Chapter 2 – Lecture 2

Molecular Mechanisms of DNA and Chromosomal Damage and Repair

9/12/2024

Repair Pathway Cheat Sheet

Repair Pathway	Major Players	Major Lesions Repaired
BER **Base Excision Repair**	Glycosylase Endonuclease XRCC1	Oxidative damaged bases
NER **Nucleotide Excision Repair**	XPA-XPG	UV pyrimidine dimers “Bulky” lesions
MMR **Mismatch Repair**	MSH2, MLH1	DNA replication error (Mismatches, Frameshifts)
NHEJ **Non-Homologous End Joining**	Ku70, Ku80, DNAPKcs	DNA double strand breaks (Error prone)
HRR **Homologous Recombination Repair**	ATM, RAD51, MRN	DNA double strand breaks (Error free)
Cross-Link Repair	FANC Proteins	DNA interstrand cross links

What's Left?

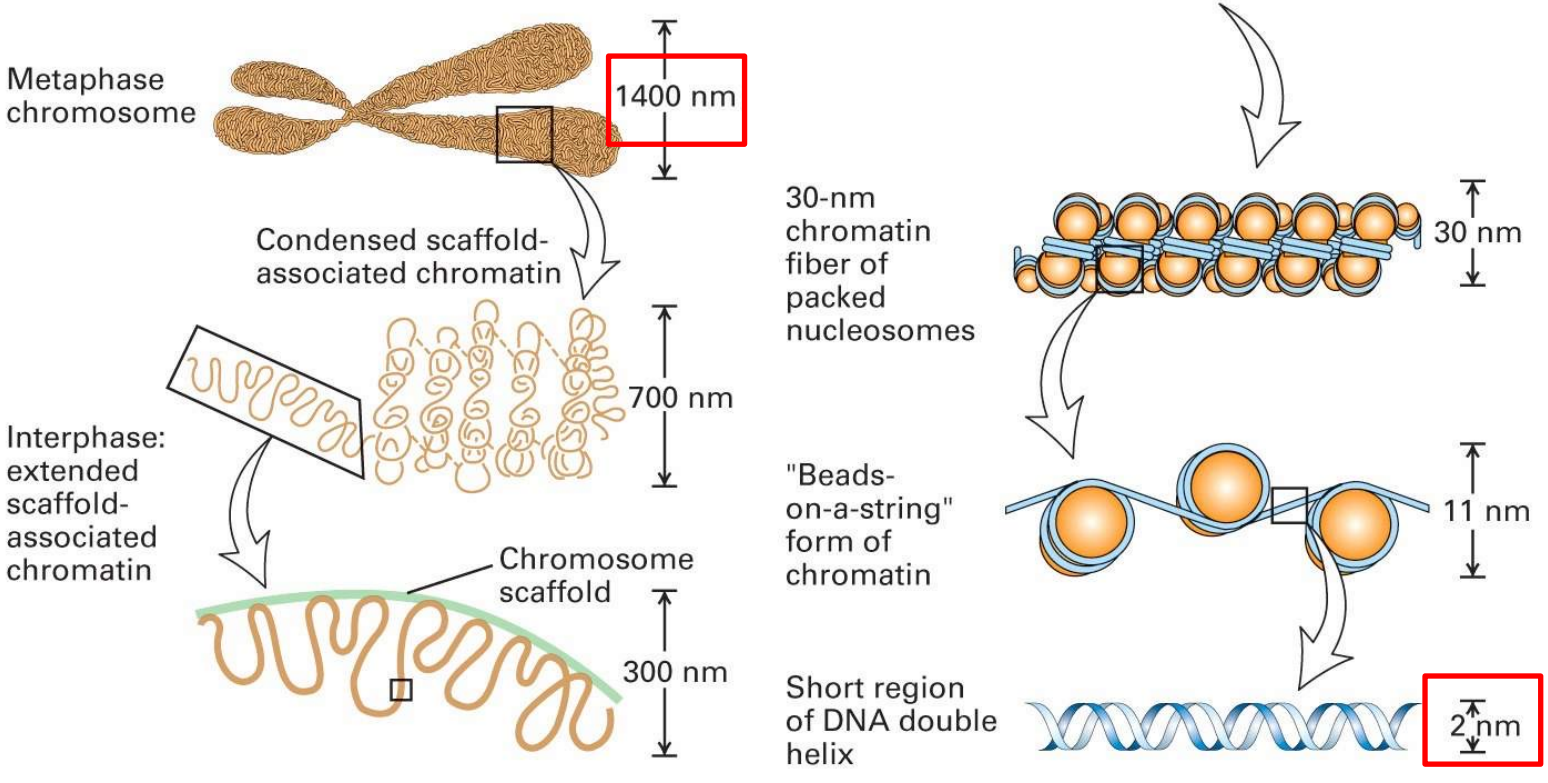
- Once all that can be repaired has been repaired, what's left?
  - … the residual damage (unrepaired and mis-joined), that can lead to mutations and chromosome aberrations
- Small mutations may or may not have consequences, but **larger chromosome aberrations** are *usually* **fatal** to the cell

The rest of this lecture will focus on the types of **chromosome aberrations** induced by ionizing radiation

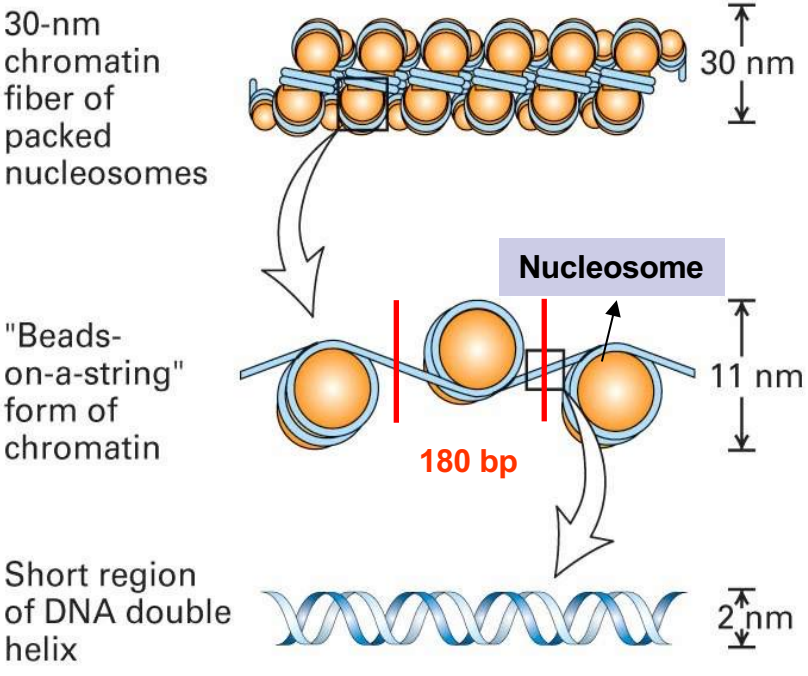
Outline

- General Overview of DNA Strand Breaks
- Measuring DNA Strand Breaks
- DNA Repair Pathways
- **Chromosomes and Cell Division**
- Radiation-Induced Chromosome Aberrations
- Chromosome Aberrations in Human Lymphocytes

