THE PROCESS OF INFECTION WITH BACTERIOPHAGE φX174, XXI. REPLICATION AND FATE OF THE REPLICATIVE FORM*

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Previous publications have described the formation of a double-stranded DNA intermediate[1] (replicative form, RF) during the replication of bacteriophage φX174, the semiconservative replication at a bacterial "site"[2] of that RF containing the parental DNA strand,[3, 4] and the persistence of the parental DNA strand at the site, accompanied by the release of the daughter RF.[5] The nascent RF molecules first appear as RFII,[6, 7] and are rapidly converted to RFI.

This paper considers the structure of the nascent RF molecules in more detail and inquires into the fate of the daughter RF molecules during the subsequent period of progeny single-strand DNA formation. A succeeding paper[8] will consider the process of formation of the progeny single-strands.

Experimental Procedure.—Strains and media: *Escherichia coli* HF4704 (HCR⁻, thymine-requiring) was used in these experiments. TPG-2A medium[9] and minimal "heavy" ($C^{13}N^{15}$) and "light" ($C^{12}N^{14}$) media[10] have been described. φXam3 is a lysis-defective mutant,[11] while φXγh is a temperature-sensitive mutant of extended host range.

Mitomycin treatment: In all experiments, HF4704 cells were pretreated with mitomycin C before infection as previously described.[12]

DNA: DNA was isolated from infected cells by lysis with lysozyme-EDTA and deproteinization with phenol as previously described.[9]

Centrifugal techniques: Separation of single-stranded φX-DNA, RFI, and RFII was achieved by zone sedimentation through a preformed cesium chloride gradient (1.20–1.35 gm/ml) at neutral pH[13] at 25,000 rpm for 6 hr. Separation of circular and linear φX-DNA from RFII was achieved by sedimentation through a similar preformed CsCl gradient at pH 12.4 at 40,000 rpm for a 5-hr period. Separation of denatured RFI, the viral strand of φX-DNA, and the complementary strand of φX-DNA was achieved by sedimentation to equilibrium in alkaline (pH 12.4) CsCl ($\rho = 1.74$–1.77 gm/ml) at 37,000 rpm (SW50 rotor) for 36 hr at 25°C.[13] Under these conditions the buoyant densities of denatured RF, viral DNA, and complementary DNA are 1.780, 1.765, and 1.753 gm/ml, respectively.[14, 15] In some experiments denatured DNA from *M. lysodeikticus* ($\rho = 1.788$ gm/ml) was added as a density marker.

Identification of "heavy" ($C^{13}N^{15}$) and hybrid ($C^{13}N^{15}$):($C^{12}N^{14}$) RF and "heavy" single-stranded DNA was achieved by sedimentation to equilibrium in neutral CsCl ($\rho = 1.73$ gm/ml) with γh-DNA (identified by infectivity) employed as a density marker ($\rho = 1.725$ gm/ml).

Results.—Structure of nascent daughter RF: Lindqvist and Sinsheimer[7] showed that H³-thymine incorporated into RF during the period of RF replication (3–12 min after infection) first appeared in RFII, which was then quickly converted to RFI. As shown in Figure 1, when the H³-label incorporated into RF during a 30-second pulse (at 37°C) at six minutes after infection (30% RFI, 70% RFII) is centrifuged to equilibrium in alkaline CsCl, approximately equal amounts of label are found in the separated viral and complementary strands (from the RFII).

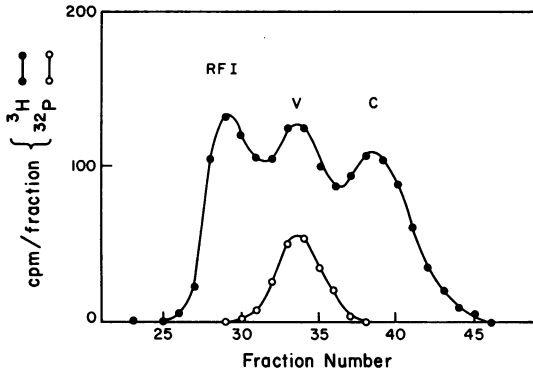As shown in Figure 2a, when a similar RFII (isolated by sedimentation

FIG. 1.—Equilibrium distribution in an alkaline CsCl density gradient of H³-label incorporated into RF-DNA in a 30-sec pulse, at 6 min after infection. A density marker of P³²-φX-DNA was added. Density increases from right to left.

