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Genetic and Physical Structure of Salmonella-coli Phage
Hybrids and Development of New Generalized
Transducing Hybrid Phages for E. Coli

Final Report

Nobuto Yamamoto, Ph.D.

May 1984

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Hahnemann University School of Medicine
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Hybrids between unrelated bacteriophages are invaluable models to study genetic evolution, gene regulation, and morphogenesis. For many years studies in my laboratory have been focused on the mechanism of hybrid formation between evolutionary diverse phages. Collaborative studies with a group of Walter Reed Army Institute of Research began searching for E. coli-S. typhimurium recombinants in 1971. After isolating E. coli-S. typhimurium		

hybrids which are common hosts for the various coli and Salmonella phages, we developed efficient selective methods for isolation of hybrids between Salmonella and E. coli phages and have isolated numerous hybrid phages in a tailor made fashion by varying the selection procedure. A variety of unusual hybrid phages such as λ immP22, P22imm λ , ϕ 80immP22 and MuimmP22 have been isolated and characterized by physical and genetic means. The genetic homologies of these hybrid phages with the parental phages, particularly P22 phage, have been extensively analyzed. In general hybrid genomes consist of the entire late genes of one parent phage and most of the early region of the other parent phage. Physical homologies of these hybrid phages and the parental phage (e. g. λ and λ immP22 hybrids) were demonstrated by heteroduplex analysis of hybrid and parental phage DNAs and correlated with the genetic maps of the hybrid phages.

Using hybrids between coliphages and P22, we studied basic problems on formation of hybrids between unrelated phages, regulation of gene expression and development of high transducing and antigen converting hybrid phages. Some hybrid phage species such as λ immP22dis and ϕ 80immP22dis phages carry bipartite immunity region (c and Im) of P22 although they inherit the entire late genes (tail and head proteins) of λ and ϕ 80 respectively. Thus these dis phages are coimmune with P22 phages and infect their dis lysogens. Since the P22 tail spike gene (9) and O-1 antigen conversion gene (a1) are located between the P22 c and Im regions, both the tail spike gene 9 and the antigen conversion gene a1 are inherited and expressed in these dis hybrid phages. Although these hybrid phages infect and lysogenize E. coli, the O-1 antigen conversion was not detectable in these lysogens. However, when E. coli recombinants carrying a small Salmonella segment for the O-antigen repeating unit were lysogenized with these phages, the O-1 antigen was efficiently expressed. This approach for intergeneric antigen conversion is medically significant. In addition since the P22 prophage attachment region (attP22) is located between the a1 and c regions of P22, these hybrid prophages are inserted at the P22 attachment site on the E. coli segment near the prdine AB genes of E. coli-S. typhimurium recombinants. Some phage particles in spontaneously induced lysates of ϕ 80immP22dis have lost the a1-9-Im segment and acquired the argF-proAB segment of E. coli. These hybrid derivatives, therefore, designated ϕ 80immP22dis⁻, are high transducing phage specific for argF and proAB although the parental phage, ϕ 80, is a specialized transducing phage for the E. coli trp operon.

Phage P22 can recombine with coli-mutator phage Mu. Genetic studies correlated with serological and host range analyses of MuimmP22 hybrids revealed that crossovers occurred at the tail fiber genes within the invertible G segment of Mu phage and the tail spike gene of P22 phage to form MuimmP22 hybrids. MuimmP22 hybrids infect hosts carrying the smooth host O-antigen (Man-Rha-Gal)_n repeating unit which is specific receptor for adsorption of P22 phage. However, anti-P22 serum is unable to neutralize the MuimmP22 hybrids. This is probably because the contribution of P22 spike peptide to the hybrid tail fiber is too short to form P22 spike like structure and antigenicity. Anti-serum prepared for the G(+) orientation of Mu phage neutralized the MuimmP22 hybrid at about a 1/10 neutralization rate of MuG(+) phage. Thus

we suggested that MuimmP22 hybrid may carry the inverted (-) orientation of the G segment of Mu phage. In fact near the distal ends of both P22 tail spike and Mutail fiber there is an accidental homology. Therefore, we conclude that MuimmP22 hybrids carry the (-) orientation of the G segment and the G(-) sequence facilitates crossovers with the P22 spike gene to yield the hybrid tail fiber.

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SUMMARY

Hybrids between unrelated bacteriophages are invaluable models to study genetic evolution, gene regulation, and morphogenesis. For many years studies in my laboratory have been focused on the mechanism of hybrid formation between evolutionary diverse phages. Collaborative studies with a group of Walter Reed Army Institute of Research began searching for E. coli-S. typhimurium recombinants in 1971. After isolating E. coli-S. typhimurium hybrids which are common hosts for the various coli and Salmonella phages, we developed efficient selective methods for isolation of hybrids between Salmonella and E. coli phages and have isolated numerous hybrid phages in a tailor made fashion by varying the selection procedure. A variety of unusual hybrid phages such as λ immP22, P22 imm λ , ϕ 80immP22 and MuimmP22 have been isolated and characterized by physical and genetic means. The genetic homologies of these hybrid phages with the parental phages, particularly P22 phage, have been extensively analyzed. In general hybrid genomes consist of the entire late genes of one parent phage and most of the early region of the other parent phage. Physical homologies of these hybrid phages and the parental phage (e. g. λ and λ immP22 hybrids) were demonstrated by heteroduplex analysis of hybrid and parental phage DNAs and correlated with the genetic maps of the hybrid phages.

Using hybrids between coliphages and P22, we studied basic problems on formation of hybrids between unrelated phages, regulation of gene expression and development of high transducing and antigen converting hybrid phages. Some hybrid phage species such as λ immP22dis and ϕ 80immP22dis phages carry bipartite immunity region (c and Im) of P22 although they inherit the entire late genes (tail and head proteins) of λ and ϕ 80 respectively. Thus these dis phages are coimmune with P22 phages and infect their dis⁻ lysogens. Since the P22 tail spike gene (9) and O-1 antigen conversion gene (a1) are located between the P22 c and Im regions, both the tail spike gene 9 and the antigen conversion gene a1 are inherited and expressed in these dis hybrid phages. Although these hybrid phages infect and lysogenize E. coli, the O-1 antigen conversion was not detectable in these lysogens. However, when E. coli recombinants carrying a small Salmonella segment for the O-antigen repeating unit were lysogenized with these phages, the O-1 antigen was efficiently expressed. This approach for intergeneric antigen conversion is medically significant. In addition since the P22 prophage attachment region (attP22) is located between the a1 and c regions of P22, these hybrid prophages are inserted at the P22 attachment site on the E. coli segment near the proline AB genes of E. coli-S. typhimurium recombinants. Some phage particles in spontaneously induced lysates of ϕ 80immP22dis have lost the a1-9-Im segment and acquired the argF-proAB segment of E. coli. These hybrid derivatives, therefore, designated ϕ 80immP22dis⁻, are high transducing phage specific for argF and proAB although the parental phage, ϕ 80, is a specialized transducing phage for the E. coli trp operon.

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FOREWORD

Though we initially planned for development of a gene cloning vector, we have not established a recombinant DNA method for these hybrid phage during this period.

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PROGRESS

I. Introduction

For many years, studies in my laboratory have been focused on the formation mechanism and genetic of hybrids between evolutionary diverse phages. During the last ten years of U.S. Army Research Contract Support collaborative studies with a group of Walter Reed Army Institute of Research on bacterial genetics enable us to develop efficient selective methods for isolation of hybrids between Salmonella phage P22 and E. coli phages. A variety of unusual hybrid phages, such as P22 λ , λ immP22, P22imm λ , ϕ 80immP22, P22imm ϕ 80, MuimmP22, and P22immMu, have been isolated and characterized by physical and genetic means. Using these hybrid phages, we studied basic problems on formation of hybrids between unrelated phages and intergeneric antigen conversion and transduction of Salmonella antigens in E. coli.

