Clustered DNA Damages

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The Plan

- Introduction to DNA Damage Clusters

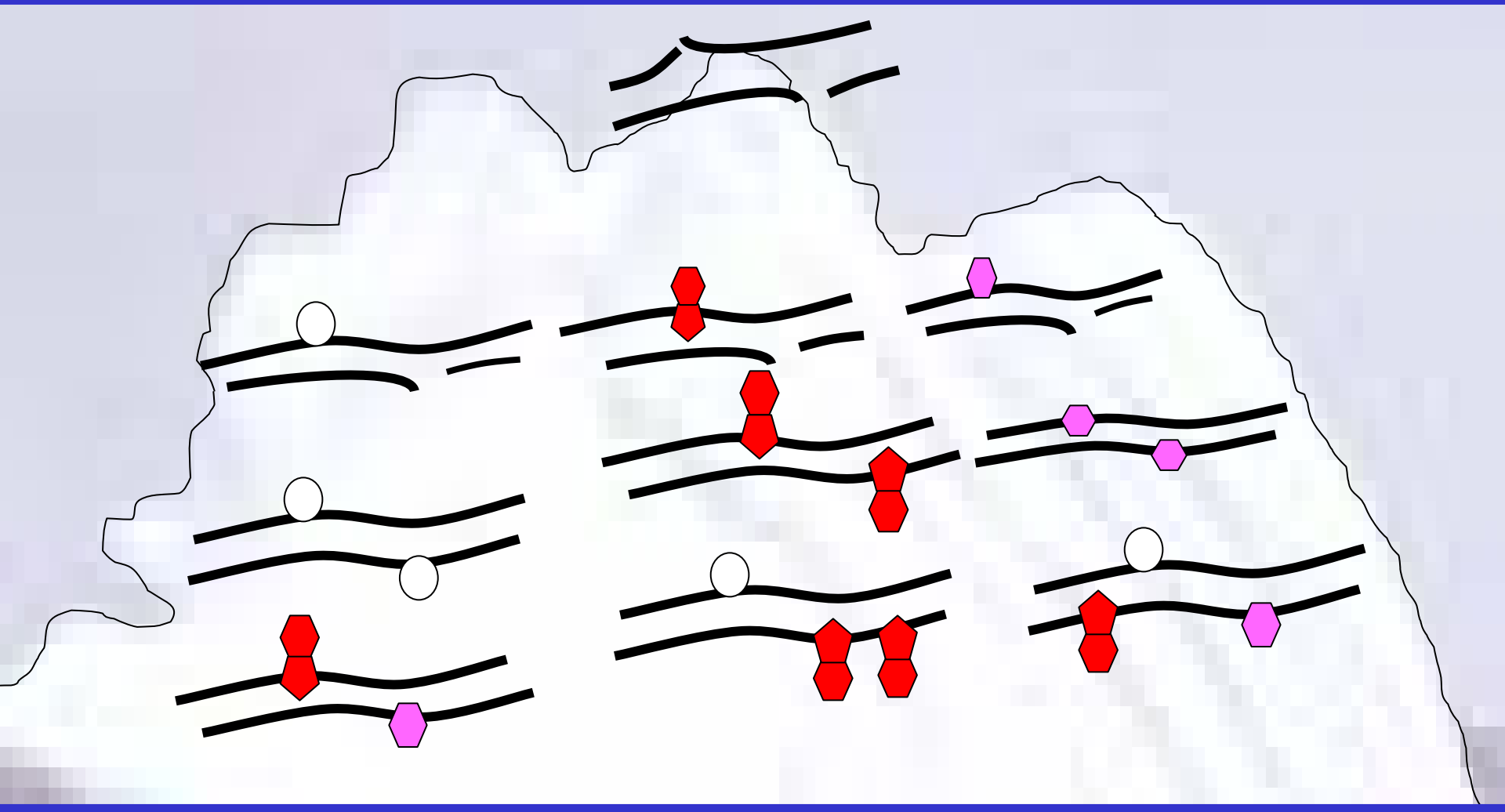
- New data

  Cluster damage spectra

  Endogenous clusters in smokers' stem cells

  Repair concerto

Double Strand Breaks: Critical Damages



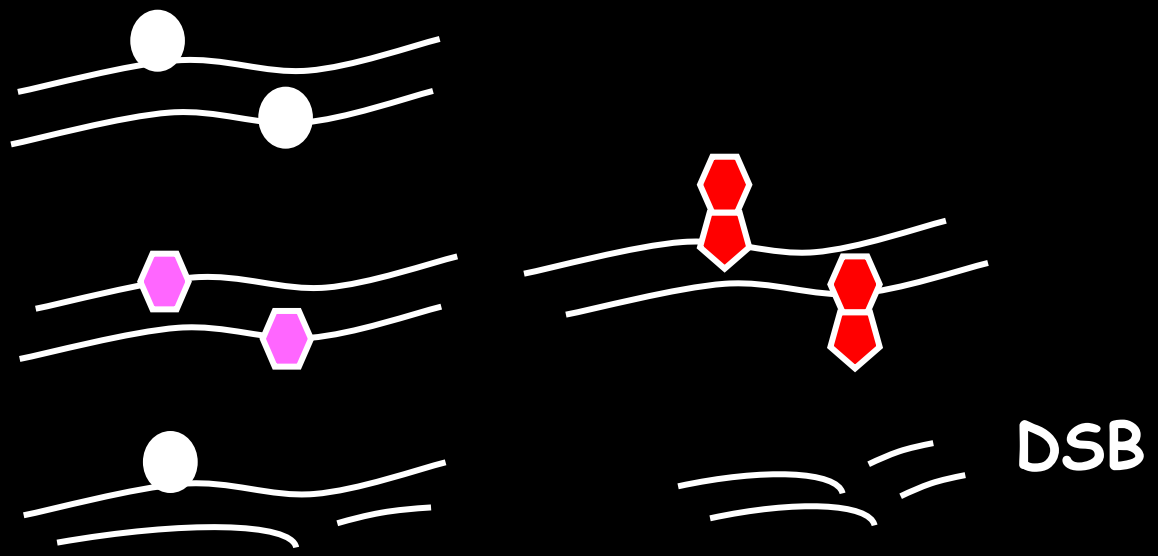
But are they only *the tip of the iceberg* of Critical Clustered Damages?

Bistranded Damage Clusters

- Two or more lesions within 1 or 2 helical turns

Rick Reynolds --CPD

- May contain
  - abasic sites
  - -oxidized bases
  - -strand breaks



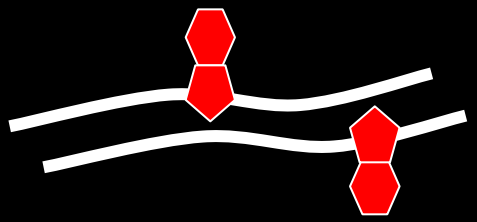
- Hypothesized as repair-resistant, critical radiation damages

(John Ward, Dudley Goodhead)

Does radiation really induce clusters in DNA?

