THE BIOSYNTHESIS OF PHAGE LAMBDA DNA:
THE STRUCTURE OF THE FIRST INTERMEDIATE*

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The mature DNA molecule which is found in normal lambda phage is a linear duplex with a molecular weight of $3.3 \times 10^7$ daltons.\(^1\) The $5'$ ends of this DNA molecule are single-stranded regions of about 15 nucleotides\(^2\) which are complementary and which can base pair with one another to form either intermolecular aggregates or intramolecular base-paired circular molecules.\(^3\)

Previous studies on the de novo synthesis of lambda DNA after lysogenic induction have shown that both covalently linked circular and large concatenated forms of the lambda DNA are produced as intermediates.\(^4\)\(^5\) The covalently linked circular lambda DNA molecules are formed in small amounts and apparently are never converted to mature lambda DNA.\(^4\) Their function is unknown. The large, concatenated lambda DNA forms are major intermediates in the formation of the mature lambda DNA.\(^5\) They seem to have molecular weights much larger than mature lambda DNA and resemble the replicative forms previously implicated in the synthesis of the DNA of the phages T4, T5, and lambda.\(^5\)\(^6\)\(^-\)\(^8\) The concatenated lambda DNA intermediates are accumulated in large quantities in induced mutant lysogenic strains which are unable to make normal phage heads and cannot break down these intermediate forms of DNA to the mature lambda DNA found in the phage particle.\(^5\)

It was previously found that the first detectable form of lambda DNA which is synthesized after lysogenic induction sediments at the same rate as mature lambda DNA in both neutral and alkaline sucrose gradients.\(^4\)\(^5\) This early intermediate is therefore probably a linear duplex of the same size as mature lambda DNA. The results in this paper will show that this early intermediate lacks the normal single-stranded ends found in the mature lambda DNA, and that it is a precursor of both the large, concatenated forms of lambda DNA and the mature phage DNA which appear later in the cell.

Materials and Methods.—Escherichia coli W3350 (λ) was obtained from Dr. Allen Campbell. Lysogenic induction was accomplished by irradiation with ultraviolet light, as previously described.\(^5\)

Radioactive labeling experiments: Three 100-ml cultures of W3350 (λ) cells were grown in synthetic media\(^5\) with aeration to a cell density of $2 \times 10^9$/ml and irradiated with ultraviolet light (200 ergs/mm\(^2\)). Forty minutes after irradiation each 100-ml culture received 1.0 mc of H\(^3\)-thymidine (New England Nuclear Corp., 10 c/mmole). After a five-minute incubation one culture was cooled to 0°C and cells were collected by centrifugation. Ten micromoles of nonradioactive thymidine were then added to the other cultures and the cells permitted to incubate for an additional 15 or 25 minutes. Addition of this large excess of nonradioactive thymidine stops the further incorporation of any significant amount of H\(^3\)-thymidine. Thus, at 45, 60, or 70 minutes after irradiation the pulse-labeled cells were collected by cen-
trifugation and the DNA was extracted as previously reported.\textsuperscript{5} Lysis of these irradiated cells occurs 75 minutes after treatment with ultraviolet light, with a phage yield of $2 \times 10^{10}$/ml.

C\textsuperscript{14}-lambda DNA was prepared from purified lambda phage by treatment with phenol.\textsuperscript{6}

Sucrose gradient centrifugations: One milliliter of the H\textsuperscript{3}-labeled DNA preparation was layered onto a linear 5–25 per cent sucrose gradient (26 ml) prepared in 0.02 $M$ Tris, pH 7.4, 10\textsuperscript{–3} $M$ EDTA. The centrifugation was carried out at 0°C for 16 hours at 18,000 rpm in the SW-25 rotor in the Spinco model L ultracentrifuge. Twenty-five-drop fractions (0.8 ml) were collected by siphon and 0.01-ml aliquots were counted in a triton-toluene scintillation mixture.\textsuperscript{9}

The peak fraction of radioactivity from the above sucrose gradient was dialyzed against 0.6 $M$ NaCl, 0.01 $M$ Tris, pH 7.4. An aliquot containing less than 2 $\mu$g of DNA per ml was then used for annealing experiments following the procedure of Hershey \textit{et al.}.\textsuperscript{3} Before and after annealing experiments the DNA preparations used were diluted with an equal volume of water and sedimented in 32 ml of a linear 5–25 per cent sucrose gradient pH 7.4. Centrifugations were performed in the SB-110 rotor (International Equipment Company B-60 ultracentrifuge) for 16 hours at 22,000 rpm. Fifteen-drop fractions (0.5 ml) were collected and counted directly in a triton-toluene scintillation mixture.\textsuperscript{9}