II. Brief Background

The generalized transducing Salmonella phage P22 can recombine with a variety of phages. One of the hybrid phage species, P22 λ , which is a hybrid between P22 and a prophage of Salmonella typhimurium LT-2 (St for short), Fels 1, has been extensively studied in our laboratory since 1961(1). After the initial discovery of P22 λ , we established a general procedure for isolation of hybrids between unrelated phages (2,5,8,11) successfully found many different types of hybrid phages (8,11). We extended this approach to isolation of hybrid phages and between P22 and coliphages (14). Some of the hybrid phages are able to confer intergeneric antigen conversion (32). The isolation procedure for hybrid phages, background information, and present status of problem are briefly described.

1. Isolation Procedure for Hybrid Phages

Salmonella phage P22 is a remarkable phage which can recombine with evolutionary distant phages if common hosts are provided. High titer stocks of P22 phage grown on a bacterium lysogenic for another phage will contain a small number of hybrid phage particles. These hybrid phages are selected by plating the P22 phage stocks on a host bacterium resistant to P22 and lysogenic for the same prophage. Plaque formers found on such a bacterium should be hybrid phages because the above selective host lacks the receptors for P22 phage adsorption and is immune to the other parental phage. These hybrid phages carry the protein coat of the prophage and the early genes, at least the immunity gene (c gene), of the P22 phage.

A general isolation procedure and selection hosts for hybrid phages are shown in Table 1. Our accumulated observations led us to the conclusion that recombination between unrelated phages requires at least the following three indispensable conditions: (i) ϕ 1, one of the parental phage, has to be a generalized transducing phage(8,20); (ii) B, a common host bacterium, has to be a wild type recA+ host (11,20); and (iii) ϕ 2, the other parental phage, has to be in the prophage state in a recA+ host, B(ϕ 2), to be used for propagation of ϕ 1 phage (4,10). The hybrid selection host, B/1(ϕ 2), effectively detects hybrid phages even at an extremely low frequency, as low as 10^{-12} . The hybrid phages carries the protein coat of ϕ 2 and have acquired the c region of the ϕ 1 phage (1,4,8,20).

Table 1. Host ranges of bacteriophages and a selective host for their hybrids.

	$\phi 1$	$\phi 2$	$\phi 1.2$ (hybrid)
B	+	+	+
B/1	-	+	+
B($\phi 2$)	+	-	+
B/1($\phi 2$)	-	-	+

$\phi 1$: includes Salmonella phages P22, #10, #20, #23, 27, ES18, ES19, and $\phi 19$

$\phi 2$: includes Salmonella phages Fels 1 and Fels 2, and E. coli phages $\phi 80$ and Mu.

$\phi 1.2$: hybrid phage between $\phi 1$ and $\phi 2$.

This general isolation method is based on our success with recombination between P22 and Fels 1 to yield the P221 hybrid and recombination between P22 and coliphages.

III. Progress During the Last Support Period (1/1/74 - 8/31/83)

A. Development of Isolation Procedure for Recombinants Between P22 and λ

a. Isolation of a S. typhimurium Hybrid with Sensitivity to Coliphage and Phage P22.

An unusual E. coli-S. typhimurium hybrid with sensitivity to both coliphage λ and Salmonella phage P22 has been recovered from mating between an Escherichia coli K12Hfr donor and an S. typhimurium recipient. We have isolated E. coli-S. typhimurium hybrid WR4027 and its derivative WR4028 which are excellent hosts for achieving genetic recombination between λ and P22 (14).

b. Isolation of Hybrid Phage Species

The dual sensitivity of S. typhimurium hybrid WR4028 to phage P22 and λ and the availability of various lysogenic derivatives of this strain enabled us to isolate hybrid phages between these two phages. Two broad classes of hybrid phages were isolated. The λ -P22 hybrid class, which has the protein coat of λ , contains at least the c region of P22. The P22- λ hybrid class has the protein coat of P22 and has inherited at least the c marker of λ (14).

B. Genetic Characterization of P22- λ Hybrids (16,18)

Phage P22 recombines with coliphage λ to yield a hybrid phage class designated P22- λ which is serologically indistinguishable from P22. By employing λ phages with different c markers (c, cI, cII, or cIII), we have shown that the P22- λ hybrids always express the inherited c markers of λ .

As shown in Figure 1, genes O and P of λ and genes 18 and 12 of P22 which function in phage DNA replication are positioned to the right of respective c regions of these phages. Our initial efforts were directed towards isolating P22- λ hybrids in which these genes had been recombined. A λ lysogen of the *E. coli*-*S. typhimurium* hybrid, WR4028(λ c⁺), was superinfected at 25° with mutant derivatives of P22c2 expressing the temperature sensitive (ts) phenotype of genes 12 or 18. After incubation at 25° to achieve lysis, the lysate was plated on WR4028/ λ at 25°. P22- λ hybrids with the λ c⁺ region were obtained by cloning the cloudy plaques which appeared.

A number of such P22- λ hybrids isolated at 25° were then scored for their ability to replicate at the elevated temperature (40°). One major group, comprised of more than 80% of such P22- λ hybrids, no longer expressed the temperature-sensitive phenotype, thus suggesting that their 18 and 12 genes had been replaced by a λ chromosomal segment containing the functionally related O and P genes. This was confirmed by marker rescue experiments with λ which indicated the presence of λ genes O and P. Similar rescue experiments with P22 show the absence of ts12 and ts18 in these hybrids. We have designated this group as P22- λ type 1 hybrids. The remaining hybrids were unable to grow at 40° because they have retained the 18 and 12 genes of P22. As determined by marker rescue, they do not carry the O and P genes of λ . We have designated these hybrids as P22- λ type 2. In addition to these methods, the presence of various λ and P22 markers in these two P22- λ types was determined by backcross with the parental phages, as shown in Figure 1.

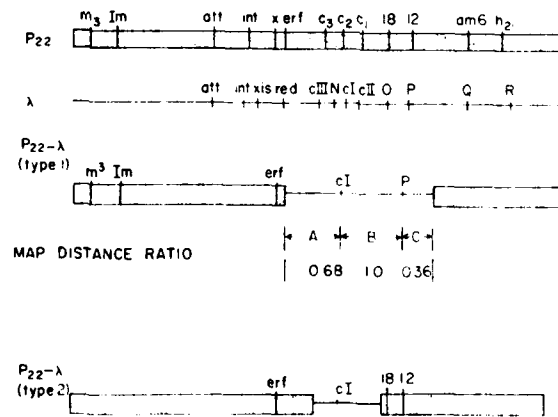


Fig. 1. Diagrammatic representation of parental and hybrid phages

C. Genetic and Physical Characterization of λ immP22 Hybrids (14,17,22,23)

1. Isolation and Genetic Characterization of λ immP22 Hybrids (14,23)

S. typhimurium phage P22 has been previously shown to recombine with coliphage λ to yield a hybrid phage λ -P22 class antigenically indistinguishable from λ . When these hybrids, now referred to as λ immP22, result from a recombina-

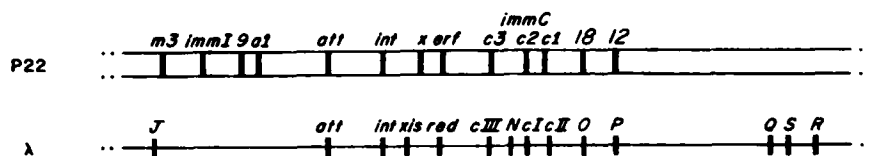
tion with P22 phages containing different c markers, i.e., either c⁺, c₁, c₂, or c₃, they express the respective c marker of P22. In the recent study, additional λimmP22 hybrids with scorable phenotypes were prepared by employing procedures similar to those previously described (Gemski, Baron, and Yamamoto, 1972 (14)). As shown in Figure 2, genes 18 and 12 of P22 and genes O and P of λ are positioned to the right of the respective c regions of these phages. Since genes 18 and 12 of P22 (Botstein and Levine, J. Mol. Biol., 34, 643, 1968) and O and P of λ function in phage DNA replication, we first attempted to isolate λimmP22 hybrids in which these genes had been recombined. S. typhimurium WR4028(λ) was superinfected with mutant derivatives of P22 carrying temperature-sensitive (ts) genes 12 and 18, and λimmP22 hybrids, selected for the inheritance of the immC marker of P22, were recovered by plating on the rough, λ immune strain, WR4027(λ). Following cloning on WR4027, such λimmP22 hybrids were scored for the inheritance of the ts18 and ts12 genes by determining whether they could replicate at the elevated temperature of 42°. All λimmP22 hybrids so tested were found to be temperature sensitive, thus indicating that their O and P genes had been replaced by a P22 chromosomal segment containing the ts18 and ts12 genes. By similar crosses, λimmP22 hybrids were recovered which express the erf and x genes of the P22 strain employed. The presence of these markers in λimmP22 hybrids was established by determining whether or not they were able to grow on the polA⁻ strain which was unable to plate λred, typical of the behavior exhibited by the polA⁻ E. coli K-12 strain WR2108. The λimmP22 hybrids selected by plating on WR4027(λ) for the inheritance of the immC marker of P22 after superinfecting WR4028(λ) with either the P22erf or P22x mutants were unable to grow in polA⁻ hosts. This result indicates inheritance of the erf-x P22 chromosomal segment by these recombinants.

