SEDIMENTATION ANALYSIS OF PHAGE T7-DIRECTED DNA SYNTHESIZED IN THE PRESENCE OF A DOMINANT CONDITIONAL LETHAL PHAGE GENE*

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It was recently shown (Hausmann et al., 1968) that during abortive infection of Shigella sonnei D_2 371-48 by coliphage T7, phage-directed DNA synthesis is initiated normally, proceeds for a few minutes, and is followed by the breakdown of newly synthesized DNA to acid-soluble material. It was also shown that this breakdown is the result of a dominant, conditional lethal function (ss^+-function, for suicide in shigella), coded for by the phage genome.

The sedimentation of phage-directed DNA, synthesized in the Shigella host by T7 wild type (T7 ss^+), and by T7 mutants lacking the "suicide function" (T7 ss^-), has now been analyzed in sucrose gradients. It will be shown that, in abortive conditions, phage-directed DNA is present mainly as fragments, not full length T7 DNA molecules, as it is the case in productive conditions.

MATERIALS AND METHODS

As host bacteria, Escherichia coli B/1, subsequently called B, or Shigella sonnei D_2 371-48, subsequently called D, were used. B is permissive for T7 ss^+ and for T7 ss^- mutants, whereas D is permissive only for T7 ss^- mutants. For details, see Hausmann et al. (1968).

Infection was carried out at 37°C, as follows. Exponentially growing broth cultures (generally 10-ml samples) of host bacteria (3×10^8 cells/ml)

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were exposed to ultraviolet light (about 10,000 ergs/mm^2) to minimize host DNA synthesis. Two minutes after irradiation, ^{3}H-thymidine (5 µc/ml culture) and phage (multiplicity of infection about 10) were added. At different times after infection, samples (1 or 2 ml) were withdrawn and (a) chilled in 10 ml of ice-cold 5% trichloroacetic acid, or (b) lysed by lysozyme, EDTA and sodium dodecyl sulfate, as described by Frankel (1966). About 10^{10} particles of ^{32}P-labeled T7 were also added to the lysis mixture, in which the phage were disrupted; the liberated DNA was used as a marker. A sample of lysed cells (generally 0.25 ml) was layered directly on a 5-20% sucrose gradient (neutral sucrose: 1.0 M NaCl, 0.01 M Tris-HCl buffer, pH 7.5, 0.01 M EDTA, 0.1% sodium dodecyl sulfate; alkaline sucrose: 0.7 M NaCl, 0.3 M NaOH, 0.01 M EDTA), and centrifuged at 25°C in a Spinco model L2 centrifuge, with the SW39L rotor, at 39,000 rpm, for 2.5 hrs. Fractions were collected through a hole in the bottom of the tubes. Carrier DNA (100 µg salmon sperm DNA) and ice-cold 5% trichloroacetic acid (5 ml) were added to each fraction. After one hour at 0°C, the precipitates were collected on membrane filters. The radioactivity retained on the filters was assayed in a dual-channel scintillation spectrometer.

^{32}P-phage was prepared as described by Hausmann et al. (1968), but carrier-free ^{32}P-phosphoric acid (20 µc/ml) was substituted for ^{3}H-thymidine.

RESULTS AND DISCUSSION

Fig. 1 shows the sedimentation profiles, in sucrose gradients, of newly synthesized DNA from lysed samples of B or D cells harvested at 6, 9 or 12 minutes after infection with either T7 ss$^+$ or with T7 ss$^-$. It can be seen that in all three instances of productive infection (infection of B by T7 ss$^+$ or T7 ss$^-$; infection of D by T7 ss$^-$), 6 min after the addition of the phage, a relatively small peak of ^{3}H-thymidine-labeled, newly synthesized material was present at the same position as a marker of ^{32}P-labeled DNA from mature T7 particles. Besides, a variable proportion of the newly synthesized DNA sedimented in a dispersed pattern; most of the dispersed DNA was faster than the mature

