Basics of Radiation Biology

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Overview

Radiation damage to cells

- **DNA**

Effects of radiation damage on cells

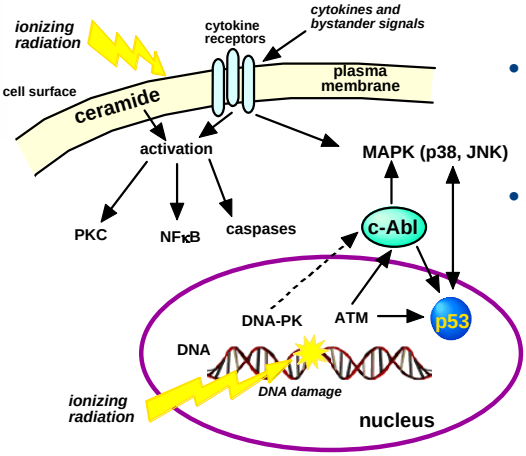
- **Cell cycle arrest**
- **DNA repair**
- **Cell death / apoptosis**

Detecting radiation damage

- **Cytogenetic assays**
- **Protein phosphorylation**
- **Changes in gene expression**
- **Changes in cellular metabolism**

Radiation causes cellular damage

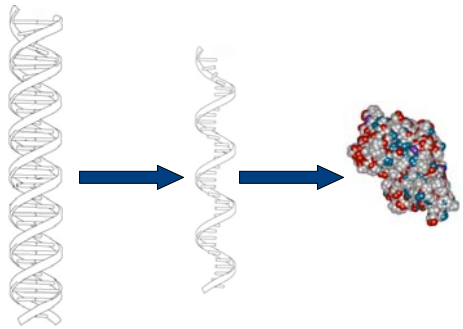
Ionizing radiation removes electrons from matter, causing molecular bonds to break.



- **Radiation damage can occur throughout the cell**
- **signaling cascades communicate radiation damage**

Radiation causes cellular damage

Radiation can damage any part of the cell, but most cellular and molecular components can be replaced.

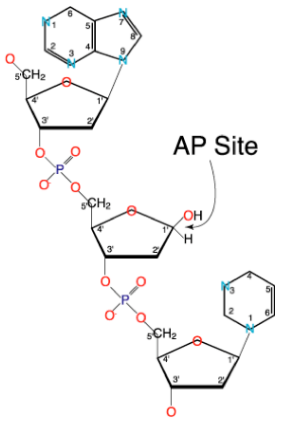


DNA **RNA** **Protein**

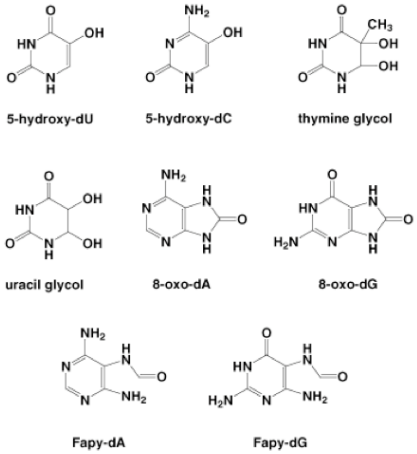
DNA damage is the most critical.
Need DNA to make everything else in the cell.

Types of radiation DNA damage

Abasic Site

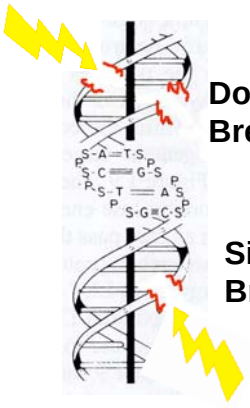


Oxidative Base Damage



Types of DNA damage cont.

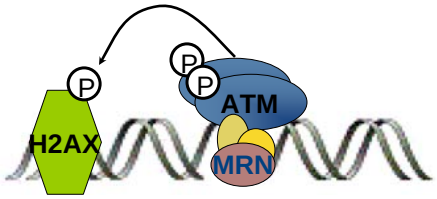
Double-strand breaks are thought to be responsible for most cell killing due to ionizing radiation



Double-strand Break (DSB)

Single-strand Break (SSB)

