Are Track Structure Simulations *Truly* Needed for Radiobiology at the Cellular and Tissue Levels

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Location: Washington D.C.

Learning Objectives

- Review (*selected*) mechanisms and processes that determine the biological effectiveness of particles relative to ^{60}Co γ-rays and MV x-rays ("particle RBE")
- Understand how the RBE for DNA damage relates to the RBE for cell survival.
- Gain insight into and learn about the putative relationship between particle RBE at the molecular, cellular and tissue levels

Acknowledgements

I've had the good fortune to collaborate with many outstanding clinicians and researchers at the UW and elsewhere, including most recently

UW and Affiliates: GA Sandison, W Smith, KRG Hendrickson, E Lee, O Gopan, M Kim, MH Phillips, S Vyas, R Ermoian, LM Halasz, U Parvathaneni, GE Laramore, CD. Bloch, J Saini, G. Moffit, D. Argento, R Emery, J Zeng, YD Tseng, R Rengan, S. St James, LA Young, N Cao, J Meyer, R Miyaoka, and JL Schwartz

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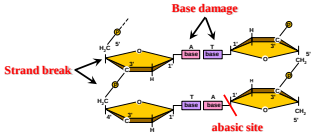
Mechanisms and Classification of Initial DNA

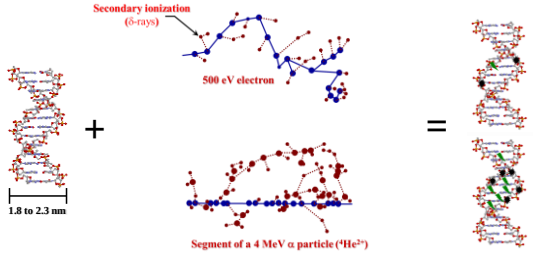
- Types of Elementary DNA Lesion
- Direct Effect
- Indirect Effect
- Classification of Clusters of DNA Lesions

Elementary Types of Damage

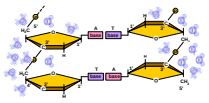
- **Individual nucleotides that become damaged are termed a *DNA lesion***
  - Abasic or AP (apurinic/apyrimidinic) sites = base loss
  - Base damage (A, T, G or C)
  - Strand breaks (damage to sugar or phosphate), usually accompanied by base loss



Direct Ionization of the DNA ("direct effect")



Ionization of H_2O close to the DNA ("Indirect Effect")

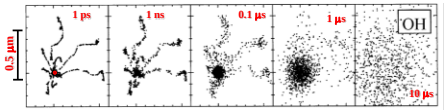


ionized water

$$\gamma + H_2O \rightarrow H_2O^+ + e^-$$

$$H_2O^+ + H_2O \rightarrow OH + H_3O^+$$

hydroxl radical



Spatial distributions of ·OH in liquid water. Red dot indicates location of a 1 µm segment of a 24 MeV $^4He^{2+}$ ion (26 keV/µm) directed into the image

Image adapted from Muroya Y, Plante I, Azzam EI, Meesungnoen J, Katsumura Y, Jay-Gerin JP. High-LET ion radiolysis of water: visualization of the formation and evolution of ion tracks and relevance to the radiation-induced bystander effect. *Radiat Res.* 165(4), 485-491 (2006).

Amount of Direct and Indirect damage after 1 Gy

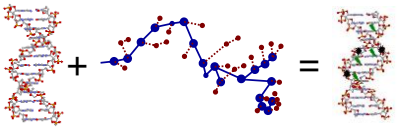
	% Volume of Nucleus	Mass (pg)	MeV per Gy	Number 50 eV events
cell	-	1,000.00	6.2422	124,843.9
nucleus	100.00	125.00	0.7803	15,605.5
human DNA	4.90	6.13	0.0382	764.8
base (A, T, G or C)	2.06	2.58	0.0161	321.8
phosphate (PO4)	1.51	1.89	0.0118	236.3
deoxyribose sugar	1.32	1.66	0.0103	206.8
sugar+phosphate	2.84	3.55	0.0222	443.0
water	70.00	87.50	0.5462	10,923.8

764 + 10,923 = 11,687 (max # of DNA lesions)

For a diploid human cell, best estimate is ~ 5,200 DNA lesions Gy^{-1}, which implies that < 10^4 nucleotides of out of 10^{10} are damaged by a 1 Gy dose of radiation (1 in 10^6).

