Regulation of bacteriophage P2 late-gene expression: The *ogr* gene

(DNA sequence/cloning/overproduction/RNA polymerase/transcriptional control factor)

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ABSTRACT The *ogr* gene product of bacteriophage P2 is a positive regulatory factor required for P2 late-gene transcription. We have determined the nucleotide sequence of the *ogr* gene, which encodes a basic polypeptide of 72 amino acids. P2 growth is blocked by a host mutation, *rpoA109*, in the α subunit of DNA-dependent RNA polymerase. The *ogr52* mutation, which allows P2 to grow in an *rpoA109* strain, was shown to be a single nucleotide change, in the codon for residue 42, that changes tyrosine to cysteine. The predicted amino acid sequence of the Ogr protein does not show similarity to DNA-binding proteins that are known to affect promoter recognition, to σ factors, or to other characterized transcriptional regulatory proteins. We have inserted the *ogr* gene into a plasmid under control of the leftward promoter and operator of bacteriophage λ . Thermal induction of *ogr* gene expression in this plasmid results in overproduction of a small protein that has been shown by complementation to possess Ogr function.

The late genes of the temperate coliphage P2 are organized into four transcription units (1, 2) and are regulated in a manner quite different from that of the lambdoid phages. During normal infection, late-gene expression requires the P2 DNA replication genes A and B (2–4) and the host RNA polymerase (5). However, transcription of the P2 late genes initiates at sites that differ in nucleotide sequence from those normally recognized by *Escherichia coli* RNA polymerase (6, 7). The product of the P2 *ogr* gene is believed to be a positive regulatory factor required for late-gene expression. Mutations in the *ogr* gene (8) define a *trans*-dominant factor that overcomes the block to P2 late-gene transcription imposed by the host *rpoA109* mutation, which causes a leucine $\rightarrow$ histidine substitution in the α subunit of DNA-dependent RNA polymerase (9). Although no amber mutations in the *ogr* gene have been isolated, it is presumed to be essential by analogy to the B gene of the P2-related phage 186. The existence of P2/186 hybrid phages in which expression of the P2 late genes is under 186 B-gene control (10) demonstrates the functional equivalence of the B and *ogr* gene products. Two amber mutations define the B gene as an essential function needed for 186 gene expression (11), and they map at a site corresponding to that of the *ogr* mutations in P2. To gain insight into the mechanisms underlying positive control of P2 late-gene transcription, we have determined the nucleotide sequence of the P2 *ogr* gene, inserted the gene into an overproduction vector, and identified the *ogr* gene product.

METHODS

Phage and Bacterial Strains. Most strains used for growth of P2 and plasmids were derivatives of *E. coli* C (12). C-1a is prototrophic (13) and C-1055 is used as a nonsuppressing indicator (14). C-2111 and C-2121 (8) carry the *rpoA109*

mutation. *E. coli* K-12 strain RR1 (15) carrying the plasmid pRK248cIts (16) was used as the host for derivatives of the plasmid pRC23 (17). P2 *vir1* carries a point mutation that blocks lysogeny (18, 19). P2 *vir22* has a 5% deletion that removes the P2 attachment site and carries a 0.5% insertion at the site of this deletion (20, 21). P2 *vir22 ogr52* is a mutant derived from P2 *vir22* by B. Sauer (E. I. DuPont de Nemours, Wilmington, DE). It carries a mutation that permits growth in *E. coli rpoA109*.

Recombinant DNA Methods. Restriction endonucleases were obtained commercially and used as recommended by the suppliers. P2 DNA fragments were resolved by electrophoresis in agarose slab gels and eluted as described (7). The single-stranded ends generated by *EcoRI* cleavage of pRC23 were filled in by incubation with deoxyribonucleoside triphosphates and DNA polymerase I Klenow fragment (New England Biolabs), and the polymerase was heat-inactivated at 65°C for 10 min prior to digestion with *Cla* I. The P2 DNA fragments were mixed with restricted plasmid DNA and treated with T4 DNA ligase (Boehringer Mannheim) overnight at 4°C.

