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CURRENT KNOWLEDGE OF THE FORMATION AND REPAIR OF DNA DOUBLE-STRAND BREAKS

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Summary

The types of double-strand breaks in DNA are described, and information on the number produced by x-rays and on the repair of such breaks is summarized.

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From the current concepts of molecular biology, it is difficult to see how DNA with random double-strand breaks could be replicated. Thus the repair of these breaks is of the greatest importance for the survival of the ability of a cell to self-duplicate, and perhaps for other cell functions.

The nature of a double-strand break. DNA double-strand breaks form in fact a rather complicated set of lesions. They may be the result of random coincidences between single-strand breaks formed by various agents; in this case the number of double-strand breaks will be very small compared to the number of single-strand breaks. Agents which produce clusters of breaks are more important biologically, and include restriction enzymes (Meselson et al, 1972) certain drugs such as bleomycin, and ionizing radiations such as x-rays. These latter release energy in discrete events with an average of 100 eV, producing clusters of radiochemical events in volumes a few tens of Angstroms in diameter.

Consider a case in which one event in a cluster has formed a break at a sugar in one chain. In the double-helical structure the closest sugars in the other chain are those 4-6 base pairs in either direction along the helix axis, favoring the formation of "sticky ends" which may or may not come apart depending on conditions. The tendency to come apart will be

somewhat greater than for similarly positioned single-strand breaks formed by enzymatic cleavage of a phosphodiester bond, for example, because in most x-ray produced breaks a base is lost, which reduces the stacking energy holding the sticky ends together.

It is presumed (but there are no measurements) that pairs of breaks between the same or adjacent base pairs, always producing a double-strand break, are an order of magnitude less common. And two orders of magnitude less common (Hutchinson, unpublished calculations) will be a situation in which ionization of a K electron, in phosphorus for example, results in such a high local energy deposition that effectively several base pairs are "deleted" from the molecule. The decay of I-125 in iodouracil incorporated in DNA probably produces such "deletions" (Krisch & Ley, 1974).

The number of double-strand breaks produced by x-rays. There are almost twenty measurements in the literature of double-strand breaks produced by sparsely ionizing radiations under well-defined conditions. In general, they show that the rate of production is far less sensitive to the solution surrounding the DNA than is the case for single-strand breaks. There are suggestions that the break rate may be increased if the irradiated DNA is exposed to low ionic strength, which would encourage sticky ends to separate. For DNA irradiated in oxygenated cells and never exposed to ionic strengths less than 0.1, the rate is 0.1-0.2 breaks per 10^9 daltons per kilorad. (Corry & Cole, 1968, 1973; Coquerelle et al, 1973; Lennartz et al., 1973; Hariharan & Hutchinson, 1973; Burrell

et al., 1971; Lett et al., 1970). In the absence of oxygen, the rate is reduced by a factor of 2-3 (Van der Schans et al., 1970; Lennartz et al., 1973).

The repair of double-strand breaks. Until recently there was strongly conflicting evidence as to whether cells could repair double-strand breaks. In many cases low x-ray doses were used, giving large DNA fragments whose sedimentation was unchanged on incubation. The conclusion that double-strand breaks were not repaired now needs re-evaluation in light of recent knowledge that large DNA's of quite different masses can sediment at about the same speed (see Hutchinson, this volume). It is significant that the unequivocal earlier demonstrations of repair were all in Micrococcus radiodurans (Kitayama & Matsuyama, 1968; Lett et al., 1970; Burrell et al., 1971). Because of this cell's ability to form colonies after massive x-ray exposures, high doses were used which reduced the DNA to pieces less than 10^8 daltons in size.

Since then repair has been shown of 2 out of 3 DNA breaks in Bacillus subtilis (Hariharan & Hutchinson, 1973) and of breaks in DNA in Chinese hamster cells (Corry & Cole, 1973). In no case, however, is there any proof that the breaks demonstrated in lysates and then repaired in cells during incubation would have actually been breaks inside the cell. For example, sticky ends which might have held together in vivo might come apart during lysis. Also, it is known that some x-ray induced lesions become single-

strand breaks only after a period of hours (Ward & Kuo, 1973), so that a damaged region, which might hold together long enough for a cell to make repairs, may separate over a time period long enough to measure strand breaks.

It is implied that repair means the joining of the appropriate ends, and not the indescriminate joining of double-strand polynucleotides without single-strand ends which has been observed for the T4 ligase (Sgaramella & Khorana, 1972). It should be noted that sedimentation could give only indirect information on such indescriminate repair.

Double-strand breaks, the ability of an organism to replicate, and repair. For a bacterial virus, Sharp & Freifelder (1971) have demonstrated directly that when a host cell did not receive a full complement of DNA from x-rayed phage, no new virus was produced.

For wild-type B. subtilis, Hariharan & Hutchinson (1973) showed that the fraction of cells which had intact genomes after incubation equaled the fraction of cells which could produce colonies.

M. radiodurans requires $\sim$ 500 kilorads to reduce colony-forming ability significantly, a radiation dose which would produce more than 100 double-strand breaks per genome. About 100 rads will stop colony formation by mammalian cells, and about 100 double-strand breaks in the genome. For mammalian cells and M. radiodurans, at least some of the double-strand breaks presumably represent actual DNA scissions in the

cell, so either these can be repaired, or broken DNA can in fact be replicated.

The conventional ideas on repair are as follows. (1) Recombinational events involving another identical DNA segment in the cell may take place; the obvious experiment, to look for repair under conditions where an identical segment is not present, has apparently not yet been done. (2) The broken pieces may be held together somehow (nucleohistones?) until repair can take place. (3) There are suspicions that repair may not take place when the DNA has been broken into pieces $\sim$1-3 x 10^8 daltons, except in exceptional cell types such as M. radiodurans, but no hard facts.

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