Bacteriophage P1 Derivatives Unaffected in Their Growth by a Large Inversion or by IS Insertions at Various Locations

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Several plaque-forming phage P1 derivatives carrying DNA rearrangements associated with IS elements are described. They have IS1, IS3 and IS5 inserted in four distinct locations, all of which are non-essential regions for phage P1 propagation. One derivative carries a genome segment, inverted relative to the one in the P1 wild-type genome, between two inverted copies of IS1. The inverted DNA segment spans about 23 kb of the 90 kb long P1 genome and it includes the invertible C segment. This phage is as viable as an isomeric P1 which carries the relevant segment in its original orientation. These results are discussed with regard to the genome organization of phage P1.

INTRODUCTION

Bacteriophage P1 is a temperate phage that propagates in its lysogenic state in Escherichia coli K12 as a plasmid of 90 kb at one to two copies per host chromosome (Yarmolinsky, 1982). The wild-type phage DNA present in the virions is double-stranded, circularly permuted and has a terminal redundancy of about 9 kb. A drug resistance transposon, shorter than the terminal redundancy, inserted into a non-essential region of the P1 genome yields a plaque-forming, specialized transducing phage for the drug resistance marker (Iida & Arber, 1977, 1980). P1 DNA carries one copy of an IS1 sequence as a natural constituent which does not affect essential phage functions (Iida et al., 1978; Meyer et al., 1983). IS1, like other mobile genetic elements, is known to promote intramolecular DNA rearrangements such as deletion and inversion (Iida et al., 1983a). Some of these processes are due to transpositional activities. In others, two identical IS elements simply provide nucleotide sequence homology for reciprocal recombination.

We describe here a plaque-forming P1 derivative carrying an inversion of about one quarter of its genome. The inverted region contains several essential functions and includes the 4.2 kb long invertible C segment (Iida et al., 1982; Yarmolinsky, 1982; Walker & Walker, 1983). This phage was isolated as one of the chloramphenicol (Cm) sensitive derivatives from a plaque-forming P1 Cm phage carrying an IS1-flanked Cm transposon (Arber et al., 1978; Iida & Arber, 1980). The P1 inversion derivative is formed through homologous recombination between the IS1 sequences. It is stable in its genome organization and shows no apparent growth disadvantage as compared with an isomeric, ‘non-inverted’ P1.

We also describe other types of plaque-forming, Cm sensitive P1 derivatives. One of them was produced by transpositional, IS1-mediated deletion, others by insertion of another IS element from the host chromosome. Although the genome structures of these P1 derivatives differ from that of wild-type P1, no phenotypic alteration in these mutants has been detected.

METHODS

Bacteria, phages, plasmids and media. Phage P1 and all its derivatives carry the thermoinducible cts225 mutation (Scott, 1968). Phage lysates of P1 derivatives were prepared by heat induction of Escherichia coli K12
Fig. 1. Restriction cleavage maps of the genomes of P1, P1Cm and P1Cm8 phages. The simplified P1 genetic map at the top represents the entire P1 genome (Yarmolinsky, 1982). The restriction maps of P1 and P1Cm70 are redrawn from published maps (Bachi & Arber, 1977; Arber et al., 1978) and those of P1Cm67 and its derivatives are aligned with the P1Cm70 map. The long bars above the lines indicate HindIII sites, those below the lines BglII sites, the short bars above the lines indicate EcoRI sites and those below the lines BamHI sites. In the immI region not all EcoRI sites are drawn. P1 DNA carries only two XhoI sites which are marked by arrowheads. The vertical lines within the boxes representing IS2 and IS3 elements give the positions of the unique PstI sites. The small boxes represent the 0.62 kb inverted repeats of the C segment (Iida et al., 1982). The numbers 4, 8, 9 and 12 on the P1 restriction map indicate the EcoRI-4, EcoRI-8, EcoRI-9 and EcoRI-12 fragments, respectively. The shaded area marks the position of fragment EcoRI-12 as probed in the Southern hybridization. The integration site of IS5 in P1Cm67-31-B was taken from Iida et al. (1983b).

Designation of IS1 elements: IS1a is the IS1 element resident in P1; IS1b and c are the flanking copies of Tn2671A70 and Tn2671A69; IS1d is a recombination product of IS1b and c; IS1e and f are recombination products of IS1a and d. IS3g is the IS3 element found on P1Cm67 and the second element IS3h is detected on P1Cm67-11-B.

strain WA921 harbouring the prophage (Iida & Arber, 1977). The phage strains used contain at least one IS1, at the site of the resident IS1 of P1 (Iida et al., 1978). This and other relevant features of the phages used are shown in Fig. 1 and their pedigrees are explained in Results and Discussion. Plasmids pRM145 carrying the EcoRI-12 fragment of P1 DNA (Mural et al., 1979) and pPP4R3::IS3, a pPP4R3 derivative with IS3 integrated into the resident IS2 (Sommer et al., 1979), were kindly provided by Dr D. Vapnek, Dept of Microbiology, University of Georgia, USA and Dr H. Saedler, Max-Planck-Institut für Züchtungsforschung, FRG, respectively. The media were as described before (Iida & Arber, 1977).
DNA rearrangements in the P1 genome

Characterization of the genome of the P1 derivatives. Cm sensitive derivatives of the P1Cm phage were isolated through the plaque centre test (Iida & Arber, 1977). Restriction enzymes were obtained from Boehringer or purchased from New England Biolabs, Mass., USA, and were used as recommended by the suppliers.

