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4 HEAVY ION INDUCED DNA-DSB IN YEAST AND MAMMALIAN CELLS (STATUS REPORT)

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Molecular changes at the DNA are assumed to be the main cause for radiation effects in a number of orgamisms. During the course of the last decades techniques have been developed for measuring DNA double-strand breaks (dsb), generally assumed to be the most critical DNA lesions. The outcome of all those different approaches portray a collection of data useful for a theoretical description of radiation action mechanisms. However, in the case of heavy ion induced DNA dsb the picture is not quite clear yet and further projects and strategies have to be developed.

The biological systems studied in our group are yeast and mammalian cells. While in the case of yeast cells technical and methodical reasons highlight these organisms mammalian cells reach greater importance when dsb **repair** studies are performed. In both types of organisms the technique of pulsed-field gel electrophoresis (PFGE) is applied, although with different modifications and evaluation procedures mainly due to the different genome sizes.

Yeast cells

Yeast chromosomes are in the size range that can be resolved by PFGE-technique. After the gel run the DNA molecules are labelled with the aid of a fluorescent dye, and the signal is recorded by a CCD camera system. The single bands, representing the different chromosomes of the yeast strains used, can be quantitated by a dedicated software and the intensity of the uppermost band, which represents the largest chromosome, can be used for the determination of the dsb induction frequency. It is assumed in this evaluation procedure that a decrease in band intensity to 37 resembles on average one break per molecule. Table I summarizes the results of several experiments performed at the UNILAC-facility in Darmstadt. The ions used were in the LET range of 100 to 11500 $keV/\mu m$ and had energies between 3 and 18 MeV/n. So far no experiments with the much faster ions at the SIS facility have been performed. Figure 1 shows the dsb induction cross section of all the experiments (with yeast and mammalian cells) as a function of LET. Clearly, the cross section and hence the probability for dsb induction rises for values up to about 300 $keV/\mu m$. This region is followed by a plateau in the LET range between 300 and 1500 $keV/\mu m$, while for even higher values the cross sections increase again. This second rise probably reflects the importance of the far reaching delta-electrons, which build up the so called "ion-penumbra". The results fit almost perfect into the picture which was generated in the last years in our group by means of the sedimentation technique.

Mammalian cells

The mammalian chromosomes are too large to be resolved by PFGE technique. In order to circumvent this problem the chromosomes are treated with rarely cutting restriction enzymes prior to electrophoresis. The endonucleases cut the DNA at specific sequences yielding for all cells the same restriction pattern which appears after electrophoresis as a restriction fragment distribution. In the described experiments the enzyme NotI was used which delivered fragments in the size range of about 0.2 to 5 Megabasepairs (Mbp). To examine only one single fragment instead of the whole distribution (analogous to the

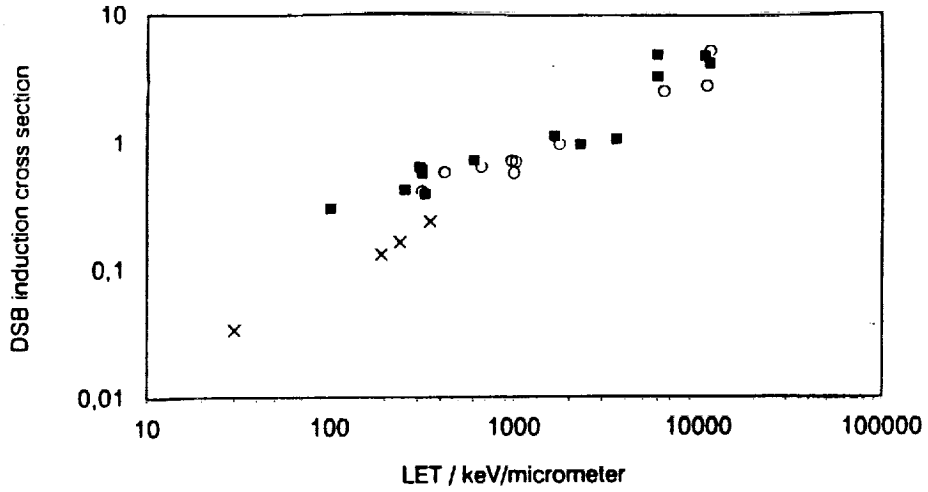

Figure 1: DNA-DSB versus LET: boxes: PFGE (Yeast), open circles: Sedimentation (Yeast), x: PFGE (Mammalian cells).

Table 1: Cross-sections for DNA-DSB for various ions, measured by means of pulsed-field gel electrophoresis.

Ion	Energy MeV/u	LET $keV/\mu m$	σ_{dsb} μm^2	RBE
C	18	100	0.3	2.3
	3.4	330	0.4	0.9
O	10.7	256	0.4	1.3
	8	316	0.6	1.6
	2.9	604	0.7	0.9
Ne	15	306	0.6	1.5
	14.4	316	0.6	1.5
Ar	5.7	1650	1.1	0.5
Kr	11.2	1600	1.1	0.2
Ni	12.1	2280	1.0	0.3
Xe	14	5980	4.9	0.6
	14	5980	3.3	0.4
Au	11.7	10980	4.9	0.3
	9.3	11600	4.2	0.3

Ion	Energy MeV/u	LET $keV/\mu m$	σ_{dsb} μm	RBE	% remaining (PFGE)	% remaining (Elution)
(X-ray)	-	0.3	-	1	32	15
Ne	425	32	0.034	0.76	41	31
Fe	600	190	0.24	0.48	70	68
Fe	400	240	0.16	0.48	-	-
Fe	250	350	0.13	0.48	-	-

Table 2: Data for DSB-induktion in mammalian cells. Cross-sections are normalized to a DNA-mass of $10^9 g/mol$ as in yeast.

situation with yeast cells) the method of "Southern hybridization" with radioactively labelled "single copy" DNA-probes was applied. Those probes bind to a DNA sequence which appears only once in the human genome and therefore only on one restriction fragment. Consequently, the restriction fragment size distribution can be reduced to a single band by analyzing the radioactive hybridization signal. The decrease of this band delivers the dsb induction rate analogous to the yeast method.

Experiments for dsb induction have been performed at the BEVALAC facility in Berkeley, CA with Ne and Fe ions inside the energy range of 250 to 600 MeV/n with the corresponding LET values of 30 to 350 $keV/\mu m$ (see table 2). The relative biological effectiveness (RBE) for dsb induction was always found to be smaller than unity (compared to X-rays) what could be explained by dsb-"cluster" inside the "ion-core" where an extremely high energy density occurs. Inside this region close to the ion trajectory the breaks are induced too close to each other to be resolved as different breaks and hence counted by all available techniques only as one. Since approximately only have of the energy is deposited inside the "core" region, the RBE is not expected to decrease below 0.5.

For dsb repair experiments the described method (called PFGE in the table) was compared with the elution approach that measures only a change in molecular weight and therefore cannot distinguish between correct and incorrect dsb rejoining events. Since the PFGE method registers only the correct rejoining and hence the real repair events (since the band with the correct molecular weight has to reappear after a certain repair time to contribute to rejoining) the respective values for remaining breaks always lie above the values for the elution approach (see table 2). As the differences between the two methods are most significant for sparsely ionizing radiation, mis-repair events take place in that case that probably serve as a "life-saving" mechanism. The fact that the proportion of unrepaired/unrejoined breaks increases with LET again reflects most likely the appearance of dsb-"clusters", since in this case all breaks of a "cluster" have to be rejoined in order to register a rejoining event.