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Genomic structure of phage F22, a hybrid between serologically and morphologically unrelated *Salmonella typhimurium* bacteriophages P22 and *Fels 2*

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Summary

Salmonella typhimurium phage P22 can recombine with a serologically and morphologically unrelated Salmonella phage *Fels 2* to yield the hybrid phage F22 at a frequency of about 10^{-12}. F22 has inherited the entire late genes (protein coat and tail structural genes) of *Fels 2* but carries some P22 early genes. P22 genes in the F22 phage were indentified by marker rescue of various P22 mutants. The F22 genome carries the *x-erf-c-18-12* segment of the P22 genome. A number of P22 amber mutants were also tested to support these data. The F22 region homologous to P22 was mapped by scoring the ratio of P22 backcross recombinant types in lysates of F22 lysogens superinfected with P22*c2ts12*. The ratio of the distances between these markers and the ends of the homologous region was determined. Furthermore a new F22 hybrid designated F22*dis*, containing both P22 immunity regions (*c* and *Im*), was isolated at a frequency of about 10^{-4} by superinfecting F22 lysogens with P22. F22*dis* phage has lost some *Fels 2* gene(s) which have been replaced by the P22 segment containing the *Im* region, resulting in formation of a defective hybrid phage.

1. Introduction

The generalized transducing Salmonella phage P22 is a circularly permuted double-stranded DNA phage with a short tail, a hexagonal base plate and six attached spikes, but no contractile sheath. It can recombine with morphologically and serologically unrelated Salmonella phages and coliphages (Yamamoto & Anderson, 1961: Yamamoto, 1969*a*; Gemski, Baron & Yamamoto, 1972; Yamamoto, Gemski & Baron, 1983). One of the Salmonella phages, *Fels 2*, is morphologically similar to *E. coli* T-even phages, having a long tail with a contractile sheath. Phage F22, a hybrid between P22 and *Fels 2*, carries the *c* immunity region of P22 and the entire late regions of *Fels 2* (Yamamoto, 1969*a*).

It has been believed that genetically unrelated bacteriophages do not recombine. Serologically and morphologically unrelated bacteriophages do not replicate in the same host, due to mutual exclusion (Luria, 1953). However, even unrelated phages without genetic homology should contain the same sequence of a few triplets in many places among the thousands of triplets in their chromosomes. If mutual exclusion is by-passed, recombination should occur at these small accidental homolgies via *recA* mediated crossovers (Yamamoto, 1969*a*, 1978). Therefore, crossovers between unrelated phages, such as P22 and *Fels 2*, occur at many different locations to yield hybrid phages at an extremely low frequency. Such hybrids can be detected only if the hybrid genomes contain all the genes essential for their viability. This hypothesis explains the variation in the lengths of the homologous regions between P22 and hybrid phages (Yamamoto & Weir, 1966; Fukuda & Yamamoto, 1972; Yamamoto *et al.* 1977, 1978, 1983). If products of crossovers between unrelated phage genomes contain a dispensable gene(s) in place of an essential gene(s), they could be detected as defective phages.

During crosses between P22 and F22 phages, we detected a new hybrid phage, F22*dis*, which carries the second immunity region (Im) of P22, but is defective in self-replicating ability. In this communication, we also report mapping of the phage F22 genome and discuss a mechanism of defective bacteriophage formation.

2. Materials and methods

(i) *Bacteriophages*

Samonella phages P22 and *Fels 2* and various P22 mutant derivatives were used for the isolation and

genetic characterization of F22. Wild-type phage P22 is temperate and forms turbid plaques. The clear plaque mutant, P22*c2*, altered in its ability to become a prophage (Levine, 1957), was employed. Temperature-sensitive mutants of P22 concerned with DNA synthesis, genes *12* and *18* (Levine & Schott, 1971), are unable to grow above 37 °C but are able to replicate at 30 °C. The wild type (P22*ts*+) is able to replicate under either condition. We also utilized P22 plaque morphology mutants detectable on colour indicator agar (*h21* and *m3*). The *h21* mutant produces a plaque with a pale green ring while the wild type (*h21*+) produces a dark green ring. The *m3* mutant produces a dark brown halo (Levine, 1957). P22*erf* mutants lack an essential recombination function and cannot replicate in either *recA* hosts (Yamagami & Yamamoto, 1970) or DNA polymerase I-deficient (*polA*) hosts. A P22*x* mutant cannot grow in *polA* hosts (Yamamoto *et al.* 1978). A number of P22 amber mutants isolated in our laboratory were also employed for analysis of P22 genes in the F22 hybrid.

