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Mutations Affecting Two Adjacent Amino Acid Residues in the Alpha Subunit of RNA Polymerase Block Transcriptional Activation by the Bacteriophage P2 Ogr Protein

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The bacteriophage P2 *ogr* gene product is a positive regulator of transcription from P2 late promoters. The *ogr* gene was originally defined by compensatory mutations that overcame the block to P2 growth imposed by a host mutation, *rpoA109*, in the gene encoding the α subunit of RNA polymerase. DNA sequence analysis has confirmed that this mutation affects the C-terminal region of the α subunit, changing a leucine residue at position 290 to a histidine (*rpoA*L290H). We have employed a reporter plasmid system to screen other, previously described, *rpoA* mutants for effects on activation of a P2 late promoter and have identified a second allele, *rpoA155*, that blocks P2 late transcription. This mutation lies just upstream of *rpoA*L290H, changing the leucine residue at position 289 to a phenylalanine (*rpoA*L289F). The effect of the *rpoA*L289F mutation is not suppressed by the *rpoA*L290H-compensatory P2 *ogr* mutation. P2 *ogr* mutants that overcome the block imposed by *rpoA*L289F were isolated and characterized. Our results are consistent with a direct interaction between Ogr and the α subunit of RNA polymerase and support a model in which transcription factor contact sites within the C terminus of α are discrete and tightly clustered.

Many eubacterial genes and operons are under positive control, and the structures and DNA-binding sites of a number of prokaryotic transcriptional activators have been well characterized (for a review, see reference 1). The precise mechanism by which these regulatory proteins catalyze the initiation of transcription remains largely obscure. The identification of lambda repressor (9, 24, 27, 28) and catabolite gene activator protein (CAP) (3, 15, 65) mutants that were defective as activators but retained DNA-binding ability suggested that these proteins function by interacting directly with RNA polymerase. A rapidly growing body of genetic evidence supports a functional interaction between the C-terminal region of the α subunit(s) of DNA-dependent RNA polymerase and specific transcriptional activators.

The earliest indication that the α subunit plays a role in positive control was provided by *Escherichia coli* mutation *rpoA109* (61), which prevents lytic growth of phage P2 and satellite phage P4 by interfering with phage late-gene expression. This mutation results in a leucine-to-histidine change in the C-terminal region of the α subunit (16). Activation of a P2 late promoter by phage-encoded late transcriptional activator Ogr (P2) or δ (P4) is severely depressed in strains carrying the *rpoA109* mutation (23). Compensatory mutations that overcame this block were shown to affect Ogr or δ (26, 61), suggesting direct communication between these proteins and the α subunit of RNA polymerase. Recently obtained evidence from a number of other systems, based on the isolation of additional *rpoA* mutants that are affected in the activation of certain positively regulated operons, is consistent with this model (for reviews, see references 33, 34, and 52). Affected activators include CAP-cyclic AMP (14, 31, 32, 66), OmpR (17, 46, 55, 58), FNR (44), AraC, MelR, and CysB (20). The general pattern that has emerged from the characterization of these mutants is that they are altered in the C-terminal region of the α polypeptide and that each *rpoA* mutation appears to be fairly specific, affecting only one or a small group of positively regulated promoters. Those mutations that affect the responsiveness of RNA polymerase to each activator tend to be tightly clustered, suggesting that each transcriptional activator interacts with a discrete domain within the C-terminal region of the α subunit to stimulate transcription.

To further define a domain of the α subunit involved in interaction with the activators of P2 late-gene expression, we have determined the precise location of the *rpoA109* mutation. In addition, we have examined other chromosomal alleles of *rpoA* for their effects on transcription from a cloned P2 late promoter. Our analysis indicates that most of these *rpoA* mutations have little effect on P2 late-promoter activation. However, one mutation, *rpoA155* (*rpoA* L289F; 44), severely impairs P2 late-gene expression and prevents P2 lytic growth. We have isolated and characterized *rpoA*L289F-compensatory P2 *ogr* mutants. Our results support a model of direct interaction of Ogr with a discrete domain of the α subunit of RNA polymerase.

MATERIALS AND METHODS

Culture media. The culture medium used was LB (45) supplemented when appropriate with antibiotics (ampicillin, 100 μg/ml; tetracycline, 15 μg/ml; kanamycin, 60 μg/ml; chloramphenicol, 25 μg/ml). For growth of P2, LB was supplemented to 0.5 mM with $CaCl_2$.

Strains and plasmids. The bacterial strains and plasmids used in this study are listed in Table 1. P1 transductions were done with P1 *cml clr* as described by Miller (47). Mutant *rpoA* alleles were introduced into strain C-1a by cotransduction with *zhc*::Tn*10*. Since the cotransduction frequency of this Tn*10* with *rpoA* is approximately 50%, the *rpoA* gene from several