Other λimmP22 hybrids were recovered from similar experiments in which the superinfecting phage carried either the h21 or m3 color indicator marker. The resulting λimmP22 hybrids were then scored for the inheritance of these color indicator markers from the superinfecting phage strains by plating on WR4027(λ). The presence of the h21 or m3 marker of P22 was not detected in such λimmP22 derivatives and also by markers rescue experiments. As shown in Figure 2, the m3 marker is distant from the immC region for which the λimmP22 hybrids had been originally selected. Additional λimmP22 hybrids were also recovered by growing P22^c⁺ on various WR4028 strains lysogenic for either λint6, λxis6, λred3, λred3gam6, λsusQ, or λsusR. Such λimmP22 hybrids were scored for the phenotype of these different λ genes. Lysogens of λimmP22 hybrids derived from the crosses between P22 and λint or λxis were inducible indicating that the int6 and xis6 phenotypes were not being expressed in the λimmP22 hybrid. Thus, it is likely that these λ markers have been replaced with functional P22 genes in these hybrids. Similarly, λimmP22 hybrids recovered from crosses between P22^c⁺ and either λred3 or λred3gam6 expressed a wild-type phenotype. These λimmP22 isolates fell into two groups, those which retained and expressed the λsusQ or λsusR allele, being unable to grow in an amber suppressor negative host (in Fig. 2) and those which were able to grow in such a host indicating that the Q and R genes of λ were replaced by P22 genes in the latter group (not shown in Fig. 2).

2. Isolation of an Unusual Hybrid Type λimmP22dis (23)

The majority of λimmP22 hybrids carry the 18 and 12 genes for DNA synthesis as well as the immC locus of P22. Although P22 and λimmP22 have identical immC regions, lysogens of λimmP22 are not immune to P22 whereas P22 lysogens are

PARENTAL PHAGES



HYBRID PHAGES



Fig. 2. Portions of the λ and P22 genome in the λ *imm*P22 hybrids are shown at the top of the figure. Genetic characteristics of the λ and the P22 genome are positioned so that similar functions in both phages are aligned as described by Botstein, Chan and Waddell (1972). The P22 genes shown in the hybrids have been determined by genetic analysis to be present. The extent of the P22 segment in the hybrids is enclosed by dashed lines to indicate that the exact extent of the P22 segments in these hybrids has not been established by genetic means.

immune to λ immP22 infection (Genski, Baron, and Yamamoto, 1972, (14); see Table 2). When a high titer P22c2 stock previously grown on WR4028(λ), however, was plated on the doubly lysogenic strain WR4027(λ , λ immP22), small, clear plaques similar to those of λ immP22 were isolated at a frequency of about 10^{-12} . These small plaque-formers were purified by stepwise cloning on WR4027(λ , λ immP22) and WR4027 and then tested for their reactivity with specific antisera against P22 and λ . Antiserum against λ neutralized these newly isolated hybrids, whereas they were unaffected by anti-P22 serum. On the basis of their tail antigen characteristics and their ability to plate on a P22 resistant bacterial strain doubly lysogenic for λ and λ immP22, we consider these hybrids to exhibit a new type of immunity system (as shown in Fig. 2). When such hybrids, designated λ immP22dis (the dis is for dismune) are used to lysogenize WR4028, the resulting lysogens are immune to superinfection with P22, and are immune also to P22dis, a phage hybrid known to possess both immunity regions of P22. In addition, λ immP22dis lysogens are immune to λ immP22, whereas λ immP22dis hybrids produce plaques on λ immP22 lysogens. These results establish that λ immP22dis is co-immune with P22, but dismune with respect to λ immP22, indicating that λ immP22dis hybrids carry the P22 immI region as well as the P22 immC region.

To determine if the same immunity pattern exhibited by λ immP22 lysogens of the *E. coli*-*S. typhimurium* hybrid WR4028 would be expressed in *E. coli* K-12, we employed K-12 strains lysogenic for the appropriate phages (Yamamoto et al., 23). We prepared a P22 lysogen of *E. coli* K-12 using the F-lac⁺-proA⁺-proB⁺-attP22⁺ plasmid according to the procedure described by Roth (Genetics, 76, 633, 1972) to compare its immunity pattern with that expressed by λ immP22dis. Moreover, the immC locus of P22, carried by the λ immP22 hybrids, and both the immC and immI loci in the case of λ immP22dis are expressed in *E. coli* K-12.

3. Determination of the Physical Length of P22 DNA in λ immP22 Hybrids (23)

The approximate length of P22 DNA that was substituted in λ immP22 hybrids could be estimated by backcross experiments performed between these hybrids and various P22 derivatives. The frequency of recombination between various markers was determined after mixed phage infection of WR4028 and plating on WR4028/ λ to select P22 recombinants. The extent of the P22 genome in λ immP22 hybrids 1 and 2 and in λ immP22dis, as inferred from genetic recombination data, is shown in Figure 2. In addition, we have employed the more direct method of electron microscope heteroduplex analysis to determine more accurately the actual physical length of the recombined segment in the individual hybrids. Thus, genetic analysis of the inheritance of unselected markers from backcross experiments could be correlated with quantitative findings from heteroduplex analyses.

Heteroduplexes formed between each of the λ immP22 hybrids and wild-type λ show which sections of the hybrid phage genomes have homology with the DNA of λ . Portions of the λ immP22 DNA molecules lacking homology to λ DNA represent those P22 gene segments substituted in the λ genome. A heteroduplex formed between λ immP22 hybrid 1 and λ , shown in Figure 3, reveals a relatively small P22 region inserted in the center of the λ genome amounting to about 15-16% of the λ genome. A block of P22 genes amounting to approximately 20-22% of the λ genome was recombined in λ immP22 hybrid 2 (Figs. 4 and 5) which supports our genetic finding of the P22 int gene in this hybrid depicted in Figure 2. Considerably more extensive substitution of P22 genes is evidenced in the heteroduplex of hybrid λ immP22dis (Figs. 5 and 6). In this hybrid, approximately 40% of the central region of λ is replaced by P22 genes.