Fels 2 and F22 produce very small or pinpoint plaques, in contrast to P22, which forms large plaques. Isolation of various plaque morphology mutants and genetic mapping of *Fels 2* and F22 have been hampered by their small plaque morphology. However, F22*c2* and F22*ts12* clones have been isolated by crossing F22 with P22 mutants.

(ii) *Bacterial strains*

The bacteria used were derivatives of a smooth *Salmonella typhimurium* stain Q1 (abbreviated *Q*). *Q* is a sensitive host for both P22 and *Fels 2*. The DNA polymerase I-deficient mutant, *QpolA*, was used for identification of P22*erf* and P22*x* mutants. An amber suppressor host, *Qsu*$^+$, was used in the detection of P22 amber mutants. Most P22-resistant *Q* derivatives isolated are also resistant to *Fels 2*. These strains were designated *Q/22*. A few strains which are P22-resistant but *Fels* 2-susceptible, designated *Q/22s*, were isolated and employed in these studies. All attempts to isolate *Q* resistant to *Fels 2* but sensitive to P22 have been unsuccessful. The following lysogenic strains were also prepared: *Q*(*P22*), *Q*(*Fels 2*), *Q*(*F22*), *Q/22s*(*Fels 2*), *Q/22s*(*F22*), *Q*(*F22*, *F22*dis) and *Q/22s*(*F22*, *F22*dis).

(iii) *Media*

Nutrient broth consisting of 8 g Difco nutrient broth and 5 g NaCl per litre of distilled water was used for 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 Difco nutrient broth per litre of distilled water. Phosphate (M/15) buffered saline contained 0·067 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 and 0·02% bromocresol purple was used to distinguish the colour markers of P22. The dyes and sugar were autoclaved separately and added to the other components just prior to preparing the agar plates. The pH of this medium was about 7·0. Soft nutrient agar was used as an overlay on the colour indicator agar.

(iv) *Preparations of P22 phage stocks.*

Confluent lysis plates were prepared by plating approximately 10^5 pfu of P22 on *Q*, using fresh nutrient agar plates. After overnight incubation, the soft agar layer was scraped under sterile conditions and suspended in 2·5 ml buffered saline. After standing at room temperature for 30 min, the sample was placed in a table-top centrifuge of 15 min at 3000 rev/min to precipitate agar and bacterial debris. The supernatant usually contained 10^{11}–10^{12} pfu/ml.

(v) *Isolation of F22 phage*

F22 phage were isolated at a frequency of about 10^{-12} by growing P22 phage in *Q*(*Fels 2*) and plating the resulting lysates on *Q/22s*(*Fels 2*), as has been reported previously (Yamamoto, 1969*a*). F22 stocks were prepared from confluent lysis plates of F22 on *Q/22s*, similar to the method employed above for P22 stock.

3. Results

(i) *Characterization of F22 hybrids*

As shown in Table 1, the smooth strain *Q* is a sensitive host for both P22 and *Fels 2*. *Q/22s* is resistant to P22 but is sensitive host for *Fels 2*. *Q/22s* is also susceptible to F22 phage, suggesting that F22 inherits the structural late genes, or at least the tail region, from *Fels 2*. This has been confirmed by serological studies (Yamamoto, 1969*a*). F22 can form plaques on *Fels 2* lysogens, *Q*(*Fels 2*) and *Q/22s* (*Fels 2*), indicating that F22 does not contain the *Fels 2* immunity region. F22 has been shown to carry the *c* markers of P22 (Yamamoto, 1969*a*). Thus F22 is unable to form a plaque on *Q*(*P22*) due to the action of the *c* repressor of P22 on the F22 genome. However, P22 can form a plaque on *Q*(*F22*), showing the second immunity (*Im*) region (Yamamoto, 1967) of P22 is not contained in the F22 phage.

