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PHYSIOLOGICAL TRANSITION OF A COLIPHAGE λ DNA REPLICATION

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Summary

The "rolling-circle" replicative intermediate (σ-type molecules) which is normally produced in the late stage of coliphage λ DNA replication can be found during the first round of λ DNA replication if cells infected with λ replication mutant *Ots28* are incubated at the nonpermissive temperature until the late stage of the latent period of λ infection. After shifting to the permissive temperature, the vast majority of replicating forms are σ-type rolling-circle even during the first round of DNA replication. Concatemeric λ DNA molecules, produced from these σ-type intermediates, are efficiently packaged into progeny phage, indicating that in the first round of λ DNA replication, double-branched θ-type molecules are not required for production of viable progeny phage.

Introduction

DNA replication of coliphage λ occurs in two stages. The first round of replication is believed to generate circular progeny DNA by double-branched θ-type replicative forms [1]. In the late stage of λ DNA replication, however, the σ-type replicative form which produce multigenomic linear concatemers are primarily found [2,5 and Bastia, D., personal communication]. It has been found that this characteristic transition of λ DNA replication from "early θ" to "late σ" mode of replication can be bypassed; when initiation of λ DNA replication is delayed until the late stage of replication by infection of a temperature-sensitive λ mutant of replication gene *O*, the vast majority of replicating molecules, even in the presumed first round of replication, are σ-molecules. As DNA replication progresses, these σ-molecules generated long DNA concatemers that are found during the late stages of λ infection; these concatemers can be encapsulated into progeny phage with good efficiency. This physiological transition is not prevented by chloramphenicol treatment during preincubation at the nonpermissive temperature suggesting that some host factors are involved in the transition.

Materials and Methods

(a) Bacterial and phage strains

Escherichia coli K12 derivative, W3102 (*gal*$^-$, *su*$^-$) was used as a host strain of density shift experiments. *E. coli* HF4704 (*thy*$^-$, *hcr*$^-$) was used as a host bacteria for experiments of radioactive precursor incorporation. The temperature sensitive (*ts*) replication gene mutant of λOts28 of coliphage λ was provided from Dr K. Matsubara, Kyushu University School of Medicine, Japan.

(b) Chemicals

($^{15}NH_4)_2SO_4$, (99 atom %; Merck, Sharp & Dohme, Montreal); Algal whole hydrolysate in ^2H_2O, (minimum 98 atom %; Merck, Sharp & Dohme, Montreal); ^2H_2O, (99.88 m %; Bio-Rad, California); [^{3}H]thymidine, (1.0 mCi (0.12 mg) in 1.0 ml H_2O; New England Nuclear, Boston); chloramphenicol, (Calbiochem, San Diego).

(c) Media

(i) *Heavy medium* contains 0.02 M potassium phosphate buffer (pH 7.3), 0.01 M $MgSO_4$, 1.6 μM $FeSO_4$, 0.5 g ($^{15}NH_4)_2SO_4$ (99 atom %), 0.4 g deuterated algal whole hydrolysate in ^2H_2O minimum 98 atom %), 2.5 g maltose per liter of ^2H_2O.

(ii) *M9 medium* contains per liter, 128 ml of salt mixture, 128 ml of 4.5% glucose, 10 ml of 0.01 M $CaCl_2$, 15 ml of 0.1 M $MgSO_4$, 100 ml of 10% casamino acid, 10 ml of vitamin B-1 (100 μg/ml), 10 ml of thymidine (100 μg/ml); salt mixture contains per liter, 70 g Na_2HPO_4, 30 g KH_2PO_4, 5 g NaCl, 10 g NH_4Cl.

