NOTES

Early Intracellular Events in the Replication of T4 Bacteriophage Deoxyribonucleic Acid

VII. ^{32}P Suicide Stabilization¹

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The relationship between ^{32}P suicide stabilization and deoxyribonucleic acid (DNA) replication during infection of *Escherichia coli* by bacteriophage T4 was reinvestigated. Replication of the parental phage DNA was detected at early stages of stabilization.

In 1955, Stent reported that infective centers formed by phage which were labeled with high specific activities of ^{32}P were increasingly resistant to the lethal effects of ^{32}P decay as a function of time after infection (4). This phenomenon was called ^{32}P suicide stabilization. Three general theories were put forth to explain this phenomenon: (i) genetic information was transferred from the parental DNA to some other macromolecule (RNA or protein), (ii) progeny DNA was synthesized at an early stage of infection, and (iii) some macromolecule, such as protein, protected the parental deoxyribonucleic acid (DNA) from lethal damage by the ^{32}P decay.

The interest generated in the mid-fifties by this phenomenon was due to the apparent lack of synthesis of DNA at a time of considerable stabilization. This conclusion was reached by comparing net synthesis as determined by Vidaver and Kozloff (6) with stabilization as determined by Stent. Kozinski and Bessman studied this relationship with CsCl analysis of the replication of parental DNA to detect the first round of replication (1). They detected little or no replication of the DNA up to 7 min after infection, a time when considerable stabilization had taken place. However, their method of extraction of the DNA from the lysates of the infected cells did not include an incubation with Pronase. At this time they were not aware of DNA lost during extraction, and they were discarding preferentially that DNA which was in the process of replication, because

FIG. 1. ^{32}P suicide stabilization of bacteriophage T4. *Escherichia coli* B23 cells were grown to 3×10^8 cells/ml in high-phosphate 5-BUdr substituted medium and were infected with T4BO_r bacteriophage labeled with ^{32}P at a specific activity of approximately 250 mc/mg of P. At intervals after infection, samples of infected bacteria were diluted into cold 3XD medium and frozen at -196°C . At intervals after freezing, samples were thawed and plated for infective centers.

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the replicative DNA is preferentially contained in a protein-DNA complex at the onset of DNA replication (2).

The following experiment once again attempted to correlate DNA replication with ^{32}P suicide

stabilization. T4 bacteriophage were labeled with ^{32}P at a specific activity of 150 mc/mg of *P. E. coli* B23 cells which had been grown for two generations in 5-BUdr medium were infected with these phage at a multiplicity of infection of 0.1. At

FIG. 2. *CsCl* density gradient analysis of parental, ^{32}P -labeled intracellular deoxyribonucleic acid (DNA). At the same time, samples were chilled for plating infective centers in the experiment described in Fig. 1, other samples of infected bacteria were chilled in NaCl-ethylenediaminetetraacetic acid and lysed with sodium dodecyl sulfate, and the intracellular DNA was extracted with Pronase and phenol. This DNA was analyzed by *CsCl* density gradient centrifugation in the usual manner.

intervals after infection, samples of infected bacteria were chilled in 3XD medium and frozen in liquid nitrogen. Immediately after freezing, a sample was thawed and plated to determine the number of infective centers (Fig. 1). The ^{32}P suicide of the parental phage which had been frozen in liquid nitrogen, also, is plotted in addition to the surviving fraction of infective centers. A hint of stabilization can be noted at very early times, but even by 6 min after infection the infective centers are only about twice as resistant to decay as the parental phage. However, by 9 min they are almost totally stabilized; so, between 6 and 9 min after infection, a dramatic change has occurred. The slopes of these curves are very similar to those obtained by Kozinski and Bessman.

At times identical to those at which samples of infected bacteria were chilled for plating, duplicate samples were chilled in NaCl-ethylenediaminetetraacetic acid, lysed with sodium dodecyl sulfate, incubated with Pronase, and extracted with phenol to purify the intracellular DNA. The extracted DNA was dialyzed against standard saline-citrate buffer, supplemented with ^3H -labeled reference DNA, and banded in a CsCl density gradient at a neutral pH (Fig. 2). During replication, the progeny DNA strands became substituted with 5-BUdr; consequently, the parental ^{32}P label was displaced toward the more dense location in the gradient as replication proceeded. The sequence of events leading to the location of the label at various times after infection has been described by Kozinski and Kozinski (3). Samples chilled later than 14 min after infection showed no further decrease in the amount of ^{32}P label found in the conservative, unsubstituted peak in the gradient. The amount of ^{32}P remaining in the conservative peak was taken to represent those phage which were dead at the time of infection or those phage which for this or another reason do not successfully replicate their DNA; the percentage of material in this peak agreed very well with the difference between the total amount of phage present, as determined by calculations from radioactivity, and the viable phage as determined by plating. Therefore, to determine the amount of parental DNA in the population which had become replicative at any time after infection, the percentage of conservative DNA at 14 min was subtracted from the amount of conservative DNA at earlier times. By 6 min about 58% of the DNA and, by 7 min, about 75% of the DNA was replicative, i.e., was displaced from the conservative peak. By 9 min almost all the molecules which would replicate had done so. This result is in distinct contrast to those of Kozinski and Bessman. In their previous experiment, incubation

with Pronase had been omitted before extraction of the intracellular DNA with phenol. Therefore, replicative DNA was being lost preferentially during the phenol extraction, and the amount of DNA replication detected at any stage of stabilization was much less than actually was the case. By 7 min in the Kozinski and Bessman experiment, almost no DNA had replicated, whereas the present results showed that at least 75% of the DNA had done so. The amount of replication at 6 min and through 9 min complemented the dramatic change in stability to suicide of the infective centers between 6 min and 9 min after infection.

It should be noted that formation of the fast-sedimenting, T4 protein-DNA complex (R. C. Miller, Jr., and A. W. Kozinski, *J. Virol.* 5:490-501, 1970) at about 4 min after infection may contribute to the stabilization process; the complex may account for the small amount of stabilization occurring before 5 min after infection. Since DNA is not liberated from the complex by ^{32}P double-strand scissions, the complex may protect the DNA by keeping the two strands of the helix in proper alignment for some repair process; this would be helpful if some ^{32}P disintegrations cause two single-strand nicks close together in the *trans* position (5).

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