Systems Biology and Individual Radiation Sensitivity

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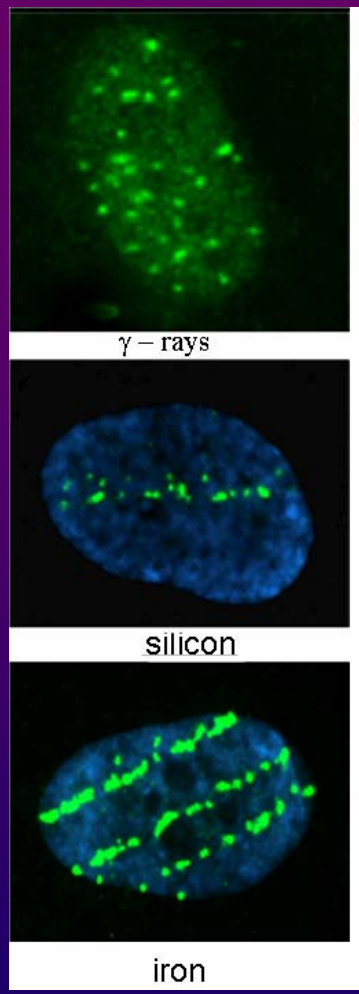
Predicting Individual Radiation Sensitivity
Columbia University, New York NY
March 18, 2008

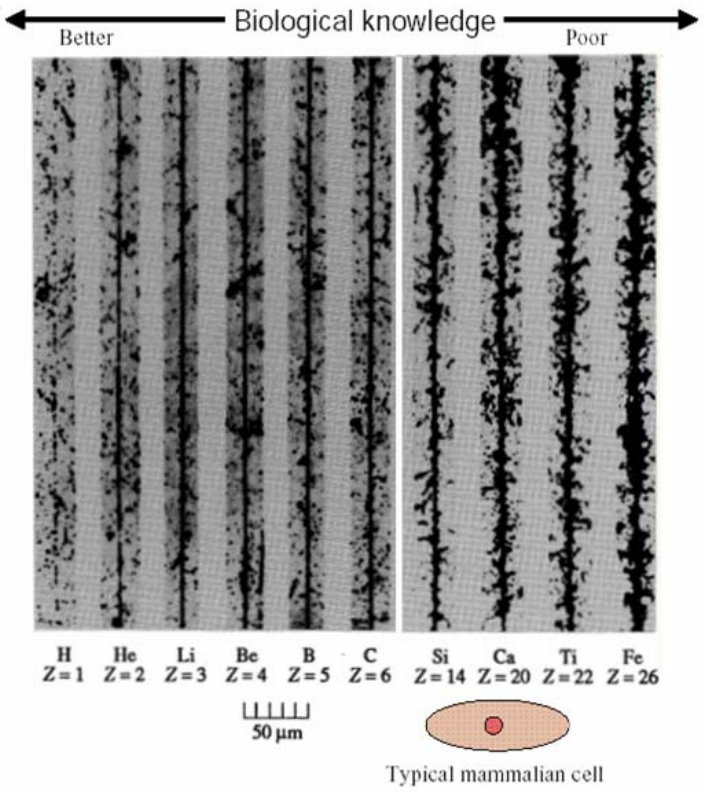
The Space Radiation Problem

Space radiation is comprised of high-energy protons and heavy ions (HZE's) and secondary protons, neutrons, and heavy ions produced in shielding

- Unique damage to biomolecules, cells, and tissues occurs from HZE ions that is qualitatively distinct from X-rays and gamma-rays on Earth
- No human data to estimate risk from heavy ions
- Animal models must be applied or developed to estimate cancer, and other risks
- Solar particle events (SPEs) can not be predicted with sufficient warning at this time
- Shielding has excessive costs and will not eliminate galactic cosmic rays (GCR)



Single HZE ions in cells And DNA breaks



Single HZE ions in photo-emulsions Leaving visible images

Cucinotta and Durante, Lancet Oncology (2006)

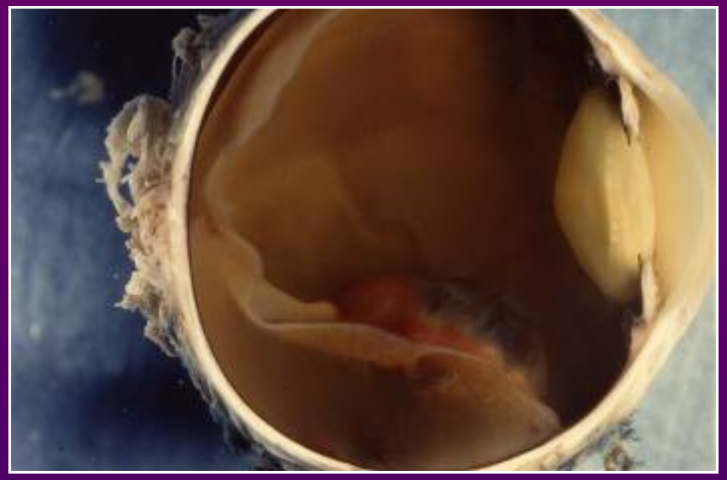
Categories of Radiation Risk

Four categories of risk of concern to NASA:

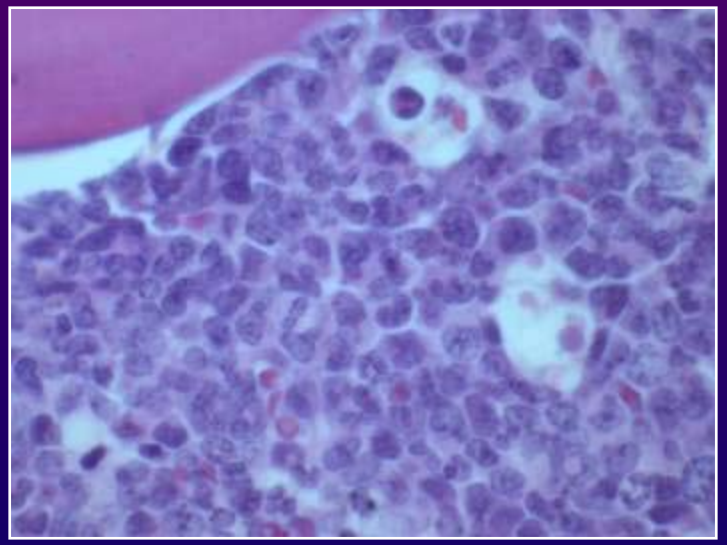
- ***Carcinogenesis (morbidity and mortality risk)***
- ***Acute and Late Central Nervous System (CNS) risks***
  - ✓ immediate or late functional changes
- ***Chronic & Degenerative Tissue Risks***
  - ✓ cataracts, heart-disease, etc.
- ***Acute Radiation Risks*** – sickness or death

Differences in biological damage of heavy nuclei in space with x-rays, limits Earth-based data on health effects for space applications

- New biological knowledge on risks must be obtained
- Are their sensitivity issues between risks?



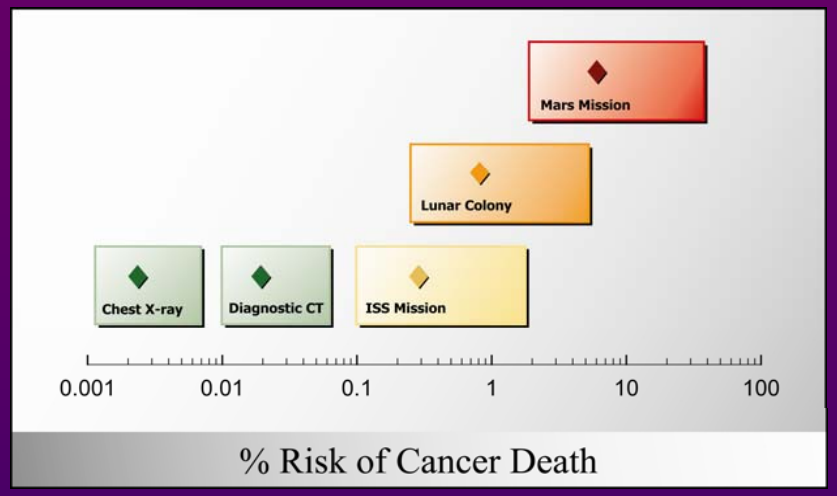
Lens changes in cataracts (E. Blakely)

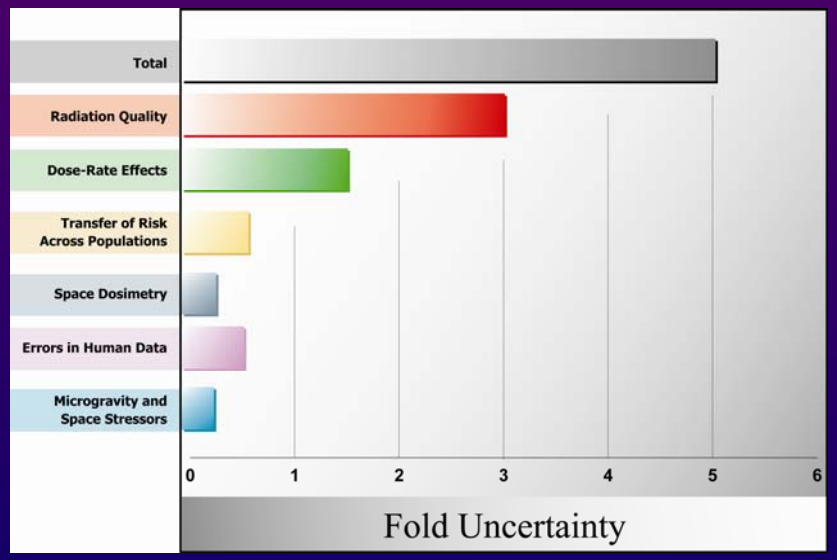


First experiments for leukemia induction with GCR (R. Ullrich)

Individual Radiation Sensitivity and Astronauts

- Small number of healthy individuals selected for a large number of mission related attributes
- Screening for high risks missions could significantly reduce costs or may be viable option based on acceptable levels of risks
- Possibility to monitor individuals over many years (entry in Corp, pre- and post-mission, continue after retirement)



