Clustered damage in DNA: implications, simulation, and detection.

- DNA damage is caused by the reactive species produced by the passage of a charged particle.

- Modeling is the only way to completely characterize the complexity of the damage including the stochastic nature of the track and its effects on damage distributions.

- Particle tracks superimposed on a model of linear DNA indicate that damage may occur in “clusters”.

- Such clusters of “locally multiply damaged sites” will vary depending on the statistical nature of the track, the random orientation of the track with respect to the DNA, and the “compactness” of the DNA.

- The biological consequences of clustered damage may be serious. Additional damaged sites near a DSB may interfere with DNA repair enzymes.

- Monte Carlo techniques are available to address the statistical nature of:
    - the location of energy deposition sites on the DNA,
    - the amount of energy deposition,
    - diffusion of water radicals.

Descriptions of Energy Deposition

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[Goodhead, 1987]

As LET increases, dsbs become “more lethal”

[Image removed due to copyright considerations]

“Simple” Double Strand Break

[Image removed due to copyright considerations]

[Goodhead, 1994]

"Complex" Damage

[Image removed due to copyright considerations]

[Goodhead, 1994]

Table 1. Suggested classes of initial subcellular damage

Class	Initial physical damage	Typical energy and target dimensions	Possible target	Frequency* of occurrence (cell^{-1} Gy^{-1})	Comment
1	Sparse	Few tens of eV within ~2 nm (~1 ionization)	DNA segment	~10^3	Little biological relevance?
2	Moderate cluster	~100 eV within ~2 nm (~4 ionizations)	DNA segment	~20–100	Characteristic of low-LET; ~repairable
3	Large cluster	~400 eV within 5–10 nm (~15 ionizations)	Nucleosome	~4–100	Characteristic of high-LET; ~unrepairable
4	Very large cluster	~800 eV within 5–10 nm (~30 ionizations)	(Nucleosome)	~0–30	Unique to high-LET; unrepairable; special relevance?

*The range of values is for different radiations, from low-LET X- and γ-rays to high-LET slow α-particles.

Track structure analysis: implications of clustered damage.

- High-LET effects are qualitatively and quantitatively **different** than low-LET.
- **All differences** between high- and low-LET radiations arise from track structures.
- Radiation produces a spectrum of damage of varying complexity.
- The complexity depends on the clustering of a track's ionizations in or very near to the DNA.
- Clustered damage occurs in DNA at sufficiently high frequency to be biologically significant.
- Some of the damage may be totally unrepairable.
- The spectrum of damage **after repair, or attempted repair**, is responsible for the permanent cellular effects.
- The **spectrum** of permanent damage may be **skewed** towards the more severe components of the initial damage.
- Assays that measure **initial** damage may be misleading.

So what kills cells?
The role of single and double strand breaks in cell killing.

How is sensitivity to killing by radiation related to DNA damage?

- Ionizing radiation produces a plethora of damages: including 1000 SSBs, 25-40 DSBs per cell per Gy.
- Variation in intrinsic radiosensitivity relates to the different repair capability of various cell lines.

Do assays to detect DNA damage measure the "important" damage?

- Sensitive methods now exist to measure SSBs, DSBs, initial yields, **and their rejoining** in individual cells, chromatin regions, or individual genes.

Misrejoining

- Unrejoined or misrejoined DSBs cause chromosome breaks or micronucleus formation.
- These lesions correlate with cell killing.

[Image removed due to copyright considerations]

- Measurement methodology is critical: sufficient time for the lesion to form, not too much time for the cell to die (apoptosis, or necrosis).

- Methodology has been developed to measure the initial yields of DSB, and the fractions rejoined correctly and incorrectly.

Complex lesions: a hallmark of ionizing radiation damage?

- Shown to be an important hallmark of radiation effects.
- More difficult to repair.
- More likely to lead to cell death, mutation or transformation.
- Complex damage is related to LET and the density of ion pairs produced at track ends.
- Explains observation that DSBs are more lethal for high-LET radiation.
- Illustrates that methods to measure DSBs cannot distinguish the "severity" of the DSB.

How does a cell define DNA damage?

- Radiosensitive cells may see both closely opposed and more widely spaced SSBs as DSBs.
- Radioresistant cells may repair the widely spaced lesions as two independent SSBs.
- The repair processes will be different, maybe the more complex repair of a DSB is more error-prone.

Does an assay define a DSB differently than a cell?

- An assay may "interpret" a DSB differently than a cell.
- Closely opposed versus widely spaced lesions handled differently in different assays.

