AUTONOMOUS REPLICATION OF SHORT DNA FRAGMENTS IN THE LIGASE NEGATIVE T4 AM H39X

A. W. Kozinski and P. B. Kozinski
Department of Medical Genetics, University of Pennsylvania, Philadelphia, Pa.

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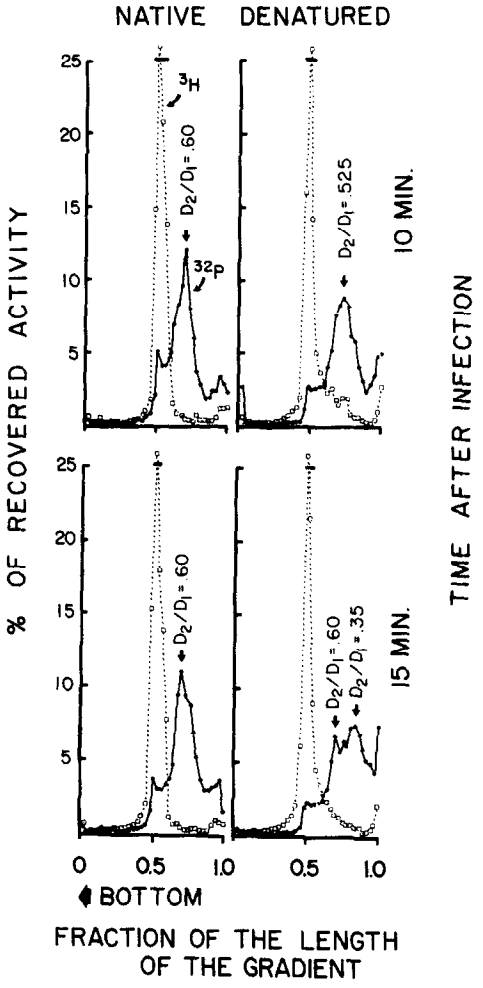
Short DNA fragments of the ligase negative T4 phage mutant are able to undergo autonomous replication in which new partner strands are repeatedly synthesized. This is shown by the experiments described below in which a non-permissive host containing heavy (5-bromodeoxyuridine substituted) DNA was infected with light ^{32}P labeled phage in the presence of bromodeoxyuridine. After the parental label had assumed a hybrid density, bacteria were sedimented, resuspended in thymidine-containing (TDR) medium and incubated further. Under these conditions the ^{32}P labeled DNA which at the time of addition of TDR resided at an intermediate replicative density (one strand light; one strand heavy) reassumed the light location (both strands light). Simultaneous analysis of the integrity of the replicative DNA at the time when the shift in the density label was performed revealed extensive breakdown of both single and double stranded DNA to fragments .2 - .1 phage equivalent length.

Experimental

Analytical methods have been described in previous papers (Kozinski et. al., 1967; Kozinski, 1968). E. coli B23 was grown at 37°C in heavy TCG medium to $3x10^8$ cells/ml. and was infected with an moi. of 3.0 of light ^{32}P labeled phage T4 AM H39X, a ligase negative mutant (Hosoda, 1967; Fareed and Richardson, 1967). At 10 and 15 minutes after infection aliquots of the suspension were quickly chilled and washed. One aliquot was kept chilled for use in DNA extraction while the second aliquot was resuspended in fresh TCG medium in which 5-BU was replaced by TDR (density reversal procedure). The incubation was continued for an additional 5 or 10 minutes to give a total of 20 minutes incubation at

37°C. At this time the remainder of the suspension was chilled, all samples sedimented and intracellular DNA extracted by the pronase-phenol method and dialyzed against CS. The extracts were supplemented with freshly prepared ^{3}H DNA, derived from mature T4 phage. The ^{3}H DNA served as a reference for



Description of Fig. #1

Sucrose Gradient Analysis of Intracellular DNA at 10 and 15 Minutes after Infection

^{32}P = parental label; ^{3}H = reference, integral DNA of size 1. Native DNA was analysed in 5-20% sucrose gradient, pH. 7.4; denatured DNA was adjusted to a final molarity of 0.2 M with KOH and sedimented in 5-20% sucrose gradient prepared in 0.2 M KOH. The condition of centrifugation: SW 39L; 27,000 rpm; 3 hr; 10°C. This sucrose gradient represents the integrity of parental label containing moieties at the moment of the density reversal procedure, and are strictly comparable with the left column of Fig. #2.

the size of one phage equivalent in sucrose gradient analysis and as a light density marker in CsCl density gradient analysis.

