Genetic and DNA Mapping of the Late Regulation and Lysis Genes of *Salmonella* Bacteriophage P22 and Coliphage λ

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Genetic and DNA heteroduplex analyses of λ imm22 hybrid phages were used to compare the *Salmonella* bacteriophage P22 and coliphage λ genes which control late gene regulation and lysis. Homologous DNA sequences were correlated with P22 gene 23 and λ gene Q (late gene regulation) and with P22 gene 13 and λ gene S (lysis control). Nonhomologous DNA sequences were correlated with P22 gene 19 and λ gene R (lysozyme and endolysin) and with the region encoding the P22 *a* and λ 6S transcripts.

The genetic organization of the related temperate bacteriophage P22 and coliphage λ is very similar (Fig. 1), especially for the early genes within and near the immunity region (8). Although analogous in function, the products of these genes nevertheless differ in specificity. For example, the early regulatory functions of P22 (gene 24) and λ (gene N) are nonhomologous in DNA sequence (3, 14), but the proteins they encode prevent transcription termination for their respective genomes (1, 15). Similarly, the P22 *immC* (*C*₂, *cro*₂₂, and *C*₁) and λ immunity (*cI*, *cro* _{λ} , and *cII*) regions, as well as their replication regions (*ori*₂₂-18-12 and *ori* _{λ} -O-P), share no major DNA homology nor do the analogous gene products complement one another (3, 11, 13-15, 27), yet *imm λ rep22* and *imm22rep λ* hybrid phages are viable (14).

We report the comparison of the nearby late genes 23, 13, and 19 of P22 and genes Q, S, and R of λ (Fig. 1) by using genetic and DNA heteroduplex analyses of λ imm22 hybrid phages. The 23 protein in P22 (4, 17) and the Q protein in λ (12) appear to control the expression of late genes by preventing transcription termination (9, 26). Our experiments show that these regulators, unlike the early regulators 24 and N, are functionally equivalent and share extensive DNA homology. In their presence small RNAs (the 6S of λ and *a* of P22) transcribed from the intergenic region between genes Q and S (16) and genes 23 and 13 (21) are believed to continue into the late genes. These RNAs differ in sequence (21), and we have correlated them with a region of DNA nonhomology between P22 and λ . By disrupting the cell membrane, the λ S protein (19) and by analogy the P22 13 protein (2) control the release of the lytic enzymes to the cell wall. Our genetic and DNA mapping has shown that genes S and 13 share a region of homologous DNA sequence. Recent cloning and characterization of the P22 lysis genes confirm this observation: the P22 13 protein shares 89% amino acid homology with the λ S protein (20). The lytic enzymes themselves have also been shown to differ (18, 20, 25), and we mapped P22 gene 19 and λ gene R to an interval of DNA nonhomology between the phage genomes.

The functional equivalence of P22 gene 23 and λ gene Q regulatory proteins was first suggested by a complementation test. Phage λ hy20(*imm22* Q λ ⁺ Aam32) with λ DNA for

the Q-S-R region was the Q⁺ donor, and λ hy18 (*imm22* 23amH335-*a*) with P22 DNA for the analogous 23-13-19 region was the 23⁻ recipient. Separate infections of *Escherichia coli* su⁻ (WD5021 *strA594*) (5) produced burst sizes of 0.1 PFU per cell (λ hy18) and 0.002 PFU per cell (λ hy20), while coinfection with both phages produced a burst size of 37 PFU per cell. This complementation suggests that the λ Q protein can recognize a site within the P22 23-13 region to stimulate late gene (A protein) expression and permit phage growth. A reciprocal complementation test was not possible because of the leakiness of λ Q amber (even double-amber) mutants.

Homologous DNA sequences between P22 gene 23 and λ gene Q were then deduced from prophage deletion mapping and phage crosses. Two different P22 23 amber mutations were mapped within adjacent deletion intervals of a λ prophage (Fig. 2). The 23 amH316 mutation lies within the proximal portion of the gene with the λ Qam73 and Qam501 mutation; the 23amH335 mutation lies within the distal portion of the gene with the λ Qam117 and Qam57 mutations. Phage crosses showed that the 23amH316 mutation recombines with Qam73 but not with Qam501 (Table 1, experiment A), suggesting that 23amH316 maps very near to or at the same site as the λ Qam501 mutation. The 23amH335 mutation was found to recombine with the Qam57 mutation (Table 1, experiment B).

