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Effect of coliphage λP gene mutations on the stability of the λO protein, the initiator of λDNA replication*

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The coliphage λ and λ plasmids derived from this phage code for two proteins, λO and λP, engaged in the assembly of the replication complex at *ori*λ, the origin of λDNA replication. At first a pre-replication complex *ori*λ-λO-λP-DnaB is formed [1], then the combined action of chaperonins, DnaJ, DnaK and GrpE, changes the structure of this complex, positioning the bacterial DnaB-helicase at the prospective replication fork, at the same time releasing this enzyme from λP-inhibition [2, 3]. The single-stranded DNA regions resulting from the action of DnaB-helicase make possible the binding of DnaG-primase. The last step in the assembly of the replication complex, the binding of holoenzyme of DNA polymerase III, makes possible the extension of RNA primer, and thus the replicative synthesis of λDNA.

We have shown recently that the replication complex does not disassemble after a round of λ plasmid circle-to-circle replication (that corresponds to phage λ "early" replication) but is inherited by one of two daughter plasmid copies [4]. The λO protein present in this complex is protected from bacterial proteases, while the excess of synthesized λO is rapidly degraded [5]. Hence the stable λO detected by immunoprecipitation in pulse-chase experiments in λ phage-infected and λ plasmid-harboring cells represents the λO present in the replication complex.

Now, our aim was to study the λO decay, under conditions when the functional replication complex assembly was inhibited by the use of λ*P* mutants.

We used two λ*P*-gene mutants, one (*Pam*3) coding for a truncated λP protein in non permissive (sup^+) cells, and another (πA66 [6], called in our laboratory *Pts*1), coding for a full-size λP protein, inactive in λDNA replication at higher temperatures. In λ*Pam*3-infected cells that are permissive (*sup*E) for *amber* mutations the phage growth corresponds to the presence of stable λO. In λ*Pam*3-infected non permissive cells the lack of phage progeny corresponds to the absence of stable λO (Table 1). In these conditions the pre-replication complex *ori*λ-λO-λP-DnaB can not be assembled and only the binding of λO protein to *ori*λ can occur; most probably λO bound to *ori*λ is not protected from bacterial proteases. The λ*Pts*1 phage does not grow at 43°C, and λ plasmid derived from this phage is eliminated from the cells growing at 43°C as efficiently as the λ*Ots* plasmid (Table 2). However, in contrast to the truncated λ*Pam*3 protein, the full-size protein coded by λ*Pts*1 behaves differently in non permissive conditions, at 43°C: stable λO is observed (Table 1). This experiment shows that λ*Pts*1 protein at 43°C is able to participate in the assembly of the pre-replication complex where λO is protected from proteases.

We have shown previously that, in λ phage-infected or λ plasmid-harboring chromosomeless minicells of *E. coli*, λO decays rapidly leaving no stable λO [7]. λ Phage DNA replication

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Table 1

Stability of λO protein in Escherichia coli cells in the presence and absence of λP replication function

Source of λO and λP[a]	Relevant *E. coli* host genotype[b]	Temperature [c]	λP replication function[d]	Percentage of stable λO protein[e]
λ wt plasmid (pKB2)	wt	30°C	+	25.6 (±5.8)
λ wt plasmid (pKB2)	wt	43°C	+	20.6 (±6.8)
λ *Pts*1t plasmid (pGW2)	wt	30°C	+	50.8 (±3.5)
λ *Pts*1 plasmid (pGW2)	wt	43°C	–	42.3 (±4.2)
λ*Pts*1phage	wt	30°C	+	39.2 (±0.6)
λ*Pts*1 phage	wt	43°C	–	32.5 (±4.5)
λ*Pam*3 phage	*sup*E	37°C	+	54.6 (±1.5)
λ*Pam*3 phage	*sup*$^+$	37oC	–	<1

[a]pKB2 is a λ plasmid carrying the wild type (wt) *Hin*dIII-*Bam*HI replication region from λ phage and a kanamycin resistance marker [9]; pGW2 is the same plasmid as pKB2 but the replication region derives from λcIb2πA66 phage (called λ*Pts*1 in the text) which carries a temperature sensitive mutation in the *P* gene [6]; pGW2 was constructed in this work; λ*Pam*3 phage carries *amber* (nonsense) mutation in the *P* gene [10];

[b]The genotypes of *E. coli* K-12 strains are as follows: QD5003 *supF* [11] ("wt" in the Table); C600 *supE44 hsdR thi*-1 *thr*-1 *leuB6 lacY*1 *tonA*21 [12] ("*supE*" in the Table); W3350 *galK2 galT*2 [10] ("*sup*$^+$" in the Table);

[c]The indicated temperatures were maintained throughout the pulse-chase experiments;

[d](+) indicates the presence of λP replication function and (–) its absence;

[e]The pulse-chase labeling experiments and immunoprecipitation of λO protein as well as the quantification of the λO protein remaining after different chase times intervals were preformed exactly as described previously [5]. In the presence of the stable fraction of λO protein in *E. coli* cells the rapid decay of λO protein was observed during the first two minutes of chase, then the level of λO became stabilized (compare with reference 5). The zero time was the time of beginning of the chase which followed a 5 min [^{35}S]methionine pulse, and the amount of λO protein at this time was taken as 100%. The data presented in the Table are average values of the results obtained from the fourth to the sixteenth minute of the chase. Standard deviation is presented in parentheses. The detectable level of λO protein was about 1% in comparison to the amount of protein at zero time.

Table 2

Maintenance of λ plasmids in E. coli cells in dependence on λO and λP functions

Plasmid[a]			Percentage of plasmid-harboring (kanamycin-resistant) cells after overnight growth at: [b]	
Name	λO allele	λP allele	30°C	43°C
pKB2	*O*$^+$	*P*$^+$	96.2 (±0.7)	92.6 (±7.4)
pGW1	*Ots*524	*P*$^+$	70.7 (±16.3)	2.6 (±1.8)
pGW2	*O*$^+$	*Pts*1	72.7 (±8.5)	2.2 (±1.8)

[a]Wild type λ plasmid (pKB2) and λ*Pts*1 plasmid (pGW2) were as described in Table 1. The λ*Ots*524 plasmid (pGW1) is an pKB2 analog carrying a temperature sensitive mutation in λ*O* gene and has been described earlier [13];

[b]Different *E. coli* strains (HB101 [14], CBO129 Hfr [9], C600 [12] or wild type MG1655) harboring pKB2, pGW1 or pGW2 plasmid were grown overnight at 30°C in LB medium containing kanamycin at final concentration of 25 μg/ml. Then the cultures were diluted 1:1000 and cultivated overnight in LB medium without any antibiotic at 30°C or 43°C. Bacterial cultures were titrated on LB plates with and without kanamycin at 30°C and the percentage of kanamycin-resistant colonies was estimated for each culture. The results are average values ± S.D. from separate experiments. Standard deviation is presented in parentheses.

does not occur in this system [8] in spite of extensive synthesis of λO and λP proteins. In the light of the present results we infer that in minicells a step leading to the assembly of the pre-replication complex can not occur. The simplest assumption is that DnaB-helicase does not segregate to minicells during asymmetric septation that produces these chromosomeless bodies.

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