(d) High temperature treatment of λOts28 infected cells

λ-sensitive *E. coli* W3102 was grown in heavy medium (or M9 medium) to log phase (A_{590nm} = 0.70, cell density = $2 \cdot 10^8$/ml) at 37° C. Non-labelled or [^{3}H]thymidine-labelled λOts28cI was infected at a multiplicity of infection of 10 in Tris-Mg (0.01 M each) and incubated for phage adsorption at 3.8—4.0° C for 40 min. At a permissive temperature, the infected complex was incubated at 31—32° C and at a non-permissive temperature the cells were incubated at 42—43° C. (At this high temperature the efficiency of plating of λOts28 mutant reduced at about 10^{-7}) [4].

(e) Preparation of intracellular λ DNA

E. coli (W3102) was grown to a cell density of $2 \cdot 10^8$/ml in heavy medium and infected with [^{3}H]thymidine-labelled λOts28 at a multiplicity of infection = 10. Intracellular λ DNA replication was terminated and purified as already described [4].

(f) Measurement of λ DNA synthesis in the infected cells

E. coli (HF4704) was grown in M9 to a cell density of $2 \cdot 10^8$/ml. They were then incubated with 50 μg/ml of mitomycin-C for 20 min to stop bacterial DNA synthesis [3], washed and resuspended in 0.1 vol of λ dilution fluid (0.01 M phosphate buffer, and 0.01 M Mg^{2+}) at 0° C and infected with λ at a

multiplicity of infection of 10. After 40 min adsorption at 0° C (over 95% of input phage adsorbed) they were diluted 10 times into prewarmed M9 containing 2 μg/ml of thymidine and then aerated vigorously. At the desired time, 1.0 ml was removed and added to an aeration tube containing 50 μCi of [*Me*-^{3}H]-thymidine and aerated 2 min, 3 ml of cold 10% trichloroacetic acid was then added to terminate the incorporation and after 60 min at 0° C the precipitate was collected on GF/A filter (Whatman) and counted in a Beckman scintillation counter.

(g) Electron microscopy

Replicating λ DNA from the CsCl density-gradient fractionation containing ^{3}H activity with a buoyant density intermediate between full-light and full-heavy was photographed, measured, and computed to normalize the length. The electron micrographs of DNA were measured by back projecting them onto a glass screen and tracing the molecules with a Numonics graphics calcula-

