Which DNA Damage Is Likely To Be Relevant In Hormetic Responses?

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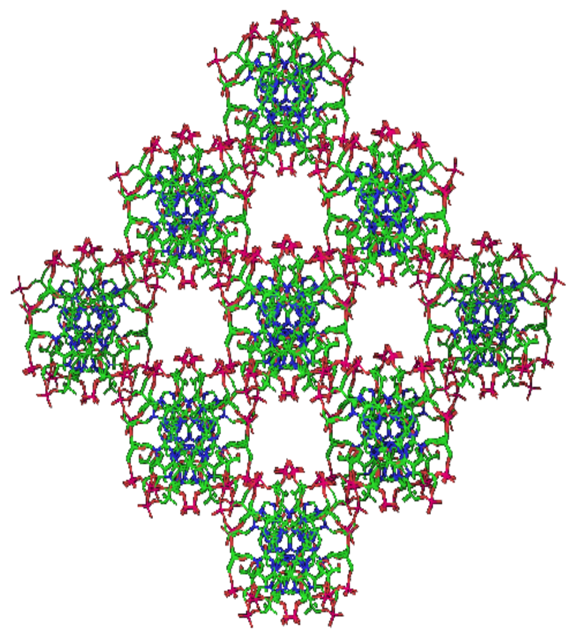


Supported by National Cancer Institute of NIH

Outline

1. Ionizing radiation triggers a cellular response by damaging DNA
2. The high toxicity of radiation damage is directly related to formation of damage in clusters
3. Damage produced by the direct effect is comparable to that produced by the indirect effect
4. Primary lesions produced by the direct effect
5. Yields of clustered damage by the direct effect
6. Predicted threshold for a biological response

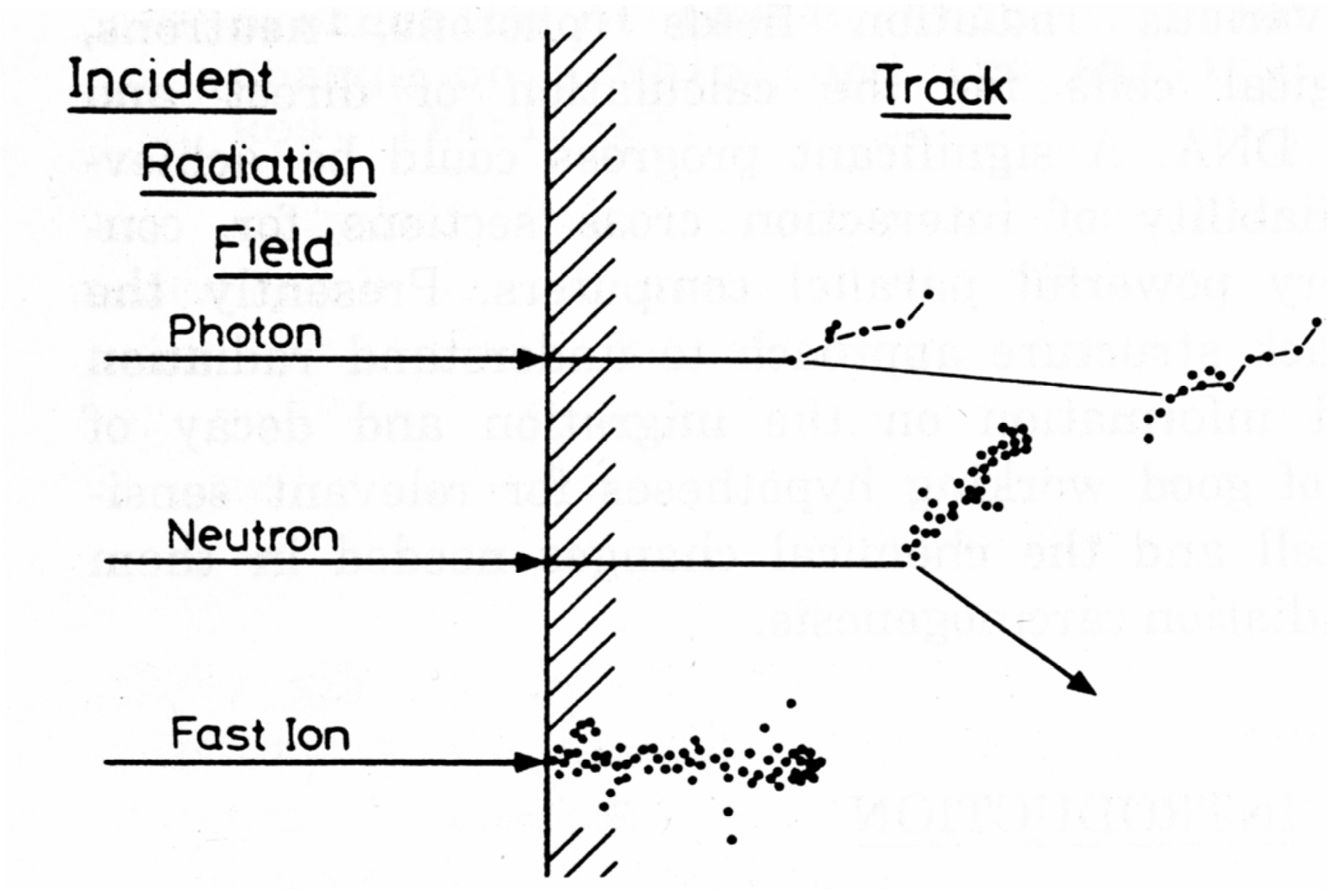
Damage produced by ionizing radiation is different

- Endogenous – 10^8 DNA damages per cell per year

- 10 mGy of radiation (~4x background) – ~2 DNA damages per cell per year

- Since 1000x background is lethal, there is something about radiation damage that makes it highly toxic.

