Effects of Radiation on DNA

Irradiation of cells causes:

- cell killing
- mutagenesis

Understanding the processes between energy deposition and expression of damage may allow for manipulation of the process to e.g.,

- increase the damage to a tumor
- decrease the damage to normal tissues.

A. What is the evidence that DNA is the target?

1. Selectively irradiate the nucleus or the cytoplasm. Results show the nucleus to be more sensitive than the cytoplasm.

To kill a cell the dose to the nucleus is 100 times smaller than the dose to the cytoplasm.

- Polonium needle: alpha particle range ~ 40μm.

[Image removed due to copyright considerations]

- Microbeams capable of delivering particles (protons or alpha particles) with μm resolution.

2. Incorporation of halogenated base analogues into DNA sensitizes cells. Iodine or bromine

[Image removed due to copyright considerations]

[Tubiana, 1990]

Spatially, the halogens are similar to the methyl group, thus “errors” are possible anad incorporation into the DNA strand.

Incorporation makes the DNA more susceptible to damage, including radiation damage.

3. Radioisotopes incorporated into DNA kill cells much more efficiently than radioisotopes in RNA or in proteins. ^{125}I in the DNA is 200-300 times more effective than ^{125}I in the cytoplasm or on the cell membrane.

4. Tritium is particularly useful: emits beta particles of 18 keV, range in tissue is less than 1-2 µm.
    - Tritiated thymidine….labels DNA
    - Tritiated uracil ….labels RNA
    - Tritiated amino acids to label proteins
    - Tritiated water (uniform distribution) is 1000 times less effective than tritiated thymidine (DNA localization).

- [^{3}H]Thd incorporation caused chromosome breaks, correlating with the point of attachment visualized by autoradiography.

5. Correlation between cellular DNA content or chromosome volume and radiosensitivity, particularly in simpler organisms such as viruses.

[Image removed due to copyright considerations]

[Tubiana, 1990]

6. Cells deficient in DNA repair enzymes are generally more radiosensitive. Drugs that inhibit DNA repair are sensitizers.

B. Types of DNA Damage

Radiation can produce a variety of lesions in DNA

- Rupture of the strand
- Alteration to bases
- Destruction of sugars
- Crosslinks and formation of dimers

But what about the background levels of DNA damage that occur every day from “natural” sources (primarily oxygen)?

We have evolved sophisticated DNA damage detection and repair mechanisms to deal with this damage.

Does radiation produce unique damage?

Clustered Damage

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Radiation damage to DNA

DNA Strand Breaks

Single strand breaks:

- Can take place at the phosphodiester bond, or at the bond between the base and the sugar.
- A large proportion of the single strand breaks are caused by hydroxyl radicals (OH•). Radical scavenging experiments have demonstrated this.

[Image removed due to copyright considerations]

[Roots, 1972]

[Image removed due to copyright considerations]

[Tubiana, 1990]

Double strand breaks:

- Involves breakage of both strands at points less than 3 nucleotides apart (there are still questions about whether further spacings are recognized and repaired as dsbs).
- Production by single particle crossing both strands?
- Production by two independent events?
- Can be measured by various techniques (e.g., sucrose gradient centrifugation)
- Double strand breaks have shown a direct proportionality to radiation dose.

[Image removed due to copyright considerations]

[Prise, 1994]

** X-ray dose of ~1 Gy produces about 1000 single strand breaks and about 50-100 double strand breaks in a typical mammalian cell.
This dose causes about 50% cell death.
DSBs are not necessarily lethal.

Base changes:
Bases can be damaged or destroyed or chemically modified by radiation.
Hydroxyl radical and byproducts can add to bases.
Pyrimidines (T, C) more sensitive than purines.

The biological significance of base damage is less than that of strand damage.