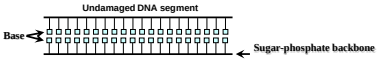
Clusters of DNA Lesions

One of the more unique characteristics of ionizing radiation is its ability to produce several DNA lesions within one or two turns of the DNA, i.e., a *cluster of DNA lesions

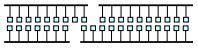


* **"Clusters of DNA lesions" are also referred to in the literature as *locally multiply damaged sites* (LMDS) or *multiply damaged sites* (MDS)**

Double Strand Break (DSB)

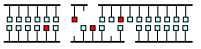


A DSB is a cluster that contains *at least* two strand breaks on opposing strands within ~ 10 bp of each other



Strand breaks formed by radiation are chemically reactive ("sticky")

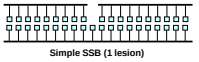
Number of lesions forming a cluster is a measure of "complexity"



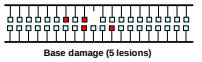
Red square (■) denotes base damage

Non-DSB Clusters

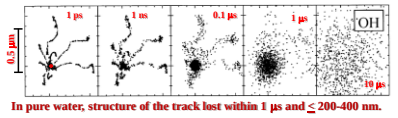
SSB (single strand break**) denotes the *family* of all types of cluster other than the DSB that contain *at least* one strand break**



Clusters that do not contain any strand breaks are referred to as "base damage"



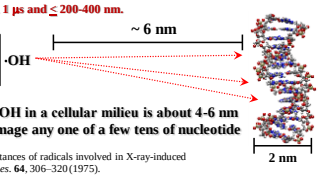
Track Structure and the Indirect Effect



In pure water, structure of the track lost within 1 µs and ≤ 200-400 nm.

Nucleus ~ 4-6 µm
Cell > 5-10 µm

Groups of ·OH not formed in close spatial proximity to the DNA mostly create *individual* lesions rather than *clusters*!



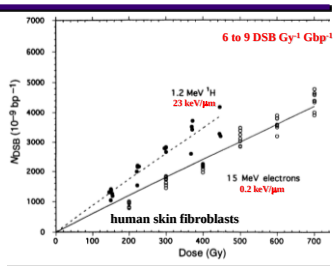
Average diffusion distance of an •OH in a cellular milieu is about 4-6 nm (Roots and Okada 1975**) – may damage any one of a few tens of nucleotide**

Roots R, Okada S. Estimation of life times and diffusion distances of radicals involved in X-ray-induced DNA strand breaks of killing of mammalian cells. *Radiat. Res.* **64**, 306–320 (1975).

DSB Induction ∝ Dose

DSB are directly or indirectly formed through the action of *individual* tracks + spatially and temporally correlated δ-rays

Not* formed by 2 or more independent tracks up to at least a few hundred Gy – would be a *linear-quadratic function of dose if DSB created by ≥ 2 tracks.

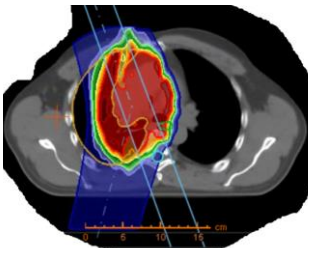


Frankenberg D, Brede HJ, Schrewe UJ, Steinmetz C, Frankenberg-Schwager M, Kasten G, Pralle E. Induction of DNA double-strand breaks by ^{1}H and ^{4}He ions in primary human skin fibroblasts in the LET range of 8 to 124 keV/microm. *Radiat Res.* **151**(5), 540-549 (1999).

Track Structure on the Tissue Level?

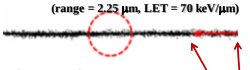
Absorbed dose at the multi-cellular (> 1 mm³) and tissue levels arise from the aggregate effects of *many* particles.

About 10^5 to 10^6 protons must pass through a 1 mm³ region of matter to deliver 1 Gy of absorbed dose.