Non-homogeneous energy deposition produces a TRACK

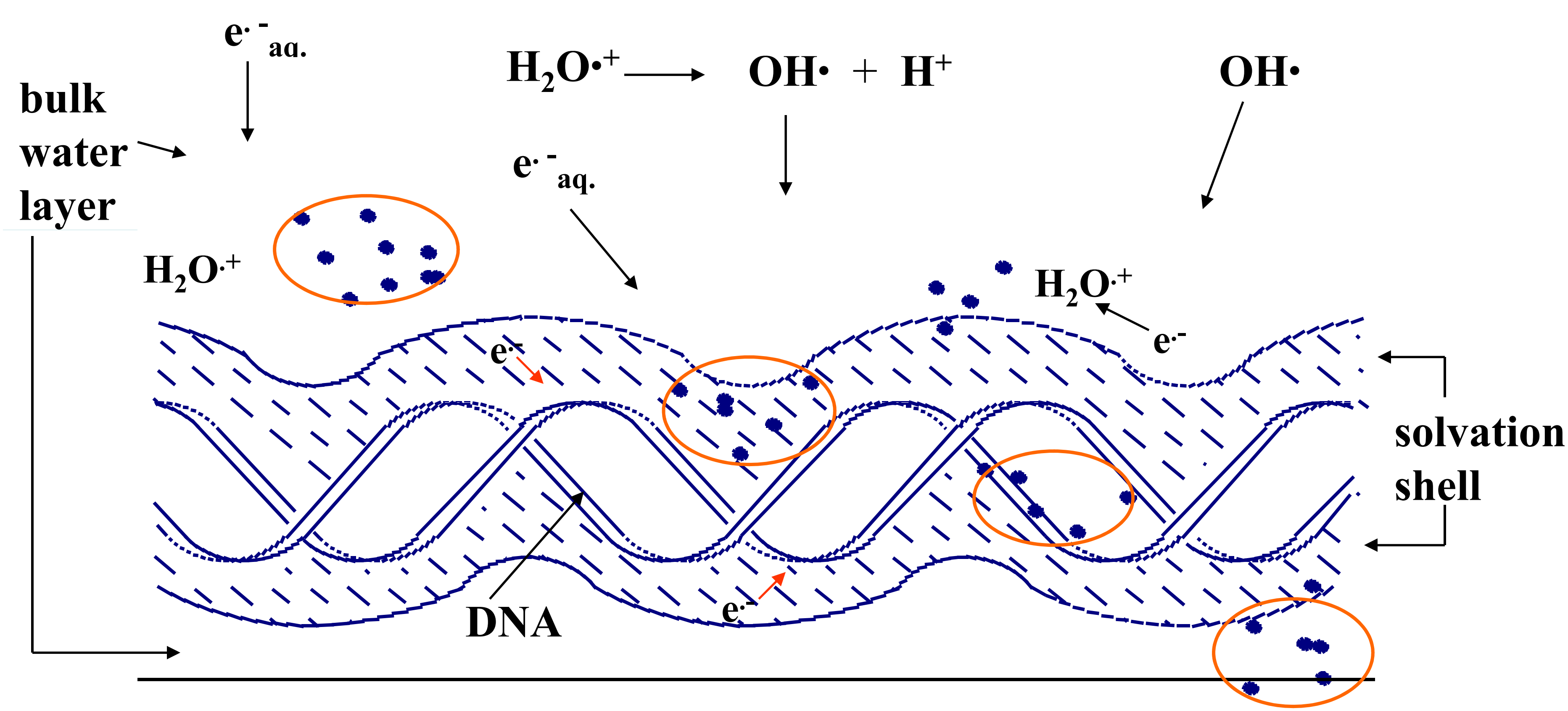


DNA damage is traditionally grouped into two categories

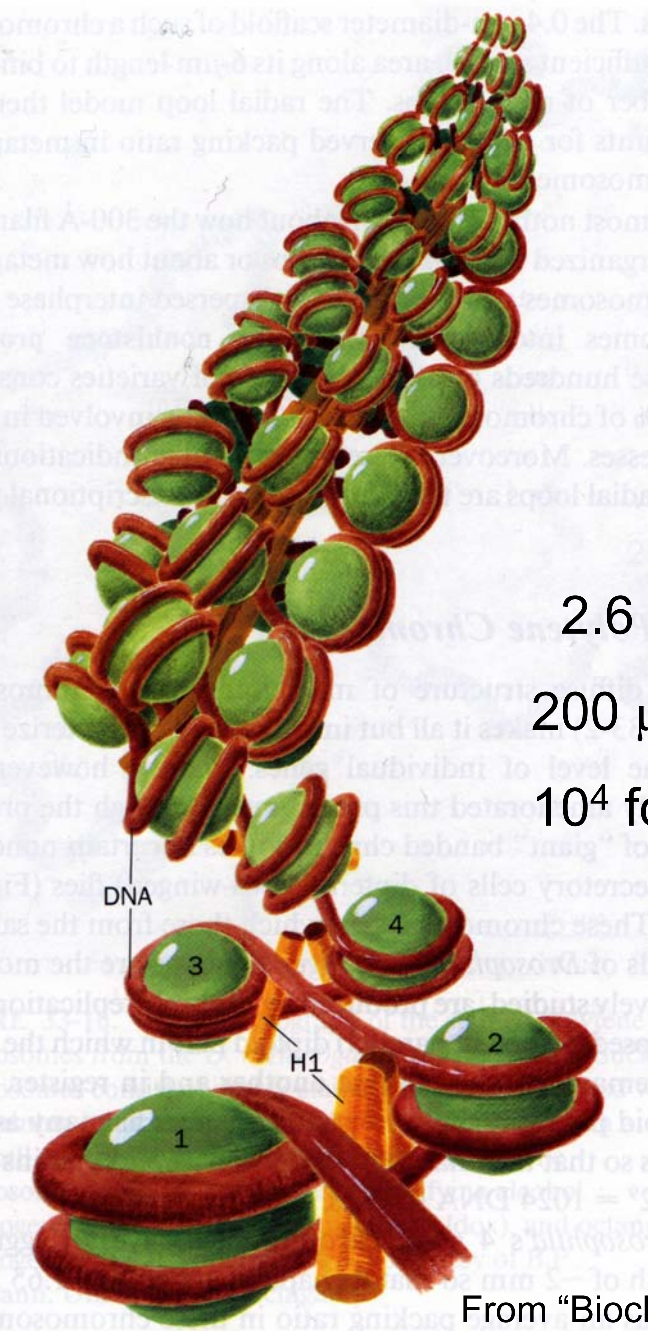
- **"Direct effect"** damage, due to the direct ionization of DNA or from the transfer of dry electrons and holes to the DNA from the hydration waters surrounding the DNA.

- **"Indirect effect"** damage due to the attack of bulk water radicals ($e^{-}_{aq.}$, $H^{\cdot}$ and $HO^{\cdot}$) on DNA.

DNA in aqueous solution



The DNA solvation shell consists of up to ~ 20 to 22 waters/nucleotide.



From "Biochemistry", Voet & Voet

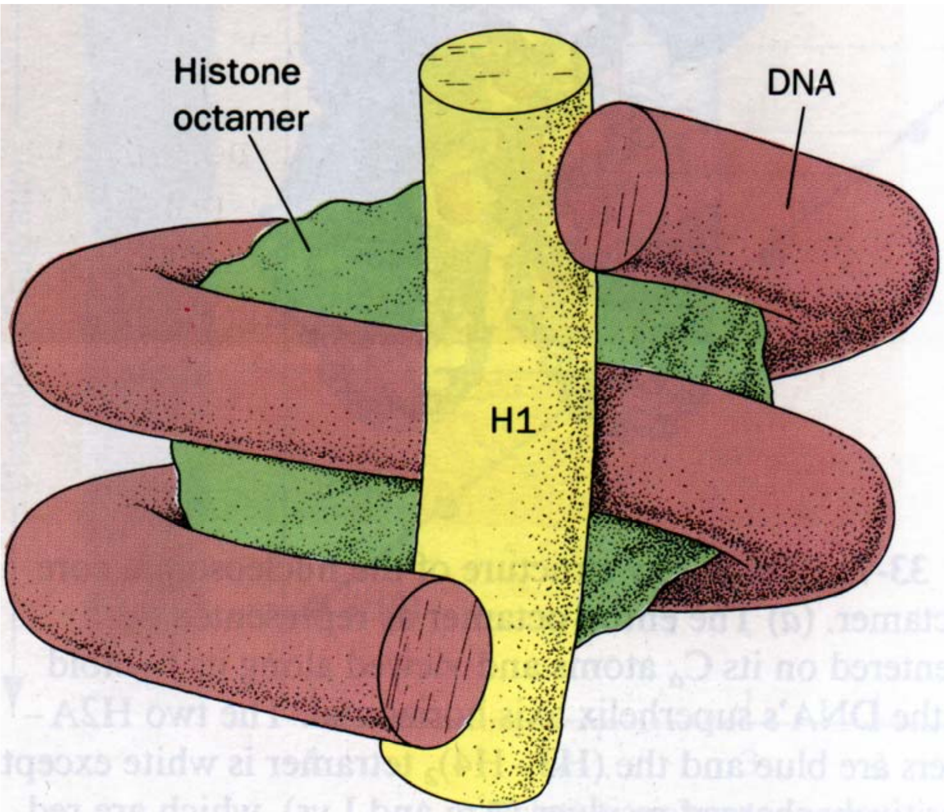
What is the relative importance of the direct and indirect effects