Organization of DNA



Organization of DNA

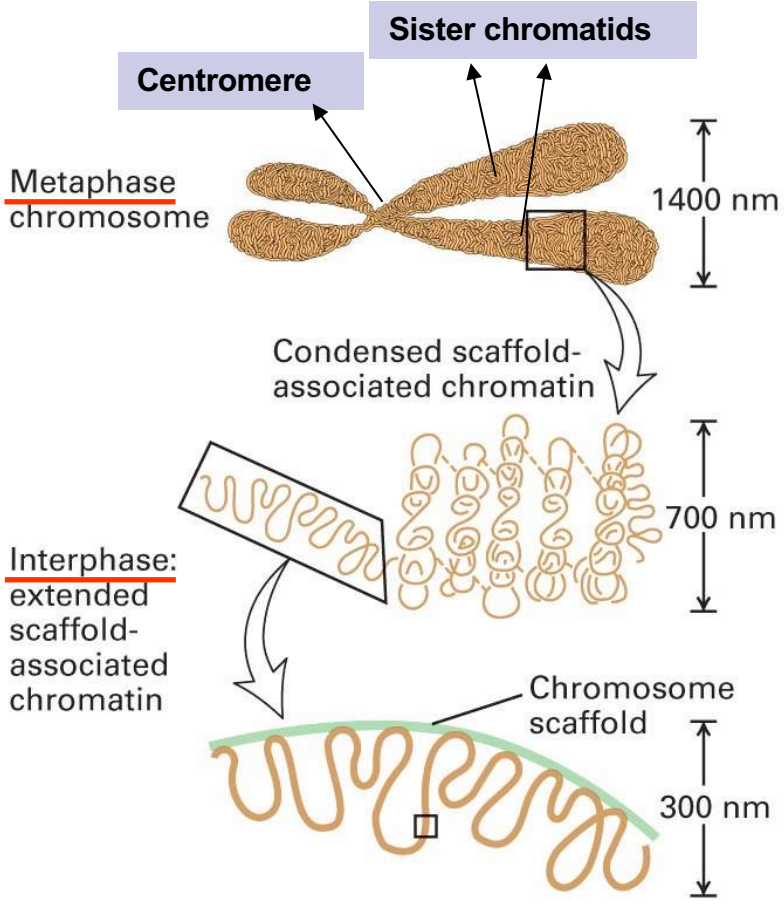


Nucleosomes are compacted together to form chromatin fibers

DNA strand is wrapped around histones
Each unit is called a nucleosome

Double helix DNA

Organization of DNA

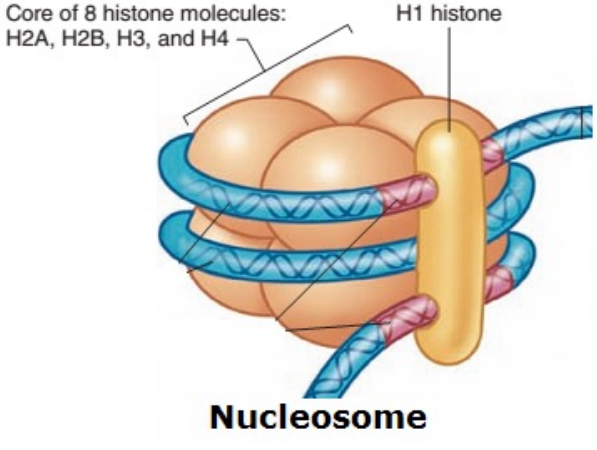


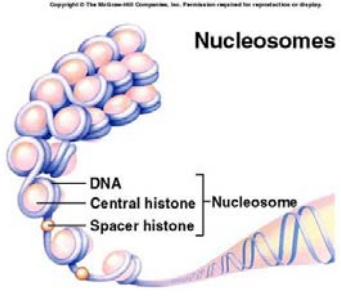
During mitosis, looped domains condense into chromatids
The chromatids are joined by a **centromere** which attach them to the mitotic spindle

Interphase chromatin are uncondensed and lightly stained and relatively active = **Euchromatin**

The chromatin fibers fold together into large **looped domain**
Each is ~ 300 kbp long, ~ 10 genes
Each domain form some sort of functional unit

Nucleosome

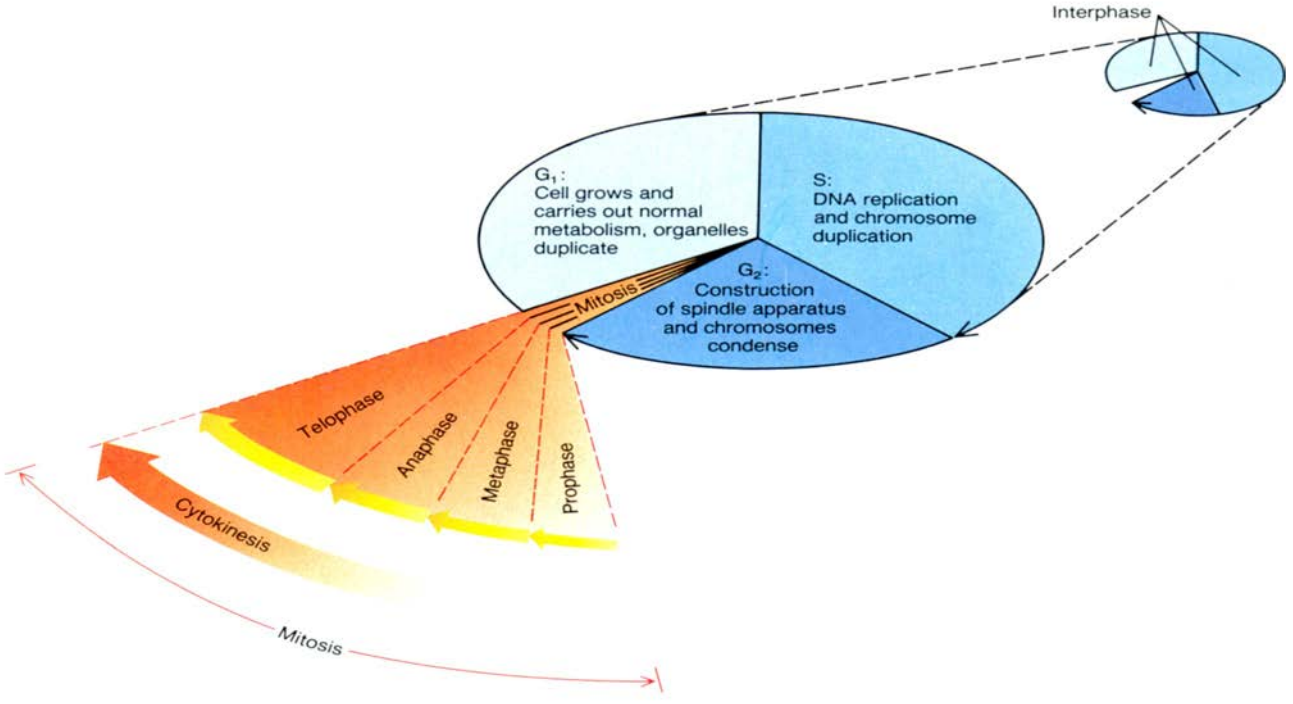




- The nucleosome core particle contains 2 copies of each histone protein (H2A, H2B, H3 and H4)
- 146 bp of DNA wrap around this histone octamer
- An external 9th histone (H1, linker histone) is added to hold the nucleosome structure together
- Represents the 1st order of DNA packaging in the nucleus

