Short Communication

ARTIFICIAL CONTROL OF THE INITIATION OF LATE-TYPE REPLICATION OF BACTERIOPHAGE λ USING TEMPERATURE-SENSITIVE *RecBC* DNase

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There are two types of replication of λ DNA (see Ref. 1). At the early stage of λ development, λ DNA replicates as a circle to produce progeny circles (θ -shaped or Carins-type replication). At a later period after infection, it replicates as a rolling circle to produce long concatemeric λ DNA, which is required for phage maturation (λ -type replication). The late type replication begins about 15 min after infection (2). The replicating intermediates of both types of replication were isolated and examined by electron microscopy (3–6). However, the molecular mechanism of the transition from θ - to λ -type replication is still obscure. To investigate the molecular mechanism of the $\theta \rightarrow \lambda$ transition is artificially controlled. In this paper, we describe the conditions under which the $\theta \rightarrow \lambda$ transition occurs when the temperature is shifted from 30° to 40°.

ENQUIST and SKALKA (7) showed that the late-type replication occurs through either recombination-dependent pathway or “replicative pathway” which is inhibited by host *RecBC* DNase. This means that the $\theta \rightarrow \lambda$ transition is controlled by the host *RecBC* DNase under recombination-deficient condition. On the other hand, TOMIZAWA and OGAWA have isolated the mutant bacteria carrying a temperature sensitive *RecBC* DNase (8). Since this temperature-sensitive *RecBC* DNase is fully active at 30° but loses the exonuclease activity for double-stranded DNA at 40° (9), this DNase may make it possible to start the $\theta \rightarrow \lambda$ transition by raising the temperature. Therefore, we tested whether the $\theta \rightarrow \lambda$ transition can be controlled by this DNase.

We employed $\lambda red_{314} gam_{210}$ (10) as the phage and *Escherichia coli* 152 *recA*[−] *recBts1* as the host bacterium. The bacterial strain was constructed by conjugation

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with 152 *recA*⁻ and Hfr strain carrying *recBtsI* (8). Under these conditions, recombination function of the bacterium (*rec*) and phage (*red*) is absent and a *gam*-gene product, and inhibitor of host *RecBC* DNase (11), is not produced, so that the production of concatemer DNA through the late-type replication must be controlled only by the temperature-sensitive *RecBC* DNase. We also introduc-

Fig. 1. Sedimentation profiles of λ DNA produced in *recA*⁻*recBtsI* host at 29° and 39°.

Escherichia coli 152 *recA*⁻*recBtsI* was grown in M9-maltose-casamino acid medium (14.7 g of NaHPO₄·12H₂O, 3 g of KH₂PO₄, 5 g of NaCl, 1 g of NH₄Cl, 1 ml of 0.1 M MgCl₂, 5 g of Difco vitamin-free casamino acid and 4 g of maltose per liter) to a concentration of 2 × 10⁸ cells/ml. Cells were washed with TM buffer (10 mM Tris, pH 7.5, 10 mM MgCl₂), resuspended in the same buffer, and irradiated by UV ray (300 erg/cm²). The bacteria were infected with the phage at a multiplicity of 1–3 at 29° for 15 min. Then, the bacteria were harvested by centrifugation, resuspended at a concentration of 2 × 10⁸ cells/ml in M9-maltose-casamino acid medium supplemented with 50 μg/ml of 2-deoxyadenosine and 0.5 μg/ml thymidine, and grown at 29° for 60 min or at 39° for 43 min. Then, bacteria were pulse labeled with 25 μC/ml of [³H]thymidine (20 Ci/mmol) for 3 min (29° sample) or 2 min (39° sample), and the labeling was stopped by the addition of Tris (pH 8.0) (final concentration of 0.1 M), EDTA (0.01 M), KCN (0.01 M), and moniodoacetate (5 mM). According to the method of ENQUIST and SKALKA (7), bacteria were lysed and the labeled DNA was analyzed by 5–20% neutral sucrose gradient in Beckman SW 50–1 rotor at 28,000 rpm for 3.5 hr, at 11°. (a), *imm*⁴³⁴*cIA*₃₂*S*₇ at 29°; (b), *imm*⁴³⁴*cIA*₃₂*S*₇*red*₃₁₄*gam*₂₁₀ at 29°; (c), *imm*⁴³⁴*cIA*₃₂*S*₇*red*₃₁₄*gam*₂₁₀ at 39°; (d), uninfected sample. ---, ¹⁴C-labeled λ DNA prepared from phage particle, —, ³H-labeled intracellular DNA.

ed two mutations in the phage, one in gene A to prevent the cutting of long concatemeric λ DNA to monomeric DNA (12) and the other in gene S to prevent lysis of the bacteria by phage lysozyme (13). These two mutations make it easier to analyze the λ DNA produced at the late stage of λ development. Using the bacterium and phage, we analyzed λ DNA produced after phage infection, by the sucrose gradient centrifugation.