??

How to measure?



Quantifying DNA Damages: Number Average Length Analysis

-T, 1 molecule

+T, 3 molecules

damages = # molecules$_{+T}$ - # molecules$_{-T}$

- *But we really want to know damage* **frequency**:

(damages/base or /kb or /Mbp or/Gbp)

$$\phi \text{ (damages/base)} = \frac{\#\text{ molecules}_{+T}}{\text{base}} - \frac{\#\text{ molecules}_{-T}}{\text{base}}$$

L_n = average molecular length of DNA population
(bases/molecule)

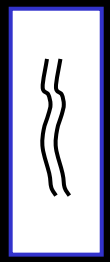
$$\phi \text{ (damages/base)} = 1/Ln_{+T} - 1/Ln_{-T}$$

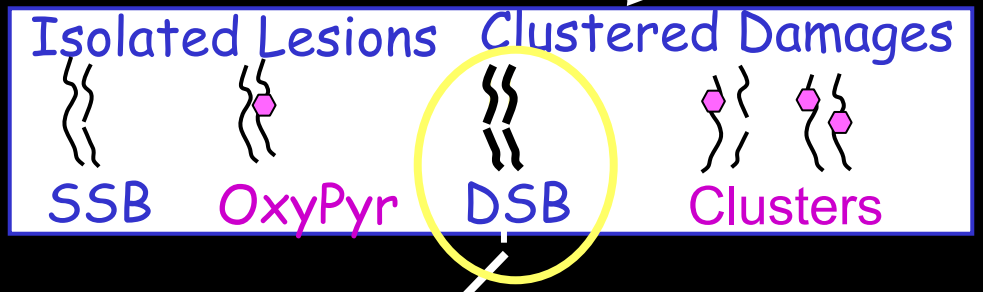
Measuring Double Strand Breaks

DNA (cells…)

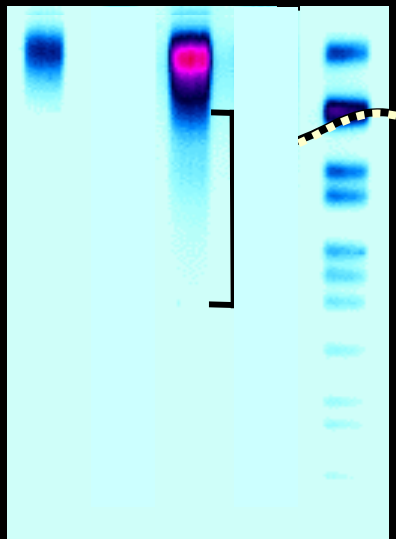
No Radiation

+ Radiation





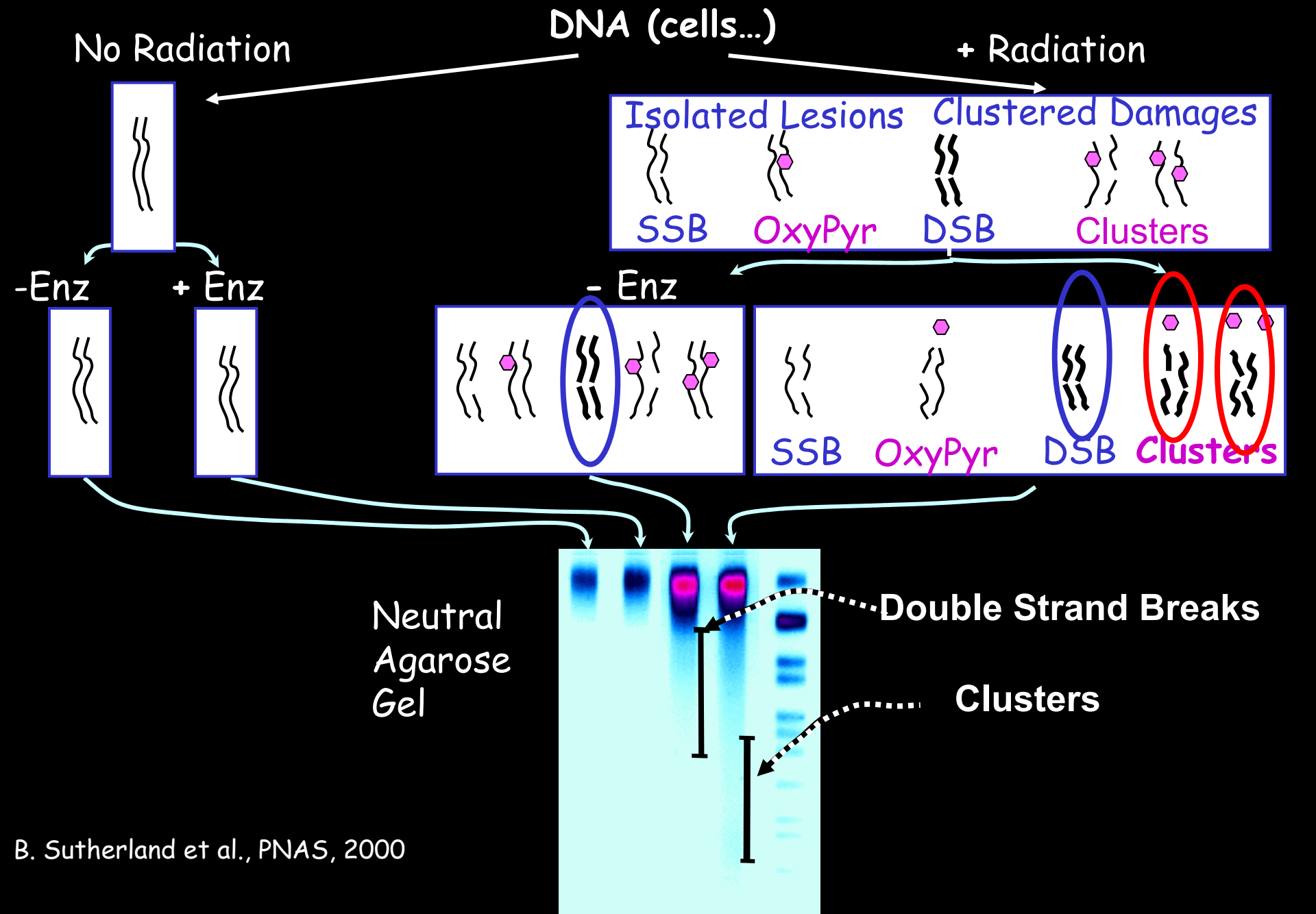
Neutral Agarose Gel



Double Strand Breaks

Chen & J Sutherland, Electrophoresis, 1989
B. Sutherland et al., Analytical Biochem, 1996