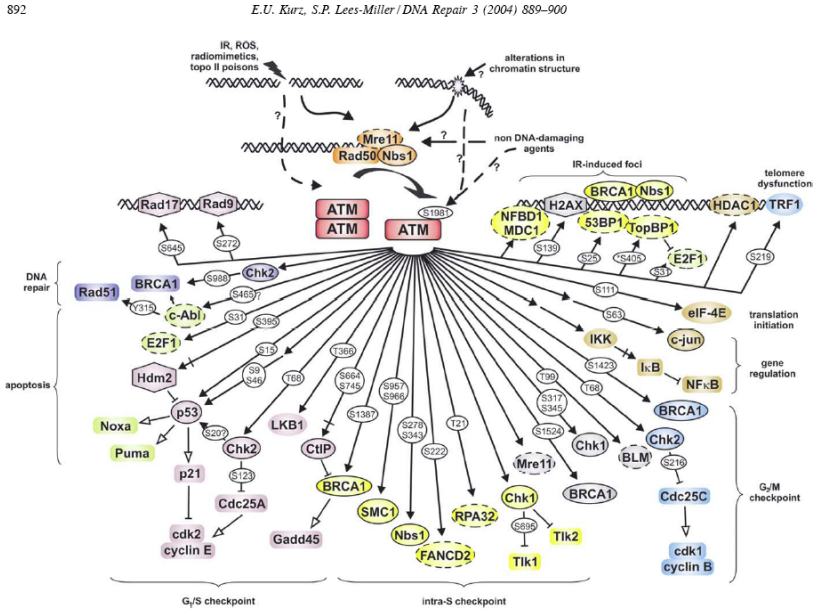
Cells can detect DSB



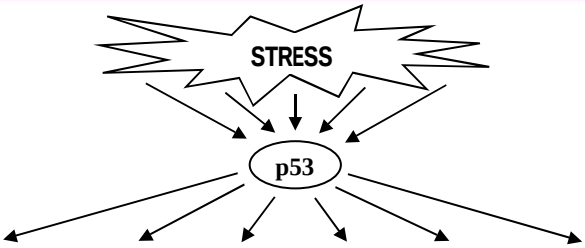
The **MRN** complex (Mre11, Rad50, Nbs1) recruits and activates ATM, which initiates damage signaling and DNA repair.

Ku70/80 also binds broken DNA ends, activates DNA-PKcs
Recruits other proteins to signal damage and initiate repair of the break.

Signaling from damage

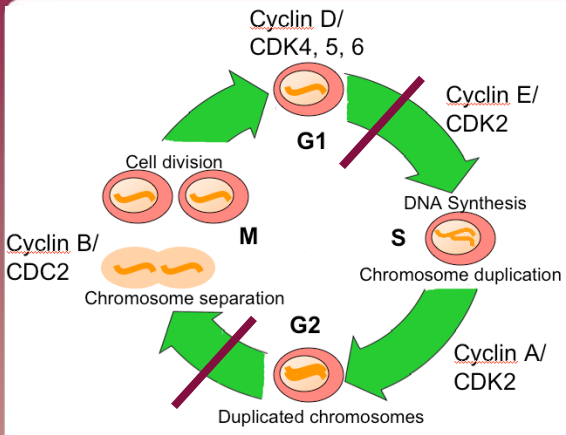


Some common p53-activated genes



Cell Cycle	Apoptosis	DNA Repair	Senescence	Anti-angiogenesis	Other
CIP1/WAF1	*BAX*	*XPC*	*CIP1/WAF1*	*VEGF*	*MDM2*
ClnG	*BCL-X*	*DDB2*	type I PACAP	*IL-8*	*FRA1*
ClnD1	*PAG608*	*GADD45A*		*HIF1α*	*ATF3*
WIP1	*FAS/APO1*	*PCNA*		*MMP2*	*14-3-3σ*
EGF-R	*KILLER/DR5*	*p53R2*		*BAI-1*	*Rb*
Rb	*TRUNDD*			*TSP1 & 2*	*c-MYC*
BTG2	*TRID*				*ninjurin*
PCNA	*SIAH*				*MAP4*
GADD45A	*IGF-BP3*				*amyloid*
14-3-3σ	*PIG1 to PIG14*				*PIR121*
TGF−α	*14-3-3σ*				*WIG1*
SIAH	*MAP4*				
IGF-ß	*TSP1 & 2*				
PIG1 to PIG14	*bFGF*				
inhibin-ß	*GML*				
GML					

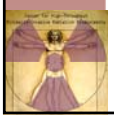
The mammalian cell cycle



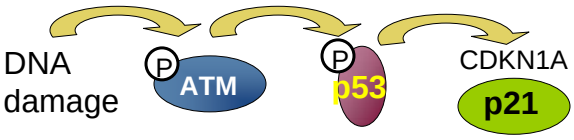
Cyclins: made and degraded each cell cycle