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Description or genotype	Reference or source
Bacteria		
Escherichia coli K-12		
GR302	GR303 *rpoA341* (*phs*)	50
GR303	*araD139* Δ(*argF-lac*)*U169 rpsL gltSo thi* Δ(*his-gnd*) *zhc*::Tn*10*	50
JMS4540	F⁻ Δ(*argF-lac*)*U169 rpsL150 relA fib-530 ptsF25 deoC*IΦ(*ompF'-lacZ*⁺)16-13 *zhc-3*::Tn*10*	58
JMS4542	JMS4540 *rpoA85*	58
JMS4543	JMS4540 *rpoA50*	58
JMS4544	JMS4540 *rpoA52*	58
JMS4545	JMS4540 *rpoA53*	58
JMS4546	JMS4540 *rpoA54*	58
Escherichia coli C		
C-1a	F⁻ prototrophic	54
C2121	F⁻ *rpoA109 rpsL*	61
C4595	F⁻ *rpsL*	23
C4613	C-1a *rpoA50 zhc-3*::Tn*10*	This study; transduction from JMS4543
C4618	C-1a *rpoA52 zhc-3*::Tn*10*	This study; transduction from JMS4544
C4620	C-1a *rpoA53 zhc-3*::Tn*10*	This study; transduction from JMS4545
C4622	C-1a *rpoA85 zhc-3*::Tn*10*	This study; transduction from JMS4542
C4624	C-1a *rpoA54 zhc-3*::Tn*10*	This study; transduction from JMS4546
C4626	C-1a *rpoA*⁺ *zhc-3*::Tn*10*	This study; transduction from JMS4540
C4628	C-1a *rpoA341 zhc*::Tn*10*	This study; transduction from GR302
C4631	C-1a *rpoA*⁺ *zhc*::Tn*10*	This study; transduction from GR303
Salmonella typhimurium		
TN2540	*metE551 metA22 ilv-452 trpB2 hisC527*(Am) *galE496 xyl-404 rpsL120 flaA66 hsdL6 hsdSA29*	M. J. Lombardo and C. G. Miller
TN1379	*leuBCD485*	44
TN3611	*leuBCD485 zcc-850*::Tn*10 rpsE rpoA151*	M. J. Lombardo and C. G. Miller
TN3612	*leuBCD485 zcc-850*::Tn*10 rpsE rpoA155*	M. J. Lombardo and C. G. Miller
TN3613	*leuBCR485 zcc-850*::Tn*10 rpsE rpoA153*	M. J. Lombardo and C. G. Miller
TN3614	*leuBCD485 zcc-850*::Tn*10 rpsE rpoA154*	M. J. Lombardo and C. G. Miller
TN3615	*leuBCD485 zcc-850*::Tn*10 rpsE rpoA*⁺	M. J. Lombardo and C. G. Miller
TN3555	*leuBCD485 zhb-1624*::Tn*10*Δ*16*Δ*17 rpoA8*	M. J. Lombardo and C. G. Miller
TN3556	*leuBCD485 zhb-1624*::Tn*10*Δ*16*Δ*17 rpoA*⁺	M. J. Lombardo and C. G. Miller
TN4235	*galE zhb-1624*::Tn*10*Δ*16*Δ*17 rpoA155*	M. J. Lombardo and C. G. Miller
TN4236	*galE zhb-1624*::Tn*10*Δ*16*Δ*17 rpoA*⁺	M. J. Lombardo and C. G. Miller
LB5010	*hsdLT6 hsdA29 hsdSB121 metA22 metE551 trpE2 ilv-452 leu-3121 xyl-4-4 galE856 rpsL120 H1-b H2-enx flaA66* (cured of Fels 2)	8
MS1439	LB5010 *zhb-1624*::Tn*10*Δ*16*Δ*17 rpoA155*	This study; transduction from TN4235
Plasmids		
pBB100	Kan^r; P15A replicon; derivative of pNB177 carrying λ p_L promoter from pRC23	This study
pBB105	Kan^r; P15A replicon; derivative of pBB100 carrying λ *c*I(ts) gene from pRK248*cIts*	This study
pBB106	Kan^r; P15A replicon; derivative of pBB105 carrying p_L-*ogr*Y42C	This study
pCV1	Amp^r; derivative of pRC23 carrying *ogr*Y42C allele	12
pGC57	Kan^r; P15A replicon; carries λ *c*I(ts) and p_L-*ogr*⁺	23
pFCAT100	Amp^r; carries *cat* under control of P2 *F* promoter	23
pGVB1	Kan^r; P15A replicon; carries λ *c*I(ts) and p_L-δ	64
pNB177	Kan^r; P15A replicon; *Bal* 31 deletion derivative of pACYC177 lacking Amp^r	23
pRC23	Amp^r; carries λ p_L and synthetic ribosome-binding site	13
pRK248*cIts*	Tet^r; expresses λ *c*I(ts)	4
pSL130	Amp^r; carries *cat* under control of *tac* promoter	42

tetracycline-resistant isolates from each transduction was sequenced to determine the presence of the desired mutation. Screening for P2-nonpermissive mutants was done with clear-plaque mutant P2 *vir1* (5). For growth of P1 and P2 on *Salmonella typhimurium*, *galE* mutant strains were used to allow phage adsorption (48).

Burst size determinations. Burst sizes were determined in one-step growth experiments, essentially as described by Six (57). Infection of *E. coli* and *S. typhimurium* strains was done at 37°C. The optical density at 600 nm of the infected cultures was monitored for at least 80 min following infection. Phage yields were assayed between 60 and 80 min after infection. For all infections that did result in mass lysis of the culture, lysis appeared to be complete at the time of assay as judged by the decrease in optical density at 600 nm.