Since P22 gene 13 and λ gene S encode functions which control the time of cell lysis, their functional equivalence could not be tested by complementation. A phage cross, however, did reveal a region of DNA homology (Table 1, experiment C). Amber-positive progeny was observed at a

FIG. 1. Partial genetic map of phages P22 and λ . The λ genes and 6S transcript are labeled on the inside of the arc, while the analogous P22 genes and *a* transcript are labeled on the outside of the arc.

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Strain	Extent of Prophage Deletion							Rescue of <i>am</i> ⁺ Allele By 23 <i>am</i> H316	Rescue of <i>am</i> ⁺ Allele By 23 <i>am</i> H335
	Pam3	Pam80	Qam73	Qam501	Qam117	Qam57	Sam7		
MS508	■	■	■	■	■	■	■	+	+
MS1147	■	■	■	■	■	■	■	-	+
MS1118	■	■	■	■	■	■	■	-	-

FIG. 2. Prophage deletion map of P22 23 amber alleles within λ gene Q. Hybrid phages λ hy13(*imm*22 23*am*H316) and λ hy16(*imm*22 23*am*H335), constructed by the method of Hilliker and Botstein (14), were spotted at 10^6 , 10^7 , and 10^8 PFU/ml on lawns of *E. coli su*⁻ strains carrying deletions of the λ prophage (solid bar indicates deletion) (22). E. Signer (Massachusetts Institute of Technology, Cambridge, Mass.) supplied λ mutants in genes P and Q which were used as controls, and all media used here have been described previously (14). After overnight incubation at 37°C, the spots were scored positive (+) when phage growth occurred (indicating that the 23*am* allele was replaced by functional allele from the prophage) and negative (-) when phage growth did not occur (indicating that the 23*am* allele was not replaced by a functional allele from the prophage).

frequency almost 400-fold above the reversion rate in a cross between λ c1857*Sam*7 and λ hy30 (which carries the P22 13*am*H101 mutation). In contrast, no evidence of recombination was obtained (Table 1, experiment D) in a cross between mutations 19*am*H736 (in the P22 lysozyme gene) and *Ram*5 (in the λ endolysin gene).

DNA heteroduplex mapping of three *imm*22 hybrid phages was carried out to correlate P22 genes 23, 13, and 19 and λ genes Q, S, and R with the predicted intervals of DNA homology and nonhomology. These hybrids were constructed to carry the P22 *immC* locus but to differ in the

TABLE 1. Phage crosses between λ and *imm*22 hybrid phages containing P22 DNA substitutions within the λ Q-S-R region^a

Expt	Phage(s)	Amber-positive progeny/total progeny	
		Without UV	With UV
A	λ hy21(<i>imm</i> λ c160 23 <i>am</i> H316)	1.3×10^{-6}	3.3×10^{-6}
	λ c1857 <i>Qam</i> 501	1.2×10^{-6}	2.2×10^{-6}
	λ cIII67 <i>Qam</i> 73	7.8×10^{-6}	
	λ hy21 $\times$ λ <i>Qam</i> 501		3.0×10^{-7}
	λ hy21 $\times$ λ <i>Qam</i> 73	6.7×10^{-4}	
B	λ hy22(<i>imm</i> λ c160 23 <i>am</i> H335)	1.0×10^{-6}	
	λ c1857 <i>Qam</i> 57	5.0×10^{-6}	
	λ hy22 $\times$ λ <i>Qam</i> 57	9.0×10^{-4}	
C	λ hy30(<i>imm</i> 22 <i>C</i> ₂ ⁵ 13 <i>am</i> H101)	2.3×10^{-8}	
	λ c1857 <i>Sam</i> 7	1.9×10^{-6}	
	λ hy30 $\times$ λ <i>Sam</i> 7	7.5×10^{-4}	
D	λ hy36(<i>imm</i> 22 <i>C</i> ₂ ⁵ 19 <i>am</i> H736)	1.5×10^{-5}	
	λ c1857 <i>Ram</i> 5	1.1×10^{-5}	
	λ hy36 $\times$ λ <i>Ram</i> 5	1.6×10^{-5}	

