Somatic 0–1 Antigen Conversion of *Salmonella typhimurium* by a Type B Phage P221dis, Hybrid between P22 and Fels I Phages

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**SUMMARY**

A type B *Salmonella* phage P22I, derived from recombination between a type A phage P22 and a type B phage Fels I, carries the protein coat of Fels I and the P22 early genes, at least the c to h21 genes. One of the P22I strains, P221dis, is disimmune over P22I lysogens and co-immune with P22. Thus it carries the Im gene (the second immunity region) of P22 to establish co-immunity with P22. Since the att region and at1 gene for somatic 0–1 antigen conversion of P22 are located between the Im and c genes, the P221dis prophage attachment site and 0–1 antigen of P221dis lysogens were analysed. P221dis prophage is integrated at the attP22 site near the pro A region of the bacterial chromosomes and expresses the somatic 0–1 antigen.

**INTRODUCTION**

The general transducing *Salmonella* phage P22 can recombine with the prophages Fels I and Fels 2 of *Salmonella typhimurium* LT-2 (abbreviated St) to evolve into new phage types at extremely low frequencies (about 10⁻¹⁰ or less; Yamamoto & Anderson, 1961; Yamamoto, 1967; Yamamoto, 1969). Thus the hybrid phage species consist of parts of P22 and the other parental phage genomes.

One of the hybrid phage species, P221 which is a hybrid between phage P22 and the prophage Fels I, has been extensively studied in our laboratory. P221 has a flexible tail without a base plate, whereas the bacteriophage P22 has a short tail with a hexagonal base plate but no contractile sheath. Neither head nor tail antigens of P221 are related to those of P22 (Yamamoto & Anderson, 1961). Fels I is serologically and morphologically indistinguishable from P221. Thus, P221 retains the protein coat of Fels I (Yamamoto, 1967). The heat stability test (Boyd, 1950) for phage particles showed that P221 and Fels I phages belong to type B phages. However, P22 and P221 carry a large genetic homologous segment containing at least the c (c1, c2 and c3), g and h21 markers. Mixed infection of a host bacterium with P22 and P221 produced particles in which there were genomic masking particles carrying P22 genomes in P221 capsids and P221 genomes in P22 capsids. Moreover, the genetic markers of P22 can be exchanged with those of P221 (Yamamoto & Anderson, 1961). A large number of these P221 strains have been isolated and mapped for genetic homology between P22 and P221. The length of the homologous region varies among P221 strains (Yamamoto, 1967; Yamamoto, 1969; Fukuda & Yamamoto, 1972).

No genetic homology between P22 and Fels I phages has been demonstrated (Yamamoto, 1967). Furthermore DNA-DNA hybridization studies showed no significant homology...
between P22 and Fels I phage DNAs (Akiyoshi & Yamamoto, 1970). Even if there were no genetic homology between phages, the same sequence of a few triplets should be found here and there among the thousands of triplets in both chromosomes. If such small accidental homologous segments (subgenic homology) provide the opportunity for recombinational crossovers, we should be able to find hybrids between a variety of unrelated phages even though these might occur at an extremely low frequency. Support for this expectation has been obtained with various phage systems including not only hybrids between the Salmonella phages but also hybrids between P22 and λ (Yamamoto, 1969; Gemski et al. 1972; Yamamoto et al. 1977; Yamamoto, 1978). The above hypothesis also suggests a possibility that crossovers between unrelated phages may occur at many different locations but with an extremely low frequency. However, the frequencies of viable hybrid phage formation may also depend on proper combinations of essential genes and their regulation for phage replication. This plausible hypothesis could explain the rarity of the creation of viable hybrid phages and the variation in length of the homologous regions between P22 and P221. All P221 strains must carry at least the c (c1, c2 and c3) genes of P22 because the isolation host system is selecting this hybrid phage by the c genes (Yamamoto, 1967). Although P22 and P221 share the c regions, most P221 lysogens are not immune to P22 infection because P22 carries the two immunity genes, c and Im. However, one of P221 groups, P221dis, confers host bacterium immunity to superinfection with P22 (Yamamoto, 1967). P221dis carries a large genetic segment containing the c (c1, c2 and c3), g, h21 and Im (2nd immunity gene) genes of P22. In addition to these P22 genes, all P221dis group phages can confer somatic o−i antigen conversion to host S. typhimurium strains.

