consequence of such DNA binding, these activators could interact with the σ^{70} holoenzyme to enhance its recognition of the poor −10 and −35 hexamers present in the late promoters or to effect its rapid isomerization during relatively transient recognition events. Purification of the δ protein by the assay described here should provide materials with which to test these ideas.

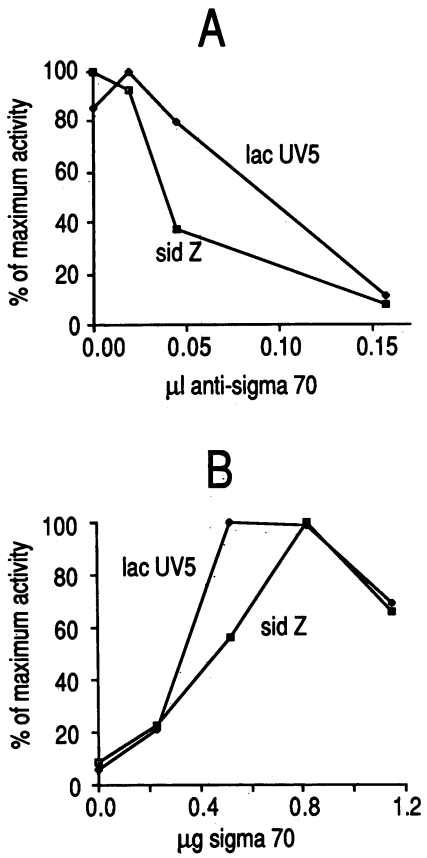

FIG. 3. Inhibition of δ-dependent transcription by a monoclonal antibody to σ^{70} (A) and restoration of transcription of purified σ^{70} (B). Expression from the P_{sid} (□; 3 μg of pSidZ per reaction) or *lac*UV5 ◆; 5 μg of pRS229 per reaction) promoter was assayed as for Table 2. Each reaction included 3 μl of a crude extract containing the δ protein, prepared as for Table 2. (A) The reactions contained the indicated amounts of the 3D3 monoclonal antibody to *E. coli* σ^{70} (M. Strickland, N. Gribskov and R. Burgess, Biochemistry, in press). The maximum activities were 3,839 pmol of ONP per min from P_{sid} and 6,172 pmol of ONP per min from the *lac*UV5 promoter. (B) The reactions contained 0.185 μl of the 3D3 monoclonal antibody and the indicated amounts of pure σ^{70}. The maximum activities were 1,876 pmol of ONP per min from P_{sid} and 7,579 pmol of ONP per min from the *lac*UV5 promoter.

TABLE 3. Inhibition of δ-dependent transcription from the P_{sid} promoter by σ^{70} antibodies[a]

μl of anti-σ^{70}	Activity (pmol of ONP/min) expressed from:		
	pSidZ (P_{sid})	pRS229 (*lac*UV5)	pJES40 (*glnA*)
0	3,910	60,500	8,710
0.018	2,070	61,800	6,130
0.038	790	7,740	6,880
0.075	340	550	12,440

[a] Expression from the P_{sid}, *lac*UV5, and *glnA* promoters was assayed as for Table 2. Each reaction contained 1 μl of the δ extract used in reactions 1 to 11 of Table 2, and it also contained the NTRA and NTRC proteins, which are necessary to activate transcription from the *glnA* promoter (10, 13). Polyclonal antiserum to the σ^{70} subunit of *E. coli* RNA polymerase was added to each reaction in the amount indicated. Plasmid pJES40 carries an in-frame protein fusion of codon 42 of *glnA* to codon 9 of *lacZ*, which places β-galactosidase production under control of the *glnA* promoter (10, 13).

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