^a Experiments: A, Crosses mapping the P22 23*am*H316 allele within the proximal portion of the λ gene Q and very near to or at the site of *Qam*501; B, Cross mapping the P22 23*am*H335 allele in the distal portion of the λ gene Q; C, Cross revealing homologous DNA sequences between P22 gene 13 and the analogous λ gene S; D, Cross demonstrating no recombination (above the level of reversion) between P22 19*am*H736 and λ *Ram*5 alleles. Hybrid phages were constructed by the method of Hilliker and Botstein (14), and all media were previously described (13, 14). Standard phage crosses (14) at a multiplicity of infection of 5 for each parental phage were carried out in permissive *E. coli* hosts (Q1, a *su*II⁺ C600 derivative [23] for experiments A and B, and QD5003, a *su*III⁺ strain [10] for experiments C and D) and assayed for amber-positive progeny on *E. coli* WD5021 *su*⁻ *str*A594 (5) and total progeny on the permissive host. The 13*am*H101 allele requires *su*II⁺ for suppression, and the *Sam*7 allele requires *su*III⁺ for suppression. In one cross of experiment A, irradiation (100 ergs per mm² at 254 nm) was used to stimulate recombination.

FIG. 3. Heteroduplexes of *imm*22 hybrid phages carrying substitutions of P22 DNA in the λ Q-S-R region. (A) Class I, λ hy36(*imm*22 19*am*H736)/ λ b2; (B) class II, λ hy37(*imm*22 *R*₁⁺/b2; (C) class III, λ hy37(*imm*22 *R*₁⁺)/ λ b519b515*nin*5; (D) class IV, λ hy17(*imm*22 23*am*H335-6S)/ λ b2. Bar, 1 μ m. The arrows indicate the deletion loops for the physical markers *b*2, *b*519, and *b*515. Hybrid phages were constructed by the method of Hilliker and Botstein (14), and lysates were purified through two CsCl equilibrium density gradients and dialyzed against 0.01 M Tris (pH 7.4)–0.01 M MgSO₄. Heteroduplex molecules prepared by the micromethod of Davis et al. (7) were photographed by using a Philips 201 transmission electron microscope.

FIG. 4. Alignment of λ imm22 heteroduplex molecules with a λ physical map. Class I, λ hy36(imm22 19amH736)/ λ b2; class II, λ hy37(imm22 R_{λ}^{+})/ λ b2; class III, λ hy37(imm22 R_{λ}^{+})/b519b515nin5; class IV, λ hy17(imm22 23amH335-6S)/ λ b2. Ten molecules of each class were measured by using a Numonics electronic graphics calculator, and the length in centimeters of each homologous and nonhomologous segment was converted to percent λ . This normalization required that the total length of each class of molecules be calculated by using the longer single strand in each nonhomologous segment whenever the length of the λ strand was not known. For this reason, the alignment of each class with the λ physical map varies slightly but does not affect the interpretation of the data.

amount of P22 DNA substituted for λ DNA within the region of interest. The righthand endpoint of the P22 substitution mapped as follows in each hybrid: (i) in λ hy36(imm22 19amH736), between the 19 amH736 mutation and λ gene A, (ii) in λ hy37(imm22 R_{λ}^{+}), between P22 gene 13 and λ gene R, and (iii) in λ hy17(imm22 23amH335-6S), between the 23amH335 mutation and the DNA sequence encoding the λ 6S transcript. These endpoints were deduced from the genotypes of parental and recombinant phages and in the case of λ hy17 from *in vitro* transcription experiments (21).

Heteroduplex molecules of these λ imm22 hybrids and λ b2 were prepared (Fig. 3A, B, and D); in addition, heteroduplex molecules of λ hy37 and b519b515nin5 were prepared (Fig. 3C) to utilize the *nin5* deletion as a physical marker between λ genes P and Q. The alignment of each class of heteroduplex molecules with the λ physical map (Fig. 4) varies slightly (due to the method of normalization) but does not affect the following analysis of the region.