The Cell Cycle

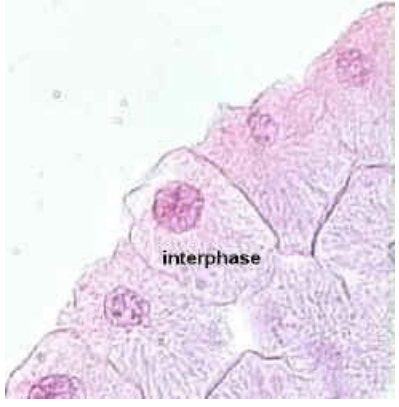
Interphase



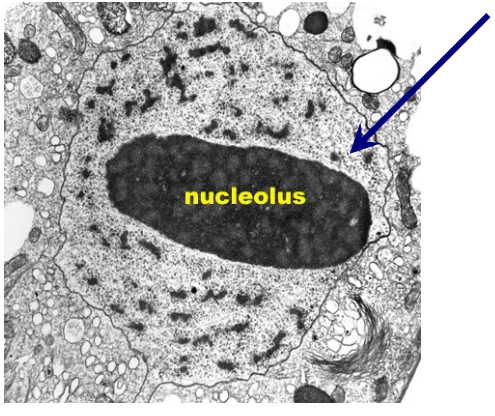
Mitosis

Interphase

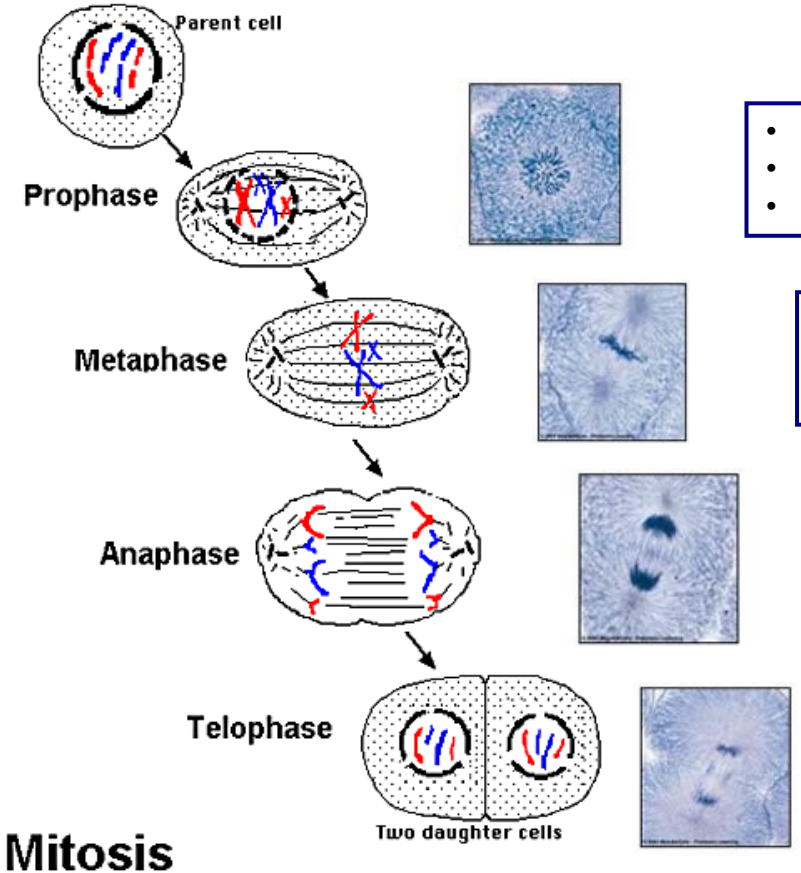
- Interphase is the phase of the cell cycle in which the cell spends the majority of time
- During interphase, DNA appears as a lacework of fine and lightly stained material
- **Nucleoli** can be seen
- DNA replication occurs during interphase and cell actively prepares itself for division



Nucleoli are sites of ribosome assembly



Mitosis



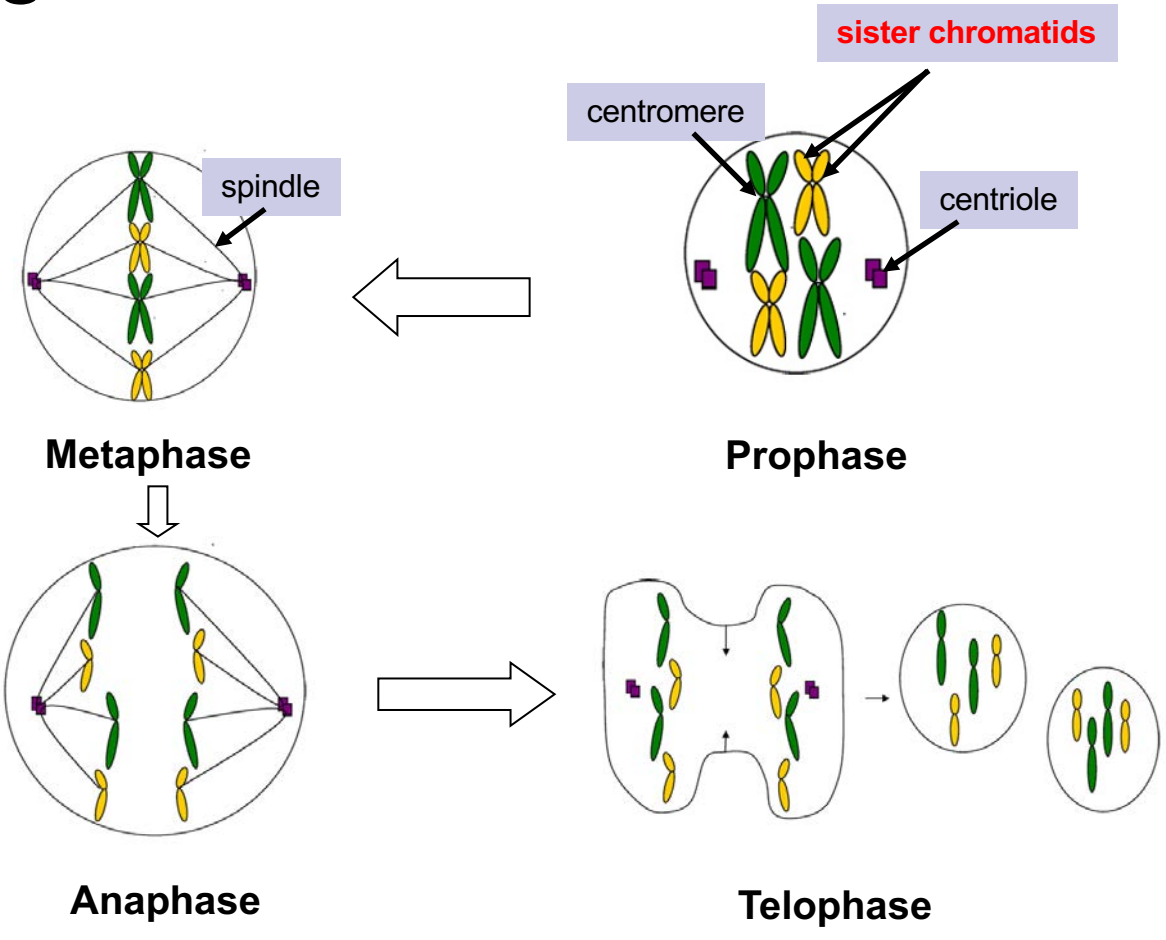
- Chromosomes condense
- Centromere appears
- Nuclear membrane & nucleoli disappear

- Chromosomes move to the equator
- Spindle forms
- Centromeres divide

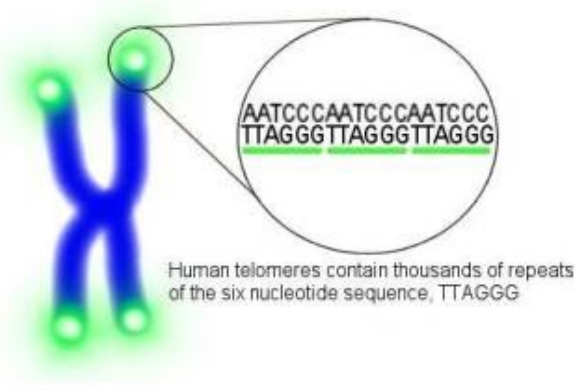
- Chromosomes move to the poles

- Chromosomes uncoil
- Nuclear membrane & nucleoli reappear

Mitosis


sister chromatids
centromere
centriole
spindle
Metaphase
Prophase
Anaphase
Telophase

Telomeres



Telomere length has been described as the “molecular clock”.

