REPAIR REPLICATION OF DNA IN BACTERIA: IRRELEVANCE OF CHEMICAL NATURE OF BASE DEFECT

Philip C. Hanawalt
Biophysics Laboratory, Stanford University, Stanford, California
and
Robert H. Haynes
Donner Laboratory, University of California, Berkeley, California

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The repair of ultraviolet (UV) damaged DNA in certain UV-resistant strains of bacteria has been shown to be initiated by the excision of intrastrand thymine dimers (Setlow & Carrier, 1964; Boyce & Howard-Flanders, 1964a) and other photoproducts (Setlow, Carrier & Bollum, 1965). Direct physical evidence that a subsequent step in the repair process consists in the replacement of the excised segments with normal bases has been obtained by Pettijohn and Hanawalt (1964b). The fact that the relative sensitivities of resistant (B/r) and sensitive (B_{s-1}) E.coli strains are similar for nitrogen mustard (HN2) and UV inactivation was taken to imply that defects other than pyrimidine dimers might be repaired by the same mechanism (Haynes, Patrick, & Baptist, 1964; Haynes, 1964). [The predominant chemical reaction of HN2, a bifunctional alkylating agent, with DNA consists in attack at the 7-nitrogen position of guanine (Brookes & Lawley, 1961).] In the present communication it is shown that a non-conservative mode of DNA replication follows treatment of UV-resistant bacteria with HN2. Thus, it would appear that a general biochemical mechanism exists for the repair of a variety of DNA structural defects.

Materials and Methods

The thymine-requiring E.coli strain TAU-bar was grown in a

glucose-salts medium at 37°C (Hanawalt, 1963). Cells were harvested in log phase by the rapid filtration method (Maaløe & Hanawalt, 1961) and then resuspended to a titer of 4×10^8/ml in tris buffer (pH 7.4). This suspension was then incubated for 20 minutes at room temperature in the presence of 0.2 mg/ml HN2 after which the cells were again harvested, washed on a filter, and resuspended in 37°C growth medium. This HN2 treatment was sufficient to reduce viability to about 10%, indicating that the HN2 sensitivity of E.coli TAU-bar is similar to that of B/r. DNA replication in control or HN2 treated cultures was observed by density labeling with 5-bromouracil (BU); the density distributions of newly-replicated DNA were analyzed by cesium chloride density gradient ultracentrifugation (Pettijohn & Hanawalt, 1964a,b).

Results

The density distribution of DNA fragments obtained when an exponentially growing culture is transferred to medium containing BU in place of thymine is illustrated in Figure 1. A ten minute

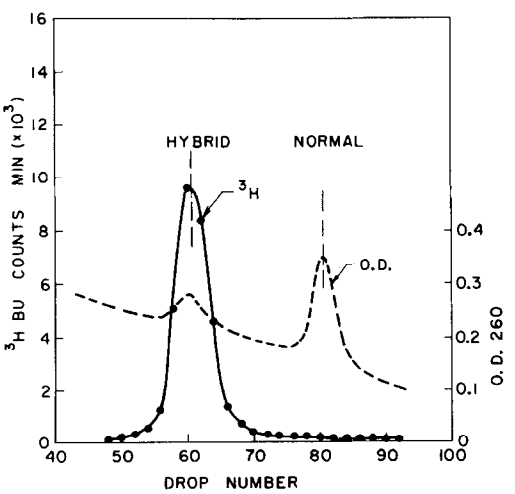

Fig. 1 Density distribution of DNA in CsCl gradient after growth for 10 minutes in BU medium followed by 10 minutes in H^3-BU medium. Optical density (O.D.) at 260 mμ indicates position of parental thymine-containing DNA fragments. Radioactivity was assayed as previously described (Hanawalt & Ray, 1964).

period of growth with non-radioactive BU preceded growth with H^3-labeled BU so that fragments which contained the growing point at the instant of transfer to BU medium would not be radioactively labeled (Hanawalt & Ray, 1964). Also, normal growing point fragments were excluded by a chloroform-octanol deproteinization step in the lysate preparation (Pettijohn & Hanawalt, 1964b). The newly-