Results.—Pulse labeling of DNA 40–45 minutes after lysogenic induction: The inducing dose of ultraviolet light used in these experiments inhibits the host cell DNA synthesis. Under these conditions, lambda DNA synthesis starts at about 40 minutes after ultraviolet irradiation of \textit{E. coli} W3350($\lambda$).\textsuperscript{5} DNA synthesis by the isogenic nonlysogenic strain irradiated under the same conditions is about one-tenth that of the lysogen. If the induced lysogenic strain is pulse labeled with H\textsuperscript{3}-

![Fig. 1](image-url)

\textit{Fig. 1.}—Sedimentation of H\textsuperscript{3}-DNA synthesized 40–45 min after ultraviolet light induction of \textit{E. coli} W3350($\lambda$). Induced cells were pulse-labeled with H\textsuperscript{3}-thymidine, and DNA preparations from these cells were sedimented in a SW-25 rotor in the Spinco model L as described in the text. Sedimentation in this and other figures is from right to left with fraction 1 representing the bottom of the tube. (\textit{A}) Pulse labeled 40–45 min and cells collected immediately. (\textit{B}) Pulse labeled 40–45 min; nonradioactive thymidine added for an additional 15 min and cells collected. (\textit{C}) Pulse labeled 40–45 min; nonradioactive thymidine added for an additional 25 min and cells collected.
thymidine from 40 to 45 minutes after induction, and the cells then immediately isolated, the newly synthesized phage DNA shows a single peak when sedimented in a sucrose gradient (Fig. 1A). As shown below, this DNA band has the same sedimentation rate as mature lambda DNA. Furthermore, this DNA peak is not formed in irradiated, nonlysogenic control cells.

Properties of the early intermediate formed at 40-45 minutes: In order to investigate the structure of the early DNA species which is synthesized at 40-45 minutes after induction, the DNA contained in the peak tube (tube 19) of Figure 1A was re-sedimented in a sucrose gradient in the presence of a marker C14-lambda DNA obtained from mature phage particles. The pulse-labeled H3-DNA sediments at the same rate (within 2-3%) as does mature lambda DNA (Fig. 2A). If this pulselabeled DNA is heated to 75°C in the presence of 0.6 M salt and annealed as described by Hershey et al., its sedimentation rate relative to linear lambda DNA is unaffected (Fig. 2B). These annealing conditions are known to convert linear mature lambda DNA to a base-paired circular molecule which sediments 1.13 times as fast as the linear mature lambda DNA. This is confirmed under the conditions of our experiments as shown in Figure 2C, where both the C14-mature lambda DNA marker and the pulse-labeled H3-DNA were mixed together in solution and annealed. After annealing, the mature C14-lambda DNA sediments about 10 per cent faster than the linear lambda DNA, as would be expected for base-paired circular molecules. These results show that the first form of lambda DNA which is synthesized de novo in induced cells is apparently a linear structure of the same size as lambda DNA but is missing the complementary 5'-single-stranded ends which are necessary for the formation of base-paired circles. The possibility that the 5'-

![Fig. 2.](image-url)
single-stranded ends are still present but altered or blocked in some way cannot be excluded. This early form of lambda DNA, lacking the single-stranded ends, will be referred to as intermediate I.

Confirmation that intermediate I is a form of lambda DNA has been obtained by hybridization experiments using the DNA-agar technique. Intermediate I shows a high degree of reaction (62%) with wild-type lambda DNA imbedded in agar. Furthermore, the thermal elution pattern obtained after such hybridization is similar to that found with whole lambda DNA itself.