A **telomere** is a region of repetitive DNA at the **end of chromosomes**, which protects the end of the chromosome from destruction

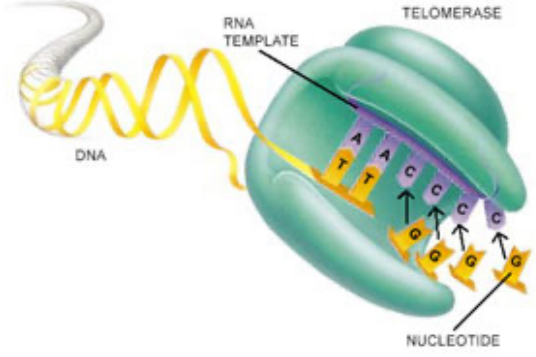
They contain long arrays of **TTAGGG** repeats

Each time a cell divides, the terminal end of the telomere is lost; successive divisions lead to progressive shortening

After 40-60 divisions, vital DNA sequences are lost

At this point, the cell cannot divide further and undergoes **senescence**

Telomerase



Telomerase is a **reverse transcriptase** that polymerizes TTAGGG repeats to offset the degradation of chromosome ends that occurs with successive cell divisions, **rendering a cell immortal**

Telomerase expression thus is implicated in carcinogenesis

TTGGGG is the telomere sequence in Tetrahymena (a protozoan)

Molecular biologist Carol Greider discovered the enzyme telomerase. This license plate, which was on her car when she worked at Cold Spring Harbor Laboratory on Long Island, New York, advertises her research interest!

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- General Overview of DNA Strand Breaks
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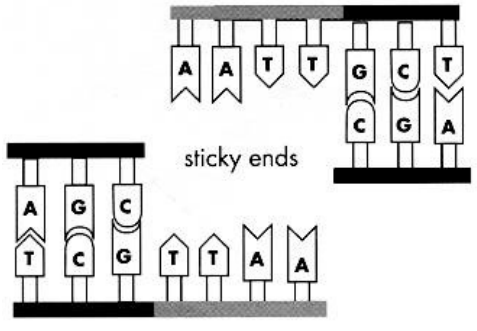
- DNA Repair Pathways
- Chromosomes and Cell Division
- **Radiation-Induced Chromosome Aberrations**
- Chromosome Aberrations in Human Lymphocytes

How Do Chromosome Aberrations Happen?

- Larger chromosome aberrations are *usually* fatal to the cell
- When cells are irradiated, double strand breaks (DSB) are produced in the chromosomes
- Broken ends appear to be "sticky" and can rejoin with any other sticky end

DSB



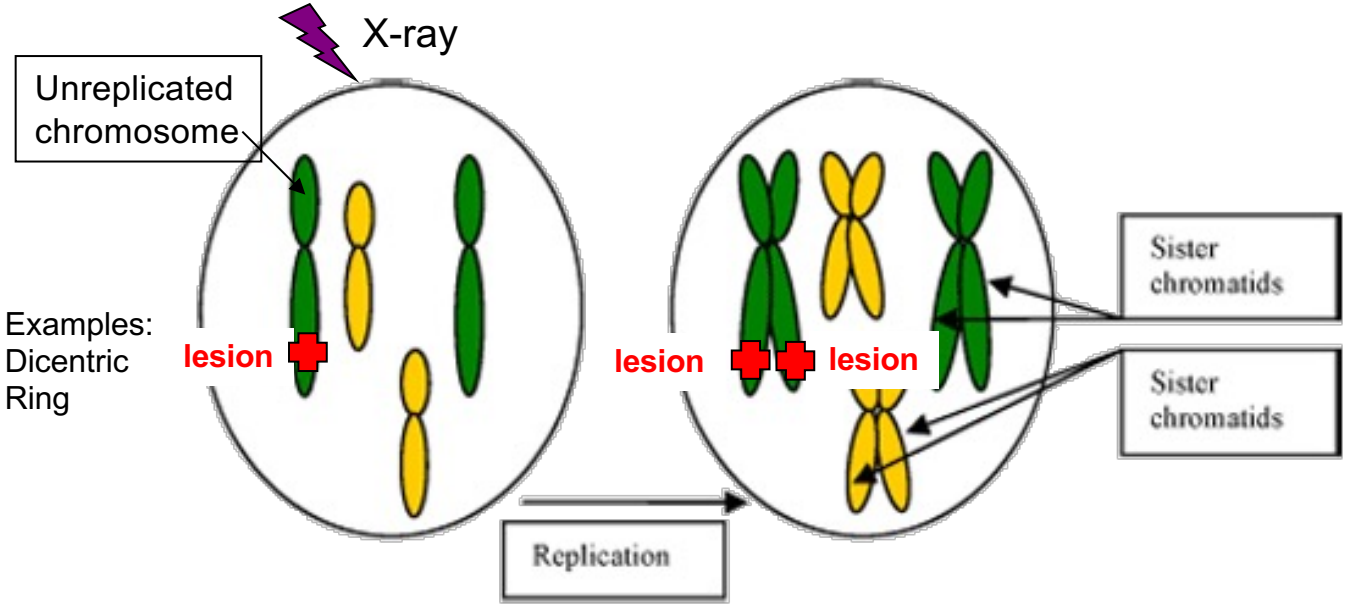
How Do Chromosome Aberrations Happen?

- Once breaks are produced, different fragments may behave in a variety of ways
- Breaks may rejoin in their original configuration
- Breaks may fail to rejoin and give rise to a deletion
- Broken ends may reassort and rejoin other broken ends and give rise to chromosomes that appear to be grossly distorted if viewed at the following mitosis

Types of Aberrations

- The aberrations seen at metaphase are of 2 classes
- **Chromosome aberrations** result if a cell is irradiated early in interphase (G_1) before chromosomal material is duplicated
  - *Aberrations occurring in chromatin will be **replicated** in S phase*
- **Chromatid aberrations** result if a cell is irradiated after S phase when DNA material has already been replicated (G_2)
  - *Radiation is likely to break one but not both chromatids*