TABLE IV
YIELDS OF DNA DAMAGE NECESSARY TO KILL 63% OF THE CELLS EXPOSED[a]

Agent	DNA lesion	Number of lesions per cell per D_{37}[b]	Reference
Ionizing radiation	ssB	1000	*17*
	dsB	40	*17*
	Total LMDS[c]	440	*68*
	DPC[d]	150	*20*
Bleomycin A2	ssB	150	*69, 70*
	dsB	30	
UV light	T<>T dimer	400,000	*71–73*
	ssB	100	
Hydrogen peroxide			
0°C	ssB	<2,600,000	*64*
37°C	?		
Benzo[*a*]pyrene 4,5-oxide	Adduct	100,000	*74*
Aflatoxin	Adduct	10,000	*75*
1-Nitropyrene	Adduct	400,000	*76*
Methylnitrosourea	7-Methylguanine	800,000[e]	*77*
	O^6-Methylguanine	130,000[e]	
	3-Methyladenine	30,000[e]	
2-(*N*-Acetoxy-*N*-acetyl)amino-fluorene	Adduct	700,000	*74*
Other similar aromatic amides produce about the same number of adducts per lethal event			

[a] This table is reproduced with permission from J. F. Ward, C. L. Limoli, P. Calabro-Jones and J. W. Evans, Radiation vs. chemical damage to DNA. *In* "Anticarcinogenesis and Radiation Protection" (O. F. Nygaard and P. Cerutti, eds.). Plenum, New York (1988).
[b] D_{37} = dose of agent required to reduce survival of cells to 37% of the number exposed.
[c] Calculated; LMDS = locally multiply damaged sites (see below).
[d] DPC = DNA–protein cross-links.
[e] D_{37} calculated from individual exposures; no survival curves available.

TABLE III
MEASURED NUMBERS OF DAMAGED SITES PER CELL PER GRAY

Type	Yield	Reference
Single-strand breaks	1000	*17*
8-Hydroxyadenine	700	*18*
T* (thymine damage)	250	*19*
Double-strand breaks	40	*17*
DNA–protein cross-links	150	*20*

D. Techniques for measuring DNA damage

Base damage: various techniques exist for measuring the release of bases, or damaged base fragments: e.g., HPLC, GC-MS, ^{3}H release from previously incorporated ^{3}H-thymidine, immunological probes.

[Image removed due to copyright considerations]

DNA strand breaks:

- Many of these techniques can measure either ssb or dsb by manipulation of the pH.
- High pH (alkaline conditions) will denature DNA (separate the two strands).
- Neutral conditions: double strands remain intact.

Sucrose gradient sedimentation:

- cells carefully lysed on top of sucrose gradient,
- centrifuged at high speed,
- the larger fragments will migrate further into the gradient

[Image removed due to copyright considerations]

[Tubiana, 1990]

Filter elution:

- DNA prelabeled by growing cells in radioactive DNA precursors (^{3}H or ^{14}C thymidine). Cells lysed gently on the filter.
- Elution through pores in a filter using a pump and fraction collector.
- The amount of DNA eluted as a function of time is proportional to radiation damage.
- Larger fragments elute more slowly.
- Neutral conditions or alkaline conditions can be used.

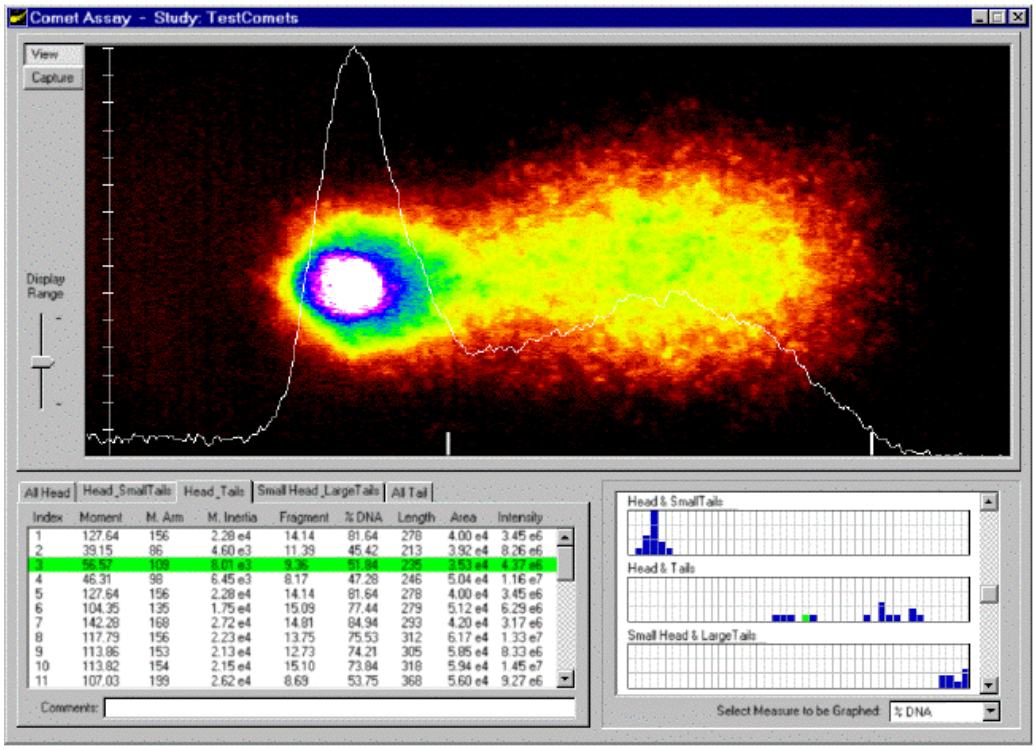
Gel electrophoresis:

