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Fusions of Bacteriophage P22 Late Genes to the *Escherichia coli lacZ* Gene

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The late genes of bacteriophage P22 were fused to *lacZ* to study their differential expression from the late operon transcript. No instances of posttranscriptional regulation were uncovered, thus supporting the model that the late genes are expressed, by and large, in fixed ratios based on their translational efficiency and message stability.

The late genes of the *Salmonella typhimurium* bacteriophage P22 are arranged as a block on the P22 genome, with the gene coding for the tail protein separated from the other late genes by the immunity I region (for a review, see reference 18). As in other lambdoid phages, the late genes are activated by an early gene, such as gene *23* in the case of P22, and are expressed as a unit from a single promoter (2, 21, 25). The various late genes are expressed at widely differing rates which reflect the levels necessary for their functions and their abundance in procapsid and capsid structures (2, 9, 13). Studies of late-gene expression in phages P22 and lambda have left unclear the control mechanisms involved in this differential expression. One explanation is that the level of each late-gene product is determined by the intrinsic rate of translation of the corresponding message, i.e., the proteins are expressed in fixed ratios. Another possibility is that the level of one or more late proteins is regulated in such a way as to optimize the efficiency of phage assembly. This study was undertaken to probe for examples of late-gene regulation.

The expression of the P22 and lambda late genes has been examined in some detail. In lambda, the large differences in the levels of protein expressed from the various late genes cannot be accounted for by either the amounts of hybridizing RNA or the functional half-lives of messages coding for each gene (14, 15). In P22 as well, the functional stability of the late-gene messages cannot account fully for their different levels (4). Accordingly, it is hypothesized that at least some of the differences in levels among the late proteins must be accounted for by the efficiency of translation of these proteins as mediated by the strength of their ribosome-binding sites, by the secondary structure of the mRNA in the region of the ribosome-binding sites, and possibly by soluble factors coded for by either the phage or the host.

Late-gene transcription in P22 has at least one feature that is not paralleled in lambda. The product of gene *8*, the scaffolding gene, is regulated in response to phage head assembly (5, 8, 9). The scaffolding protein is present in the prohead but is released and recycled when proheads are converted to heads. Mutations that block this conversion lead to an increase in the expression of gene *8*. The regulation of gene *8* can be explained by the observation that the functional stability of the gene *8* message increases in vivo when the conversion of proheads to heads is blocked (4). In an in vitro transcription-translation system, the expression of gene *8* is posttranscriptionally repressed by the addition of purified gene *8* protein (24).

The object of this study was to examine P22 late-gene expression for additional cases of regulation. The use of gene fusions was chosen for two primary reasons. (i) Expression can be assayed with ease, and (ii) one can assay the expression of a protein in its absence, providing the potential for uncovering cases of autogenous regulation that respond only to that absence. No new examples of late-gene regulation were discovered, lending support to the idea that the late genes are, by and large, expressed in fixed ratios determined by their rates of translation. However, the large collection of fusions obtained in this study provides information on the fidelity with which β-galactosidase activity expressed from gene fusions reflects the level of expression of the native target gene. In addition, the method by which the fusions were constructed provides a well-defined series of deletion intervals, allowing the alignment of the physical and genetic maps of P22 to be refined.