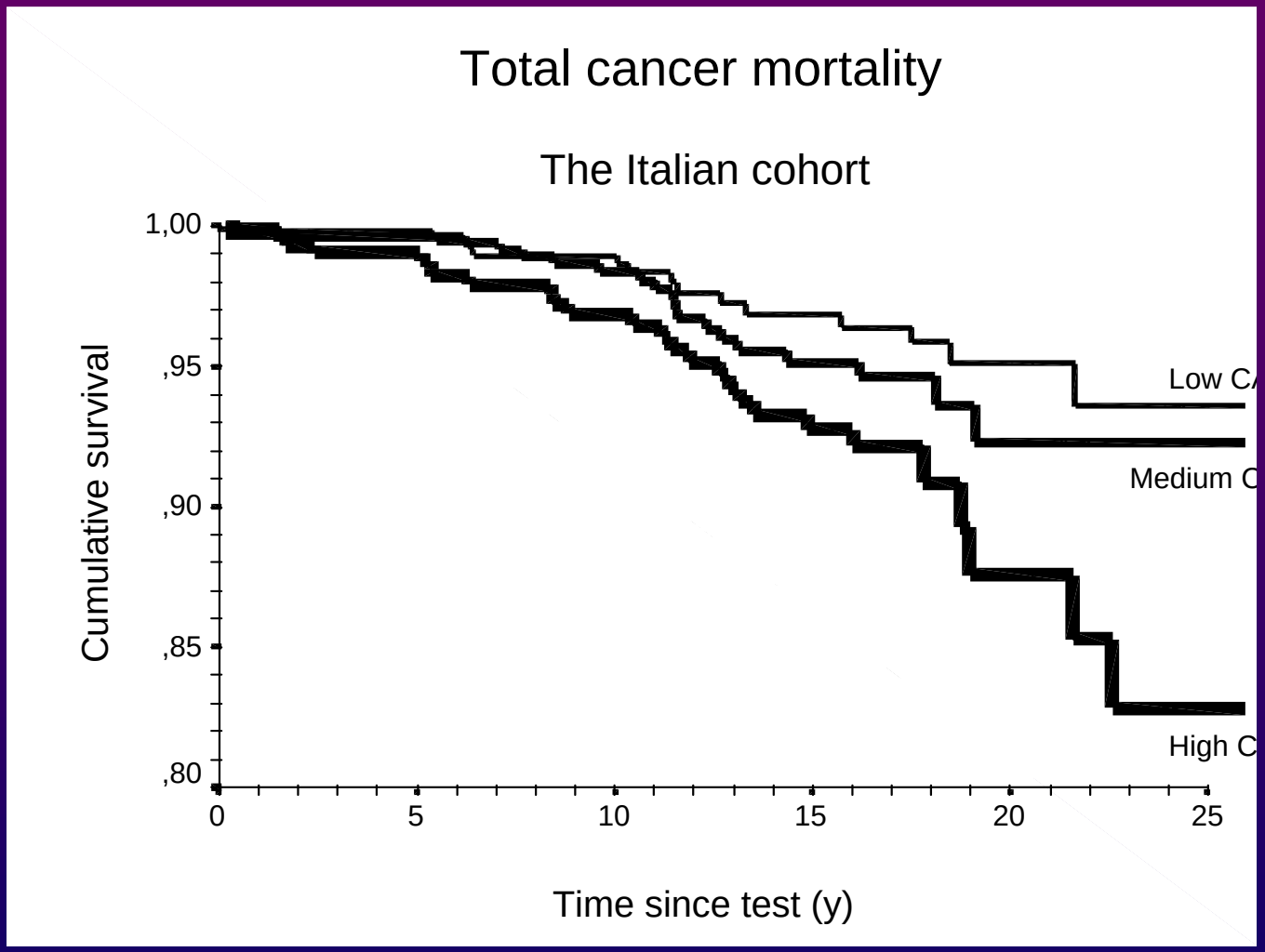


Durante and Cucinotta, Nature Rev Cancer (2008)

Systems Models to Integrate Across Approaches

- As new knowledge and technologies are developed systems biology approaches will be needed to improve understanding of data and integrate across radiation sensitivity measures
  - Chromosomal Aberrations
  - Telomere Changes
  - γH2AX residual levels
  - cDNA arrays
  - Protein arrays
  - Micro-satellite mutations, etc.

Correlation between Chromosomal Aberrations and Cancer



Bonassi et al Cancer Res

International Space Station (ISS) Biodosimetry

Use multi-color FISH painting to count frequency of specific aberrations (PCC vs. Metaphase spreads)

Pre-flight blood draw exposed to low doses of gamma-rays to determine individual calibration curve

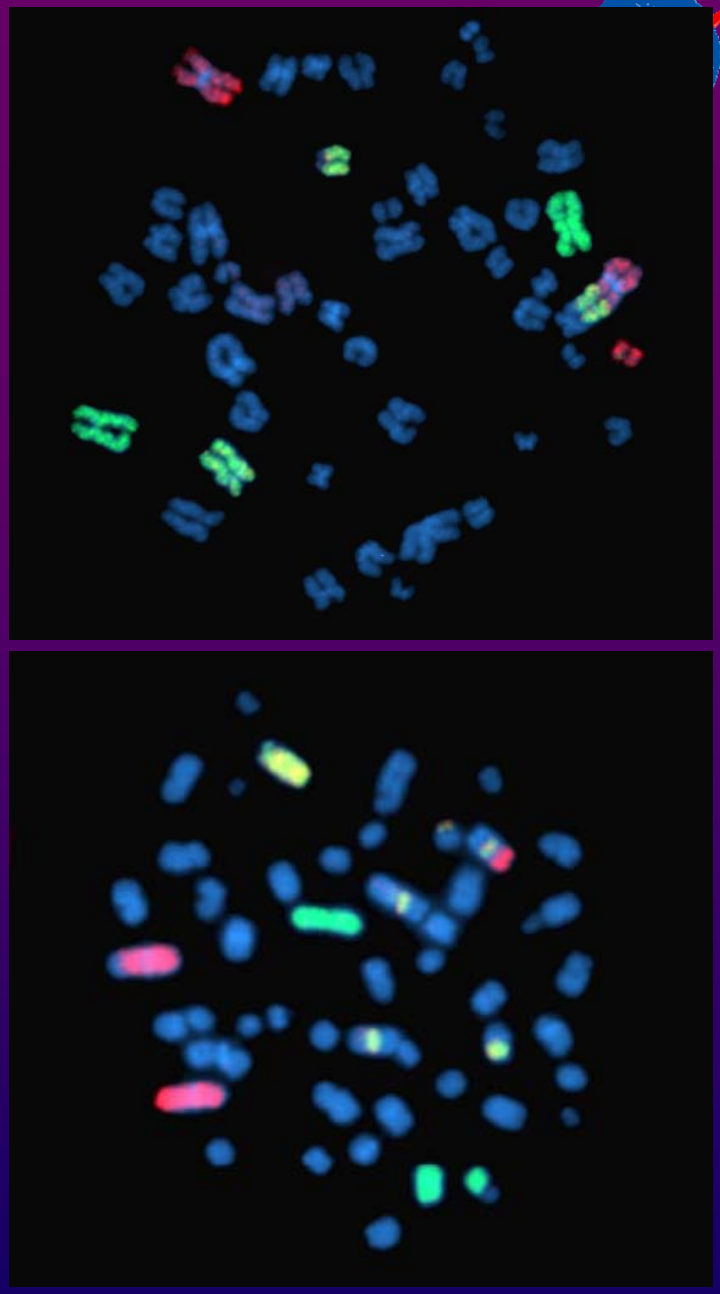
Post-flight blood draw used as comparison and for determination of mGy-Eq. dose

Weighted linear regression

$$Y_{pre} = A + B\ Dose$$

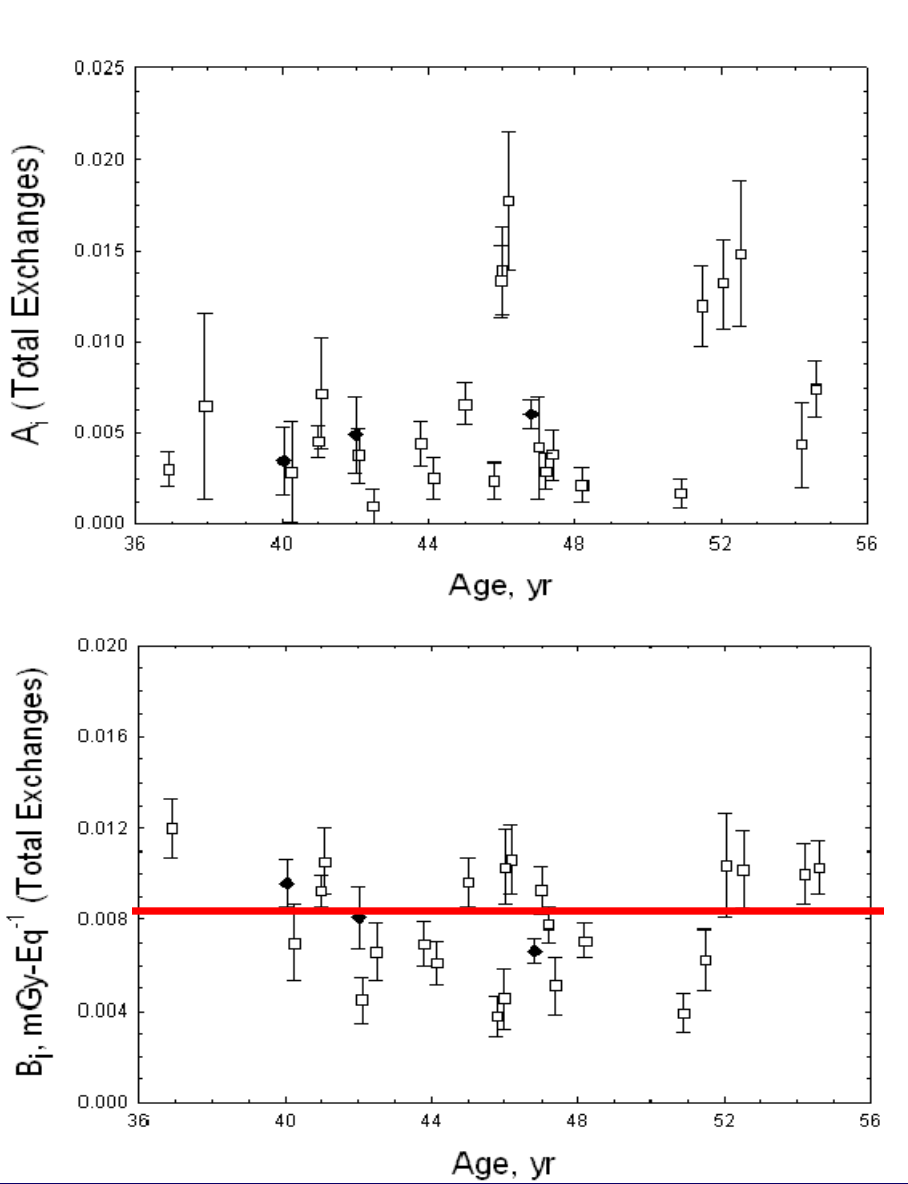
Post-flight biological dose eq.