DNA Sequence Analysis. Phage DNA was prepared as described by Ljungquist *et al.* (19). Restriction fragments were labeled at their 5' ends with [γ -³²P]ATP and polynucleotide kinase or at their 3' ends with cordycepin 5'-[α -³²P]triphosphate and terminal deoxynucleotidyltransferase, as previously described (6, 19). DNA sequence was determined by the method of Maxam and Gilbert (22).

Marker Rescue. Isolates of *E. coli* C-2111 containing plasmids carrying fragments of P2 *vir22 ogr52* DNA were infected by P2 *vir1* at a multiplicity of 10, following irradiation of bacteria and phage as described (6). The lysate was assayed on C-2111 to detect any P2 *ogr52* recombinants and compared to the titer on C-1055, which measures phage of the input genotype as well.

Complementation. *E. coli* strains carrying pRK248cIts and the Ogr-overproducing plasmids pRF5 or pCV1 (see below) were grown to a density of 10⁸ cells per ml at 30°C in LB broth containing tetracycline (15 μ g/ml) and ampicillin (50 μ g/ml). Cultures were shifted to 40°C for 30 min, concentrated 10-fold in LB broth supplemented with 5 mM CaCl₂, and infected with P2 *vir1* at a multiplicity of 10. Following preincubation at 37°C for 10 min, unabsorbed phage were inactivated by treatment with antiserum (standard titer *K* = 5) for 5 min at 37°C. The infected cells were diluted 10,000-fold into prewarmed medium and titered on C-1055 for infective centers. After incubation for 90 min at 37°C, the burst was titered on C-1055.

Protein Analysis. Bacteria containing pRK248cIts and the Ogr-overproducing plasmids were grown in TPG medium (23) supplemented with 0.2% glucose, 0.5% Casamino acids, and 1 μ g of thiamine per ml, to a density of 2 $\times$ 10⁸ per ml at 30°C and then shifted to 40°C to derepress the λ P_L promoter. Samples were removed at various times after the temperature shift, centrifuged, resuspended in 0.05 volume of 0.125 M Tris Cl, pH 8/10% (vol/vol) glycerol/2% NaDodSO₄/5% (vol/vol)

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FIG. 1. (a) Restriction map of P2 *vir22* in the *ogr* region. Distances from the left end of the wild-type P2 map are indicated as percentages of genome length. The location of the *vir22* deletion/insertion is indicated by the heavy line; the box indicates the *ogr* gene. bp, Base pairs; att, attachment site. (b) Sequencing strategy for the *ogr* gene. Filled circles designate 5'-labeled ends; open circles designate 3'-labeled ends. The lengths of the lines correspond to the extent of sequence obtained from each labeled end.

2-mercaptoethanol and boiled for 2 min. Before electrophoresis, aliquots (2–10 μ l, adjusted to correspond to the density of the culture at the time of induction) were diluted with 10 μ l of sample buffer (24) and boiled for 3–4 min. Proteins were separated by electrophoresis in NaDodSO₄/15% polyacrylamide gels and stained with Coomassie blue.

RESULTS

Nucleotide Sequence of the *ogr* Gene. The *ogr* gene has been mapped to the right of the *D* gene and to the left of the P2 attachment site (8). In order to locate the gene more precisely, restriction fragments spanning this region were isolated from P2 *vir22 ogr52*, cloned in pBR322, and tested for their ability to rescue P2 *vir1* in an *rpoA109* host. Marker rescue was obtained with a plasmid, pRF1, which carries the 420-base-pair *Nru* I-*Cla* I fragment (Fig. 1). Sequence analysis of P2 *vir22* DNA revealed a short open reading frame in this region (Fig. 2), including two potential initiation codons that would result in polypeptides of 72 and 84 amino acids. Only the initiation codon for the 72-residue protein is pre-

ceded by sequence complementary to the 3' end of 16S rRNA. In addition, the upstream methionine codon is proximal to the 5' end of *ogr* mRNA (see below). The 72 amino acid polypeptide must therefore be the *ogr* gene product. Sequence analysis of *ogr52* DNA revealed an A→G transition in the codon for amino acid 42, substituting cysteine for tyrosine. This change is identical to the independently isolated *ogrI* mutation (25).