Clear plaque mutants of P22, P22*c2*, produce turbid (*c*+) plaques on *Q*(*F22c*+) at a frequency of about 10^{-3}–10^{-4}. This type of phenomenon is explained by recombination of P22 with the F22 prophage during superinfection. In mixed infection of *Q* with P22*c2* and F22*c*+, the frequency of P22*c*+ recombinant detection was less than 10^{-4}. This may be explained by some mutual exclusion due to differences in adsorption rates and latent period of these phages. Therefore,

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Table 1. *Host range and immunity relationships between Salmonella phages*

Bacterium	P22	*Fels 2*	F22	F22*dis*
Q	+ (S)	+ (S)	+ (S)	− (N)
Q(P22)	− (I)	+ (S)	− (I)	− (I)
Q(*Fels 2*)	+ (S)	− (I)	+ (S)	+ (S)
Q(F22)	+ (S)	+ (S)	− (I)	+ (S)
Q(F22, F22*dis*)	− (I)	+ (S)	− (I)	− (I)
Q/22s	− (R)	+ (S)	+ (S)	− (N)
Q/22s(*Fels 2*)	− (R)	− (I)	+ (S)	+ (S)
Q/22s(F22)	− (R)	+ (S)	− (I)	+ (S)
Q/22s(F22,F22*dis*)	− (R)	+ (S)	− (I)	− (I)

−, Forms no plaque and is immune (I), resistant (R) or non-permissive (N).
+, Forms plaques and is sensitive (S).

genetic crosses between these phages were performed by superinfecting F22 lysogens with P22 phages.

(ii) *Genetic homology between F22 and P22: P22 genes found in F22 hybrid*

We previously reported that F22 hybrids carry some early genes, at least the *c* region, of P22 phage (Yamamoto, 1969*a*). Since the plaque size of F22 is extremely small, inheritance of P22 colour indicator markers *h21* and *m3* is not demonstrable in colour indicator agar. Thus the presence of these markers in F22 hybrids was analysed by back crossing with P22 colour indicator mutants. We superinfected *Q(F22)* with P22*h21* or P22*m3* mutants. The resulting P22 lysates were then scored for the wild-type colour indicator markers, *h21+* or *m3+*, by plating with *Q* on colour indicator agar. Numerous attempts of this kind gave no evidence for the inheritance of these markers by the F22 hybrid.

Levine & Schott (1971) reported that genes *12* and *18*, the regulatory genes of P22 phage DNA replication, are located to the right of the *c* region, and between *c2* and *h21* genes as shown in Fig. 1. *Q(F22)* was superinfected at 30 °C with mutant derivatives of P22*c2* carrying the temperature-sensitive (*ts*) phenotypes for genes *12* or *18* (*ts12* or *ts18*). These backcrossed lysates were plated on *Q* at 30 °C. Turbid plaque-forming P22 recombinants were cloned and tested for their ability to replicate at 40 °C. A large fraction of the recombinants were able to grow at this non-permissive temperature, showing that P22 genes *12* and *18* are present in the F22 hybrid. These results suggest that the homology between P22 and F22 ends between genes *12* and *h21* on the right side of the *c* region.

We then performed another backcross by superinfecting an amber suppressor strain, *Qsu*$^+$, lysogenic for F22 with a number of P22 amber mutants located adjacent to the DNA regulatory genes (*12* and *18*) and *h21*. When the resulting lysates were plated on wild-type *Q* (no suppressor) recovery of all wild-type phenotypes for amber mutants situated between genes *c2* and *12* was detected, and they are thus contained in the homologous region between F22 and P22. Despite numerous efforts, wild-type P22 recombinants were not recovered for amber mutants to the right of gene *12*. From this we conclude that the homologous region between F22 and P22 ends between the *H14* and *H6* amber mutation alleses, as shown in Fig. 1.

Similarly, the *erf* and *x* genes located to the left of the *c* region were examined. Since the *erf* and *x* mutants are unable to grow in *polA* mutants of *Q*, presence of these P22 markers was demonstrated by marker rescue of their wild-type phenotypes from F22 lysogens detected as plaque formers on *QpolA* in the backcrossed lysates. This suggests that both *erf* and *x* genes of P22 are inherited by the F22 hybrid.

