JOURNAL OF VIROLOGY, Mar. 1978, p. 730-737
0022-538X/78/0025-0730$02.00/0

Vol. 25, No. 3
Printed in U.S.A.

Roles of Bacteriophage Lambda Gene Products *O* and *P* During Early and Late Phases of Infection Cycle

JÖRG KLINKERT AND ALBRECHT KLEIN*
Microbiology, University of Heidelberg, 6900 Heidelberg 1, West Germany

Received for publication 26 September 1977

Ring-to-ring (early) replication of bacteriophage λ DNA was blocked after heat inactivation of the *P* protein. Rolling circle (late) replication continued for several rounds at the rate reached when the temperature shift was carried out. The same differential effect was observed after inhibition of RNA or protein synthesis during the two different phases of replication. In contrast, inactivation of the *O* protein resulted in a fast stop of λ DNA synthesis at early and late times after infection. The results were consistent with the following interpretations. (i) The λ *P* gene product plays a role in the initiation of the ring-to-ring replication. (ii) Ring-to-ring replication continues parallel to rolling circle replication, possibly diminishing with time after infection. (iii) The *O* function is stable in and necessary for the structural integrity of an elongation complex. It is unstable in free form and probably released from such a replication complex after each round of replication at the ring-to-ring stage.

Two modes of replication of bacteriophage λ DNA are observed after lytic infection of an *Escherichia coli* host cell (2; see reference 9 for review). Covalently closed circular molecules formed after the injection of the linear DNA replicate bidirectionally from a fixed origin (16). New ring molecules are created in the process. After one or a few rounds of ring-to-ring replication, at approximately 8 min after infection at 37°C, rolling circle structures of replicating DNA are formed. The switch to the rolling circle mode of replication is dependent on the product of the λ gene *gam* (4). This protein inhibits the cellular *recBC* nuclease (13), which otherwise destroys nascent rolling circle structures of replicating λ DNA. In wild-type host cells, the replication of *gam*-defective λ mutants is thus confined to the ring-to-ring mode (15). Rolling circle DNA replication of λ *gam* mutants does occur in *recBC*-defective cells.

Numerous protein factors are involved in the λ replication process (7, 20), most of which are contributed by the host cell. Only two phage-specific replication proteins, the products of genes *O* and *P*, are directly required for λ DNA replication. All host factors known to be needed are active in DNA chain elongation (including priming of Okazaki fragments). Three cellular initiation factors, the gene products of genes *dnaA*, *dnaC*, and *dnaP*, are dispensable for λ DNA replication (5, 21), suggesting that the phage controls its replication initiation by elements coded for by its own genome.

It is, in fact, known that the *O* protein interacts specifically with the origin of replication (M. E. Furth, C. McLeester, and J. L. Yates, submitted for publication). The *O* protein is unstable (22). It has a half-life of less than 5 min in vivo, as tested by complementation of *O*-defective superinfecting λ phage. In addition to its role in initiation, the *O* protein has an at least indirect function in chain elongation (17): heat inactivation of thermolabile *O* product in vivo leads to an immediate stop of chain elongation, with the formation of extended single-stranded gaps in the vicinity of the replication fork.

The role of the *P* protein, whose physical interaction with both the *O* protein (19) and the cellular *dnaB* (6) protein has been inferred from genetic analysis, is less clear.

Initiation of λ DNA replication is directly dependent on active RNA polymerase, which may serve two functions: (i) synthesis of a primer RNA (8) and (ii) transcriptional activation (3) of the origin, which might be understood as a change in the secondary structure of this DNA sequence.

In this paper we try to further elucidate the mode of action of the λ replication functions in the process of λ DNA replication.

MATERIALS AND METHODS

Media and buffers. The following media and buffers were used: Penassay broth (Difco Laboratories, Detroit, Mich.), supplemented with 2 μg of thymine per ml; λ medium (1% tryptone [Difco]-2 μg of thymine per ml-0.4% maltose); nutrient agar plates (1% tryptone-0.1% yeast extract-0.8% sodium chloride-

0.1% D-glucose-1% agar [Difco]); top agar (1% tryptone-0.1% yeast extract-0.8% sodium chloride-0.1% D-glucose-0.6% agar [Difco]-2.5 mM calcium chloride); phage buffer (0.01 M Tris-hydrochloride [pH 7.4]-0.01 M magnesium chloride-0.01% gelatin).

Unless specified otherwise, all chemicals where purchased from E. Merck AG, Darmstadt, West Germany.

Bacterial and bacteriophage strains. All bacterial strains used are listed in Table 1. The following phage strains were used: λ*cI b2* (referred to as wild type in the text), λ*cI b20am8* λ*cI b2Pam3*, λ*cI b2Pam80*, and λ*cI b20ts28*. Additional λ mutants and recombinants are described below.