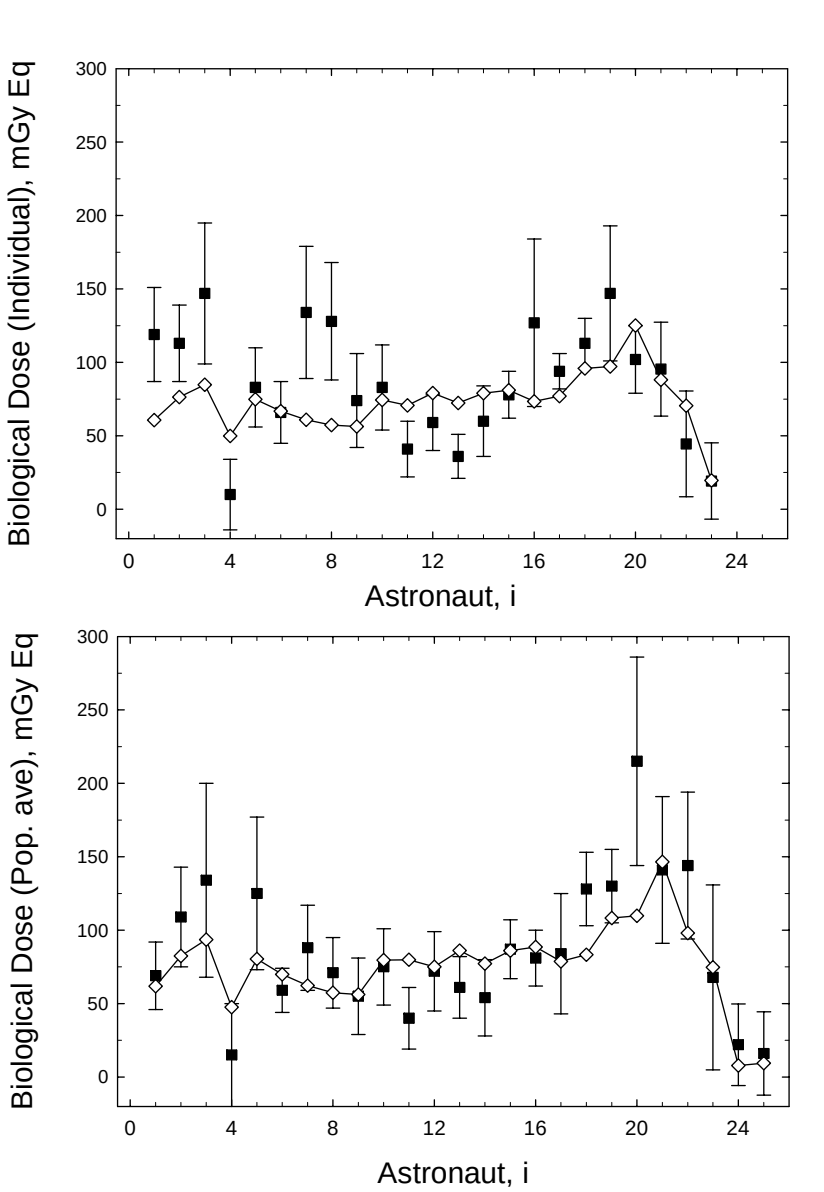
$$BDE = (Y_{post} - A)/B$$



Complex Aberrations post ISS

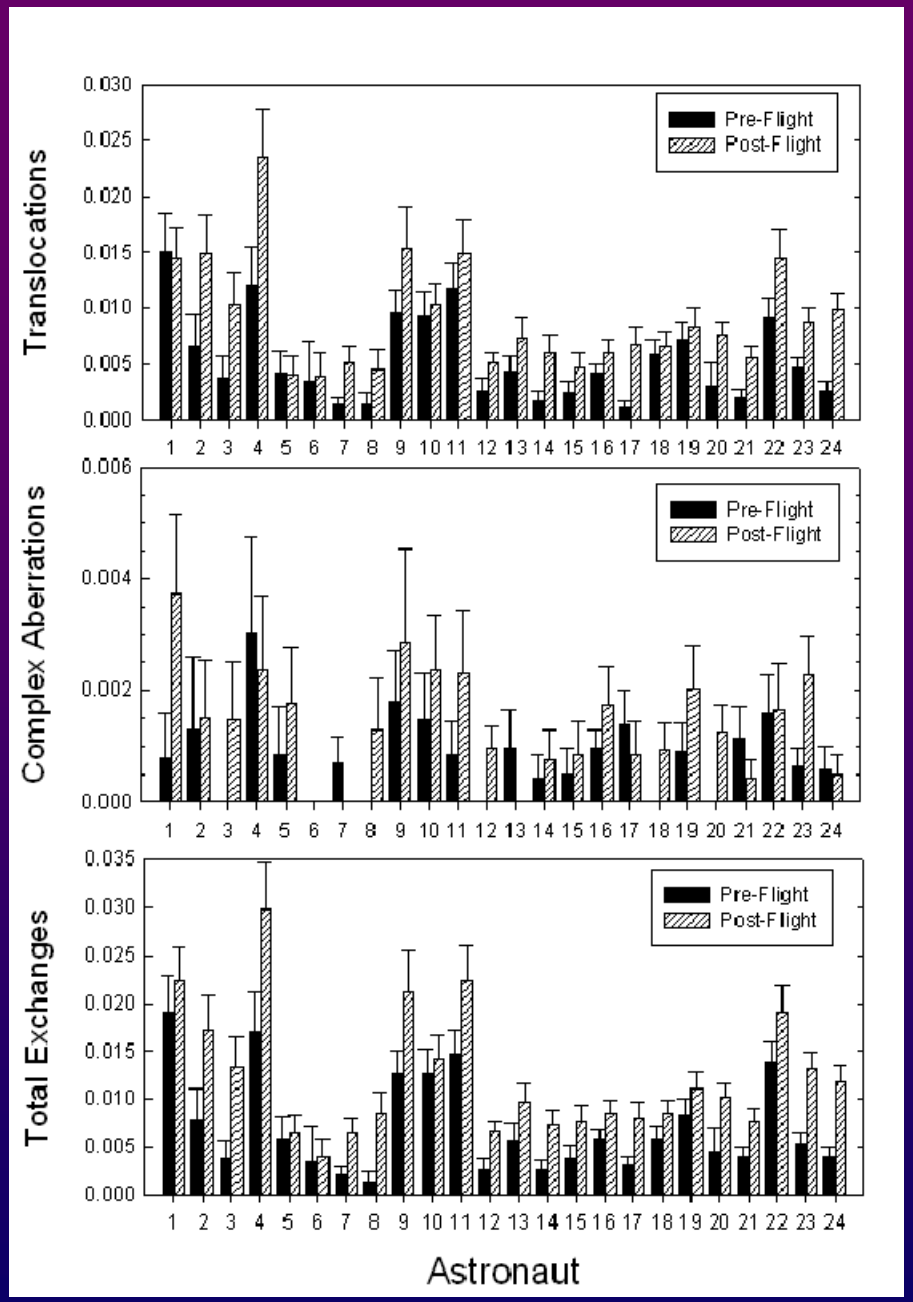
Individual vs Population Biodosimetry



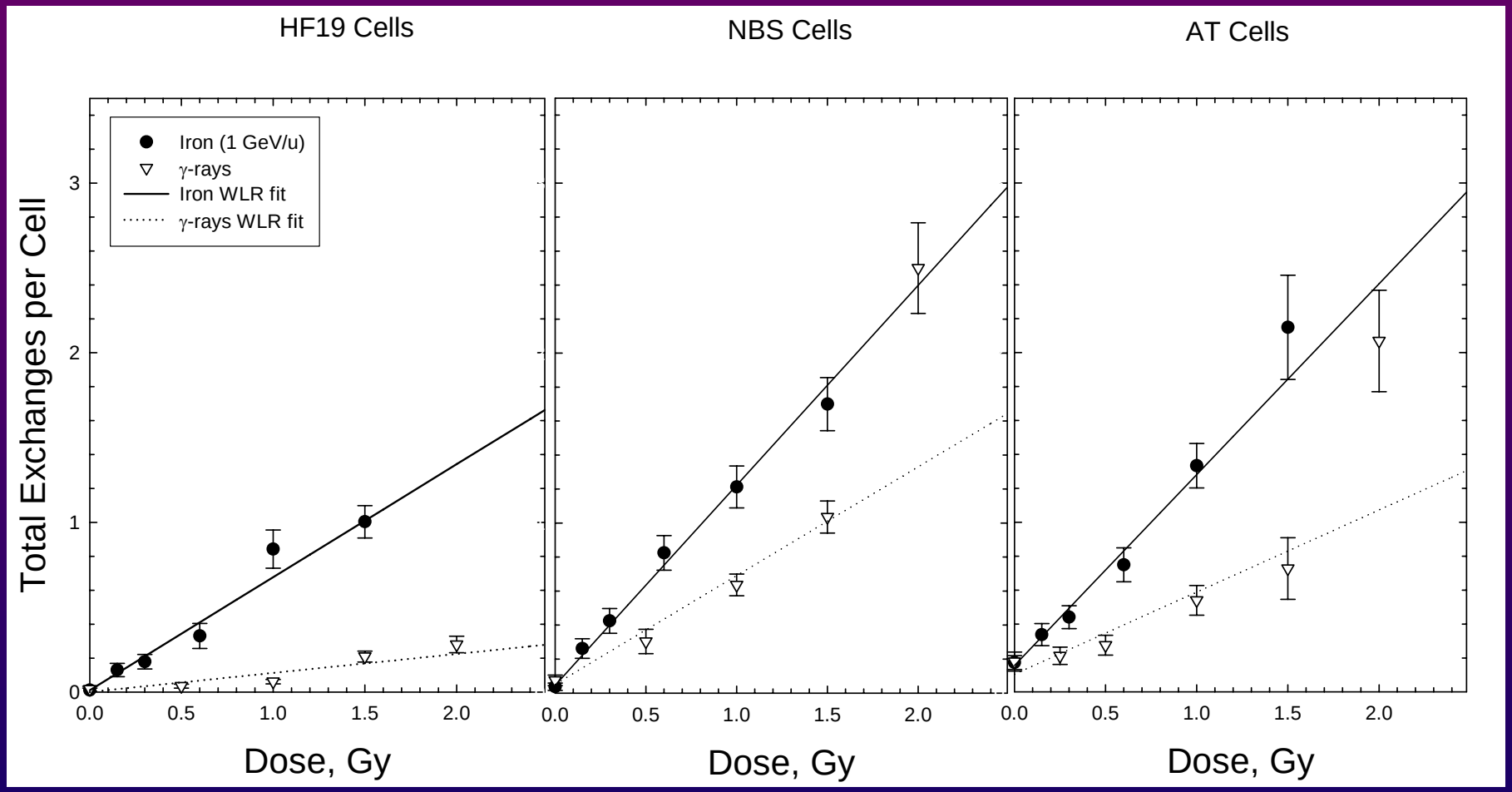


NASA Results

- 24 assessments to date
  - Mir Station 4 Crew
  - Shuttle 2 Crew
  - ISS 19 Crew
- Total exchanges increased in all cases
- Translocations and complex aberrations increased in majority of cases



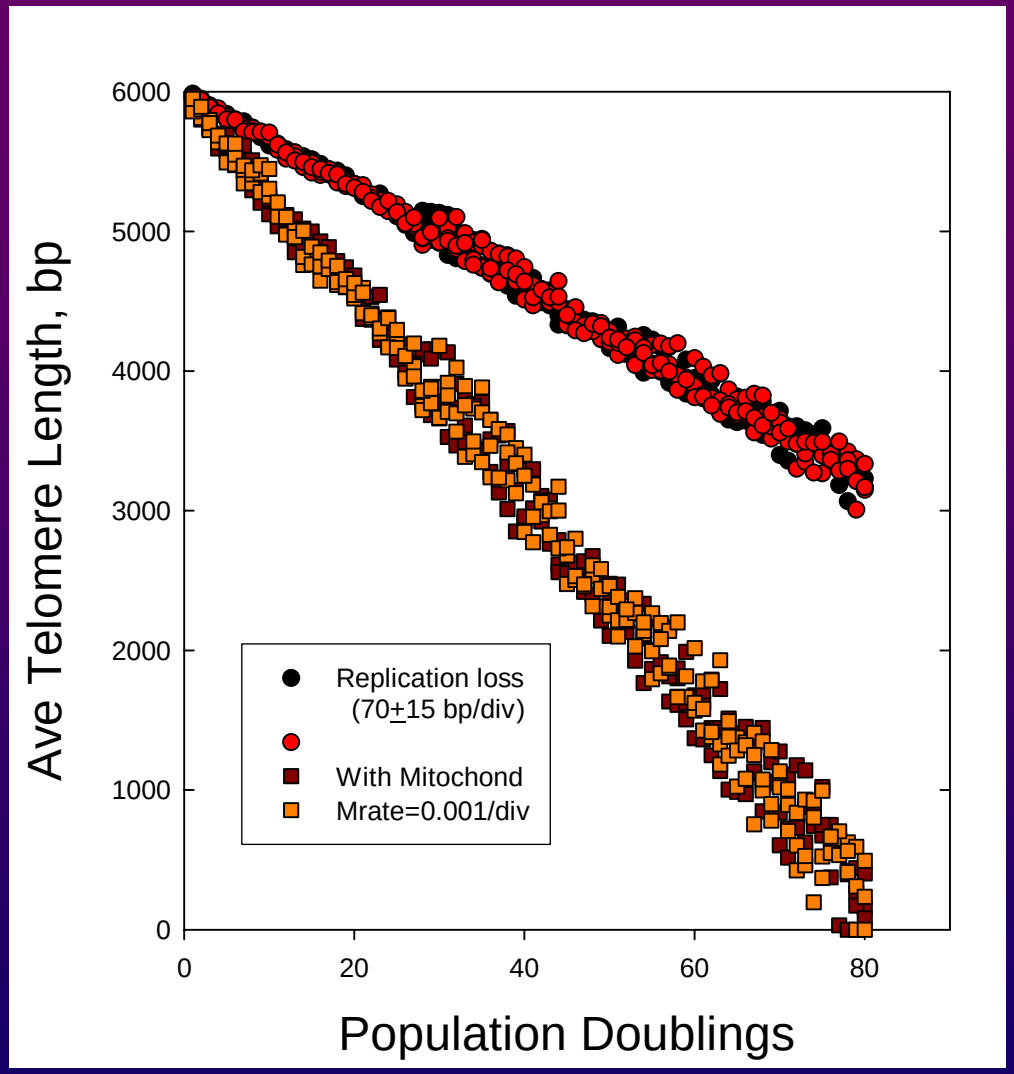
DNA Repair Defects and Radiation Quality