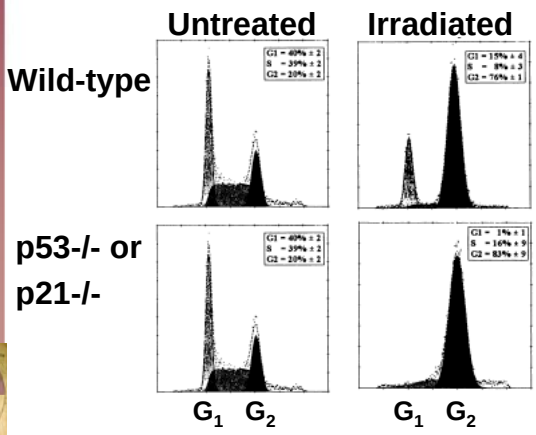
Cyclin-dependent kinases: drive cell division

Radiation exposure triggers checkpoints that halt cell cycle progression.



G1 arrest



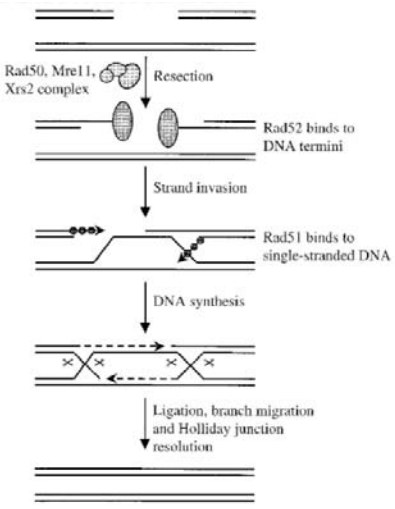


p21 binds to G1 cyclin/cdk complexes and inhibits kinase activity

Arrest can be transient or permanent

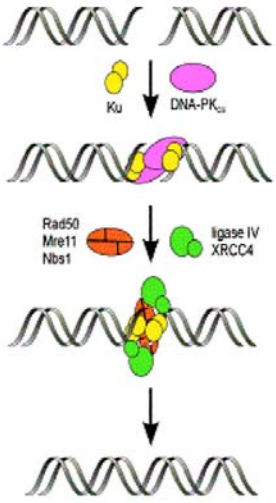
Repair of DSB

Homologous recombination



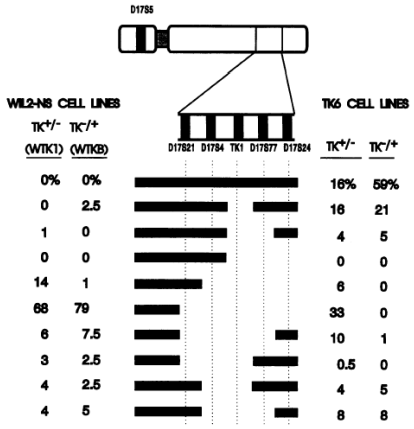
Most accurate

Non-Homologous End joining (NHEJ)



Most common

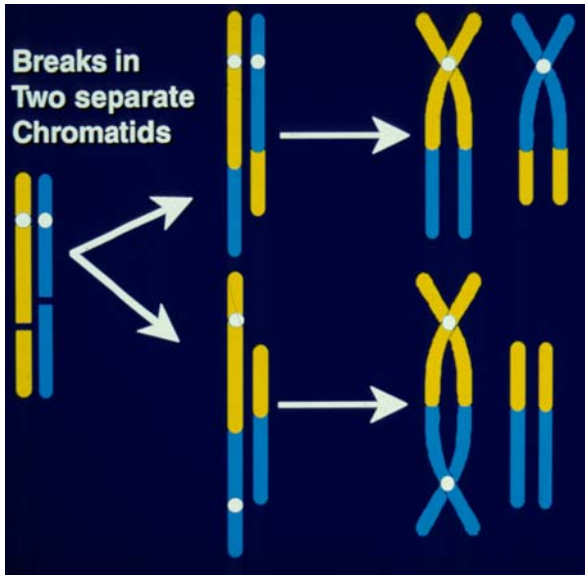
Incorrect repair - mutation



Hprt mutants per 10^6 cells

Mutation type	Spont.	2 Gy gamma
point	2.3	2.8
Partial gene del.	2.2	8
Total gene deletion	0.5	8.8
Multiple loci del.	.05	5.5

