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Lysogenization of *Escherichia coli* by Bacteriophage Lambda: Complementary Activity of the Host's DNA Polymerase I and Ligase and Bacteriophage Replication Proteins *O* and *P*

USHARANJAN RAY[1] AND A. SKALKA*
Roche Institute of Molecular Biology, Nutley, New Jersey 07110

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When bacteriophage lambda DNA replication is blocked by mutation in phage genes *O* or *P*, the efficiency of lysogenization drops to a very low value unless high multiplicities of infecting phage are used. Our results show that even at high multiplicity, lambda *O* or *P* mutants cannot efficiently lysogenize some hosts that are defective in either DNA polymerase I or DNA ligase. Covalent closure of infecting DNA molecules, a preliminary step for insertion according to Campbell's model and an obvious candidate for this lysogenization defect, appears to occur normally under our conditions. In addition, prophage excision as measured by the frequency of curing O^- and P^- lysogens seemed normal when tested in the *polI*$^-$ strain. These results suggest that the *Escherichia coli* enzymes DNA polymerase I and ligase, and phage proteins *O* and *P*, are able to provide some complementary activity whose function is required specifically for prophage integration.

Infection of a sensitive *Escherichia coli* host with bacteriophage lambda results in either a lytic or a lysogenic response, depending on the physiological conditions. A schematic of the steps leading to lysogeny is shown in Fig. 1. Phage DNA, which is linear inside the virus, is injected into the cell and circularizes by hydrogen bonding of complementary single-stranded sequences at its ends. Integration is a site-specific recombination event that presumably acts on such a circle. The reaction requires the phage locus marked PP', the bacterial locus BB', and the phage protein *int* (see reference 10 for review). Various lines of evidence indicate that other phage or bacterial functions may be involved in this reaction (15, 26). Excision, the reverse of the integration process, requires not only the *int* protein but also a second phage protein, *xis*. In addition, this reaction involves loci BP' and PB', which are genetically distinct from those involved in integration (14).

When phage DNA replication is blocked by mutations in genes *O* or *P*, the frequency of lysogenization is very low. This defect can be overcome by infection with O^- or P^- phages at high multiplicity and has therefore been attributed to a gene-dosage effect (2). It has been suggested that an increased number of chromosome copies might facilitate the integration reaction either through the influence of mass action or by affecting the concentration of cI repressor (4, 8, 18, 23, 27).

In the course of attempts to construct O^- and P^- *polA1* lysogens for biochemical studies in our laboratory, unexpected difficulties were encountered. The experiments reported here, which were the result of an attempt to understand the source of these difficulties, indicate that lysogenization may depend on *O* and *P* function in a way that is distinct from the effects of gene dosage mentioned above. We find that high-multiplicity infection does not compensate for the loss of *O* and *P* in lysogenization of polymerase I- or ligase-defective hosts. Thus, it appears that some activity of these phage proteins may be able to complement an unsuspected role normally played by these host functions during lysogenization.

MATERIALS AND METHODS

Phage and bacterial strains. *E. coli* strains used in this investigation and their relevant genetic markers are as follows: JW149 *su*$^-$*met*$^-$*E*$^-$*thy*$^-$, a W3110-derived strain from JG108 (24) provided by J. Wechsler; JW149 *su*$^-$*thy*$^-$*polA1*, constructed by us by P1 transduction from JG112 (19), a strain that carries the original *polA* amber mutation of DeLucia and Cairns (3); KS268 *thy*$^-$*lig*ts7 *su*$^-$ (17), provided by D. Freifelder; N2668 *lig*ts7 *su*$^-$ (11), obtained from M. Gottesman; and QD5003 *su*III$^+$, permissive for λ *S*am7 mutants, obtained from E. Signer.

Lambda mutants were λcI857*S*am7, λcI857*O*-am29*S*am7, λcI857*P*am80*S*am7, λcI857*O*am29*P*-am80*S*am7, λ*ind*$^-$, and λ*h*80*del*9c (31), which was provided by L. Enquist.

[1] Present address: Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Mass. 02115.

