THE FORMATION OF CIRCULAR DNA AFTER LYSOGENIC INDUCTION

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When lysogenic or sensitive cells are infected with phage λ, the entering linear duplex λ DNA is rapidly converted to a new DNA species (1,2). Bode and Kaiser (2) have presented evidence that this new phage DNA species is a circular (nonended) DNA which has the characteristics of the twisted circular DNA obtained from polyoma or SV40 viruses (3,4) and of the replicating form of ϕ X 174 DNA (5).

Since much, if not all, of the infecting linear λ phage DNA seems to be converted to a circular form, the significance and function of this DNA species are of some interest. Campbell (6) has previously postulated a circular DNA form as an intermediate in the formation of prophage from the infecting phage genome. In this connection it is worth noting that a circular DNA species is also found soon after infection of cells by the C_I, C_{II}, C_{III} and virulent mutants of λ (7). These mutants are deficient or lacking in the ability to lysogenize a host cell.

The experiments reported in this communication demonstrate the formation of a circular DNA species after lysogenic induction of E. coli K12λ and show it to have characteristics similar to those of the circular DNA formed from λ phage DNA immediately after infection. Thus the formation of a circular λ DNA is not limited to that derived directly from an infecting phage DNA but is also formed during the normal replication of the λ DNA after lysogenic induction.

MATERIALS AND METHODS

Escherichia coli CR-34 (S) (nonlysogenic) and CR-34 (λ) Leu⁻Thr⁻Thy⁻B_1^- were obtained from Dr. M. Meselson. Each strain was grown with rapid aeration at 37° to a cell population of 2 x 10^8 cells per ml in 250 ml of synthetic medium containing 60 mM thymine (7). The culture was then induced with ultraviolet light and immediately thereafter 0.25 millicurie methyl-H^3-thymine (New England Nuclear Corp.) was added. A 10-ml aliquot was removed and observed for lysis and phage yield. At 1, 10, 20, 30, 35, 45, and 60 minutes after induction, 250 ml of the irradiated culture were rapidly cooled to 0° and the cells collected by centrifugation. The cells (0.5 g) were washed once with 0.01 M Tris, pH 8.0, and the DNA isolated from the induced cells by a modification of the procedure of Bode and Kaiser (2,7). One milliliter of these DNA preparations was layered on 28 ml of linear 10-30% sucrose gradients (sucrose dissolved in 0.3 N NaOH, 10^{-2} M Tris, pH 7.4, 10^{-3} M EDTA). Alkaline sucrose gradients were used in these experiments because of the high sedimentation coefficient of double-stranded cyclic DNA above pH 12 (2,3). Centrifugation was performed in a Spinco SW-25 head for 16 hours at 18,000 rpm at 0°. One-milliliter fractions were collected and the acid-insoluble material was assayed for radioactivity as described previously (7).

RESULTS

At 1, 10, 20, 30, and 35 minutes after ultraviolet light induction of CR-34 (λ) only one DNA peak is noted in the alkaline sucrose gradient (Fig. 1). This peak sediments at about the same rate as DNA obtained either from mature λ phage or the host cell. Forty-five minutes after induction of the lysogen, however, one can detect a second DNA species sedimenting at 3-4 times the rate of the slow moving peak (Fig. 2). This new DNA species is also seen at 60 minutes after ultraviolet irradiation of the lysogen. Control experiments using the same ultraviolet irradiation and the nonlysogenic CR-34 (S) strain failed to reveal any fast sedimenting DNA species appearing within 60 minutes.