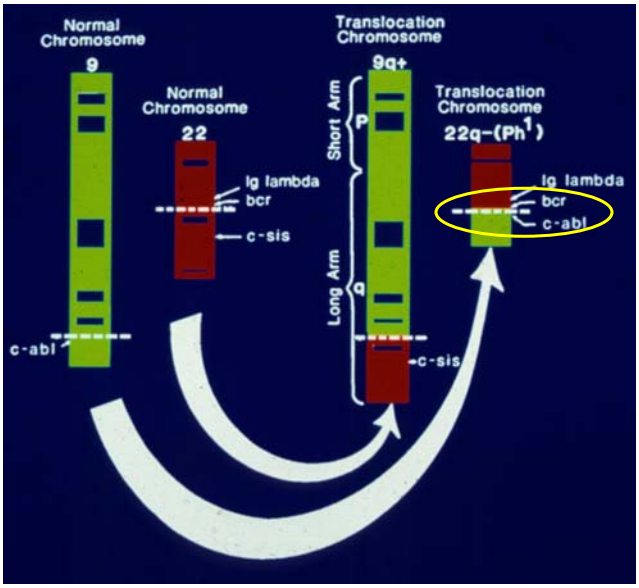
Incorrect repair - cytogenetic damage



Translocation: not lethal, but may activate an oncogene

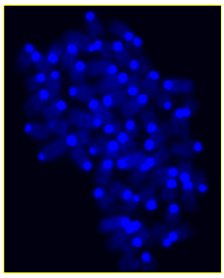
Dicentric and fragment: usually lethal

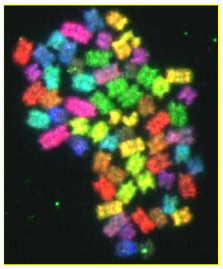
Translocation in Chronic Myeloid Leukemia



Multiplex FISH

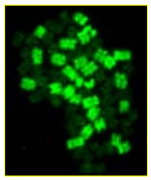
Paint each chromosome a different color



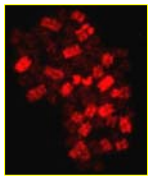


Combined

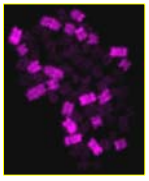
FITC



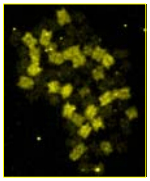
SPECTRUM O



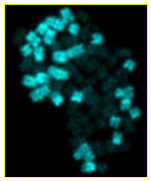
TEXAS RED



Cy5

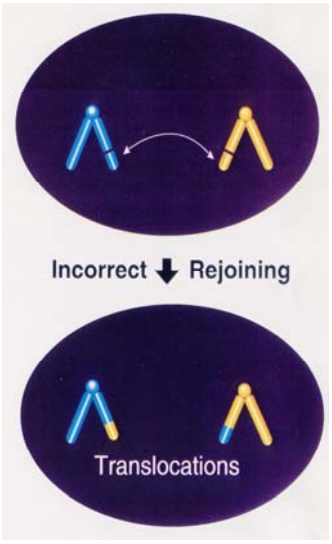


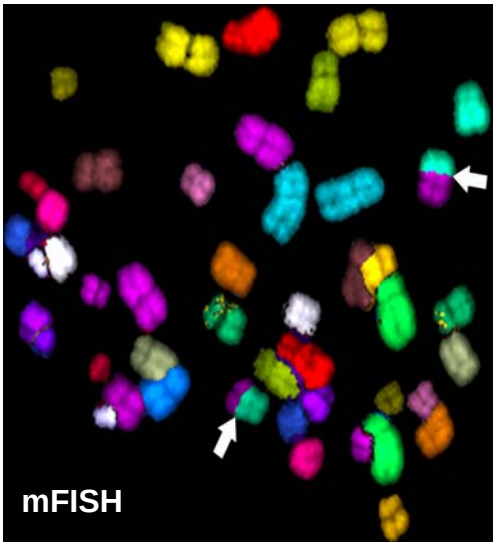
DEAC



"Two break" stable aberrations

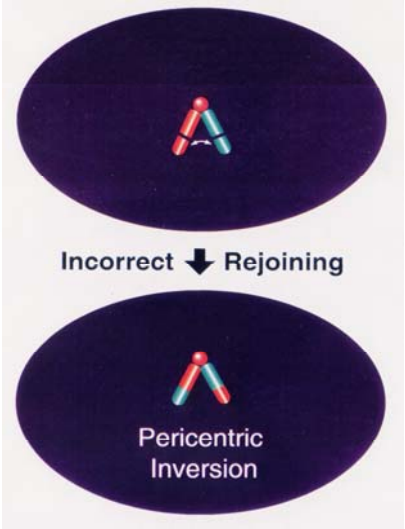
Inter-arm (translocation)

