Free Rad. Res. Comms., Vol. 6, No. 2-3, pp. 177-178
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RADIATION INDUCED CLUSTERED DAMAGE IN DNA

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This is a review about research work of the last years dealing with radiation induced clustered damage in DNA. Sites where the DNA base pairing was interrupted or lost were of special interest. Therefore, we did not study structural alterations of single bases, but the ability of base pairing in irradiated DNA by reannealing kinetics. Gamma-irradiation of DNA was performed either in aqueous solution (*in vitro*) or in λ phage particle (*in situ*) where diffusible radicals were scavanged by a competent medium. DNA, irradiated *in vitro*, showed typical mismatch patterns dependent on the gamma-dose. For DNA, irradiated *in situ*, it was found, that the DNA strands matched complementarily, except scattered patches which were unable to reanneal and remained conserved. We assume that this effect was caused by a direct absorption of radiation energy within the DNA.[1]

The latter observed effect was supposed to be due to a local denaturation at multiple, clustered base damage. This was confirmed by a sensitivity of these sites to S1-nuclease, specific for single-stranded DNA: The enzyme split these sites to double strand breaks. These "S1-sites" were only observed in DNA, irradiated in-situ not in-vitro.[2-4] A single lesion on one strand of the DNA chain, as a single strand break or an apurinic site, was no substrate for S1-nuclease.[2]

S1-sites were detected in DNA of irradiated organisms as bacteriophage, yeast or mammalian cells.[5] There was a linear rise of S1-sites with dose, the yield equaled about that of double strand breaks.[5,6] This means, that about half of the effective direct absorption events of radiation energy was leading to dsb and half to "locally multiple damaged sites",[7] which can be described also as a clustered damage of bases within the DNA strand.

The distribution of the S1-sites in the phage-DNA seems to be at random, there was no preference for regions of high or low GC-content.[8] However, some structural factors seem to prevent the formation of S1-sites in DNA, as they were not found in supertwisted DNA nor in certain non-transcribed genes in yeast cells.[6]

Information about the nature of S1-sites was obtained studying the action of gamma endonuclease from M. luteus in DNA of irradiated λ — phages. This enzyme splits the DNA strand at the position of an oxidized pyrimidine base. It was found, that the gamma endonuclease cuts double strand breaks in half of the S1-sites, indicating base damage on both strands within the S1-site.[9] However, half of the S1-sites were not converted into double strand breaks by gamma endonuclease, indicating multiple base damage only on one strand within the unpaired region. The mechanisms of formation of such sites by the direct radiation action has to be discussed.

The repair of S1-sites apart from that of double strand breaks was followed in yeast chromosomes, using the Orthogonal Field Alternation Gel Electrophoresis to separate unbroken DNA from individual chromosomes.[6] In all chromosomes of

diploid wild-type yeast cells there was a repair of dsb as well as of S1-sites within 20–30 hours. In haploid yeast and in rad 50/2 n mutations, defective in recombination repair, there was no repair of dsb or S1-sites. In excision repair deficient cells (rad 3/2 n), dsb and S1 are more or less repaired. However, in rad 18/2 n yeast cells, defective in mutagenic repair, there was only repair of dsb, not of S1-sites. It was concluded, that the repair of S1 sites required recombinational repair and in addition also enzymatic steps involved in mutagenic repair.

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