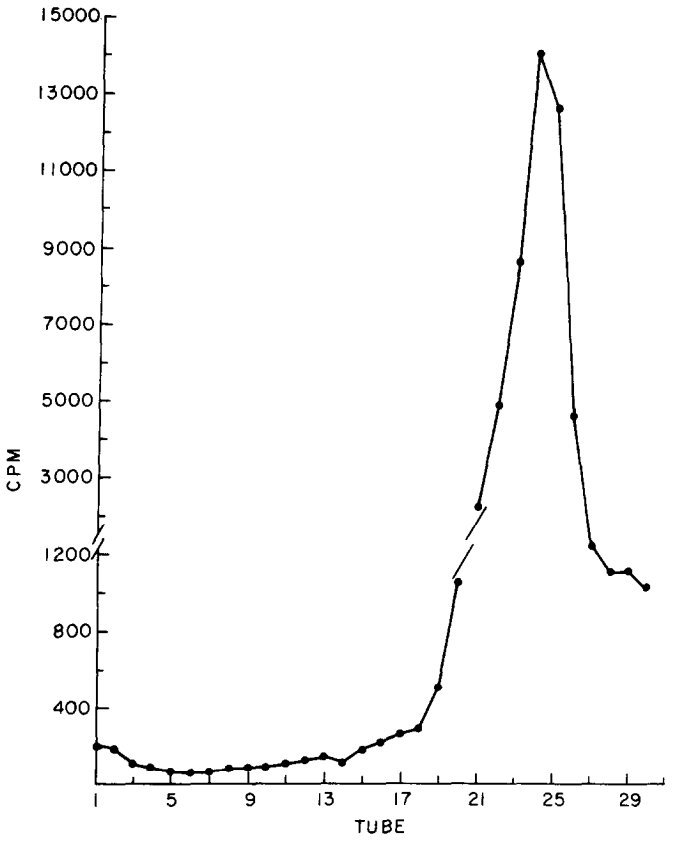

Fig. 1. Sucrose gradient centrifugation at pH 12.2 of DNA obtained from CR-34 (λ) 30 minutes after ultraviolet induction. Essentially the same pattern is obtained with DNA isolated from cells from 1-35 minutes after ultraviolet irradiation. The bottom of the gradient is in tube 1 and sedimentation is from right to left.

The appearance of the fast sedimenting DNA species in the induced lysogenic strain occurs at the time that phage DNA synthesis begins. As shown in Figure 3, ultraviolet irradiation of CR-34 (λ) stops almost all DNA synthesis for approximately 30-45 minutes, after which time synthesis resumes at a rapid rate. Cell lysis and release of phage are pronounced by 75 minutes.

This new fast sedimenting DNA species observed after lysogenic induction has been isolated and its properties compared with those of the purified circular λ DNA formed directly from the infecting λ genome (2,7). As shown in Table I, the properties of these two DNA species are identical. They both

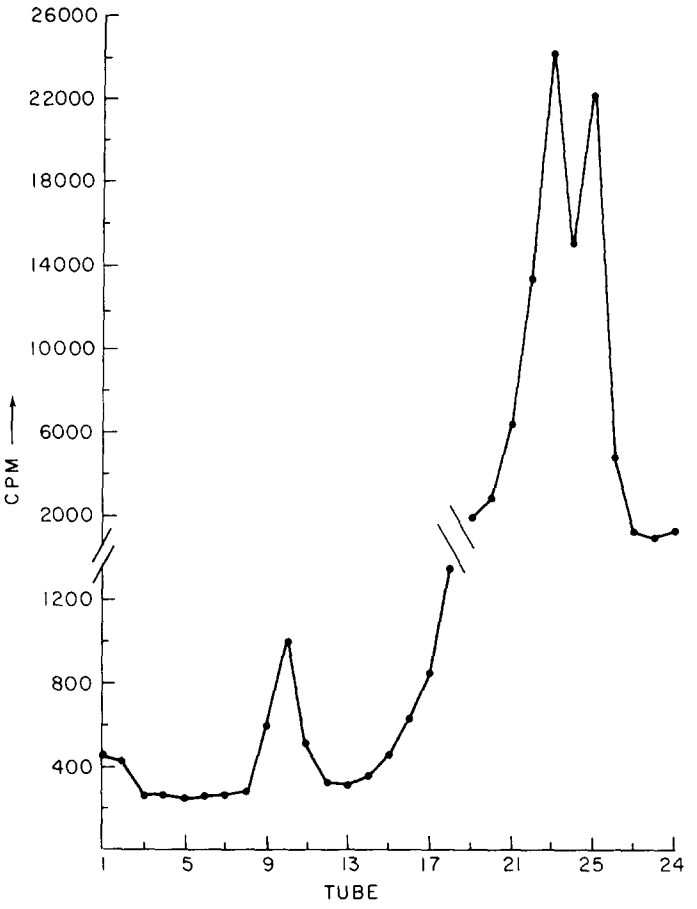

Fig. 2. Sucrose gradient centrifugation at pH 12.2 of DNA obtained from CR-34 (λ) 45 minutes after ultraviolet induction. Direction of sedimentation is as described in Figure 1.