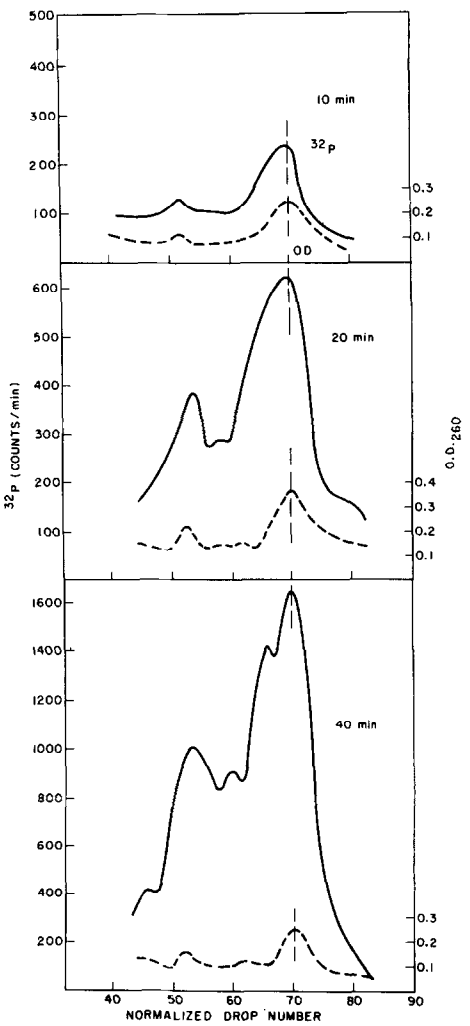

Fig. 2 Density distribution of DNA in CsCl gradient after HN2 treatment, and different periods of BU incorporation in presence of P^{32} orthophosphate. 10 minutes growth in unlabeled BU medium preceded the HN2 treatment. (Similar results were obtained when H^3BU was used instead of P^{32} + BU.)

replicated hybrid DNA fragments are seen in a symmetrical band of H^3, clearly separated from the normal density parental DNA.

Following the HN2 treatment the density distribution of newly-replicated DNA is qualitatively quite different, as seen in Figure 2. Most of the newly-synthesized DNA after 10 minutes, 20 minutes, and even 40 minutes of density labeling is unshifted from the normal density position although a hybrid band is also evident.

Rebanding of an intermediate density fraction from the 40 minute sample is shown in Figure 3, along with a diagramatic representation of the expected distribution of BU in the DNA. Heat denaturation of this material allows only the expected shift to higher density but no release of homogeneous BU strands which would have appeared in the first few density fractions at the bottom of the gradient. [The latter would have been expected if the BU incorporation were only in one

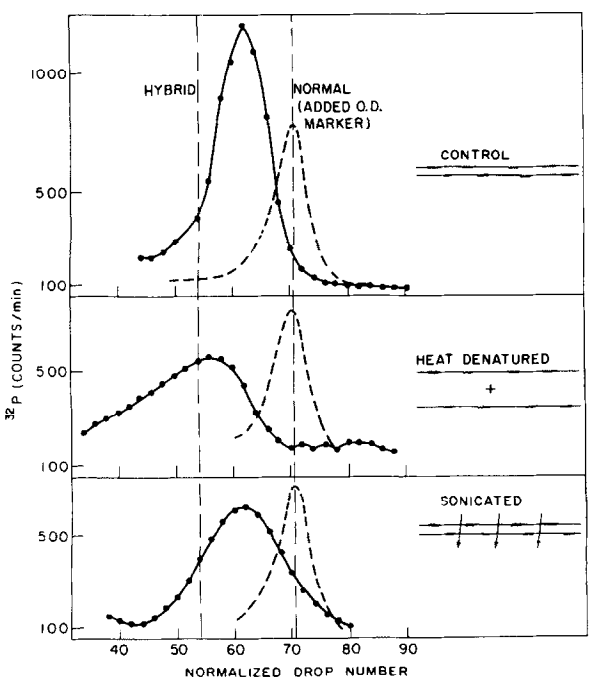

Fig. 3 Rebanding of an intermediate density fraction from Fig. 2 (40 min). O.D. marker is normal thymine containing E.coli DNA. See text.

strand, or if there were single strand breaks separating the regions of BU incorporation from adjoining thymine containing segments.] Lateral scission by sonication reduces the molecular weight of the fragments as seen by the broadening of the band but it does not shift the density position. This is consistent with the idea that the BU is incorporated into very short single strand segments of the bacterial chromosome. These features have been previously reported as characteristic of the non-conservative replication of DNA which follows UV irradiation of this bacterial strain (Pettijohn & Hanawalt, 1964b).

Discussion

The fact that HN2 induces the same non-conservative mode of DNA replication as that observed following UV treatment of bacteria suggests that a general "error correcting" mechanism exists in which it is not the precise nature of the base damage that is recognized, but rather some associated secondary structural alteration in the phosphodiester backbone. Independent evidence that other DNA defects can be repaired comes from studies on DNA breakdown following mitomycin treatment of UV-sensitive and UV-resistant bacterial strains (Boyce & Howard-Flanders, 1964b). The recognition step in the repair mechanism could be formally equivalent to threading the DNA through a close fitting "sleeve" which gauges the closeness-of-fit to the Watson-Crick structure. Such a mechanism might even be able to detect accidental mispairing of bases after normal replication. Strains defective in repair would then exhibit an abnormally high spontaneous mutation rate. Current studies should determine the significance of the repair replication system for the maintenance of genetic stability.

Acknowledgements

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