Table 2. Host range, sensitivity, and immunity patterns exhibited with λ , P22, and hybrid phages

Phages	WR4028 (P22)	WR4255	WR4028 (<i>zimm</i> 21)	WR4027 (<i>zimm</i> 21)	WR4028 (<i>zimm</i> P22) hybrid 1	WR4027 (<i>zimm</i> P22) hybrid 1	WR4028 (<i>zimm</i> P22) hybrid 2	WR4027 (<i>zimm</i> P22) hybrid 2	WR4028 (<i>zimm</i> P22) <i>zimm</i> P22/ <i>dis</i>)	WR4027 (<i>zimm</i> P22) <i>zimm</i> P22/ <i>dis</i>)	WR4028 (<i>zimm</i> P22) <i>zimm</i> P22/ <i>dis</i>)	WR4027 (<i>zimm</i> P22) <i>zimm</i> P22/ <i>dis</i>)
P22	-	+	+	R	+	R	+	R	-	-	-	R
λ	+	- ^a	+	+	+	+	+	+	+	+	+	+
<i>zimm</i> P22 hybrid 1	-	+	-	-	-	-	-	-	-	-	-	-
<i>zimm</i> P22 hybrid 2	-	+	-	-	-	-	-	-	-	-	-	-
<i>zimm</i> P22/ <i>dis</i>	-	+	+	+	+	+	+	+	+	+	+	+

P22 was propagated on WR4028 to yield over 10^{10} pfu/ml; λ and the hybrid phages were propagated on WR4027 to yield over 10^9 pfu/ml

Symbols: +, sensitive to lysis; -, immune; R, resistance due to inability to absorb phage; (), lysogenic for phage

^a Lack of lysis due to improper functioning of λ N gene in *Salmonella* (Baron et al., 1970; Baron et al., 1972)

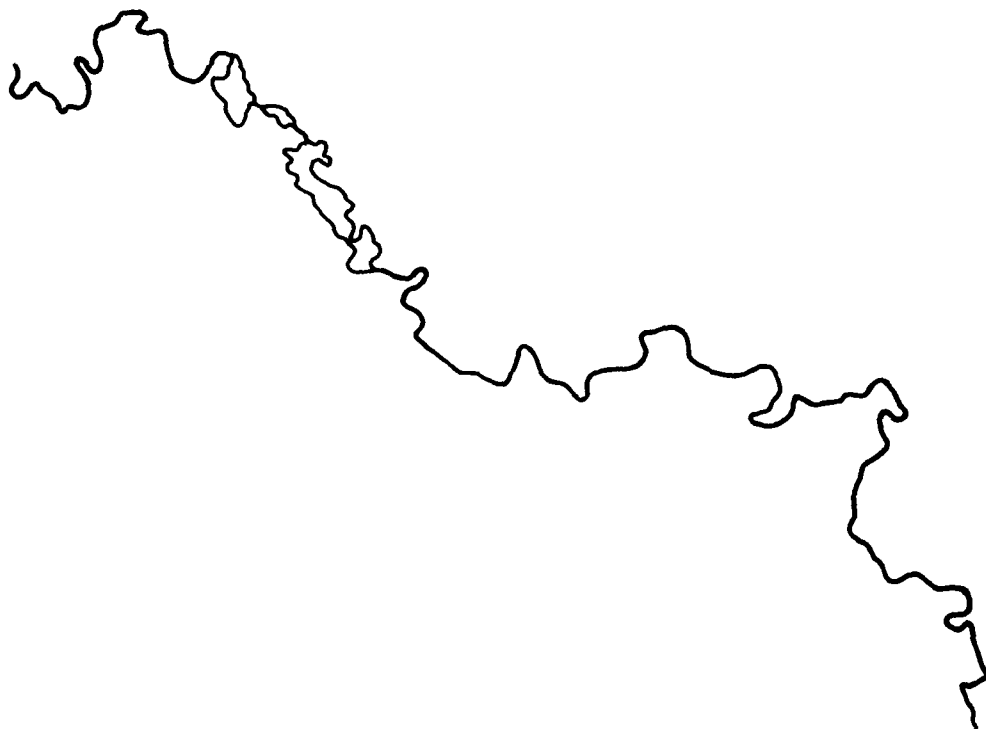
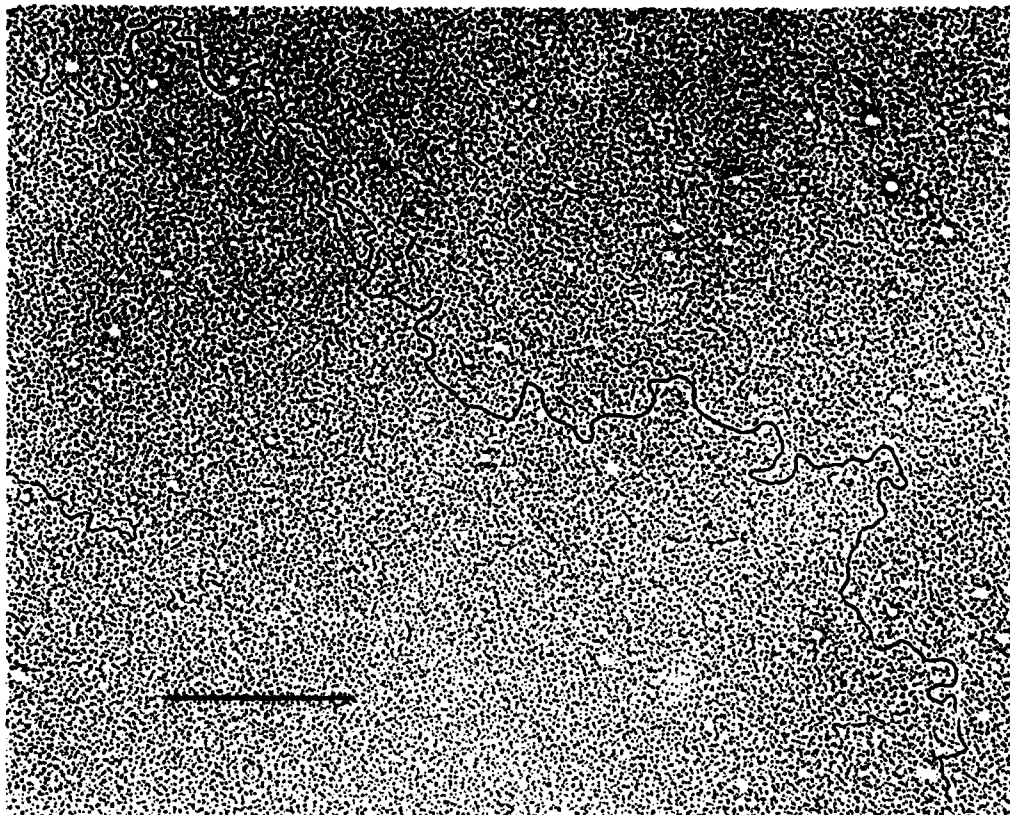


Fig. 3. An electron micrograph of the heteroduplex formed between the DNA of wild-type *z* and *zmmP22* hybrid I is shown in the top portion of the figure. Below is a line drawing of the heteroduplex molecule. Homologous regions are indicated by a thick line and unhybridized single-stranded regions are indicated by thin lines. The bar in the electron micrograph represents 1.0 μ m.

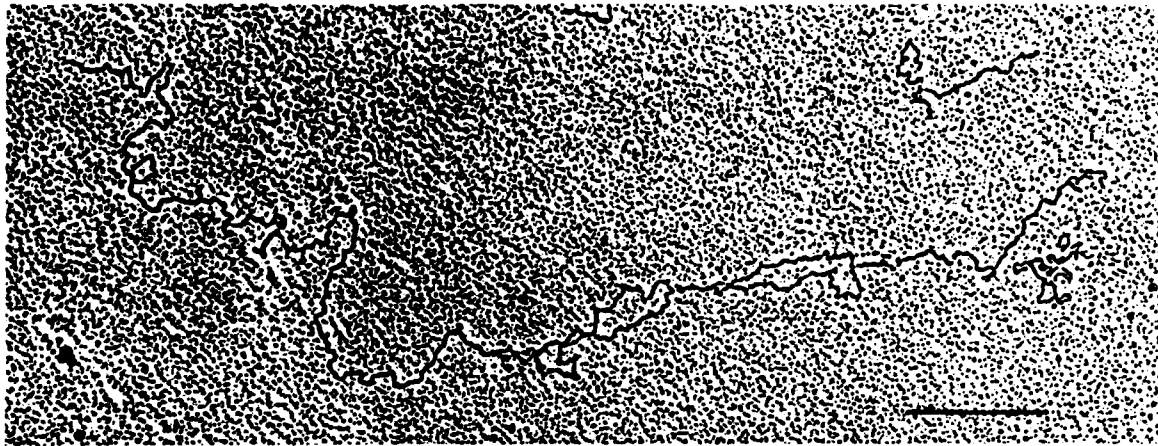


Fig. 4. The heteroduplex formed by DNA from λ and *zimm*P22 hybrid 2. The bar represents 1.0 μ m