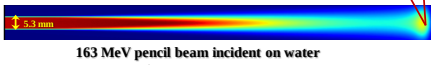


Tracks Structure at the CT voxel level (~ 1 mm³)

0.15 MeV $^{1}H^{+}$
(range = 2.25 µm, LET = 70 keV/µm)



On a CT voxel-sized scale, fine features of even very high LET tracks are *not* visible.



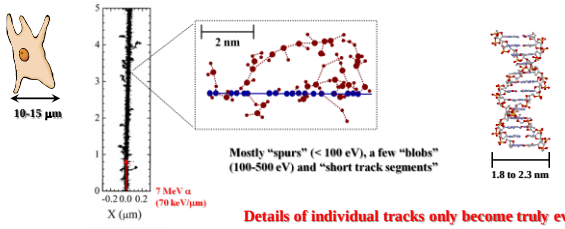
163 MeV pencil beam incident on water
(*structure of individual tracks not visible***)**

About 50 to 100 protons must pass through a cell (diameter ~ 5-10 µm**) in order to deliver 1 Gy**

10-15 µm

2D proton dose distribution courtesy S. Streitmatter

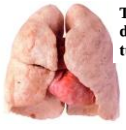
Track Structure on the Molecular Scale



Details of individual tracks only become truly evident only at the molecular level (*few tens of nanometers*).

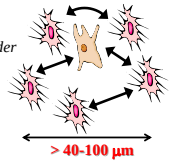
Image adapted in part from Muroya Y, Plante I, Azzam EI, Meesungnoen J, Katsumura Y, Jay-Gerin JP. High-LET ion radiolysis of water: visualization of the formation and evolution of ion tracks and relevance to the radiation-induced bystander effect. *Radiat Res.* 165(4), 485-491 (2006).

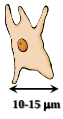
Macro- to the Microscale – Dosimetry and Track Structure



Track structure *not* very important, except possibly for very low doses of high LET particles, e.g., outer space, OARs distant from tumor target, …

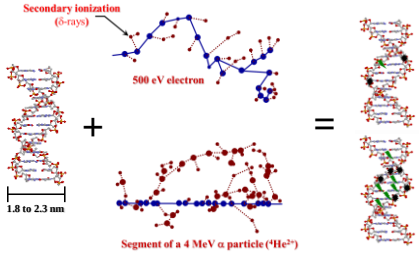
Cell-to-cell communication (*bystander effects, adaptive responses, …***) cell-microenvironment interactions**





Track structure *most important* at the cellular and sub-cellular levels – small and large doses of all types of ionizing charged particle

Ionization Density ("LET effects") and Cluster Complexity



Effects of Track Structure on DNA Clusters

- **# of lesions per cluster tends to increase with increasing LET**

As # of lesions per cluster increases, more likely to have at least 2 opposed strand breaks within 10 bp, i.e., a DSB.

SSB (*less likely*) **DSB (*more likely*)**

A cluster of DNA lesions can only be a DSB, a SSB or a cluster of damaged bases, i.e., mutually exclusive cluster categories.

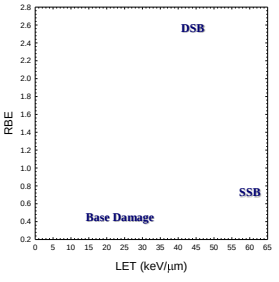
- **DSB ↑ SSB ↓ Base Damage ↓**
- **Ratio of SSB to DSB goes from about 20:1 ("low LET") to about 3:1 ("high LET")**

Trends in RBE_{DSB} with proton LET (*same overall trends other ions*)

Filled Yellow Symbols: ***Track Structure Simulation*** **(**Nikjoo *et al.* 1997, 2001, 2002**)**

Filled Red Symbols: ***Track Structure Simulation*** **(**Friedland *et al.* 2003**)**

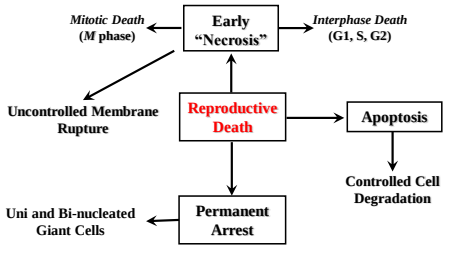
Lines: ***Monte Carlo Damage Simulation**** **(MCDS)**