Construction of late-gene fusions. The fusions described here were constructed by cloning regions of the late operon on a plasmid containing the *lacZ* gene and making deletions with *Bal*31 that fused the various late genes to *lacZ* (16). In vitro constructions were performed by using standard recombinant DNA techniques (7, 11). Bacterial and bacteriophage strains are given in Tables 1 and 2. Plasmids pRB252 and pRB253, derivatives of pRB248, were linearized for *Bal*31 digestion with *Bgl*II; pRB251 was linearized with *Sal*I (Fig. 1). Plasmids containing in-frame fusions (as indicated by a weak Lac$^+$ phenotype of cells bearing them on plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) were mapped by restriction enzyme analysis (data not shown). The fusion plasmids were then transformed into *S. typhimurium* DB1185, and the fusion was crossed onto the resident P22 phage by making a UV-induced lysate and using it to transduce DB7136 to ampicillin resistance. Lysogens obtained in this manner contain the fusion on a defective prophage. The position of the fusion junction was mapped genetically by testing for the presence of the wild-type allele of various amber mutations by marker rescue (6). In cases in which a fusion junction was flanked by amber mutations in a single gene, the fusion was assigned to that gene. Fusion junctions that were flanked by amber mutations in different genes were assigned to a gene on the basis of complementation tests for the most promoter-distal gene product that

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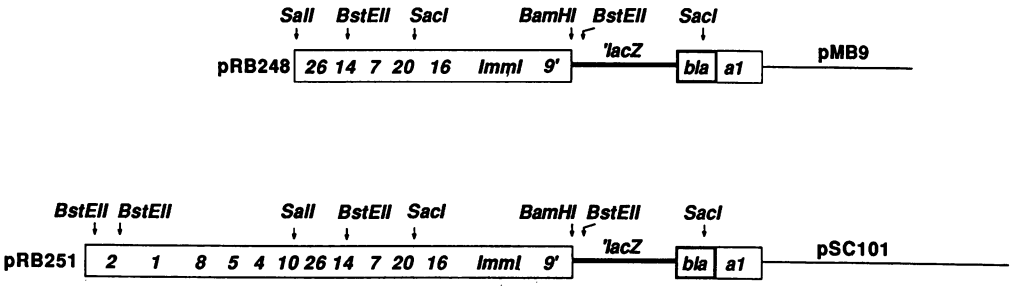


FIG. 1. Structure of pRB248 and pRB251. The boxed portions of the plasmids correspond to DNA fragments from P22, with the numbers indicating the positions of the late genes. The fragment containing the *bla* gene (ampicillin resistance) and a portion of the P22 *a1* region originated as a *Bam*HI fragment of P22Ap2, a phage with a Tn*1* insertion in the *a1* region. The *Bam*HI site on the counterclockwise side of the *a1* region was destroyed by localized mutagenesis by the method of Shortle and Nathans (17). The *Bam*HI-*Bgl*II fragment containing the *lac* region came from pMC931 (3). To create convenient sites for linearizing pRB248 within the late genes, a *Bgl*II linker was inserted in the *Bst*EII site in the region of gene *14*, creating pRB252, and in the *Sac*I site in gene *20*, creating pRB253 (20).

could be provided in *trans* to a superinfecting phage (data not shown) (1). A summary of the physical and genetic mapping of the deletions associated with the fusions constructed is presented in Fig. 2.

Expression of β-galactosidase activity from fusions. To allow the assay of these late-gene fusions in a well-synchronized lytic cycle, the *c2-ts30* allele was crossed onto the prophage in a subset of the fusion lysogens. β-Galactosidase was then assayed after temperature induction of lysogens of these phage (Fig. 3). The activity of different fusions to the same gene could vary up to threefold, indicating differences in either the specific activity or the stability of the various hybrid proteins. In general, the levels of β-galactosidase activity are in rough agreement with the estimated levels of the corresponding gene products (2, 9, 13).

Because the genetic content of each of the fusion phage is different, the following experiments were carried out to measure the expression of the fusions under conditions in which all gene products could be provided. Simultaneous induction of a fusion prophage and superinfection with wild-type (*c1-7*) or amber-mutant phage (carrying a c1-7 or *c2-5* allele) was performed, with subsequent assay for β-galactosidase activity. Since the fusion prophage is defective for all genes located downstream from the fusion junction, infections in which the superinfecting phage carries an amber mutation in one of the downstream genes lack that gene product. Some of the superinfecting phage carried the *h* plaque morphology marker (in gene *13*), which confers an early-lysis phenotype. Infections with these phage led to a twofold reduction in the level of β-galactosidase activity that accumulated before lysis, compared with h^+ phage. No other differences in the expression of a fusion protein in

