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Chapter 6

A model treating the DNA double-strand break repair inhibition by damage clustering

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ABSTRACT: A microdosimetric model for the interpretation of radiation induced irreparable DNA double-strand breaks was applied on the biological endpoint of chromosomal aberrations. The model explains irreparable DNA double-strand breaks as a result of break clustering in DNA subunits. The model predicts quite good chromosomal aberrations in gamma- and X-ray irradiated V79 cells and human lymphocytes. In the case of α-particle irradiation the presumption had to be made, that only the cells with indirect events in the nucleus (due to delta-electrons) reach the metaphase and are analysed. With the help of this model we are able to explain the peculiar effectiveness of ultrasoft C-X-rays in human lymphocytes. Furthermore a interpretation of the experiments with accelerated and spatially correlated particles is given.

1. INTRODUCTION

DNA double-strand breaks (DSBs) have been identified as the most important initial radiation damage for the expression of various biological endpoints as there are cellular inactivation, chromosomal aberration, mutation and transformation. Studying the induction and subsequent repair of radiation induced DSBs in vivo one finds, that after incubation there remains a fraction of not repaired DSBs which depends on the radiation dose and quality. This is why one would expect, that these not repaired (residual) DSBs are closer related to the observable biological effects than the over-all amount of initially induced breaks. Studying DNA-release in irradiated mammalian cells we found, that the number of DSBs in subchromosomal DNA-units (subunits) determines the amount of released DNA. From the dose dependence of released DNA Erzgräber and Lapidus (1988) concluded, that the subunits consist of $2.4*10^9$ dalton DNA (V79 cells). Studying the fragment distribution of irradiated mammalian DNA similar conclusions were drawn (Regel,Günther,Kampf 1983). The existence of these subunits and their importance for radiation effects was prooved by a lot of other investigators, too. Cole et al (1974) interpreted results of sedimentation studies at irradiated and repair incubated CHO-DNA by the

postulate, that a 10^9 dalton DNA-subunit can form only one DSB-repair enzyme complex, leaving all additional DSBs unrepaired. Ostashevsky (1989) found a good theoretical prediction of survival curves when assuming irreversible DNA fragment loss due to multiple DSB induction in chromatin subunits. Interpreting the occurence of chromatid aberrations in Chinese Hamster cells by different radiation qualities in accordance to the TDRA-theory, Brenner (1988) could predict an interaction substructure with the diameter of some hundreds of a nanometer. Bohr and Hanawalt (1984) collected further experimental results which support the idea, that only one damage can be repaired in a distinct chromatin (or repair) subunit.
The ideas of DSB-repair inhibition by the clustering of the initial breaks in subunits were put here together to a microdosimetric model for the calculation of irreparable DNA double-strand breaks. It should be mentioned, that the original purpose of the model was the comparison of theoretical predictions with the the results of experiments, which are more biophysical than biological experiments(sedimentation studies of DNA fragments, nucleoid sedimentation studies on the restoration of DNA-supercoiling). Nevertheless, the first tests of applying the model to calculate irreparable DSBs per cell nucleus after gamma-irradiation resulted in a surprising comparability with the observed yields of chromosomal aberrations (in human lymphocytes and V79 cells, resp.).

2. THE DSB-REPAIR MODEL

The model bases on the prooved existence of DNA-subunits in the mammalian nucleus. As the leaves of a tree are distinct in shape and size for every species, the DNA-subunits show a characteristic geometrical shape and DNA-contènt for every cell type. Going a step further in our analogy, the DNA-subunits are considered to represent relatively autonomous structures in relation to the repair of damages. The DSB-repair process in a subunit is supposed to depend on the number of its own breaks rather than on the whole number of damages in the nucleus. For the characteristic repairabilities of DSB-clusters k in a subunit a function R(k) has been introduced, which must be cell typical and valid for any irradiation condition (dose, quality, dose-rate). The value R(k) represents the probability for the repair of all k DSBs in subunit. Since R(k) is a unknown function and reflects mechanisms of very complex enzyme-substrat interaction, the repair function must be fitted from experimental results. The only statement which can be made a priori is that R(k) decreases with growing k, since all investigations have shown, that the more

inhomogenous the initial damages are distributed in the nucleus (as the result of increasing LET), the bigger is the fraction of residual (non repaired) DSBs.

