High Frequency Transduction by Phage Hybrids Between Coliphage $\phi 80$ and Salmonella Phage P22

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SUMMARY

$\phi 80$immP22dis, a hybrid between $\phi 80$ and P22, carries all the late genes of $\phi 80$ and most of the P22 early region including the immC and immI bipartite immunity loci. The presence of the immI region allows this hybrid to grow on lysogens of $\phi 80$immP22 hybrids which have the immC locus, but not the immI locus. In addition to these P22 immunity regions, $\phi 80$immP22dis contains the P22 att marker so that the prophage can be inserted into the chromosomal P22 attachment site adjacent to the proA–proB region of the host. Unlike its $\phi 80$ parent which performs specialized transduction of the trp region, $\phi 80$immP22dis transduces markers located adjacent to its attachment site to Escherichia coli K12 recipients at high frequencies (0.3% for argF and 0.18% for proA). Induction of $\phi 80$immP22dis lysogens yields new hybrid phage clones which have incorporated E. coli K12 chromosomal segments in place of the P22 immI to att segment. Having lost the immI region, the new hybrids no longer grow in $\phi 80$immP22 lysogens. These new hybrids, termed $\phi 80$immP22dis−, possess specialized transducing properties, transferring the argF and proA markers at higher frequencies (21% for argF and 12% for proA) than previously obtained with the $\phi 80$immP22dis phage.

INTRODUCTION

The availability of Escherichia coli–Salmonella typhimurium hybrids susceptible to Salmonella phage P22 and various coliphages, along with techniques for conversion between the smooth and rough antigenic states of these hosts, have enabled us to isolate hybrids between P22 and coliphage $\phi 80$ (Yamamoto et al., 1983).

Two types of $\phi 80$–P22 hybrid phages were identified: $\phi 80$immP22, which carries a large P22 early gene segment including the x–erf–immC–12 genes, and $\phi 80$immP22dis, a dismune hybrid which carries the immI region in addition to the P22 early region of $\phi 80$immP22 and thus can plate on $\phi 80$immP22 lysogens. The $\phi 80$immP22dis hybrid possesses the P22 att region, so that its prophage is inserted near the pro region of the host chromosome (Yamamoto et al., 1983), rather than at the trp region as is the case with $\phi 80$ (Matsushiro, 1961). Induction of $\phi 80$immP22dis creates new phage types carrying various lengths of the bacterial chromosome which have replaced portions of the hybrid phage genome. In this communication, we report on the characteristics of these phages and their ability to act as specialized transducing phages.

METHODS

Bacteriophages. Coliphage $\phi 80$, Salmonella phage P22 and various P22 mutant derivatives were used in the genetic characterization of $\phi 80$immP22 hybrid classes. Pertinent P22 markers are shown in Fig. 1(a). Wild-type phage P22, hereafter designated P22c+, is temperate and produces turbid plaques. Three clear-plaque mutants of P22c−, (c1, c2, c3) altered in their ability to become prophages (Levine, 1957), were employed in genetic analyses. P22c1 and P22c2 behave like virulent phage, whereas the c3 mutant remains temperate, lysogenizing at frequencies lower than that of P22c+. Amber and temperature-sensitive (ts) conditional lethal mutations of P22

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defective in DNA synthesis genes 12 and 18 (Levine & Schott, 1971) and plaque morphology mutations distinguishable on colour indicator agar were also used as genetic markers for construction of genetic maps and for identification of the hybrid phage homology with P22 (Yamamoto & Weir, 1966). P22 erf mutants, which lack an essential recombination function and do not replicate either in recombination-deficient (recA) strains or in DNA polymerase I defective (polA) hosts, and a P22 x mutant which cannot grow in polA hosts were also employed (Yamamoto et al., 1978). The second immunity region immI, tail gene 9, and antigen conversion gene a1 of P22 were used for characterization of \( \phi 80 \text{immP22dis} \) hybrids.

Besides these P22 mutants, a mixture of high titre stocks of rough-specific Salmonella phages designated R phages (Wilkinson et al., 1972) were used to select P22-sensitive smooth mutants from WR4027 and its lysogenic strains.