-Iron Nuclei and Total Exchanges



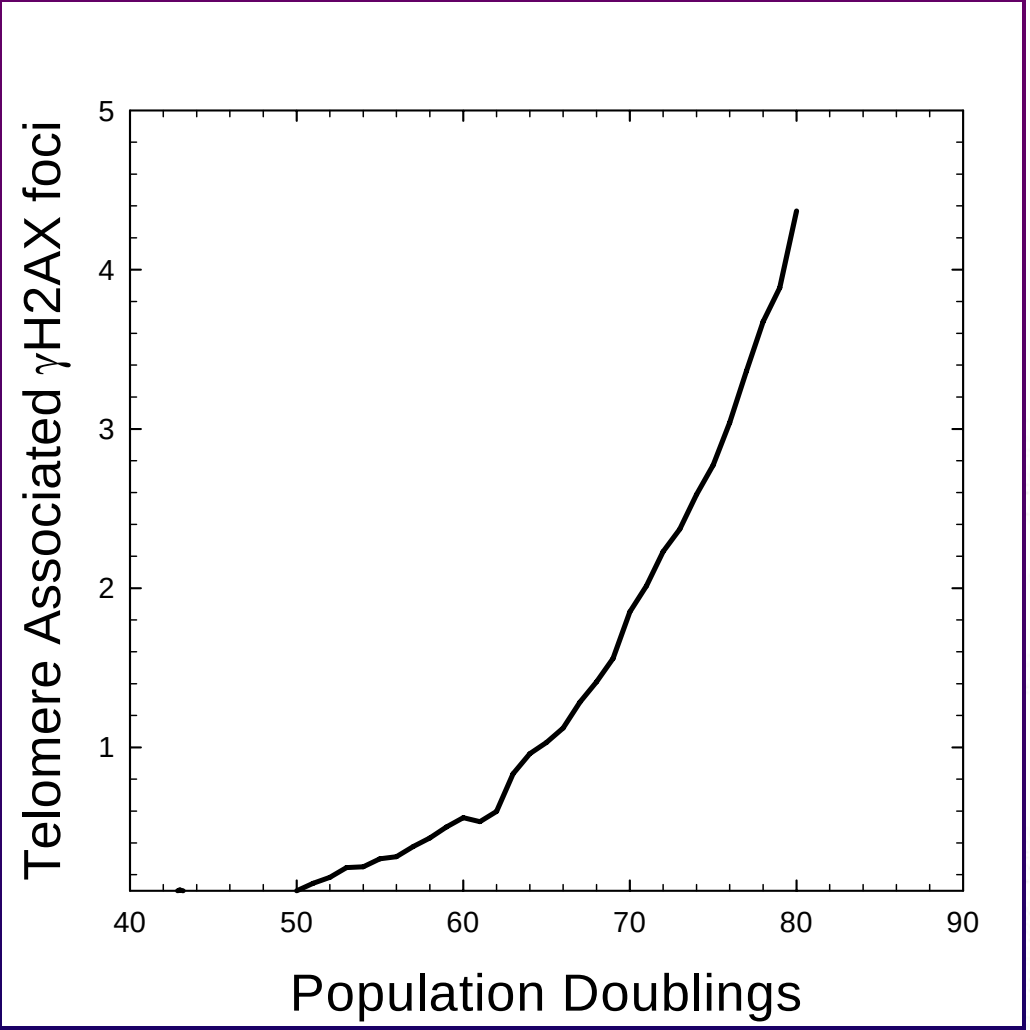
RBE = 7 1.8 2.3

Telomere Length Changes

- Telomere length associated with aging, disease, and damage repair
- Mechanisms of loss
  - Replication loss
  - ROS with Mitochondria leading to replication stress
  - Also considered, somatic mutation and radiation mutation (terminal deletion)
- Missing data
  - ROS vs PD
  - Mutation rates
  - Additive vs Multiplicative Mechanisms?
- For astronauts telomere length over career (entry, pre- and post-flights) of interest