Isolation of bacteriophage mutants and recombinants. (i) λcI b2πA66. A set of λ*Pts* mutants including λ*cI b2πA66* was isolated by screening λ*πA* mutants isolated on *E. coli groPA15* for thermosensitivity of plaque formation. λ*π* mutants are known to be missense mutants in gene *P* (6). The phenotype of the mutants was confirmed by checking the temperature sensitivity of the phage-specific DNA synthesis. Mutants exhibiting different degrees of temperature sensitivity were obtained. λ*cI b2πA66* is one of the most temperature-sensitive mutants isolated.

(ii) λcI857gam5red113. λcI857*gam5red*113 was constructed in a cross of λcI857*gam5red*113*S7* and λ*cI b2P80*. Recombinants form normal plaques on MO676 *recBC*, tiny plaques on W3350 *rec*$^+$, and no plaques on E1081 *recA recBC*$^+$.

(iii) λgam5red113πA66. λ*gam5red*113*πA66* was obtained in a cross of λcI857*gam5red*113 and λ*cI b2πA66*. The recombinant forms small plaques on W3350 and grows on MO676 at 37°C but not at 42°C. Unexpectedly, it does not form plaques on the *groPA15* strain, possibly because of the compounded effects of the *gam* and *π* mutations. It does, however, complement λ*cI b2P80* in *groPA15* strains and can thus be shown to carry a *π* allele.

Assay of bacteriophage DNA synthesis. E1081 or E1081 *recBC* bacteria were grown in λ medium to exponential phase (2×10^8 cells per ml), spun down, and suspended in λ medium prewarmed to 37°C, containing 16 μg of mitomycin C (Kyowa Hakko Kogyo Co. Ltd., Tokyo, Japan) per ml, at a concentration of 2×10^9 cells per ml. After the suspension had been incubated for 20 min at 37°C in the dark, a fivefold excess of ice-cold phage buffer containing 2 mM KCN was added. The mitomycin treatment abolishes host cell DNA synthesis. The cells were centrifuged, suspended in phage buffer at a density of 2×10^9 cells per ml, infected with λ phage (multiplicity of infection, 5), and incubated at 0°C for 20 min. A control sample was left uninfected. The cells were then transferred into glass tubes containing prewarmed λ medium and 10 μCi of [^{3}H]thymine (New England Nuclear Corp., Boston, Mass.) per ml. The final concentration was 2×10^8 cells per ml. Samples (0.2 ml) were taken at different times, poured on glass filters of 24-mm diameter (Whatman GF81) that had been soaked with 0.2 ml of 0.1 N NaOH-0.2% sodium dodecyl sulfate (SERVA, Heidelberg)-0.02 mg of calf thymus DNA (SERVA) per ml-2% saturated sodium PP_i, and subsequently dried again before use. At the end of the experiment, the filters were washed once in 0.3 M trichloroacetic acid-1% saturated sodium PP_i, twice in 0.06 M trichloroacetic acid, and finally twice in methanol. The filters were dried, and radioactivity was determined by scintillation counting.

In certain experiments, temperature shifts were performed by transferring infected cells from the 37°C water bath to prewarmed glass tubes in a 43°C water bath. In experiments in the course of which chloramphenicol (Boehringer Mannheim Corp., New York) or rifampin (SERVA) was applied, the procedure was as follows. The infected cells were placed into glass tubes containing fresh solutions of 5 mg of rifampin per ml of 0.1 M Tris-hydrochloride (pH 7.4) or 1.25 mg of chloramphenicol per ml of the same buffer. The final concentration of rifampin was 500 μg/ml, and that of chloramphenicol was 125 μg/ml. When the effects of chloramphenicol, rifampin, and a temperature shift were to be compared, the tubes used for the temperature shift contained the same amount of buffer as did those to which inhibitors had been added.

RESULTS

Heat inactivation of *O* and *P* gene products leads to different λ DNA synthesis patterns at the rolling circle stage. Takahashi (17) observed that the inactivation of thermolabile *O* or *P* proteins led to inhibition of λ DNA synthesis during rolling circle replication. Whereas the inhibition of DNA synthesis was complete with heat-labile *O* mutants, the rate of synthesis was merely reduced with *P* mutants.