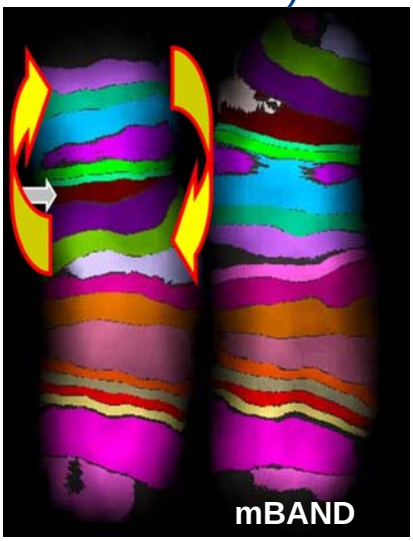




"Two break" stable aberrations

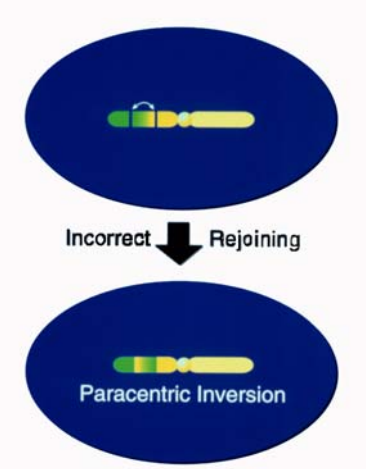
Inter-arm (pericentric inversion)

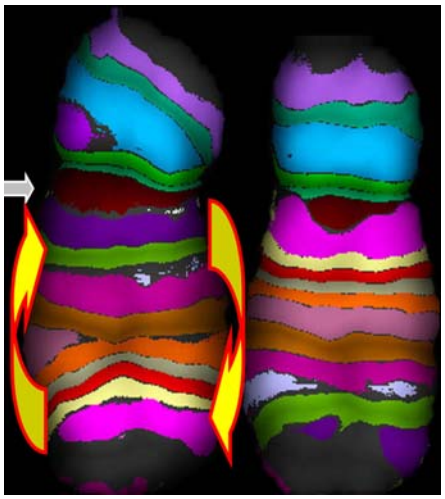




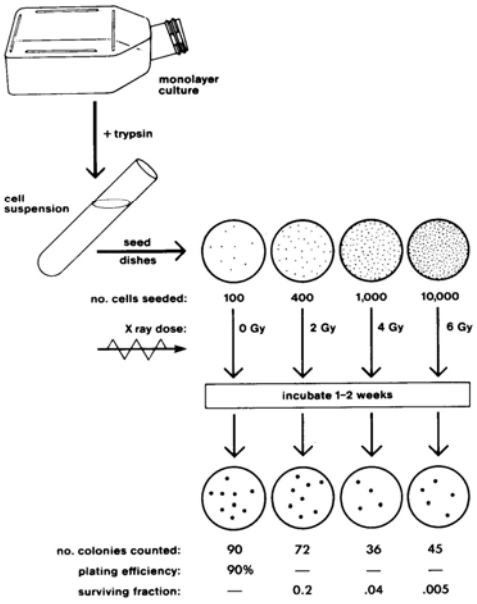
"Two break" stable aberrations

Intra-arm (paracentric inversion)