The sequence upstream of the *ogr* coding region contains a near-consensus promoter sequence for *E. coli* RNA polymerase. This promoter is functional *in vitro* (26), and nuclease S1 protection experiments indicate that this promoter corresponds to the 5' end of *ogr* mRNA made *in vivo* as well (G.E.C., unpublished observations). Just distal to the *ogr* gene is a characteristic ρ -independent terminator: a G+C-rich region of dyad symmetry followed by a run of uridines in the transcript. This sequence has also been shown to function *in vitro* (26).

Overproduction of Ogr Protein. The strategy for constructing an Ogr overproducer is shown in Fig. 3. The *Nru* I site immediately preceding the *ogr* coding region allowed convenient isolation of the *ogr* gene without its promoter sequence.

FIG. 2. DNA sequence of the *ogr* gene and flanking regions. The location of the *ogr52* point mutation is indicated. The predicted amino acid sequences for the *ogr* gene product (Ogr protein) and the C-terminal end of the *D* gene product are given. The assignment of the coding region for the *D* gene is based on extensive homology to the *D* gene of the P2-related phage 186, in which two *D* amber mutations have been located (B. Kalionis, M. Pritchard, & J. B. Egan, personal communication). P_{ogr} designates a near-consensus promoter sequence that serves as the initiation signal for *ogr* gene transcription; t_{ogr} designates a region of dyad symmetry that has been shown to function *in vitro* to terminate *ogr* gene mRNA (M. Pritchard & J. B. Egan, personal communication). The sequence complementary to the 3' end of 16S rRNA is overlined.

FIG. 3. Construction of an overproduction plasmid for the P2 Ogr protein. The pRC23 vector (17) is a derivative of pBR322 carrying the phage λ P_L promoter and a synthetic ribosome binding site. The scheme for inserting the *ogr* gene is illustrated. Ends produced by *Cla* I cleavage are not shown. Boxed region indicates the *ogr* coding sequence; lines above the sequence preceding the *ogr* gene indicate regions complementary to the 3' end of 16S rRNA.

This fragment also lacks the necessary signals for initiation of *ogr* mRNA translation. The vector pRC23 (17) provides the leftward promoter (P_L) of bacteriophage λ and a synthetic Shine-Dalgarno sequence (ribosome binding site). By filling in the overhanging end generated by *Eco*RI cleavage and ligating it with the blunt end of the *ogr* fragment generated by restriction with *Nru* I, the *ogr* initiation codon was positioned an appropriate distance from the nucleotides complementary to the 3' end of 16S rRNA. The resulting wild-type *ogr* plasmid, pRF5, directed the synthesis of a new protein of ≈ 9 kDa when the λ P_L promoter was derepressed (Fig. 4A). In order to determine whether this small polypeptide was functional Ogr protein, the construction was repeated using the *Nru* I-*Cla* I fragment carrying the *ogr52* allele. This plasmid, pCV1, was tested for complementation of P2 *vir* I in *E. coli rpoA109*. When C-2111 carrying pRK248cIts and pCV1 was infected at 30°C with P2 *vir* I, a P2 "burst" of less than 0.2 phage per infected center was observed. In contrast, when the cells were infected 30 min after a shift to 40°C, the burst size was increased, on the average, 150-fold. Eighty-five individual plaques from the progeny of two such experiments were tested for growth on C-1055 and C-2111; all were of the input (*ogr*⁺) genotype. Therefore, the increased production of P2 was due to complementation. Induction of pCV1 resulted in synthesis of a protein of the same apparent size as that observed with the wild-type pRF5 plasmid (Fig. 4B).

Although a large amount of *ogr52* gene product was seen after induction of pCV1 in C-2111 (Fig. 4B), little *ogr* gene product was seen after induction of wild-type *ogr* from pRF5 in the same strain (Fig. 4C). It seems unlikely that plasmid copy number or *ogr* expression from λ P_L are affected by the *ogr52* mutation; a more plausible explanation is that *ogr* gene product is unstable in the *rpoA109* strain. To investigate whether the low steady-state level of Ogr protein is (i) specific to the presence of the *rpoA109* mutation or (ii) possibly due to an additional UV-induced mutation in C-2111,