Plasmid constructions and transformation. Standard tech-

TABLE 2. Oligonucleotides used for amplification and sequencing

Name	Sequence[a]	Location
DJA3	5′-TTCGgATCcGTCTGCGGACATTAA-3′	~80 bases upstream of *rpoA* start site
DJA4	5′-GCGggtACCTGTGATCCGGTTACTC-3′	*rpoA*, base 985 and through stop codon
DJA5	5′-ACGAggtACCAGCTCGATGTAAGC-3′	372 bases beyond *rpoA* stop
DJA6	5′-CGGTCCGATACAAGGCGTTATA-3′	91 bases beyond *rpoA* stop
LC2	5′-GCAGGAGTCTGATGCGA-3′	Near end of P2 gene *D*
OGR3RK	5′-CACATTGACCACATCGAC-3′	Just distal to *ogr* terminator
TX	5′-AgTcAACTAAAATGGCACCATCAAC-3′	Within *ogr* gene promoter

[a] Underlined bases designate restriction sites, lowercase letters represent mismatches, and boldface bases indicate the location of the *rpoA* stop codon.

niques for recombinant DNA methodology were employed (45), and enzymes were used as recommended by the suppliers. Plasmid pBB106 is identical to p_L-*ogr* plasmid pGC57 (23), except that it carries the *rpoA109* compensatory *ogr* allele, which replaces Tyr-42 with Cys (*ogr*Y42C). To construct this plasmid, a 640-bp *Bam*HI-*Bgl*II fragment carrying the lambda p_L promoter from pRC23 (13) was ligated with pNB177 (23), which had been cleaved with *Bam*HI. The resulting plasmid, pBB100, was cleaved with *Bam*HI and ligated with a 2.4-kb *Bgl*II fragment carrying the lambda *c*I(ts) gene from pRK248*cIts* (4) to yield pBB105. A 1-kb fragment carrying the *ogr*Y42C gene was isolated from plasmid pCV1 (12) following digestion with *Eco*RI and *Eco*RV and ligated with pBB105, which had been cleaved with the same two enzymes.

Competent cells were prepared by the rubidium chloride technique described in technical manual TM001 (Promega Corp., Madison, Wis.). Plasmid DNA isolated from *E. coli* was passed through strain TN2540, which modifies DNA but is defective in restriction, prior to transformation of other *Salmonella* strains.

CAT enzyme assay. Lysates of cultures containing reporter gene plasmid pFCAT100 and a compatible plasmid encoding Ogr (pGC57 or pBB106) or δ (pGVB1) were assayed for chloramphenicol acetyltransferase (CAT) by the spectrophotometric method of Shaw (56) as described previously (23). Protein concentrations were determined by the method of Bradford (7), with the Bio-Rad (Richmond, Calif.) protein assay kit. Cells were grown to an optical density at 600 nm of 0.5 at 30°C in LB supplemented with the appropriate antibiotics, shifted to 42°C to derepress expression of plasmid-encoded Ogr or δ from p_L, and assayed after an additional 30 min of incubation. Cultures of strains carrying *tac* promoter plasmid pSL130 were grown at 37°C and assayed 45 min after addition of 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG). CAT activity was determined for at least two independent single colony isolates of each plasmid-containing strain.

PCR amplification and DNA sequence analysis. Primers used for the PCR and DNA sequencing (Table 2) were purchased from Oligos Etc., Inc., Wilsonville, Oreg. For sequence analysis, the entire *rpoA* gene was amplified by using primers DJA3 and DJA4, DJA5, or DJA6. Amplification was done with bacterial colonies as described by Joshi et al. (36). Colonies were picked with an inoculating loop into 50 μl of H_2O and vortexed vigorously. A 5-μl aliquot was used as the DNA template, and 1 μg of each of the appropriate primers was used in an otherwise standard PCR with reagents and protocols supplied by the manufacturer (Perkin-Elmer Cetus, Norwalk, Conn.). Prior to sequencing, the amplified products were separated by agarose gel electrophoresis and purified by subjecting the appropriate gel slice to several freeze-thaw cycles, followed by phenol extraction and ethanol precipitation. Sequencing was done with reagents and protocols supplied with the ABI dye terminator kit, and reactions were analyzed on an ABI Automated Sequencer. Flanking primers DJA3 and DJA5 (Table 2) and internal primers APN2, APN3, APN4, APC2, APC3, and APC4 (29) were used to sequence the entire gene.

Isolation and characterization of *rpoA155*-compensatory P2 mutants. P2 mutants able to form plaques on lawns of *S. typhimurium* strains carrying the *rpoA155*(L289F) mutation were isolated in two ways. P2 *vir1* modified to escape restriction by *S. typhimurium* was obtained either by plating a high-titer phage stock directly on TN4236 or by plating on r^- m^+ strain LB5010. Phage purified from the plaques which appeared on these plates were able to plate on TN4236 with an efficiency of plating of 1. Aliquots containing 3×10^9 to 3×10^{10} phage from such a stock were plated on lawns of TN4235 and incubated overnight at 37°C. Extremely tiny plaques appearing at a frequency of 4×10^{-9} to 1×10^{-10} were picked, and phage were purified by plating on TN4235. Alternatively, approximately 10^8 P2 *vir1* phage (unadapted to *S. typhimurium*) were plated on lawns of r^- m^+ *rpoA155* mutant strain MS1439, a derivative of LB5010 obtained by P1 transduction from TN4235. Small plaques appearing at a frequency of 10^{-6} on MS1439 turned out to be P2 *old* mutants, and these phage were unable to grow on TN4235. These findings will be reported elsewhere. Larger plaques appearing at a frequency of approximately 10^{-8} contained phage able to grow on TN4235; these were purified by plating on MS1439.

The *ogr* gene from mutant phage stocks derived from each method was sequenced as follows: 1 μl of lysate (approximately 10^{11} phage per ml) was diluted into 100 μl of H_2O; 3 μl was then used as the template in an otherwise standard PCR. The *ogr* gene was amplified by using flanking primers LC2 and OGR3RK (Table 2), and the amplified fragment was gel purified and subjected to DNA sequence analysis as described above, with primers OGR3RK and TX (Table 2). The sequence of the *ogr* gene from each mutant was determined in its entirety on both strands from two independent PCRs.