The second crucial quantity in the frame of the model is the distribution of the initial DSBs, i.e. the distribution of break-cluster in the subunits immediatly after the irradiation. This distribution can be deduced with the help of microdosimetry, if one knows the geometric shape of the subunits and the pattern of energy transfer from the radiation field onto the structure of the subunit.

1 Mathematical formulation of the model

We start with the densitiy function of the specific energy z after one absorption event in the target volume (subunit)

$$f_1(z)$$

This one-event distribution is characteristic for the radiation quality and for the shape of the target volume. For the $f_1(z)$-distribution we haven taken the data form (Günther, Schulz 1983).

The number of absorption events can be regarded as poisson distributed acoording

$$p_n = e^{-D/\bar{z}_f} \frac{(D/\bar{z}_f)^n}{n!}$$

The weighted summation of the convoluted one-event distributions of z (to takes into account the multiple absorptions) leads to the distribution of z for a macroscopic dose D

$$f(z,D) = \sum_{n=0}^{\infty} f_n^{*n}(z) \cdot p_n$$

Now we assume, that a distinct value of z in a subunit induces double-strand breaks there, whose numbers are poisson-distributed . i.e.

$$U_k(z) = e^{-M_0 \cdot s \cdot z} \frac{(M_0 \cdot s \cdot z)^k}{k!}$$

(M_0: DNA content of a subunit, s: number of DSBs per M_0 and dose).

Proper integration over all possible z results in the distribution of initial break numbers (cluster distribution) in the subunit under macroscopic irradiation conditions

$$U_k^{init}(D) = \int f(z,D)\, U_k(z)\, dz$$

The distribution of irreparable DSBs is equal the initial break distribution times the probability of non-repair, i.e.

$$U_k^{irrep}(D) = \int \left[1 - R(k)\right] f(z,D)\, U_k(z)\, dz$$

The ratio of the mean numbers of both distributions gives the fraction of irrepared DSBs

$$\bar{k}_{irrep}(D) = \frac{\sum_k k \cdot U_k^{irrep}(D)}{\sum_k k \cdot U_k^{init}(D)}$$

Relating this to the whole nucleus we tried to find a correlation to observable chromosomal aberration

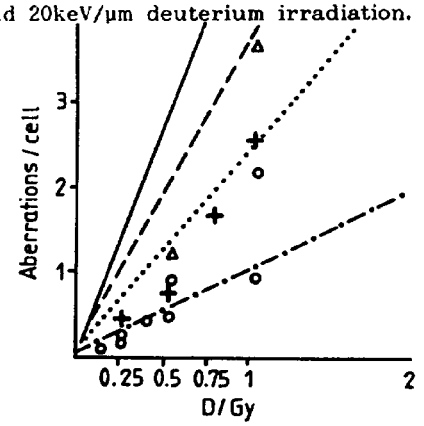
$$N_{irrep}^{DSB}(D) = s \cdot D \cdot M_{nucl.}^{DNA} \cdot k_{irrep}(D)$$

($M^{DNA}_{nucl.}$ DNA content of the whole nucleus). In the case of dicentrics and ring aberrations, resp., we compared the value $N_{irrep}^{DSB}(D)/2$ with the aberration yield to take into account that these aberrations may consist of two DNA-breaks.

3. COMPARISON WITH EXPERIMENTAL DATA

3.1 Chromosomal Aberrations in proton-, deuteron- and alpha-particle irradiated V79 cells (B1)

Since our own experiments with V79 cells (gamma-irradiated in G2) allowed us to asses the repair function for this cell type, we restricted this consideration to the yields of G2-irradiated cells from (Geard 1985), too.
As can be seen in fig.1, the prediction of the model well coincides for the 10keV/µm proton and 20keV/µm deuterium irradiation.