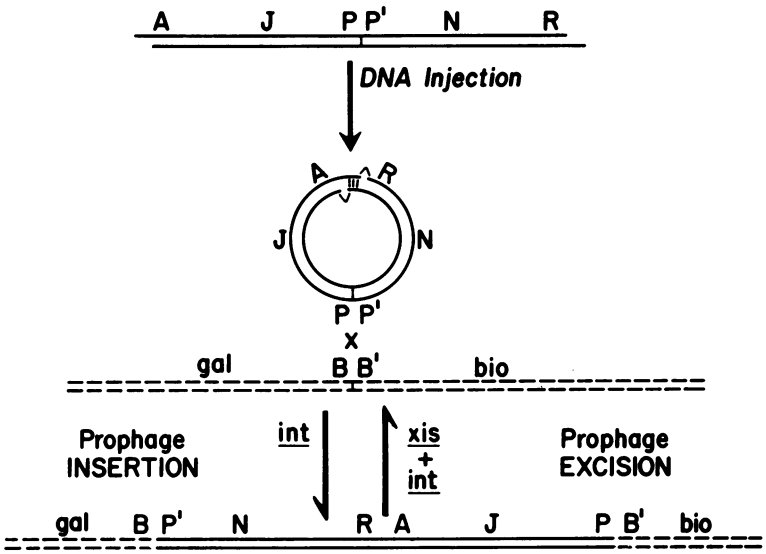

FIG. 1. *Adaptation of Campbell's model for integration and excision from Gottesman and Weisberg (10).*

Media. Tryptone broth and minimal medium were as described by Enquist and Skalka (6).

Measurement of lysogenization frequency. These methods were essentially as described by Hoffmann and Rubenstein (16). Cells were grown in tryptone + 0.2% maltose broth to late log phase ($\sim 10^9$ cells/ml), pelleted, and resuspended in 0.02 M $MgSO_4$. The cells were starved by incubation with aeration at 37 C for 60 min. Aliquots were placed at 30 C and infected with phage at multiplicities of 10 or more per bacterium. After 15 min at 30 C, the infected cells were washed to remove unadsorbed phage and then resuspended in twice their original volume of tryptone broth. They were incubated at 30 C for 20 min and plated at 30 C after appropriate dilution. Survivors (usually 10 to 30%) were checked for λ immunity by one of two ways: (i) by plating on agar and cross-streaking 2-day-old surviving colonies against λ*c*I phage, or (ii) by plating on agar previously seeded with 10^8 λh80*del*9c/plate (this phage, which is *int*⁻ and *red*⁻, allows growth of only lysogens or λ-resistant colonies) and then checking for immunity by cross-streaking.

Curing frequencies. Curing after heat induction of lysogens was measured as described by Guarneros and Echols (14).

DNA measurements. Extraction and measurement of covalently closed circular λ DNA after infection with labeled phage was done according to Freifelder et al. (9).

RESULTS

Lysogenization of DNA polymerase I-defective *E. coli* by λ *O* and *P* mutants. We used two *E. coli* strains (JW149) that are isogenic except for a small region surrounding the *polA* gene and tested for frequency of lysogenization after infection with wild-type λ or phages carrying mutations in genes *O* and/or *P*. To overcome the potential gene-dosage defect with the *O* and *P* mutants (2), and to otherwise facilitate the lysogenic response (16), we used multiplicities of 10 or more phage per bacterium.

As expected (Table 1), at high multiplicity λ *O* and *P* mutants were only slightly less efficient in their ability to lysogenize the *pol*⁺ host than was wild-type lambda. However, with the *polI*⁻ strain, lysogenization frequencies for *O* and *P* mutants were approximately 100 times lower than with wild type. In addition to the JW149 strains shown in Table 1, we examined several others containing the same *polA1* mutation: JG112, a JW149 derivative constructed by P1 transduction in the laboratory of Julian Gross; the original mutagenized W3110 strain, P3478, of DeLucia and Cairns (3), obtained from the laboratory of E. Signer and called WA5023 in their collection; and strain JW151, which, unlike the others, is not a W3110 derivative, and was constructed by J. Brockes in the laboratory of N. Murray (obtained from J. Wechsler). In all cases, results similar to those in Table 1 were obtained.