RESULTS

Sequence of *rpoA109*. Fujiki et al. (16) isolated the altered α subunit from an *rpoA109* mutant and identified a tryptic peptide containing a histidine in place of leucine. The composition of this tryptic peptide, compared with the amino acid sequence encoded by the *rpoA* gene, is consistent with a mutation affecting one of a pair of leucine codons at residues 289 and 290. The sequence of the *rpoA109* gene was determined directly from PCR-amplified genomic DNA of strain C2121. A single T-to-A transversion at nucleotide 868 in the *rpoA* coding sequence (CTT→CAT) was found. This mutation results in a substitution of histidine for leucine at amino acid residue 290 in the α polypeptide (*rpoA*L290H).

The *ogr*Y42C mutation restores expression from a P2 late promoter in a strain carrying the *rpoA109* (L290H) mutation. The *rpoA*L290H mutation prevents P2 lytic growth by inhibiting P2 late-gene expression, and P2 *ogr* mutants that overcome this block have been isolated (61). Sequence analysis of six independent compensatory *ogr* mutations revealed a single change, resulting in substitution of Cys for Tyr at amino acid 42 (*ogr*Y42C; 6, 12, 18). We have previously described a reporter plasmid system in which expression of *cat* from a cloned P2 late promoter, in pFCAT100, is detected only when Ogr or δ is provided in *trans* from a compatible plasmid (23) and have shown that the *rpoA*L290H mutation inhibits activation of *cat* expression in this system (23; see also Table 3). This decrease in expression is not due to a general defect in transcription, since a similar construct with *cat* under control of the *tac* promoter had comparable levels of expression in *rpoA*$^+$ and *rpoA*L290H strains (Table 3). When Ogr was supplied by a plasmid encoding the compensatory *ogr*Y42C mutant, a slight reduction in CAT activity was seen in an *rpoA*$^+$ strain, but 40% of this level of expression was retained in a strain with the *rpoA*L290H mutation (Table 3).

Effects of other chromosomal alleles of *rpoA* on expression from P_F: a severe reduction is conferred by the *rpoA155* (L289F) mutation. Mutations in *rpoA* that affect positive control have been isolated in both *E. coli* and *S. typhimurium*. The location of each of the *rpoA* mutations tested in this study is shown in Fig. 1.

Each of the chromosomal *rpoA* mutations reported to affect positive control in *E. coli* K-12 was moved by transduction into prototrophic C strain *E. coli* C-1a, and activation of *cat* expression from pFCAT100 by Ogr (pGC57) or δ (pGVB1) was assayed (Table 3). Although it has been reported previously that some of these mutations do not affect P2 growth (58), we felt that it was important to analyze the effects of these *rpoA* alleles on a cloned P2 late promoter in a well-defined background for several reasons. First, the reduction of operon-specific gene expression reported for some of these *rpoA* mutants is quite modest. For example, even the most severe of the *dip* mutations only reduces *ompF* expression to about 40% of that seen in an *rpoA*$^+$ strain (58). A similar-magnitude reduction in P2 late transcription would most likely not prevent plaque formation. In addition, a number of *E. coli* K-12 laboratory strains have recently been shown to contain cryptic P2-like prophages with functional *ogr* gene analogs (2, 59). This raises the possibility that such genes, if present in the strains in which the *rpoA* mutations were tested, could mask an effect of the polymerase mutation on normal P2 late-gene expression.

Some of the *E. coli rpoA* mutations we examined had no effect on expression from the cloned P2 late promoter, as discussed below, while others had small effects similar in magnitude to those previously reported for other promoters. None of the mutations conferred a defect severe enough to affect phage growth noticeably (data not shown).

The *rpoA341* (*phs*; *rpoA*K271E) mutation confers on *E. coli* a pleiotropic growth defect and leads to impaired transcription from at least three unlinked sets of genes, *melAB*, *cysA*, and the *ara* regulon, each of which requires a regulon-specific transcriptional activator (20, 21, 50, 51). The reported levels of expression of the genes affected by the *rpoA*K271E mutation are 7% of the wild-type level for *melAB* and *araBAD* and 14% for *cysA*, as measured in *lacZ* fusions (20). The *rpoA*K271E mutation did not cause a significant reduction in *cat* expression from P2 late promoter P_F or from P_{tac}, in either an *E. coli* C strain (Table 3) or the original *E. coli* K-12 mutant (data not shown).

Several mutations that affect *E. coli* porin gene expression have been shown to result in amino acid substitutions in the C-terminal part of α (17, 58; Fig. 1). We examined mutants isolated on the basis of a decrease in transcription from the *ompF* promoter (*dip*; 17), as well as one that suppresses the defect conferred by a mutation in *envZ* (*sez*; 58). The reported effects of the *dip* mutations on transcription from the *ompF* promoter are fairly modest, ranging from about 40 to 66% of that seen in an *rpoA*$^+$ strain (58). Similar modest effects on activation of P_F by Ogr or δ were seen for *rpoA52* (P323S): i.e., 60 and 64%, respectively, of that seen in an *rpoA*$^+$ strain (Table 3). The adjacent *rpoA54* mutation (P322S) did not impair Ogr- or δ-dependent transcription from P_F. Activation of P_F by δ was also unaffected by *rpoA53* (G3S) and *rpoA50* (L28F and P240S), but modest effects on activation by Ogr were seen for both mutants. Ogr-dependent expression was reduced to 33% of the wild-type level by the *rpoA*G3S mutation, while a twofold increase in expression was seen for *rpoA50* (Table 3). The *sez* mutation, *rpoA85* (P323L), caused a slight reduction in expression from P_F in the presence of Ogr or δ. A similar modest reduction was reported for *ompF* expression in an *envZ*$^+$ background (58); as shown in Table 3, the same results were obtained for expression from the *tac* promoter. This mutation thus appears to affect transcription in general rather than interfere with positive control.