From these results we conclude that the F22 hybrid carries a segment of P22 DNA for early regions containing at least *x-erf-c-12*.

(iii) *Growth behaviour of conditional mutants of P22 on Q(F22)*

When P22*c2ts12* was plated on *Q(F22)*, an equal plating efficiency was observed at both permissive and non-per-

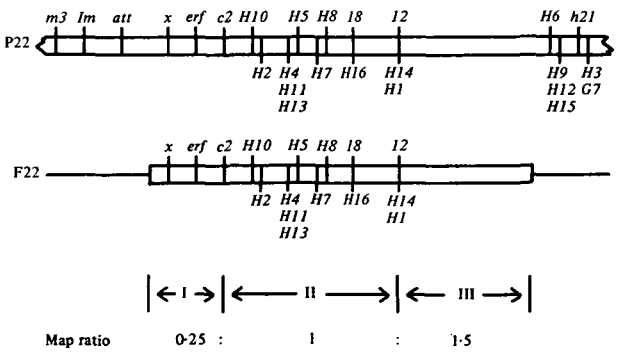

Fig. 1. Diagrammatic representation of P22 and F22 genomes. H1–H16 and G7 indicate amber mutant alleles. Presence of F22 genes was detected by marker rescue of P22 mutants by F22 lysogens. Map ratios were determined by scoring recombinants from lysates of P22*c2ts12* superinfection of wild-type F22 lysogens. Segment II was arbitrarily assigned the value of 1.

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Table 2. *Recombinant classes from infection of Q(F22c+ts+) with P22c2ts12*

	Procedure 1: *c* marker phenotype of *ts+* recombinants		Procedure 2: *ts* marker phenotype of *c+* recombinants	
Phage class	(A) P22*c+ts+*	(B) P22*c2ts+*	(C) P22*c+ts12*	(A) P22*c+ts+*
No. of recombinants	247	1002	131	201
Crossover regions	I+III	II+III	I+II	I+III
Ratio of recombinants*	0·25 :	1	1 :	1·5

* Ratio of the three segments I : II : III = 0·25 : 1 : 1·5.

missive temperatures. This suggests that the gene *12* product of the F22 prophage was expressed in *Q(F22)* cells superinfected with P22. Therefore a number of P22 amber mutant isolates within the early region could be tested by plating on *Q(F22)*. Amber mutants located between *H10* and *H14* formed plaques on *Q(F22)* but not on *Q*, confirming the previous experiments showing that these genes are contained in the F22 genome. The amber mutants located between *H6* and *G7* could not form plaques either on the F22 lysogen or on non-lysogenic strains, indicating that they are not found in F22. An amber mutant for the positive regulator gene *24* was also able to form plaques on *Q(F22)*. These results also support the backcross analysis for genes in homologous region.

(iv) *Length of homologous region between P22 and F22*

To approximate the length of the P22 homologous region in the F22 hybrid, backcrosses were performed between an F22 hybrid and P22 derivatives. A wild-type F22 lysogen of *Q* was superinfected with P22*c2ts12*. The resultant lysate was plated on *Q*, and P22 phage were scored for *c2* or *c+* plaque morphology at permissive and non-permissive temperatures. By means of a procedure similar to that employed to map the homologous region between P22 and P221 (Yamamoto & Weir, 1966), the length of the homologous region between P22 and F22 was estimated by determining the frequency of recombination between various markers of P22.

Analysis of recombinants resulting from infection of a wild-type F22 lysogen with P22*c2ts12* is shown in Table 2. The total recombination frequency was approximately 2×10^{-4}. All recombinant types could be explained on the basis of double crossover events within the homologous region. Relative map distances were determined rather than map units due to 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 *c2*, from *c2* to gene *12*, and from gene *12* to the right end of the homologous region. Since all recombinants are due to double crossovers within the region of homology, the frequency of recombination should be proportional to the products of the lengths of the two segments in which the crossover events occurred. The relative lengths of segments I and II were computed from the frequencies of P22*c+ts+* and P22*c2ts+* recombinants, both of which had a second crossover in segment III. Similarly, relative lengths of segments II and III were calculated from the frequency of P22*c+ts12* and P22*c+ts+* recombinants, both of which had a second crossover in segment I. To compare the lengths of the three segments, segment II was arbitrarily assigned the value of one.