We investigated this differential behavior in more detail. Figure 1 confirms that heat inactivation of the *O* function led to an almost immediate and complete stop of λ DNA synthesis, regardless of the time after infection at which the inactivation was carried out. In contrast,

TABLE 1. *E. coli K-12 strains and their chromosomal markers*

Strain	Chromosomal markers	Source
C600	*thr1 leu6 thi1 lacY1 tonA21 supE44*	Our own collection
E1081	*thyA recA uvrB RNase*$^-$	Our own collection
E1081 *recBC*	*thyA recA uvrB RNase*$^-$ *recBC*	Our own collection
groPA15	*dnaBts arg met sm*^{r} (supports growth of λ*πA* and λ*πB* phage mutants)	I. Herskowitz
MO676	*recB21 recC22 sbcb15 endB supE*	M. Oishi through H. Hoffmann-Berling
W3350	*gal*	Our own collection
204 (λcI857*gam5red*113*S7*)	*thy recA*	A. Skalka

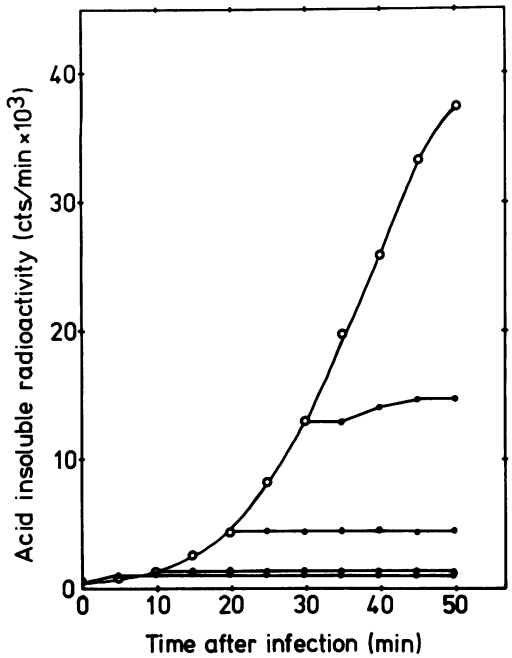

FIG. 1. *Effect of a temperature shift on DNA synthesis in λOts28-infected E1081 bacteria at different times after infection. Mitomycin-treated E1081 bacteria were infected with λOts28 phage and incubated in [³H]thymine-containing medium at 32°C. Samples (0.2 ml) were taken at intervals and assayed for acid-insoluble radioactivity as described in the text. At different times after infection, portions of the culture were transferred to test tubes at 42°C. Symbols: ○, DNA synthesis at 32°C; ●, DNA synthesis after a temperature shift to 42°C.*

with a temperature-sensitive *P* mutant, the residual rate of replication depended on the time of heat inactivation (Fig. 2), dropping off very gradually after that time. At the permissive temperature the rate increased up to 25 min after infection and became apparently linear afterwards before it decreased toward the end of the latent period (after 40 min). These results were repeatedly obtained with 10 different temperature-sensitive *P* mutants from a set isolated as described above.

Inhibitors of RNA and protein syntheses produce a pattern of DNA synthesis comparable to that found after inactivation of the *P* function. The data described above might be interpreted as an inhibition of DNA chain elongation in the case of the inactivation of the *O* function (17). In the case of the *P* function, the results indicate a block of initiation of ring-to-ring replication (i.e., formation of new replication forks). To test this notion, we inhibited RNA polymerase by adding rifampin at different times after the infection with wild-type λ, thus blocking initiation of DNA replication. (It is known that the drug does not affect chain elongation in the course of the cellular DNA synthesis [21].) The effects of rifampin on DNA synthesis were indeed similar to those obtained after inactivation of the *P* protein (Fig. 3).

This effect could be due to a direct effect on RNA polymerase, i.e., elimination of transcriptional activation necessary for initiation of replication (3). On the other hand, blocking RNA synthesis might indirectly affect protein synthesis and thereby inhibit the resynthesis of a labile protein necessary for initiation of ring-to-ring replication. Figure 4 shows results of experiments similar to those shown in Fig. 3, with protein synthesis instead of RNA synthesis inhibited by the addition of chloramphenicol. Again, the rate of DNA synthesis was reduced to values correlated with the times at which the inhibitor was employed. Thus, the effect of rifampin may be due to either direct action on RNA synthesis or the indirect effect on protein synthesis.

Inhibition of RNA or protein synthesis or inactivation of the *P* function leads to a complete stop of replication at the ring-to-

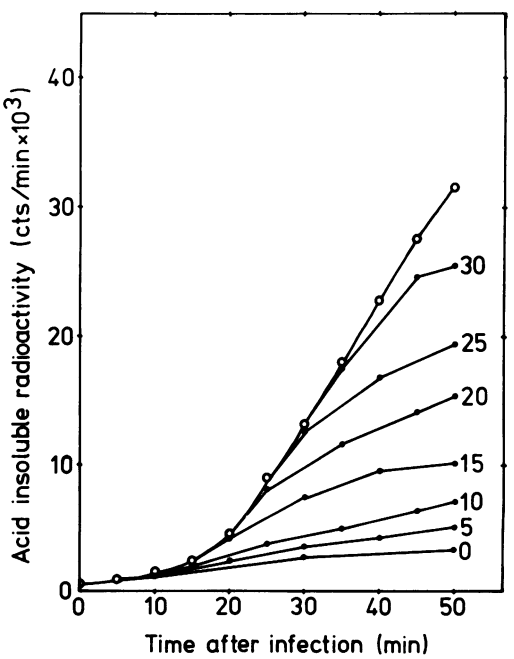

FIG. 2. *Effect of temperature shifts on DNA synthesis in E1081 bacteria infected with the Pts mutant λcIb2A66. Thymine incorporation of 0.2-ml samples was determined as described in the text. The times of the temperature shifts (in minutes after infection) are indicated by the numbers inserted in the figure. Symbols: ○, DNA synthesis at 37°C; ●, DNA synthesis after a temperature shift to 43°C.*