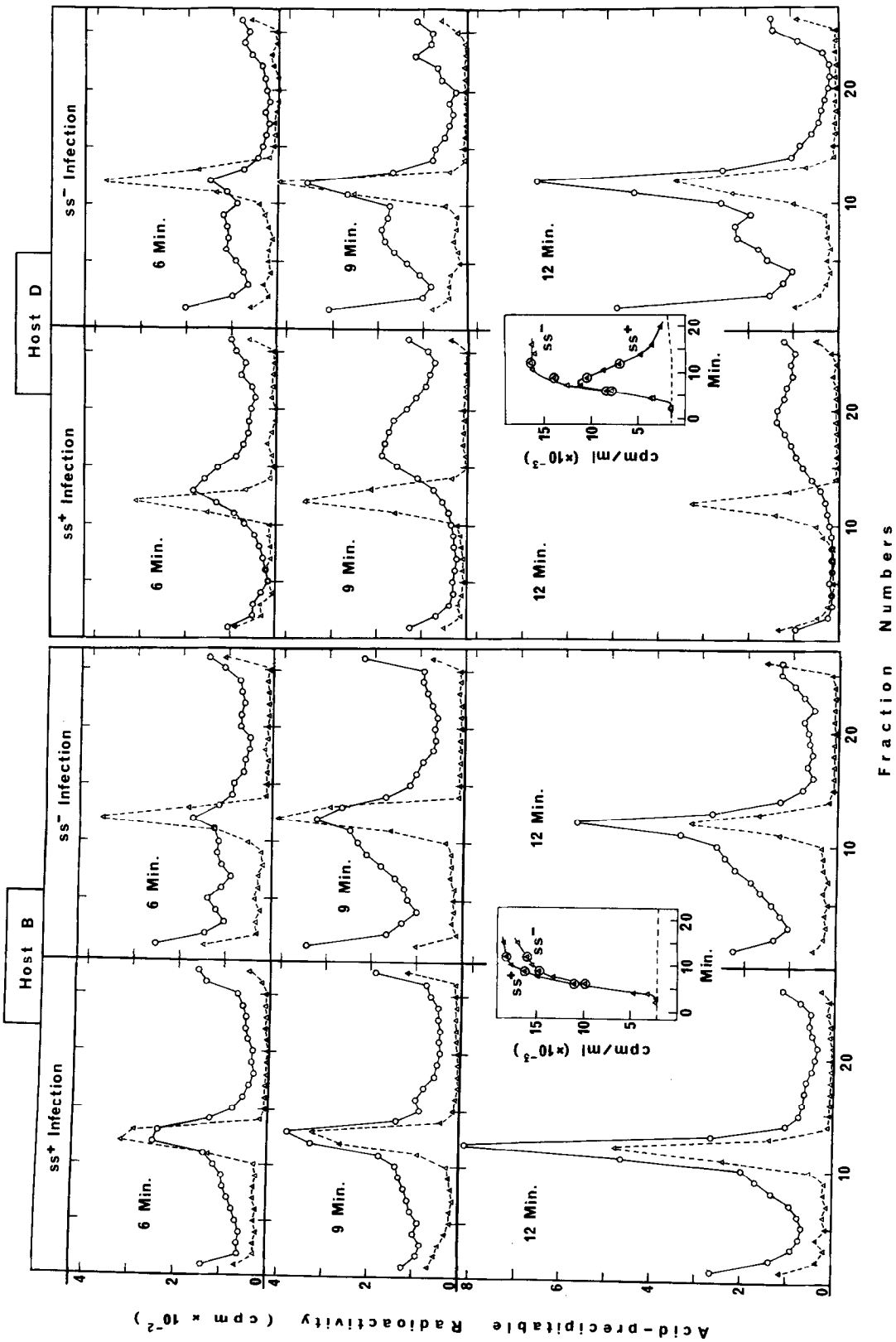
Host B
Host D
ss+ Infection
ss− Infection
6 Min.
9 Min.
12 Min.
Acid-precipitable Radioactivity (cpm × 10⁻²)
Fraction Numbers
cpm/ml (×10⁻³)
ss+
ss−
Min.