Two additional *rpoA* mutations that were isolated on the basis of a decrease in transcription from the *ompF* promoter have been described more recently (55), *rpoA203* (A272T) and *rpoA207* (E215K). Neither of these mutations impairs Ogr- or δ-dependent expression from P_F (12a). We also tested the two characterized temperature-sensitive alleles of *rpoA* (29, 35) to see whether they affected P2 late-promoter activation at the permissive temperature. Both mutations affect the N-terminal region of the α subunit and, as expected, did not impair P2 growth or show a significant reduction in late-promoter activation (data not shown).

Five *rpoA* mutations in *S. typhimurium* that affect the induction of *pepT* and other anaerobically regulated *oxrA* (*fnr*)-dependent genes have been described (44); these mutations also change amino acids in the C-terminal part of the α subunit (Fig. 1). Plasmids pFCAT100 and either pGC57 (Ogr) or pGVB1 (δ) were introduced into *Salmonella* strains carrying each of these *rpoA* mutations and tested for levels of expression (Table 3). Since the *rpoA* genes from *S. typhimurium* and *E. coli* encode α subunits with the same amino acid sequence (44), the same interaction(s) between Ogr or δ and the *Salmonella* α subunit as seen with *E. coli* would be predicted. Not surprisingly, levels of CAT expression from the P_F promoter in the *rpoA*$^+$ *Salmonella* strain were very similar to those observed in *E. coli*. The effects of most of the *oxrB* alleles on FNR-dependent activation of the *pepT* promoter are relatively modest. Two mutations, *rpoA154* (W321Ter) and *rpoA153* (R317H), reduced activity to about two-thirds of the wild-type levels (44). A similar-magnitude reduction was seen in Ogr- or δ-mediated expression of P_F (Table 3). Two mutations affecting amino acid residue 311, *rpoA151* (G311E) and *rpoA8* (G311R), had a somewhat more severe effect on *pepT* expression, reducing it to 31 and 27%, respectively, of the levels seen in *rpoA*$^+$ strains (44). These mutations had relatively little effect on activation of P_F by Ogr or δ (Table 3). All four of these *rpoA* mutations were reported to have little effect on the growth of *S. typhimurium* or on expression of the *E. coli lac* operon on an F′ plasmid (44); consistent with this, we saw little effect on transcription from the cloned *tac* promoter. In contrast, the *rpoA155* (L289F) allele conferred a modest reduction in expression from the *tac* promoter, while expres-

TABLE 3. P2 late-promoter activation in strains carrying mutant *rpoA* alleles

Strain	Allele	Mutation	Promoter	Regulatory protein	CAT activity	
					Units[a]	% of wt[b]
C4595	*rpoA*$^+$	None (wt)	P_F	Ogr	2.1 ± 0.1	100
			P_F	δ	22.7 ± 1.1	100
			P_F	Ogr1	1.0 ± 0.4	100
			P_{tac}		54.0 ± 7.7	100
C2121	*rpoA109*	L-290→H	P_F	Ogr	<0.01	<0.5
			P_F	δ	0.8 ± 0.1	3.5
			P_F	Ogr1	0.4 ± 0.2	40
			P_{tac}		50.6 ± 14.0	94
C4631	*rpoA*$^+$	None (wt)	P_F	Ogr	1.5 ± 0.6	100
			P_F	δ	12.0 ± 3.1	100
			P_{tac}		47.1 ± 5.0	100
C4630	*rpoA341*	K-271→E	P_F	Ogr	1.0 ± 0.1	67
			P_F	δ	10.4 ± 2.0	87
			P_{tac}		42.3 ± 15.6	90
C4626	*rpoA*$^+$	None (wt)	P_F	Ogr	1.5 ± 0.4	100
			P_F	δ	10.6 ± 2.0	100
			P_{tac}		51.6 ± 2.6	100
C4618	*rpoA52*	P-323→S	P_F	Ogr	0.9 ± 0.1	60
			P_F	δ	6.8 ± 0.3	64
			P_{tac}		53.9 ± 1.7	104
C4622	*rpoA85*	P-323→L	P_F	Ogr	1.0 ± 0.2	67
			P_F	δ	9.2 ± 0.5	87
			P_{tac}		42.9 ± 3.4	83
C4624	*rpoA54*	P-322→S	P_F	Ogr	1.7 ± 0.5	113
			P_F	δ	12.1 ± 1.3	114
			P_{tac}		58.6 ± 4.5	114
C4613	*rpoA50*	P-240→S L-28→F	P_F	Ogr	3.2 ± 0.8	213
			P_F	δ	10.1 ± 1.7	95
			P_{tac}		58.3 ± 1.6	113
C4620	*rpoA53*	G-3→S	P_F	Ogr	0.5 ± 0.1	33
			P_F	δ	10.1 ± 1.1	95
			P_{tac}		42.1 ± 2.9	82
TN3615	*rpoA*$^+$	None (wt)	P_F	Ogr	1.0 ± 0.2	100
			P_F	δ	13.0 ± 1.4	100
			P_F	Ogr1	0.5 ± 0.2	100
			P_{tac}		47.1 ± 5.2	100
TN3612	*rpoA155*	L-289→F	P_F	Ogr	<0.01	<1.0
			P_F	δ	1.1 ± 0.2	8.5
			P_F	Ogr1	0.02 ± 0.01	2.0
			P_{tac}		31.2 ± 4.9	66
TN3614	*rpoA154*	W-321→ter	P_F	Ogr	0.6 ± 0.1	60
			P_F	δ	7.6 ± 1.4	58
			P_{tac}		42.3 ± 4.9	90
TN3613	*rpoA153*	R-317→H	P_F	Ogr	0.5 ± 0.1	50
			P_F	δ	9.4 ± 2.3	72
			P_{tac}		40.0 ± 2.4	85
TN3611	*rpoA151*	G-311→E	P_F	Ogr	0.6 ± 0.1	60
			P_F	δ	12.0 ± 1.0	92
			P_{tac}		40.1 ± 7.0	85