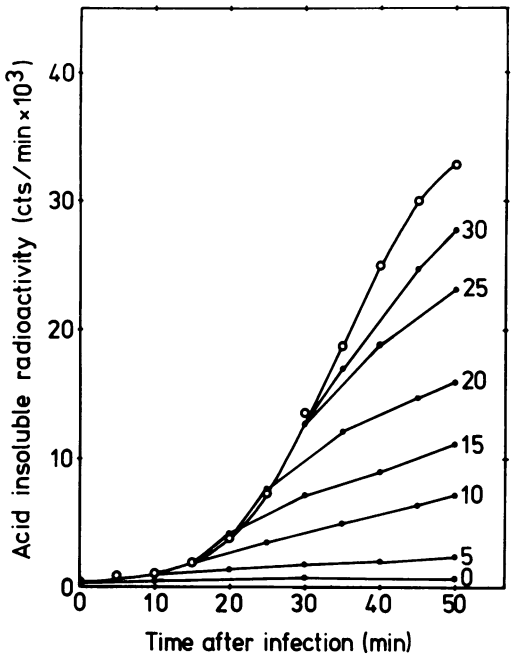

FIG. 3. *Influence on DNA synthesis in λcIb2-infected E1081 bacteria of rifampin addition at different times after infection. A 500-μg amount of rifampin was added per ml of infected bacteria at the times (in minutes) indicated by the numbers inserted in the figure. Thymine incorporation of 0.2-ml samples was determined as described in the text. Symbols: ○, DNA synthesis without rifampin; ●, DNA synthesis after application of rifampin.*

ring stage. We assumed that the effect of inactivation of the *P* function at late times after infection reflects a stop in the initiation of the ring-to-ring replication, i.e., that no new replication forks are formed. If this assumption were correct, denaturation of the heat-labile *P* protein at the ring-to-ring stage should lead to a fast stop of DNA synthesis since the completion of an initiated replicating ring molecule takes 2 min or less. The same argument is valid for the interpretation of the RNA or protein synthesis inhibition. By using *gam* mutants in a *recBC*$^+$ host, we can show (Fig. 5 and 6) that DNA synthesis is indeed much more sensitive to the abolition of the *P* function or to inhibition of RNA or protein synthesis during ring-to-ring replication than it is at the rolling circle stage (compare Fig. 2, 3, and 4).

Elimination of the *recBC* nuclease restores the rolling circle mode of replication with a *gam* mutant. The ring-to-ring mode of λ DNA replication is maintained by *gam* mutants in wild-type host cells due to the lack of inhibition of the cellular *recBC* nuclease (4). *recBC* mutant host cells allow λ*gam* phages to carry out normal rolling circle replication. It can therefore be expected that the DNA synthesis pattern after heat inactivation of a *gam*-deficient λ*Pts* mutant in a *recBC*-deficient host would be identical to the one seen after the inactivation of λ*gam*$^+$*Pts* in *recBC*$^+$ cells. Figures 7 and 8 show a comparison of results of temperature-shift experiments employing λ*gamP*$^+$ and λ*gamPts* phage in a *recBC* strain, on the one hand, with results obtained with a pair of *P*$^+$ and *Pts* phages both carrying the wild-type *gam* allele in the same host, on the other hand: DNA replication of both λ*gam*$^+$ and λ*gam* phage continued (at a reduced rate) after heat inactivation of the *P* function in the *recBC* host late after infection. This must be compared with the results obtained in the otherwise isogenic *recBC*$^+$ host described above (compare Fig. 2 and 5).