We conclude that the host DNA polymerase I and the phage gene products *O* and *P* play some complementary role in lambda lysogenization and that this role is distinct from the one dependent on gene dosage.

Lysogenization of DNA ligase-defective *E. coli* by λ *O* and *P* mutants. Similar tests were performed on two independently derived strains of *E. coli* that carry the same ligase allele. These *lig*ts7 mutants are defective for growth at 42 C (11, 17). At 30 C, the permissive temperature, these strains have sufficient li-

TABLE 1. *Lysogenization frequency of λ mutants in pol⁺ and polI⁻ E. coli*

Infecting phage	% Lysogens[a]	
	pol⁺	*polI⁻*
λ wild type	59	51
λ *O⁻*	27	≤0.6
λ *P⁻*	24	≤0.5
λ *O⁻P⁻*	20	NT[b]

[a] Each value represents the average of two to three independent tests of 100 to 500 survivors. Multiplicity of infection was 10 to 20, and bacterial survial 10 to 30%.

[b] NT, Not tested.

gase to grow, but the in vivo activity is still somewhat deficient as judged by the inefficient plating of λ *red* mutants (the *feb⁻* phenotype) (17). In our plating tests (data not shown), the efficiency of plating for λ *red⁻* was an order of magnitude lower for KS268 than for the N2668 strain. Tests for lysogenization by λ *O* and *P* mutants (Table 2) showed that the frequency was low for KS268 but almost normal for N2668. The results with KS268 are similar to those obtained with the *polA* strain. As with *polA*, they suggest that phage *O* and *P* functions can complement for a defect in the hosts' DNA ligase. However, in contrast to results with *polA*, it appears that the genetic background has a profound influence on the response of these ligase ts7 mutants. This strain-dependent feature suggests that other factors in the cell may also exert an influence on lysogenization.

Studies of λ *O⁻* and *P⁻* prophage excision from DNA polymerase-defective lysogens. We considered the possibility that polymerase + ligase and/or phage *O* and *P* function might be directly required at the *int*-mediated recombination step of lysogenization. Since the *int* protein is also required in the excision reaction, it seemed possible that the excision of λ *O⁻* and *P⁻* prophage might also be defective in the *polI⁻* strain. As Table 1 illustrates, it is difficult to make *polI⁻* lysogens by infection with λ *O⁻* or *P⁻* phage. To circumvent this problem and eliminate the possibility that the *O⁻* and *P⁻* *polI⁻* lysogens that can be obtained by infection might be abnormal, lysogens were first made from the *pol⁺* JW149 strain, and *polI⁻* derivatives were then constructed by P1 transduction. Our measurements of curing frequencies with these lysogens (Table 3) showed no significant difference between the *pol⁺* and *polI⁻* derivative. In each case, curing frequencies were close to the 20% value previously reported for wild type (14).

Test for formation of covalently closed circles of λ DNA after infection of DNA polymerase I- and ligase-defective strains. Successful lysogenization requires covalent closure of the cohered mature ends of infecting DNA (10). Such closure is presumably catalyzed by the host's DNA ligase (34) and, in some cases, might conceivably be aided by repair of damaged ends by DNA polymerase I. The following experiments were designed to determine whether the low frequency of lysogenization of the defective strains by *O* (or *P*) mutants might be the result of a failure in ligation of the mature ends of infecting DNA. The bacterial strains, JW149, JW149 *polI⁻*, N2668, and KS268, were first lysogenized with λ *ind⁻* so that all expression of the infecting λ DNA would be repressed and the contribution of bacterial functions could be determined independently. Covalent closure (i.e., ligase and possibly *polI* activity) was measured by the formation of supercoils from infecting DNA as follows: the lysogenic cultures were divided into two portions, and each portion was separately infected with labeled [^{32}P]λ *O⁻* or [^{3}H]λ *O⁺* (wild type) phage at a multiplicity of 10 phage/

TABLE 2. *Lysogenization frequency of λ mutants in ligase-defective (ligts7) hosts at permissive (30 C) temperature[a]*

Infecting phage	N2668	KS268
λ wild type	60	36
λ O⁻	24	≤2
λ P⁻	46	≤0.1
λ O⁻P⁻	NT[b]	≤1

[a] Conditions as described in Table 1.