Measuring Clusters



B. Sutherland et al., PNAS, 2000

Quantifying DNA Damages: Pulsed field Gel Electrophoresis, Quantitative Electronic Imaging & Number Average Length Analysis

John Sutherland

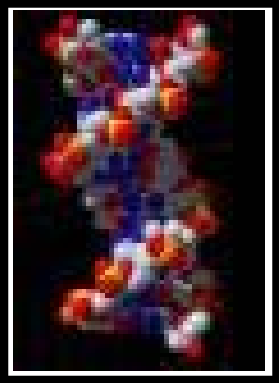
John Trunk

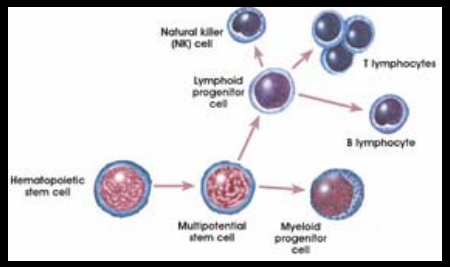
Denise Monteleone

- No specific distribution of damages required
- No radiolabelling of DNA
- Uses nanograms of DNA per measurement
- Current sensitivity = 1-2 damages/ 10^9 base pairs (Gbp)

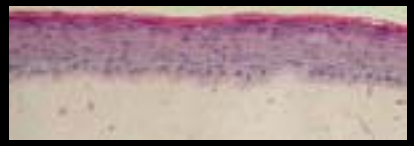
DNA Damages Can Be Quantified in Simple & Complex Systems

Human tissue



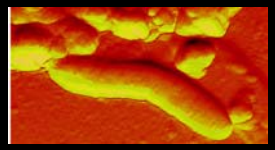


Human hematopoeitic stem & progenitor cells

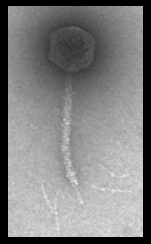


3D human tissue models

Field samples



Microorganisms



Bacteriophage

Higher plants

Now we can measure clusters
IF they are present!

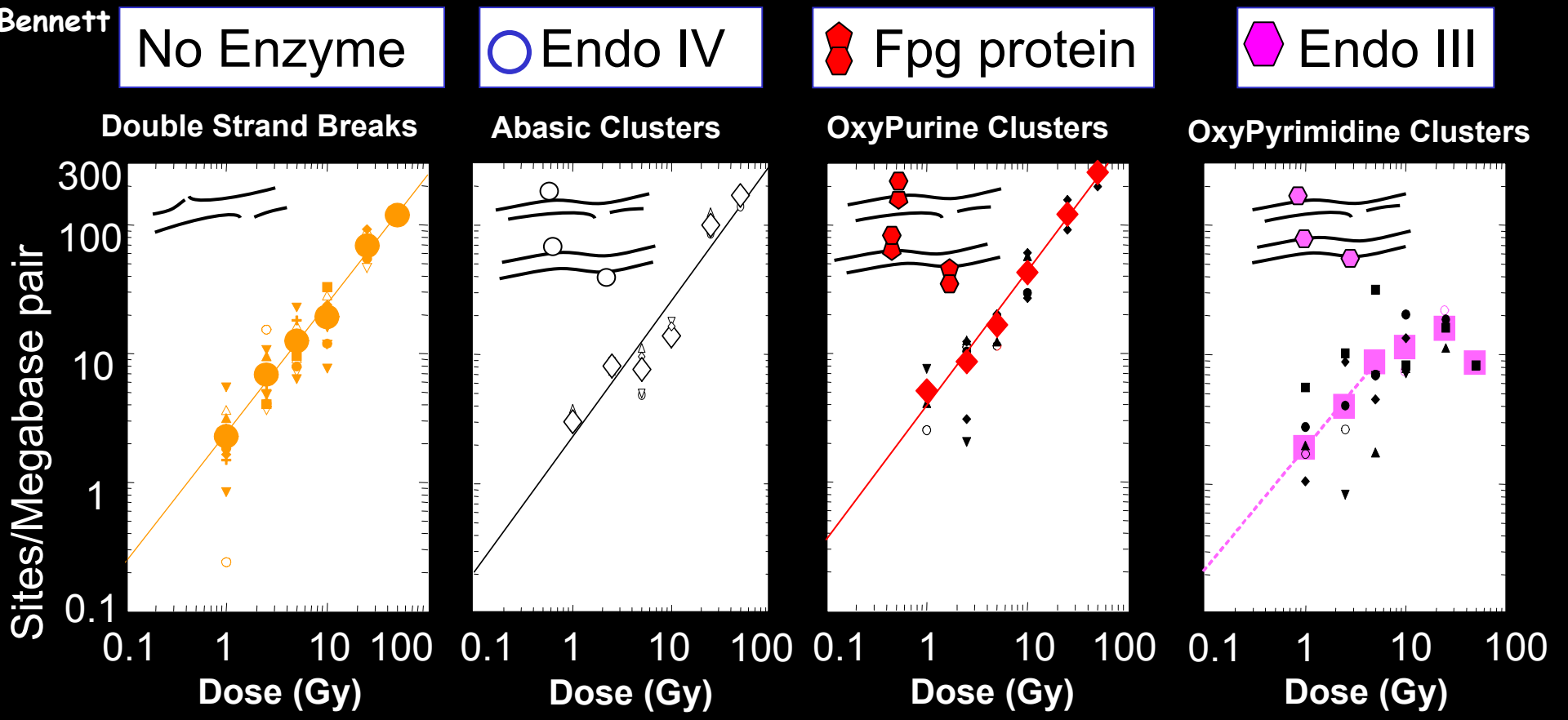
Does radiation induce clusters?

Experimental Design

- DNA in dilute phosphate buffer
- Expose to ^{137}Cs γ rays
- Immediately transfer to enzyme buffer (HEPES)
- Treat with one of the following:
  - -- Nth protein: mainly oxidized pyrimidines
  - -- Fpg protein: mainly oxidized purines
  - -- Nfo protein: abasic sites
- Agarose gel electrophoresis (native conditions)
- Stain DNA with DNA-binding fluorophore
- Quantitative electronic image
- Number average length analysis