FIG. 4. Induction of Ogr protein synthesis. All host strains carried the λ plasmid pRK248cIts (16). Cultures were grown at 30°C and were sampled after they were shifted to 40°C to inactivate the λ cIts repressor. Extracts of total cellular proteins were subjected to NaDodSO₄/15% PAGE and stained with Coomassie brilliant blue. Lanes 0–3: samples taken at 0, 1, 2, and 3 hr after the shift to 40°C, respectively. (A) pRF5 in RR1. (B) pCV1 in C-2111. (C) pRF5 in C-2111. (D) Molecular weight standards.

we tested both plasmids in C-1a and in C-2121, a C-1a transductant carrying the *rpoA109* allele (8). These results are shown in Fig. 5. Wild-type Ogr protein can be detected in C-2121 (Fig. 5C), although in lesser amounts than in C-1a (Fig. 5B). Induction of pCV1 results in accumulation of a large amount of Ogr52 in both strains (Fig. 5D and E). The *ogr52* mutation thus appears to increase the stability of the Ogr polypeptide, even in a wild-type host.

This raises the formal possibility that the ability of P2 *ogr52* to grow in *rpoA109* strains can be accounted for by a general increase in Ogr protein stability conferred by the *ogr52* mutation. If this were true, the wild-type Ogr protein overproduced from pRF5 should complement P2 *ogr*⁺ in an *rpoA109* host, although possibly to a lesser extent than complementation by the Ogr52 protein from pCV1. *E. coli* C-2121 carrying pRK248cIts and pRF5 was infected with P2 *vir* I at 30°C or after a 30 min shift to 40°C, and the burst was compared to that obtained under the same conditions when

FIG. 5. Comparison of Ogr overproduction in wild-type and *rpoA109* strains. Each host strain carried pRK248cIts (16), and samples were treated as described for Fig. 4. Lanes 0–2: samples taken 0, 1, and 2 hr, respectively, after the shift to 40°C. (A) Molecular weight standards. (B) pRF5 (*ogr*⁺) in C-1a. (C) pRF5 in C-2121 (*rpoA109*). (D) pCV1 (*ogr52*) in C-1a. (E) pCV1 in C-2121.

C-2121 carrying pRK248cIts and pCV1 was used. No detectable burst was obtained after infection with P2 *vir1* at 30°C with either plasmid present. When the cells were shifted to 40°C for 30 min prior to infection with P2 *vir1*, C-2121 carrying pCV1 yielded a burst of 20–30 phage per infected center, comparable to what had been observed before. The burst size in C-2121 carrying pRF5, however, was less than 0.16 phage per infected center. Thus, the overproduced wild-type *ogr* gene product does not complement P2 *ogr*⁺ in an *rpoA109* strain. Since the steady-state levels of *ogr* gene product produced from the plasmid after induction are demonstrably higher than the levels present after P2 infection (G.E.C., unpublished observations), we would have expected to see some complementation if the wild-type Ogr protein were functional in the *rpoA109* strain.

DISCUSSION

We have determined the nucleotide sequence of the P2 *ogr* gene and its 5' and 3' flanking regions. The *ogr* gene encodes a polypeptide of 72 amino acids, with a predicted molecular mass of 8383 daltons. The mobility of Ogr in polyacrylamide gels gives an apparent size closer to 9000 daltons, but due to the poor resolution in this size range and the basic character of the protein, migration could be aberrant. The overall percentage of charged residues, 24%, is similar to that found for the average protein, but the charge distribution is quite different: Ogr contains a striking excess of positively charged amino acids (18% basic residues, 5.5% acidic), as compared to 13.5% basic and 11.6% acidic on the average (27). The pI predicted from the amino acid sequence is 11.2, and there is clustering of the charge. Of the NH₂-terminal 30 amino acids, 8 (27%) are basic residues.

The amino acid sequence of Ogr was compared by computer to other known transcriptional effectors and to all the proteins in the National Biomedical Research Foundation Protein Sequence Database. Although the distribution of charged residues in Ogr suggests that it may bind to DNA, the primary sequence of Ogr shows no homology to any of the known DNA binding proteins that regulate transcription. In addition, neither inspection of the amino acid sequence flanking the two glycine residues nor prediction of the secondary structure by the method of Chou and Fasman (28) reveals any trace of the characteristic α -helical domains that are thought to be involved in the DNA binding of a number of transcriptional regulatory proteins (29).