2.6 m = length of DNA in 46 human chromosomes

200 μm = length of 46 chromosomes in metaphase

10^4 fold = compaction due to DNA packaging

Direct vs. Indirect DNA Damage



From “Biochemistry” , Voet & Voet

30% (DNA + its solvation shell)

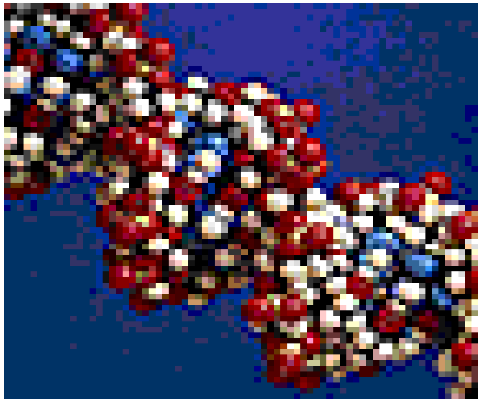
30% (Protein + its solvation shell)

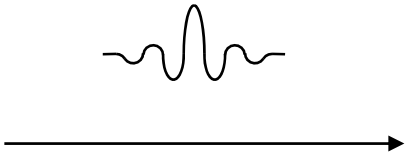
40 % free water

Based on partitioning of energy deposition by mass ratios one predicts

~60% direct damage
~40 % indirect damage

Research Objective



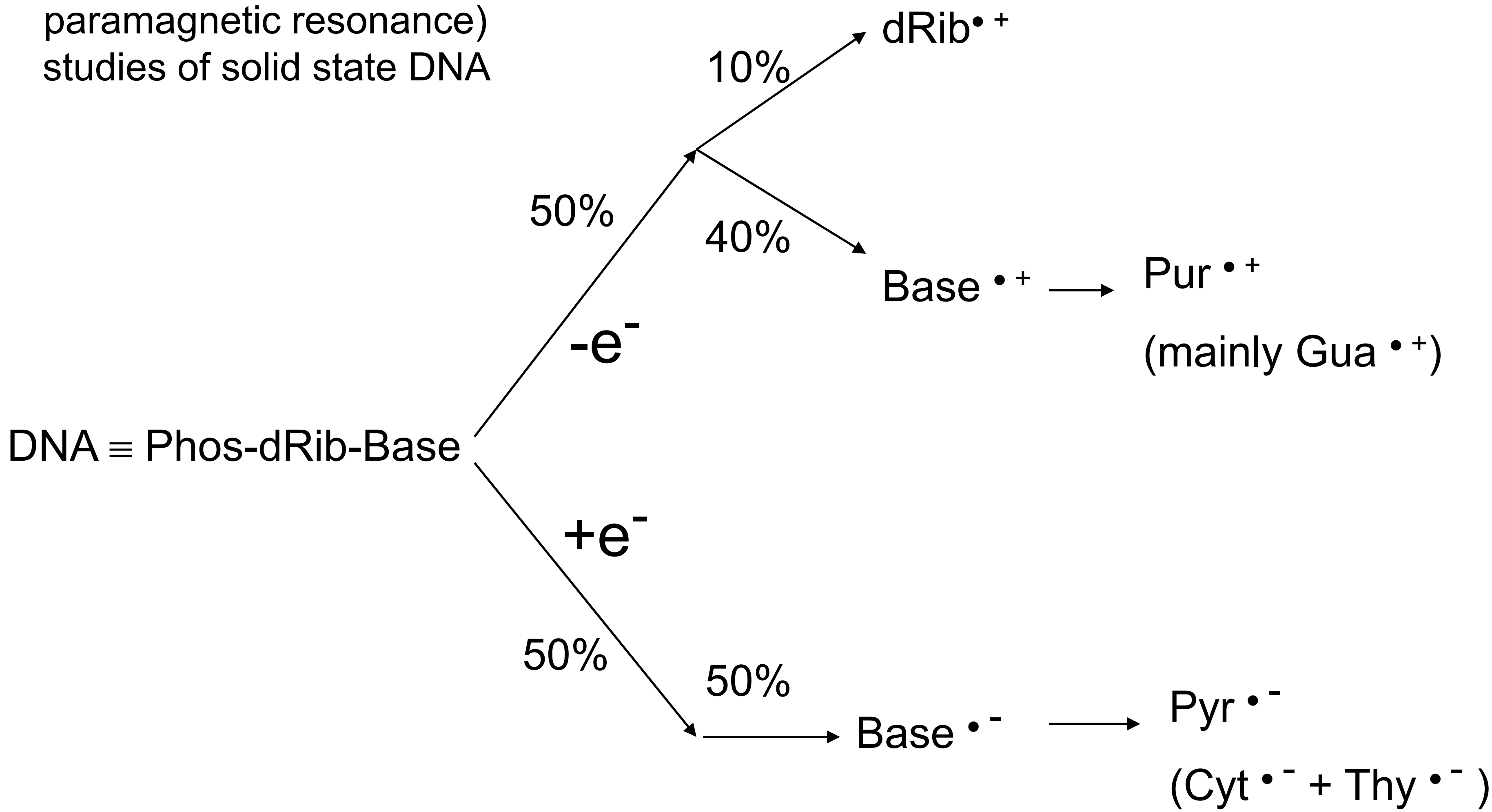


DAMAGE

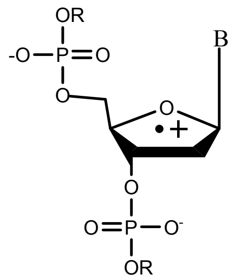
- Type
- Yield
- Spatial distribution

Model – Initial Radical Ion Distribution

From **EPR** (electron paramagnetic resonance) studies of solid state DNA

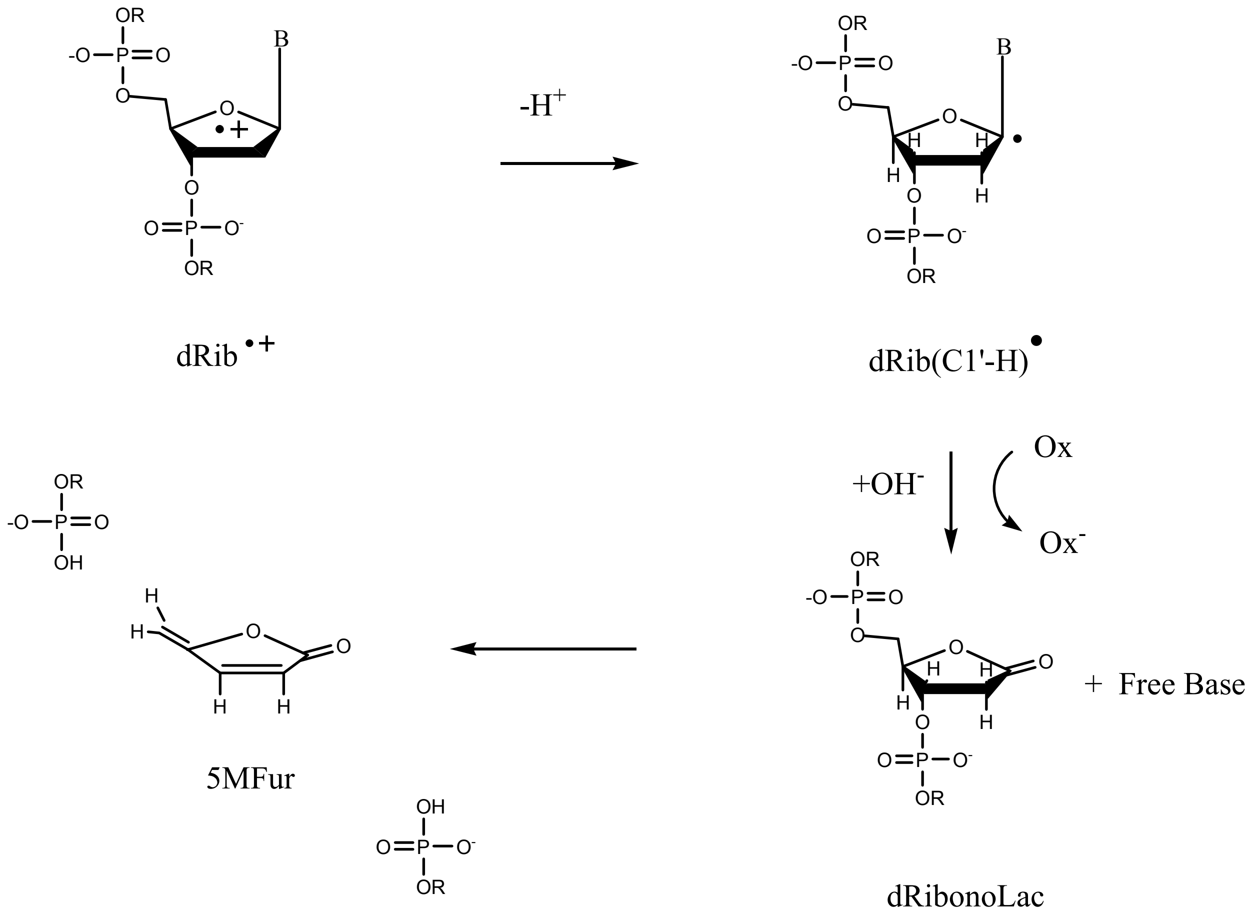


Deoxyribose-phosphate Oxidation mechanism for single strand break formation



