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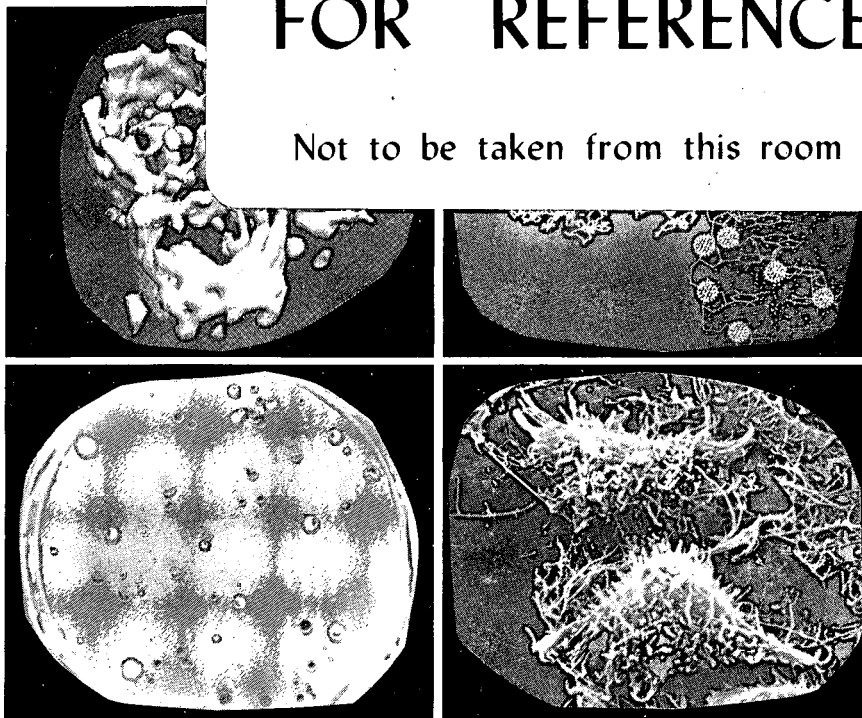
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**Problems in Mechanistic Theoretical Models for
Cell Transformation by Ionizing Radiation**

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PROBLEMS IN MECHANISTIC THEORETICAL MODELS FOR CELL TRANSFORMATION BY IONIZING RADIATION

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ABSTRACT: A mechanistic model based on yields of double strand breaks has been developed to determine the dose response curves for cell transformation frequencies. At its present stage the model is applicable to immortal cell lines and to various qualities (X-rays, Neon and Iron) of ionizing radiation. Presently, we have considered four types of processes which can lead to activation phenomena: (i) point mutation events on a regulatory segment of selected oncogenes, (ii) inactivation of suppressor genes, through point mutation, (iii) deletion of a suppressor gene by a single track, and (iv) deletion of a suppressor gene by two tracks.

1. INTRODUCTION

An understanding of the molecular mechanisms associated with radiation-induced mutagenesis and carcinogenesis is of fundamental importance. With respect to carcinogenesis it is now known that it is a multi-step process and at least three distinct stages seem to be involved: (i) initiation, (ii) promotion, and (iii) progression. In the initiation stage, a normal cell undergoes neoplastic transformation to become a pre-malignant cell as a result of an irreversible mutation (or several mutations) in the DNA. In the promotional stage, the initiated cell converts from a pre-malignant to malignant phenotype. In addition, it is thought that tumor promoting agents (TPA) act in this stage to reduce the growth inhibitory effects of neighboring cells on the initial cell. In the stage of progression, more vigorous growth takes place and invasive potential is increased (Renan 1990).