In this communication we describe the characterization and genetic structure of P221dis and discuss its capacity for somatic o−i antigen conversion in Salmonella typhimurium.

METHODS

Bacteriophages. Salmonella phages P22 and Fels I and various P22 mutant derivatives were used in the isolation and genetic characterization of hybrids, P221, between P22 and Fels I phages. Pertinent P22 markers are shown in Fig. 1. Wild-type phage P22, hereafter designated P22c+, is temperate and produces cloudy plaques with readily discernible haloes. Three clear plaque mutants of P22, c (c1, c2 and c3), altered in ability to become prophage (Levine, 1957), were employed in genetic analysis. P22c1 and c2 behave like virulent phages, whereas the c3 mutant remains temperate, lysogenizing at frequencies lower than that of P22c+. Temperature sensitive (ts) conditional mutants of P22 concerned with DNA synthesis (genes t2 and t8; Levine & Schott, 1971) and plaque morphology mutants distinguishable on colour indicator agar, were also used for constructing hybrid phages. P22h21 produces a pale green plaque on colour indicator agar whereas the presence of the wild-type h21+ marker results in a dark green plaque (Levine & Curtiss, 1961). The m3 mutation in P22 produces plaques with a brown halo (Levine & Curtiss, 1961). P22erf mutants (Yamagami & Yamamoto, 1970), which lack an essential recombination function and do not replicate either in recombination deficient (recA−) strains or in DNA polymerase I (polA−) host, and a P22x mutant which cannot grow in polA− hosts, were also employed (Yamamoto et al. 1977). In addition, integration (int−) mutant derivatives (Smith & Levine, 1967) and P22Im mutants, which are unable to grow in P221 lysogens, were used.

Bacterial strains. The bacterial strains used were Salmonella typhimurium Q1 (abbreviated Q) and its P22-resistant mutants, Q/22, and P221-resistant mutant, Q/221 (Table 2). Bacterial
Salmonella antigen conversion by phage P22idis

strains lysogenic for Fels I or P221 and their double lysogen, Q(Fels 1), Q/22(Fels 1), Q(P221), Q(Fells 1, P221), Q/22(P221) and Q/22(Fells 1, P221) were prepared for this study. Q/22, Q/22(Fells 1) and Q/22(Fells 1, P221), are resistant to both P22 and its previously described host range mutant P22h (Yamamoto & Anderson, 1961). Thus, they are useful for cloning new hybrid phage species. Recombination deficient (recA-) mutants of Q and S. typhimurium LT-2 (abbreviated St) and a polA- mutant of Q, described in this paper, were used for the study of the erf and x genes of P22 and a pol- derivative of Q/22 for characterization of these P22 markers in P221 hybrids.

An S. typhimurium LT-7 mutant in which the P22 attachment site is deleted, proAB47 (Smith & Levine, 1965) and its derivative carrying a plasmid Flac + pro + attP22, kindly supplied by Dr L. S. Baron, were used to analyse the prophage attachment site for P221 and P22idis.

For somatic O-1 antigen conversion analysis, St, its semi-rough (rfaK) mutant St/22 (Yamamoto & Anderson, 1961; Naide et al. 1965), and deep rough rfa mutant TA1535 (Ames et al. 1973) were used. In addition, Q and LT-7 and their P22-resistant mutants Q/22 and LT 7/22 were also employed.

Media. Nutrient broth consisting of 8 g of Difco nutrient broth and 5 g sodium chloride per litre of distilled water was used for the preparation of phage lysates and bacterial cultures. For phage titrations, we used an agar base containing 23 g Difco nutrient agar and 5 g NaCl per litre with an overlay of soft nutrient agar consisting of 7.5 g Difco bacto-agar, 5 g NaCl and 8 g of Difco nutrient broth per litre of distilled water. Phosphate buffered saline contained 0.067 M-phosphate in 0.1 M NaCl at pH 7.0.