We also compared Ogr to known bacterial and phage-encoded σ subunits of RNA polymerase. Again, no homologies were found either to the *E. coli* σ factors (30, 31) or to those encoded by phages SPO1 (32, 33), T4 (34), or ϕ 29 (35). In addition, Ogr is not homologous to the product of SPO1 gene 27 (36), which is not a σ factor but is required for SPO1 late transcription.

The product of the *B* gene of the P2-related coliphage 186 is functionally homologous to the *ogr* gene product and can substitute for the *ogr* gene product in P2 late transcription (10). Like the Ogr protein the 186 B protein contains 72 amino acids, and the two proteins exhibit an overall homology of 62.5% (B. Kalionis, M. Pritchard, and J. B. Egan, personal communication). The NH₂-terminal portions of the molecules are highly conserved, with differences at only 7 residues out of the first 39.

When the normal P2 pathway for late-gene expression is blocked by mutation in one of the P2 early genes, late-gene expression can still be turned on by the satellite phage P4. This transactivation depends on the P4 δ gene (37, 38) and uses the same late promoters as the normal P2 pathway (6, 7). The product of the δ gene, as deduced from the nucleotide sequence reported by Lin (39) and corrected by C. Halling (personal communication), is a basic protein of 166 amino

acids. There is some amino acid homology between the NH₂-terminal domains of Ogr and the P4 δ protein (B. Kalionis, M. Pritchard, and J. B. Egan, personal communication). The first 40 residues of the *ogr* and δ gene products are 47% homologous, although there is only one place where more than two consecutive amino acids are identical. This region, where the two proteins share five consecutive residues, is adjacent to the site of the *ogr52* mutation. It is tempting to speculate, therefore, that this region is involved directly in interaction of these proteins with the α subunit of RNA polymerase. Defective mutations of the P4 δ gene, δ 35 and δ 6 (38, 40) lie in the carboxyl-terminal portion of the δ protein (ref. 38; C. Halling, personal communication). The P4 *org4* mutation, which allows P4 to grow with a wild-type P2 helper in *E. coli rpoA109*, also maps to the carboxyl-terminal region of the δ gene (M. Sunshine, K. Lane, and E. Six, personal communication). The NH₂-terminal domain of Ogr is also homologous to a region in the carboxyl-terminal half of the δ protein. This homology includes a stretch of 5 out of 7 identical amino acids that contains the sites of the δ 6 and δ 35 mutations. When the sequences of the *ogr* and δ gene products are aligned at these homologous residues, the P4 *org4* mutation lies in a region corresponding to the location of the P2 *ogr52* mutation (C. Halling, personal communication). This reinforces the idea that these proteins interact in a specific way with the host RNA polymerase α subunit. The P2 *ogr*, 186 B, and P4 δ gene products thus appear to represent a class of effectors that differ from other known positive transcriptional regulators.

How might the expression of Ogr itself be regulated? If the Ogr protein were the sole positive effector of normal P2 late-gene expression, we would expect it to be under control of the P2 early genes. Such regulation has been proposed, based upon the inability of P2 *A⁻ogr1* to complement P2 *vir1* in an *rpoA109* host (41). However, transcription of *ogr* initiates *in vivo* at a site that is similar to a normal *E. coli* promoter (G.E.C., unpublished observation) and that is recognized by *E. coli* RNA polymerase *in vitro*. The *ogr* promoter does not contain the P2 operator sequence (19); hence, it is not under P2 repressor control. If the *ogr* gene can be transcribed constitutively, as is suggested by these observations, an additional factor must either regulate *ogr* expression posttranscriptionally or participate directly in control of late-gene transcription.

In summary, we have deduced the primary structure of a positive transcriptional regulatory protein that is required for P2 late-gene expression. The Ogr protein does not show homology to other phage or bacterial transcriptional effectors, except for those encoded by the P2-related phage 186 and satellite phage P4. Elucidation of the mechanism of Ogr action should thus provide new insights into the ways in which promoter specificity of *E. coli* RNA polymerase can be modulated.

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