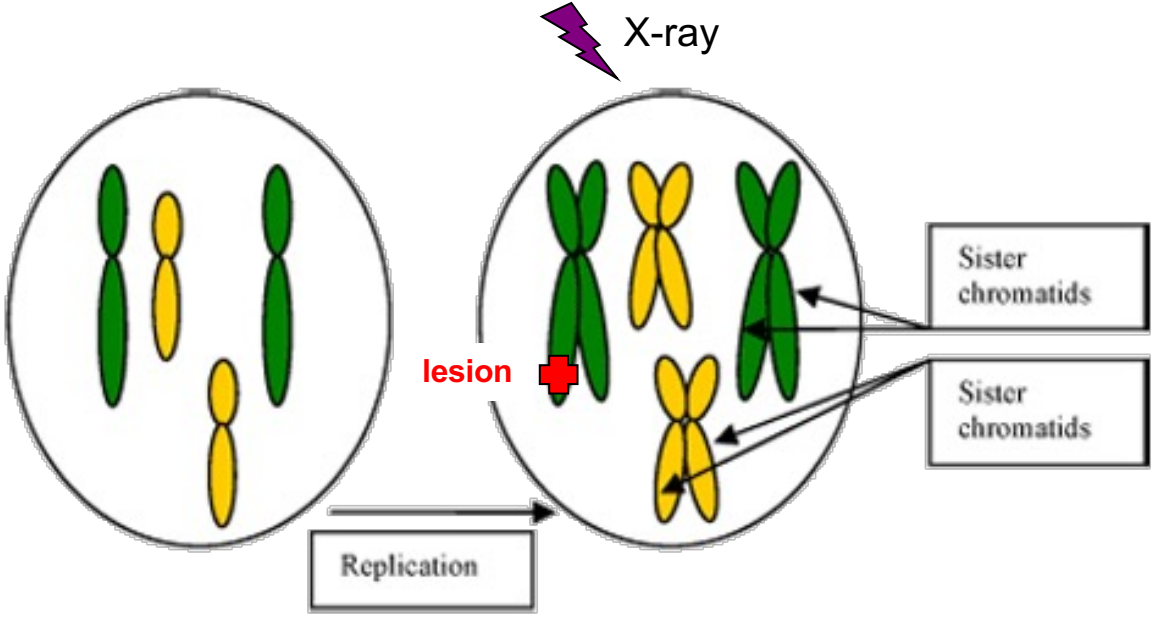
Chromosome Aberrations



Aberrations occurring in chromatin will be **replicated** in S phase

Damage in G1 results in **chromosome aberration**

Chromatid Aberrations

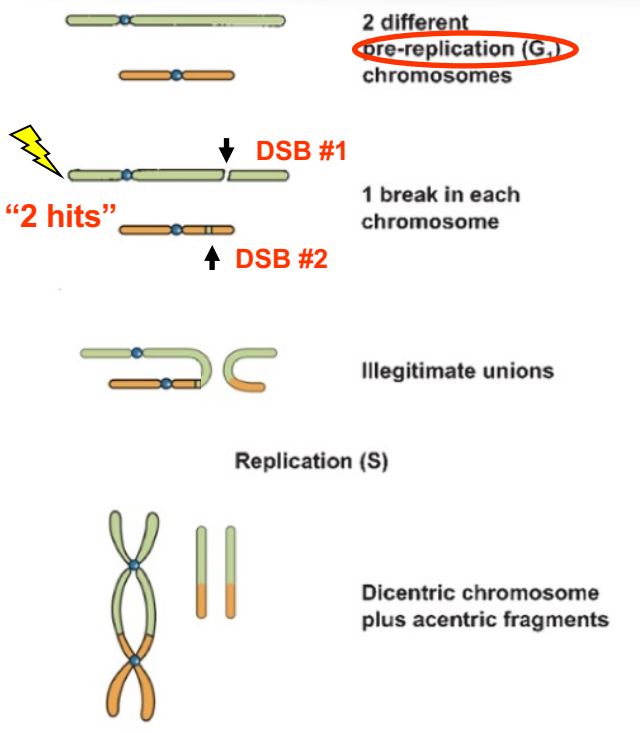


Damage in G2 results in **chromatid aberration**

Examples of Radiation-Induced Aberrations

- Many types of aberrations and rearrangements are possible
- **Lethal aberrations**
  - *Dicentric*, *Ring* – chromosome aberration
  - *Anaphase Bridge* – chromatid aberration
- **Non-Lethal aberrations**
  - *Symmetric translocations*
  - *Small interstitial deletions*
  - *Terminal deletions*

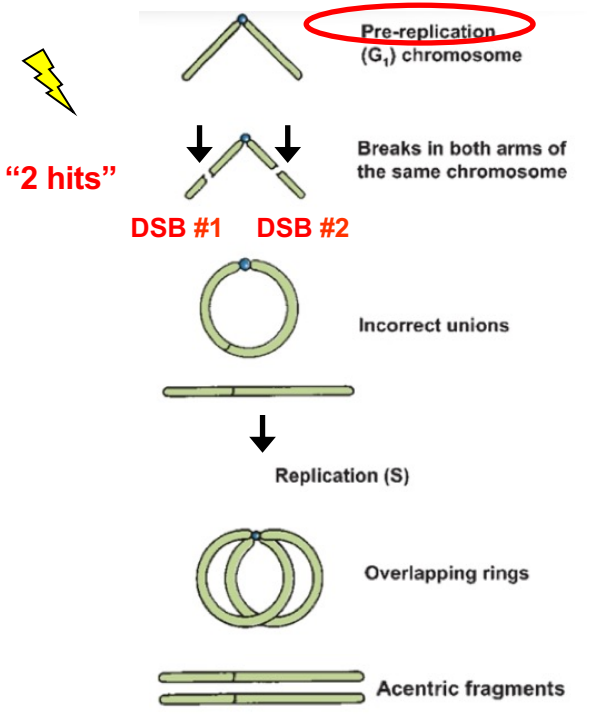
Dicentric



- Involves interchange between 2 separate chromosomes
- If break occurs in each one early in interphase and sticky ends are close together, they may rejoin
- This interchange is replicated during DNA synthesis and results in a distorted chromosome with **2 centromeres** and 2 acentric fragments

This is an example of **chromosome aberration**

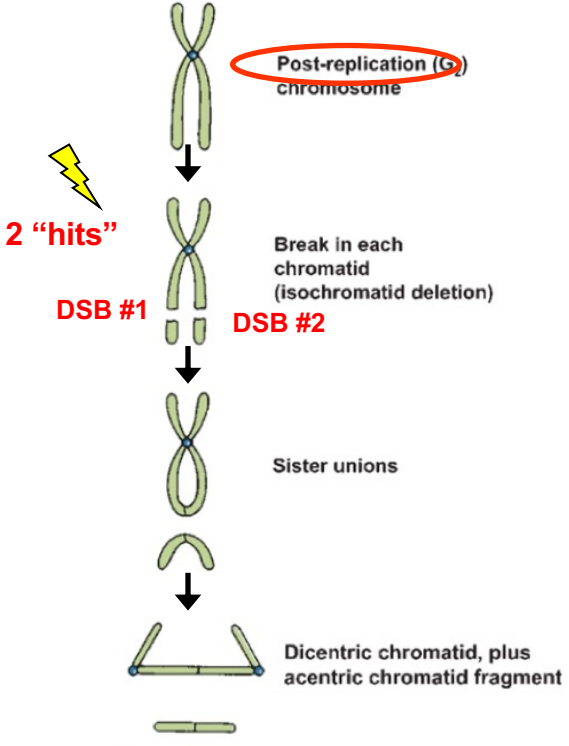
Ring



- Break induced by radiation in each arm of the same chromosome early in cell cycle
- The sticky ends may rejoin to form a ring with a centromere and an acentric fragment
- Later during DNA synthetic phase the chromosome is replicated