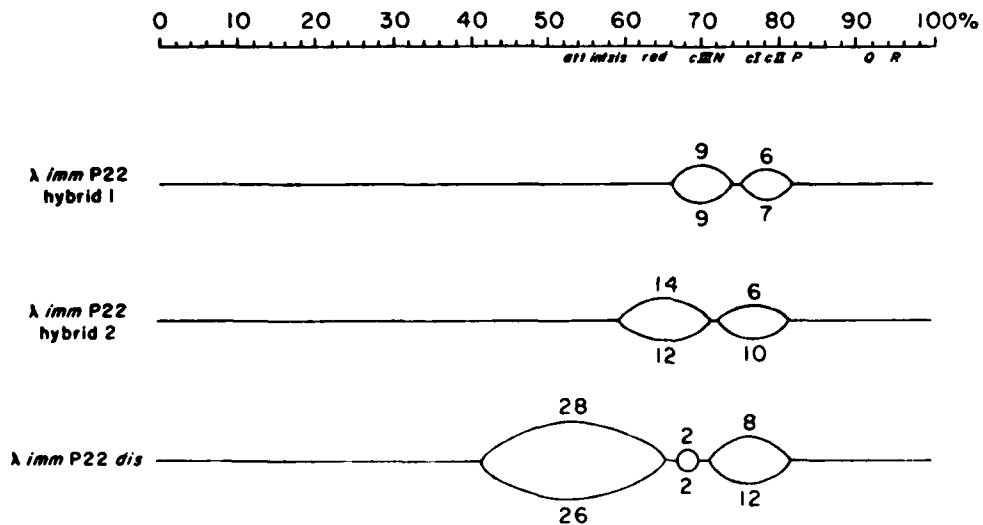


Fig. 5. The results of heteroduplex analysis of the *zimm*P22 hybrids are summarized diagrammatically. The regions of homology between wild-type λ and the *zimm*P22 hybrids are indicated by a single line. Nonhomologous portions of the molecule are indicated by loops. These loops correspond to single-stranded non homologous regions of the DNA heteroduplex molecules. The combined results from at least ten molecules of each heteroduplex type were used to establish the duplex regions in the representations of the molecules.

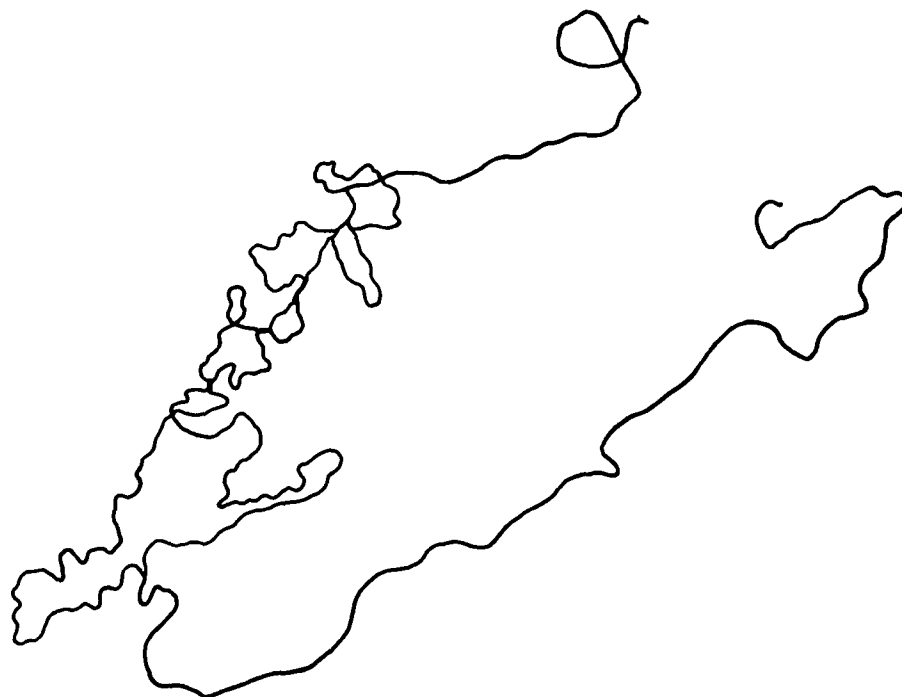
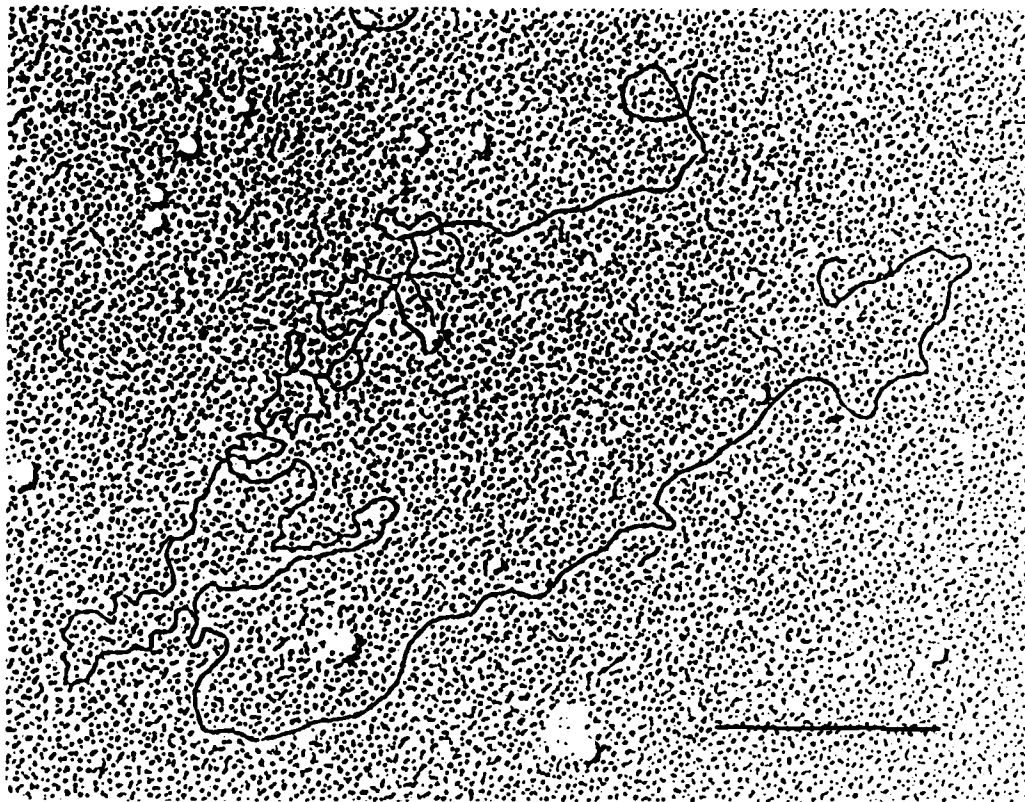


Fig. 6. The heteroduplex formed by DNA from *z* and *zmmP22lys*. The bar represents 1.0 μ m

The results of these heteroduplex studies are presented in diagrammatic form (Fig. 5) in which a percentage scale is used to locate map positions on the λ genome. Thus, the nonhomologous region in λ immP22 hybrid 1 can be defined as extending from a map position of 66 to 82. The two regions of nonhomology are connected by a small segment of homologous DNA. In λ immP22 hybrid 2, the P22 segment extends from map position 60 to 82 of the λ chromosome. Again, two regions of nonhomology connected by a short segment of homologous DNA are evident. The λ immP22dis phage DNA has a nonhomologous region that starts at map position 42 and extends to position 82. Three regions of nonhomology in this hybrid are separated by two small regions of homology. The nonhomologous chromosomal region of this hybrid, extending further leftward on the λ genome than do those of the other two hybrid types, goes well beyond the site of the att gene of λ and thus contains both the immI and immC regions of P22. This finding is in agreement with the results of our genetic experiments illustrated in Figure 2.