Colour indicator agar, containing 1% Bacto-tryptone, 0.3% yeast extract, 0.5% NaCl, 1.5% Difco bacto-agar, 0.6% glucose, 0.006% aniline blue (Matheson, Coleman and Bell, Norwood, Ohio) and 0.02% bromocresol purple (Harman-Leddon Co., Philadelphia, Pa.) was used to distinguish the colour markers of P22. The dyes and sugar were autoclaved separately and added to the other components just before preparing agar plates. The pH of this medium was about 7.0. Soft nutrient agar was also used as an overlay on this colour indicator agar (Yamamoto & Weir, 1966 a).

Preparation of high titre stocks of P22 grown on Q or Q(Fells 1). Between 5000 and 50000 P22 particles were mixed with 10^8 bacterial cells of Q or Q(Fells 1) and plated on fresh nutrient agar plates. After incubation at 37°C for 12 h, the soft agar was scraped under sterile conditions and placed into 3.0 ml buffered saline. After standing for 30 to 60 min at room temperature, the sample was centrifuged in a table top centrifuge at 2500 rev/min for 15 min to precipitate agar and bacterial debris. The supernatant usually contained about 0.5 to 2 × 10^12 P22 phage particles per ml.

Antiserum. Anti-P22, anti-P221 and anti-Fels 1 sera were previously prepared (Yamamoto & Anderson, 1961; Yamamoto, 1967). Anti-P221 neutralizes Fels 1 as well as P221 and Fels I is antigenically indistinguishable from P221 (Yamamoto, 1967). Dr Tseng-Tong Kuo kindly supplied us with anti-Salmonella O-1, 3, 19 serum which was prepared by the Communicable Disease Center, Atlanta, Georgia. This serum was diluted 1:10 in 0.7% NaCl for detection of the O-1 antigen conversion by slide agglutination.

RESULTS

Selective isolation and characterization of P22idis hybrids

S. typhimurium phage P22 has been previously shown to recombine with Fels 1 to yield a hybrid phage (termed P221) class antigenically indistinguishable from Fels 1 (Yamamoto,
Using Fels I, lysogenic strains of *Salmonella typhimurium* strains Q and Q/22, abbreviated Q(Fels I) and Q/22(Fels I), respectively, were prepared. When P22 stocks grown on Q(Fels I) were plated on Q/22(Fels I), small plaque forming P22I phage were found at a frequency of about $10^{-10}$. A number of these P22I strains were isolated and characterized. These P22I strains were subdivided into a few groups by means of host range, immunity response and their inheritance of P22 markers (Yamamoto & Weir, 1966b; Yamamoto, 1967). The genetic homology between P22 and P22I has been analysed by recombination experiments. The length of the homologous region between P22 and P22I varies from strain to strain of P22I. The majority of P22I strains carry the c1, c2, c3, g, and h2I markers of P22 (Yamamoto & Weir, 1966a). Although P22 and P22I share the c regions, P22I lysogens are not immune to P22 infection whereas P22 lysogens are immune to P22I infection (Yamamoto, 1967; see Table I).

However, when P22c2 phage stocks grown on Q(Fels I) were plated on a doubly lysogenic strain Q/22(Fels I, P22c) a few small and clear plaques similar to those of P22IC2 were found at a frequency of about $10^{-12}$. These small plaque formers were purified by stepwise cloning on Q/22(Fels I) three times and on Q/22(threetimes to free them of Fels I and P22I and tested for their antigenicity. Anti-P22I serum inactivated these new clones and P22I with about the same rate constant, $k$, of 122 min$^{-1}$ whereas these clones were unaffected by anti-P22 serum which inactivates P22 with a rate constant of 1150 min$^{-1}$. Due to their antigenicity and capacity to plate on P22-resistant bacterial strains and their P22I lysogens, we considered these clones to represent a new group of P22I, henceforth designated as P22Idis. Table I shows that Q lysogenic for P22Idis is immune to super-infection with P22 and P22I lysogens are also immune to P22Idis phage. In addition P22Idis lysogens are immune to P22I whereas P22I lysogens produce plaques of P22Idis. Since P22Idis is disimmune over P22I lysogens and co-immune with P22, P22Idis should carry the Im gene (the second immunity region) in addition to the c genes of P22 to establish co-immunity with P22 (Yamamoto, 1967).