DSB are *only* major category of *initial* DNA damage that increases with increasing particle LET

* **Semenenko and Stewart 2004, 2006, Stewart *et al.* 2011**

Cell Death Modes and Kinetics



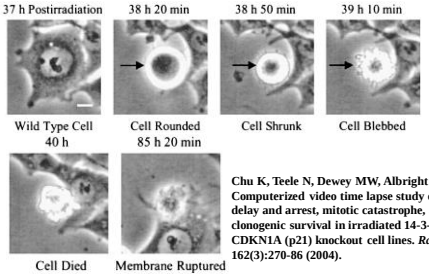
Necrosis

- Intracellular contents released in uncontrolled fashion (**membrane *ruptures***)

200X phase-contrast images of EJ30 human bladder carcinoma cell undergoing necrosis 45-48 h after exposure to 6 Gy of 220 kVp x-rays

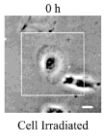
Chu K, Leonhardt EA, Trinh M, Prieur-Carrillo G, Lindqvist J, Albright N, Ling CC, Dewey WC. Computerized video time-lapse (CVTL) analysis of cell death kinetics in human bladder carcinoma cells (EJ30) X-irradiated in different phases of the cell cycle. *Radiat Res.* 158(6):667-77 (2002).

Apoptosis (*HCT116 colorectal carcinoma*)



Chu K, Teele N, Dewey MW, Albright N, Dewey WC. Computerized video time lapse study of cell cycle delay and arrest, mitotic catastrophe, apoptosis and clonogenic survival in irradiated 14-3-3sigma and CDKN1A (p21) knockout cell lines. *Radiat Res.* 162(3):270-86 (2004).

Unitnucleated Giant Cells (*arrested cell*)

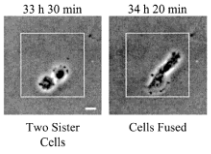


200X phase-contrast images of EJ30 human bladder carcinoma cell after exposure to 6 Gy of 220 kVp x-rays

Chu K, Leonhardt EA, Trinh M, Prieur-Carrillo G, Lindqvist J, Albright N, Ling CC, Dewey WC. Computerized video time-lapse (CVTL) analysis of cell death kinetics in human bladder carcinoma cells (EJ30) X-irradiated in different phases of the cell cycle. *Radiat Res.* 158(6):667-77 (2002).

Binucleated giant cells

- **Cell completes mitosis but progeny fuse back together**



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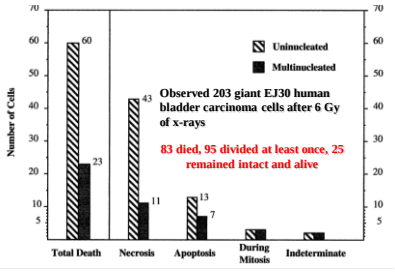
Ultimate Fate of Giant Cells

Prieur-Carrillo G, Chu K, Lindqvist J, Dewey WC. Computerized video time-lapse (CVTL) analysis of the fate of giant cells produced by X-irradiating EJ30 human bladder carcinoma cells. *Radiat Res.* 159(6):705-712 (2003).



What's the connection between DNA Damage and Cell Survival?

- Are all DSB lethal? What about SSB? Base Damage?
- Breakage and reunion therapy, a.k.a., what happens when pairs of DSB interact?

Are all DSB Lethal?

After 1 Gy dose of low LET radiation, a typical human cell sustains 45 ± 10 DSB Gy^{-1} cell^{-1}. If DSB are *always* lethal, the fraction of cells that will survive a 2 Gy dose is

$$S = \exp(-45\ \text{DSB Gy}^{-1} \cdot 2\ \text{Gy}) = 10^{-40}\ (10^{-31}, 10^{-48})$$

Only those cells that do not sustain a radiation-induced DSB survive
(Poisson distribution of DSB among irradiated cells**)**

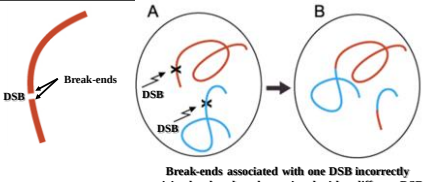
For comparison, *many* published studies indicate a surviving fraction of 0.1 (repair compromised**) to 0.9 (*repair proficient*) cells after a 2 Gy dose of radiation. Only way to reconcile observations is**

< 2% of initial DSB formed in a cell are lethal

∴ cells must be *really* good at repairing/rejoining DSB!