Paula Bennett

γ-rays Induce Clusters in DNA in Solution



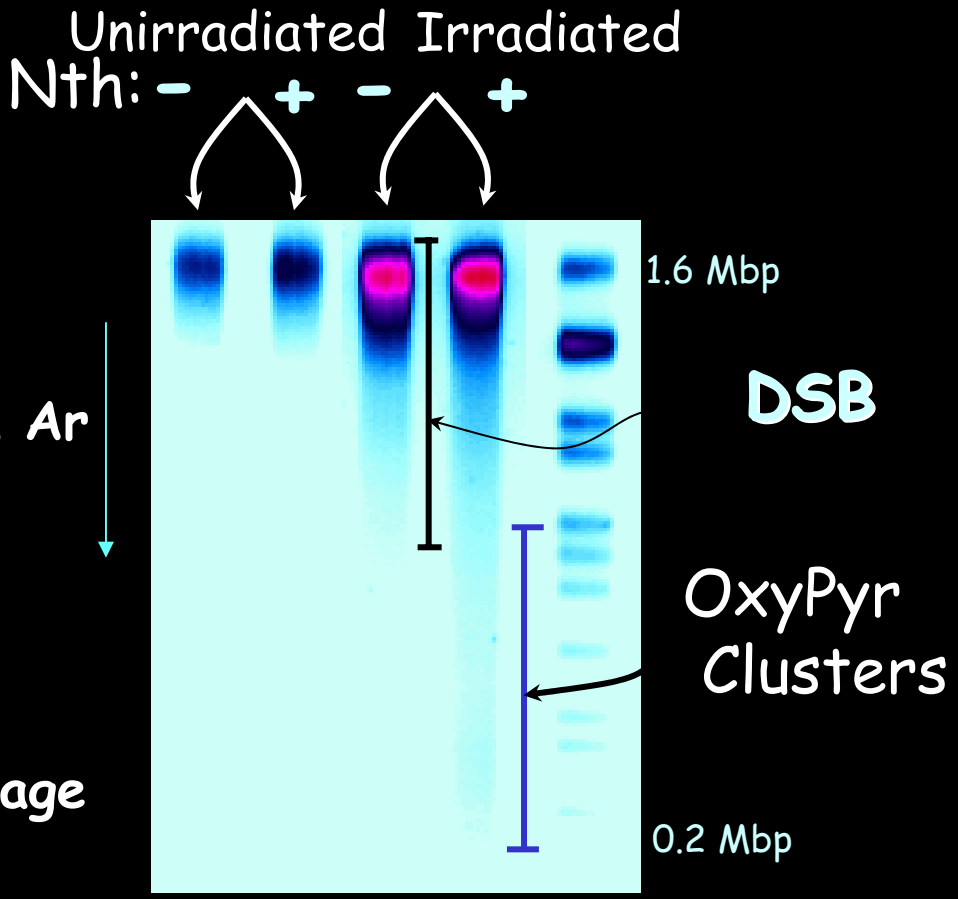
Dose-responses on log-log plot:
straight line, slopes ≅ 1:
damage cluster is formed by a single radiation hit

Sutherland et al., PNAS 2000

Paula Bennett

OxyPyrimidine Clusters in Human Cells

- Irradiate cells cold
- Harvest immediately
- Isolate DNA in agarose in Ar
- NotI restrict
- +/- Nth protein
- Neutral TAFE gel
- Stain with ethidium
- Quantitative electronic image
- Calculate number average length
- Calculate cluster frequency



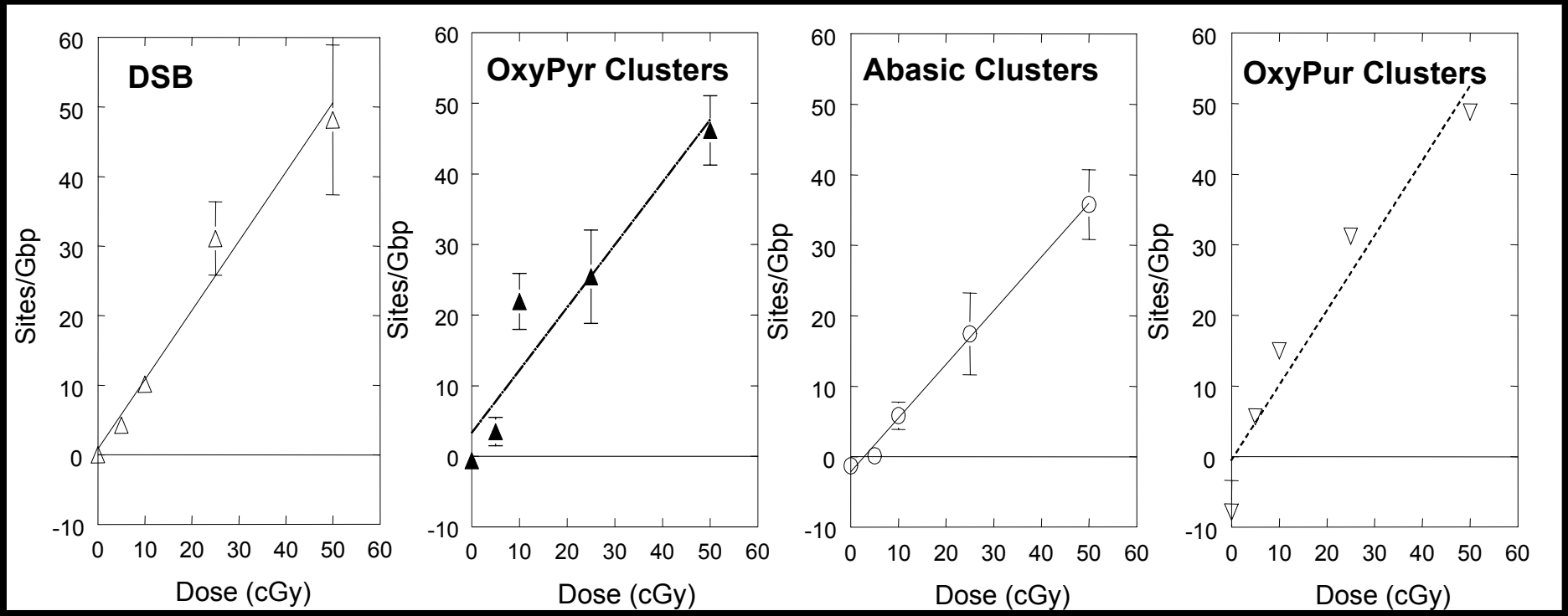
Electronic image of human DNA on electrophoretic gel

Sutherland et al., PNAS 2000

Paula Bennett

X-rays Induce Clusters in Human Cells

100 kVp X-rays, 28SC monocytes



Sutherland et al., Radiation Research, 2002

Conclusions

- Radiation induces DSBs, OxyPyrimidine clusters, OxyPurine clusters & Abasic clusters.
- Clusters are induced at very low radiation doses.
- Linear dose response for cluster induction.
- Single radiation 'hits' induce damage clusters.

What factors affect cluster induction?

- Radiation dose determines cluster LEVELS.
- DNA environment affects cluster SPECTRUM.
  (ratio of cluster types)
- Other factors?

Does Radiation Species Determine

- cluster yields?

- DNA damage spectrum?
(relative levels of specific complex damages)

Where to find radiation
of different LET, energy & Z?