4. Two Dispensable P22 Phage Genes in λ immP22dis

It is well established that control of λ phage gene expression is efficient and involves no waste. The genome of λ immP22dis hybrid carries two P22 phage genes which are dispensable for λ immP22dis phage. Therefore it became of interest to test whether these dispensable genes are expressed.

a. Antigen Conversion by λ immP22dis Hybrids (19,23,32)

The *S. typhimurium* somatic 0-1 antigen conversion gene al of P22 has not been precisely mapped, being located in the large genome segment between the immC and m3 loci on the P22 chromosome (Young et al., Virology, 23, 299, 1964). Through the use of λ immP22 hybrids, we have been able to position the P22 al gene in proximity to the m3 locus. Most of the λ immP22 hybrids that we have characterized have a P22 segment that ends between the int and att gene of P22 (see Fig. 2). Such hybrids retain the att and other genes of the λ genome as indicated in this figure. Therefore, λ immP22 hybrids 1 and 2, typical of many that we have examined, do not possess the al gene and are unable to confer antigen conversion when the host bacterium WR4028 is lysogenized with these hybrids. In contrast, hybrids of the λ immP22dis type carry the al gene because evidence from the genetic and physical mapping studies indicates that the P22 segment carried by such hybrids always contains the immI region and can extend very close to the m3 region even though this locus does not appear in λ immP22dis hybrids. Accordingly, various independent isolates of λ immP22dis were used to lysogenize WR4028, and such lysogens were tested for somatic antigen 0-1 conversion of the host. The WR4028(λ immP22dis) lysogens were tested in slide agglutination tests for the expression of *Salmonella* somatic antigen 0-1 using single factor 0-1 antiserum. The results establish that the λ immP22dis hybrid carries the al gene and confers 0-1 antigen production on the host bacterium WR4028.

However, λ immP22dis hybrids do not convert *E. coli* K12 because *E. coli* K12 does not have 0-1 antigen acceptor sites which are (Man-Rha-Gal) repeating units of *Salmonella typhimurium* cell surface. When *E. coli* K12 derivatives carrying a small *Salmonella* genetic segment for the (Man-Rha-Gal) repeating units were examined, the above λ immP22dis strains conferred the antigen 0-1 conversion (19,32).

b. Production to Free P22 Tails by λ immP22dis (23)

Based on our genetic mapping and heteroduplex studies, we would expect that the λ immP22dis hybrid phages also possess tail gene 9 of P22. To determine whether or not tail gene 9 is expressed during λ immP22dis replication, we employed an in vitro self-assembly method described by Israel et al. (Proc. NAS 57, 282, 1967). A cell suspension of *S. typhimurium* strain Q at a concentration of 2×10^8 cells/ml was infected with P22ts9, a temperature sensitive tail gene 9 mutant that does not produce functional tails at elevated temperatures and thus gives rise to tail-less heads. This P22ts9-infected cell suspension was then cultured for 1 hour at the restrictive temperature of 39°C in Penassay broth with aeration to allow lysis. This lysate was examined by electron microscopy and found to contain approximately 2×10^{10} per ml of P22 heads without tails. Such heads contain the entire P22 genome and can be made into infective particles by adding functional tails. Should such a preparation of tail-less heads be added to a λ immP22dis lysate, an increase in P22ts9 plaque-forming ability would only be detected if free P22 tails were being produced by this phage hybrid. In fact, when the head preparation was added to a lysate of λ immP22dis, incubated for 1 hr and then plated on strain Q at 25°C, a 10,000-fold increase in P22 plaque-forming activity was observed. This increase in plaque formation was completely neutralized by using a λ immP22dis lysate pretreated with anti-P22 serum, but was not inhibited by a λ immP22dis lysate pretreated with anti- λ serum. These observations establish that tail gene 9 of P22 is being expressed by the λ immP22dis hybrid, although the protein coat and tail of this hybrid phage are those of λ .

5. Restriction Endonuclease Analysis of DNA from λ immP22 Hybrid Phages (22)

The λ immP22 hybrid phages in which segments of the P22 genome have replaced portions of the λ genome were examined by restriction endonuclease analyses. The extent of substitution of P22 into the λ genome was also determined by electron microscope hetero-duplex studies. All of the hybrids examined have the P22 segment substituted into the central region of the λ genome. Fragments that result from EcoRI restriction enzyme digestion of the DNA from these λ immP22 hybrids were compared to the restriction fragments obtained from λ and P22 DNA. Most of the fragments from the hybrids are identical to those obtained from λ , but at least one or more correspond to P22 fragments. Thus, the substitution of a P22 segment into λ results in the loss of the EcoRI fragments of λ and the appearance of P22 fragments. A correlation can therefore, be made between map position of λ fragments and the corresponding P22 fragments that replace them. One of the hybrids, λ -P22dis, contains a large segment of substituted P22 DNA and restriction analysis was used to order the P22 fragments in this segment (20).

D. Isolation and Characterization of Hybrid Phages between *E. coli* Phage ϕ 80 and *Salmonella* Phage P22.

1. Isolation of ϕ 80immP22 Hybrid Phage (24,26,29).

E. coli-*S. typhimurium* hybrid strain WR40207 is a rough bacterium and sensitive to coliphage ϕ 80 for its replication but insensitive to P22 phage because of lack of P22 phage adsorption. Therefore WR40207 lysogenic for phage ϕ 80, WR4027(ϕ 80), is insensitive to P22 phage. By infecting WR4027(ϕ 80) with a mixture of high titer stocks of rough specific *Salmonella* phages (designated R phages), we were able to isolate R-phage resistant derivatives of WR4027(ϕ 80), designated WR4027(ϕ 80)/R, which are smooth and fully sensitive to P22 phage. Phage P22 stocks grown on this

smooth derivative of the $\phi 80$ lysogen give rise to recombinants between P22 and $\phi 80$. Such recombinants were recovered by plating on a P22 resistant host which is immune to $\phi 80$, namely WR4027 ($\phi 80$). They retain the protein coat of $\phi 80$ but have acquired the immC region of P22. In addition these $\phi 80$ immP22 recombinant carries P22 DNA replication genes 12 and 18 as well as the x and erf genes of P22 (Fig. 7). Some $\phi 80$ immP22 recombinants, designate $\phi 80$ immP22dis, contain the immI region as well as the immC region, the two widely separated loci involved in the bipartite immunity system of P22.

2. Characterization of Unusual $\phi 80$ immP22dis Hybrid Phages (29)

Although the 0-1 antigen conversion gene a1 and tail gene 9 of P22 are located between immC and immI genes, no $\phi 80$ immP22dis hybrids carry both the a1 and 9 genes. Some hybrids carry the gene a1 and others carry the gene 9 (Fig. 7).

Although λ immP22dis hybrids carry both genes 9 and a1, $\phi 80$ immP22dis hybrids carry only one of these genes (i.e. gene 9 or a1). Both λ and $\phi 80$ phage genomes contain physically corresponding and functionary similar genes. These phage genomes also carry genetically inert DNA segments which are located between their respective att and tail (J) genes. However, the entire physical length of $\phi 80$ phage genome is about 92% of the size of λ phage genome. This seems to be reflection of difference in sized of the inert segments: $\phi 80$ carries an inert DNA segment smaller than that of λ (Fig. 7). Since the inert segments can be replaced by genes 9 and a1 to form dis hybrid phages, we concluded that the $\phi 80$ immP22dis hybrid phages are unable to accommodate both genes 9 and a1 simultaneously.