See also the classic review: DT Goodhead. Initial events in the cellular effects of ionizing radiations: clustered damage in DNA. IJRB 65(1): 7-17 (1994).

Breakage and Reunion Theory

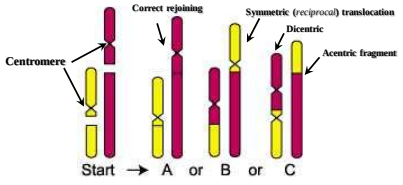


Break-ends associated with one DSB incorrectly rejoined to break-end associated with a *different* DSB

Proximity Effects: *pairs* of DSB formed in close spatial *and* temporal proximity are more likely to rejoin incorrectly than *pairs* of DSB separated in time and\or space (→ *dose rate* and *LET effects*)

R.K. Sachs and D.J. Brenner, Chromosome Aberrations Produced by Ionizing Radiation: Quantitative Studies
http://web.ncbi.nlm.nih.gov/books/bv.fcgi?call=bv.View..ShowTOC&rid=mono_002

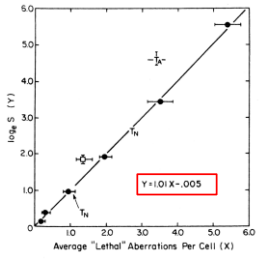
Lethal and Non-Lethal Aberrations



Dicentrics and acentric fragments are usually lethal in the reproductive sense because segregation of chromosomes at mitosis is disturbed. In contrast, correct DSB rejoining and symmetric (*reciprocal*) translocations are consistent with continued cell division

R.K. Sachs and D.J. Brenner, Chromosome Aberrations Produced by Ionizing Radiation: Quantitative Studies
http://web.ncbi.nlm.nih.gov/books/bv.fcgi?call=bv.View..ShowTOC&rid=mono_002.TOC&depth=10

1:1 relationship between lethal aberrations and cell survival



AC 1522 normal human fibroblasts irradiated by x-rays

$$S = e^{-Y}$$

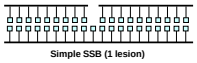
S = fraction that survive

***Y* = average number of lethal aberrations per cell**

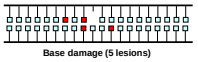
Source: Cornforth and Bedford, *Rad. Res.*, 111, p 385-405 (1987). See also Figure 3.4 in Hall (p. 37)

What about SSB? Base Damage?

After 1 Gy dose of low LET radiation, a typical human cell sustains 1000 ± 200 SSB Gy^{-1} cell^{-1}. Using the same logic as for DSB, ≤ 0.1% of initial SSB are involved in cell killing, i.e., *cells are even better at repairing SSB than DSB. Same argument applies to base damage.*


Simple SSB (1 lesion)


Complex SSB (6 lesions)


Base damage (5 lesions)

Small-scale (*point*) mutations are far easier for a cell to tolerate than larger-scale chromosome aberrations

A Mechanism-Inspired RBE Model

RADIATION RESEARCH 169, 447–459 (2008)
0033-7587/08 $15.00

Combined Use of Monte Carlo DNA Damage Simulations and Deterministic Repair Models to Examine Putative Mechanisms of Cell Killing

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Combines a kinetic repair-misrepair model very similar to the earlier RMR (Tobias 1985), LPL (Curtis 1986), and MK (Hawkins 1996, 1998, 2003) models with an independently tested Monte Carlo Damage Simulation (MCDS) for initial DSB induction (Stewart *et al.* 2004, 2006, 2011, 2015).

Connection between RBE_{DSB} and the RBE for cell survival?