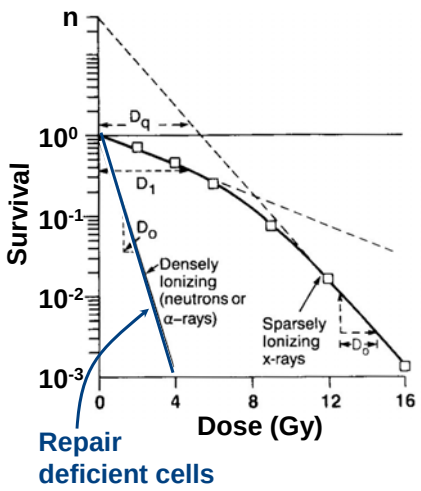




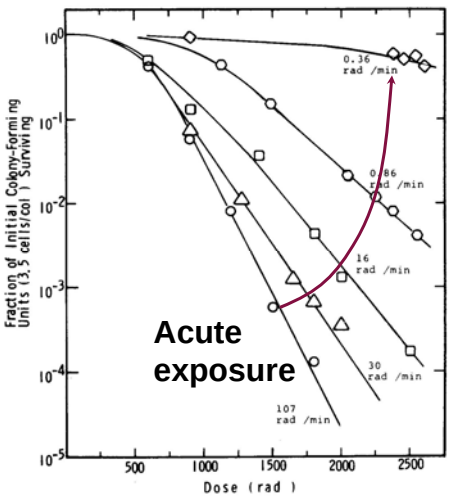
Cell killing - clonogenic survival



Radiation survival curves



Low dose-rate protects cells



Low
Dose-rate

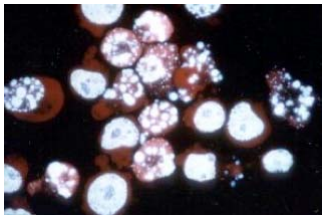
Acute
exposure

Cell killing by radiation

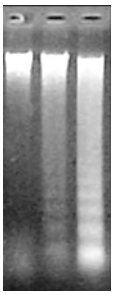
- Apoptosis
  Complex genetic program triggering cellular "suicide," or "programmed cell death."
- Necrosis
  Rapid depletion of ATP, breakdown of cell membrane, inflammation, nuclei shrink and condense, random degradation of DNA
- Mitotic catastrophe
  Abnormal mitosis with cytogenetic damage, conflicting signals, checkpoint failure

Hallmarks of apoptosis

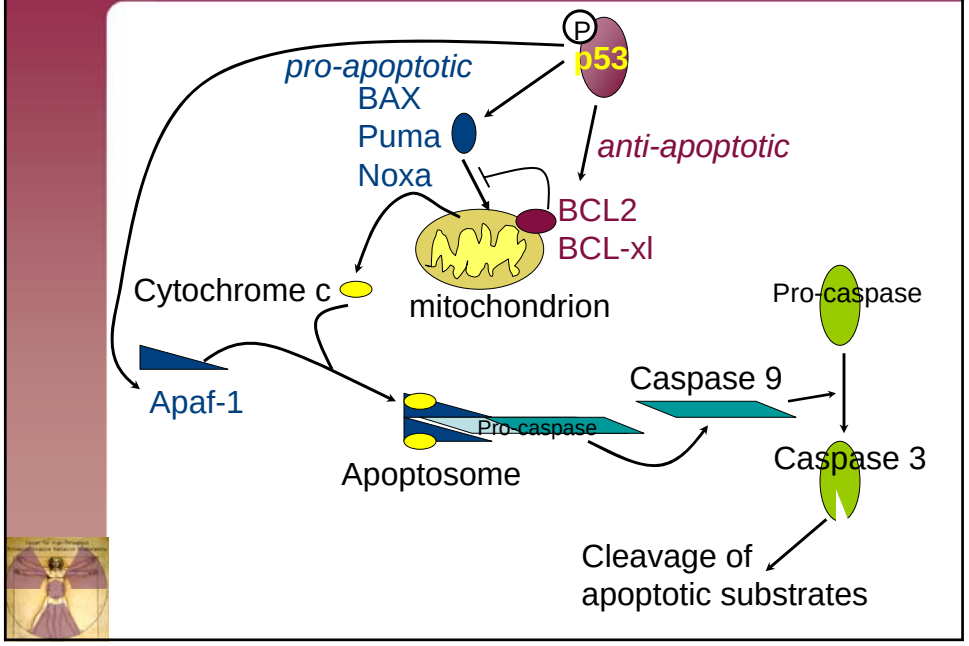
Programmed Cell Death



- Chromatin condensation
- Phosphatidylserine translocates from inner to outer cell membrane
- Loss of mitochondrial membrane potential
- Caspase activation, protein cleavage
- DNA laddering - nucleosome fragments



p53-dependent apoptotic pathway



Application to Biodosimetry

Cellular responses to radiation provide opportunities for biodosimetry.

