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# Characterization and Identification of the Bacteriophage P4 Mutant Suppressing *sir* Mutations of Bacteriophage P2

**Kyoung-Jin Kim*, Melvin G. Sunshine[1], and Erich W. Six[1]**

*Department of Microbiology, Sunmoon University, Choongnam 336-840, Korea, and*
*[1]Department of Microbiology, University of Iowa, Iowa City, IA, 52242, USA*

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**Bacteriophage P4 *ost1* was isolated as a suppressor mutant of P2 *sir3* and identified by restriction enzyme site analysis. The mutant DNA turned out to be an imperfect P4 trimer containing deletions. It was suggested that the deletion resulted from *int*-mediated site-specific recombination. CsCl equilibrium density gradient experiment confirmed the genome size of P4 *ost1*.**

**Key words: Bacteriophage P2-P4, *E. coli*, packaging, site-specific recombination, integrase.**

Bacteriophage P2-P4 system provides a unique opportunity to study the mechanism of viral capsid assembly. Bacteriophage P2 is a temperate phage of *Escherichia coli*. It has an icosahedral capsid of about 60 nm in diameter within which 33 kb long P2 DNA resides and has a straight tail of length 135 nm (1). Bacteriophage P4 is a satellite phage of P2. It uses P2 genome as a helper to provide all the late gene products including those for capsid proteins and tails (1). Consequently the phage particles of P2 and P4 are same in appearance, but they differ in their sizes. The P4 capsid is smaller than that of P2. Their DNAs show few sequence homology (less than 1%) except 19 bp long *cos* cleavage site (6). And the 11.6 kb long P4 DNA can be replicated and maintained as a plasmid in host cell in the absence of P2 (3).

Some mutant P4 phages having large, P2-sized, heads filled with two or three P4 genomes were isolated. From the subsequent studies, it was concluded that the *sid* gene of P4 is responsible for the determination of head size (8, 11). The gene product of *sid* turned out to be an outer-scaffolding protein which guides the small capsid assembly with same capsid protein, the gene product of *N* (gpN), of P2 (7). As both genes have been proposed to be involved in the head size determination in P2-P4 system, genetic studies of the capsid assembly in P2-P4 system have focused on the isolation and the characterization of mutations in *N* gene of P2 and *sid* gene of P4.

Six *et al.* isolated the P2 mutants which can still assemble the large head in the presence of *sid* gene of P4 (13). Those mutations were named as *sir* (*sid* responsiveness) and so far, thirteen *sir* mutations were identified in the middle region of *N* gene of P2 (*sir* segment). Most of them (twelve out of thirteen) turned out to be missense mutation. In comparing the burst size of P4 *sid*$^+$ with P2 lysogen and that of P4 *sid*$^+$ with P2 *sir* lysogen, unexplainable discrepancy was observed. With P4 *sid*$^+$ infection, P2 and P2 *sir* lysogens are expected to assemble the large head, and two or three genomes of P4 *sid*$^+$ are expected to be packaged into the large capsid. But the burst size of P4 *sid*$^+$ with P2 *sir* lysogen was smaller than that of P4 *sid*$^+$ with P2 lysogen (see Table 1). As one of P 2 *sir* mutants, P2 *sir3*, shows very strong Sir phenotype and does not allow the plaque formation of P4 (13), the isolation and characterization of P4 suppressor of P2 *sir3* would give valuable information for the capsid assembly and packaging.

In this communication, we isolated and characterized a P4 suppressor for *sir3* mutation of P2, P4 *sid71 ost1* (*ost* means overcome sir three and abbreviation form is P4 *ost1*) to explore the mechanism of phage head assembly and packaging in P2-P 4 system. P4 *ost1* was isolated as a rare plaque former by plating P4 *sid71* (8) phage stock (about $10^8$ phages) with P2 *sir3* lysogen.