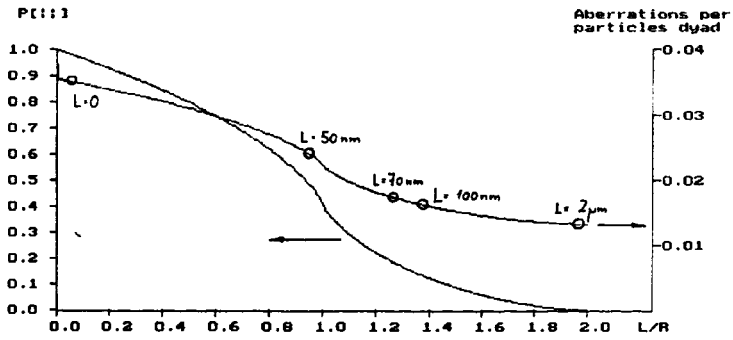
Chromosomal aberrations in V79 cells irradiated with 10keV/µm protons (●), 20keV/µm deuterons (○), 40 keV/µm deuterons and 40keV/µm α-particles△.Model calculations for $M^{DNA}_{nucl}=3.5*10^{12}$ dalton, $N_0=2.4*10^9$d, ($\bar{z}_r$=22 Gy, $\bar{z}_d$=28 Gy, $s=4*10^{-12}$/Gy*d for 10kev/µm protons — · —), ($\bar{z}_r$=45Gy, $\bar{z}_d$=52 Gy, $s=5*10^{-12}$/Gy*d für 20keV/µm deuterons ····), ($\bar{z}_r$=83Gy, $\bar{z}_d$=112Gy, $s=5.8*10^{-12}$/Gy*d for 40keV/µm deuterons – –), ($\bar{z}_r$=118 Gy, $\bar{z}_d$=153 Gy, $s=7.5*10-12$/Gy*d for 80keV/µm α-particles —).

However, an increasing LET leads to an overestimation of residual breaks (thought to result in chromosomal aberrations). The reason may be the non-equal distortion of the cell cycle of the differently damaged cells. It seems probable, that the fraction of cells which recieved direct traverses of densely ionizing particles through the nucleus may be inhibited from reaching metaphase and that the overwhelming part of analyzed cells represent a subpopulation with indirect events. The separation in a fraction of ion-like cell inactivation and gamma-like cell inactivation (Katz et al

1971) predicts, that repair of sublethal damages, plays a role only in the gamma-term of inactivation. A more precise assesment of these effects is given in the part treating human lymphocytes.

3.2 Aberrations in V79 Cells irradiated with associated particles (B 1)

The effects of associated particle irradiation were analyzed as follows: If two particles have the fixed separation L, than the probability P(¦¦) that a absorption event is due to a simultanous traverse of both of the particles is a pure function of the ratio L/R (R: projective radius of the spherical target). The absorption event at two passing particles could be described by its $f_1(z)$-distribution, which corresponds the self-convoluted $f_1(z)$-distribution of the one particle absorption event. Calculating the mean fractions of irreparable DSBs for both absorption events and weighting them with the P(¦¦) and (1-P(¦¦), resp. leads to the dependence shown in fig.2. there is a characteristic part of the plot, at which the line changes from convex to concave behaviour. This takes place when the particle separation became larger than the radius of the target. If we plot the experimental yields into the same figure, we can asses the mean projected diameter of the sensitive target (around 100nm), what corresponds quite well with the geometrical data of the subunits.