DISCUSSION

We observed a differential change in the rate of λ DNA synthesis after inactivation of the *P* function as well as after inhibition of RNA or protein synthesis at the early (ring-to-ring) com-

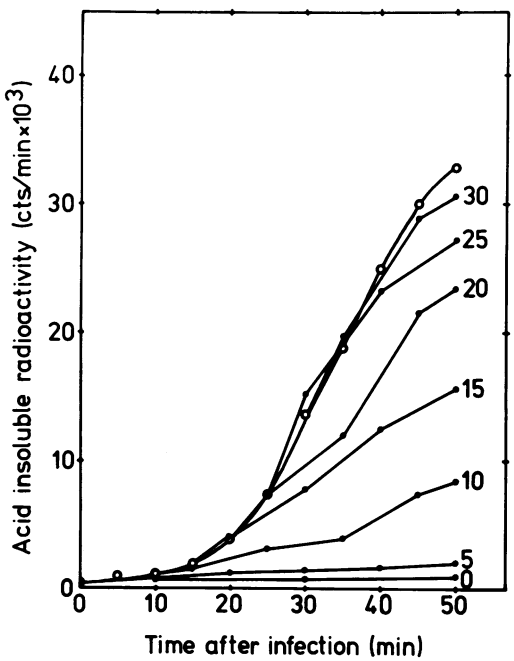

FIG. 4. *Influence of chloramphenicol on λcIb2-infected E1081 bacteria at different times after infection. A 125-μg amount of chloramphenicol was added per ml of infected bacteria at the times (in minutes) indicated by the numbers inserted in the figure. Thymine incorporation of 0.2-ml samples was determined as described in the text. Symbols: ○, DNA synthesis without chloramphenicol; ●, DNA synthesis after application of chloramphenicol at 25 min after infection.*

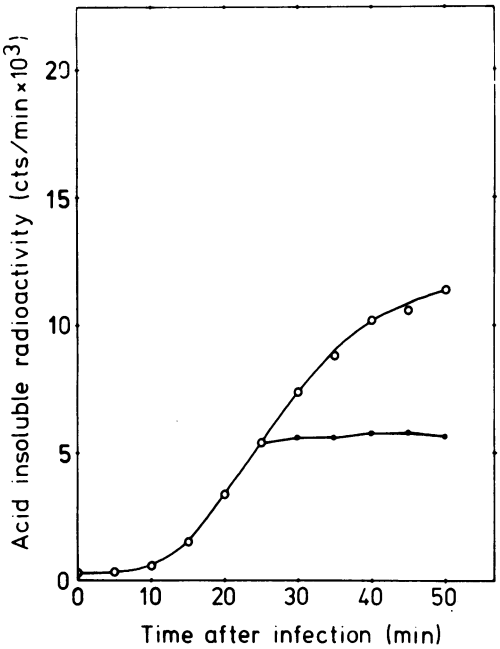

FIG. 5. *Effect of a temperature shift on λ DNA synthesis in λgam5red113πA66-infected E1081 bacteria. Thymine incorporation of 0.2-ml samples was determined as described in the text. Symbols: ○, DNA synthesis at 37°C; ●, DNA synthesis after a temperature shift to 43°C at 25 min after infection.*

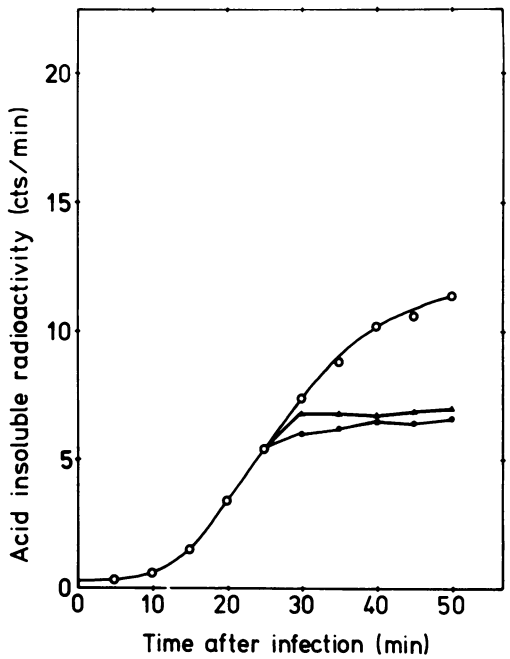

FIG. 6. *Effect of rifampin or chloramphenicol on DNA synthesis in λgam5red113πA66-infected E1081 bacteria. A 500-μg amount of rifampin or 125 μg of chloramphenicol was added per ml of cell suspension at 25 min after infection. Thymine incorporation of 0.2-ml samples was determined as described in the text. Symbols: ○, DNA synthesis in the absence of antibiotic; ●, DNA synthesis after application of rifampin; ▲, DNA synthesis after application of chloramphenicol.*

pared with the late (rolling circle) stage of replication. The results obtained with the inhibitors of transcription and translation are used below to help interpret the experiments involving *P* inactivation.

As mentioned above, it can be assumed that inhibition of protein and RNA syntheses specifically blocks initiation of ring-to-ring replication. Our results then suggest that, after a short phase during which the incoming molecule is replicated as a ring, the mode of replication is changed to the rolling circle mechanism for which initiation at the origin of replication is no longer necessary. Parallel to the rolling circle replication, which probably depends only on elongation factors, ring-to-ring replication continues, and new forks are thus formed by repeated initiation events.