A vast amount of information on each of these stages is available from experimental measurements (Little 1981, Savage 1989, Fry 1981, Hall and Hei 1986). In order to strengthen our understanding and examine correlations between various critical phenomena observed experimentally, a theoretical approach to complement the present understanding is necessary. In this paper, we have presented our initial attempt to develop a theoretical model based on mechanisms. In this model we have addressed the initiation stage only by considering neoplastic transformation of C3H10T1/2 cells by different qualities of ionizing radiation. The choice of this cell line seems reasonable because of the availability of a large amount of experimental data and also because this is the only system where measurements have been made with heavy charged particles (Yang 1985).

DNA has been considered to be the critical target in the model and of all the possible kinds of damage that can be produced by ionizing radiation, double strand breaks have been assumed to be the only lesions that are associated with oncogenic activation. A single double strand break can cause a point mutation and two double strand breaks are needed to cause a deletion event. If one of these events, point mutation or deletion, takes place at an appropriate location of the genome, then there is potential for neoplastic cell transformation.

In this model, there are several constants which are characteristic of a cell. These constants are not treated as adjustable parameters. Based on our present knowledge, the various constants have been assigned reasonable values.

2. CALCULATION PROCEDURE

2.1 Assumptions:

- i. Double strand breaks are the critical lesions for activation or deletion events.
- ii. In partially transformed cell lines (such as C3H10T1/2) one oncogene activation, directly or indirectly, is sufficient to cause neoplastic cell transformation.
- iii. A point mutational event (dsb) on a suppressor gene can lead to an oncogenic activation.
- iv. A deletion event (two dsbs) leading to the removal of a suppressor gene can lead to oncogenic activation.

2.2 Method:

2.2.1 Basic Set-Up

Let Y be the yield of double strand breaks per gray per base pair. For X-rays, Ne (400 MeV/n), and Fe (400 MeV/n), the respective values are 2.0×10^{-9} , 2.6×10^{-9} , and 4.3×10^{-9} . The corresponding LET values respectively are 0.9 keV/ μ , 32.3 keV/ μ , and 240.7 keV/ μ . The numerical values of Y have been obtained from a previously developed computational procedure (Chatterjee and Holley 1991; Holley, Chatterjee and Magee 1990). This procedure is mechanistically based, and is related to $\cdot\text{OH}$ damage and direct ionization damage. It was found that on average it takes about 60 eV to create a double strand break.

The overall probability, P , for cell transformation is conceptually written as:

$$P(\text{total}) = P(\text{point mutation on a regulatory segment of a gene}) + P(\text{point mutation on a suppressor gene}) + P(\text{deletion of a suppressor gene by a single track}) + P(\text{deletion of a suppressor gene by two tracks}) \quad (1)$$

2.2.2 Point Mutation (Regulatory Segment)

It has been suggested (private communication, Brian P. Endlich, Memorial Sloan-Kettering Cancer Center) that a possible truncation mechanism for oncogenic activation needs to be explored. Under this hypothesis, truncation of tyrosine kinases (e.g., src, yes, syn, kit, hck, abl, fes, etc.), serine/threonine kinases (e.g., mos, raf/mil, pks, etc.), and certain receptor proteins (c-erbB/EGF receptor, neu/erbB2, c-fms/CSF-1, c-ros/insulin receptor, PDGF receptor, etc.) results in the loss of sites for regulation mediated by phosphorylation and is a potentially activating lesion. The phosphoryla-

tion site is usually situated approximately 5-10% of the distance upstream of the c-terminus. For truncation by deletion to occur, one dsb must occur in the corresponding region of the gene and a second dsb somewhere downstream. We believe that this has a lower probability than a single dsb event on this regulatory coding segment of the oncogene resulting in a point mutation frame shift. Hence, according to our single dsb point mutation mechanism,

$$P(\text{point mutation-regulatory}) = \eta_{\text{pm}} N_G \langle S_t \rangle f_{\text{ur}} Y D \quad (2)$$

η_{pm} = efficiency = 0.67, based on frame shift events only.

N_G = total number of genes which can be activated by a point mutation affecting a regulatory region of the resultant protein = 10 (assumed in the absence of a definite value).