Continued on following page

TABLE 3—*Continued*

Strain	Allele	Mutation	Promoter	Regulatory protein	CAT activity	
					Units[a]	% of wt[b]
TN3556	*rpoA*$^+$	None (wt)	P_F	Ogr	1.2 ± 0.3	100
			P_F	δ	19.2 ± 3.3	100
			P_{tac}		43.1 ± 4.7	100
TN3555	*rpoA8*	G-311→R	P_F	Ogr	0.9 ± 0.2	75
			P_F	δ	16.7 ± 3.8	87
			P_{tac}		45.6 ± 4.6	106

[a] Micromoles of chloramphenicol acetylated per minute at 37°C per milligram of protein in cell extract. Averages of at least two determinations plus or minus the standard errors are shown.

[b] Percent activity compared with that in the otherwise isogenic *rpoA*$^+$ strain assayed for each mutant (C4595, C4631, C4626, TN3615, and TN3556) is shown. wt, wild type.

sion from P_F in the presence of either Ogr or δ was reduced drastically (Table 3). Levels of CAT activity in the presence of Ogr were too low to be measured, while δ-mediated activity was less than 10% of that obtained in the *rpoA*$^+$ strain. This mutation affects the amino acid residue immediately adjacent to that altered by the *rpoA*L290H mutation. This region thus appears to be critical for the interaction of the α polypeptide(s) with the P2- or P4-encoded positive regulatory proteins.

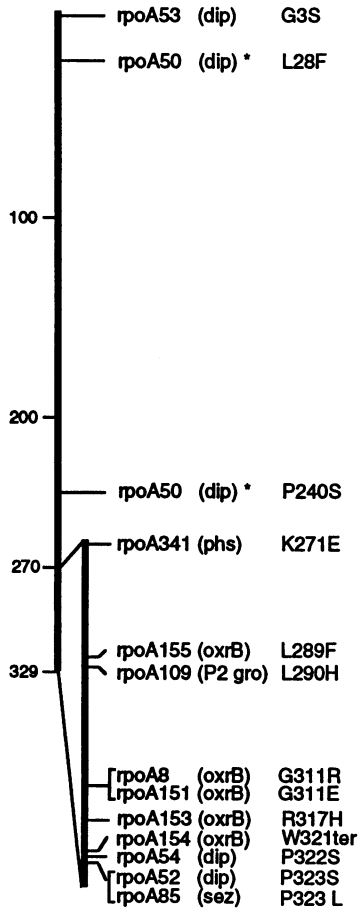

FIG. 1. Map of the α subunit showing the locations of and residues altered by the mutations examined in this study. The original name (or phenotype) of each mutant allele is indicated in parentheses; asterisks highlight the two changes caused by *rpoA50*. References: *sez*, 17; *dip*, 58; *phs*, 63; *oxrB*, 44; *rpoA109*, 16 and this work.

As described above, the compensatory *ogr*Y42C mutation restored expression from P_F in a strain carrying the *rpoA* L290H mutation to 40% of that seen in an *rpoA*$^+$ strain. In contrast, this *ogr* mutation was not able to restore even partial expression in a strain carrying the *rpoA*L289F mutation (Table 3). This suggests that the block to interaction with the Ogr protein imposed by mutation of each of these adjacent residues is distinct and that the *ogr*Y42C mutation does not simply result in a more effective activator.

An *ogr* mutation overcomes the block imposed by the *rpoA155* (L289F) mutation. The failure of *ogr*Y42C to restore transcription in a strain carrying the *rpoA*L289F mutation prompted us to isolate *rpoA*L289F-compensatory P2 mutants. We initially attempted to transduce the *rpoA155* allele from *S. typhimurium* into *E. coli*. Repeated attempts to transduce *rpoA155* into *E. coli* by using a P1 lysate prepared on *galE* mutant strain TN4235 were unsuccessful, despite the fact that we could transduce the allele into *rpoA*$^+$ strains of *S. typhimurium*. This may be due to the fact that despite identity at the amino acid level, the nucleotide sequences of the *rpoA* genes of the two species differ by 2.4%, interfering with homologous recombination. However, P1 and P2 are serologically related and share adsorption specificity, and P2 has also been reported to grow on *galE* mutants of *S. typhimurium* (48). We therefore carried out analysis of the effects of the *rpoA*L289F mutation on P2 and subsequent isolation of compensatory mutants in *S. typhimurium*. P2 stocks that would plate efficiently on *galE* mutants of *S. typhimurium* were generated by picking plaques of phage that had escaped restriction and then passaging them through the same strain. Such phage grow equally well on restriction-proficient *S. typhimurium* TN4236 and on derivatives of *E. coli* C, which are naturally restriction deficient. We also plated unmodified P2 stocks on MS1439, an *rpoA*L289F derivative of restriction-defective *S. typhimurium* LB5010. The *rpoA*L289F mutation blocks the growth of P2 in both backgrounds (Table 4 and data not shown), consistent with the transcriptional defect observed in the CAT assays (Table 3). Spontaneous P2 mutants that overcome the block imposed by the *rpoA*L289F mutation were isolated by plating the *Salmonella*-modified P2 on restriction-proficient *rpoA155* mutant strain TN4235 and by plating a standard P2 lysate (grown in *E. coli* C) on nonrestricting *rpoA*L289F mutant derivative MS1439. The *ogr* genes of several independent mutants obtained from each selection contained the same C-to-T transition at position 38; this changes codon 13 from Ala to Val (*ogr*A13V).