Formation of other lambda DNA species from intermediate I: The data in Figure 1 show that intermediate I is converted, in part at least, to the faster-sedimenting DNA species which, as described previously, have the properties of large, concatenated molecules. Thus, at 60 minutes after induction much of the pulse-labeled DNA formed at 40–45 minutes has been converted to larger molecules (concatemers) which sediment considerably faster than mature lambda DNA (Fig. 1B). By 70 minutes after induction (Fig. 1C), just before lysis occurs, the fast-sedimenting concatemers have disappeared and the early pulse-labeled DNA is, once again, sedimenting at the rate of mature lambda DNA. This is shown in detail in Figure 3A where the H\(^2\)-DNA in the peak tube (tube 20) of Figure 1C was resedimented in a sucrose gradient and found to migrate with lambda DNA marker. When annealing experiments are carried out, it is found that most of the original pulse-labeled H\(^2\)-DNA, which is still present in the cells 70 minutes after induction, can now be annealed and then sediments at the rate of base-paired circular lambda DNA molecules (Fig. 3B and C). This is in contrast to the results shown in Figure 2 where intermediate I formed from 40 to 45 minutes after induction and immediately isolated could not be annealed to form circular molecules. Thus, between 45 and 70 minutes after induction intermediate I, which lacks complementary 5'-single-stranded DNA.

![Fig. 3](image-url)

**Fig. 3.**—Annealing experiments with H\(^2\)-DNA present in induced cells just prior to lysis. Aliquots of the H\(^2\)-DNA in tube 20, Fig. 1C, were sedimented before and after annealing experiments. **Solid line**, H\(^2\)-DNA; **dashed line**, C\(^14\)-lambda DNA. (A) No annealing. (B) H\(^2\)-DNA annealed as in Fig. 2, and C\(^14\)-mature lambda DNA added after the finish of the annealing. (C) Both H\(^2\)-DNA and C\(^14\) marker lambda DNA annealed together. Approximately 60 fractions were collected after each gradient centrifugation, and no radioactivity was found in the upper 30 fractions.
ends, can be converted to the mature lambda DNA. Figure 1 indicated that the fast-sedimenting concatenated DNA species probably play a role in this conversion. It should be pointed out that approximately one third of the early pulse-labeled DNA, even at 70 minutes after induction, still cannot be annealed to form circular molecules (Fig. 3).

Discussion.—The biosynthesis of lambda DNA after lysogenic induction has been shown to involve several intermediate structural forms of the DNA molecule. From the work reported in this paper it is possible to set up a tentative scheme for the de novo synthesis of lambda DNA in lysogenically induced cells, as follows:

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Prophage---⟩ Intermediate I------⟩ Concatenated----⟩ Mature
    template                      (linear DNA intermediates      lambda
        (lacking 5'-single-stranded ends)
                        ↓
                         Circular lambda DNA
                            (covalently linked)
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Several points about this scheme should be discussed. Intermediate I, once formed, can be a precursor of mature lambda DNA. This occurs by way of the intermediate formation of larger, concatenated molecules which may then be broken down to mature lambda DNA. This hypothesis is very similar to that described by Frankel for the synthesis of phage T4 DNA. However, part of intermediate I, which is formed 40–45 minutes after induction, is stable and is neither converted to concatenated forms (Fig. 1) nor to mature lambda DNA (Fig. 3). Whether this intermediate as such is even packaged into a phage partial remains to be answered. The mechanism of formation of intermediate I also remains obscure, although it is possible that it represents a direct copy of the prophage in the chromosome. A determination of the gene order of intermediate I would be of significance in this respect.

Although, as indicated in scheme (A), covalently linked circular lambda DNA molecules are known to be synthesized after lysogenic induction, it is unclear how they are formed or what their direct precursors are. They represent only a few percent of the total lambda DNA which is made and available evidence strongly indicates that they are formed after intermediate I when induction is accomplished by thymine deprivation or ultraviolet light. At present the function of the various intermediate forms of lambda DNA shown in scheme (A) is a matter of speculation, but it is possible that these different DNA species determine the transcription of early and late phage-specific messenger RNA.

Summary.—The first lambda DNA molecules which are synthesized 40–45 minutes after lysogenic induction are the size of mature lambda DNA but lack the normal 5'-single-stranded ends. This early intermediate form of lambda DNA can be converted to large, concatenated forms of DNA and then eventually to mature lambda DNA. Part of the early intermediate DNA seems to maintain its structure and can be detected in the induced cells just before lysis occurs.

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Wu, R., and A. D. Kaiser, these PROCEEDINGS, 57, 170 (1967).

Hershey, A. D., E. Burgi, and L. Ingraham, these PROCEEDINGS, 49, 748 (1963).


The DNA hybridization experiments were carried out in collaboration with Dean Cowie and Lowell Belin of the Carnegie Institution of Washington and will be the subject of a future report.