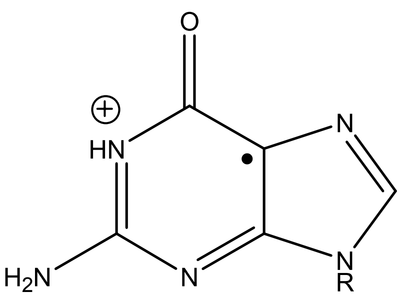
dRib $^{\bullet+}$

Deoxyribose-phosphate Oxidation mechanism for single strand break formation



dRib•+ · dRib(C1'-H)• · dRibonoLac · 5MFur

Guanine Oxidation
mechanism for 8oxoGua formation



Gua $^{\bullet +}$

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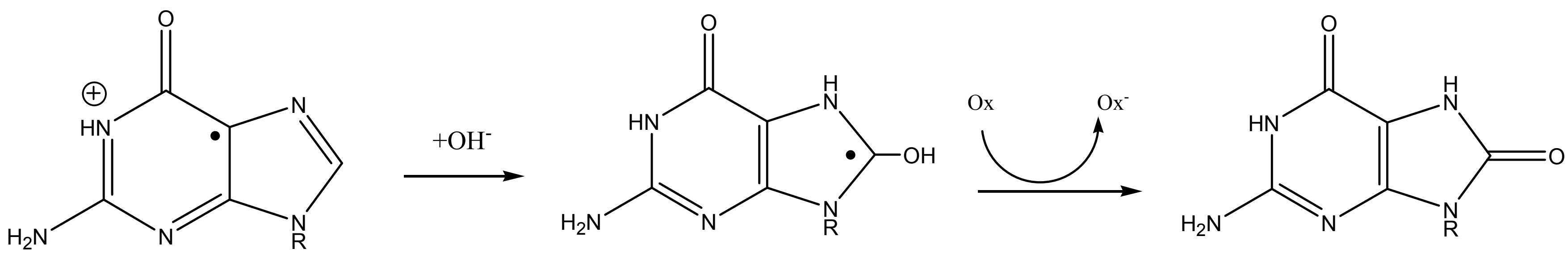
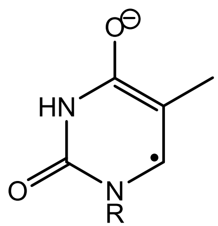


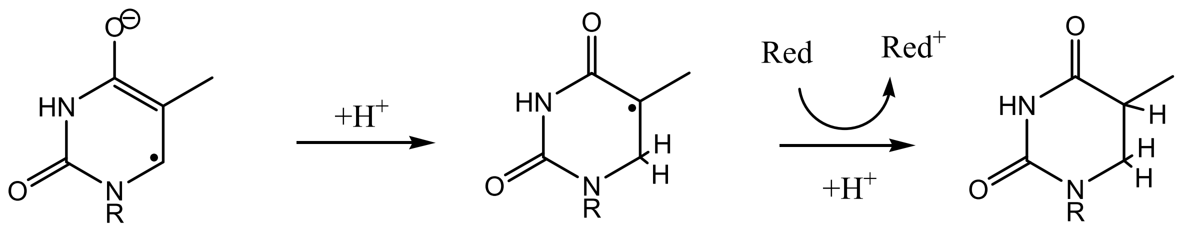
Gua •+   Gua(C8+OH)•   8OxoGua

Thymine Reduction
mechanism for dihydrothymine formation



Thy•−

Thymine Reduction
mechanism for dihydrothymine formation



$Thy^{\bullet -}$ $Thy(C6+H)^{\bullet}$ DHThy

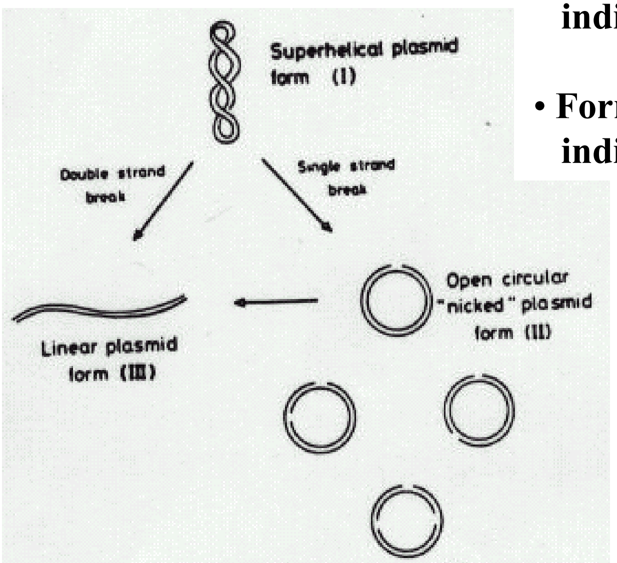
Similarly for cytosine

$$Cyt^{\bullet -} \xrightarrow{+H^+} Cyt(C5+H)^{\bullet} \xrightarrow[+H^+]{Red \to Red^+} DHCyt \xrightarrow[-NH_3]{+H_2O} DHUra$$