3. Isolation of Specialized Transducing Phage Type from $\phi 80$ immP22dis Hybrid type (26,31)

$\phi 80$ immP22dis hybrid type carries a large P22 segment (Im - att - erf - c - h21). Thus this hybrid type also can carry the P22 tail gene 9 and the antigen conversion gene a1 both of which are located between the Im and att regions (29). Since the genes in the Im - 9 - a1 - att portion of the P22 segment of the hybrid are dispensable for replication or viability of the hybrids, loss of this hybrid DNA segment carrying such dispensable genes by improper excision during prophage induction of *E. coli*-*S. typhimurium* lysogenic for $\phi 80$ immP22dis should result in the hybrid acquiring the bacterial DNA segment adjacent to the prophage attachment site (31). These resultant (dis⁻) phages which have lost the second immunity region (Im) are unable to grow in $\phi 80$ immP22 lysogens thus screened by its inability to grow in $\phi 80$ immP22 lysogen and found to carry *E. coli* chromosomal segment of *E. coli*-*S. typhimurium* hybrid. When these $\phi 80$ immP22dis⁻ derivatives were tested with various *E. coli* auxotrophic mutants for transducing ability, most $\phi 80$ immP22dis⁻ hybrids were found to be high transducing phages for arg F and proAB and some $\phi 80$ immP22dis⁻ hybrids were able to transduce met D. However, they transduce neither pro C nor lac. These observations suggest that the attachment site of P22 prophage in *E. coli* is located between argF and lac genes. Thus, the P22 prophage attachment site in *E. coli* which was previously mapped between pro AB and argF (Hoppe et al Genetics 76, 633, 1974) should be revised (31).

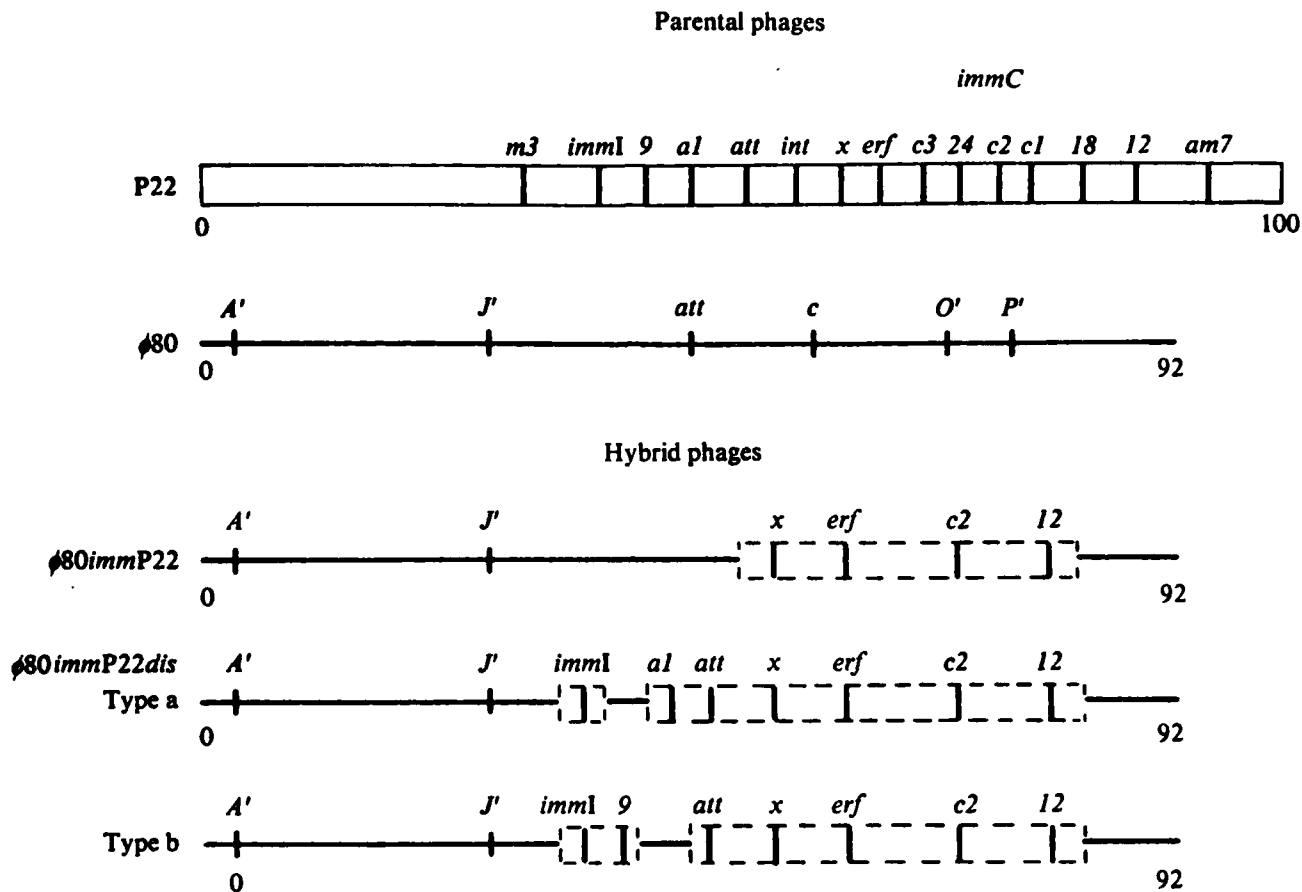


Fig. 7. The P22 and ϕ 80 genomes are depicted at the top of the figure. The P22 genes in the ϕ 80*imm*P22 hybrids have been shown to be present by genetic analysis. The extent of the P22 segment is enclosed in dashed lines to indicate that the exact P22 segments in the hybrids vary. The relative sizes of P22 and ϕ 80 are represented by 100% and 92% respectively. Markers on the ϕ 80 map, which correspond to those of λ , are indicated as *A'*, *J'*, *O'* and *P'*. Gene *h21* of P22, mentioned in the text but not indicated in the figure, is located to the left of the *am7* marker.

Similarly $\phi 80immP22dis^-$ derivatives were isolated from $\phi 80immP22dis$ lysogenic K37 derivatives of E. coli K12. When various E. coli K12 auxotrophic mutants were infected with the above $\phi 80immP22dis^-$ derivatives, high frequency of transduction; 2% for argF, and 0.9% for pro A, observed confirming that the attP22 site of E. coli lies between argF and lac genes.

4 Characterization of $\phi 80immP22$ Hybrid Type

As previously discussed, $\phi 80immP22$ carries the att-erf-c-12-h21 segment of P22. Detailed map analysis of the P22 homology by back-cross with various P22 mutants showed that the left arm of the P22 segment ends at the att region(29). Since $\phi 80immP22dis$ is found ten times more frequently than $\phi 80immP22$ hybrid type, the latter type would be $\phi 80immP22dis^-$ type derived from $\phi 80immP22dis$ hybrid type. When transducing activity of $\phi 80immP22$ was tested, they are all found to be high specialized transducing phages for argF and proAB. This observation proved our hypothesis that $\phi 80immP22$ hybrid is a $\phi 80immP22dis^-$ derivative.

E. Characterization of MuimmP22 Hybrids (27,33,34)

Employing an approach used for isolating hybrids between P22 and coliphage or 80, we have isolated MuimmP22 hybrids after intensive efforts. Difficulty of identifying MuimmP22 is due to the fact that the host range of the hybrids is different from that of Mu phage though the late genes of the hybrids are derived from those of Mu phage.

1. The Host Range of Muimm P22 Hybrids (23,24)

MuimmP22 hybrids carry the att-erf-c-12 segment of the P22 early regions whereas the late regions are derived from Mu phage. However, the hybrids infect smooth and semi-rough strains of E. coli-S.typhimurium (33) while Mu phage infects rough hosts exclusively. Since semi-rough strains carry a reduced amount of Salmonella smooth O-antigenic (Man-Rha-Ga) repeating units while such a repeating unit is not present in rough strains, the receptor of the MuimmP22 hybrid could be smooth O-repeating unit which is the specific receptor for P22 infection.

2. Tail Antigen for Plaque Neutralization of MuimmP22 Hybrid (23,24)

Since the host range of MuimmP22 differs from that of Mu phage, serological analysis of the hybrid became desirable. Although the MuimmP22 hybrids infect host via P22 specific receptor, anti-P22 serum showed no effect on plaque forming ability of the MuimmP22 hybrid. However, anti-Mu serum neutralized the MuimmP22 hybrid at about a 1/10 neutralization rate of the parental Mu phage. This reduced serological cross-reactivity with anti-Mu serum could be due to the antigenicity of tail fiber coded by the inverted orientation (-) of the G segment. Since inversion of the G segment is known to produce new tail fiber antigens different from those of the MuG(+).