**Bacterial strains.** *E. coli*-S. typhimurium recombinant strain WR4028 (Gemschi et al., 1972) served as a host for P22 and various P22 mutants. Strain WR4027 (Gemschi et al., 1972), which is rough and thereby resistant to P22 but sensitive to \( \phi 80 \), and NY4027(\( \phi 80 \)), a \( \phi 80 \) lysogen sensitive to P22, were used for isolation of \( \phi 80 \text{immP22} \) hybrids (Yamamoto et al., 1983). These hybrid bacterial strains contain the lac-argF-metB genes acquired from E. coli K12 (Gemschi et al., 1972; Yamamoto et al., 1978; N. Yamamoto et al., unpublished results).

The *E. coli* K12 strains employed as recipients in transduction experiments were: AB1133 and AT3141, for scoring proA transductants; PC0950 and H11238, for scoring argF transductants; CD4, for scoring metD transductants. These strains were obtained from B. Bachmann, *E. coli* K12 Culture Collection Center, Yale University, New Haven, Conn., U.S.A.

*S. typhimurium* strain Q and its rough derivative, Q/22 (Yamamoto & Weir, 1966) were employed for host range analysis.

**Media.** Nutrient broth for growth of bacteria, agar plates for bacterial colony formation and soft agar for plaquing techniques have been described previously (Yamamoto & Anderson, 1961; Gemschi et al., 1972). Colour indicator agar for scoring phage colour markers was as described by Yamamoto & Weir (1966). Minimal medium supplemented with the required growth factors was used for scoring transductants (Gemschi et al., 1972). M9 minimal medium was used for transduction analysis (Adams, 1959).

**Transduction.** Phage lysates of WR4027 lysogenic for \( \phi 80 \text{immP22dis} \) and phage stocks of \( \phi 80 \text{immP22dis} \) derivatives were diluted to about 10⁶ particles/ml (to reduce any colicin activity present; *E. coli*-S. typhimurium hosts are colicinogenic) and used to infect the appropriate *E. coli* recipient to determine the transduction of various markers. Phage-infected cultures were diluted 10⁻¹, 10⁻² and 10⁻³ and 0.1 ml was spread on minimal agar plates. After 48 h incubation at 37 °C, transductants were scored as growing colonies and tested for the presence of the \( \phi 80 \text{immP22dis} \) prophage.

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**Fig. 1.** Genome structures of the parental phages \( \phi 80 \) and P22 and their hybrids.


RESULTS

Selection and confirmation of φ80immP22dis hybrid type

Hybrids between coliphage φ80 and P22 were isolated after superinfection of NY4027(φ80) by P22. The two types of φ80–P22 hybrids, designated φ80immP22 and φ80immP22dis possess the φ80 protein coat and tail genes (Yamamoto et al., 1983) and carry at least the x–erf–immC–12 segment of the P22 genome. The φ80immP22dis hybrid type carries an additional immunity (imm1) region of P22. This phage is therefore able to plate on host cells lysogenic for φ80immP22 (Table 1). The ability of the φ80immP22dis hybrid to produce plaques on WR4027(φ80immP22) provides a method for selection of this hybrid type.

Host range of φ80immP22 hybrid phages

As shown in Table 1, both φ80immP22 and φ80immP22dis infected not only WR4027, but could also infect E. coli K12. In the case of the E. coli K12(φ80) host, both phage hybrids produced plaques, whereas K12(φ80immP22) lysogens would only support growth of φ80immP22dis, showing that expression of their P22 immunity responses occurred in E. coli. These hybrids, however, were unable to infect either the smooth or the rough strains of S. typhimurium (Q and Q/22) as such strains lack the φ80 adsorption site (Table 1).