The rate of DNA synthesis normally still increases while rolling circle replication is already going on. This increase is abolished after rifampin or chloramphenicol treatment (Fig. 3 and 4).

These observations can be interpreted as follows. With initiation blocked, DNA synthesis at the rolling circle stage first continues at a constant rate before slowly dropping off. This decrease in rate could be due to the withdrawal of replicating molecules from the pool during packaging or to the slow inactivation of replication forks. In the situation of continued ring-to-ring replication (after infection of *recBC*$^+$ host cells with *λgam* phage), which needs repeated initiation, inhibition of RNA and protein syntheses blocks DNA replication, presumably due to the abolition of initiation. It is known from studies of the replication of the *E. coli* chromosome, which is also a circular DNA molecule replicating bidirectionally, that its initiation, too, is sensitive both to protein synthesis inhibition and, directly, to RNA synthesis inhibition (11, 12).

The results of our experiments involving the inactivation of the *P* function parallel those obtained after RNA and protein synthesis inhibition and, therefore, suggest a role of the λ*P* protein in initiation.

This statement, however, must be qualified in two ways. First, it is based on the assumption that the chain elongation mechanisms are identical at the early and late stages of replication, and, second, there remains the possibility that

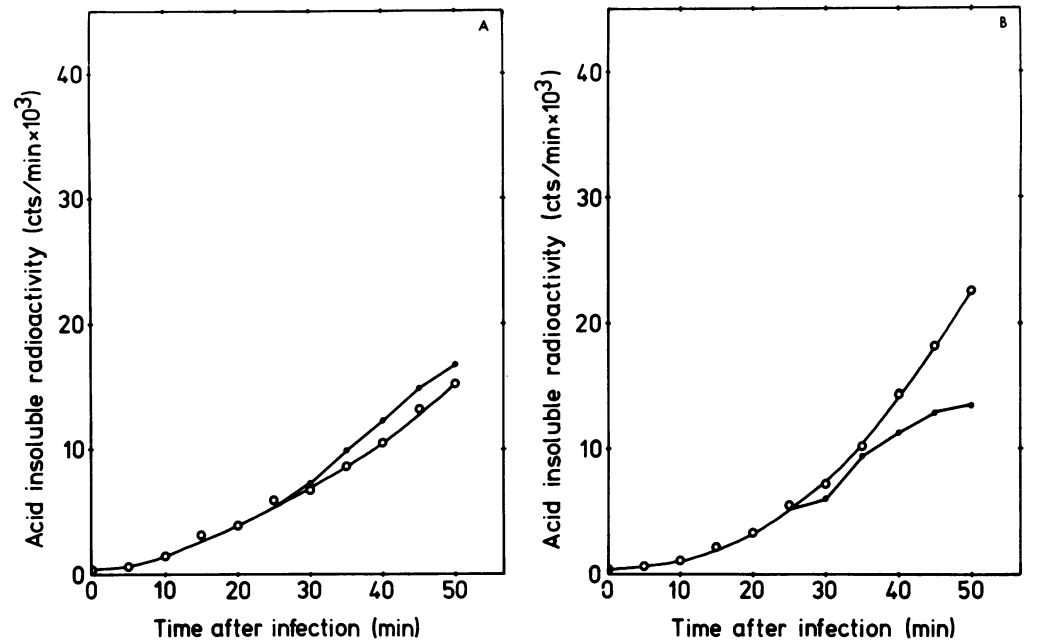

FIG. 7. *Effect of temperature shifts on the DNA synthesis in E1081 recBC cells infected with (A) λgam5red113P⁺ or (B) λgam5red113mA66. The infected cells were incubated at 37°C. They were shifted to 43°C at 25 min after infection. Thymine incorporation of 0.2-ml samples was determined as described in the text. Symbols:* ○, *DNA synthesis at 37°C;* ●, *DNA synthesis at 43°C.*