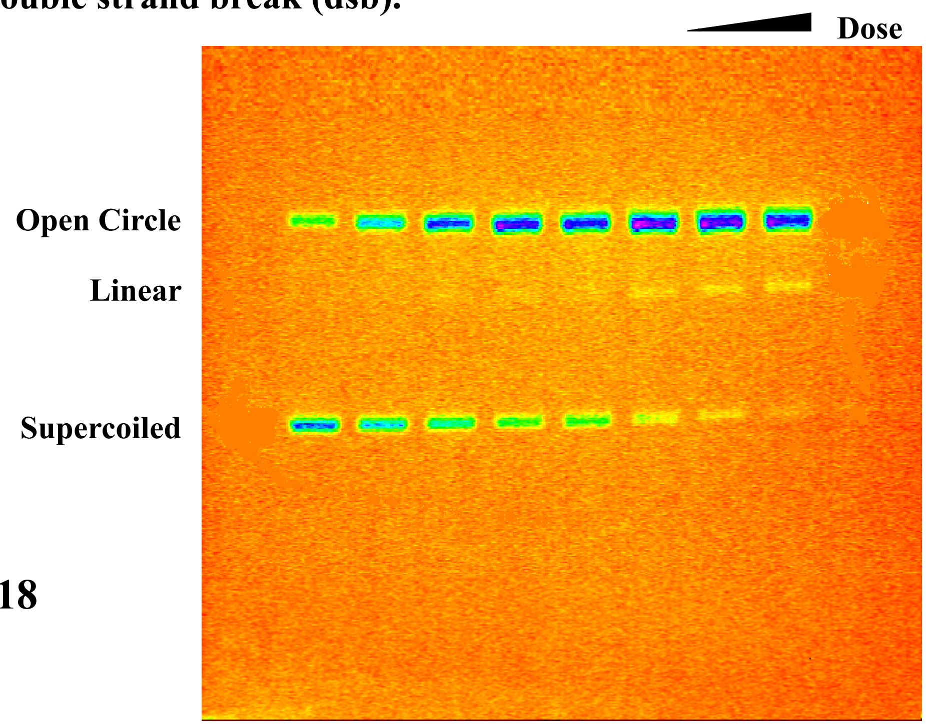
Measurement of Clustered Damage in DNA

- Films were prepared from pUC18 plasmids hydrated to Γ = 2.5, 7.5, 11.5, 15, and 22.5 waters/nucleotide.
- X-irradiated at 0.3-2.9 kGy/min (70 kV, tungsten target)
- Strand break yields were measured by agarose gel electrophoresis at 4 ^{0}C.

Quantification of strand break yields in X-irradiated plasmid using agarose gel electrophoresis.

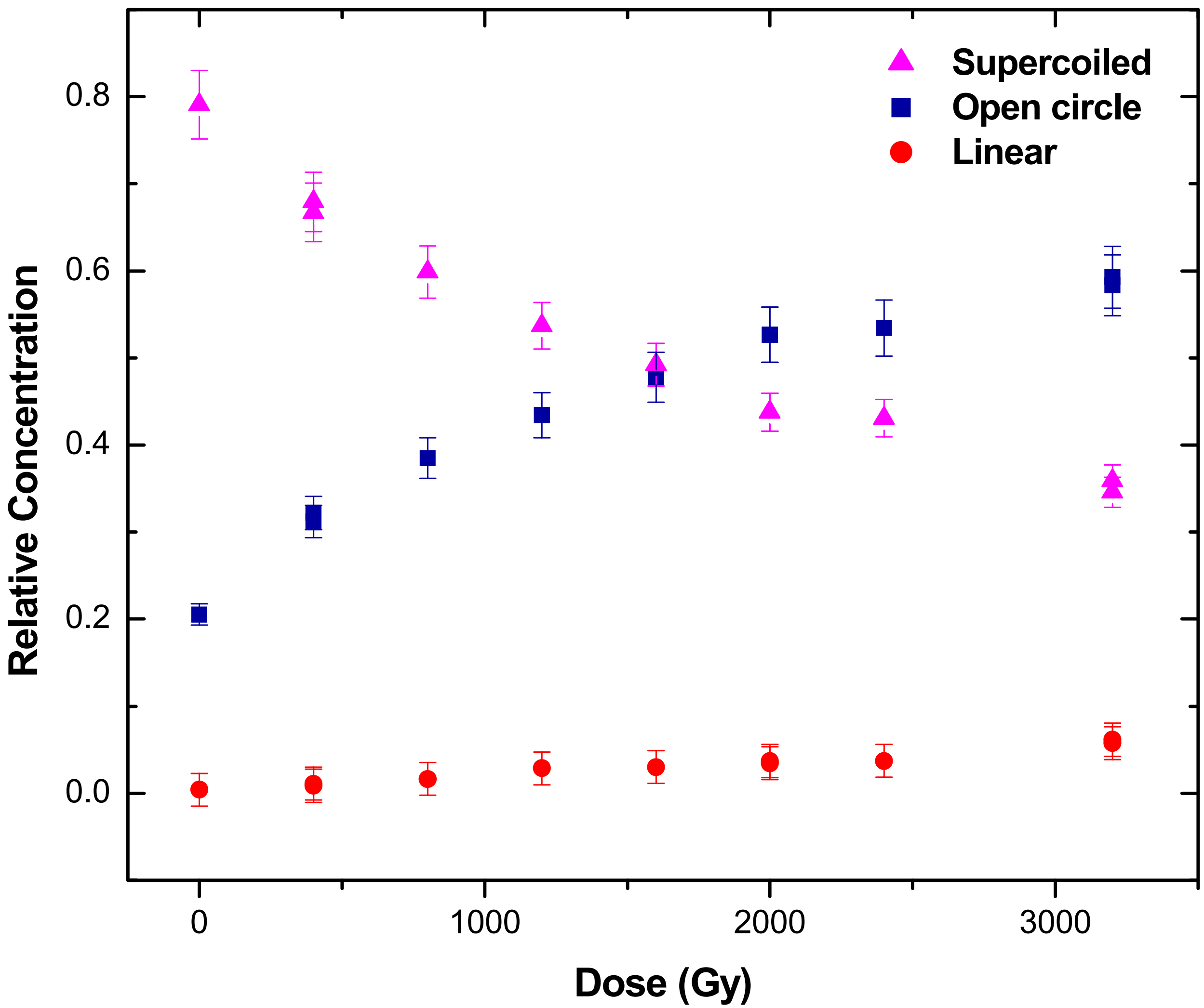


- **Form I – Supercoiled**
- **Form II – Open Circle**
  indicates a single strand break (ssb).
- **Form III - Linear**
  indicates a double strand break (dsb).