$\langle S_t \rangle$ = average target size (in number of base pairs) = 150, which is about 10% of an average gene size of 1500 base pairs.

f_{ur} = fraction unrepaired.

D = dose in Gray.

2.2.3 Point Mutation on a Suppressor Gene

A point mutation through the formation a dsb on a substantial region of a suppressor gene can lead to an activation of an oncogene. The corresponding probability can be written as:

$$P(\text{point mutation on a suppressor gene}) = \eta_{\text{SG}} N_{\text{SG}} \langle S_{\text{SG}} \rangle f_{\text{ur}} Y D \quad (3)$$

η_{SG} = efficiency = 0.67, based on frame shift events only.

N_{SG} = total number of suppressor genes involved in this mechanism = 20 (assumed in the absence of a definite value).

$\langle S_{\text{SG}} \rangle$ = average effective target size of a suppressor gene (which may not be the total gene) = 1000 base pairs (assumed in the absence of a definite value).

2.2.4 Deletion of a Suppressor Gene by a Single Track

For this mechanism, two dsb events are needed, one on each side of a suppressor gene. The total target size can be quite large. However, the probability of these events induced by a single track is LET dependent. For low-LET, the probability is quite low.

The probability is given by,

$$P(\text{deletion of a suppressor gene by a single track}) = \eta_{\text{del}} N_{\text{SG}} \frac{\langle n_d \rangle^2}{n_t} f_d \langle N_b \rangle Y D \quad (4)$$

η_{del} = efficiency = 1.0.

N_{SG} = total number of suppressor genes involved = 20 (assumed in the absence of a definite value).

- $\langle n_d \rangle$ = average number of base pairs in a large deletion containing a suppressor gene: two calculations are done, one with 9×10^5 base pairs and another with 1×10^6 base pairs (this quantity is also unknown).
 n_t = total number of base pairs in a cell nucleus = 6×10^9 .
 $\langle N_b \rangle$ = average number of dsb per track through a cell nucleus of 5μ diameter.
 f_d = the fraction rejoined following deletion.

2.2.5 Deletion of a Suppressor Gene by Two Tracks

For this mechanism, dsbs are created on each side of a suppressor gene by two tracks. This process leads to a quadratic dependence on the dose.

The relevant equation is:

$$P \text{ (deletion of a suppressor gene by two tracks)} = \eta_{\text{del}} N_{\text{SG}} \langle n_d \rangle^2 f_d Y^2 D^2 \quad (5)$$

- η_{del} = efficiency factor = 1.0.
 N_{SG} = total number of suppressor genes involved = 20 (assumed in the absence of a definite value).
 $\langle n_d \rangle$ = average number of base pairs in a large deletion containing a suppressor gene.

2.2.6 Total Probability

$$p = \eta_{\text{pm}} N_G \langle S_t \rangle f_{\text{ur}} Y D + \eta_{\text{SG}} N_{\text{SG}} \langle S_{\text{SG}} \rangle f_{\text{ur}} Y D + \eta_{\text{del}} N_{\text{SG}} \frac{\langle n_d \rangle^2}{n_d} f_d \langle N_b \rangle Y D + \eta_{\text{del}} N_{\text{SG}} \langle n_d \rangle^2 f_d \langle N_b \rangle Y^2 D^2 \quad (6)$$

2.2.7 Unrepaired Fraction

Almost nothing is known with respect to repairability of double strand breaks by different qualities of radiation. Based on Premature Chromosome Condensation techniques, Goodwin *et al.* (1988) have measured rejoining kinetics of chromatin breaks for several charged particles (Fig. 1). In this model we have assumed that most double strand breaks are not repaired correctly even if they are rejoined. Ideally one has

$$f_{\text{total rejoin}} = f_{\text{correct rejoin}} + f_{\text{incorrect rejoin}}$$

where $f_{\text{incorrect rejoin}} = f_{\text{ur}}$.