[b] NT, Not tested.

TABLE 3. *Curing frequency of lambda lysogens[a]*

Prophage in lysogen	*polI* phenotype of lysogen	Frequency of cured cells
λ wild type	+	0.14
	−	0.08
O⁻	+	0.6
	−	0.45
P⁻	+	0.19
	−	0.18
O⁻P⁻	+	0.12
	−	0.10

[a] Lysogens were grown overnight in tryptone broth at 30 C. Cells were diluted fivefold in tryptone broth and grown for 1.5 h. The cells were then induced by heating at 45 C for 5 min, diluted 10-fold in Tryptone broth at 30 C, grown for 120 min, and plated for cell survival at 42 C (cured cells) and 30 C (total cells). Survival of cells after induction was 30 to 40%.

bacterium. After removal of unadsorbed phage and a designated period of growth, the two independently infected portions were combined and the cells were concentrated immediately. Lysates were made from the concentrated cells, and the infecting DNA was analyzed immediately by alkaline sucrose sedimentation. Differential labeling permitted comparisons of the fate of λ O^- and λ O^+ infecting DNA in the same preparation.

Results from a typical experiment are shown in Fig. 2. Depending on the phage preparation, 15 to 40% of the infecting DNA was found at the position of covalently closed circles. This value is similar to that found by others who used the same technique (21). A smaller percentage of the DNA was recovered from the dense pad at the bottom of the tube; this probably represented covalently closed molecules that traveled faster than normal down the walls of the nitrocellulose tube during centrifugation, but was not included in our estimates of percentages of supercoils. The remainder of the DNA, consisting of single-stranded linear and circular molecules, was recovered in a slower sedimenting fraction near the top of the tube.

Table 4 summarizes the results from 12 separate tests of this kind. In all cases ligation was somewhat more efficient for the O^- DNA than for O^+ DNA. Since the presence of cI protein can be assumed to result in repression of both chromosomes, this is probably not a significant difference. The ratio of O^- to O^+ supercoils was about the same for all strains at all three time points tested. The only significant exception was with the *polI*$^-$ strain at 10 min after infection. In this case the value for O^+ (and not O^-) supercoils seemed abnormally low. Furthermore, although the percentage of supercoils was slightly higher in *pol*$^+$ as compared with the *polI*$^-$ strain, the difference was always less than a factor of two. There was no significant difference between the two ligase-defective strains. It appears from these results that the enzyme levels in these defective strains are not so low as to limit sealing of the mature ends of infecting λ DNA. In addition, the sealing reaction seems to be very rapid and essentially completed by 10 min after infection.

The data in Table 5 summarize the results of a test for the covalent closure of O^- DNA in nonlysogenic *pol*$^+$ and *pol*$^-$ cultures. No significant difference was detected at any of three time points, and the percentages of supercoils were similar to those found for O^+ with the corresponding lysogens. This suggests that the O^- defect in lysogenization does not reflect a requirement for DNA polymerase (or ligase) brought about by expression of phage (or bacterial) genes during transcription or abortive replication in the absence of *O* protein.

DISCUSSION

Our findings confirm results from earlier work which showed that for wild-type *E. coli*, defects in lysogenization by λ *O* or *P* mutants can be overcome by infection at high multiplicity. However, even at high multiplicity, O^- and/or P^- phage did not readily lysogenize some DNA polymerase I- or DNA ligase-defective strains. Thus, we can distinguish between two effects attributable to the phage *O* and *P* proteins. The first is an indirect effect and is probably due to influences of gene dosage. The second is an effect that is only observed in the absence of the host's polymerase I or ligase and may not even be important when these enzymes are present.