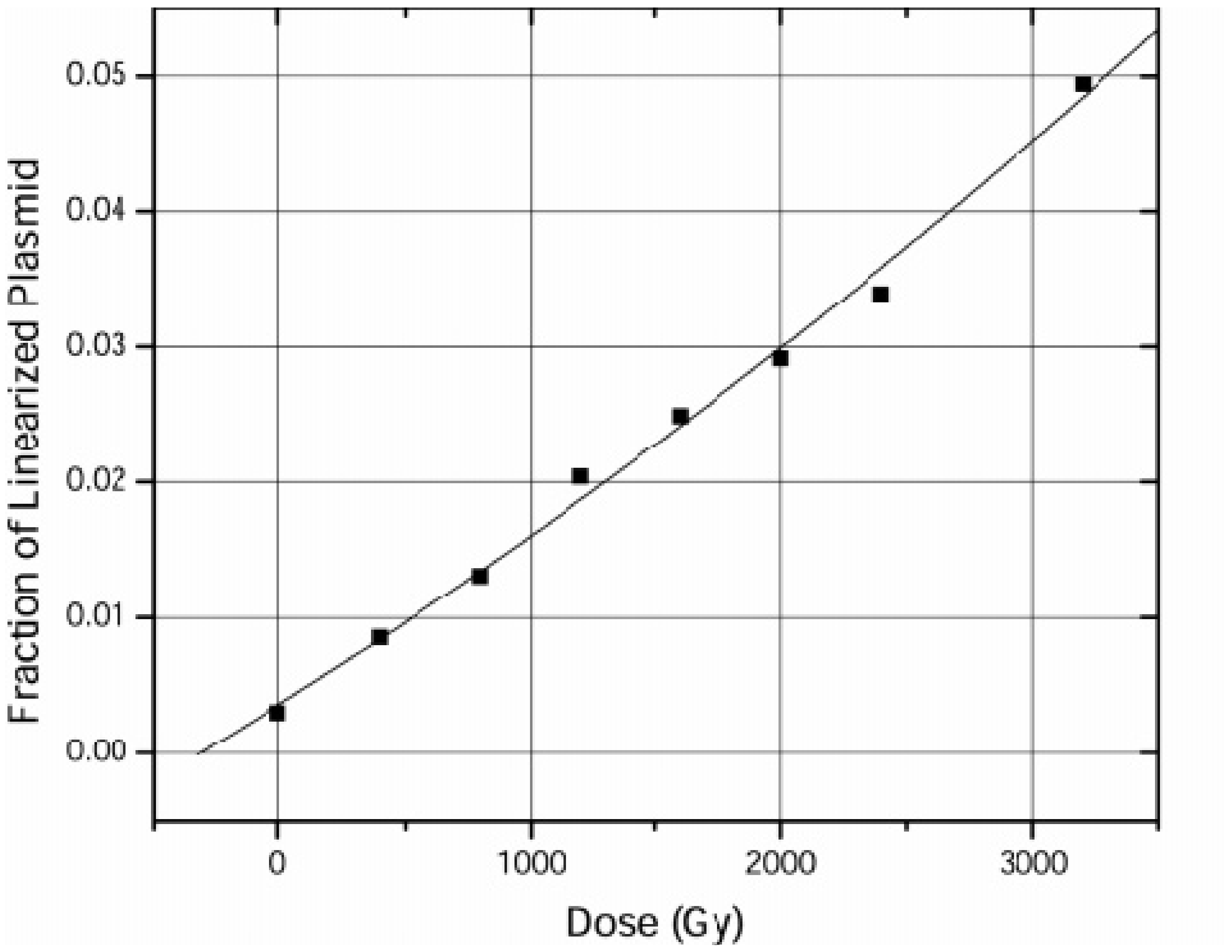


Gel image of x-irradiated pUC18 as a function of dose, 0-3300Gy.

Dose Response for Loss of Supercoiled and Formation of Open Circle and Linear Plasmid



Formation of linearized pUC18 DNA



Three types of clusters were measured

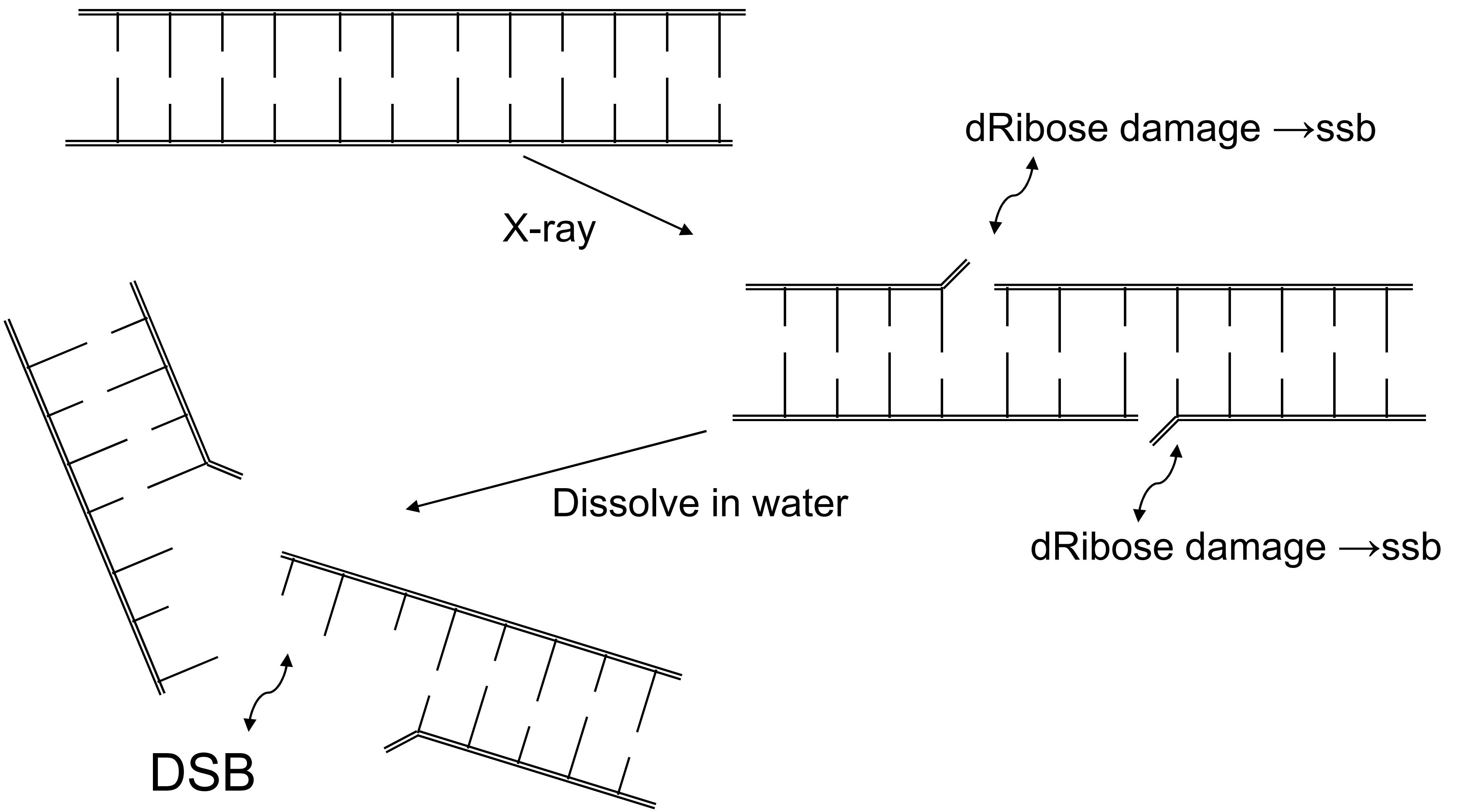
1. Containing dRibose damage on opposing strands (within ~10 bp of each other)