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exhibit characteristic sedimentation rates in sucrose gradients at pH 7.4 or 12.2. After equilibrium banding of these DNA preparations in CsCl and re-isolation, both still show the original sedimentation rates in alkaline sucrose gradients. They are converted by exposure to 0.1 M acetic acid at room temperature for 2 minutes to a slower sedimenting form. In addition, both the known circular λ DNA and the fast sedimenting DNA species isolated from induced cells are unaffected by heating at 75° for 10 minutes at pH 7.4.

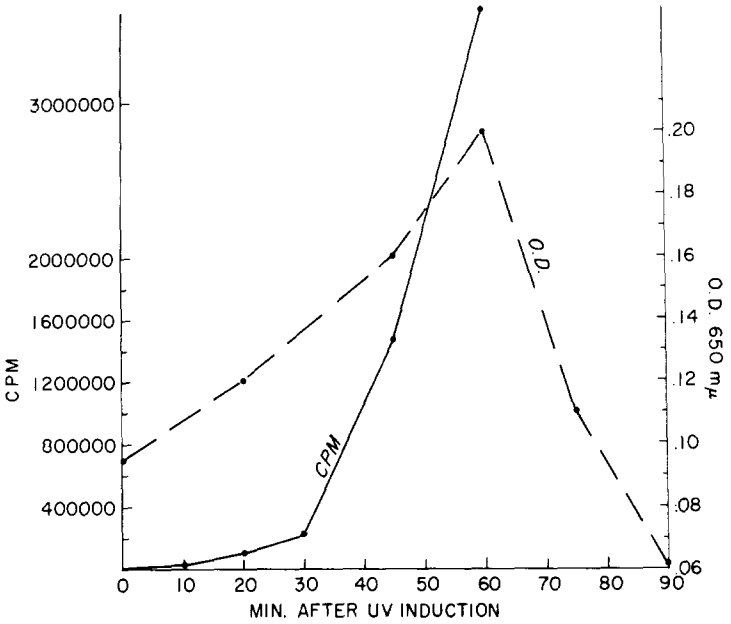

Fig. 3. The incorporation of H^3-thymine into DNA by E. coli CR-34 (λ) and cell lysis (as measured by optical density at 650 mμ) after lysogenic induction with ultraviolet light.

Table I. Properties of fast sedimenting DNA species isolated after lysogenic induction

	Circular λ DNA†	Fast sedimenting DNA obtained after induction
Mobility relative to linear DNA in sucrose gradients, pH 12.2	3.5-4.0	3.5-4.0
Mobility relative to linear duplex DNA in sucrose gradients, pH 7.4	2-3	2-3
Bands as does linear duplex λ DNA in CsCl, pH 7.4	+	+
Retains initial mobility in alkaline sucrose after banding in CsCl	+	+
Altered by incubation in 0.1 M glacial acetic acid at 25°	+	+
Stable on heating at 75° for 10 minutes at pH 7.4 in 0.6 M NaCl	+	+

†Obtained from infecting E. coli W3350(λ) with λ^+ as previously described (7)

DISCUSSION

These data satisfy the criteria used by previous workers (2,3) to establish the presence of circular duplex DNA molecules and strongly suggest that the fast sedimenting DNA species observed after lysogenic induction is a twisted circular (nonended) λ DNA. The appearance of this circular λ DNA 45 minutes after lysogenic induction coincides with the onset of DNA synthesis in the induced cells. The amount of this new DNA species detected at any time after induction does not exceed 1-2% of the total DNA being synthesized. Experiments with CR-34 (λ) cells labeled with H^3-thymine before lysogenic induction show no formation of a radioactive λ DNA circle. Thus, this circular DNA is not derived directly from the prophage or any part of the bacterial chromosome. With the present data it is impossible to evaluate the significance of the circular λ DNA form or whether it is a precursor or product of the linear λ DNA molecule. Since it occurs both after lysogenic induction as shown here, and after infection of immune (lysogenic) or sensitive E. coli K12 cells (2,7), it may have some role in the formation of λ phage.

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