Fig. 1. Sedimentation profiles in sucrose gradients, of phage-directed DNA synthesized after infection of B cells (first and second columns), or D cells (third and fourth columns), by T7 ss^+ (first and third columns), or by T7 ss^- (second and fourth columns). Exponentially growing cells were ultraviolet irradiated to minimize host DNA synthesis. ^{3}H-thymidine (5 μc/ml) and phage (multiplicity of infection ~10) were then added. At intervals, culture samples were (a) chilled in 5% trichloroacetic acid, or (b) lysed with lysozyme, EDTA, and sodium dodecyl sulfate. ^{32}P-labeled mature T7 particles were also added to the lysis mixture; the liberated T7 DNA was used as a marker (dashed lines). Samples lysed at 6, 9, or 12 min after infection (corresponding to the circled points of the insets) were layered on sucrose gradients, pH 7.5, and centrifuged (Spinco SW39L rotor, 150 min, 39,000 rev/min). The bottom fractions are to the left. In the insets, total acid-precipitable ^{3}H-thymidine per ml culture, at different times after infection, was plotted. The dashed lines in the insets correspond to ^{3}H-thymidine incorporated by irradiated uninfected cells.

T7 DNA. The nature of this faster sedimenting material has not been further investigated. Without changing their position, the peaks increased in size in the course of the latent period, which was about 17 min in our conditions. (The total amount of ^{3}H-thymidine per ml of culture, incorporated into an acid-insoluble form during infection is plotted in the insets of Fig. 1.) In the case of abortive infection (host D plus phage T7 ss^+), the faster sedimenting dispersed material, and the peak corresponding to full length T7 genomes could not be found at any time. Instead, 6 min after infection, i.e. at a time when the rate of phage-directed DNA synthesis was still normal (see Fig. 1, inset at right), the incorporated ^{3}H-thymidine was distributed over a broad band, with a peak sedimenting at about a 0.9-fold speed of the T7-DNA marker. At 9 min after infection, although net DNA synthesis had proceeded, the fragmentation was more extensive; by use of an empirical equation derived by Hershey, Burgi and Ingraham (1963), the molecular weight of the fragments in the peak fraction (fraction 16) was calculated to be about 10^7 daltons, i.e., about 1/3 of the molecular weight of the T7-DNA molecule [considered to be about 2.6×10^7 daltons (Studier, 1965; Richardson, 1966)]. And at 12 min, the fragments were much smaller.

The breakdown to acid-soluble material of DNA newly synthesized during abortive infection of D by T7 ss^+ could be entirely prevented by the addition of chloramphenicol (80 μg/ml), 4 min after infection (Hausmann et al., 1968).

The patterns of ^{3}H-thymidine incorporation directed by T7 ss^{+} or by T7 ss^{-} were then virtually identical (Fig. 2, inset). However, after lysed samples of cultures infected under these conditions were analyzed by centrifugation in sucrose gradients, a striking difference between the T7 ss^{-}-directed and the T7 ss^{+}-directed DNA became evident. In the case of T7 ss^{-}-infection, chloram-