Under our conditions, the requirement for polymerase I and ligase in the absence of *O* and/or *P* function does not seem to be explained by failure in ligation of cohered mature ends. We observed only a small difference in the efficiency of sealing after infection of *pol*$^+$ and *polI*$^-$ strains. We found no difference between sealing in two lysogenic ligase (ts7)-defective strains, even though one (but not the other) of the corresponding nonlysogens gave poor efficiency of lysogenization with the λ *O* and *P* mutants. Ligation of mature ends is clearly necessary for stable lysogenization (10), although it is not known at what stage this step occurs. Under our conditions, even in the ligase-defective strains, covalent closure seemed very rapid and would probably be completed before integration started (10 to 20 min after infection, according to references 7, 25, and 26). We must point out, however, that our physical tests show what happens to most of the infecting DNA in these experiments. Despite the fact that there is no evidence to support it, the possibility that something else happens to the few molecules involved in events in the 10 to 30% of the bacteria that survive to be scored as lysogens cannot be excluded.

Although under intensive study (8, 12, 13, 25, 26, 33), complete details concerning the molecular mechanism of *int*-promoted recombination are not yet available. Even though it appears that normal levels of the host's ligase may not be required (33) and extensive DNA replication need not take place (25), a requirement for some ligase activity or a small amount of "repair replication" cannot be excluded. More recent results (1) suggest that concentrations of NMN used to inhibit ligase in earlier tests of *int* activity (33) may have been too low to inac-

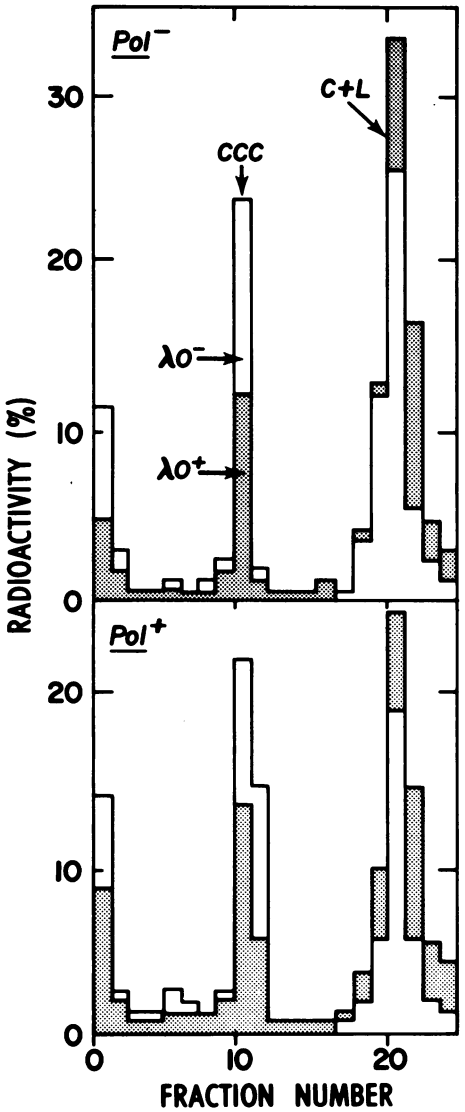

FIG. 2. *Formation of covalently closed circles (CCC) after infection of polI⁻ and pol⁺ (λind⁻) lysogens. Conditions for growth of cells and infection were as described in the text. The two cultures were grown at the same time: after they had reached a cell density of approximately 10⁹, each culture was divided into two portions and each portion was infected separately with H³-labeled O⁺ (λcI857 Sam7 at 10⁻⁵ counts/min per PFU) or P³²-labeled O⁻ (λ Oam29cI857Sam7 at 1.4 × 10⁻⁴ counts/min per PFU) at a multiplicity of 10 PFU/bacterium. After the adsorption period (20 min at 30 C), cells were washed with cold broth and resuspended in broth at 30 C at what is designated time 0. The cultures were incubated at 30 C with aeration for various lengths of time. At each time point, 2 ml of the O⁺-infected portions and 0.4 ml of the O⁻-infected portions of each culture were combined and placed in ice. The cells were then washed, and DNA was extracted and analyzed in alkaline sucrose gradients as described by Freifelder et al. (9). The figure shows results with the polI⁻ and pol⁺ lysogens 30 min after infection. The clear histogram shows O⁻ DNA; the stippled shows O⁺ DNA. Each gradient contained from 5,000 to 15,000 counts/min of each isotope. C + L points to the position of single-stranded linears and circles. A dense pad on the bottom, fractions 1 and 2, collected fast-sedimenting material.*