At first, we characterized this phage by the burst size determination with wild type P2 lysogen (C295), P2 *sir2* lysogen (C2142) and P2 *sir3* lysogen (C2143) (12, 13). All strain used in this experiments were derived from C1a, a type strain of *E. coli* C (10). To

* To whom correspondence should be addressed.
(Tel) 0418-530-2273; (Fax) 0418-541-7425
(E-mail) kyjkim@omega.sunmoon.ac.kr

**Table 1.** Burst sizes of P4 and its derivatives with various P2 lysogens

| Phage | Burst size with C1a lysogenic for P2 *sir*⁺ | P2 *sir2* | P2 *sir3* |
|---|---|---|---|
| P4 | 111 | 1.4 | 0.2 |
| P4 *sid71* | 41 | 4.4 | 2.0 |
| P4 *ost1* | 91 | 93 | 22 |

determine the burst size, one-step growth experiments were done according to Six and Klug (12). As shown in Table 1, P4 *ost1* showed large burst size not only with P2 *sir3* lysogen but also with wild type P2 lysogen.

The identification of P4 *ost1* was carried out by conventional restriction enzyme site analysis. P4 phage DNA was prepared by phenol extraction according to Lindqvist (5). Agarose gel electrophoresis of *Bam*HI-digested P4 *ost1* DNA showed the expected P4 DNA fragments and an extra 1.06 kb long fragment. Subsequent enzyme digestion studies revealed that the 1.06 kb *Bam*HI fragment resulted from a deletion in the 5.2 kb *Bam*HI fragment of P4 DNA (Fig. 1). Overall the band intensity of deletion-containing fragments was stronger than that of counterpart fragments without deletion. This suggested that more than one copy of identical deletion-containing fragment might be present in P4 *ost1*. Based on these findings, we deduced a P4 *ost1* genome as an imperfect P4 *sid71* trimer consisting of one complete P4 genome and two P4 genomes with the same deletion (Fig. 2).

The deletion-containing 1.06 kb *Bam*HI fragment

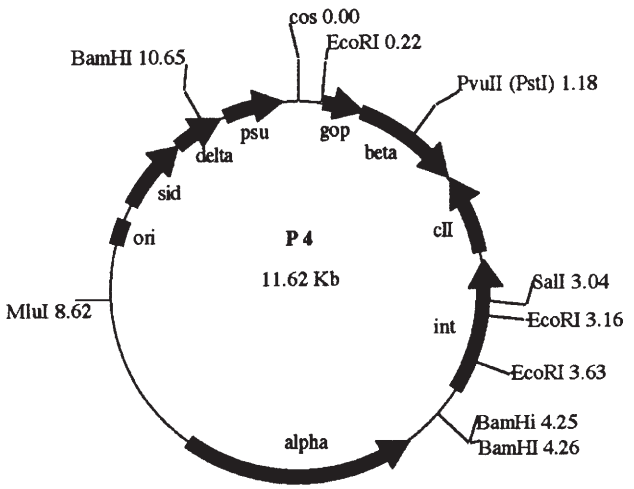


**Fig. 1.** The physical and genetic map of P4. The positions of restriction enzyme sites are given in kb according to the P4 sequence (GenBank accession number; X51522). Some genes and the *ori* of P4 are shown. The DNA segments containing genes *gop, beta, cII*, and *int* are regarded as the nonessential regions of the P4 genome (4).

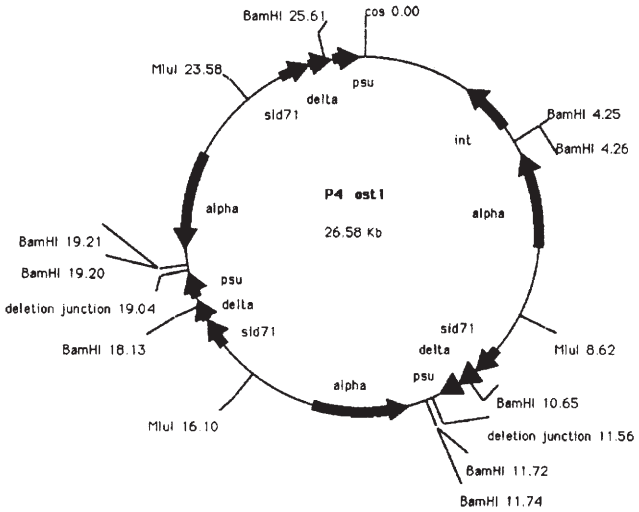


**Fig. 2.** The physical and genetic map of P4 *ost1*. This map shows the *Bam*HI and *Mlu*I sites. The deletion junction and *cos* site are also shown. One complete P4 genome is shown in the upper part of the map (i.e.: from *Mlu*I at 23.58 kb to *Mlu*I at 8.62 kb). Two deleted P4 genomes are shown in the remaining part of the map (i.e.: from *Mlu*I at 8.62 kb to *Mlu*I at 16.10 kb and from *Mlu*I at 16.10 kb to *Mlu*I at 23.58 kb respectively). The *sid71* mutation which is the point mutation in *sid* gene (A at the position of 9,682 of P4 DNA changes to C) allows P4 to be packaged into the large head (11).