v = Viral strand.
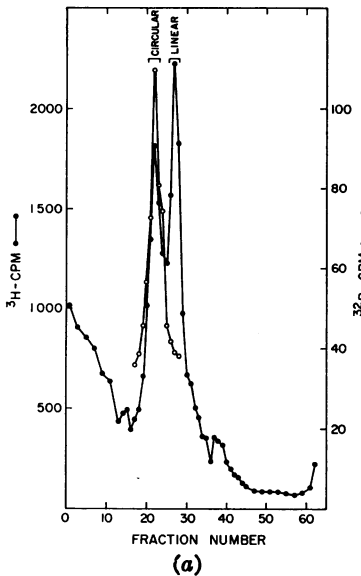
C = Complementary strand.

(a)

FIG. 2.—Analysis of RFII labeled during period of RF replication (5–8 min after infection) and isolated from a zone sedimentation through a neutral CsCl gradient.

(a) Distribution of H³-label after sedimentation through an alkaline CsCl gradient (P³²-φX-DNA added as marker). Sedimentation from right to left.

(b) Equilibrium distribution in an alkaline CsCl density gradient of the "circular" component from (a). (M. lysodeikticus DNA (A_{260}) and P³²-φX-DNA added as markers.)

(c) Equilibrium distribution in an alkaline CsCl density gradient of the "linear" component from (a). (M. lysodeikticus DNA (A_{260}) and P³²-φX-DNA added as markers.)
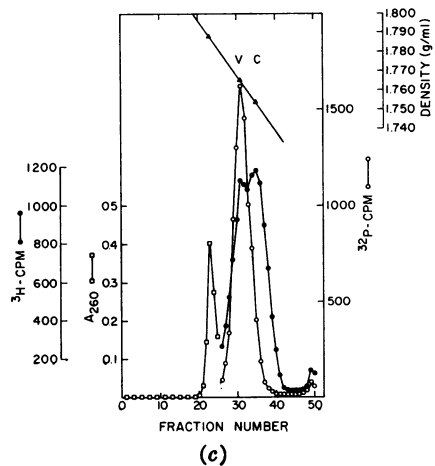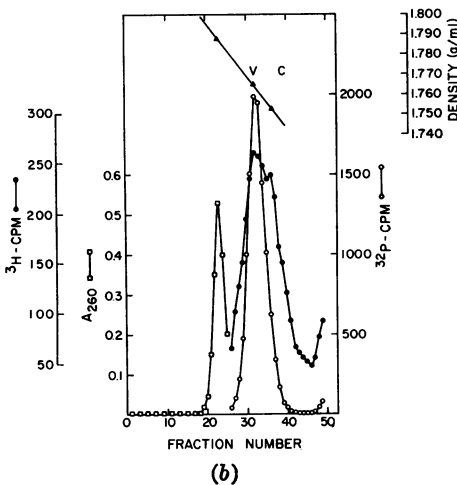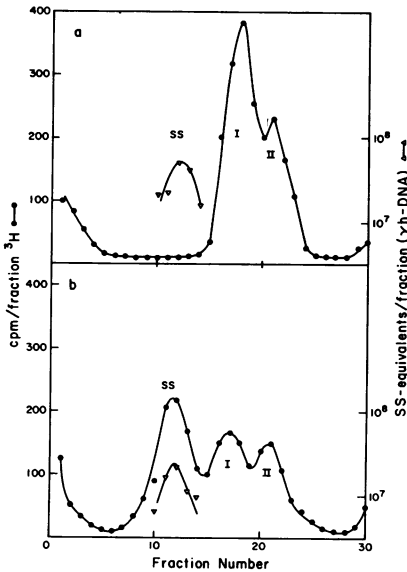

(b)

(c)