- Separation of DNA fragments according to size (and shape) in a gel of acrylamide or agarose when exposed to an applied electric field.
- Cannot resolve very large fragments.
- A variation known as pulsed field gel electrophoresis can resolve much larger fragments. The electric field is pulsed and alternated in orientation.
- Methods to quantitate the amount of DNA migrating out of the well either radiolabeling or fluorescence (expressed as fraction of activity released, FAR).

[Image removed due to copyright considerations]

Comet assay:

- Single-cell gel electrophoresis.
- Cells embedded in gel, lysed to remove proteins, then subjected to electric field.
- Smaller DNA fragments migrate further making a “tail” that can be stained and viewed under a fluorescent microscope.



E. DNA repair

Repair of damage to DNA is of central importance to all cells, and is an ongoing process.

Existing repair mechanisms eliminate most radiation-induced lesions.

- Restores viability
- But viable cells may still harbor mutations or chromosomal aberrations

Distinguish repair as

- Error-free: restores DNA to its original state
- Misrepair: non-lethal errors are incorporated and passed on to daughter cells. This could lead to genomic instability and carcinogenesis.

DNA repair is governed by a multitude of genes, and executed by DNA repair enzymes.

Mutants deficient in DNA repair genes have helped elucidate these complex systems and their control.

Question: are there radiation-induced lesions that are unique? Lesions that the sophisticated repair system that has evolved to handle oxidative damage cannot handle?

Example of DNA repair.

Excision-repair:

- Principle mechanism of repair
- Operates on single strand breaks, or damage.
- Stepwise repair involves at least four different repair enzymes.
    - Recognition of the presence of a lesion
    - Excision of the damaged area
    - Resynthesis by **copying** from the adjacent strand
    - Resealing the break

[Image removed due to copyright considerations]

[Tubiana, 1990]

Repair of double strand breaks:

- Mechanisms not clear.
- Repair by recombination with the homologous strand is possible.
- Large (complex) lesions may not be repaired correctly
- Some double strand breaks may not be repairable at all.
- Misrepair or deletions may lead to chromosome aberrations, mutations or carcinogenesis.

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So What Kills Cells?

Chromosome aberrations correlate with dose.

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[Sachs, 1993]

High LET radiation is more effective than low-LET for production of aberrations.

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Chromosome aberrations correlate with cell death.

[Hall, 2000]

Cornforth: observed individual cells following irradiation.
If a micronucleus was present in the daughter cell, no colony was formed.

N.B., Aberrations present at mitosis, may be far removed from initial damage.
Repair, cell cycle, changes in chromatin structure all can affect the results.

Chromosome aberrations also show the LET dependency with a maximum at about 100 keV/μm. At high LET, the damage could be too severe for survival.

[Image removed due to copyright considerations]

Chromosome aberrations require a dsb.

The fact that the dsb do not correlate with cell killing is troubling.

Recent work indicates that *clustered damage* is involved.