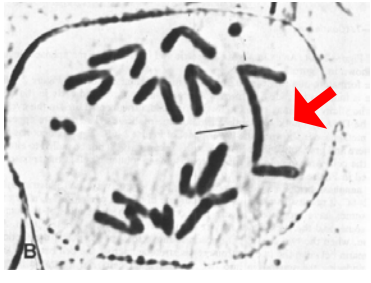
This is an example of **chromosome aberration**

Anaphase Bridge

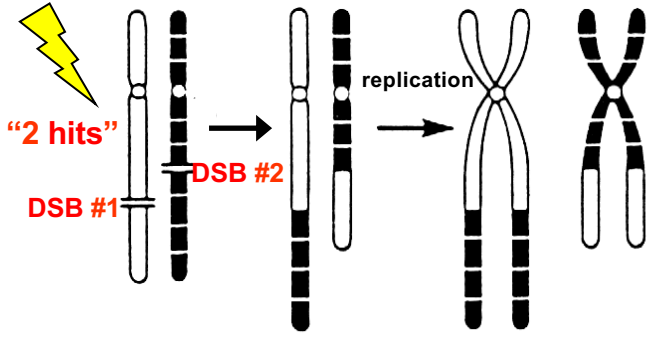


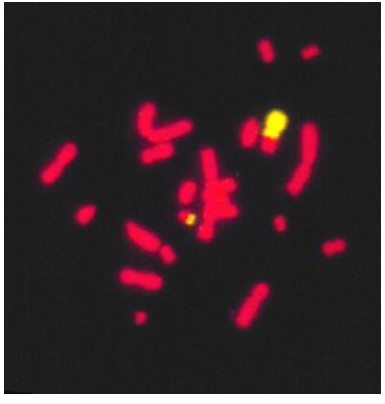
This is an example of **chromatid aberration**

- Results from breaks that occur late in cell cycle (G_2), after chromosome has replicated
- Breaks may occur in each chromatid of the same chromosome
- Sticky ends may rejoin incorrectly to form a sister union
- At anaphase, when the 2 sets of chromosomes move to opposite poles, the section of chromatin between the centromeres is stretched across between the poles, hindering separation into new daughter cells



Symmetric Translocation

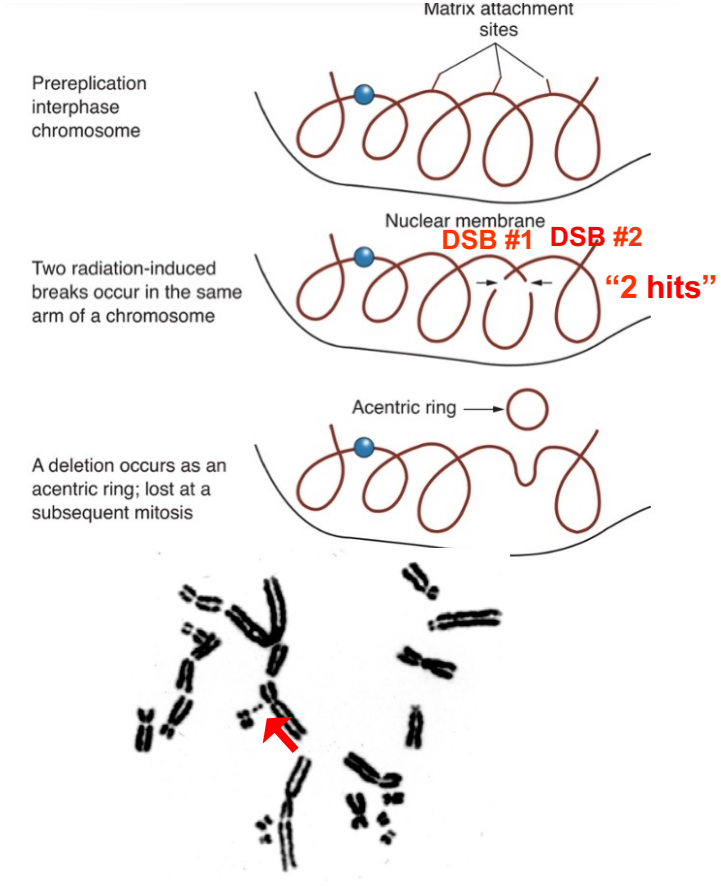




FISH

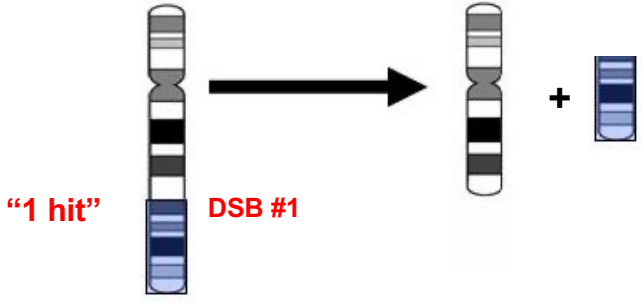
- Involves break in 2 pre-replication chromosomes, with broken ends being exchanged between the 2 chromosomes
- Difficult to see in conventional preparation, but easy to observe with **fluorescent *in situ* hybridization (FISH)**
- Non-lethal, but are associated with several human malignancies caused by the activation of an **oncogene**

Small Interstitial Deletion



- Result from 2 breaks in the same arm of the same chromosome
- The "sticky" ends rejoin
- A loop of DNA is isolated = **acentric ring**
- May be associated with carcinogenesis if the lost genetic material includes a **tumor suppressor gene**

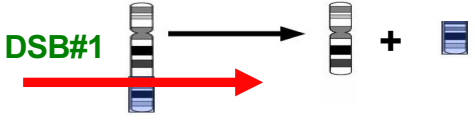
Terminal Deletion



- Involves one break **near the end** of a pre-replication chromosome
- Results in loss of genetic material from end of chromosome
- May be associated with carcinogenesis if loss of material includes a **tumor suppressor gene**

Dose Response – Single Hit

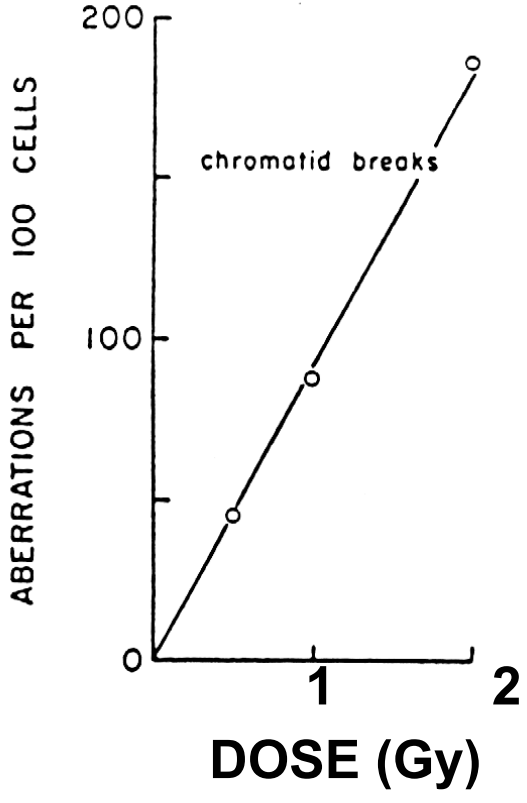
Example: Terminal deletion = “1 hit”



single e^- track

$$Y \text{ (Yield)} = \alpha D$$

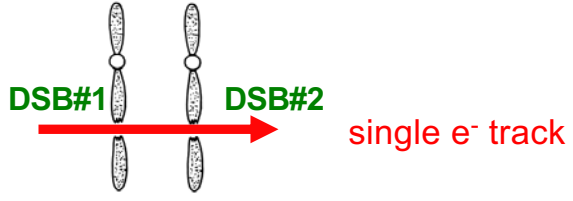
α = proportionality



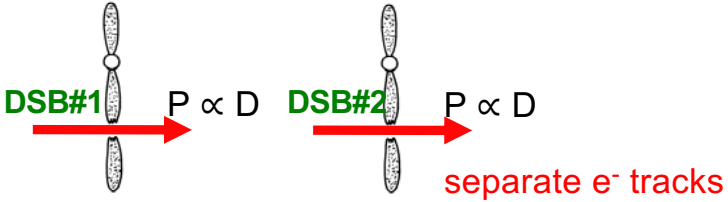
Dose Response – “2 Hits”