**P22 genes found in P22Idis hybrids**

Although P22Idis plaques appear very small on Q/22 and selective indicator hosts, it was evident that the c markers of P22Idis hybrids mimic those of the P22 strain employed in preparing lysates on Fels I lysogens, Q(Fels I). For example, when a wild type P22c+ strain is used for infection, the P22Idis hybrids exhibit turbid plaques. In contrast, infection with the c1, c2 or c3 clear plaque mutants of P22 result in P22Idis derivatives which express the corresponding degrees of clearness typical of the P22 mutant employed. These findings thus confirmed that the P22Idis hybrid class contains the c locus of phage P22 and conserves the protein coat of Fels I.

P22Idis hybrid carrying P22Im mutant phenotype cannot be isolated because both P22Idis Im and P22I should show the same immunity response and be unable to grow in P22I lysogens. Thus the presence of the Im+ phenotype in P22Idisc+ was tested by being backcrossed with P22Imc2. Some of the P22 recombinants carrying c+ phenotype were found to be also Im+ phenotype, indicating that P22Idis has the Im gene of P22.

As shown in Fig. 1, genes I8 and I2 of P22 have been positioned to the right of the c regions of P22 phage. Moreover, since genes I8 and I2 of P22 (Levine & Schott, 1971) function in phage DNA replication, we first attempted to isolate P22Idis hybrids in which these genes had been recombined. *S. typhimurium Q(Fels I)* was superinfected at 25 °C with mutant derivatives of P22 carrying the temperature sensitive (ts) genes I2 or I8 and P22Idis hybrids selected for the inheritance of the c marker of P22 were recovered by
Table 1. Host range and immunity relationships between Salmonella phages

<table>
<thead>
<tr>
<th>Bacteriophage</th>
<th>P22</th>
<th>Fels 1</th>
<th>P221</th>
<th>P221dis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Q(P22)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Q(Fels 1)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Q(P221)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Q(P221dis)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Q/22</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Q/22(Fels 1)</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Q/22(P221)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Q/22(P221dis)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* - , Forms no plaques and is immune or resistant; +, forms plaques and is not immune.

Fig. 1. Diagrammatic representation of parental and hybrid phages.

Salmonella antigen conversion by phage P221dis

By similar crosses, P221dis hybrids recovered after superinfecting Q(Fels 1, P221) with either the P22erf or P22x mutants were unable to grow in Q/22polA−, thus indicating inheritance of the erf-x P22 chromosomal segment. In addition, 13 P221dis hybrids were recovered from crosses in which the superinfecting P22 phage expressed the ts phenotypes of genes erf and x. The presence of these markers in the phage hybrids was established by testing for ability to grow on Q/22polA− at an elevated temperature (39 °C). None of the 13 phage hybrids was able to grow at the non-permissive temperature, thus again indicating the inheritance of the erf-x chromosomal segment of P22.

Other P221dis hybrids were recovered from similar experiments in which the superinfecting phage carried either the h21 or m3 colour indicator marker. Due to the small plaque size of the P221dis hybrids on colour indicator agar, the h21 and m3 phenotypes
could not easily be distinguished. As a consequence, the presence of the markers in P22\textit{dis} hybrids was established in backcrosses with P22. The resulting P22 recombinants were then scored for the inheritance of the colour indicator markers from the P22\textit{dis} hybrid phage strains by plating on \textit{Q/22t}. The results of such analysis indicated that P22\textit{dis} hybrids with the \textit{h2t} allele could be readily isolated. In contrast, inheritance of the P22 \textit{m3} marker was not detected in such P22\textit{dis}.

Various P22\textit{dis} hybrids were also recovered by growing P22\textit{c+int} on \textit{Q/Fels I}. Such P22\textit{dis} hybrids were scored for the phenotype of the P22\textit{int} gene. Lysogens of P22\textit{dis} hybrids derived from the crosses between P22\textit{c+int} and \textit{Fels I} were not inducible, indicating that the \textit{int} phenotype was being expressed in the P22\textit{dis} hybrid. This result suggests that the corresponding \textit{Fels I} marker has been replaced by recombination with the \textit{int} gene of P22.

\textit{Length of the homologous regions between P22\textit{dis} hybrid and P22}

To approximate the length of P22 DNA in P22\textit{dis} hybrids which is homologous to P22, backcrosses were performed between these hybrids and various P22 derivatives. The length of the homologous region between P22 and P22\textit{dis} was estimated by determining the frequency of recombination between various markers after mixed infection of \textit{Q} with P22\textit{dis} \textit{c+ts} and P22\textit{c2tsI2} phages followed by plating on \textit{Q/22t} to select P22 recombinants.