If $f_{\text{correct rejoin}}$ is much smaller than $f_{\text{incorrect rejoin}}$, then $f_{\text{ur}} \approx f_{\text{total rejoin}}$ (7)

The values of $f_{\text{total rejoin}}$ can be obtained from Fig. 1 and hence we have an estimation of f_{ur} . For f_d one can write,

$$f_d = (1 - f_{\text{rejoin}})^2 f_{\text{rejoin}} \quad (8)$$

For X-rays, Neon (400 MeV/n) and Iron (400 MeV/n), the respective values of $f_{\text{total rejoin}}$ are 0.8, 0.6, and 0.4.

3. RESULTS

The results of our calculation have been compared with the experimental data of Tracy Yang (1985). His data were obtained with confluent cultures of the C3H10T1/2 mammalian cell line. These cells are immortal and hence partially transformed. Each figure caption (Figs. 2-5) describes the results.

4. DISCUSSION

The model presented here needs further development and the major portion of the development will depend upon how accurately one can estimate the values of the various constants as they have appeared in the equations. These constants cannot be and should not be treated as adjustable parameters. For the model described here, perhaps the first challenge is to narrow down the range within which variations in the presently estimated values are allowed.

Within the context of the present model for low-LET radiation at low doses, point mutation of a suppressor gene is the dominant mechanism for cell transformation. This is in indirect agreement with Little *et al.* (1991), who reported that in Southern blot analysis of X-ray irradiated mouse 10T1/2 cells, they could not find any change in banding patterns for 15 known oncogenes including ras. Hence it appears that activation of oncogenes as observed in human and animal tumors may be a late phenomena. For high-LET radiation, based on the present model, deletion process seems to be an important mechanism.

In conclusion, the model may have the potential to provide an understanding of the mechanisms associated with cell transformation. The main problems faced by any model of this general class is to determine all of the important oncogenic mechanisms and to obtain reliable estimates of the relevant constants.

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6. ACKNOWLEDGMENTS

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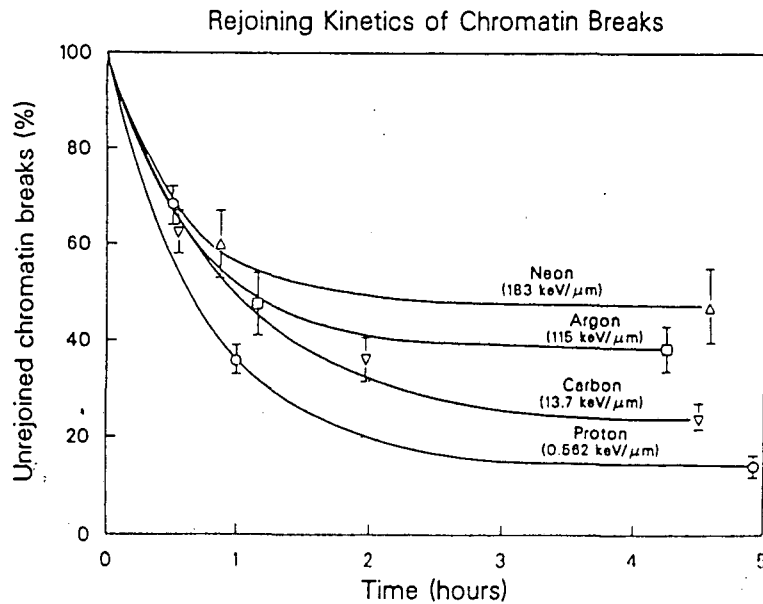


Figure 1. Rejoining Kinetics of Chromatin Breaks for heavy charged particles (Goodwin 1988) for CHO cell lines. In the absence of any repair information on strand breaks in 10T1/2 cells by these particles, these data were used. At present the application of such an approximation is highly questionable, unless it can be proven that the fraction of rejoined breaks is independent of either the cell line or the secondary and higher order structure of DNA.