Exchange-type
Rearrangements = “2 hits”

Dependent upon
SPACE = proximity
TIME = interaction



$Y = \alpha D$



$P = D \times D = D^2$

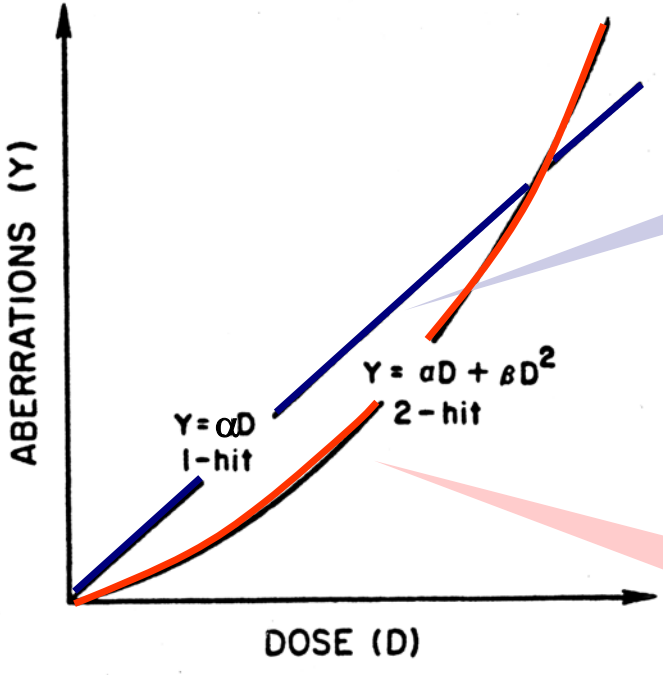
$Y = \beta D^2$

$Y = \alpha D + \beta D^2$

Linear component

Quadratic component

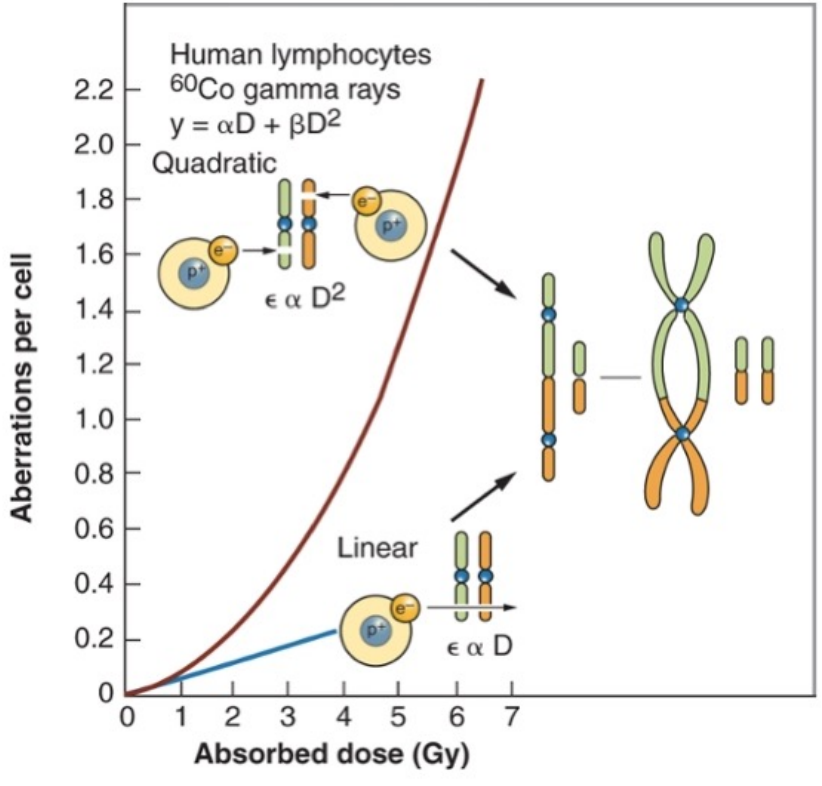
“1-Hit” vs. “2-Hits”



1-Hit
Linear function of Dose
$Y = \alpha D$

2-Hit
Linear-quadratic function of Dose
$Y = \alpha D + \beta D^2$

Dose Response – “2 Hits”



At Higher Doses

The two breaks are more likely to be caused by separate electrons → the probability of an exchange aberration is **proportional to square of dose**

At Low Doses

Both breaks may be produced by the same electron → the probability of an exchange aberration is **proportional to dose**

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- **Chromosome Aberrations in Human Lymphocytes**

Human Lymphocytes as a Biomarker

- Irradiated cells may display a number of types of aberrations including dicentrics, centric rings, acentric fragments and translocations, all of which may be related to radiation dose
- Chromosomal aberrations in peripheral lymphocytes have been widely used as biomarkers of radiation exposure
- The dose can be estimated by comparison with *in vitro* cultures exposed to known doses

Dicentrics as a Biomarker

- For biological dosimetry, ***dicentrics*** historically has been the aberration of choice
- Most reliably scored, easily seen in chromosome spread
- 10x more common than rings and about as common as excess fragments
- Low level of occurrence in unirradiated persons (about 1/1000 cells)

Scoring Lymphocyte Aberrations

Lymphocytes in the blood sample may be **stimulated to divide** *in vitro* by adding phytohemagglutinin (PHA)

⇩

Stopped at their 1st metaphase by addition of Colcemid after about 45 hrs of culture

⇩

Slides containing metaphase spreads stained with Giesma or FISH probes and scored

In suspected exposure case, typically 500 cells are scored

Can detect a recent total-body exposure of as low as **0.25 Gy**

Useful in distinguishing “real” vs. “suspected” exposures

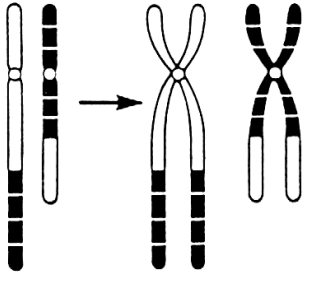
Limitations of Scoring Dicentrics

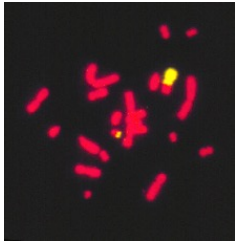
- Major limitation of using dicentrics for dosimetry is loss of lymphocytes from the blood
  - **Mature T lymphocytes** (nondividing) have a finite life span of ~ 1,500 days and are eliminated slowly
  - Consequently, the yield of dicentrics observed in peripheral lymphocytes declines with time

- When replaced by **stem cells** (dividing), dicentrics (and other asymmetric aberrations) will be lost in subsequent mitosis, because these are lethal, hence **"unstable" aberrations**