In recombination experiments with P22\textit{c2tsI2} and P22\textit{dis} \textit{c+ts}, the total recombination frequency in P22 was about 2\%, ranging from a minimum of 0.2\% to a maximum of 3\%. All recombinant types could be explained on the basis of double crossover events. Relative map distances rather than absolute map distances were determined because of the low recombination frequency. As shown in Fig. 1, 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 \textit{c2}, from \textit{c2} to \textit{tsI2}, and \textit{tsI2} to the right hand end of the homologous region. Since all recombinants are due to double crossovers within the region of homology, the frequencies of recombination should be proportional to the products of the lengths of the two segments in which crossover events occur.

Table 2 summarizes such an analysis of recombinants resulting from mixed infection of \textit{Q} with P22\textit{c2tsI2} and P22\textit{dis} \textit{c+ts}. By means of a procedure similar to that employed to map the homologous region between P22 and P22 (Yamamoto & Weir, 1966a, b), we have determined the ratio of the genetic lengths of these segments (Fig. 1) by detecting the number of backcross recombinants found in P22 (Table 2). P22 recombinants, able to grow on \textit{Q/22t} at the non-permissive temperature (39 °C), exhibiting the wild type gene \textit{I2} phenotype were scored for the presence of the \textit{c+} or \textit{c2} marker. Of 3930 such P22 recombinants which acquired the \textit{ts} \textit{phenotype} of P22\textit{dis} \textit{c+ts}, 3061 also obtained the \textit{c+} phenotype. The remaining 869 carried the \textit{c2} phenotype. The 3.52:1:0 ( = 3061:869) ratio of these recombinants represents the size ratio of segments I and II. We also isolated 1914 P22 recombinants carrying the \textit{c+} phenotype by plating the lysate of the above mixed infection on \textit{Q/22t} at room temperature (25 °C). Each of these recombinants was cloned and examined for the ability to grow on \textit{Q/22t} at 39 °C to determine the number of P22\textit{c+} recombinants carrying the \textit{ts} \textit{phenotype}. A total of 1298 clones carry \textit{c+ts} \textit{phenotypes}. The remaining 616 clones carry \textit{c+tsI2} \textit{phenotypes}. The 1:0:2:10 ( = 616:1298) ratio of the recombinants is equivalent to the size ratio of segments II and III. The relative sizes of the three segments are listed in Table 2 and shown in Fig. 1. The absence of scorable markers other than the \textit{c} marker in \textit{Fels I}, used in the present system for P22\textit{dis} hybrid isolation, precluded a definitive measurement of the extent of the \textit{Fels I} segment which P22\textit{dis} hybrids contain.
Table 2. Recombinant classes from mixed infection of *S.* typhimurium Q with *P* 22c2ts12 and *P* 221disc+ts+

<table>
<thead>
<tr>
<th>Procedure 1:</th>
<th>Procedure 2:</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>c</em> marker phenotype <em>tst</em> recombinants</td>
<td><em>ts12</em> marker phenotype of <em>c</em>+ recombinants</td>
</tr>
<tr>
<td>Phage class*</td>
<td>(A) P22c+ts+</td>
</tr>
<tr>
<td>No. of recombinants</td>
<td>3061</td>
</tr>
<tr>
<td>Crossover</td>
<td>I×III</td>
</tr>
<tr>
<td>Crossover ratio</td>
<td>I : II</td>
</tr>
<tr>
<td>Ratio of recombination frequencies</td>
<td>3.52 : I</td>
</tr>
<tr>
<td>Ratio of three segments</td>
<td>I : II : III = 3.52 : I : 2.10</td>
</tr>
</tbody>
</table>

* Predictable recombinant classes: (A) P22c+ts+ = I×III; (B) P22c2ts+ = II×III; (C) P22c+ts12 = I×II.