Preparation of phage and plasmid DNA, restriction cleavage analysis, electron microscopic heteroduplex studies and hybridization of the restriction cleavage fragments with a 32P-labelled DNA probe were performed as described before (Bachi & Arber, 1977; Meyer & Iida, 1979; Iida & Arber, 1980). DNA sequencing was according to Maxam & Gilbert (1977).

Test for stability of P1Cm67 genomes. The genomic stability of P1Cm67 during vegetative phage growth was tested as follows. Subclones of strain WA921, lysogenic for the P1Cm in question, were heat induced. Each resulting lysate was plated with indicator bacteria, strain WA921 to obtain plaques. Starting from single plaques, two parallel secondary phage lysates were prepared by the confluent plate lysate method. The resulting plate stocks were used for infection of strain WA921, and one WA921(P1Cm) lysogen was isolated from each infection. These lysogens were grown up and heat induced. Phage was harvested from the resulting lysates and its genome structure examined by restriction analysis of the extracted DNA.

RESULTS AND DISCUSSION

The genome structures of P1Cm67 and P1Cm70

Plaque-forming P1 phage derivatives P1Cm67 and P1Cm70 were isolated as specialized transducers of the Cm resistance marker which had been derived from the R plasmid NR1-Base1 (Iida & Arber, 1977; Arber et al., 1978). They carried IS1-flanked Cm transposons which are now called Tn2671A67 and Tn2671A70, since these transposons are IS1-mediated deletion derivatives of Tn2671, the IS1-flanked r-determinant of NR1-Base1 (Arber et al., 1978; Iida & Arber, 1980; Iida et al., 1981). Restriction analysis revealed that these two transposons were the same size and were integrated into the same site within the EcoRI-9 segment of the P1 genome and in the same orientation (Fig. 1). Since these P1Cm phages were isolated from the same lysate enriched for plaque-forming P1Cm, the transposons may be of the same origin. However, P1Cm67 carries an additional 1.3 kb insertion which contains one PstI and two HindIII sites (Fig. 1). The internal HindIII-PstI fragments as well as the HindIII fragment produced from this insertion proved to be identical in size to those from IS3 on the plasmid pPP4R3: :IS3 (data not shown), indicating that the insertion is probably IS3.

Cm sensitive derivatives of P1Cm67

P1Cm phages carrying Cm transposons flanked by directly repeated IS1 elements segregate P1Cm derivatives (Iida & Arber, 1977; 1980). Recombination between the IS1 sequences results in the loss of the Cm segment, leaving one copy of IS1 at the insertion site of the Cm transposon. The plaque centre test revealed that such P1Cm derivatives appeared with a frequency of about 1% in the lysates prepared by heat induction of cells lysogenized with P1Cm67 (Iida & Arber, 1977). Judging from the restriction cleavage patterns, 19 out of 21 independently-isolated P1Cm67 derivative had identical and expected genome structures, represented by P1Cm67-11 (Fig. 1). They carry one copy of IS1 at the site where Tn2671A67 had originally been integrated. The two other P1Cm67 derivatives, P1Cm67-30 and P1Cm67-31, are different from P1Cm67-11 and also from each other.

The genome structure of P1Cm67-31 and its stability

Structural analysis revealed that P1Cm67-31 has lost most of the Cm' determinant by IS1-mediated deletion, but still carries the two directly repeated IS1 sequences close together (Fig. 1). Sequencing revealed the DNA between these two IS1 elements to consist of 77 bp (data not shown). This DNA must have originated from the Cm' segment next to IS1c, since the sequence of the IS1c end and the adjacent 77 bp is identical to the sequence carried on a related Cm transposon derived from Tn2671 (Marcoli et al., 1980). Therefore, the deletion was caused by IS1b and removed all the coding sequences of the Cm' (cat) gene together with its promoter (Le Grice et al., 1982).

Like IS1-flanked Cm transposons (Iida & Arber, 1977, 1980; MacHattie & Jackowski, 1977; Meyer & Iida, 1979), the IS1-77 bp-IS1 structure is relatively stable even in rec+ cells.
Nevertheless, among progeny phages prepared by lytic infection we obtained some P1Cm\textsuperscript{67-31} derivatives with a genome structure identical to that of P1Cm\textsuperscript{67-11} (Fig. 1). Thus, recombination between the direct repeats IS\textsubscript{Zb} and IS\textsubscript{Zc} resulted in removal of the 77 bp Cm segment together with one copy of Cm. This also occurred in the prophage state. From subclones of a P1Cm\textsuperscript{67-31} lysogen that had been stored in a stab culture at room temperature for three years, we recovered P1 phages with the parental genome structure as well as others with a genome identical to P1Cm\textsuperscript{67-11}. In addition, we also obtained P1Cm\textsuperscript{67-31-B} (Fig. 1) which carried two copies of IS\textsubscript{I} elements, one IS\textsubscript{J} and one IS\textsubscript{5} insertion. Apparently, this IS\textsubscript{5} had been transposed from the host chromosome into the non-essential res(P1) gene (Iida et al., 1983b).