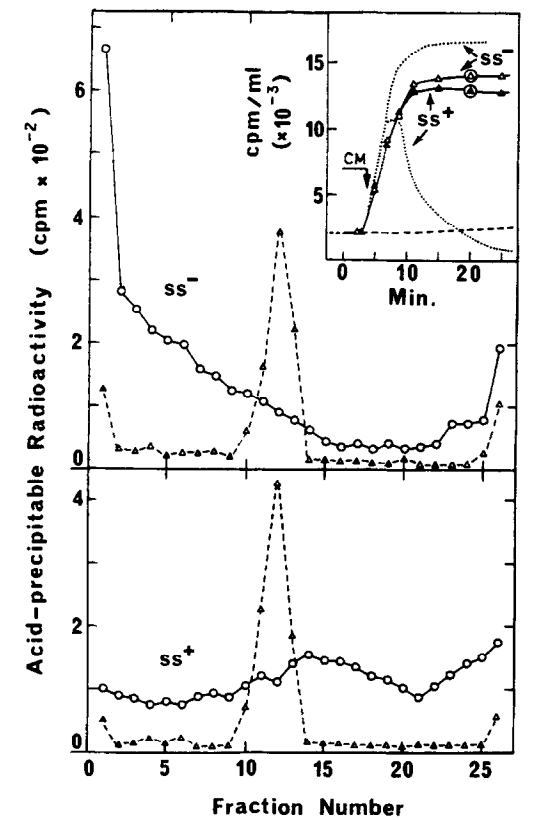

FIG. 2. Sedimentation profiles of DNA synthesized after infection of D cells by T7 ss^{-} (upper part), or by T7 ss^{+} (lower part), in the presence of chloramphenicol (CM). Conditions were as described in the legend to Fig. 1, but 4 min after infection CM (80 µg/ml) was added to the cell cultures. The samples used for centrifugation were harvested 20 min after infection (circled points in the inset). The dotted lines refer to control experiments without CM. See also legend to Fig. 1.

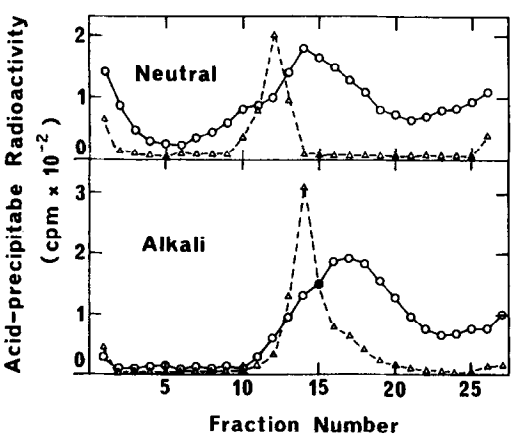

FIG. 3. Sedimentation profiles in neutral and alkaline sucrose of DNA synthesized after infection of D cells by T7 ss^{+}. Cells were harvested 8 min after infection. See also legend to Fig. 1.

phenicol, added 4 min after the phage, inhibited the formation of a peak sedimenting like T7 DNA, although the rate of ^{3}H-thymidine incorporation was not markedly depressed (Fig. 2); the phage-directed DNA synthesized in the presence of chloramphenicol sedimented faster than mature T7 genomes, in a dispersed pattern.* In the case of T7 ss^{+}-infection, the newly synthesized DNA sedimented in a very broad band; most of it sedimented slower than T7 marker-DNA. It was thus shown that, although no DNA was broken down to acid-soluble material when chloramphenicol was added 4 min after infection, the ss^{+}-function had been at least partially expressed at that time. Considering that chloramphenicol prevented T7-directed DNA synthesis when added less than 4 minutes after the phage, it seems possible that the ss^{+}-function is expressed simultaneously with the functions required for phage-directed DNA synthesis, and not at the time where net phage-directed ^{3}H-thymidine incorporation stops.

To check the existence of single-strand breaks in the fragments of DNA in T7 ss^{+}-infected D cells, such DNA (from cells harvested 8 min after infection) was alkali-denatured (0.3 M NaOH) and centrifuged in an alkaline sucrose gradient. The results, shown in Fig. 3, suggest that no, or relatively few single-strand breaks existed within the fragments, since no sharp decrease in the average size of the fragments occurred in alkaline conditions.

Concluding, it seems that the ss^{+}-directed breakdown of intracellular T7-DNA is primarily due to endonucleolytic double-strand breaks; however, it is unknown whether the ss^{+} gene product of phage T7 has a direct endonucleolytic activity in the D host, or whether it sets up intracellular conditions

* The nature of this DNA has not been further investigated. It is possible that it represents a replicative intermediate of T7 DNA. The existence of such an intermediate, whose maturation to normal-sized T7 DNA molecules could be inhibited by chloramphenicol has been reported in a preliminary communication (Kelly, 1968). A report by Thomas (1967) indicates, however, that parental T7 DNA does not change its sedimentation pattern after infection. Fast sedimenting replicative intermediates have been found for other double-stranded DNA phages, such as λ (Young and Sinsheimer, 1967; Salzman and Weissbach, 1968) and T4 (Frankel, 1968).

which more or less indirectly render the phage DNA accessible to nucleases coded for by the host, or by other phage genes. The degradation to acid-soluble material may be considered a secondary effect, in which nucleases coded for by the host, and by the phage (Hausmann and Gomez, 1968), may possibly play a role.

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