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Bacteriophage P22 Gene *23* Product Acts Preferentially in *cis*

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The expression of the P22 late operon was measured while the activator of the late operon, the product of gene *23*, was provided in *cis* or in *trans*. It was found that expression of the late operon, assayed from a late-gene–*lacZ* gene fusion, was reduced by more than twofold when the only functional copy of gene *23* was present in *trans*.

Salmonella typhimurium bacteriophage P22 is a temperate phage in the lambdoid family (for review, see reference 14). P22 genes can be divided into two broad groups based on the temporal order of gene expression upon infection. The early genes are those involved in the decision between lysis and lysogeny, replication, integration into the bacterial chromosome, and the control of late-gene expression. The late genes code for proteins necessary for phage morphogenesis during the lytic cycle, including all of the structural proteins of the phage capsid. There is good evidence that the late genes are expressed as a single transcriptional unit under the positive control of early gene *23*. In phages carrying a *23* mutation, expression of all the late genes is deficient (2, 17). In both lambda and P22, there is a promoter located at the upstream end of the late genes that makes a short transcript in vitro (12). In lambda, this transcript can be extended in vitro in the presence of the purified *Q* protein (8). Both gene *Q* of lambda and gene *23* of P22 have been sequenced (5; M. Kroger and G. Hobom, unpublished data), and the amino acid sequences, inferred from the DNA sequence, are 95% homologous. Echols et al. (7) and Burt and Brammer (3) have shown that in vivo the *Q* protein acts most efficiently in *cis*. The subject of this paper is the test of the *cis* specificity of the P22 gene *23* protein in phage carrying a gene fusion to the most promoter-distal late gene, gene *9*.

A list of phage and bacterial strains used appears in Table 1. The fusions used in this study were constructed by ligating a fragment containing the *Escherichia coli lac* operon in place of the 2.6-kilobase *Bam*HI fragment of P22 2063 (Fig. 1). The *lac* regions used were the *Bam*HI-*Bgl*II fragments of pMC903, containing the *lac* operon without its promoter, and pMC931, which lacks the *lacZ* ribosome-binding site and the first 22 base pairs of the *lacZ* gene (4). P22 2063 contains deletion 155 (about 5 kilobases), which allowed P22 to accept the larger *lac* fragments in place of its own 2.6-kilobase internal *Bam*HI fragment while retaining a genome length that could be packaged in a head with terminal repetition. The deletion of the P22 *Bam*HI fragment inactivates gene *9*, the P22 tail gene; phage lacking tails can be converted into infectious phage in vitro by the addition of purified tail protein (9, 18). The ligation mixtures were used to transfect *E. coli* DB5142 *recBC sbcB*, and transfected cells were plated on a lawn of *S. typhimurium* DB4566 *hsdSA hsdL* in the presence of 10^{11} P22 tail equivalents and the chromogenic β-galactosidase substrate 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside. In the construction producing the operon fusion, Lac^+ plaques were purified, and the phage isolated were used to prepare DNA. Isolates containing the *lac* operon in the orientation appropriate to produce a late-operon fusion were identified by restriction analysis (data not shown). One such isolate was labeled P22 2065. In the construction producing the *9-lacZ* protein fusion, it was predicted that an out-of-frame protein fusion would be produced (see references 4 and 13 for the predicted sequence of this fusion). Plaques produced by this transfection were transferred to nitrocellulose and probed with ^{32}P-labeled pMC931 (6). Plaques that hybridized to the *lac* probe were detected at a frequency of about 1%, and approximately half of these plaques were Lac^-. lac^+ and *lac* phage were purified, and DNA was isolated and analyzed by restriction digestion (data not shown). The lac^+ phage carried the insert in the orientation in which the *lac* genes would be read in the direction opposite to that of the late operon. Presumably *lacZ* is fused to a nonessential gene in the *a1* region that is interrupted by the *Bam*HI site near *att*. Lac^- phage contain the *lac* insert in the orientation that creates the *9-lac* fusion. One of these phage was subsequently passaged through *S. typhimurium* DB7136 in the presence of ICR-191 (15), and a Lac^+ phage was isolated (P22 2068). The expression of β-galactosidase from the late-operon fusion and the late-gene fusion was qualitatively similar with respect to timing of expression and dependence on gene *23* (data not shown).

TABLE 1. Phage and bacterial strains

Phage or bacterium	Genotype	Source or reference
P22 10	*C1-7 h21*	
P22 2063	*sieA44* Δ155(*int*)	Youderian and Susskind (17)
P22 2065	*sieA44* Δ155(*int*) *9-lac*$^+$ (operon fusion)	This work
P22 2068	*sieA44* Δ155(*int*) *9-lac*$^+$I1 (protein fusion)	This work
P22 2078	*23am*H79, *am*H335 *c1-7*	Laboratory collection
P22 2082	*sieA44* Δ155(*int*) *9-lac*$^+$I1 *c1-7*	This work
P22 2083	*23am*H79, *am*H335 *sieA44* Δ155(*int*) *9-lac*$^+$I1 *c1-7*	This work
S. typhimurium DB4566	*hsdSA hsdL proC90* Δ *his-2253 dhuA1 purF145 galE503*	Laboratory collection
E. coli DB5142	*recB21 recC22 sbcB15 leu-6 ara-14 his-4 trpBC27 thr-1 lacY1 mtl-1 xyl-5 galK2 proA2 argE3 str-31*	Laboratory collection
S. typhimurium DB7136	*leuA414*(Am) *hisC527*(Am)	Winston et al. (16)