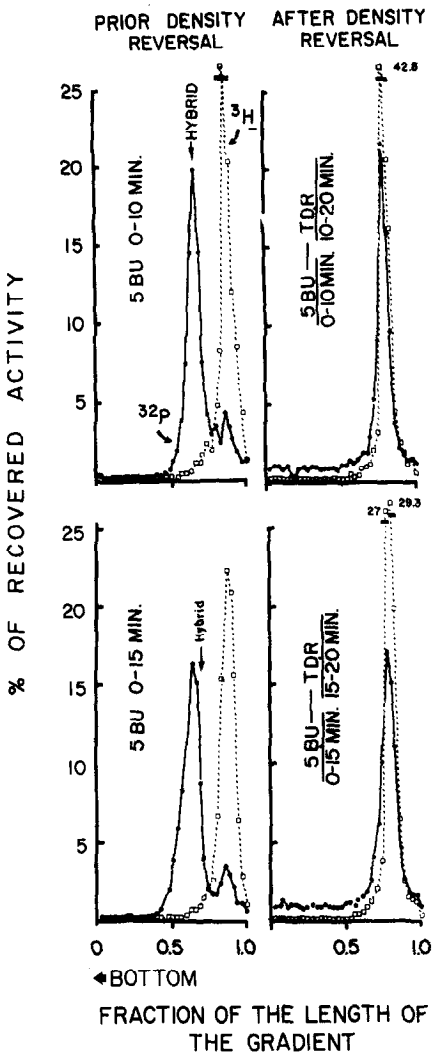
The results of the sucrose gradient analysis are shown in Figure #1. Both at 10 and at 15 minutes after infection the injected parental DNA underwent extensive breakages demonstrable both in neutral and in alkaline sucrose gradients. This can be seen by comparing the locations of ^{32}P and the intact ^{3}H moieties.

The observed breakage of the parental DNA does not arise as an artefact of the extraction procedure. Identical patterns of distribution in alkaline sucrose gradients were obtained when the sampling procedure was the following: small samples of bacterial suspensions at 10 and 15 minutes after infection were transferred directly from the water bath in which the experiment was performed to a 0.2M KOH solution. This was followed by incubation at 37°C for 20 minutes and successive analysis in a 5-20% alkaline sucrose gradient. This approach eliminates the possibility of shearing during extraction and enzymatic breakdown in the lapse of time between sampling and phenol extraction.

The observed breakdown is due to the activity of phage-induced endonucleases in situ and can be inhibited by the addition of chloramphenicol (CM) at early times after infection with T4 H39X. Indeed, it was demonstrated that the addition of CM at approximately 3-5 minutes after infection prevents endonucleotic breakages, assures the integrity of parental DNA, and permits synthesis of progeny strands of one phage equivalent unit length.

The fact of replication of DNA as integral strands in Am T4 H39X in the presence of CM and the fact of the similarity in sizes between parental and newly synthesized progeny DNA assures us that the role of ligase is a repair of nicks generated by phage coded DNAse rather than repairing a discontinuous product generated by polymerase. A full discussion of this problem is presented in Kozinski, 1968.

Figure #2 shows a CsCl analysis of intracellular DNA at the moment of the shift in the density label (10 and 15 minutes respectively) and after



Description of Fig. #2

CsCl Density Gradient Analysis of Intracellular DNA

^{32}P = parental label (originally light); ^3H = reference light DNA.

The left column represents the fate of parental DNA at the moment of the density reversal; the right column represents the fate of the same DNA after further incubation in the presence of thymidine. Note that the parental moiety was almost completely replicated and assumes the hybrid density location; note also that after incubation in the TDR medium the light parental radioactive strand replaced its partner heavy strand for a newly synthesized light strand, and bands again at the light location.

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further incubation in TDR medium. Virtually all of the parental DNA replicates by 10 minutes after infection and bands at an intermediate density, not over-

lapping with light ^{3}H reference DNA. After the density reversal the parental label returns quantitatively back to the light location.

We conclude that short fragments of DNA are able to be efficiently replicated semiconservatively. Thus, for continued synthesis of DNA in infected bacteria, neither the integrity of parental strands nor the integrity of the double helical structure is necessary. It is not excluded, however, that the integrity of the parental strands might be necessary for the initiation of enzymatic processes involved in successful intracellular replication of phage.

Acknowledgments

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