- The larger the dose, the greater the biological response

Needed in the event of large-scale radiological event

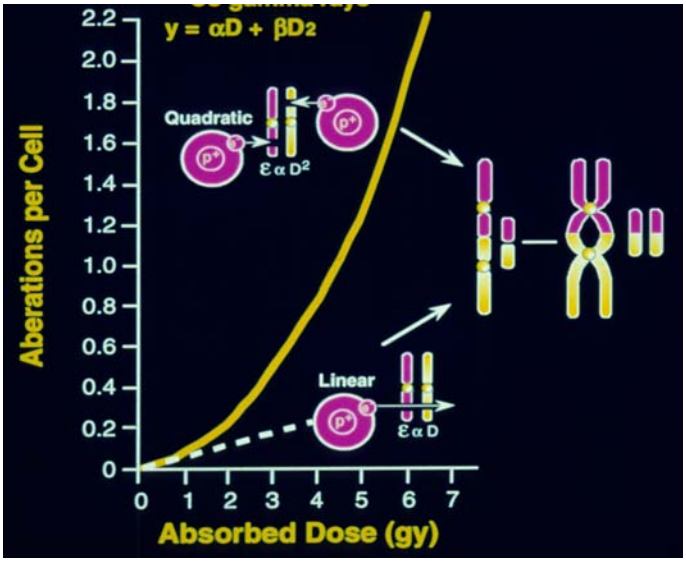
- Medical Triage
- Active reassurance - reduce panic

Detection of radiation damage to cells can be translated into an estimate of exposure

- Cytogenetics
- Protein phosphorylation
- Gene expression
- Metabolic changes

Cytogenetics - Dicentrics

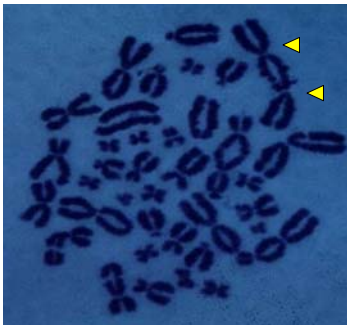
Assayed in peripheral lymphocytes



Cytogenetics - Dicentrics

"Gold standard" for radiation biodosimetry

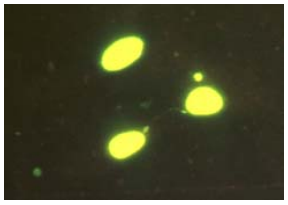
- Specific for *radiation* damage
- Stable to about 6 months after exposure
- Informative for doses 0.2-5 Gy
- Used for biodosimetry in many accidents (Chernobyl, Goiânia, Istanbul, Bangkok etc.)



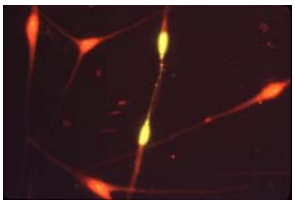
Cytogenetics - Micronuclei

Simpler assay with great automation potential

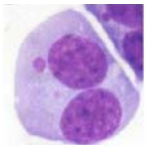
- Stable to about 6 months after exposure
- Informative for doses 0.3-5 Gy
- International standards for scoring

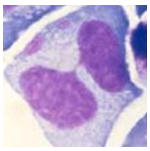


Micronuclei



Cytoplasmic bridges

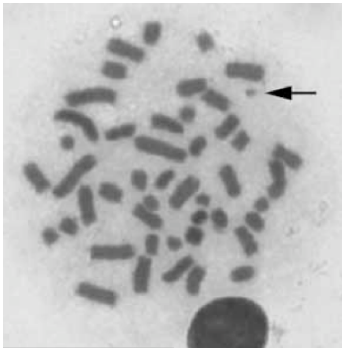


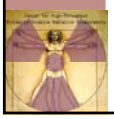


Cytogenetics - PCC

Premature Chromatin Condensation

- Informative for doses 0.2-10 Gy
- Potential for automation
- Without cell division
  - Requires fusion with mitotic cells to force condensation of chromatin
- With cell division
  - Condense chromosomes using Calyculin A

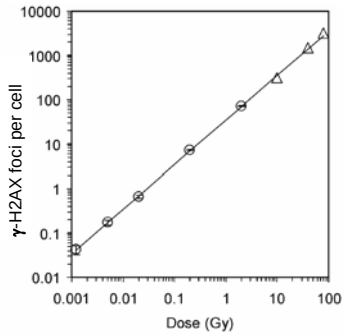


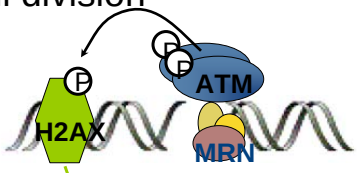


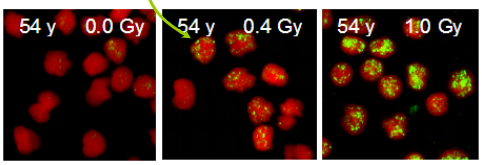
Protein phosphorylation

Phospho-γH2AX forms foci in irradiated cells

- Linear over broad dose range
- Informative for first day after exposure
- Can be automated for high-throughput
- does not require cell division