Prophage attachment site for *P* 221dis

The homology between *P* 221dis and *P* 22 covers a large *P* 22 chromosomal segment containing the *h21*, DNA synthesis (i2 and i8), *c* (c1, c2 and c3), *erf*, *x*, *int* and *In* genes. This large homologous group should also include the *att* region of *P* 22, because the *att* region is adjacent to the *int* gene (Smith, 1968). Therefore, prophage *P* 221dis might be integrated at the (preferred) prophage integration site for *P* 22, near *proA* (Smith & Levine, 1965; Smith & Stocker, 1966; Young & Hartman, 1966). An LT-7 mutant in which a segment including the *proAB* region and the *P* 22 attachment site is deleted, *proAB47* (Smith & Levine, 1965), could not be lysogenized by *P* 221dis. In addition, surprisingly, *P* 221 also could not lysogenize *proAB47*. From these results, it may be suggested that *P* 221 and *P* 221dis phages share the *P* 22 prophage attachment site *attP* 22 of the host bacterial chromosome.

To check this conclusion, a derivative of LT-7 *proAB47*, which carries a plasmid *F lac*+ *pro*+ *attP* 22 was therefore tested for susceptibility to lysogenization by *P* 22, *P* 221 and *P* 221dis. As shown in Table 3, this plasmid carrier was readily lysogenized by any of these phages. Eight *P* 221dis lysogenic derivatives of the plasmid carrier were isolated with eight independent *P* 221dis strains. They were then cultured in nutrient both for 20 h, and plated on MacConkey agar containing 0.1% lactose. After overnight incubation at 37 °C, the total of 25 *lac*− segregants, average of three segregants from each of these lysogens, were isolated and tested for *lysogeny* and *pro*− phenotype. All 25 segregants were found to be *pro*− and not lysogenic for *P* 221dis, suggesting that those which lost the plasmid also lost *P* 221dis prophage. Similar results were also observed with *P* 22 and *P* 221 phages as shown in Table 3. Therefore it is unequivocally concluded that *P* 221dis and *P* 221 carry the *att* region of *P* 22 and thus their prophages are integrated near the *proA* gene of host bacterial chromosome.

Antigen conversion of *S.* typhimurium by *P* 221dis

The somatic o−1 antigen conversion gene *a1* of *P* 22 is located between the *c* and *m*3 genes (Young *et al.* 1964; Gough & Scott, 1972). The homology between *P* 22 and *P* 221 does not carry the *a1* gene. However, *P* 221dis might carry the *a1* gene because calculations from the mapping experiments showed that the left end of the homology between *P* 221dis and *P* 22 extends to beyond 6/7 of the *c2-m*3 distance. Moreover, Gough & Scott (1972) showed that the *a1* gene of *P* 22 is situated at the central region of the *c2-m*3 linkage. Accord-
Table 3. Isolation and characterization of lac- segregants from S. typhimurium LT-7 proAB47 deletion carrying a plasmid Flac+pro+attP22 and lysogenic for P22, P22I or P22idis

<table>
<thead>
<tr>
<th>No. lysogenic strains tested</th>
<th>Deletion</th>
<th>Plasmid</th>
<th>(Prophage)</th>
<th>No. clones examined</th>
<th>Markers lost</th>
<th>Cell type</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 proAB47</td>
<td>Flac+pro+attP22</td>
<td>(P22)</td>
<td></td>
<td>10 pro⁺, P22</td>
<td>proAB47</td>
<td></td>
</tr>
<tr>
<td>5 proAB47</td>
<td>Flac+pro+attP22</td>
<td>(P22I)</td>
<td></td>
<td>58 pro⁺, P22I</td>
<td>proAB47</td>
<td></td>
</tr>
<tr>
<td>8 proAB47</td>
<td>Flac+pro+attP22</td>
<td>(P22idis)</td>
<td></td>
<td>25 pro⁺, P22idis</td>
<td>proAB47</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Somatic 0–1 antigen conversion by P22idis

<table>
<thead>
<tr>
<th>Bacterial strain lysogenic for P22idis</th>
<th>S. typhimurium factor I serum*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smooth S. typhimurium strains</td>
<td></td>
</tr>
<tr>
<td>Q</td>
<td>+++</td>
</tr>
<tr>
<td>St = LT-2</td>
<td>+++</td>
</tr>
<tr>
<td>LT-7</td>
<td>+++</td>
</tr>
<tr>
<td>P22-resistant or semi-rough strains</td>
<td></td>
</tr>
<tr>
<td>Q/22</td>
<td>+</td>
</tr>
<tr>
<td>St/22 (rfak)</td>
<td>+</td>
</tr>
<tr>
<td>LT-7/22</td>
<td>+</td>
</tr>
<tr>
<td>A deep rough LT-2 mutant</td>
<td>rfα</td>
</tr>
</tbody>
</table>

* Anti-Salmonella 0–1, 3, 19 serum was used. The serum was diluted 1:10 in 0.7% NaCl for slide agglutination. ++++, Rapid agglutination in a few seconds (about 5 s); +, slow agglutination (10 to 30 s); -, no agglutination.