P22 recombinants able to replicate at 37 °C, exhibiting the wild-type *ts+* phenotype, were scored for the presence of the *c+* or *c2* phenotype. Of 1249 P22 recombinants with the *ts+* phenotype, 247 also obtained the *c+* phenotype, while 1002 retained the *c2* phenotype. The ratio of 0·25 : 1 (247 : 1002) of these recombinants represents the ratio of the length of segments I and II. When we plated at 30 °C, we isolated 332 recombinants which expressed the *c+* phenotype. Each of these was cloned and tested for their ability to replicate on *Q* at 37 °C, with comparative 30 °C assay, to determine how many had also acquired the gene *12* wild-type (*ts+*) phenotype. A total of 131 clones retained the *ts12* phenotype while the remaining 201 exhibited the *ts+* phenotype. The ratio of 1 : 1·5 (131 : 201) of these recombinants represents the ratio of the lengths of segments II and III. Therefore, the relative lengths of these segments I : II : III are 0·25 : 1 : 1·5 (Fig. 1).

(v) *Isolation of a defective hybrid: F22dis*

We attempted to isolate F22 hybrids carrying an expanded P22 homology which contains the second immunity *Im* region of P22 in addition to the *c* region. When P22 stocks previously grown on *Q(F22)* were planted on *Q/22s(F22)*, such F22 hybrids, designated F22*dis* (*dis* for 'dismune'), were isolated at a frequency of about 10^{-4}. F22*dis* is also able to form plaques on F22 lysogens of a smooth host *Q(F22)* (Table 1). F22*dis* is unable to form a plaque on *Q(P22)* and P22 is also unable to form a plaque on the F22*dis* lysogen, *Q(F22, F22dis)*. These findings indicate that F22*dis* carries both immunity regions (*c* and *Im*) of P22. Although F22*dis* is able to form plaques on F22 lysogens, it is unable to form plaques on non-lysogenic

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strains, *Q* and *Q/22s* (Table 1). This observation suggests that F22*dis* is defective for an F22 gene(s) which was replaced by a new extended P22 segment in F22*dis*. We expected that this F22 gene(s) was in the *Fels 2* portion of the F22 hybrid. This was confirmed when F22*dis* was able to form plaques on *Q*(*Fels 2*). Thus we concluded that F22*dis* is defective for a *Fels 2* gene(s) contained in the homologous region between F22 and *Fels 2*, which was replaced by the P22 segment on the left of the *x-erf* region extended to the *Im* region.

4. Discussion

In the present study, we mapped the P22 homologous segment of the F22 hybrid phage by analysing the frequency of backcross recombinants with various P22 mutants. The entire span of P22 homology carries the *x-erf-24-c-18-12* genes. Expression of these genes should be controlled by the positive regulatory gene *24* and the negative regulatory gene *c* for bidirectional transcription. Since P22 phage carries the antirepressor (*ant*) gene in the *Im* region, superinfection of F22 lysogens with P22 inactivates the *c* repressor, resulting in expression of all genes in the P22 homologous segment. Like P22 and λ phages, inactivation of the *c* repressor induces the F22 prophage (unpublished observation). This finding suggests that the genes responsible for prophage induction (i.e. *int* and *xis* from *Fels2*) of F22 may be linked to the P22 homologous segment.

The frequency of F22 hybrid formation is extremely low, less than 10^{-11}, suggesting that recombination must take place at extremely small homologous sites (Yamamoto 1969*a*). Since the parental P22 and *Fels2* phages share no genetic homology, they appear to cross over at accidental homologies (Yamamoto, 1969*a*). Such crossovers can occur between various unrelated phage genomes having different organization of functionally comparable genes. This hypothesis is proven by the finding of hybrids between P22 and a coli mutator phage Mu (Yamamoto *et al.* 1981; Yamamoto *et al.* in preparation). Some crossover products between unrelated phages may carry dispensable gene(s) which results in the deletion of essential gene(s) since hybrid genomes must be packaged in the limited space of the hybrid phage head. Such defective hybrids can be detected on hosts lysogenic for a parental phage which supplies the defective function(s). One such defective phage, F22*dis* offers us a new concept for understanding host ranges and host tropism of viruses in general.

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