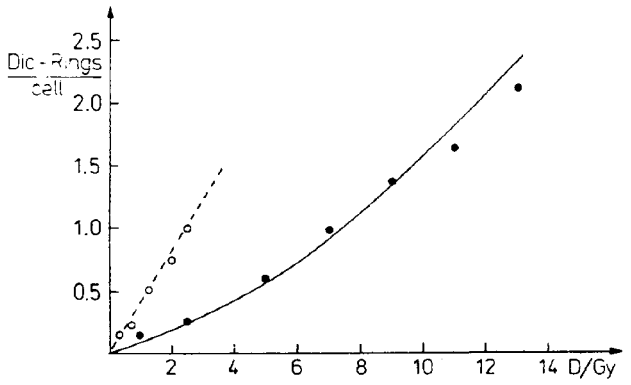


P(¦¦):Probability, that an absorption event in a sphere of radius R consists of a particle dyad, if associated particles have the fixed separation L. Right scale: associated numbers of chromosomal aberrations, if a particle dyad ($\bar{z}_f$=90Gy, $\bar{z}_d$=104Gy, $s=5*10^{-12}/Gy*d$) yields 18% irreparable DSBs, a travers of a single particle ($\bar{z}_f$=45 Gy, $\bar{z}_d$=52 Gy, $s=5*10^{-12}$) yields 8% irreparable DSBs. Experimental values with deuterium dyads (2 * 20keV/µm) are plotted (O) together with the associated particle separation L.

3.3. V79-cells irradiated with ultrasoft X-rays (B 20)

A calculation was carried out for the irradiation with ultrasoft C X-rays. These cause densily ionizing tracks of electrons, which have short ranges

and are statistically independent in the whole irradiated object. This means, that ultrasoft X-rays behave in the short range (some 10 of nm) like medium LET radiation, on the other hand like low LET radiation in longer dimension. We therefor concluded, that a s-value (initial DSB-rate) according to 30 keV/µm particles may be valid, although the $f_1(z)$-distribution is similar to ordinary X-rays. The results of the model calculation are shown in fig.3.

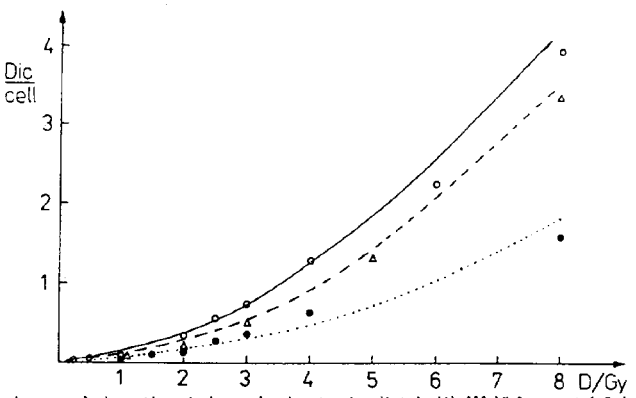


Mean number of dicentric and ring aberrations in V79 cells irradiated with ultrasoft C-K-rays (○ data from B20) and 250 kV X-rays (● data from B20), resp. Lines denote the results of the model calculations with the following parameters: $N_0=2.4*10^9$ d, do=150 nm, $N^{DSB}_{nucl}=3.7*10^{12}$ d, ($\bar{z}_r$= 10 Gy, $\bar{z}_d$= 11 Gy, $s=6*10^{-12}$/Gy*d (- - -) for C-K-ray), ($\bar{z}_r$= 1.8 Gy, $\bar{z}_d$= 3.4 Gy, $s=4.5*10^{-12}$/Gy*d (——) for 250 kV X-ray). For the model calculations the value $N^{DSB}_{irrep}/2$ is plotted to take into account, that a dicentric or a ring aberration consists of two breaks.

3.4 Dicentric Aberrations in human Lymphocytes (B3,B6,B7,B8,B10,B13)

The prediction of gamma- or X-ray induced irreparable DSBs with the help of the model shows hopeful correlations with the measured yield of chromosomal aberrations, if we assume that two irrepaired DSBs form a dicentric or ring type aberration (fig.4). To calculate the values under chronic irradiation (0.003 Gy/min) , the model was expended to taking into account the kinetic of repair, i.e. the possibility, that breaks which are induced one after the other in a subunit are repaired one after the other, too. Only if the temporal separation between their induction is significantly less than the repair half-time, they may combine to an irreparable break cluster. If we tried to predict irreparable DSBs after irradiation with heavy particles, we find a considerable overestimation. That was most astonishing , because we yield quite good results interpreting experiments on residual DSBs. The reason may be, that the analysis of chromosomal aberrations represents a much more biological experiment, i.e. effects of interphase death or changes in the cell cycle distribution play a more important role.