Telomere Associated γH2AX foci



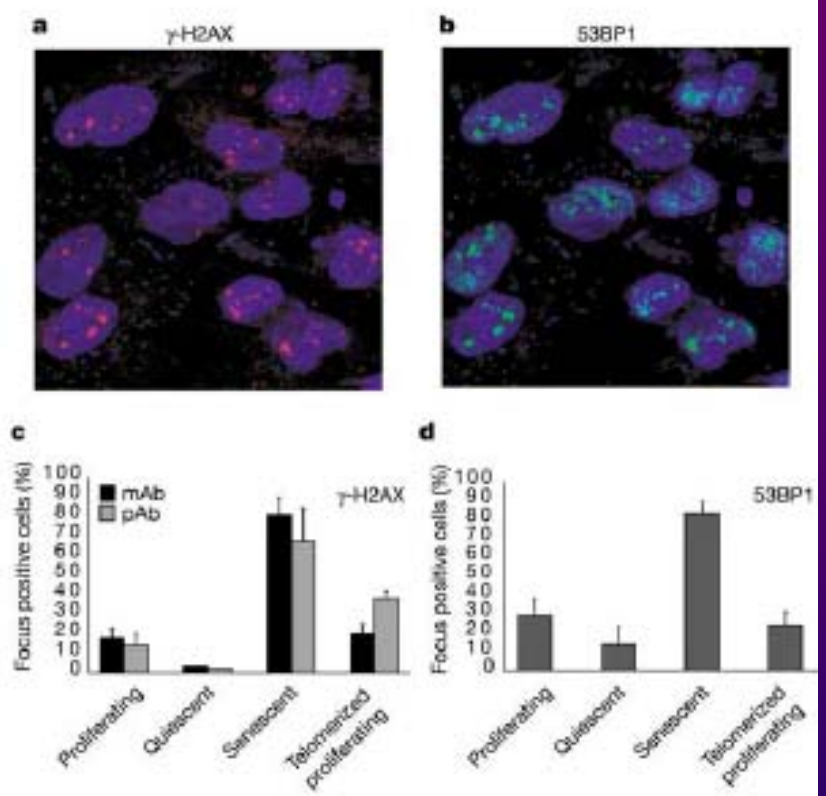


Figure 1 A DNA damage response in senescent cells. a, b, γ-H2AX and 53BP1 foci in

A DNA damage checkpoint response in telomere-initiated senescence

Fabrizio d'Adda di Fagagna[1]*, Philip M. Reaper[1], Lorena Clay-Farrace[1], Heike Fiegler[2], Philippa Carr[2], Thomas von Zglinicki[3], Gabriele Saretzki[3], Nigel P. Carter[2] & Stephen P. Jackson[1]

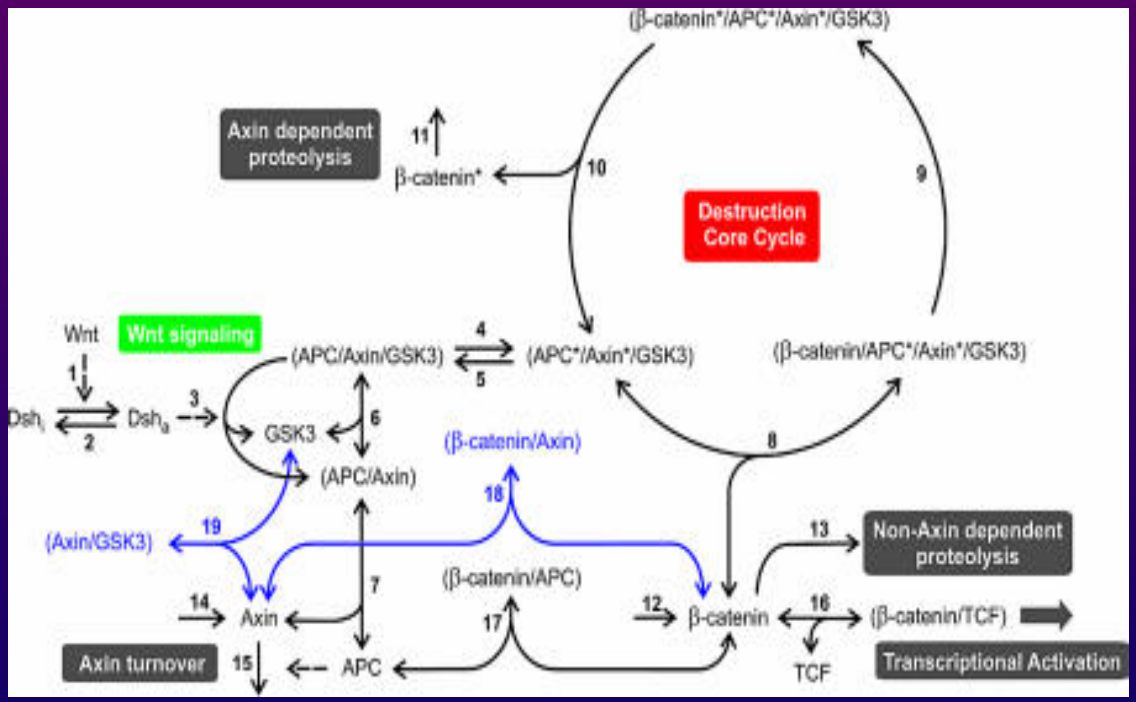
Systems Biology and Radiation Effects

- Systems biology "the Science that discovers the principles underlying the emergence of functional properties of living organisms from interactions between macromolecules" -Alberghina and Westerhoff (Topics in Current Genetics, 2005)
- Radiation presents several classes of substrates that perturb biological systems
  - Simple and complex DNA damages
  - Intra- and extra-cellular oxidative damage
- Pathway modeling of interest for DSB repair, telomere regulation, etc related to cancer risk
- Useful concepts from systems biology of pathways
  - Flux and Control coefficients
  - Robustness

Control coefficients in WNT/β-Catenin Pathway

Metabolic control coefficients characterize effects of Enzymatic steps on a “Flux” (Kacser and Burns):

$C_k^F=(k/F)dF/dk$; $\Sigma C_k^F=1$



Lee et al. PLOS biology, vol 1

Table 3. Control Coefficients for the Total Concentrations of β-Catenin and Axin and Parameters Quantifying the Sensitivity and the Robustness of the Wnt/β-Catenin Pathway

	Parameter of step j	$C_j^{\beta cat}$	C_j^{axin}
Kinase/ phosphatase module	k_4	−0.89	0.50
	k_5	0.89	−0.50
β-catenin module	k_9	−0.89	−0.08
	k_{10}	$-(10^{-5})$	−0.10
	k_{11}	−0.03	0
	v_{12}	0.93	0.19
	k_{13}	−0.01	−0.01
Axin module	v_{14}	−0.89	0.82
	k_{15}	0.89	−0.82
Binding, dissociation	k_6, k_{-6}	±0.89	±0.74
	k_7, k_{-7}	±0.89	±0.79
	k_8, k_{-8}	±0.89	±0.02
	k_{16}, k_{-16}	±0.11	0
	k_{17}, k_{-17}	±0.08	±0.02
	σ	0.66	0.44
	ρ	0.60	0.70

The control coefcients (Equation 7) were obtained by numerical determination of the response to a change of the rate constants of all steps by 1%. Using relative changes of rate constants less than 1% does not lead to a signicant improvement of the precision of the C values. Coefcients are given for the reference state. Horizontal lines separate the coefcients for distinct modules of the pathway. The last block contains the coefcients for parameters that enter the systems equations as binding rate constants k_{+j} and dissociation rate constants k_{-j} via dissociation constants $K_j = k_{-j}/k_{+j}$. The upper signs of these coefcients refer to changes in k_{+j} and the lower sign to changes in k_{-j}. The sum of the control coefcients in each column is zero. Additional summation rules hold true for the rate constants within each module as well as for the two rate constants of each binding equilibrium. The standard deviation σ of the concentration control coefcients and the robustness ρ for β-catenin and axin are calculated by applying Equations (8) and (9).

DOI: 10.1371/journal.pbio.0000010.t003

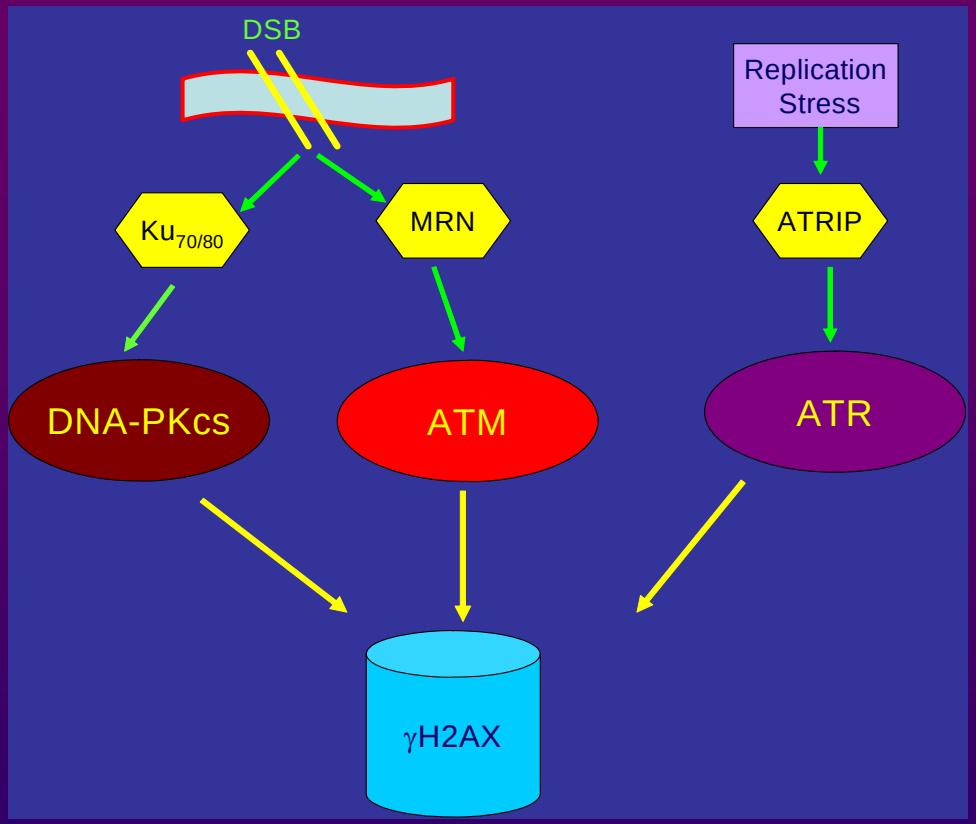
Table 4. Concentration Control Coefficients for the Total Concentrations of β-Catenin and Axin Relative to Changes in the Concentrations of Pathway Components

	$W = 0$		$W = 1$	
	$C_j^{\beta cat}$	C_j^{axin}	$C_j^{\beta cat}$	C_j^{axin}
APC	−0.83	0.79	−0.87	0.52
GSK3β	−0.89	0.74	−0.94	0.40
PP2A	0.89	−0.50	0.94	−0.30
TCF	0.20	0	0.07	0
Dsh	0	0	0.78	−0.33
β-catenin	1.00	0.20	1.00	0.44
Axin	−1.08	1.00	−1.59	1.00

The control coefcients were obtained by numerical determination of the response to a change of total concentrations by 1%. Coefcients are given for the reference state and for the standard stimulated state.