Interestingly, 16 P22idis strains were tested for somatic 0–1 antigen conversion of its propagating host bacterium S. typhimurium Q/22. All P22idis strains tested carry the at gene for antigen conversion suggesting that the at gene lies somewhere between c3 and Im genes. We further tested the capacity of 0–1 antigen conversion in various bacterial hosts. P22idis can convert semi-rough (rfak) hosts St/22 and LT-7/22. However, the 0–1 antigen conversion of their smooth parental bacterial strains St, Q, and LT-7 is much more readily demonstrable than that of P22-resistant bacterial strains such as Q/22, St/22 and LT-7/22 as shown in Table 4. This is simply because those smooth strains carry readily accessible attachment sites (i.e. galactose of the smooth 0-specific repeating unit, [Gal-Rha-Man]; Lindberg, 1973) for 0–1 antigen conversion via glucosylation of the galactose (Mäkelä & Stocker, 1969). When a deep rough (rfα) mutant of St, TA1535 (Ames et al. 1973) was examined, we were unable to convert it. Since the deep rough strain lacks the smooth or repeating unit, the somatic 0–1 antigen conversion is a consequence of glucosylation of the galactose moiety of the repeating unit but not the galactose of the core structure.

DISCUSSION

P22I phage carries the c (c1, c2 and c3) genes of P22. Although P22I phage has the protein coat of Fels I, P22I forms plaques on Fels I lysogens. Similarly, P22I lysogens of S. typhimurium are sensitive to Fels I because this hybrid phage contains the P22c region in place of Fels Ic region. In addition, P22 lysogens are immune to P22I. However P22I lysogens are not immune to P22. Thus we established the hypothesis that the P22c gene
Salmonella antigen conversion by phage P22\textsuperscript{dis} 375

product alone cannot repress P22 phage replication. Consequently we also hypothesized that another immunity related gene is responsible for establishing immunity to P22 infection. The discovery of P22\textsuperscript{dis} phage proved the existence of the second P22 immunity gene \textit{Imm} (Yamamoto, 1967). This phage group that we characterized, is co-immune with P22 and dismune (Cohen, 1959) over P22\textsubscript{I} lysogen, thus designated P22\textsuperscript{dis}. Similar phenomena have been reported by others (Gough, 1968; Bezdek & Amati, 1968; Suskind \textit{et al.} 1971), confirming the second immunity region of P22.

The above finding implies that the length of homology between the P22 and P22\textsuperscript{dis} groups is larger than that between P22 and P22\textsubscript{I}. Furthermore, a new P22\textsubscript{I} strain containing the c (c1, c2, and c3) region but lacking the g and h2\textsubscript{I} region of P22 has been isolated. Thus it is concluded that P22 supplies various lengths of its genetic segment to form a variety of P22\textsubscript{I} types.

The present studies on prophage attachment chromosomal sites showed that five P22\textsubscript{I} strains tested are inserted near the \textit{proA} gene. However, Young & Hartman (1966) showed that P22\textsubscript{I} phage integrated between a cluster of tryptophan and a cluster of galactose genes. These data also confirmed the variation of P22 segment in P22\textsubscript{I} phage.

Smooth specific A type phages (Boyd, 1950) such as P22 confer somatic antigen \textit{a} to smooth \textit{Salmonella} hosts but not to P22\textsubscript{I} resistant non-smooth or rough strains. Therefore transfer of various antigen conversion genes to B type phages, which are rough specific or infect both smooth and rough bacteria, provides the opportunity to test various antigen conversions with a variety of bacterial cell types. The use of B type phage carrying antigen conversion genes promises to be a powerful assay method to study specificity of the receptors (e.g. sugar linkage) for antigen conversion by using various colony morphology mutants with well characterized LPS.

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REFERENCES


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