The Space Radiation Environment

Galactic Cosmic Rays (GCR)

high energy protons
highly charged, energetic atomic nuclei
(HZE particles)

not effectively shielded
(break up into lighter, more penetrating pieces)

abundances and energies quite well known

MAIN PROBLEM:

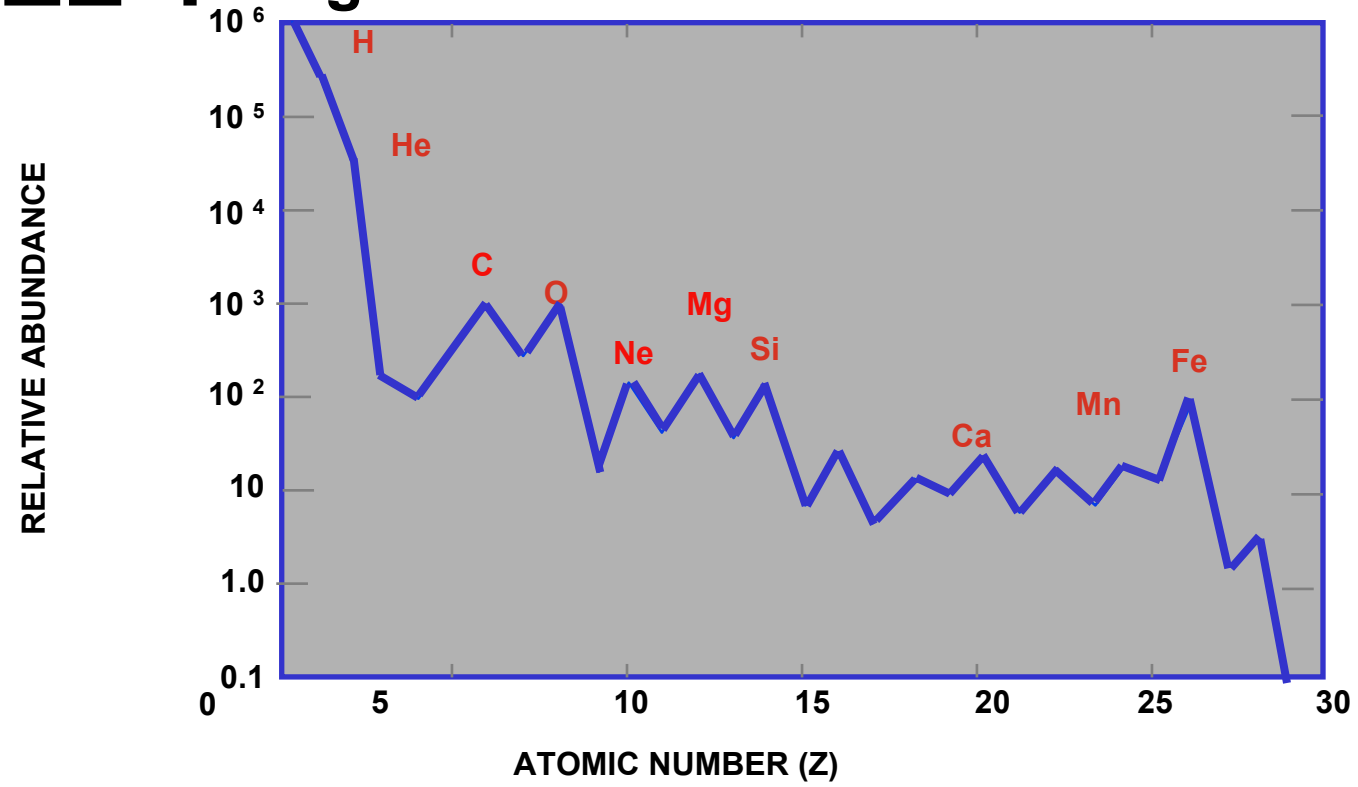
biological effects poorly understood

but known to be most significant space radiation hazard

Slide thanks to Dr. Walter Schimmerling, NASA

Galactic Cosmic Rays (GCR)

"HZE": High Z



~85% protons
~14% helium
~ 1% heavier particles

Booster Operating Parameters:

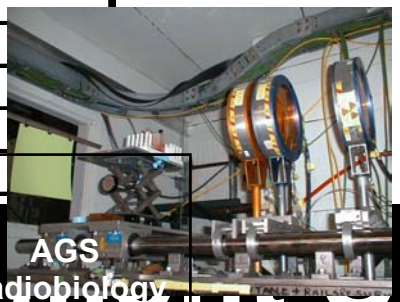
SPECIES (Z,A)	ENERGY RANGE (MeV / nucleon)	TYPICAL DOSE RATES (Gy/min)
H(1,1))	100-3070	54-16
Si (14,28)	90-1230	114-31
Fe(26,56)	100-1100	146-43
Cu(29,63)	100-1040	n.a.
Au(79,97)	40-300	n.a.

Radiation Environment Simulation

NASA Irradiation Facilities

Brookhaven National Laboratory

RELATIVISTIC HEAVY ION COLLIDER (RHIC)



AGS Radiobiology Experiments

NASA Space Radiation Laboratory (NSRL)

TANDEM VAN DE GRAAFFS

ALTERNATING GRADIENT SYNCHROTRON (AGS)