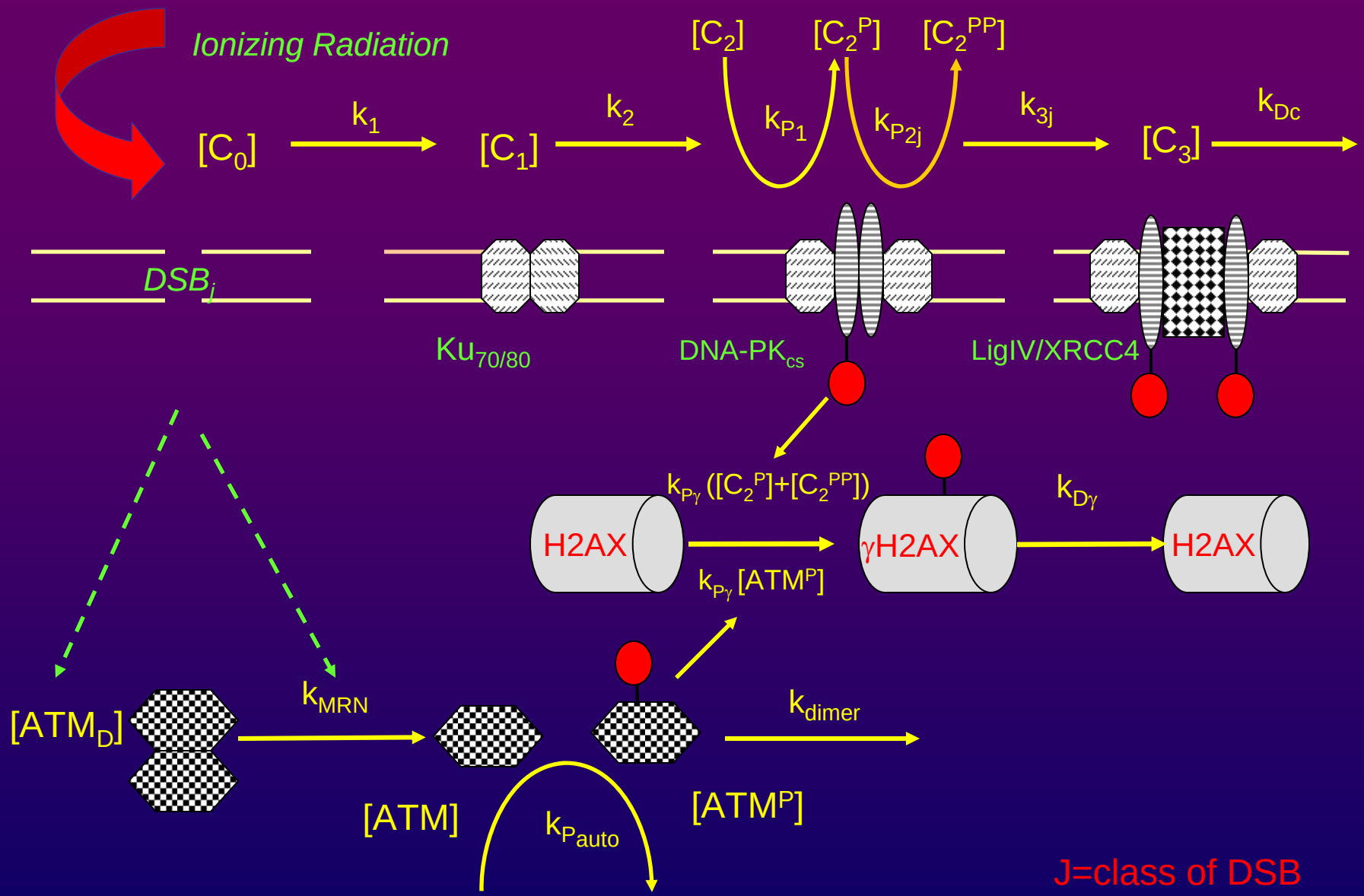
Multiple Mechanisms for γH2AX Activation

- There are multiple mechanisms of γH2AX involved in both early radiation responses and pre-neoplasia DDR
  - Family of PIKKs and acting on DSBs
  - Replication stress
  - Chromosomal aberrations or interstitial deletions?
  - Uncapped telomeres (signal many other DDR response proteins)



Members of the phosphoinositide 3 kinase-related protein kinase (PIKK) family activate γH2AX in an overlapping manner. γH2AX is an important indicator of early and residual damage; however not all of the downstream controls have been elucidated.

NHEJ Reaction Pathway



J=class of DSB

Systems Equations for NHEJ

$$(1)\quad \frac{d[C_0]}{dt} = \alpha\frac{dD}{dt} - k_1[Ku_{70/80}][C_0]$$

$$(2)\quad \frac{d[C_1]}{dt} = k_1[C_0][Ku_{70/80}] - k_2[DNAPK_{cs}][C_1] \qquad (3)\quad \frac{d[C_2]}{dt} = k_2[DNAPK_{cs}][C_2] - k_{P_1}[C_2]$$

$$(4)\quad \frac{d[C_2^{\,P}]}{dt} = k_{P_1}[C_2] - k_{P_2}[C_2^{\,P}] - k_{res}[C_2^{\,P}] \qquad (5)\quad \frac{d[C_2^{\,PP}]}{dt} = k_{P_2}[C_2^{\,P}] - k_3[LigIV][C_2^{\,PP}]$$

$$(6)\quad \frac{d[DSB_{res}]}{dt} = k_{res}[C_2^{\,P}] \qquad (7)\quad \frac{d[C_3]}{dt} = k_3[LigIV][C_2^{\,PP}] - k_{D_C}[C_3]$$

γH2AX Foci

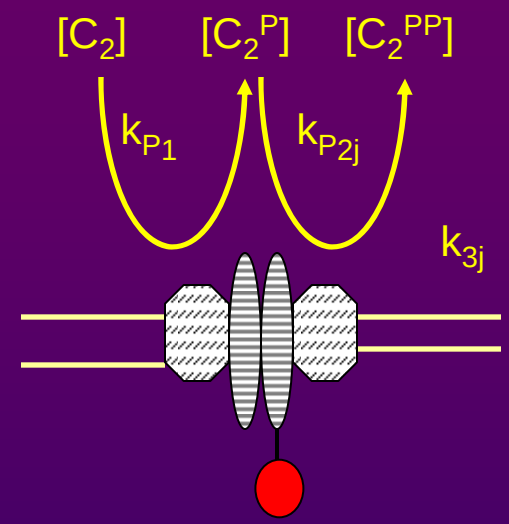
$$\frac{d\gamma(t)}{dt} = \kappa_{P_\gamma}[c_2^{\,P}(t) + c_2^{\,PP}(t) + c_{atm}^{\,P}(t)](1-\gamma(t)) - k_{D_\gamma}\gamma(t)$$

Rejoining Kinetics

$$DSB_{remaining}(t) = H_1[c_0(t) + c_1(t) + c_2(t) + c_2^{\,P}(t) + c_2^{\,PP}(t) + c_3(t)]$$

Ku induction (e.g. Live Cell Experiments)

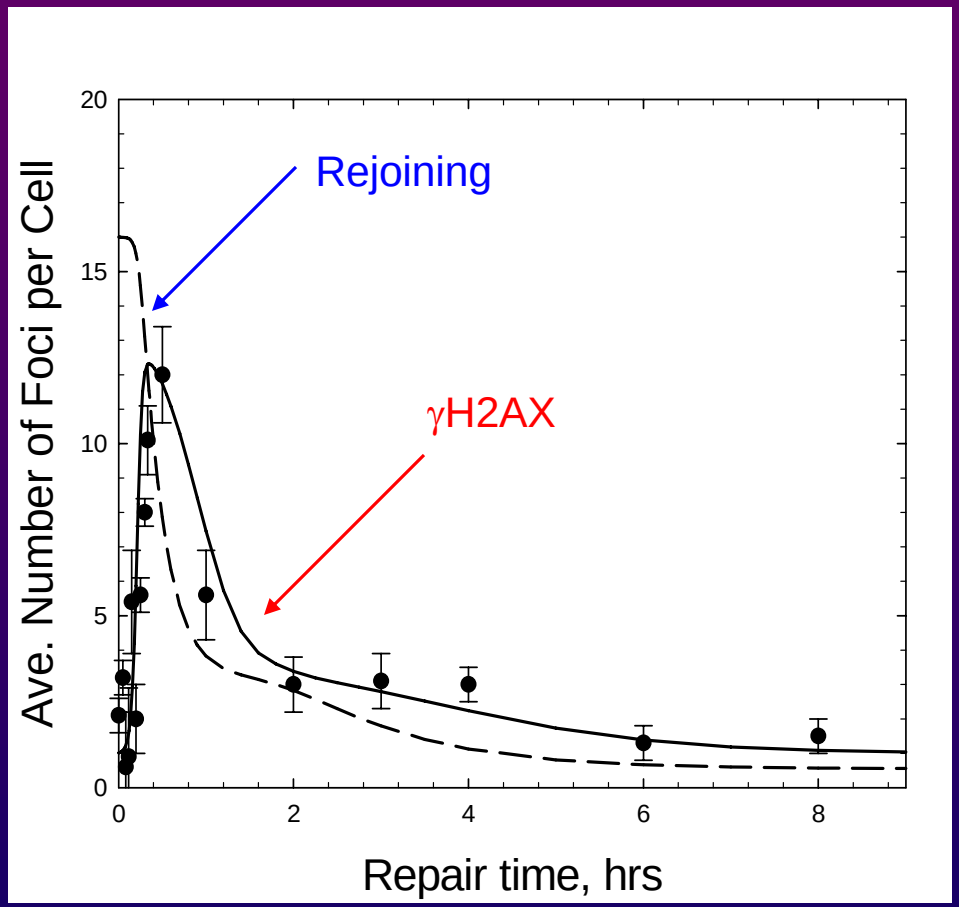
$$C_{Ku_{70/80}}(t) = H_1[c_1(t) + c_2(t) + c_2^{\,P}(t) + c_2^{\,PP}(t) + c_3(t)]$$