was cloned into pUC18 (14) and sequenced to identify the deletion event. Plasmid DNA was isolated by the alkaline lysis method of Sambrook *et al.* (9) and sequenced with universal primers of pUC18, using an automated DNA sequencer (model 373A DNA sequencer; Applied Biosystems, Foster City, CA USA) at the University of Iowa DNA Core Facility.

The deletion started at nucleotide number 11,565 of the P4 DNA sequence, extending past *cos* and the nonessential region of P4 (4), and ended at the core of the P4 *att* site at nucleotide number 4,089 of the P4 sequence (15). Total 4,148 bp of P4 DNA including *cos* and all of the nonessential region of P4 were deleted. Accordingly, the calculated size of P4 *ost1* DNA was 26.576 kb.

To confirm the deduced structure and size of P4 *ost1*, we analyzed P4 *ost1* and P4 *sid71* phage stocks with the CsCl buoyant equilibrium density gradient ultracentrifugation. Each phage stock ($10^7$ to $10^9$ plaque forming units in 50 to 100 μl) was mixed with 12 ml of CsCl lution whose average density was adjusted to 1.38 g/ml, and each mixture was put into 14×95 mm Ultra Clear Beckman tubes. Each tube was centrifuged with a Beckman Ultracentrifuge (model L2-65B) at 55,000 g (SW 41.1 Ti rotor at 21,000 rpm) for 60 hours at 4°C. After centrifugation, the tubes were punctured at the bottom, and 8 drops were collected per fraction. Each fraction was titrated for P4 plaque

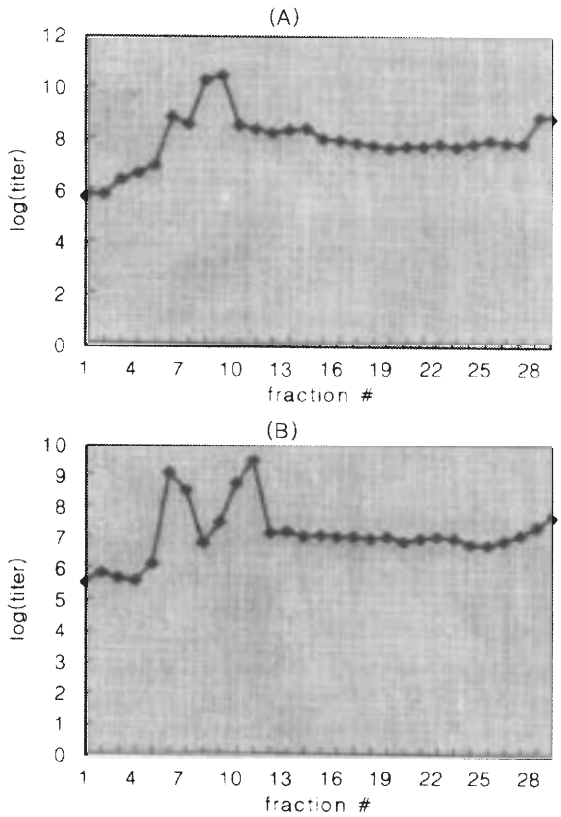


**Fig. 3.** CsCl buoyant equilibrium density gradient profiles of P4 *ost1* (A) and P4 *sid71* (B). The ordinate of this profile shows the P4 titer of each fraction expressed in log scale, and the abscissa shows the number of fraction. The fraction number 1 is the heaviest one (the bottom of the tube) and appears at the left end of the profile. In the profile of P4 *sid 71*, the first peak from the left end is composed of P4 with three genomes (34.87 kb) in the large head, and the second peak is composed of P4 with two genomes (23.5 kb) in the large head. The peak composed of P4 *ost1* appears between two peaks of P4 *sid71*.

forming units using C353 as an indicator (13). The CsCl buoyant equilibrium density gradient profile of P4 *ost1* showed a single peak (Fig. 3A), and that of P4 *sid71* showed two peaks (Fig. 3B).