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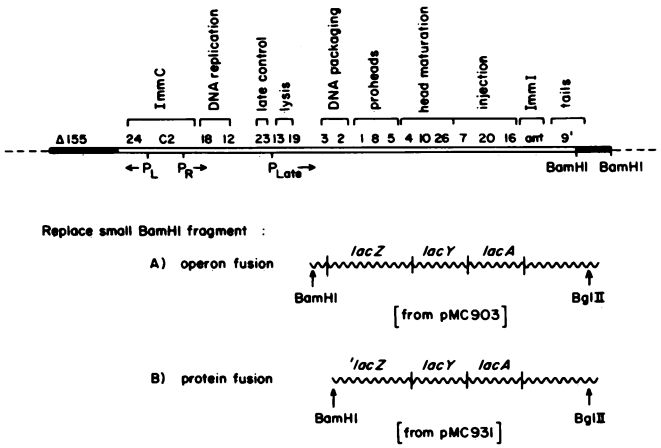

FIG. 1. Construction of P22 2065 and P22 2068. The upper portion of the figure shows the prophage map of P22 2063. ▬, Regions deleted in the fusion phage. The fusion phage were constructed by replacing the small P22 *Bam*HI fragment with a fragment containing *lac* from the appropriate plasmid (see text). P_L, Major leftward early promoter; P_R, major rightward early promoter; P_{Late}, late promoter.

Because of the possibility of posttranscriptional control of late-gene expression, the gene fusion was chosen for further study.

The dependence of expression of the fusion on gene *23* was tested in single infections of fusion phage carrying a wild-type *23* and a *23* double-amber allele (Fig. 2). As expected, expression of the fusion was dependent on a functional gene *23*. To test whether the *9-lac*$^+$ protein could be activated in *trans*, cells were mixedly infected with *9-lac*$^+$ and Lac$^-$ phage with and without the *23* double-amber allele (Fig. 2). The expression of the p9–β-galactosidase hybrid protein is significantly reduced when p23 is provided in *trans*, indicating that p23 acts preferentially in *cis*. The effect ranges from fivefold at 40 min to a little more than twofold at 60 min. The reduction in magnitude of the effect with increasing time seems to be due to the fact that β-galactosidase expression does not shut off as soon when it is in *cis* to a *23* allele as when it is in *cis* to a *23*$^+$ allele. In addition, the fact that this experiment is conducted under recombination-proficient conditions (P22 requires a recombination function to circularize after infection) (1) makes it probable that the measurement of expression of the hybrid protein when it is in *trans* to a functional *23* gene is an overestimate. The burst from this experiment was found to contain approximately 10% *23*$^+$ *9-lac* recombinants. Thus the preference of p23 for acting in *cis* is probably more pronounced than is indicated in this experiment.

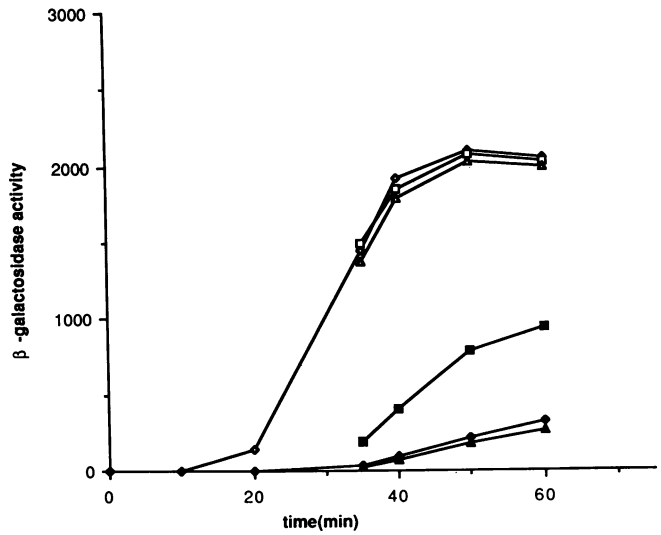

FIG. 2. Effect of gene *23* on expression of the *9-lac* fusion. *S. typhimurium* DB7136 cells suspended in buffer saline were infected with each phage at a multiplicity of 5. It was observed that the fusion phages, which are *9* and depend on tail protein that is added exogenously, adsorb poorly in the presence of wild-type phage. Accordingly, the *9* phage was allowed to adsorb for 20 min at room temperature in the absence of the coinfecting *9*$^+$ phage. The *9*$^+$ phage was then added and allowed to adsorb for an additional 5 min, and M9CAA was added so that the final cell density was 2×10^8/ml. The infected cells were incubated at 37°C with aeration, and portions were removed for β-galactosidase assay, as described by Miller (11), at the times indicated. ◇, P22 2082; □, P22 10 and P22 2082; △, P22 2078 and P22 2082; ◆, P22 2083; ■, P22 10 and P22 2083; ▲, P22 2078 and P22 2083.

There have been two studies done with phage lambda that tested the *cis* specificity of its analogous late-gene activator, gene *Q* (3, 7). Echols et al. (7) measured the expression of the *R* gene (an endolysin), while Burt and Brammer (3) constructed an operon fusion downstream from the *J* gene at the promoter-distal end of the operon. In the first study, expression was found to be about 1/10 of that of wild type when *Q* was provided in *trans*, while in the second study, expression was much less than that. These experiments, unlike the one described above, were done under recombination-deficient conditions. The considerably greater *trans* activity of p23 found in this study is unlikely to be due entirely to recombinants that put a functional *23* gene *cis* to the fusion. This activity may reflect a difference in the action of gene *23* relative to the lambda gene *Q* in either the concentration of the late-gene activator or in its affinity to its site of action (10).

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