We supposed, that especially those cells which experienced direct absorptions by a He-ion or an α-particles do not reach the metaphase and by this are absent when chromosoms are studied . So we tried to calculate the irreparable DSBs in those nuclei, which only have had indirect events (delta-events) (fig.5).



Mean number of dicentric chromosomal aberrations in human lymphocytes irradiated with 250 kV X-rays at 1 Gy/min (○ data from B3), Co-gamma-rays at 0.5 Gy/min (△ data from B3), 250 kV X-rays at 0.003 Gy/min (● data from B6) and Co-gamma-rays at 0.003 Gy/min (● data from B3 and B6).

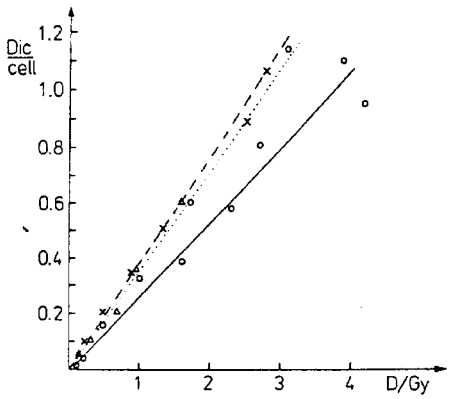
Lines denote the results of the model calculations with the following parameters: $N_0=1*10^9$ d, $d_0=150$ nm , $s=5*10^{-12}$/Gy*d, $N^{DNA}_{excl}=3.5*10^{12}$ d,

$\bar{z}_f$= 1.3 Gy, $\bar{z}_d$= 3.4 Gy (—— 250 kV X-rays)

$\bar{z}_f$= 0.9 Gy, $\bar{z}_d$= 1.8 Gy (– – – Co-Gamma rays acut)

$T^{1/2}$ = 45 min (· · · · Co-Gamma rays chron.)

For the model calculations the value $N^{DSB}_{irrep}/2$ is plotted to take into account, that a dicentric consists of two breaks.



Mean number of dicentric chromosomal aberrations in human lymphocytes irradiated with Cm-α-particles (○ data from B7), Pu-α-particles (△ data from B8) and 23.5 MeV He-Ions (× data from B13) .

Lines denote the results of the model calculations with the following parameters: $N_0=1*10^9$ d, $d_0=150$ nm , $N^{DNA}_{excl}=3.5*10^{12}$ d,

$\bar{z}_f$= 18.7 Gy, $\bar{z}_d$= 26.8 Gy, s=7 $*10^{-12}$/Gy*d, p_d=23 % (—— Cm-α)

$\bar{z}_f$= 20.5 Gy, $\bar{z}_d$= 28.5 Gy, s=7.2*10^{-12}/Gy*d, p_d=20 % (· · · · · · Pu-α)

$\bar{z}_f$= 9.0 Gy, $\bar{z}_d$= 15.2 Gy, s=6.5*10^{-12}/Gy*d, p_d=28 % (– – – 23.5 MeV He)

For the model calculations the value $N^{DSB}_{irrep}/2$ is plotted to take into account, that a dicentric consists of two breaks.

The microdosimetric quantities are related to the delta-ray-fraction of absorption events.

Since the freqency fraction $\mu\hat{f}$ of indirect events corresponds to a dose fraction $\mu\hat{d}$, only the dose ($D*\mu\hat{d}$) is applied in the model, when D is the dose used for the experiment. Similar, the $\hat{f}1(z)$ distribution for the

delta fraction of specific energy were used (Olko and Booz 1990) with its mean value $\bar{z}_\delta^2$. The experimental yields could only be interpreted, if we assume a rather large dose fraction of delta-events. They exceeded the available data (Günther and Schulz 1983, Kellerer 1971, Olko and Booz 1990) by a factor of 3. Nevertheless, the model predicts the characteristic linear dose dependence for higher LET radiation in opposition to the linear-quadratic behaviour for low-LET radiation.

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Used data sources according to the Workshop Benchmark Data

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B3 Lloyd et al, Int. J. Radiat. Biol. 28, 1975, 75-90
B6 Edwards et al, Int. J. Radiat. Biol. 37, 1980, 89-92
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