DSB rejoining kinetics may hold clues to the relevant DNA damage.

- Rejoining of DSB shows both fast and slow components.
- The fast component may be: simple lesions that are more readily rejoined, more accessible lesions, or SSBs.
- Data suggest that initial rate is correct rejoining, the slower component is misrejoining.

Is residual damage the key?

- If unrejoined DSBs are responsible for cell killing, then residual damage should correlate with radiosensitivity.

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Clusters of DNA Damage Induced by Ionizing Radiation: Formation of Short DNA Fragments. I. Theoretical Modeling

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The 30-nm chromatin model

- A right-handed "super-solenoid" structure with 20 turns.
- Each turn is 11 nm long; the total length of the solenoid is 220 nm.
- One turn contains 6 helical nucleosomes.
- Each nucleosome consists of 171 base pairs of DNA wound in a left handed helix two full turns around a histone protein core: 146 bp wound around the histone and 25 bp as linker to the next nucleosome.
- The coordinates of all atoms in the DNA sugars and bases are known.

[Image removed due to copyright considerations]

One turn of the 30-nm chromatin fiber model is shown.

- The complete model contains ~ 20.7 kbp.

- Two hydration layers of water are included;
  - Tightly bound: 15 water molecules per nucleotide; participates in a charge transfer to the DNA. (charge transfer cross sections not known).
  - Loosely bound: 18 water molecules per nucleotide; serves as a source of •OH.

- Model includes only the second hydration layer in the water radiation chemistry.

Calculations:

The chromatin is situated in an aqueous environment. (It is impossible to accurately model the complete intracellular environment.)

A hydroxl radical scavenger is added to approximate the scavenging potential of the intracellular environment. The scavenger concentration is adjusted to give a mean •OH diffusion length of about 3 nm.

Charged particle tracks are positioned at random orientation to the chromatin model and at random distances from the chromatin.

- The energy deposition events are simulated,
- the generation of water radicals are followed,
- the water radicals are allowed to diffuse,
- each radical is followed until it either reacts with another radical, a protein, or the DNA.

Direct effects are scored when DNA sites on the chromatin fiber are close enough to be targets of direct energy deposition events.

Indirect effects are scored when a hydroxyl radical originating in a water molecule reaches the DNA.

- Model the creation of the radicals by energy deposition events in the track and in the delta rays.
- The mean amount of energy required to produce a hydroxyl radical is 17 eV.
- Up to 6 hydroxyl radicals can be created in glancing collisions (100 eV cutoff for the glancing collisions).
- Each radical is tracked through migrations in a series of random-walk jumps.
- Each •OH radical is tracked until it meets one of 4 fates:
    - Recombination with another radical
    - Scavenged by another molecule in the nucleus
    - Diffusion into a region of histone protein
    - Reaction with a DNA sugar or base
- Spherical reaction radii are defined for the DNA bases and the sugars

DNA damage is scored from direct and indirect effects.

DSBs are scored when SSBs occur on opposite strands within 10 bp of each other.

Between 10^4 and 10^7 tracks are scored for each particular energy and Z.

Track Structure

Two types of particle interactions are used: **glancing collisions** and **" knock-on"** or close collisions.

- Glancing collisions generate a core to the track; the radius of which is dependent on the particle **velocity**, but not the charge.
- 100 eV is selected as the cutoff between the glancing collisions and the close collisions.
- The close collisions are further divided as those involving energy transfer events of 100 ev – 2 keV, and those > 2 keV.
- Each delta ray is modeled explicitly as low-LET radiation acting independently of the original particle track.
- All interactions are tracked and DNA damage is scored.

Approximate scale: 0.01 µm

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Clustered Damage

Damage from three particles was simulated:

- 10 MeV/n helium: LET = 18.7 keV/µm
- 30 MeV/n nitrogen: LET = 93 keV/µm
- 10 MeV/n iron: LET = 2,470 keV/µm.

The iron particles show clear signs of significant damage clustering.

The intermediate LET nitrogen particle shows potential clustering.

The helium ion track shows a relatively sparse damage pattern.

Damage clustering depends to a large extent on the track ionization density, but also on the orientation of the track with respect to the chromatin.

[Image removed due to copyright considerations]

Mapping of the damage

- The entire linear sequence is known.
- Any sequence with a DSB (two SSBs within 10 bp) is examined for the presence of additional breaks, or base damage sites, nearby (i.e., clustering of the damage).
- The degree of damage clustering is evident when projected onto linear maps of the damage sites.

[Image removed due to copyright considerations]

Inspection of linear sequences on a larger scale to look for patterns of damage.