Mutations *ogr*Y42C and *ogr*A13V differ in the ability to overcome the effects of the *rpoA*L290H and *rpoA*L289F mutations. Table 4 summarizes the burst sizes of P2 derivatives on *E. coli* and *S. typhimurium* strains carrying the *rpoA*L290H and

TABLE 4. Burst sizes of *rpoA*-compensatory P2 *ogr* mutants

Strain	*rpoA* mutation	Burst size[a] of:		
		P2 *vir1*	P2 *vir1* *ogr*Y42C	P2 *vir1* *ogr*A13V
C4595	None (*rpoA*$^+$)	153	248	159
C2121	*rpoA*L290H	0.05[b]	274	113
LB5010	None (*rpoA*$^+$)	101	163	101
MS1439	*rpoA*L289F	0.48[b]	0.014[b]	82

[a] Average of at least two determinations.
[b] No visible lysis occurred in these infections.

*rpoA*L289F mutations, respectively. Neither of the *ogr* mutations impaired the growth of P2 in *rpoA*$^+$ strains; P2 *ogr*Y42C actually appeared to grow better than P2 *ogr* in *rpoA*$^+$ *S. typhimurium*. The *ogr*Y42C mutation restored growth in strains with the *rpoA*L290H mutation but not in strains with the *rpoA*L289F mutation, consistent with the CAT fusion plasmid data reported in Table 3. In fact, the burst size data suggest that the *rpoA*L289F mutant is more restrictive for P2 *ogr*Y42C than for P2 *ogr*$^+$. The *ogr*A13V mutant, in contrast, overcomes the block imposed by either the *rpoA*L290H or the *rpoA*L289F mutation.

DISCUSSION

We have demonstrated that point mutations affecting two adjacent amino acid residues in the C terminus of the α subunit, *rpoA*L290H and *rpoA*L289F, block activation of P2 late-gene transcription by both P2 Ogr and P4 δ. Point mutations in *rpoA* affecting activation by specific regulators are generally clustered into discrete and nonoverlapping regions (33, 34, 52; Fig. 1), and the two mutations that we describe here are consistent with this general pattern. Ogr and δ thus fit the definition of class I transcription factors proposed by Ishihama (34). Like other class I transcription factors, these phage-encoded activators appear to exert their effects via an interaction involving a discrete site within the C-terminal region of α. The effect of the *rpoA*L290H mutation appears to be highly specific; resistance to P2 and P4 is the only apparent phenotype associated with this change. The *rpoA*L289F mutation confers a broader range of defects, including a modest reduction in FNR-dependent *pepT* expression, partial auxotrophy for cysteine and methionine, and inability to be lysogenized by phage P22 (44). The pleiotropic nature of this mutation, the fact that it confers a modest defect, and its location in a region of the C terminus of α different from that of those mutations with the strongest effects on *pepT* expression led to the suggestion (52) that the *rpoA*L289F mutation may affect transcription indirectly rather than disrupt a specific interaction between FNR and RNA polymerase. In contrast, the effect of *rpoA*L289F on P2 late transcription, as measured by expression from the P_F-CAT fusion plasmid (Table 3) or by phage burst size (Table 4), is quite severe. Furthermore, this effect appears to be due to disruption of an interaction involving the transcriptional regulatory protein, as evidenced by the fact that we have isolated a compensatory Ogr mutant.

Both *rpoA*L290H and *rpoA*L289F severely impair activation of P2 late promoters by Ogr, as well as δ. These transcription factors are related proteins (6, 12, 26) that activate P2 late transcription from the same sites (10, 11). Nevertheless, there are some major differences in structure and function between the two proteins. δ, a much larger protein, contains two tandem Ogr-like domains (26). Activation of transcription by Ogr, but not that by δ, appears to require a replicating template (19, 40, 43, 60). Of special interest, therefore, are mutations that appeared to have differential effects on activation of transcription by the two phage-encoded proteins. The *rpoA50* allele, which carries two mutations (P240S and L28F), increased transcription in the presence of Ogr, while the *rpoA*G3S mutation caused a modest decrease in Ogr-mediated expression from P_F without affecting activation by δ. Although these are small effects, they suggest that we may find differences in the way these two proteins interact with RNA polymerase.

The *rpoA*P323S, *rpoA*R317H, and *rpoA*W321ter mutations caused modest differences in P2 late transcription without a concurrent reduction in expression from P_{tac}. These reductions were similar in magnitude to the effects that have been described for these *rpoA* mutations on other activators. All three mutations affect residues close to the C terminus of α; this may indicate that conformational changes in this region can have small and relatively nonspecific effects on the ability of α to respond to a variety of activators.