tivate it by more than 40%. It is known that different pathways in DNA metabolism require different levels of DNA ligase and polymerase; DNA repair, as measured by the *feb*⁻ phenotype, is defective at levels of polymerase I and ligase that are sufficient for DNA replication (32). It may be significant that the response of the ligase-defective strains to lysogenization by *O* and *P* mutants parallels their expression of the *feb*⁻ phenotype. Perhaps DNA replication and covalent closure of mature ends can proceed with very low levels of enzyme activity, whereas integration and repair require somewhat higher amounts. It is not obvious how *O* and *P* functions might compensate for such defects in repair, especially since Freifelder et al. (8) have presented evidence that in normal *E. coli* phage replication per se seems to inhibit insertion. We note, however, that similar complementation between components of recombination and replication pathways have been observed in studies of repair of UV damage (22).

The data from our curing experiments suggest that the excision reaction does not require either of the complementing activities, but these tests do not measure kinetic differences. We have, in fact, noticed what seems to be an abnormally high rate of "spontaneous" curing during routine maintenance of the *O*⁻ and *P*⁻ *polI*⁻ lysogens. This could signify that our measurements of curing frequency were insufficiently sensitive. Alternatively, we suppose that if (as has been suggested [29, 30]) maintenance of lysogeny involves a normal equilibrium between the integrated and nonintegrated prophage state, this high rate of "spontaneous" curing could very well reflect an integration defect. Measurement of excision by other methods, for example following infection by *O*⁻ or *P*⁻ derivatives of the λ *att*² phage described by Gottesman and Gottesman (12) or by lysogenization in the "reversed" reaction described by Echols (5), could provide a more satisfactory test for this aspect of the problem.

We note here that a probably analogous distinction between the effects of proteins 18 and 12 (assumed to be equivalent to λ *O* and *P*) on DNA replication and lysogenization of P22 has

TABLE 4. *Test for covalent closure of infecting DNA in lysogens*[a]

Time (min) after infection	% Supercoils			
	pol$^+$ (λind^-)		*polI*$^-$ (λind^-)	
	λO^+	λO^-	λO^+	λO^-
10	15.4	32.3	8.8	27.5
20	18.4	36.7	12.6	25.6
30	20.8	38.8	14.7	27.7
(B) *ligts7* lysogens				
Time (min) after infection	N2668 (λind^-)		KS268 (λind^-)	
	λO^+	λO^-	λO^+	λO^-
10	15.3	22.7	16.7	30.1
20	22.6	31.6	17.4	31.1
30	25.2	36.6	17.2	31.1

[a] See Fig. 2 for conditions.

TABLE 5. *Test for covalent closure of λO^- infecting DNA in nonlysogenic pol$^-$ cells*[a]

Time (min) after infection	% Supercoils	
	pol$^+$	*pol*$^-$
10	17.8	17.8
20	19.8	17.2
45	14.2	14.5

[a] Conditions were as in Fig. 2 except that the O^- phage was labeled with ^3H (λ *Oam29cI857Sam7* at 10^{-6} counts/min per PFU).

been described. As a result of their studies, Levine and collaborators (20, 28) concluded that these phage proteins have dual functions, with one activity required in replication and another during prophage integration. As with our results, the phage functions did not appear to be required in the excision reactions. It is striking that these authors hypothesized a possible complementarity between the phage proteins and polymerase and ligase activities, the very activities that have been independently implicated in our studies. Furthermore, they, as we, proposed that either covalent closure of infecting DNA or the integration reaction per se could be the critical step. In our system covalent closure seems to be normal; this implies (admittedly by default) that it is the site-specific integration step that requires the complementing activities.

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