FIG. 3.—Distribution after zone sedimentation through neutral CsCl gradient of H³-label incorporated into DNA in a period 2–11 min after infection in "heavy" (C¹³N¹⁵) medium. (*a*) Immediately at end of label period (11 min). (*b*) After a 40-min chase in unlabeled, light (C¹²N¹⁴) medium (γh-DNA added as marker, detected by specific infectivity, in both centrifugations).
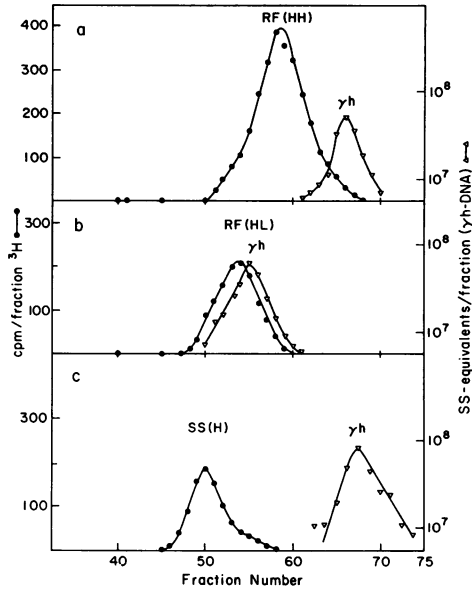
FIG. 4.—Equilibrium distribution in neutral CsCl density gradient of fractions from Fig. 3 (γh-DNA added as density marker).
(*a*) RF fractions of Fig. 3*a*.
(*b*) RF fractions of Fig. 3*b*.
(*c*) SS fractions of Fig. 3*b*.

through a neutral CsCl gradient of DNA extracted after a three-minute pulse of H³-thymidine, from 5 to 8 min after infection) is centrifuged through an alkaline CsCl gradient, equal amounts of label are found in the circular and linear forms. When both forms are centrifuged, separately, to equilibrium in alkaline CsCl (Figs. 2*b*, *c*), in both instances equal amounts of label are found in the viral and complementary strands.

It is evident from these results that two forms of nascent RFII are present in essentially equal amounts; in one, the viral strand is open, and in the other the complementary strand.

Fate of the daughter RF molecules: During the period 3 to 12–15 minutes after infection, some 15–20 daughter RF molecules accumulate in the infected cell.   After this period, net RF synthesis effectively stops[7] and progeny single-strand DNA synthesis begins.   The fate of the progeny RF during this latter period has been uncertain.

In the experiment leading to the results shown in Figures 3 and 4, cells were grown and infected in "heavy" (C¹³N¹⁵) medium.   H³-thymine was added at two minutes after infection.   At 11 minutes after infection, the cells were transferred to "light" (C¹²N¹⁴) medium.   As shown in Figure 3*a*, at this time

the label was present entirely in RFI and II. This RF was fully heavy (Fig. 4a). After 40 minutes in the "light" medium (in the presence of unlabeled thymine), approximately one half the label had moved from RF to single-stranded DNA (Fig. 3b), while the remainder was partly in RFI, partly in RFII. As shown in Figures 4b and c, the label remaining in RF at this time is now to be found at a hybrid density, while the label moved to single-strand DNA is at a fully heavy density.

In a similar experiment, cells were labeled from 0 to 14 minutes after infection with H³-thymidine and then transferred for 20 minutes to unlabeled media. After this chase, as shown in Figure 5, approximately half the incorporated
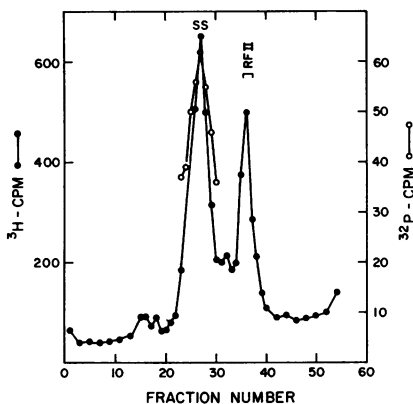

FIG. 5.—Distribution after zone sedimentation through neutral CsCl gradient of H³-label incorporated into DNA in a period 0–14 min after infection and then chased for 20 min in unlabeled medium. (P³²-φX-DNA added as marker.)

label is to be found in single-stranded DNA, while half is still in RF, mostly RFII in this instance.

These results suggest that the viral strands of the daughter RF molecules formed during the period of RF replication are subsequently to be found among the single-stranded progeny DNA molecules. By inference, the complementary strands remain in the RF.   More direct evidence for this conclusion and evidence as to the role of the daughter RF in viral single-stranded DNA synthesis will be presented in a succeeding paper.[8]

Summary.—The nascent daughter RFII molecules synthesized during the period of RF replication are of two kinds, present in equal amounts. In one, the viral strand is open; in the other, the complementary.

During the period of single-strand DNA formation, the viral strands of the daughter RF molecules are released and become part of the accumulation of progeny single-stranded DNA molecules (in virus particles).

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[6] RFII is the more slowly sedimenting ring form of ϕX RF, in which one or both (at nonadjacent sites) of the polynucleotide strands is open; RFI is the more rapidly sedimenting form in which both strands of the ring are covalently closed.

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