2. Containing DHPyr on one strand and DHPyr or dRibose damage on the opposing strand

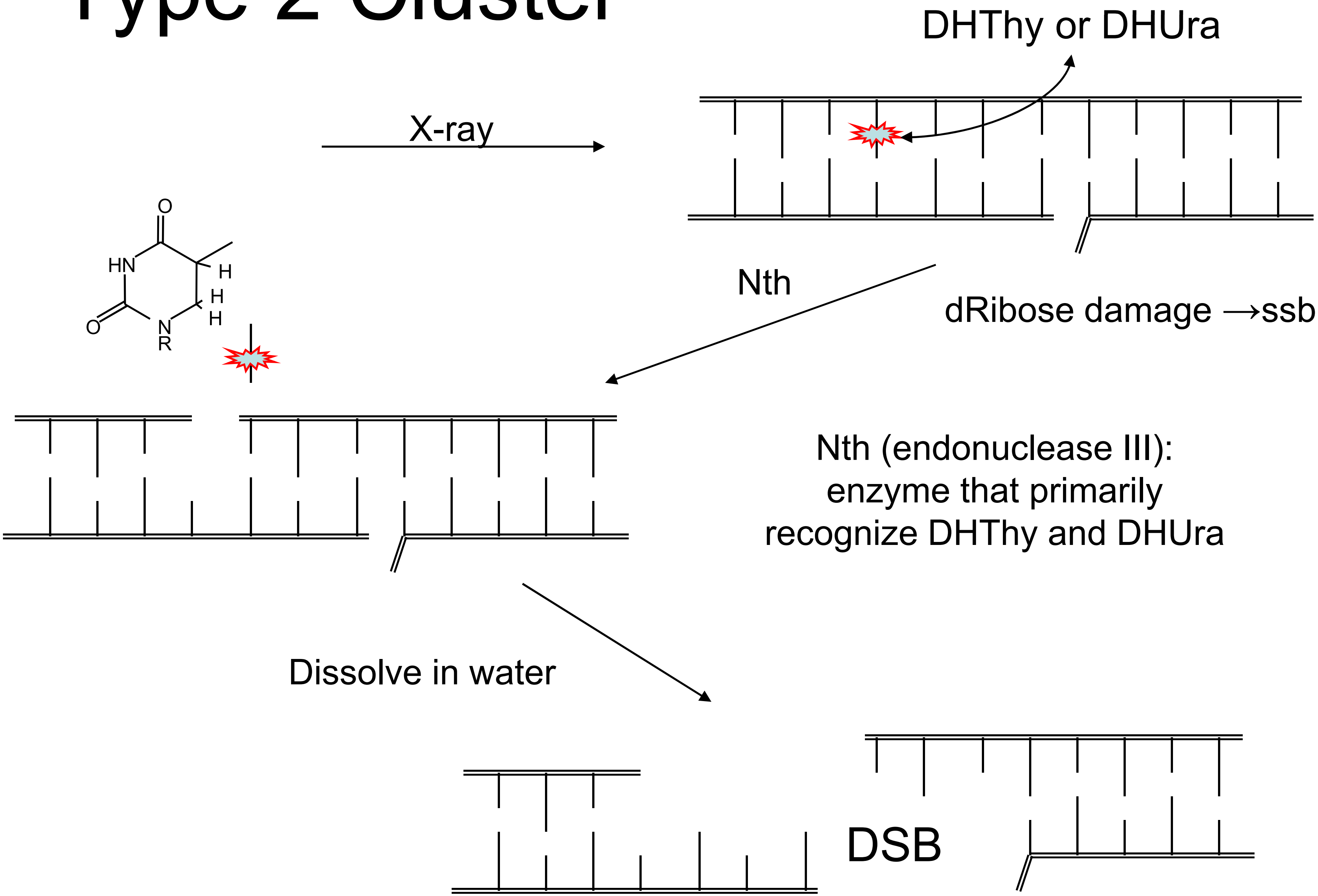
3. Containing 8-oxoGua on one strand and 8-oxoGua or dRibose damage on the opposing strand