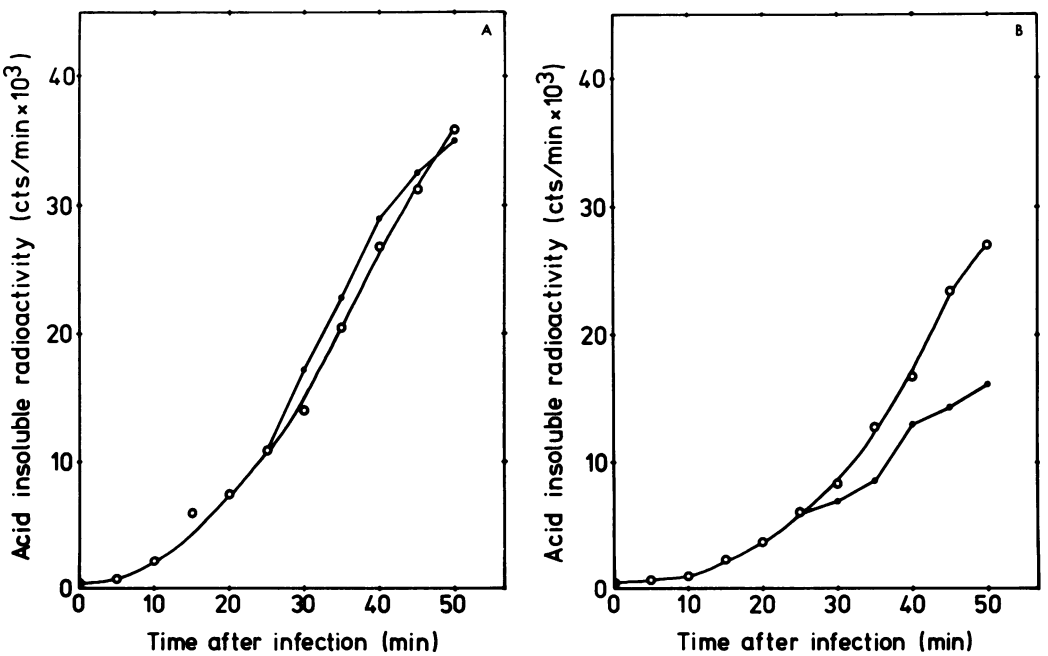

FIG. 8. *Effect of temperature shifts on the DNA synthesis in E1081 recBC cells infected with (A) λcIb2P⁺ or (B) λcIb2A66. The infected cells were incubated at 37°C. They were shifted to 43°C at 25 min after infection. Thymine incorporation of 0.2-ml samples was determined as described in the text. Symbols:* ○, *DNA synthesis at 37°C;* ●, *DNA synthesis at 43°C.*

all of our *Pts* mutants represent a class in which only one replication function, namely, the one necessary for initiation, is modified, whereas an elongation function of the same protein might still be heat resistant.

Dual roles are in fact known with other replication proteins, as will be discussed below. The inactivation of temperature-sensitive *O* mutants leads to a fast shutdown of λ DNA synthesis (17; Fig. 1; and unpublished data), even at late times after infection when mainly rolling circle replication is taking place. This has been interpreted as indicating a role of the *O* protein in chain elongation. On the other hand, it is known, as discussed above, that the *O* protein interacts with the origin of replication and with the *P* protein, which, according to the data presented here, has a role in initiation. A comparable situation exists with the cellular replication function *dnaB* (23): one *dnaB* allele (*dnaB252*) leads to a phenotype, which suggests that the *dnaB* product is involved in the initiation of DNA replication at the origin of the bacterial chromosome, whereas an analysis of other *dnaB* alleles indicates a role in chain elongation (7).

These experimental results on the dual roles of replication proteins can be reconciled if one assumes that the proteins are parts of replication complexes, which is most likely for the *O*, *P*, and *dnaB* proteins as discussed above.

A converse situation has been found (14) for the *dnaC* product of *E. coli*, which, although normally classified as an initiation factor, exhibits a behavior reminiscent of elongation functions when the *dnaB* function is replaced by an analogous protein coded for by prophage P1.

In the case of the *O* function, it would appear that the protein has an essential role in the replication complex, which would result in the inactivation of the whole complex upon the inactivation of the *O* protein and thus stop chain elongation.

The observed effects (Fig. 4 and 6) of protein synthesis inhibition on λ DNA synthesis at the early and late phases of replication suggest that a labile protein is absolutely required for ring-to-ring replication. This points to its involvement in the creation of new replication forks.

The most likely candidate for such a component is the *O* protein. It is involved in initiation, interacting with the origin as discussed above, and has a short half-life when assayed as complementing activity of *O* mutants in vivo (22). It is part of the replication machinery throughout the lytic cycle (Fig. 1). All of these properties are compatible with a model describing the *O* protein as a stable component of a chain elongation complex. However, if this complex is rearranged after each ring-to-ring replication, the *O* protein might be liberated and decay. Therefore, its continuous resynthesis would be essential for the ring-to-ring mode of replication.

Consequently, protein synthesis inhibition would block the formation of new replication forks and thus abolish the increase in replication rate late after infection.

It is noteworthy in this context that the limitation of the amounts of the *O* and *P* proteins in the cell leads to almost exclusive rolling circle replication (1, 18), which is consistent with our ideas about the essential roles of these factors in the initiation of ring-to-ring replication.

ACKNOWLEDGMENTS

We thank our colleagues for the gift of bacterial strains.

This work was supported by the Deutsche Forschungsgemeinschaft.