In the MCDS+RMF system of models, the RBE for cell survival in the limit of low dose (RBE_{LD}) and high dose (RBE_{HD}) is related to RBE_{DSB} by

$$RBE_{LD} \equiv \frac{\alpha}{\alpha_\gamma} = RBE_{DSB}\left(1+\frac{2\bar{z}_F RBE_{DSB}}{(\alpha/\beta)_\gamma}\right) \qquad RBE_{HD} \equiv \sqrt{\frac{\beta}{\beta_\gamma}} = RBE_{DSB}$$

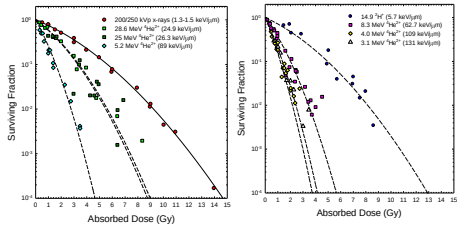
Unrepaired and Mis-repaired DSB

Intra-track DSB-DSB interactions

Inter-track DSB-DSB interactions

Adjust α_γ and $(\alpha/\beta)_\gamma$ to fit cell survival data for reference radiation. Then, use MCDS to compute RBE_{DSB} as a function of particle type and energy (*no adjustable parameters*).

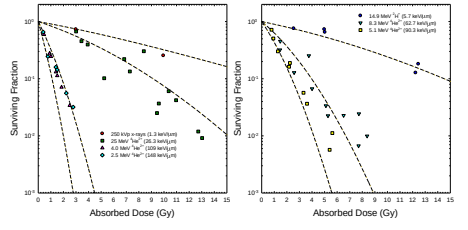
Human Kidney T1 Cells (*aerobic*)



$$\alpha = \alpha_\gamma \cdot RBE_{DSB}\left(1+\frac{2\bar{z}_F RBE_{DSB}}{(\alpha/\beta)_\gamma}\right) \qquad \beta = \beta_\gamma RBE_{DSB} RBE_{DSB}$$

Measured data from Barendsen circa 1968-1966

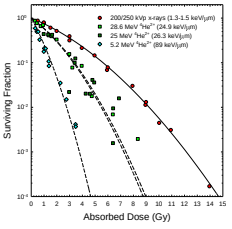
Human Kidney T1 Cells (*anoxic*)



RMF model predictions (dashed lines) based on reference radiation α_γ and $(\alpha/\beta)_\gamma$ values *for aerobic conditions* and MCDS estimates of RBE_{DSB} for anoxic conditions (i.e., no fitting to measured data in these figures)

Power of a Mechanism-Inspired Biophysical Model

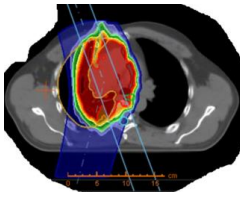
- **LQ analysis** (15 × 2 = **30 adjustable parameters**)
- **MCDS+RMF** analysis (**2 adjustable parameters**, RBE_{DSB} from first principle MCDS simulations)
- **Models and measurements in about equally good agreement given the number of adjustable parameters!**



Radiobiology – cell to tissue level?

Well known that *equal doses* of low and high LET radiations do *not* cause the same level of biological damage in the near- and longer term. Hence the need for an RBE concept…

Thought Experiment: *If* during and just after a course of radiotherapy the larger-scale temporal and spatial pattern of dead and dying is *exactly* the same for a low and high LET radiation, would the longer-term *clinical outcome* be the same?



OAR Dose-Volume Constraints for MV-xrays and Particles

Poster #SU-F-T-128 (*Stewart et al.*)

(*1*) Illustrates how to use estimates of RBE_{DSB} and the RBE for cell survival to determine plausible dose-volume constraints for protons, fast neutrons and other ions.

(*2*) Tabulates tolerance dose constraints (*arbitrary fractionation schedule*) and reference radiation (α/β) values for 30 different OAR and clinical endpoints

(*3*) Provides some evidence that, if the spatial and temporal distribution of dead and dying cells is about same for low and high LET radiations, longer-term clinical impact on OAR toxicity is about the same.

Supplemental Slide (OAR tolerance dose)