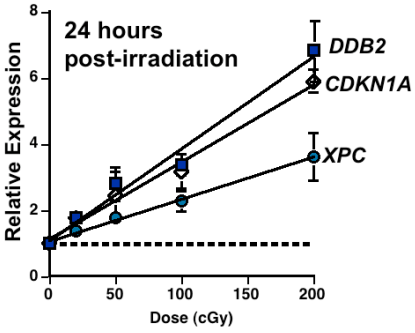


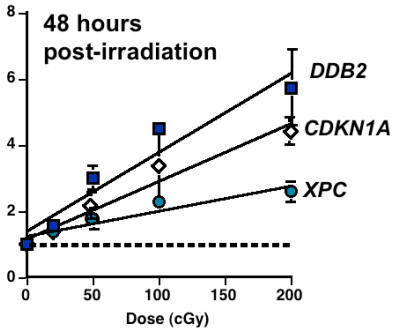
Rothkamm & Lobrich (2003)
PNAS 100:5057

Gene expression

Potential new approach

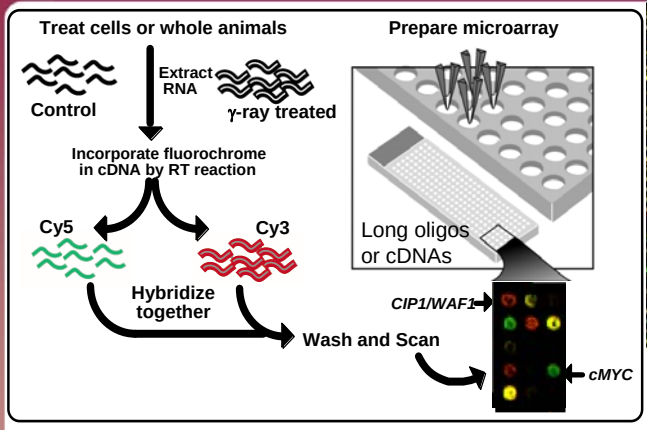
- Informative for doses 0.2 - 8 Gy
- Useful in first 2-3 days after exposure
- Specificity for radiation needs testing

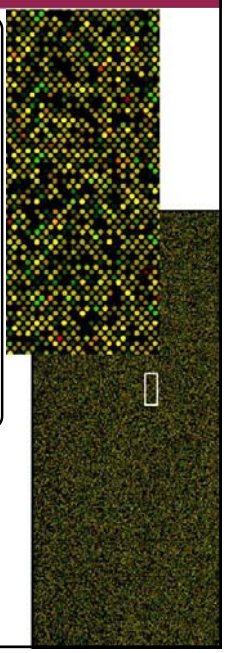




Amundson *et al*., (2000)
Radiation Research, 154 (3): 342-346

Gene expression





Screening with microarrays allows rapid discovery of potential radiation exposure markers

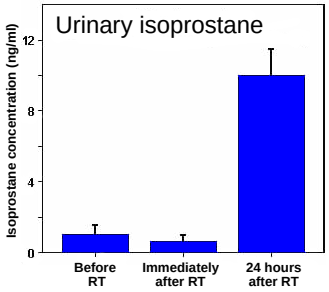
Gene expression

Advanced nanofluidics are being developed for self-contained “biochips” for rapid radiation dose assessment in emergencies

Metabolomics

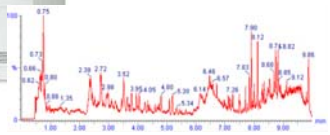
Potentially most rapid and least invasive

- Cellular changes in response to radiation result in changes in metabolism
- Results in changes in small molecules secreted in urine, saliva, sweat etc.
- Specificity for radiation specificity and dose dependence need testing



Metabolomics

Marker discovery and testing using UPLC-MS(TOF)



Current technology could easily be adapted to rapidly screen for a radiation signature

Summary of biological effects

- Radiation causes damage to all cellular molecules, but DNA damage is most critical
- DNA damage starts signaling cascades that result in
  - Cell cycle arrest
  - DNA repair
  - Apoptosis or other cell death
- Radiation damage can be detected by
  - Cytogenetics
  - Changes in gene expression
  - Changes in protein expression or phosphorylation
  - Changes in metabolic products