At first, we tested whether a temperature-sensitive *RecBC* DNase is active enough to prevent the production of concatemer λ DNA at 30° and inactive enough to permit the late-type replication. Host bacteria were irradiated with UV before infection to block the host DNA synthesis (7). Then, the bacteria were infected with λ^+ or $\lambda_{red^-gam^-}$ and grown in the presence of [3 H]thymidine. At the late stage of λ development, the bacteria were lysed and the labeled DNA was extracted, analyzed by sucrose gradient centrifugation. As shown in Fig. 1, long concatemeric λ DNA which sediments 2–3 times faster than monomeric λ DNA (7) was produced by wild type λ at 29°, but hardly produced by $\lambda_{red^-gam^-}$ phage. The $\lambda_{red^-gam^-}$ was however, able to produce the concatemeric λ DNA at 39° (Fig. 1c). Since the labeled DNA which sediments faster than monomeric λ DNA was not observed in uninfected cells (Fig. 1d), and most of the labeled DNA was thought to be λ DNA. We further confirmed this point by DNA-DNA hybridization study. As shown in Table 1, at least 60% of the labeled DNA which sediments faster than monomeric λ DNA (fraction number 1–15) preferentially hybridized to λ DNA. These results indicate that the labeled DNA which sediments faster than monomeric λ DNA is actually λ DNA. Thus, the temperature-

Table 1. DNA-DNA hybridization analysis of labeled DNA.^a

Tested DNA (Fraction number ^b)	Hybridization (%) with	
	<i>E. coli</i> DNA (20 μ g)	λ DNA (5 μ g)
1+2+3	12.4	33.5
4+5+6	11.7	29.8
7+8+9	13.6	25.6
10+11+12	10.4	40.2
13+14+15	10.8	34.6
16+17+18	10.7	37.1
19+20+21	8.7	48.3
¹⁴ C- λ DNA from phage	2.3	54.9
<i>E. coli</i> ³ H-DNA	34.9	1.0

^a DNA-DNA hybridization was made by using an adaptation of the method of DENHARDT (14) DNA-filter technique described by IMAE and KURODA (15).

^b *red^-gam^-* was grown in *recA^-recBts* bacteria at 29° as described in the legend to Fig. 1. At 60 min, the growth temperature was shifted to 44° and DNA was labeled with [3 H]thymidine for 3 min. Then, the phage was grown for 10 min (5 min at 44° and 5 min at 39°) in the presence of excess amount of unlabeled thymidine. The labeled DNA was subjected to sucrose gradient centrifugation as described in Fig. 1. Position of λ monomer DNA was fraction number 22.

sensitive *RecBC* DNase is able to control the production of concatemeric λ DNA. Under our conditions, the labeled DNA which sediments much slowly than monomeric λ DNA was observed. This small DNA comes from the host bacteria, because such DNA was also observed in uninfected bacteria (Fig. 1d). The nature of such a small DNA is unknown so far.

Next, we tested whether the production of concatemer DNA can be started at appropriate time of the late stage of λ development by raising temperatures. Bacteria infected with the $\lambda red^- gam^-$ phage were grown at 29° and at the late stage of λ development the growth temperature was shifted to 44° to inactivate the temperature-sensitive *RecBC* DNase. At the same time, the bacteria were pulse-labeled with [3 H]thymidine for 2 min and then grown at 39° for 15 min in the presence of excess amount of unlabeled thymidine. As shown in Fig. 2, the

Fig. 2. Sedimentation profile of λ DNA produced in *recA^- recBts* hosts infected with $\lambda red^- gam^-$.

Cells were grown at 29°, washed, and irradiated with UV ray as described in the legend to Fig. 1. After infected with $\lambda red^- gam^-$, the cells were grown at 29° for 60 min in M9-maltose-casamino acid medium supplemented with 2-deoxyadenosine and thymidine, and then, the temperature was shifted to 44°. At the same time, DNA was pulse-labeled with [3 H]thymidine (final concentration of 25 μ C/ml) for 2 min. Half of the sample was further grown at 44° for 3 min and at 39° for 15 min in the presence of 2 mg/ml of unlabeled thymidine. Bacteria were lysed and the DNA was analyzed as described in the legend to Fig. 1. (a), Intracellular DNA just after pulse labeling; (b), intracellular DNA after chasing out. —, 3 H-labeled intracellular DNA; ---, 14 C-labeled λ DNA as a marker.

DNA extracted just after pulse-labeling hardly contained concatemer DNA, but that at 15 min after chasing out contained a large amount of concatemer DNA. These results indicated that the production of concatemer DNA was initiated by the rise in temperature and that the λ DNA labeled just after the temperature shift is a precursor of long concatemer DNA. Thus, under our conditions, the production of concatemer DNA through the late-type replication can be initiated at an appropriate time only by raising the temperatures. We believe that this system is useful for studying the molecular mechanism of the $\theta \rightarrow \lambda$ transition.

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