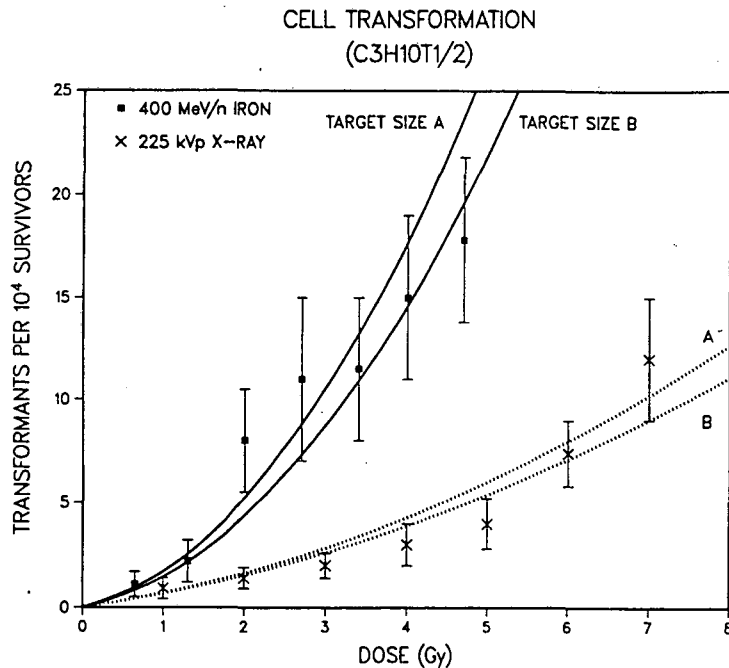


Figure 2. The solid curves and the dotted curves are calculated results for 400 MeV/n iron particles and 225 kVp X-rays respectively. The experimental data and errors are also shown. For target size A, the maximum size of a deleted segment containing a suppressor gene is 2×10^6 and for target size B it is 1.8×10^6 . Based on high-dose experimental values, it is difficult to evaluate the proper size of a target. At low doses, there seems to be not much difference for both qualities of radiation.

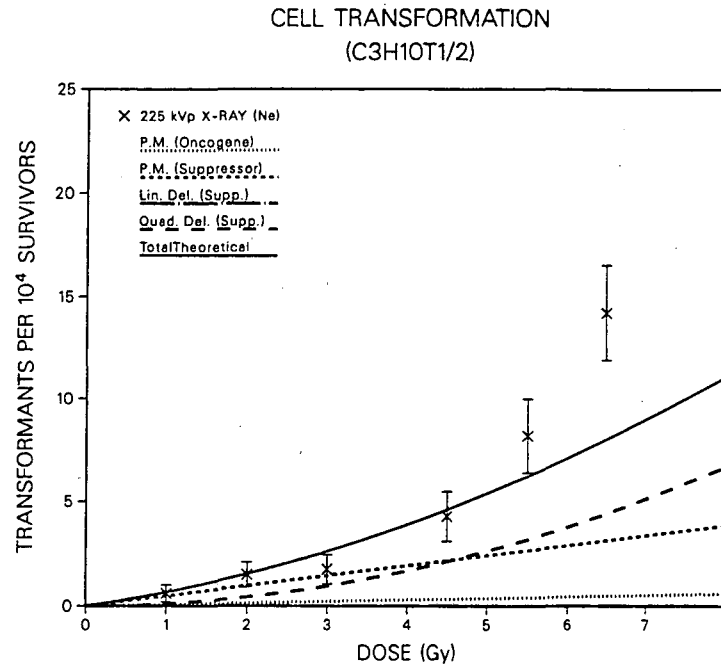


Figure 3. Theoretical results have been plotted showing the contributions from each of the four terms in equation (6) for 225 kVp X-ray. Experimental data are from Yang (1985). At low doses, the dose response is linear and the major contribution is due to point mutations of suppressor genes.