The relative proportion of direct vs. indirect damage events is relatively constant over a wide range of LET. All of these are considered high-LET.

Fraction of DSB's by Direct, Indirect and Combined Effect

	E/n	LET	direct	indirect	combined
He	10	18.7	.44	.31	.25
	3	48.9	.50	.30	.20
	1	107.	.55	.30	.15
Ne	1000	22.4	.42	.40	.18
	200	45.4	.41	.40	.19
	100	73.8	.43	.38	.19
	50	126.	.48	.31	.21

[Image removed due to copyright considerations]

Prediction of fragment length distribution patterns

- The entire sequence is known.
- The locations of all DSBs are known.
- Fragment lengths can be calculated and fragment length distributions predicted.

The prediction of fragments of ~80 bp and ~1000 bp was **new** and related to the structure of the chromatin model.

Interpreted as one turn around the histone and one turn around the 30-nm chromatin solenoid, respectively.

Extension of the fragment prediction to genomic DNA

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Prediction of fragment yields in the 100 bp to 2 kbp range.

Actual measurements show good agreement (Rydberg, 1996).

Technically difficult: must go to large numbers of cells, and a large dose.

Summary:

The simulation of track effects in the 30-nm chromatin model produced 3 previously unknown results.

- Prediction of small fragments with non-random fragment size distribution that reflects the structural features of the chromatin model.
    - Standard DSB assays do not measure small fragments.
    - If the smaller fragments are unaccounted for, RBEs for initial DSB will be ***underestimated***.

- The model agrees with the general concept of clustered damage ("locally multiply damaged sites") and shows various degrees of clustering up to 40 bp.

- Multiple local clusters are predicted over extended regions of up to several kbp. New term coined to describe this observation: **"regionally multiply damaged sites"**.
    - Fragments in the intermediate size range were not modeled but could result from higher order chromatin structure, i.e., loops attached to the nuclear matrix.

Biological implications

- If small fragments are lost, DNA information will be compromised.
- Clustered damage is probably harder to repair, and a more relevant metric of biological effects.

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Clusters of DNA Damage Induced by Ionizing Radiation: Formation of Short DNA Fragments. II. Experimental Detection

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Objective: development of methods to test the Holley and Chatterjee predictions of non-random generation of small DSB fragments.

Approach:

- Label cells (GM38 primary human fibroblasts) with [^{3}H]thymidine 4 days before the experiment. Change to fresh, unlabeled medium 24 hrs before the experiment.
- Harvest cells when close to confluency, i.e., > 90% are in the G_0 stage of the cell cycle.
- Embed labeled cells in agarose plugs.
- Expose plugs to x rays or high-LET radiation at 0º (or 4ºC).
- Lyse the cells in the agarose plugs.
- Gel electrophoresis under conditions designed to separate fragments in the 0.1-2 kb size range.
- Electrophoresis under neutral conditions to measure DSBs, and under alkaline conditions to measure SSBs.
- Correlate the counts with fragment size, express as fragment size distribution.

[Image removed due to copyright considerations]

X rays

DNA size markers included in the gel are indicated by the arrows.

The gel was sliced and counted.

Increase in short fragments as a function of dose was linear, and greater than expected from random breakage.

Sensitivity of the method limited by counts per 3 x 10^6 cells in these small fragments. Count rates are about 2 x background.

Total counts in small fragments ~ 1 x 10^{-4} of the total radioactivity in the DNA.

The peak at 0.5 kbp is of unknown origin and was *present in the unirradiated cells.*

High-LET particles

The total yield of fragments is greater than for x rays.

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(a) Primary data is fragment weight distribution. This is proportional to both the number of fragments and the span of sizes in the gel slice.
Weight distributions were converted to number distributions.

(b) Theoretical distribution of fragments from 30 MeV/n nitrogen (from Holley and Chatterjee. 1996).

The measured and theoretical distribution patterns **appear different**.

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Total yield of fragments shows good agreement between measurements and theory.

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JMB

Chromatin Conformation in Living Cells: Support for a Zig-Zag Model of the 30 nm Chromatin Fiber

Björn Rydberg, William R. Holley, I. Saira Mian and Aloke Chatterjee*

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“Zigzag” model of DNA winding on the nucleosomes in the chromatin fiber produces a much better fit between theory and experiment.

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Heavy charged particle track interacting with the 30 nm solenoid fiber. Damaged sugars and bases represented by pink and yellow spheres, respectively.

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A Model for Interphase Chromosomes and Evaluation of Radiation-Induced Aberrations

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