There is considerable genetic evidence in support of direct protein-protein interactions between the C terminus of α and certain transcription factors (see references 33, 34, and 52 for reviews). In addition to the *rpoA* alleles examined in this study, point mutations have been isolated in plasmid-borne copies of *rpoA* that impair CAP-dependent transcription from *lac*P1 and affect amino acid residues between 261 and 270 (14, 66). The properties of two C-terminal α deletion mutants (30) that have been characterized extensively in vitro are also consistent with involvement of this region in positive control. An RNA polymerase holoenzyme carrying these truncated α subunits recognized promoters such as *lacUV5 trp*, or *rplJ*, expression of which is independent of positive regulatory proteins, but failed to respond to transcription activation of the *lac*P1 promoter by cyclic AMP-CAP (32). Subsequent studies demonstrated that mutant RNA polymerases containing these truncated α subunits were impaired in activation by OmpR (31), λ CII (25), *Pseudomonas* TrpI (25), Ada (53), and OxyR (62). Stimulation of the λ p_L promoter by integration host factor was also abolished (22). In the cases of CAP at *lac*P1 (38) and OxyR at *katG* (62), loss of cooperative binding of the activator and RNA polymerase was associated with the truncated α subunits. These results provide strong evidence that the carboxyl-terminal region is involved in interaction with at least some transcriptional regulatory proteins.

Igarashi and Ishihama (32) have proposed that the discrete clusters of *rpoA* mutations affecting different activators identify amino acids involved in direct contact between each transcriptional effector and RNA polymerase. It is also possible, however, that the effects of these mutations are more indirect. They could alter the conformation of RNA polymerase in such a way that interaction with the regulatory protein is affected or block an allosteric transition within RNA polymerase in response to the activator. Furthermore, recent evidence indicates that the C-terminal third of α can participate directly in promoter recognition, making specific contacts with an upstream sequence element in the *rrnB* P1 promoter that is also present in a number of other strong promoters (49). Therefore, at least some of these α mutants may be affected not in an interaction with the regulatory protein but in a specific interaction with the DNA template that is dependent on activator binding. Strong genetic evidence of a direct protein-protein interaction would be provided by the existence of activator mutants that are allele-specific suppressors of the defects conferred by the *rpoA* mutations. Such evidence has recently been obtained in support of a direct interaction between the λ repressor and RNA polymerase, but in that case allele-specific

suppressors of a cI^{pc} mutant were found in *rpoD*, suggesting that for the λ repressor, the contact site for activation is on the σ subunit rather than an α (41).

The P2-P4 system provided the first characterized examples of *rpoA*-compensatory mutations in the transcriptional regulatory proteins, although mutations affecting FNR have recently been isolated as suppressors of *rpoA*G311R and *rpoA*G311E (44a). Previous sequence analysis of spontaneous *rpoA*L290H-compensatory Ogr mutants (6, 12, 18) revealed an identical Tyr-to-Cys change at amino acid residue 42. The four characterized *rpoA*L290H-compensatory δ mutants replace a Thr at residue 127 with Ala; this position is in the second Ogr-like domain of δ at a position equivalent to residue 44 in Ogr (26). The proximity of these mutations makes this part of the activators an attractive candidate for the activation domain. The ability of phages carrying the *rpoA*L290H-compensatory mutations still to plate on $rpoA^+$ strains as well suggested that the defect conferred by the *rpoA*L290H mutation was not due to loss of a specific contact. Replacement of Tyr-42 in Ogr with every other amino acid revealed that only substitution of Ala or Gly in addition to the naturally occurring Cys yielded Ogr that was active in an *rpoA*L290H mutant strain, while many residues allowed function in an $rpoA^+$ background (37). This is consistent with the notion that the block to activation imposed by the *rpoA*L290H mutation is due to steric hindrance of the interaction with Ogr, which can be relieved if the residue at 42 is replaced by one with a small side chain. Ogr protein with the Y42C substitution is able to interact with wild-type polymerase as well, although there is an approximately twofold reduction in CAT activity. The *rpoA*L289F-compensatory P2 *ogr*A13V mutant is also able to activate transcription with wild-type RNA polymerase, suggesting that like *rpoA*L290H, the *rpoA*L289F mutation does not disrupt a specific protein-protein contact that is restored directly by the compensatory mutation in the activator. Despite the fact that the two *rpoA* mutations affect adjacent residues, the constraints imposed on the interaction with Ogr must be different, since the *ogr*Y42C mutation can compensate for *rpoA*L290H but not *rpoA*L289F. The *ogr*A13V mutation results in a protein that appears to be able to function with both mutant and wild-type RNA polymerases; this compensatory mutation may simply result in a more effective activator. While the residues affected by the two *rpoA*-compensatory mutations are not near one another in the primary sequence, Ogr is believed to contain a Cys_4-coordinated Zn^{2+} binding domain (39) involving Cys residues at positions 4, 7, 35, and 39. It is possible, therefore, that amino acid residues 13 and 42 are in close proximity in the Ogr polypeptide. The fact that mutations in either of these residues can compensate for the *rpoA*L290H mutation is consistent with this model.

The analysis reported here provides genetic evidence consistent with a direct interaction between the P2- and P4-encoded transcriptional activators and the α subunit of RNA polymerase. We have characterized two point mutations in *rpoA* that appear to be severely impaired in interaction with Ogr, and one of the *rpoA*-compensatory *ogr* mutants exhibits some allele specificity. Isolation and analysis of additional mutants should allow us to define the specific contacts between Ogr and α that are required for activation of P2 late transcription.

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