200 ft

HEAVY ION TRANSFER LINE

BOOSTER

200 MeV LINAC

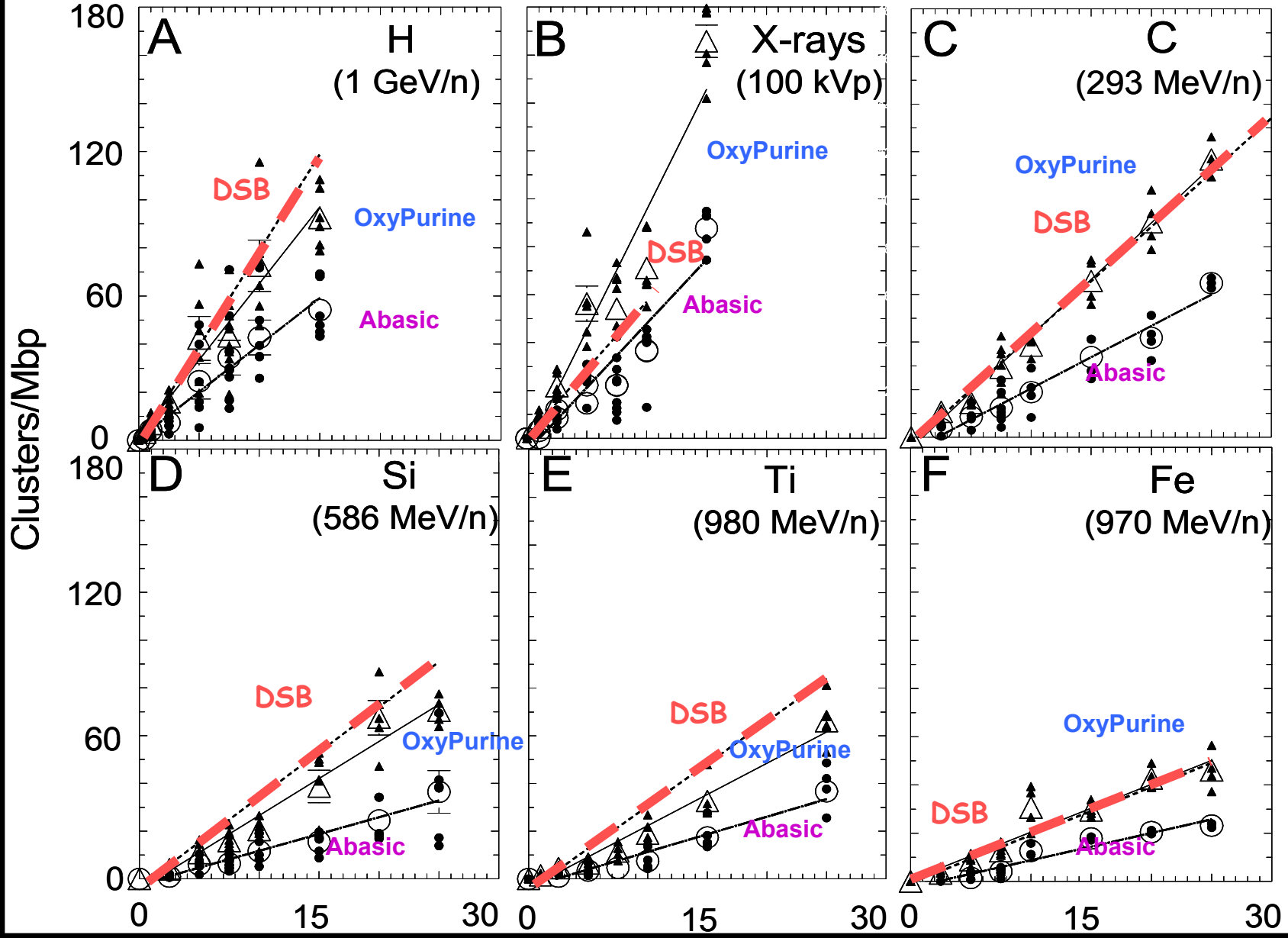
Slide thanks to Dr. Walter Schimmerling, NASA

Experimental Design

- DNA in dilute phosphate buffer
- Expose to charged particles [H (1 GeV/n) to Fe (1 GeV/n)]
- Immediately transfer to enzyme buffer (HEPES)
- Treat with one of the following:
  - -- Nth protein: mainly oxidized pyrimidines
  - -- Fpg protein: mainly oxidized purines
  - --Nfo protein: abasic sites
- Agarose gel electrophoresis (native conditions)
- Stain DNA with DNA-binding fluorophore
- Quantitative electronic image
- Number average length analysis