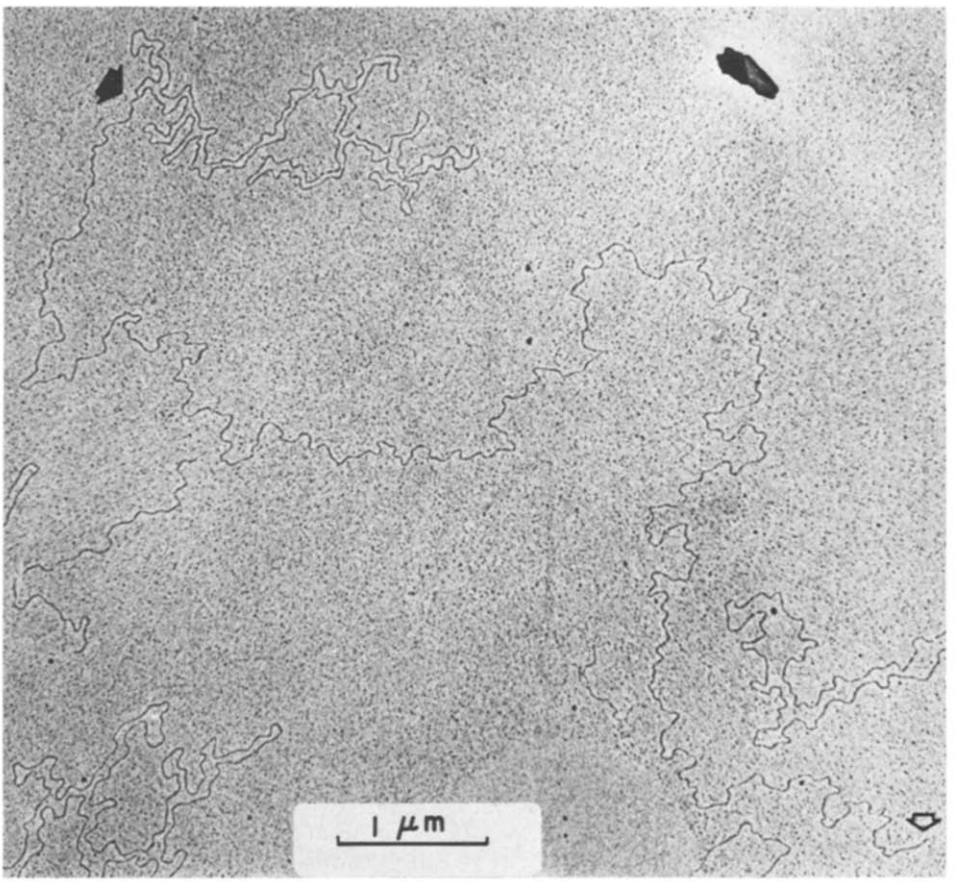

Plate 1. Electron micrograph of a rolling-circle replicative intermediate found after temperature shift. This molecule was found in fraction 19 of Fig. 1-(H): left upper circle is branched (shown by black arrow) with long tailed linear molecules. This daughter tail replicated over 100% of parental circular λ DNA. A free end of the tail is shown by a white arrow.

tor (Numonics Corp., Pa.), a digitizing device, coupled to a Newlett-Packard Model 9820 A calculator.

Results and Discussion

(a) Comparison of density shift profiles of parental λ DNA

80 ml of log phase culture of λ-sensitive *E. coli* W3102 in a heavy $N^{15}/^{2}H_2O$ Medium [1,4] was infected with [^{3}H]thymidine-labelled light λOts28 at a multiplicity of infection of 10. Adsorption of the phage proceeded for 40 min in an ice bath; 94% of the input phage were adsorbed. After centrifugation to remove unadsorbed phage, the culture was divided into eight 10-ml fractions. Four of these fractions (A, B, C and D in Fig. 1) were incubated at the permissive temperature (30° C) for 0 min (A), 15 min (B), 30 min (C) and 60 min (D), respectively. The other four tubes were incubated at the nonpermissive temperature (42° C) for 30 min and then shifted down to the permissive temperature (30° C) in order to allow DNA replication for 0 min (E), 15 min (F), 30 min (G) and 60 min (H).

At the end of each incubation, the culture was poured into an equal volume of stop solution containing 0.01 M EDTA, 0.001 M KCN, and 15% pyridine (v/v). After freezing (with liquid nitrogen) and thawing (in running water) three times, the lysates were treated with Sarkosyl, lysozyme and