The genome structure of P1Cm\textsuperscript{67-30} and its stability

Restriction cleavage patterns and Southern hybridization with the \textsuperscript{32}P-labelled EcoRI-12 fragment indicated that the region between the IS\textsubscript{Je} and IS\textsubscript{Jf} in P1Cm\textsuperscript{67-30} is inverted (Fig. 1). This was confirmed by hybridizing the P1Cm\textsuperscript{67-30} DNA to P1 DNA in the original configuration and visualizing the two types of heteroduplex molecules in the electron microscope (data not shown). Thus the inversion in the P1Cm\textsuperscript{67-30} genome can be explained by reciprocal recombination between IS\textsubscript{I} elements. The inverted P1 segment is 23 kb long, spans about one fourth of the entire P1 genome and includes the invertible C segment (Fig. 1).

The inverted region between the two inverted IS\textsubscript{I} sequences on P1Cm\textsuperscript{67-30} is known to code for some P1 functions essential for phage growth (Fig. 2). Although P1Cm\textsuperscript{67-30} carries a large inversion affecting one-fourth of its genome, its burst size upon induction as well as infection was found to be comparable to that of the isomeric P1Cm\textsuperscript{67-11} and of P1 wild-type. The genome structure of P1Cm\textsuperscript{67-30} is stable and we could not detect any enrichment for the isomeric P1Cm\textsuperscript{67-11} genome during vegetative phage growth. Indeed, phage DNA from all four studied P1Cm\textsuperscript{6} phage lysates from two independent experiments, as described in Methods, gave restriction patterns identical to those of the parental P1Cm\textsuperscript{67-30} DNA. The same is true for P1Cm\textsuperscript{67-11}. These results are consistent with the notion that P1Cm\textsuperscript{67-30}, although carrying a large inversion, grows normally and has no significant disadvantage in vegetative growth relative to P1Cm\textsuperscript{67-11}. However, inversion between IS\textsubscript{Je} and IS\textsubscript{Jf} in the P1Cm\textsuperscript{67-30} genome to yield the P1Cm\textsuperscript{67-11} genome was observed in subclones of a P1Cm\textsuperscript{67-30} lysogen kept at room temperature for three years in a stab culture.

Among the subclones of a P1Cm\textsuperscript{67-11} lysogen stored in a stab culture, we found P1Cm\textsuperscript{67-11-B} carrying an additional IS element. Further restriction and electron microscopic analysis
DNA rearrangements in the P1 genome

We have demonstrated here that a phage P1 mutant, P1Cm67-30, carrying a stable inversion of about a quarter of its genome, including the invertible C segment, shows no significant deficiency in its vegetative phage propagation. Many IS insertions in this inverted region result in P1 mutants affected in vegetative phage propagation, and essential genes for P1 growth were mapped in this region (Arber et al., 1980; Iida et al., 1982; Iida, 1984) (Fig. 2). Since the inversion studied does not affect phage growth, it cannot affect transcription of the essential genes carried on this segment of the P1 genome. This is consistent with the idea that transcription of the late genes of P1 occurs in relatively short units, in contrast to late genes of some other temperate phages such as λ (Szybalski & Szybalski, 1979; Sanger et al., 1982). Indeed, the λ-encoded anti-terminator is able to relieve the polar effect caused by IS insertions within an operon (Iida et al., 1983a), and plaque-forming λ derivatives carrying ISI-flanked Cm transposons in the late operon have been isolated (Iida et al., 1980). In the C segment region of the P1 genome, two apparently independent operons of late genes for internal proteins and for phage tail fibres are shown in Fig. 2. The site-specific recombinase gene cin between the two operons shown in Fig. 2 is not a late gene and it is transcribed in the direction opposite to the adjacent late operons (Iida et al., 1982; Hiestand-Nauer & Iida, 1983; Iida, 1984).

The P1 mutants described here carry an inversion or insertion associated with IS elements. These mutants grow well both as lytic phage and in the lysogenic state. Non-lethal genomic rearrangements are of great evolutionary relevance. Indeed, the P1 related, non-defective phage P7 carries several substitutions and insertions including an ampicillin transposon, Tn902 (Yarmolinsky, 1982). Large inversion that affects the alignment of related genomes in general recombination is regarded to be a critical step in evolutionary diversification. Therefore, the observations described here indicate that IS elements play an important role in restructuring replicons (Iida et al., 1983a), thereby acting as one of the principle factors for the biological evolution of bacteria and their episomes.

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REFERENCES