Phage particles lacking the dis function occur in φ80immP22dis phage stocks

During successive cloning of the φ80immP22dis hybrid type, about 5% of the phage particles in φ80immP22dis clones were found to have lost the dis function as demonstrated by their inability to form plaques on WR4027(φ80immP22). Those which have lost the dis function, designated as φ80immP22dis−, were also found to have lost gene 9 or a1. Since the dis− derivatives had lost the imm1–a1 or imm1–9 segment, we attempted to separate the dis− derivatives from the parental φ80immP22dis phage particles by caesium chloride density gradient centrifugation. These dis− phage particles, however, were distributed over a wide range of the gradient. Some dis− particles were denser than the parental φ80immP22dis hybrid type, which suggests that the dis− derivatives are not deletion mutants of the φ80immP22dis type, but

<table>
<thead>
<tr>
<th>Host strain</th>
<th>Phage</th>
<th>P22</th>
<th>φ80</th>
<th>φ80immP22</th>
<th>φ80immP22dis</th>
<th>φ80immP22dis−</th>
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<tr>
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<tr>
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<td>S</td>
<td>I</td>
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<td>NY4027(φ80immP22dis−)</td>
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<tr>
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<td>R</td>
<td>R</td>
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<tr>
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<td>R</td>
<td>R</td>
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<td>R</td>
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</table>

* S. Phage growth and plaque formation; I, immune; R, resistant (no adsorption).
N. YAMAMOTO AND OTHERS

Fig. 2. Formation of the high frequency transducing phage hybrid φ80immP22dis- from φ80immP22dis.

rather are formed by replacement of the lost segment with host DNA. This event could have occurred during the prophage insertion–induction process, because some of the temperate phage particles always underwent the lysogenic cycle during propagation of the phages.

Isolation of φ80immP22dis- derivatives from lysates of WR4027(φ80immP22dis)

Since φ80immP22dis carries the att region of P22, its prophage is stably inserted in the P22 attachment site near the pro region of the host chromosome (Yamamoto et al., 1983). More than 10% of phage particles released from WR4027(φ80immP22dis) have lost the capacity to form plaques on WR4027(φ80immP22). These newly isolated φ80immP22dis- clones had also lost the genes between the immI and att regions as measured by self-assembly with tail-less P22 head particles (Israel et al., 1967) defective in gene 9 protein and by testing O-1 antigen conversion of their lysogens. Since the replacement of a sizeable piece of lost phage DNA by extragenomic DNA is required for phage viability, it seems likely that the host chromosomal segment adjacent to the attP22 region is incorporated to form the φ80immP22dis- hybrid type during prophage induction of φ80immP22dis lysogens. As shown in Fig. 2(d), the bacterial chromosomal segment to the left of the attP22 region would have substituted for the prophage immI–att segment.

Genome structure of φ80immP22dis-

Since the dis- hybrid type appeared to have lost P22 genes between immI and att of φ80immP22dis, the extent of the remaining homology between P22 and φ80immP22dis- was analysed. A single host for both smooth-specific P22 phage and rough-specific φ80immP22dis-
High frequency transduction by \(\phi 80-P22\) hybrids

Table 2. Transducing ability of \(\phi 80\)imm\(P22\)dis\(^-\) and \(\phi 80\)imm\(P22\)dis

<table>
<thead>
<tr>
<th>Phage lysate</th>
<th>argF</th>
<th>proA</th>
<th>metD</th>
<th>Trp</th>
</tr>
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<tbody>
<tr>
<td>(\phi 80)imm(P22)dis(\dagger)</td>
<td>0.3%</td>
<td>0-18%</td>
<td>0.11%</td>
<td>(&lt;1 \times 10^{-6})</td>
</tr>
<tr>
<td>(\phi 80)imm(P22)dis(\dagger)</td>
<td>21-0%</td>
<td>12-0%</td>
<td>7-0%</td>
<td>(&lt;1 \times 10^{-7})</td>
</tr>
</tbody>
</table>

* All transductants were lysogenic for \(\phi 80\)imm\(P22\)dis\(^-\).
† A spontaneous lysate of a \(\phi 80\)imm\(P22\) dis lysogen.
‡ A \(\phi 80\)imm\(P22\)dis\(^-\) derivative from the above lysate.