LITERATURE CITED

1. **Bastia, D., N. Sueoka, and E. C. Cox.** 1975. Studies on the late replication of phage lambda: rolling circle replication of the wild type and a partially suppressed strain, *Oam29 Pam80*. J. Mol. Biol. **98**:305–320.
2. **Carter, B. J., B. D. Shaw, and M. G. Smith.** 1969. Two stages in the replication of bacteriophage λ DNA. Biochim. Biophys. Acta **195**:494–505.
3. **Dove, W. F., H. Inokuchi, and W. F. Stevens.** 1971. Replication control in phage lambda, p. 747–771. *In* A. D. Hershey (ed.), The bacteriophage lambda. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
4. **Enquist, L. W., and A. Skalka.** 1973. Replication of bacteriophage λ DNA dependent on the function of *red, gam* and *rec*. J. Mol. Biol. **75**:185–212.
5. **Gefter, M.** 1975. DNA replication. Annu. Rev. Biochem. **44**:45–78.
6. **Georgopoulos, C. P., and I. Herskowitz.** 1971. *Escherichia coli* mutants blocked in lambda DNA synthesis, p. 553–564. *In* A. D. Hershey (ed.), The bacteriophage lambda. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
7. **Gross, J. D.** 1972. DNA replication in bacteria. Curr. Top. Microbiol. Immunol. **75**:39–74.
8. **Hayes, S., and W. Szybalski.** 1975. Role of *oop* RNA primer in initiation of coli phage λ DNA replication, p. 486–512. *In* M. Goulian, P. Hanawalt, and C. F. Cox (ed.), DNA synthesis and its regulation. W. A. Benjamin, Inc., Menlo Park, Calif.
9. **Kaiser, A. D.** 1971. Lambda DNA replication, p. 195–210. *In* A. D. Hershey (ed.), The bacteriophage lambda. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
10. **Klein, A., and A. Powling.** 1972. Initiation of λ DNA replication *in vitro*. Nature (London) New Biol. **239**:71–73.
11. **Lark, K. G.** 1972. Evidence for the direct involvement of RNA in the initiation of DNA replication in *Escherichia coli 15T⁻*. J. Mol. Biol. **65**:47–60.
12. **Messer, W.** 1972. Initiation of deoxyribonucleic acid replication in *Escherichia coli* B/r: chronology of events and transcriptional control of initiation. J. Bacteriol. **112**:7–12.
13. **Sakaki, Y., A. Karu, S. Linn, and H. Echols.** 1973. Purification and properties of the γ-protein specified by bacteriophage λ: an inhibitor of the host *recBC* recombination enzyme. Proc. Natl. Acad. Sci. U.S.A. **70**:2215–2219.
14. **Schuster, H., M. Schlicht, E. Lanka, M. Mikolajczyk, and C. Edelbluth.** 1977. DNA synthesis in an *Esche-*

richia coli dnaB dnaC mutant Mol. Gen. Genet. **151**:11-16.

15. **Sogo, J. M., M. Greenstein, and A. Skalka.** 1976. The circle mode of replication of bacteriophage λ: the role of covalently closed templates and the formation of mixed catenated dimers. J. Mol. Biol. **103**:537-562.
16. **Stevens, W. F., S. Adhya, and W. Szybalski.** 1971. Origin and bidirectional orientation of DNA replication in coliphage lambda, p. 515-533. *In* A. D. Hershey (ed.), The bacteriophage lambda. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. **Takahashi, S.** 1975. Role of genes *O* and *P* in the replication of bacteriophage λ DNA. J. Mol. Biol. **94**:385-396.
18. **Takahashi, S.** 1975. Physiological transition of a coliphage λ DNA replication. Biochim. Biophys. Acta **395**:306-313.
19. **Tomizawa, J.-I.** 1971. Functional cooperation of genes *O* and *P*, p. 549-552. *In* A. D. Hershey (ed.), The bacteriophage lambda. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. **Truit, C. L., and J. R. Walker.** 1974. Growth of phage λ, φX174, and M13 requires the *dnaZ* (previously *dnaH*) gene product of *Escherichia coli*. Biochem. Biophys. Res. Commun. **61**:1036-1042.
21. **Wada, C., and T. Yura.** 1974. Phenethyl alcohol resistance in *Escherichia coli*. III. A temperature sensitive mutation (*dnaP*) effecting DNA replication. Genetics **77**:199-220.
22. **Wyatt, W. M., and H. Inokuchi.** 1974. Stability of λ *O* and *P* replication functions. Virology **58**:313-315.
23. **Zyskind, J. W., and D. W. Smith.** 1977. Novel *Escherichia coli dnaB* mutant: direct involvement of the *dnaB252* gene product in the synthesis of an origin-ribonucleic acid species during initiation of a round of deoxyribonucleic acid replication. J. Bacteriol. **129**:1476-1486.