Double-Strand Scissions in Bacteriophage T4 Deoxyribonucleic Acid as a Result of ^{32}P Decay¹

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Received for publication 5 November 1969

Double-strand scissions produced by decay of ^{32}P incorporated into T4 deoxyribonucleic acid (DNA) were detected in cross-linked DNA and DNA containing ^{32}P in only one strand of the double helix.

Some results have indicated that double-strand scissions due to accumulated decays of incorporated ^{32}P in fact can be detected in deoxyribonucleic acid (DNA; 3). However, it was possible that the double-strand scissions were due to a shearing or local "melting" effect between two *trans* single-strand breaks during manipulation of the DNA rather than primarily due to ^{32}P decay. To decide between these two possibilities, cross-links were introduced into DNA at a frequency far greater than the number of single-strand scissions introduced by ^{32}P decay; cross-linked and control uncross-linked DNA were then tested for double-strand scissions after ^{32}P decay by analysis in sucrose gradients at a neutral pH. If the cross-linked and uncross-linked DNA were equally sensitive to ^{32}P decay, then the hypothesis that double-strand scissions arise as a primary event of ^{32}P decay would be supported. This idea is illustrated in the diagrams of Fig. 1.

The first step in the approach was to establish the frequency and stability of cross-links introduced into the DNA by formaldehyde. T4 bacteriophage were grown in low-phosphate TCG medium containing ^{32}P at a specific activity of 20 mc/mg of P. These phage were used to infect *Escherichia coli* B which had been grown for two generations in 5-bromodeoxyuridine medium. The infected bacteria were sedimented at 6 min after infection, washed to eliminate unadsorbed phage, and were resuspended in fresh 5-bromodeoxyuridine medium. The infected bacteria were incubated for 90 min, lysed with chloroform, and the progeny phage were purified. The DNA was extracted from the progeny phage and sonically treated. Because the progeny DNA contained ^{32}P in a single-strand piece, approximately 15% of the

length of a T4 phage single-strand (2), and, because these sonication procedures yielded double-stranded DNA of a molecular weight of about

FIG. 1. Scheme demonstrating the effect of cross-links in between two *trans* single-strand breaks due to ^{32}P decay.

500,000 daltons (1), the resulting labeled DNA consisted of hybrid molecules containing one cold, heavy strand and one light ^{32}P -labeled strand of about 250,000 daltons each. Part of this DNA was dialyzed against standard saline-citrate buffer con-

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FIG. 2. Double-strand scissions in normal and cross-linked T4 deoxyribonucleic acid (DNA) due to decay of incorporated ^{32}P . DNA was purified from T4 bacteriophage labeled with ^{32}P at a specific activity of 150 mc/mg of P. One part of the DNA was incubated for 72 hr at 4 C with 1% formaldehyde. Another part was stored at 4 C without further additions. Parental phage were stored at 4 C also, and plated daily to determine the suicide rate of this preparation of bacteriophage. At intervals samples of the two types of DNA were analyzed by neutral sucrose gradient sedimentation.

Parental Light ^{32}P Fragment in Heavy Progeny Molecule

Banding of ^{32}P
in CsCl gradient

FIG. 3.

taining 1% formaldehyde, and part was dialyzed against standard saline-citrate without further additions. Both samples were stored at 4 C for 72 hr, heated for 5 min at 100 C, and banded in a CsCl density gradient containing 1% formaldehyde supplemented with ^3H reference DNA. Prior to denaturation, both DNA preparations assumed a hybrid location in the gradient as expected. After heating at 100 C for 5 min, the strands of the DNA not treated with formaldehyde did separate; the ^{32}P label did return to the light location. On the other hand, the strands of the DNA which had been incubated with 1% formaldehyde for 72 hr at 4 C did not separate; all of the ^{32}P label remained at the hybrid location even after heating for 5 min at 100 C. This indicates that formaldehyde produces more than one cross-link

FIG. 3. Scheme showing excision of hybrid molecules by double-strand scissions occurring in progeny phage deoxyribonucleic acid.

per 500,000 daltons of T4 DNA under these conditions of storage.

The next phase of the experiment involved testing the integrity of cross-linked and uncross-linked DNA after ^{32}P decay. The results of this experiment (Fig. 2) indicate that cross-linked and uncross-linked DNA are equally susceptible to ^{32}P decay. The series of graphs in Fig. 2A₁–2A₄ represents sucrose gradient analysis of uncross-linked DNA. The series of graphs in Fig. 2B₁–2B₄ represents sucrose gradient analysis of cross-linked DNA. Each successive graph in the series represents a sucrose gradient of the DNA at an increasing time of storage. Each sucrose gradient of both series was run simultaneously in the same rotor. The displacement with time of the ^{32}P -labeled DNA toward the top of the gradient seems to proceed at the same rate independently of whether the DNA had been cross-linked or not. This conclusion supports the hypothesis that the double-strand scissions produced in the DNA are a primary result of ^{32}P decay.

The theory that double-strand scissions arise in DNA as a primary effect of decay of incorporated ^{32}P would be supported further if double-strand scissions could be detected in DNA which contained ^{32}P in only one strand of the double helix. One can produce such a molecule of bacteriophage T4 DNA by extracting the DNA from progeny phage produced in "heavy" (5-bromodeoxyuridine substituted) bacteria infected in "heavy" medium by "light" radioactive parental phage. Since the progeny phage contain one piece of light ^{32}P labeled parental DNA covalently bonded to the heavy progeny DNA (2), one could assay double-strand scissions in this DNA by CsCl density gradient analysis. The scheme of this experiment is illustrated in Fig. 3. If two double-strand scissions occur in this DNA, a hybrid molecule of DNA, will be excised; one strand of the excised DNA will be heavy, and one strand will be light. Therefore, as double-strand scissions accumulate in the DNA, an increasing amount of material bands at a hybrid density when the DNA is analyzed by CsCl density gradient centrifugation. The results of this experiment are illustrated in Fig. 4. CsCl analysis of the purified progeny DNA revealed no material banding at the hybrid density on day zero of the experiment,

FIG. 4. CsCl density gradient analysis of progeny DNA liberating hybrid fragments after excision by ^{32}P double-strand scissions. *Escherichia coli* B23 was grown to 3×10^8 cells/ml in high-phosphate heavy TCG and infected with T4BO₁ bacteriophage labeled with ^{32}P at a specific activity of 300 mc/mg of P. Progeny phage were purified, and their DNA was extracted with phenol. The DNA was stored at 4 C, and, at intervals, samples were analyzed in CsCl density gradients.

but, as the time of storage increased, an increasing amount of the ^{32}P label was found at the hybrid density (Fig. 4). The only way hybrid molecules could be produced under the conditions of this experiment was if two double-strand scissions arose as a primary result of the decay of ^{32}P incorporated in the parental contribution to the progeny phage.

This investigation was supported by National Science Foundation grant GB 13408 and Public Health Service grant CA 10055 from the National Cancer Institute, awarded to A. W. Kozinski.

The computer evaluation of data was performed in the University of Pennsylvania Medical School Computer Center, supported by the Public Health Service grant FR15-06, Division of Research Facilities and Resources.

I acknowledge support by Public Health Service grant GM-

006-94-09 from the National Institute of General Medical Sciences awarded to the graduate group of Molecular Biology, University of Pennsylvania.

I thank A. W. Kozinski for his guidance in this research and for his review of this manuscript.

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