Phage is not available. Therefore, a smooth lysogenic derivative, NY4027(\(\phi 80\)imm\(P22\)dis\(^-\)), was isolated by superinfecting WR4027(\(\phi 80\)imm\(P22\)dis\(^-\)) with rough-specific Salmonella phages (R phages). Superinfection of NY4027(\(\phi 80\)imm\(P22\)dis\(^-\)) with P22c2ts12 phage produced a number of P22 recombinants detectable when assayed on the smooth host, WR4028. The total recombination frequency in P22 was about 2\(^%\), ranging from 0-7\(^%\) to a maximum of 2-5\(^%\), depending on the \(\phi 80\)imm\(P22\)dis phage used. The frequencies of P22 recombinant types (c+ts\(^+\), c2ts\(^-\) and c+ts12) are explicable on the basis of double crossover events. Relative map distances rather than absolute map distances were determined because of the low frequencies obtained.

As shown in Fig. 1, the length of the homologous region was divided into three segments, I, II and III, corresponding respectively to the distances from the left end of the homologous region to c2, from c2 to ts12, and from ts12 to the right end of the homologous region. Relative crossover frequencies in I and II are computed from counts of P22c+ts\(^+\) and P22c2ts\(^+\) recombinants, both of which had the second crossover in region III. Relative crossover frequencies in II and III were calculated from counts of P22c+ts12 and P22c+ts\(^+\) recombinants, both of which had the second crossover in region I. To compare these three segments, segment II was arbitrarily assigned the value of 1, since segment II has the same physical length in all \(\phi 80\)imm\(P22\)dis\(^-\) hybrids.

Such a recombinational analysis of results from crosses between P22c2ts12 and three \(\phi 80\)imm\(P22\)c+ts\(^+\)dis\(-\) strains (no. 5, no. 9 and no. 16) showed that the relative sizes of the three P22 segments I:II:III in the three \(\phi 80\)imm\(P22\)dis\(^-\) strains were 2:1:4.45 for strain no. 5, 1.96:1.4:50 for strain no. 9 and 2.36:1.2:25 for strain no. 16. From these results, it may be concluded that segment III, the right arm of the P22 region, varies in size, whereas segment I, the left arm of the P22 region, is about twice as long as segment II. These ratios suggest that the left end of the P22 homology ends at about the att region of P22 in all \(\phi 80\)imm\(P22\)dis\(^-\) phages so far tested. The \(\phi 80\)imm\(P22\)dis\(^-\) phages had therefore lost the immI-att segment of P22 which was replaced by host chromosomal DNA. This mapping procedure is similar to the method previously used to map the homologous region between P221 and P22 (Yamamoto & Weir, 1966) and between P22 and \(\lambda\)imm\(P22\) (Yamamoto et al., 1978).

High frequency transduction by \(\phi 80\)imm\(P22\)dis\(^-\) hybrid types

Our findings indicate that \(\phi 80\)imm\(P22\)dis\(^-\) resulted from loss of the right arm of the \(\phi 80\)imm\(P22\) prophage and acquisition of portions of the bacterial chromosomal segment situated at the left of the att\(P22\) region (Fig. 2c,d). Therefore, it seemed likely that these newly constructed phage hybrids contain pro and adjacent E. coli K12 genes. Consequently, we anticipated that these new \(\phi 80\)imm\(P22\)dis\(^-\) hybrids should be high frequency of transduction phages. Since \(\phi 80\)imm\(P22\)dis\(^-\) hybrids are able to infect E. coli K12, a transduction assay system was employed with E. coli K12 auxotrophic mutants. The results of such transduction experiments, summarized in Table 2, indicate that both spontaneously induced lysates of \(\phi 80\)imm\(P22\)dis lysogens and \(\phi 80\)imm\(P22\)dis\(^-\) derivative phage stocks grown in WR4027 were able to transduce genes at an extremely high frequency. A \(\phi 80\)imm\(P22\)dis-induced lysate tested was able to transduce arg\(F\) at a frequency of 0.3\(^%\) and pro\(A\) at 0.18\(^%\) to E. coli recipients, but as expected, did not transduce the unlinked K12 trp gene. A phage stock from a \(\phi 80\)imm\(P22\)dis\(^-\) plaque was able to transduce the E. coli auxotrophs at even higher frequencies: 21\(^%\) for arg\(F\) and
12% for proA (Table 2). These transductants were all lysogenic and the transduction frequencies of $\phi80immP22dis^{-}$ corresponded to the frequency of lysogeny exhibited by the $\phi80immP22dis^{-}$ hybrids. These results show that the transduction frequency is close to 100% when compared to the occurrence of stable lysogens. Thus, we can conclude that the $\phi80immP22dis^{-}$ derivatives carry chromosomal genes situated to the left of the attP22 region and act as transducing phages, but are not defective phages. As shown in Table 2, some of the $\phi80immP22dis^{-}$ hybrids tested were able to transduce metD in addition to the argF and proA markers.