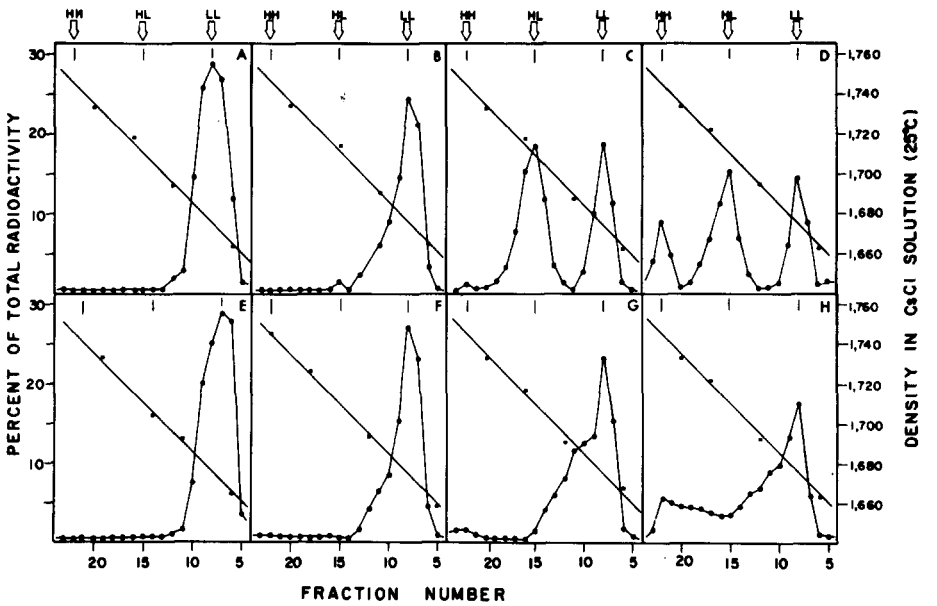

Fig. 1. Density shift profiles of parental λ DNA in normal infection and in shift down experiments. 80 ml of *E. coli* W3102 are grown in a ($^{2}H_2O$ and ^{15}N)-containing medium [1,4] to a log phase (A_{590nm} = 0.70, $2 \cdot 10^{8}$/ml) and then infected with ^{3}H-labelled λOts28 at a multiplicity of infection = 10. The infected cells are divided into eight separate tubes. Four of them A (0 min), B (15 min), C (30 min) and D (60 min) were incubated at 30° C; the other four tubes are preincubated at 42° C for 30 min and then shifted down to 30° C for E (0 min), F (15 min), G (30 min) and H (60 min). Intracellular λ DNA was purified as described previously [1,4] and was subjected to two successive CsCl density-centrifugation. The recovery of radioactivity was about 90%.

pronase. CsCl was added to each lysate (ρ = 1.67) and centrifuged (Beckman Ti-60, 30 000 rev./min at 7°C for 3 days). After the run, the centrifuged samples were fractionated from the top; fractions containing trichloroacetic acid-isoluble radioactive counts, as determined by scintillation counting, were pooled and subjected to a second CsCl density gradient run. No density shift is observed in 0 min samples at permissive temperature (A of Fig. 1) as well as 0 min samples of the shift down experiment (E). This results indicates that the pretreatment of λOts28 infected cells at high temperature blocked λ DNA replication. The 15-min samples of the normal infection (B) and the shift down experiment (F) had started replication, changing the density of their DNA toward half-heavy position. These two profiles showed no striking difference; however, samples (C) and (G) differed in their density shift profiles at the half-heavy position; sample (C) separated into a discrete half-heavy fraction, indicating parental circles replicated semiconservatively to produce progeny circles. On the other hand, the 30 min sample from the shift down experiment (G), gave continuous shift in density rather than to form a discrete peak at the half-heavy position. This characteristic difference between the density profiles of the normal infection and the shift down experiment is much more distinctive in 60 min samples (D and H of Fig. 1). These results suggest that the mode of λ DNA replication in these two different experiments may differ; this possibility can be determined by examining the replicating DNA molecules in an electron microscope.

(b) Replicating forms (RF) after temperature shift

DNA from fractions located between fully-light and half-heavy or between half-heavy and fully-heavy positions were prepared for viewing in the electron microscope.

No replicating molecules were observed in preparation from the 0 min sample at 30°C (A) or from the 0 min sample of shift down experiment (E). In the 15-min preparation at 30°C (B), 70 molecules (90%) out of 78 replicative forms were double-branched θ-forms and 8 molecules (10%) were single-branched σ-forms: In contrast, the 15 min sample from the shift down experiment (F) had 86 (95%) single-branched σ-type replicating molecules out of a total of 91 replicative forms. The DNA in fractions (G) and (H) also showed predominantly σ-type forms. It was observed, however, that 30 min (C) and 60 min (D) samples from the samples held at the permissive temperature from the beginning the incubation changed their mode of replication from θ to σ-type replicative forms as in a normal infection [1,2 and Bastia, D., personal communication]. This observation indicates that the θ is primarily formed during the first round of replication and that in the later stage of DNA replication, the σ replicative form is formed. However, preincubation of λOts28 infected cells can start the σ replicative form from the beginning of DNA replication (during the presumed first round) [5]. It is also important to note here that about 10—48% of σ replicative forms contained tails longer than one unit length of the λ genome in the late stage of latent periods in both the normal infections and the shift down experiments (30 min and 60 min samples) [2 and Bastia, D., personal communication].