Class I. The heteroduplexes of λ hy36(imm22 19amH736)/ λ b2 give an overview of the entire region of interest between 90 and 95% λ . Within this region, two intervals of DNA homology and two intervals of DNA nonhomology are found. The remainder of the molecule contains five intervals of DNA nonhomology (the largest of which contains early regulation, immunity, and DNA replication genes) and the deletion loop created by the *b2* mutation.

Class II. The heteroduplexes of λ hy37(imm22 R_{λ}^{+})/ λ b2 map the location of genes 19 and R. Constructed in a cross between λ hy36 and λ Sam7, λ hy37 is identical to λ hy36 except it carries λ gene R instead of P22 gene 19. Since the λ hy37/ λ b2 heteroduplex molecules no longer have the rightmost interval of nonhomology (93.6 to 94.6% λ) observed in λ hy36/ λ b2 heteroduplex molecules, this DNA sequence must correspond to genes 19 and R whose products differ in chemical specificity.

Class III. The heteroduplexes of λ hy37(imm22 R_{λ}^{+})/b519b515nin5 map the location of genes 23 and Q,

genes 13 and S, and transcripts *a* and 6S. In this class the *nin5* deletion (between λ genes P and Q at 83.7 to 89.5% λ) (22) has become continuous with the immunity and replication gene region nonhomology, leaving only one small nonhomologous interval to its right. Genes 23 and Q must map immediately to the right of *nin5* and, therefore, lie within the 1.8% λ homologous interval on this molecule. Since the P22 *a* transcript and the λ 6S transcript differ in sequence (19), they probably lie within the nonhomologous interval (91.4 to 92.1% λ) to the right of genes 23 and Q. This places genes 13 and S at least partly within the 1% λ homologous sequence seen in heteroduplexes of class I (λ hy36/ λ b2).

Class IV. The above gene assignments were supported by heteroduplexes of λ hy17(imm22 23amH335-6S)/ λ b2. This hybrid contains the P22 23amH335 mutation but encodes the λ 6S transcript instead of the P22 *a* transcript (21). Accordingly, these heteroduplex molecules have lost the nonhomologous interval that correlated with the region encoding these transcripts.

These gene assignments are compatible with the known or estimated molecular weights of the encoded proteins. First, the λ Q protein (molecular weight, 22,500 [22.5K]) (6) and the P22 23 protein (about 24K) (28) require a DNA sequence of about 1.3% λ which is within the 1.6 to 1.8% λ homologous sequence we report. Second, the P22 *a* transcript (about 50 to 60 nucleotides) (21) and the λ 6S transcript (201 nucleotides) (16) require DNA sequences of about 0.14 and 0.4% λ , respectively, and are within the 0.4 to 0.6 and 0.7 to 0.8% λ nonhomology we measured. Third, although the molecular weight of the λ S protein has not been determined, the P22 13 protein (about 10.5K) (27) would require a DNA sequence of about 0.6% λ , which is well within the 1% homologous sequence we observed. Last, the λ R protein (about 18K) (24) and the P22 19 protein (about 15K) (28) would require DNA sequences of about 1 and 0.8% λ , respectively, which is consistent with the 1% λ nonhomology we obtained.

This study demonstrates that the similar genetic organiza-

tion of P22 and λ late control and lysis genes reflects not only structural analogy, as in the case of their early genes, but also structural homology for genes 23 and Q and genes 13 and S. This homology is interrupted, however, by nonhomologous sequences encoding the *a* and 6S transcripts. Why should there be different transcripts in an otherwise homologous region of the genome? The transcription termination signals which terminate these RNAs differ in the amount of Q/23-independent readthrough transcription they permit. The presence of the 6S transcript (and its terminator) in a λ imm22 23am mutant confers a leaky phenotype (burst size of ≥ 10 PFU per cell in *E. coli su*⁻), characteristic of λ Qam mutants. Alternatively, the presence of the *a* transcript (and its terminator) in the same phage confers a tight phenotype (burst size ≤ 0.1 PFU per cell in *E. coli su*⁻), characteristic of P22 23am mutants. Perhaps optimal control of the P22 late operon requires that readthrough transcription of the late genes is limited early in infection.

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