TABLE 1. Bacterial strains

Strain	Genotype	Source or reference
Escherichia coli		
DB6329 (HB101)	*hsdR hsdM recA13 supE44 lacZ4 leuB6 proA2 thi1* Sm^r	7
DB1085	*hsdR hsdM*$^+$ *met supE44 supF* $B1^-$ *proC*::Tn*5* ΔU169(*lac*)	M. Lichten
DB1185	*hsdR hsdM*$^+$ *met supE44 supF* $B1^-$ *proC*::Tn*5* ΔU169(*lac*)/F′ *lac pro* (P22 *sieA44*)	DB1184 × DB1085
Salmonella typhimurium		
DB6282	*leuD798* (*ara*) *fol-101 supQ274*(*pro*) *trpA50 his-1099 supE20*/F′ *lac pro*	Laboratory collection
DB1184	*leuD798* (*ara*) *fol-101 supQ274*(*pro*) *trpA50 his-1099 supE20*/F′ *lac pro* (P22 *sieA44*)	Lysogenized DB6282 with P22 *sieA44*
DB7136	*leuA414*(Am) *hisC527*(Am)	23

TABLE 2. Phage alleles

Gene	Allele(s)	Source or reference
c1	7	10
c2	5	10
1	*am*N10, *am*N18, *am*N32, *am*N101, *am*H201, *am*H1023, *am*H1034, *am*H1081, *am*H1097, *am*H1107, *am*H1178, *am*H1221, *am*H1230, *am*H1252	Laboratory collection
8	*am*N123, *am*H202, *am*H304, *am*H1060, *am*H1115, *am*H1136, *am*H1281, *am*H1284, *am*H1348	Laboratory collection
5	*am*N3, *am*N8, *am*H312, *am*H1037, *am*H1055, *am*H1075, *am*H1133, *am*H1169, *am*H1203, *am*H1318, *am*H1364	Laboratory collection
10	*am*N33, *am*N107, *am*H70, *am*H310, *am*H1021, *am*H1027, *am*H1029, *am*H1071, *am*H1076, *am*H1077, *am*H1079, *am*H1305	Laboratory collection
26	*am*H85, *am*H204, *am*H326, *am*H1048, *am*H1054, *am*H1104, *am*H1214, *am*H1266, *am*H1300	Laboratory collection
7	*am*H1205, *am*H1375, *am*H1363	Laboratory collection
20	*am*N106, *am*N126, *am*H318, *am*H319, *am*H1025, *am*H1030, *am*H1031, *am*H1032, *am*H1051, *am*H1059, *am*H1096	Laboratory collection
16	*am*N121, *am*H60, *am*H210, *am*H1016, *am*H1067, *am*H1087, *am*H1125, *am*H1153, *am*H1192, *am*H1215, *am*H1271, *am*H1289, *am*H1374	Laboratory collection
sieA	44	19
a1 region	Ap2 (Tn*1* insertion)	22