Phage (Grundy & Howe, Virology in Press), anti-MuG(-) was prepared. Anti-MuG(-) serum neutralized MuimmP22 hybrids at about the same rate as MuG(-) phage. It should be discussed here that as shown in Fig. 8 S and S' genes would consist of a common part coded for by a region outside the G segment and a variable part located inside the G segment. Thus both orientations, G(+) and G(-), of Mu phages share some common antigenic peptide sequences. Since antibodies against various parts (not limited to adsorption binding or its adjacent sites) of tail fiber contribute to neutralization of plaque forming ability (Grundy & Howe), slight serological reactivity of MuimmP22 phage with anti-MuG(+) serum supports the G(-) orientation in the MuimmP22 hybrid. When anti-MuimmP22 serum was prepared, it neutralized MuG(-) phage at the same rate as MuimmP22 hybrid. Therefore we concluded that MuimmP22 hybrid carries the (-) orientation of the G segment. However, these MuimmP22 hybrids never gave rise to variants with the G(+) host range and serological phenotype of Mu phage.

3. Genetic Test for the Orientation of the G Segment in MuimmP22 Hybrids (33)

As shown in Fig. 8 the G segment of Mu phage harbors four tail fiber genes: S, U, S', and U'. Since this area is transcribed from left to right, the first set of genes S and U are expressed and the second set of genes S' and U' are silent in MuG(+) phage. On the other hand the S' and U' genes are expressed and the S and U genes are silent in the MuG(-) phage.

Our accumulated evidence suggested that during the course of hybrid formation between Mu and P22 phages, the inverted G(-) orientation is required to cross the P22 genome. Thus the S and U genes are no longer transcribed or are removed by crossovers. To test this possibility MuimmP22 hybrids were isolated by recombining P22 phage with Mu phage carrying an amber mutation in the S gene. The resultant MuimmP22 hybrids were able to grow in both wild type and suppressor hosts, supporting the hypothesis that the S proteins are not produced. Since there is a good serological cross reactivity between MuG(-) phage and MuimmP22 phage, it may be concluded that the (-) orientation of the MuG segment facilitates crossovers with the P22 genome to yield MuimmP22 hybrids, as shown in Fig. 9.

4. Serological Relationships between Mu and P2 Phages

The invertible C segment of P1 encodes tail fibers and is homologous to the G segment of Mu phage. Therefore P1 and Mu phages serologically cross-react. Since bacteriophage P1 and P2 are closely related, serological relationships between P2 and MuimmP22 were analysed. Anti-P2 serum neutralized MuimmP22 hybrids at a 1/10 fold reduced rate of P2 neutralization. This observation suggests that anti-P2 serum contains antibodies which cross-react with the G(-) products of MuimmP22 hybrids. Detection of a possible Mu like invertible segment in P2 requires serological reactivity between P2 and MuG(+) or MuG(-) phages.

5. Map Structure of MuimmP22 Hybrids (33)

Since MuimmP22 hybrid never gives rise to variants with the G(+) host range and serological phenotype of Mu phage, loss of gin gene or the S and U genes by replacing with P22 DNA during hybrid formation may be considered as

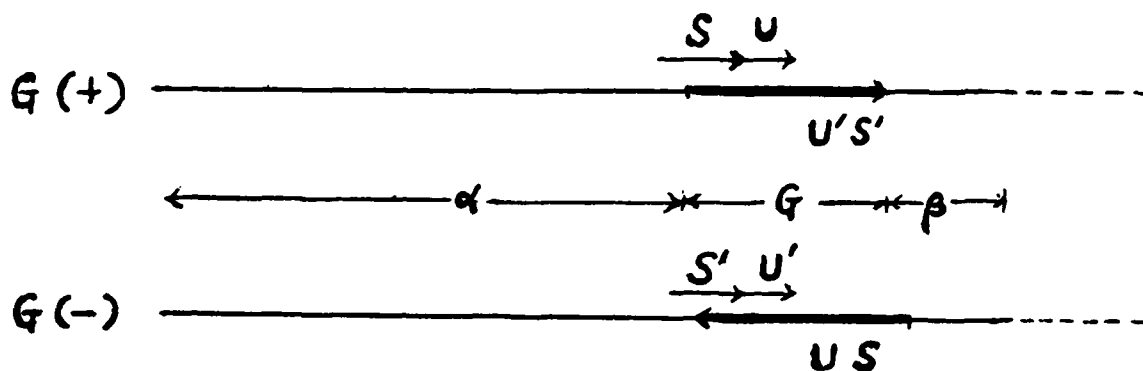


Fig. 8 The invertible G segment of Mu phage contains four tail fiber genes S, S', U and U'. In the + orientation of the G segment, the S and U genes are transcribed while the S' and U' genes are silent. In the - orientation of the G segment, however, the S' and U' genes are transcribed while the U and S genes are silent.

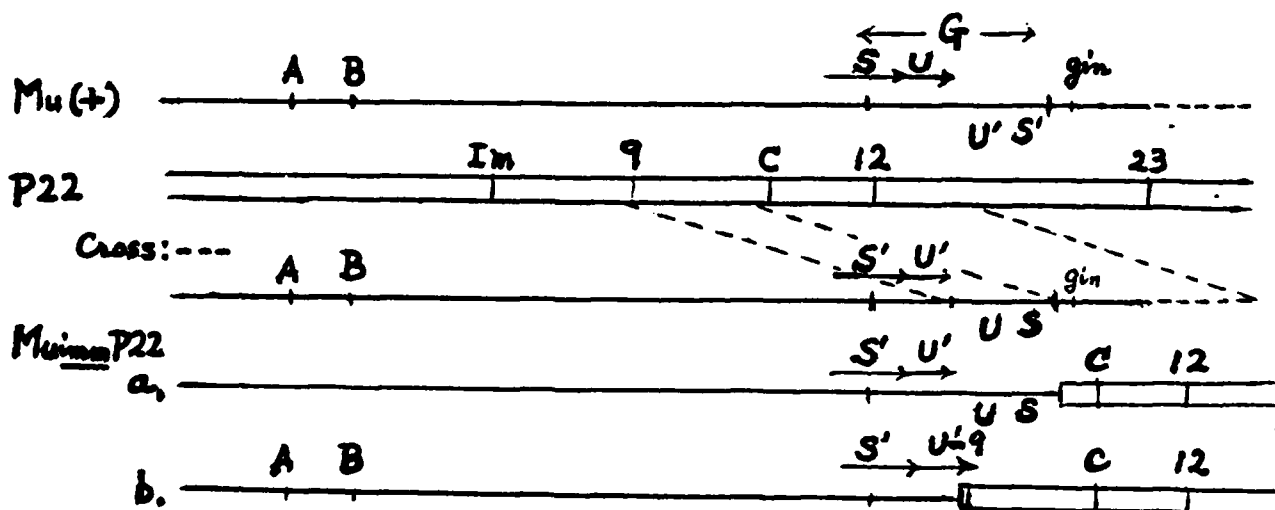


Fig. 9, Formation of hybrids between Mu and P22 phages. MuimmP22 hybrid model a or b.

shown in Fig. 9. However, Fig. 9a model is unlikely because the host control gene (hin) for flagellar phase variation (Silverman et al PNAS 76 , 391, 1979) transacts with the gin gene of Mu phage (Kutsukaki & Ino, PNAS 77, 7338, 1980) and thus should be able to invert the G segment in the MuimmP22 hybrid model of Fig. 9a even in the absence of the gin gene.

Furthermore, inheritance of P22 host range specificity to the hybrids suggests that a short P22 tail spike c-terminal peptide replaced the c-terminal segment of the U' gene products. In fact there are a small accidental homology near the right ends of the P22 tail gene 9 and Mu tail fiber segment U'. Therefore the left arm of the P22 segment is ended at the P22 spike gene 9 and situated near the right end of the U' gene. Backcross experiments with various P22 mutants revealed that the right arm of MuimmP22 ends with the att - erf - c - 12 segment of P22 without interruption by Mu DNA as shown in Fig. 9b.

IV. Partial List of Our Publications

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