Base damage is revealed using base excision repair enzymes

Type 1 Cluster

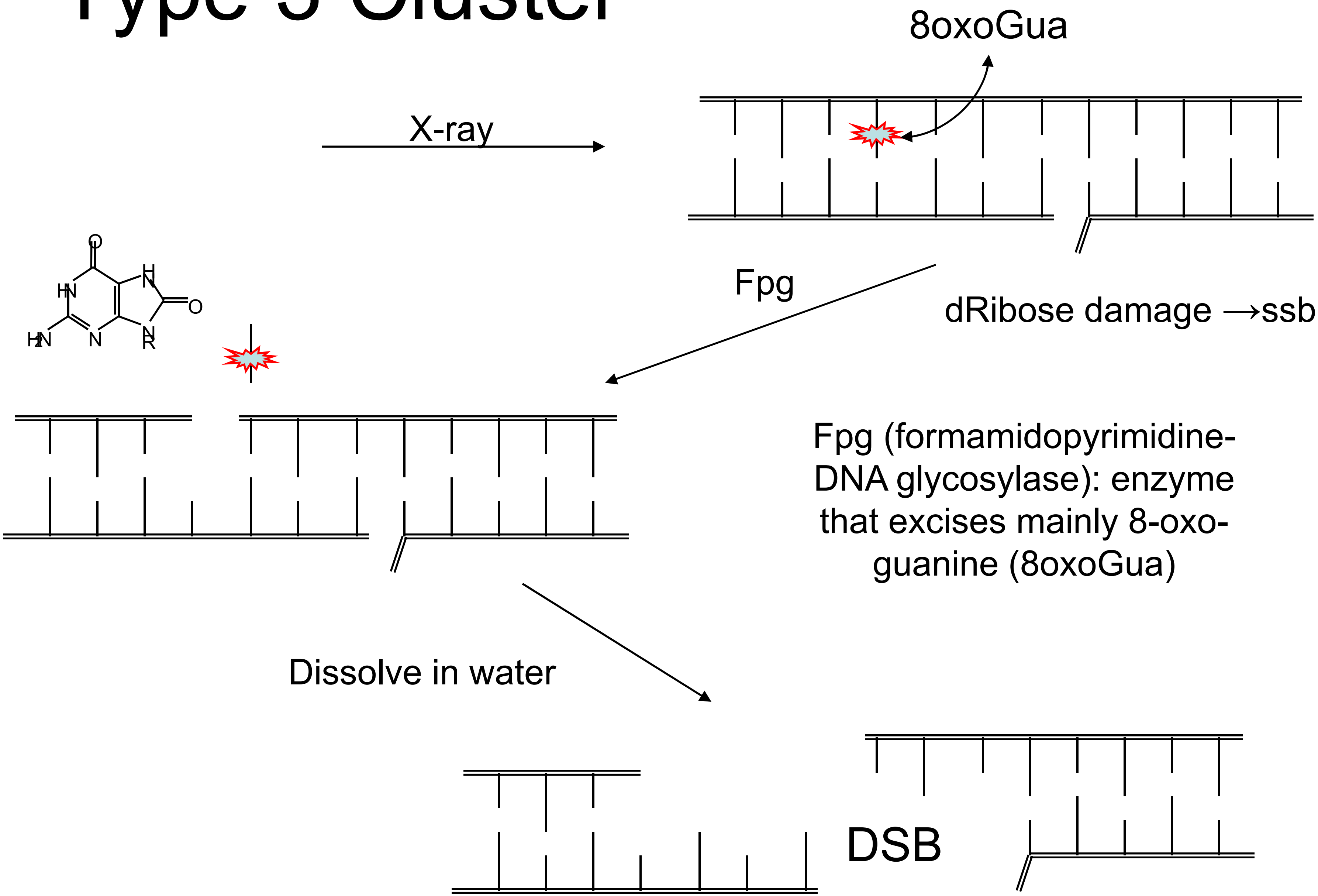


Type 2 Cluster



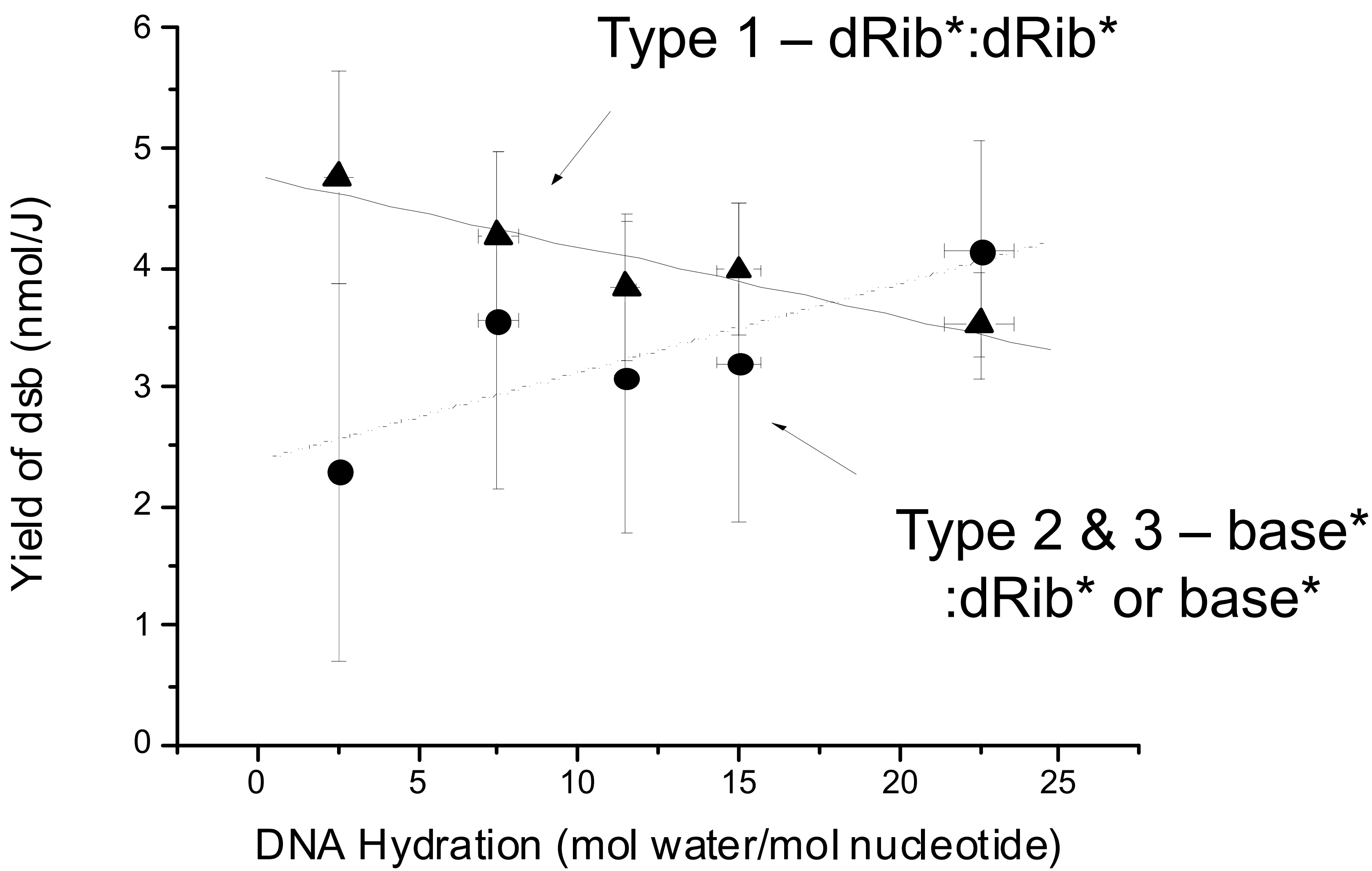
Nth (endonuclease III):
enzyme that primarily
recognize DHThy and DHUra

Type 3 Cluster



Fpg (formamidopyrimidine-DNA glycosylase): enzyme that excises mainly 8-oxo-guanine (8oxoGua)

Chemical Yields of Clusters Leading to DSB



Yields of clustered damage in films of pUC18 DNA

Type 1: G'_{noEnz}(dsb)	3.5 ± 0.5 nmol/J
Type 2: G'_{Nth}(dsb)	2.3 ±0.9 nmol/J
Type 3: G'_{Fpg}(dsb)	3.2 ±0.9 nmol/J

Total clustered damage :

direct effect only	~9 nmol/J
direct + indirect	~20 nmol/J

S. Purkayastha, et al.,
Radiat. Res., in press

Predicted Threshold for a Biological Response

- Human genome: 46 chromosomes = $7.8x10^{9}$ bp = $5.4x10^{12}$ Da
- Yield of clusters ~ 20 nmol/J
- 1 mGy gives ~1 cluster / 10 genomes
- ~1000 clusters are formed per dicentric chromosome
- If damage to 1% of the cells is required for eliciting a biological response, then the threshold dose is ~ 0.1 mGy.
- Isolated lesions are between 10x and 100x more prevalent than clustered lesions, but these are repaired with high fidelity.