The profile of P4 *sid71* coincided with the published profile of other Sid-defective P4, P4 *sid1* (11). Since the Sid-defective P4 phage stocks are the mixture of the phage particles containing dimer or trimer of P4, the first peak from left in the profile of P4 *sid71* is composed of the phage particles with trimer of P4 (34.87 kb), and the second peak is composed of the phage particles with dimer of P4 (23.5 kb) (11). The single peak of P4 *ost1* appeared at the fraction number 9 which was between the two peaks of the fractions of P4 *sid71*. Therefore the genome size of P 4 *ost1* had to be between P4 dimer (23.5 kb) and P4 trimer (34.87 kb). This ruled out the possibility that P4 *ost1* might be composed of one complete P4 and

| | |
|---|---|
| ***att* core** | TGGTGC**C**G**A**AGG**CCGG**ACTC |
| **target in P4 *ost1*** | ACCGCG**C**C**A**TTC**CCGG**CGCG |
| **target consensus** | CG CCG |

**Fig. 4.** The sequences encompassing the deletion joint in P4 *ost1* and the consensus sequence in P4-plasmid recombinants. The underlined letters indicate the conserved nucleotides between the *att* core, the core of P4's *att*P, and the target site in P4 *ost1*. The deletion is mediated by the recombination of those two sequences. The target consensus was defined by Six through the studies of P4 and plasmid recombinants (Six, unpublished results).

one deleted P4 (in this case, the total genome size would be less than P4 dimer). Instead, this result supported the deduced genome structure (one complete P4 and two deleted P4) and calculated genome size of P4 *ost1*.

The P4 sequences encompassing the two deletion end points were aligned in Fig. 4. A tetra-nucleotide, CCGG, was present in both DNA sequences. Two more identical nucleotides were found 4 and 6 bp upstream of the CCGG sequences, respectively. According to Six's study of the site specific recombination between P4 and several plasmids, a CCG triplet and a CG positioned five nucleotides upstream of the CCG turned out to be the consensus sequences for P4 site specific recombination (unpu- blished results). As this CCG triplet (in the tetra-nucleotide, CCGG) was involved in the deletion creating P4 *ost1*, it likely appears that this deletion results from a site specific recombination mediated by P4 integrase.

P4 *ost1* and two or three genomes of P4 (dimer or trimer of P4) to be packaged into the large capsid differ not only in the size of DNA to be packaged but also in the number of *cos* site. P4 *ost1* has only one *cos* site, and dimer or trimer of P4 has two or three *cos* sites, respectively. The number of *cos* site in DNA molecule to be packaged has been considered as a factor for affecting the efficient packaging in P2-P4 system (2). More than one *cos* site containing molecules (such as P4 dimer and P4 trimer) were expected to have reduced packaging efficiency with the large capsid, so they showed low burst size with P2 *sir* lysogens. It is natural that one *cos* site containing P4 *ost1* can be packaged very well with the large capsid assembled by P2 *sir*.

P4 *ost1* is the only P4 derivatives isolated so far, whose genome size is between that of P4 dimer and P4 trimer. From our study, P4 *ost1* turned out to package very well with the large capsid assembled by P2 *sir3*. We propose that the genome size of P4 *ost1* may be responsible for the suppression of *sir3* mutation of P2. Unfortunately, nothing has been known about the effect of the genome size on

packaging in phages, such as P2-P4 and lambda, whose packaging is mediated by the *cos*-cleavage mechanism. Next we will focus the genome size of P4 and packaging in P2-P4 system.

In conclusion, we isolated and characterized the P4 suppressor of P2 *sir3*, P4 *ost1*. Identification of P4 *ost1* showed that rare *int*-mediated site-specific recombination and deletion produced the deleted P4 trimer, P4 *ost1*. We suggest that the number of *cos* site (one) in P4 *ost1* may be responsible for the suppression of *sir* mutation of P2. In addition, we cannot exclude the possibility that unique genome size of P4 *ost1* (between P4 dimer and P4 trimer) rendering P4 *ost1* to suppress *sir* mutations of P2.

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