**DISCUSSION**

The isolation of two types of $\phi80$–P22 hybrids further extends our previous studies on genetic recombination between P22 and coliphages. As was previously observed from analyses of hybrids between $\lambda$ and P22, the $\phi80$–P22 hybrids result from an interchange of functional gene clusters. Thus, both hybrid classes ($\phi80immP22$ and $\phi80immP22dis$) were shown to have inherited the x-erf-immC–12 gene cluster of P22, but retain the protein coat properties of $\phi80$. The $\phi80immP22dis$ hybrid also carries the second P22 immunity (immI) region (Table 1). In addition, this class also gained the P22 att region as evidenced by its ability to lysogenize hosts with incorporation of the hybrid phage genome into the pro-linked P22 insertion site of the host chromosome rather than the native $\phi80$ site near the trp operon.

Unlike defective P22 phage (Hoppe & Roth, 1974), $\phi80immP22dis$ lysogens were found to produce viable $\phi80immP22dis^{-}$ derivatives with specialized transducing abilities following induction. As shown in Table 2, a spontaneous lysate from a $\phi80immP22dis$ lysogen and a $\phi80immP22dis^{-}$ derivative phage stock were able to transduce genes at an extremely high frequency. The high transducing frequencies of the induced $\phi80immP22dis$ lysates are due to the presence of $\phi80immP22dis^{-}$ particles. These transductants were all found to be lysogenic, indicating the transducing frequency with $\phi80immP22dis^{-}$ to be almost 100% when compared to the occurrence of stable lysogens. The results thus show that $\phi80immP22dis^{-}$ derivatives are viable and carry host genes situated at the left of the attP22 region. Since argF and proA are co-transducible, $\phi80immP22dis^{-}$ derivatives should carry at least argF and proA (Fig. 2d). This observation implies that the P22 attachment site is situated to the right of argF (Fig. 2a) on the E. coli K12 map (Bachmann, 1983). Illegitimate crossing-over during excision of the $\phi80immP22dis$ prophage is likely to result in formation of specialized transducing phage, $\phi80immP22dis^{-}$ carrying the host argF and proA genes in place of the a1, 9 and immI regions. Specialized transduction for proC and for lac has not been detected among $\phi80immP22dis^{-}$ derivatives.

These co-transduction analyses of argF and proA suggest that in the E. coli segment the argF and proA genes are situated on the left side of the attP22 site which is located between the argF and lac markers. To confirm this result in E. coli K12, we lysogenized K12 strains with $\phi80immP22dis$ and isolated $\phi80immP22dis^{-}$ phages which were able to transduce argF at 2% and proA at 0.9% to K12 recipients. These co-transductions with viable phages suggest that the attP22 marker is located between argF and lac in E. coli K12, in agreement with the attP22 position relative to argF and proA in the E. coli–S. typhimurium hybrid. Hoppe & Roth (1974) reported that attP22 in K12 is located between proAB and argF. The difference in the map location of attP22 as determined by Hoppe & Roth (1974) and as reported here by us could be due to an inversion of the attP22–argF segment among the K12 strains studied (J. Roth, personal communication).

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