DNA-PK$_{cs}$ regulation dependent on the structure of DSB; however
Repair components modeled through DSB class specific rates for DNA-PKcs regulatory steps

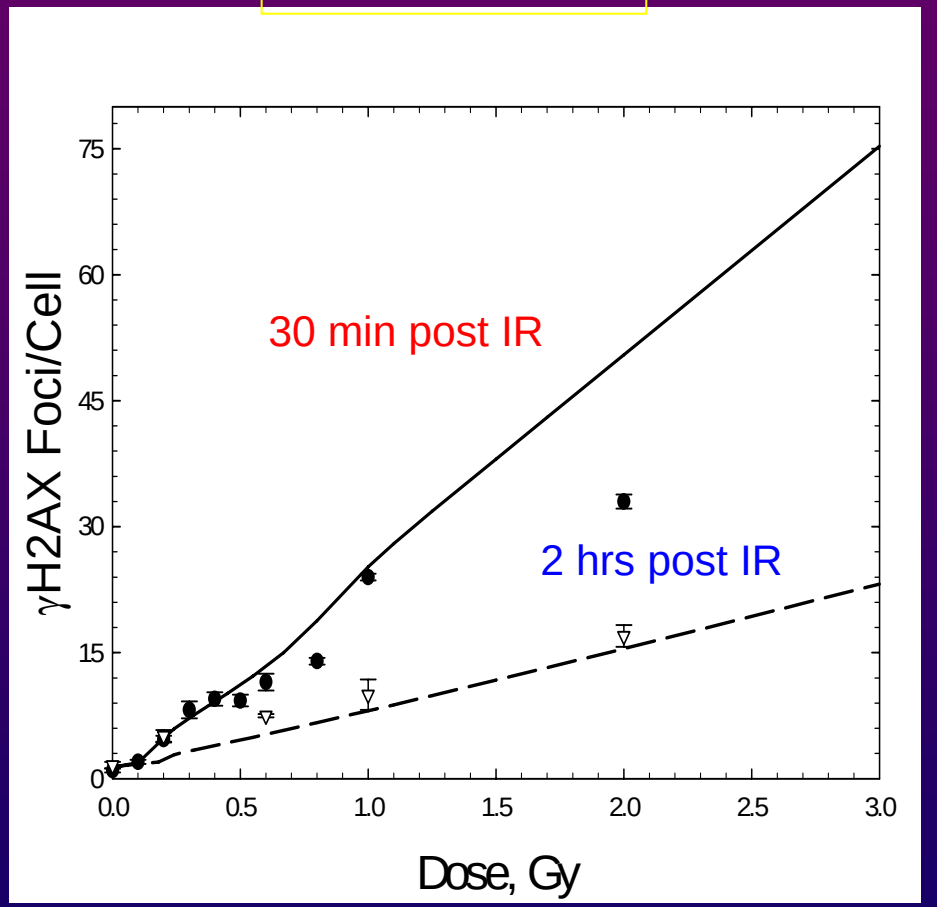
γH2AX Foci- Time Course and Dose Response

V79 Cells 1 Gy



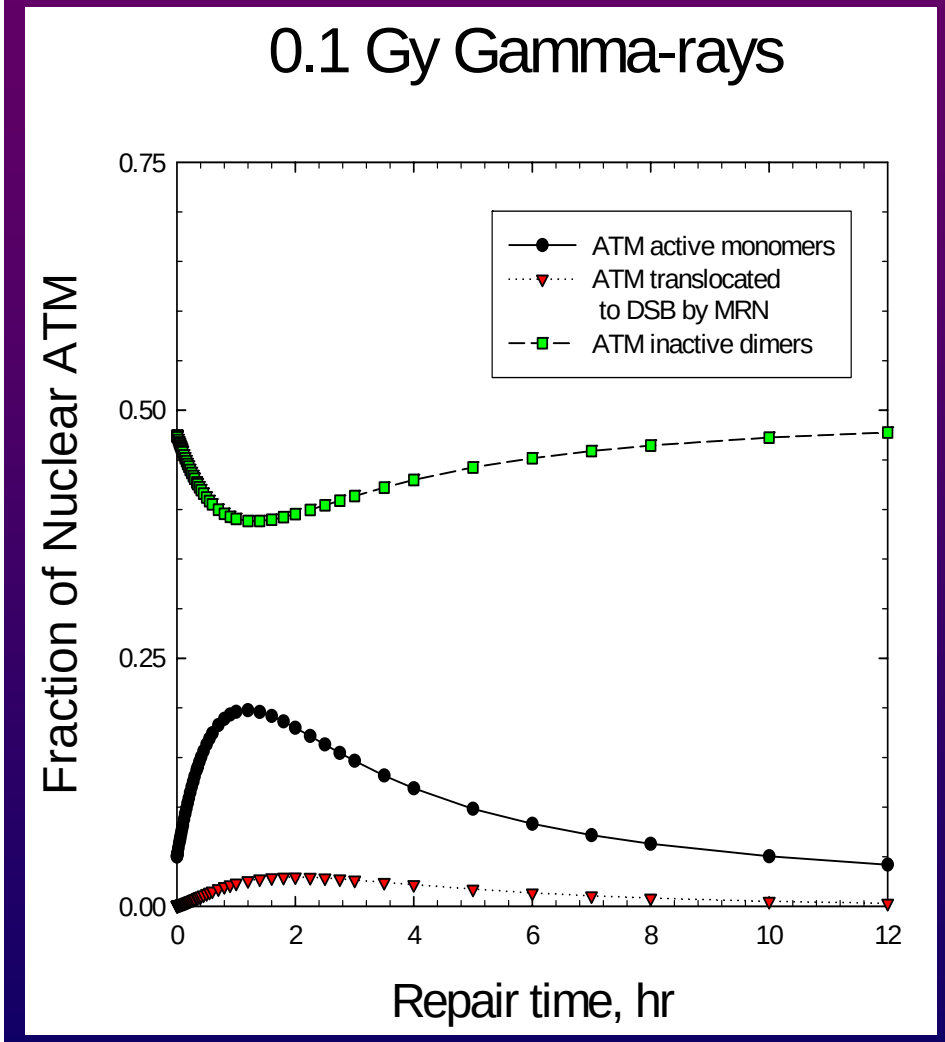
Expts of Leatherbarrow et al. (2006)