Cluster Yields Depend on the Radiation Type

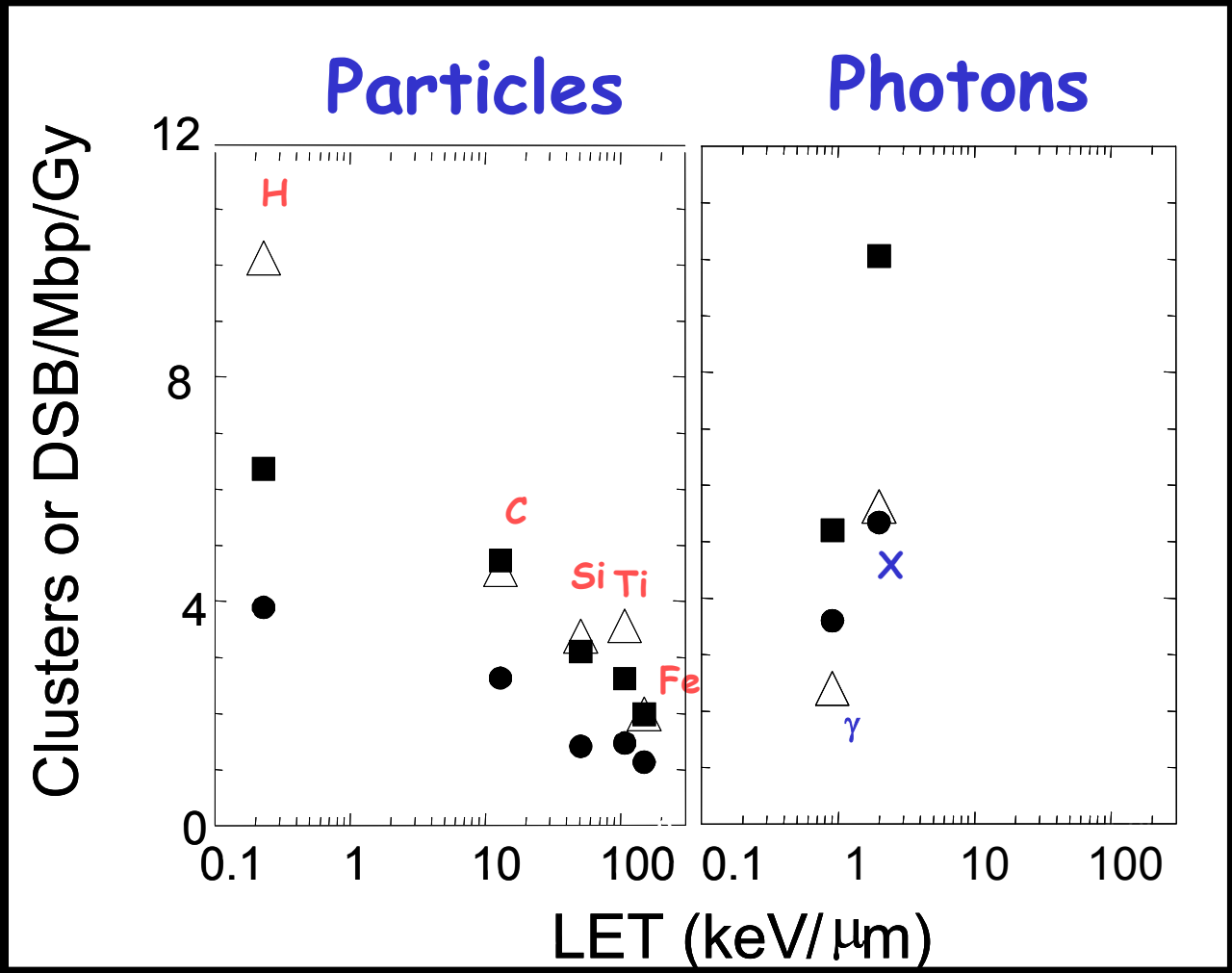
Megumi Hada



Hada & Sutherland, Rad Res. 2006

Megumi Hada

Radiation Type Determines DNA Damage Spectrum



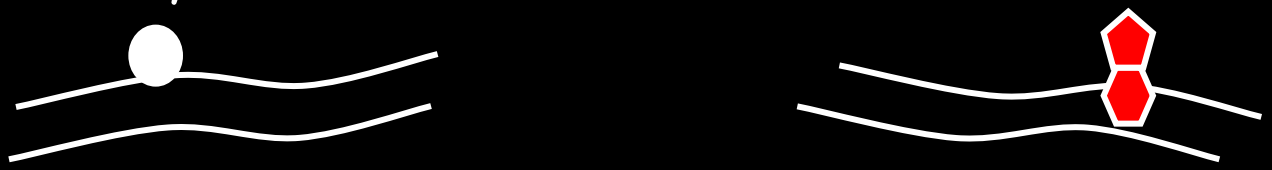
Conclusions

- Radiation type determines DNA damage spectrum.
- 1 GeV/n protons produce high levels of DSBs and other clusters in DNA in solution and in cells.

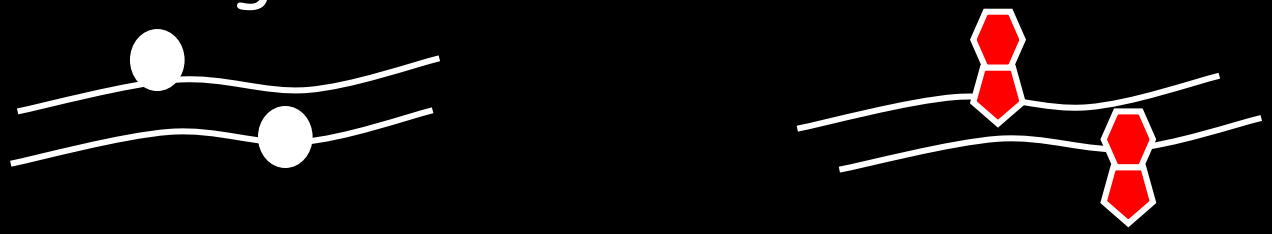
Clusters in Unirradiated Cells?

Endogenous Clustered Damages in Human Cells?

- Isolated oxidized lesions are induced in cells.
  - ~ 10,000 oxidized lesions induced per cell per day.
  - Steady state levels of ~ 2000/cell.



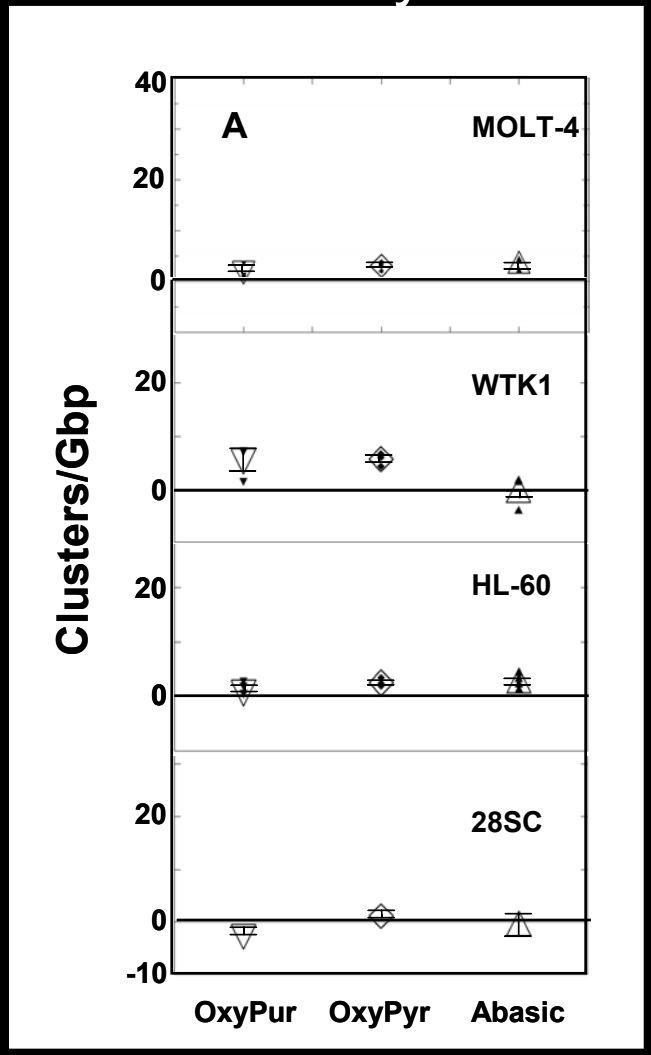
- Are Endogenous Clusters induced in cells?



Endogenous Clusters in Human Cell Lines?

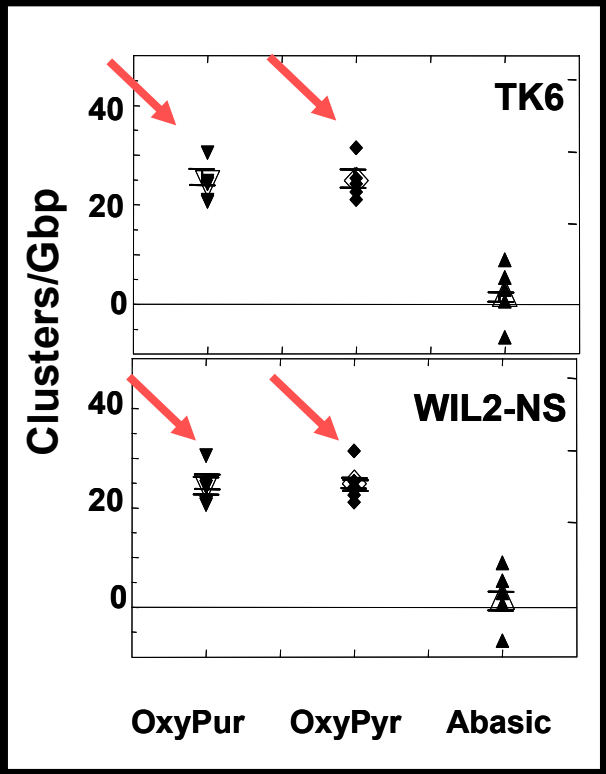
Paula Bennett

Most human cell lines don't accumulate any clusters.