TABLE I

SUMMARY OF ELECTRON MICROSCOPIC OBSERVATION OF TRANSITION OF λ DNA REPLICATIVE FORM

Replicative forms of λ DNA derived from various times after infection (i) or after shift down from 42°C to 30°C (ii) are investigated. The number shown in brackets in the 30 min and 60 min preparations indicate the numbers of molecules which possessed tail longer than one unit of the λ genome (about 17.2 μM).

Experiments	Type of (RF*)	No. of RF* at permissive temp.			Ratio of σ/θ (%)	
		15 min	30 min	60 min	Early (15 min)	Late (60 min)
(i) Permissive temperature (30°C)	(θ)	70	16	3	10	97
	(σ)	8	70 (18)	89 (34)		
(ii) Shift-down (42°C—30°C)	(θ)	5	7	2	95	97
	(σ)	86	79 (8)	56 (27)		

* RF, replicative form.

(c) Inhibition of viral protein synthesis during pretreatment

To investigate which of the factor(s) of bacteria or λ bacteriophage is required to form σ-molecules in the transition between the first and second round of λ DNA replication, we have used chloramphenicol in the pretreatment experiment. If chloramphenicol blocked the production of σ-type replicative form in shift down experiment, it suggests that a λ-encoded protein is involved in this transition. The result of chloramphenicol-treatment in shift down experiment shows that CM-treatment does not inhibit the appearance of σ-type molecules, suggesting some bacterial factors are involved in the transition; if λ encoded gene(s) is involved in σ replicative form formation, it would be inhibited by chloramphenicol during preincubation at 42°C. The result shown in Fig. 2 indicates that the transition is not blocked by chloramphenicol treatment, because the parental label shifted continuously suggesting that σ replicative form is formed. However, this experiment does not exclude a possibility of the involvement of λ-coded proteins, because it is possible that some factor(s) of λ which may involved in the transition might be synthesized immediately after chloramphenicol removement. Since in normal infection this transition occurred in the second round of λ replication, we must estimate here that some host factor(s) is involved in the transition. It is also possible that both phage λ and host factor(s) are involved.

(d) Kinetics of progeny phage production after temperature shift

As shown in Fig. 3 release of progeny phage after infection under the permissive condition starts around 30 min after infection and then increase until cell lysis (about 60 min). In the shift experiment, however, phage production was delayed 30 min after the shift down from the nonpermissive temperature; the progeny phage were then released at a rate similar to that observed in a normal infection. This result indicates that during the preincubation of λOts28 infected cells the λ genome remains in a condition ready to start DNA replication.