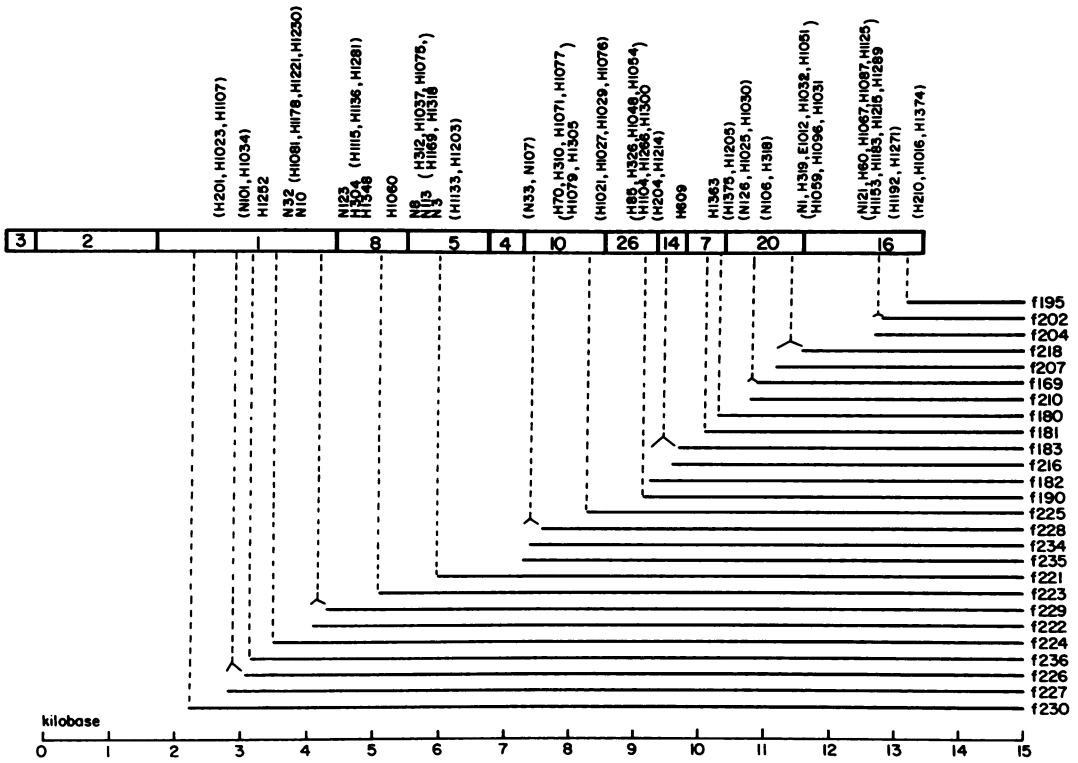
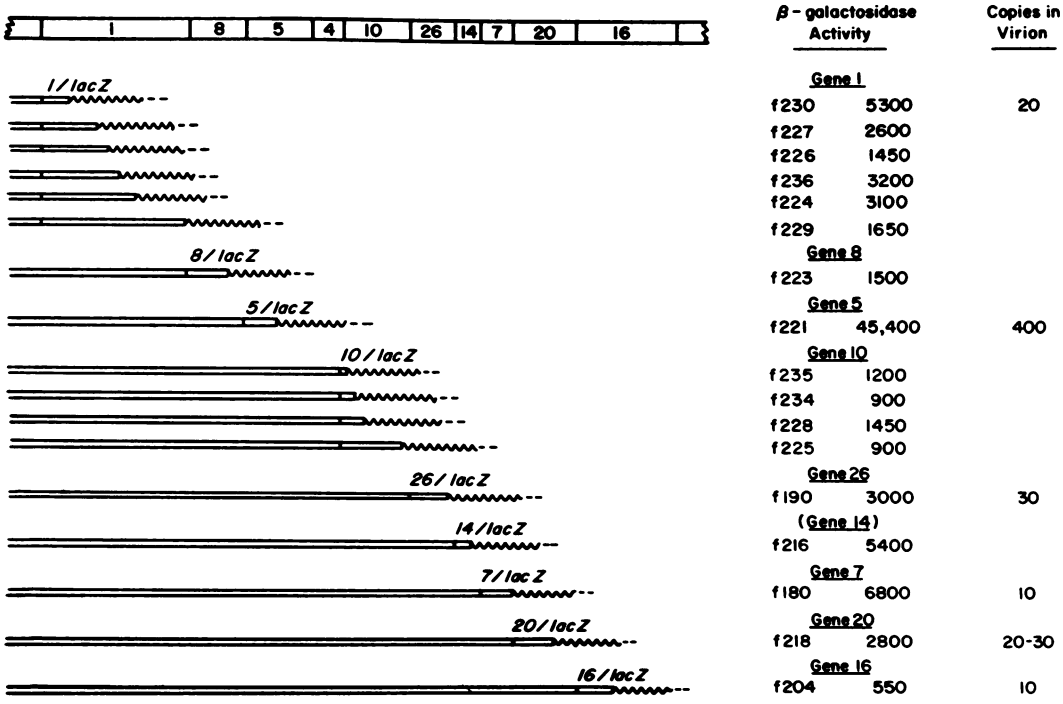