Bennett et al., FRBM, 2005

Two lines accumulate Oxidized Base clusters BUT not Abasic Clusters



Expectation for Human Primary Cells:

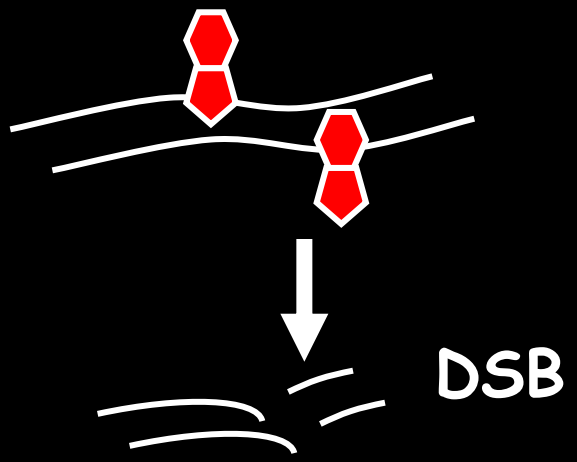
- No endogenous clusters of any type

OR

- Low levels of oxidized base clusters only
  & No abasic clusters

Cluster Repair In Human Cells

Expectation: Attempted Cluster Repair Would Produce DSBs



Reality: What do repair-proficient cells do?

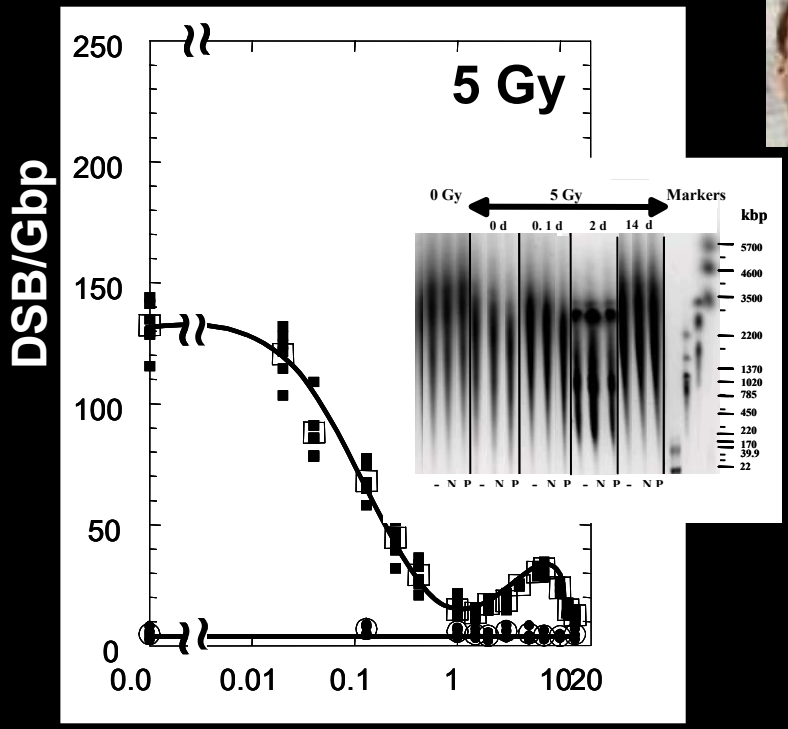
In vitro (synthetic oligonucleotides with defined cluster) : Michael Weinfeld, Susan Wallace, Peter O'Neill
In heavily irradiated cells: Susan Wallace

Experimental Design for Repair Studies

- Irradiate cells warm.
- Post-irradiate incubation at 37°C.
- Harvest at increasing times after irradiation.
- Isolate DNA, measure DSBs & clusters as usual.

Double Strand Break Rejoining in Human Cells

Alex G



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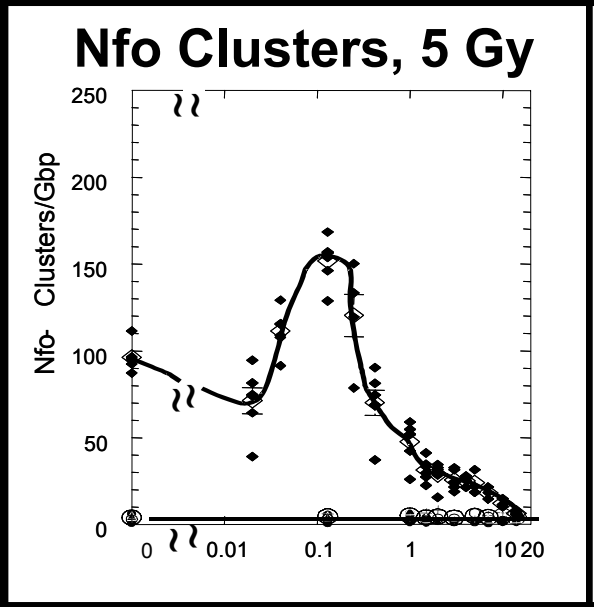
Time After Irradiation (Days)

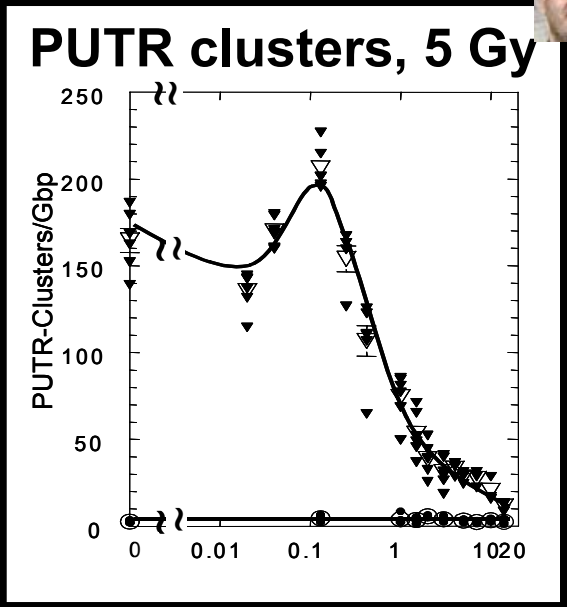
- Few if any detectable *de novo* early DSBs.

Georgaklias et al., NAR, 2004
Bennett et al, in preparation

If cells don't produce DSBs in repairing clusters, WHAT do they do?

Repair of Abasic Clusters in Human Cells





- ***De novo*** **abasic clusters appear.**
- **Presumably repair intermediates.**

Georgakilas et al., NAR, 2004
Bennett et al., in preparation

Conclusions

- Cluster repair
  - for repair-proficient cells, lower radiation doses:
  - avoids DSB production.
  - produces cluster intermediates.

The Crew

- Paula Bennett
- Jim Jardine
- Pat Hein
- Deborah Keszenman
- Mamta Naidu
- Brigitte Paap
- Stefan Tafrov
- John Trunk
- Denise Monteleone

Other Cluster Contributors

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- Nela Cintron
- Noelle Cutter
- Biraj Das
- Alex Georgakilis
- Megumi Hada
- Sunirmal Paul
- Joon Song
- Emily Weinert

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- Murat Saparbaev, IGR
- Alan Gewirtz, UPenn
- Kathryn Held, MGH
- Ann Kennedy, UPenn