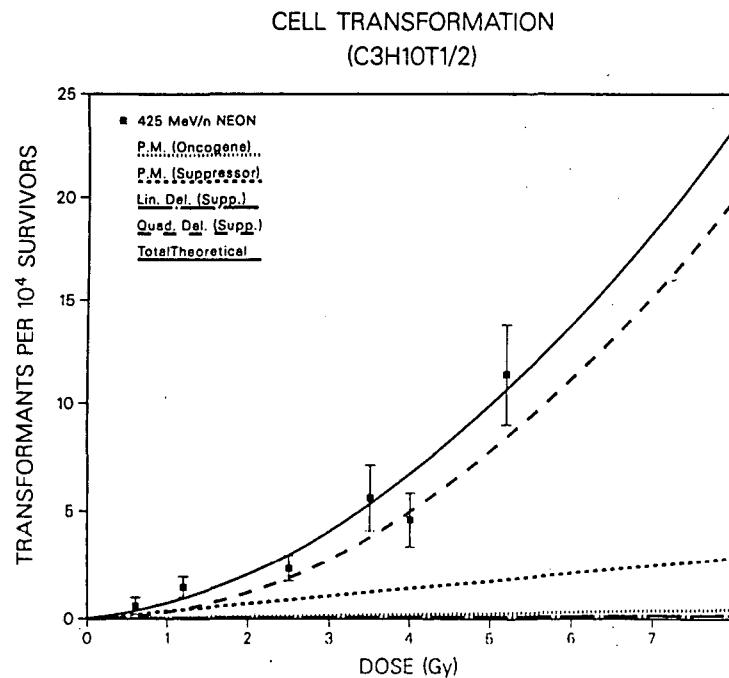


Figure 4. Theoretical results have been plotted showing the contributions from each of the four terms in equation (6) for 425 MeV/n Neon Ions. At low doses, quadratic deletions as well as point mutations of the suppressor gene seem to be the dominant processes.

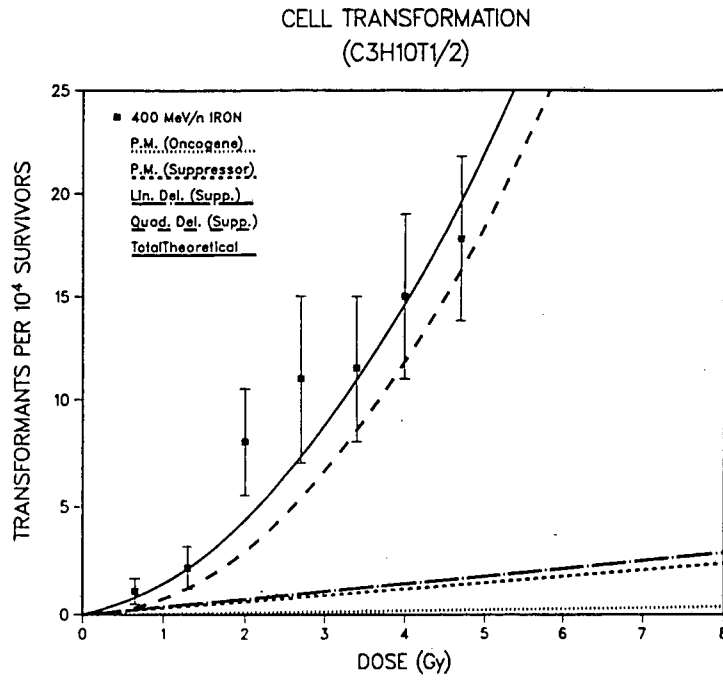


Figure 5. Theoretical results have been plotted for 400 MeV/n Iron showing the contributions from each of the four terms in equation (6). At low doses, point mutation of suppressor genes, linear deletion and quadratic deletions contribute almost equally.

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