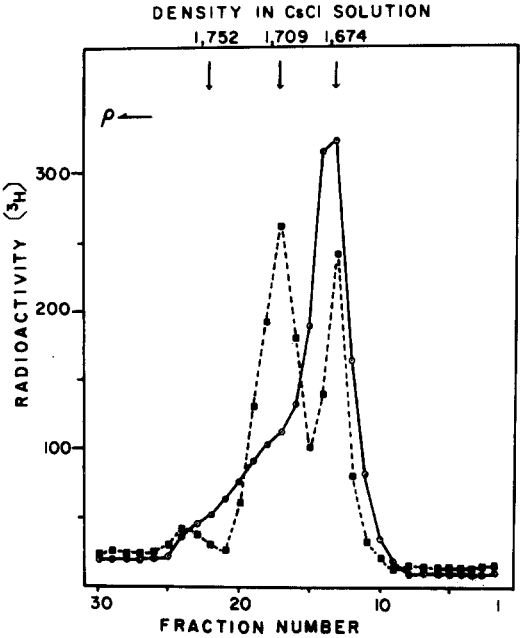

Fig. 2. Density shift profiles of parental λ DNA pretreated with chloramphenicol. 20 ml of *E. coli* was grown in a heavy medium to a density of $2 \cdot 10^8$/ml and ^{3}H-labelled λOts28 was infected at a multiplicity of infection = 10. Phage adsorption proceeded in an ice bath for 30 min. The culture was then divided into two tubes (A and B). One of them (B) was resuspended in a heavy medium containing 100 μg/ml of chloramphenicol and incubated at 42° C for 30 min. The chloramphenicol was then washed away by filtration. This culture was resuspended in heavy medium and then shifted down to 30° C for 30 min to allow DNA replication. The other culture (A) was grown in a heavy medium for 30 min at 30° C. These two lysates were lysed with sarkocyl, lysozyme and pronase and then subjected to CsCl centrifugations. ■- - - - -■; experiment (A), ○———○; experiment (B).

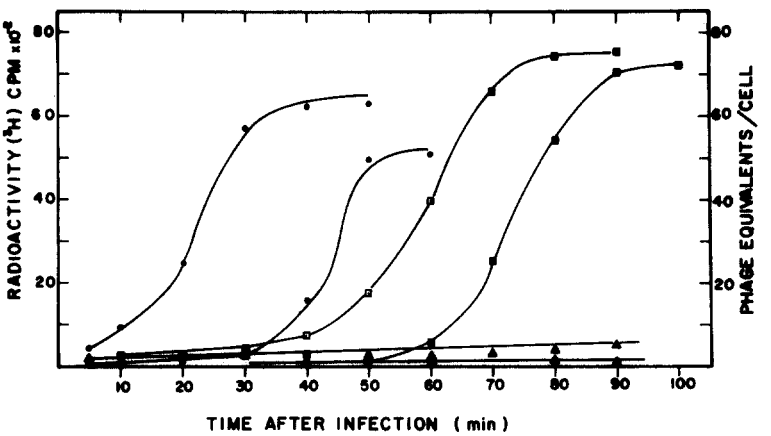

Fig. 3. Kinetics of λ DNA synthesis and progeny phage production. *E. coli* HF 4704 was grown in M9 medium to a cell density of $2 \cdot 10^8$/ml. These cells were treated with mytomycin-C to inhibit host cell DNA synthesis [3]. After washing away the mitomycin, the cells were infected with λOts28 at a multiplicity of infection = 5, incubated in an ice bath for 35 min, and then the free phage were removed by centrifugation. The cells were resuspended in fresh M9 containing [*Me*-^{3}H] thymidine (10μCi/ml) and the culture was divided into two tubes. One of them incubated at 30° C for 60 min. The other tube was first preincubated at 42° C for 30 min, and then transferred to a 30° C bath for an additional 70 min. Several times during the incubation, the incorporation of [^{3}H] thymidine and the production of progeny phage are measured. (○———○); trichloroacetic acid-insoluble cpm for normal infection, and the phage production (●———●), trichloroacetic acid-insoluble cpm for shift down experiment (□———□), and phage production (■———■), trichloroacetic acid-insoluble cpm for non-infected systems (i) (△———△) and (ii) ▲———▲).

Conclusion

From these experiments, we conclude that: The transition of the λ DNA replicative structure from the θ-type replicative form to the σ-type replicative form that is observed in normal vegetative growth is bypassed under these physiological conditions. Rolling circle replicative forms are produced directly in these experiments even in the first round of λ DNA replication; a normal level of progeny phage can be produced under these conditions.

References

1 Ogawa, T., Tomizawa, J. and Fuke, M. (1968) Proc. Natl. Acad. Sci. U.S. 60, 861—865
2 Takahashi, S. (1974) Biochem. Biophys. Res. Commun. 61, 607—613
3 Young, E. and Sinsheimer, R. (1968) J. Mol. Biol. 33, 49—59
4 Takahashi, S. (1975) J. Mol. Biol., in the press
5 Furth, M. and Takahashi, S. (1975) J. Mol. Biol., in preparation