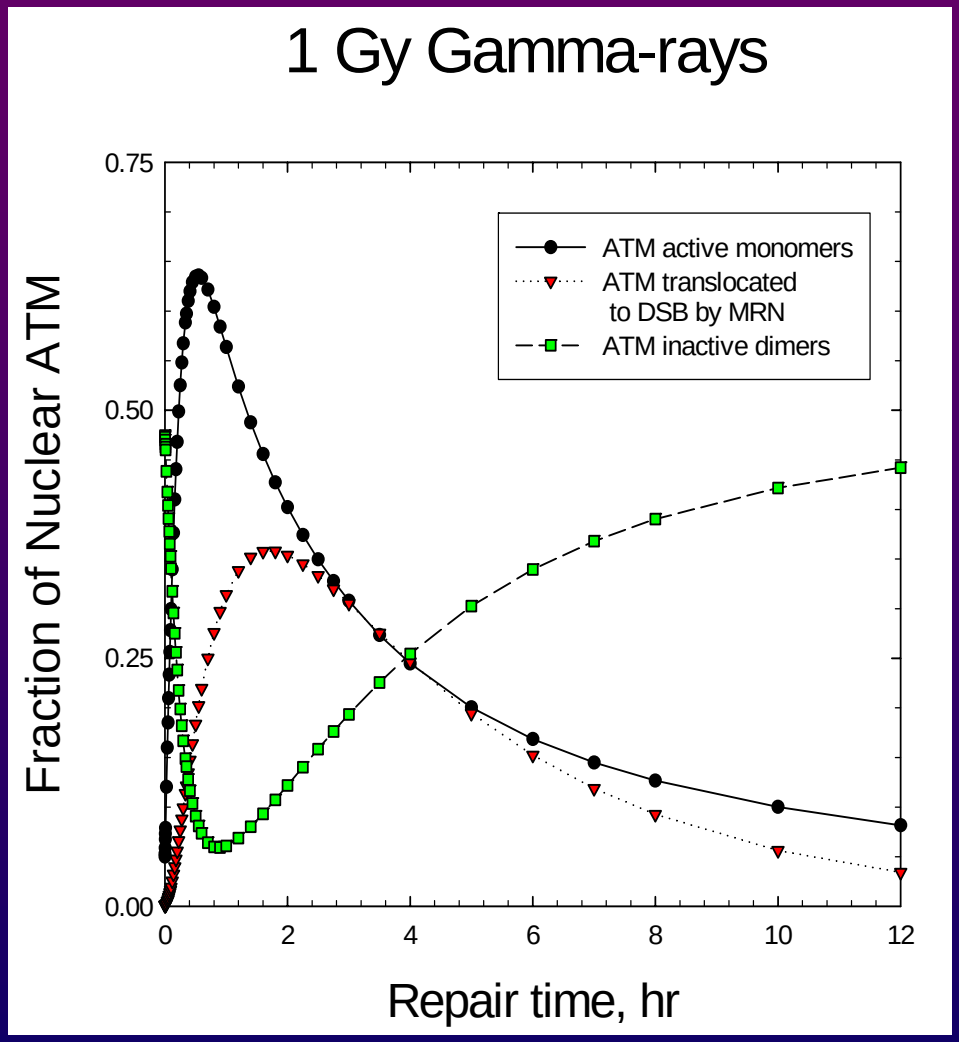
Human TG cells



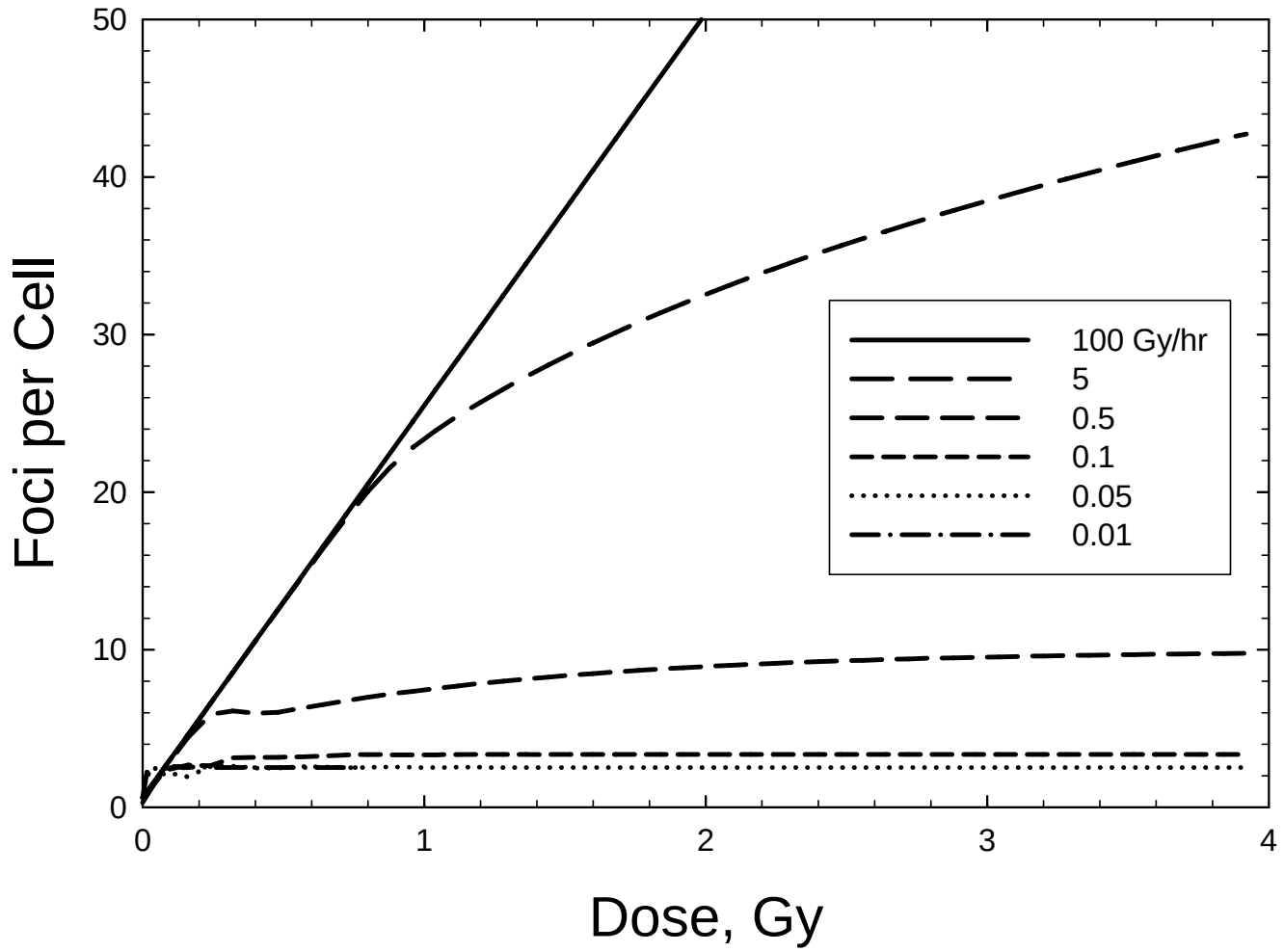
Expts of Short et al. Rad Res (2005)

ATM Activation and Translocation by MRN





Dose-Rates and Repair Foci



Residual foci
Measure of capability to Repair damage?

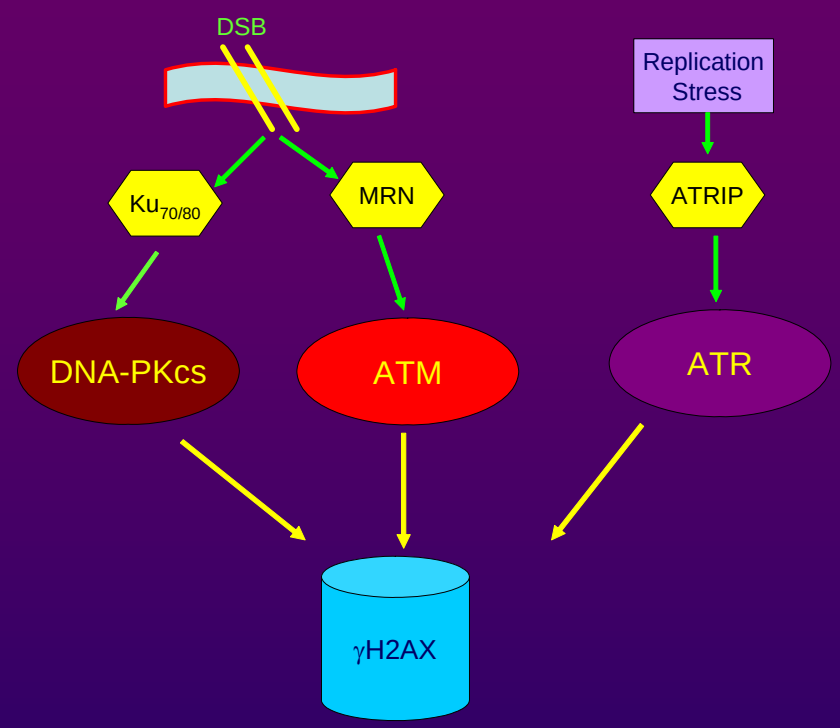
γH2AX Foci and System Responses

- Properties of gH2AX response curves have been associated with important biological outcomes
  - Radiation sensitivity
  - Genomic instability
- Signaling time and duration, measure γH2AX persistence for different radiation qualities/doses
- Signal time total amount kinase activated

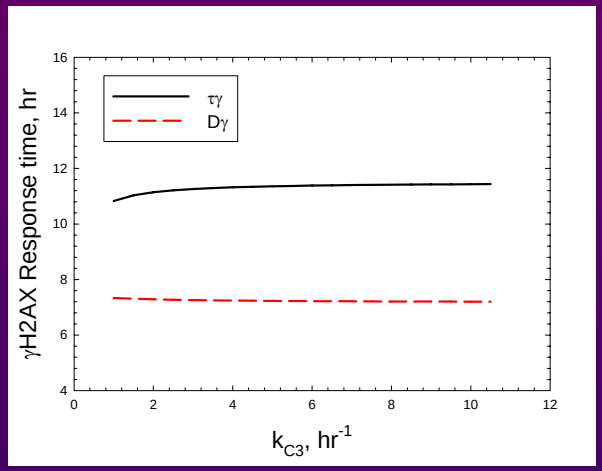
$$\tau_\gamma = \frac{\int t\gamma(t)dt}{\int \gamma(t)dt}$$

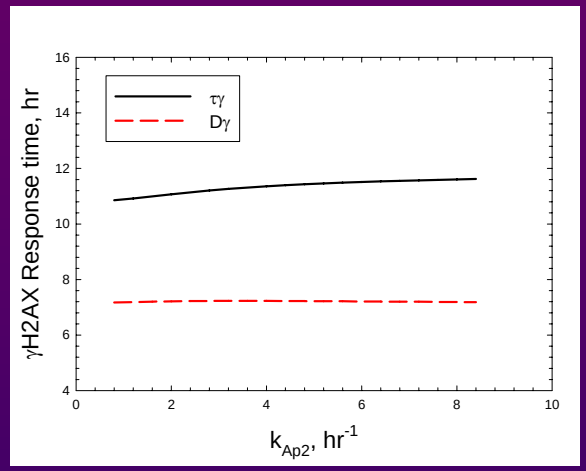
- γH2AX signal duration measure of variance about the mean

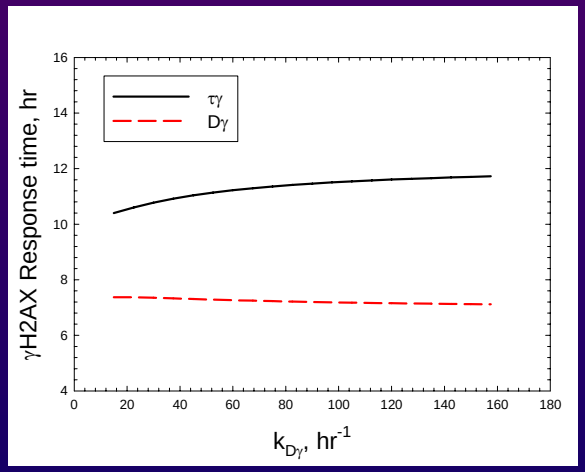
$$D_\gamma = \sqrt{Q_\gamma - \tau_\gamma^{\,2}} \quad with \quad Q = \frac{\int t^2\gamma(t)dt}{\int \gamma(t)dt}$$

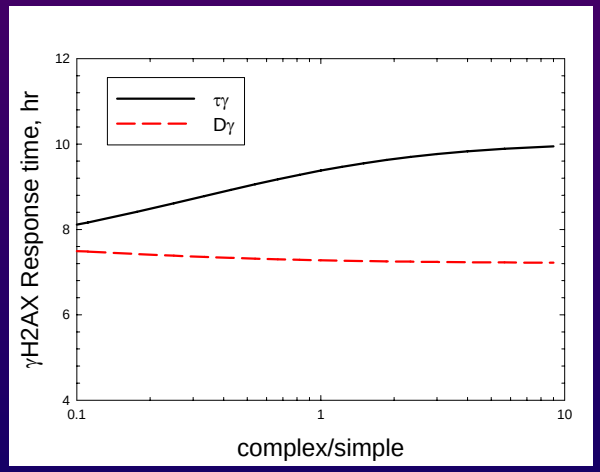


γH2AX response sensitivity (0.2 Gy)









Preliminary

Summary

Space radiation is a major challenge to exploration:

- Risks are high limiting mission length or crew selection
- Large mission cost to protect against risks and uncertainties

NASA approach to solve these problems:

- Probabilistic risk assessment framework for Exploration
- Systems biology approaches to integrate biodosimetry/biomarker data as new methods become feasible and are integrated into Lunar and Mars programs