FIG. 2. Physical and genetic map of fusions to the late genes. Distances are drawn to physical scale, with each line representing the extent of deleted material in a fusion. The physical extent of each deletion was measured by restriction mapping of the corresponding plasmid. The scale at the bottom of the figure starts at the *Hin*dIII site approximately 0.8 kilobases on the late promoter-proximal side of *pac*. At the top is the genetic map of a large portion of the late operon. The size of each late gene is calculated from the apparent molecular weights given by Youderian and Susskind (25). The allele numbers given above the genetic map indicate the results of marker rescue from the fusion lysogens; alleles that are ordered within a single deletion interval are as previously determined (6, 9; F. Winston, unpublished results). Alleles in parentheses are not ordered with respect to one another and could represent one or more sites within a gene.



	β-galactosidase Activity	Copies in Virion
Gene 1		
f230	5300	20
f227	2600	
f226	1450	
f236	3200	
f224	3100	
f229	1650	
Gene 8		
f223	1500	
Gene 5		
f221	45,400	400
Gene 10		
f235	1200	
f234	900	
f228	1450	
f225	900	
Gene 26		
f190	3000	30
(Gene 14)		
f216	5400	
Gene 7		
f180	6800	10
Gene 20		
f218	2800	20-30
Gene 16		
f204	550	10

FIG. 3. Maximum accumulation of β-galactosidase activity in heat-induced lysates of fusion lysogens. The left-hand portion of the figure is a representation of the genetic material remaining upstream of each fusion junction. The right-hand portion includes the maximum level of β-galactosidase activity, assayed by the method of Miller (12), in samples taken 70 to 80 min after heat induction of the fusion lysogen. The rightmost column is the number of monomer protein subunits present in the capsid, as previously determined (2, 13).

response to a defect in a late-gene product were observed (data not shown). Although it is possible that these experiments did not provide the signal for some hypothetical regulatory network, no evidence for any such network was observed.

These experiments did not reveal any change in the expression of the fusion to gene *8* in response to a mutation in genes *1*, *2*, or *3* (data not shown). This difference from the results of Casjens, King, and co-workers (5, 8, 9) could be rationalized in several ways. These workers measured the level of ^{35}S-labeled gene *8* protein on sodium dodecyl sulfate-polyacrylamide gels pulse-labeled at late times in lysis-defective phage infections. The measure of gene *8* expression used here is the maximum accumulation of β-galactosidase activity produced from the gene *8* fusion before lysis. It is possible that a change in the rate of synthesis that happened late in infection would be difficult to detect by the protocol used here, whereas it would be amplified by assaying after the normal lysis time. In addition, it is conceivable that the hybrid protein produced by the fusion to gene *8* could have some effect on the proposed regulation, as has been hypothesized for long amber fragments of gene *8* (9).

The results of the experiments described here support the model that the late genes are expressed in fixed ratios determined by their efficiencies of translation. The behavior of these late-gene fusions indicates that this efficiency is defined by sequences 5′ to the fusion joint, most probably by effects in the vicinity of the ribosome-binding site. Possible mediators for these effects include the inherent strength of the ribosome-binding site, secondary structure of this region, and hypothetical soluble factors. These fusions provide a tool for the genetic or biochemical study of the efficiency of ribosome loading in this system.

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