Symmetric Aberrations as a Biomarker

- Stem cells that sustain a **symmetric nonlethal aberrations** survive and pass on the aberration to their progeny
- These are referred to as **“stable” aberrations**, because they persist for many years
- The frequency of translocations can be detected using FISH technique
- Dosimetry is possible even at 50 years after total-body exposure (e.g. Hiroshima and Nagasaki)





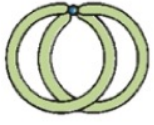
Review Questions

Question 1

An example of an asymmetrical chromosome-type aberration is:

A. a sister chromatid exchange
B. a reciprocal translocation
C. an inversion
D. a chromatid break
E. a dicentric chromosome

Lethal Aberrations



Chromosome Aberration

Chromatid Aberration

All require 2 DSBs

Asymmetric exchange-type Aberrations

Lethal during mitosis

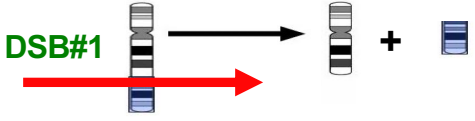
Question 2

Radiation-induced chromatid terminal deletions increase as a:

A. linear function of dose
B. quadratic function of dose
C. linear-quadratic function of dose
D. exponential function of dose
E. sigmoidal function of dose

Dose Response – Single Hit

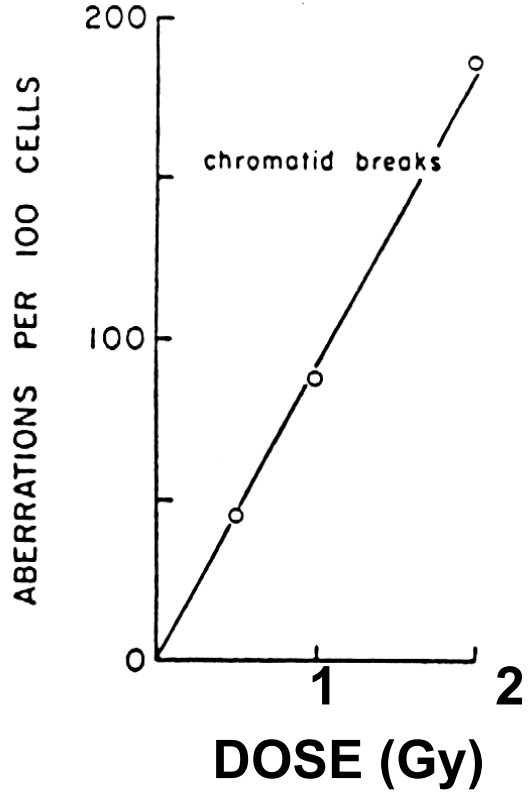
Example: Terminal deletion = “1 hit”



single e^- track

$Y\ (Yield) = \alpha D$

α = proportionality



Question 3

Measurement of which one of the following chromosomal aberrations in peripheral blood lymphocytes many years following a putative whole body radiation exposure would provide the best estimate as to the dose received?

A. reciprocal translocations
B. rings
C. dicentrics
D. chromosome breaks
E. chromatid breaks

Symmetric Aberrations as a Biomarker

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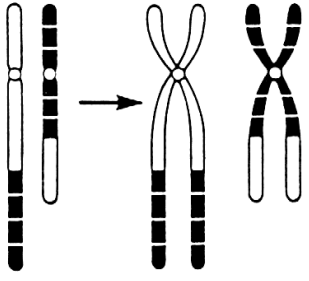
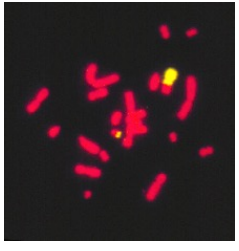




Question 4

The formation of dicentric chromosome aberrations follows a linear-quadratic dose response curve. This has been interpreted to mean that the production of dicentric chromosomes results from:

A. two chromosome breaks, produced either by one or by two separate radiation tracks
B. two chromosome breaks produced by two separate radiation tracks
C. two chromosome breaks produced by a single radiation track
D. one chromosome break produced by two separate radiation tracks
E. one chromosome break produced by a single track of radiation

Dose Response – “2 Hits”

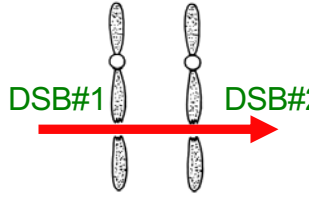
Exchange-type
Rearrangements = “2 hits”

Dependent upon
SPACE = proximity
TIME = interaction

$Y = \alpha D + \beta D^2$

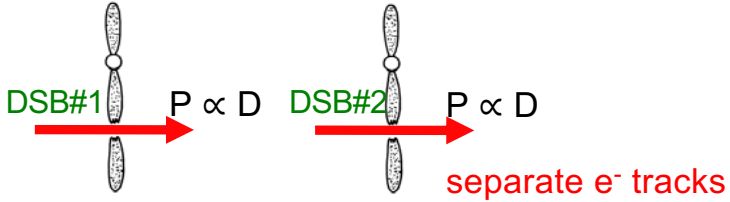
Linear component

Quadratic component



single e⁻ track

$Y = \alpha D$

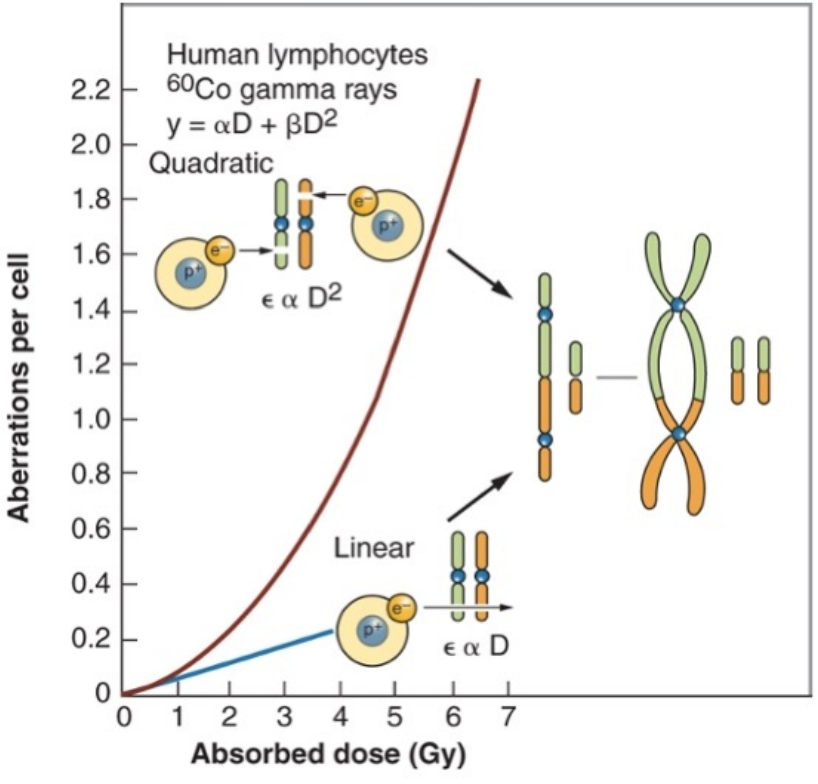


separate e⁻ tracks

$P = D \times D = D^2$

$Y = \beta D^2$

Dose Response – “2 Hits”



At Higher Doses

The two breaks are more likely to be caused by separate electrons → the probability of an exchange aberration is proportional to square of dose

At Low Doses

Both breaks may be produced by the same electron → the probability of an exchange aberration is proportional to dose

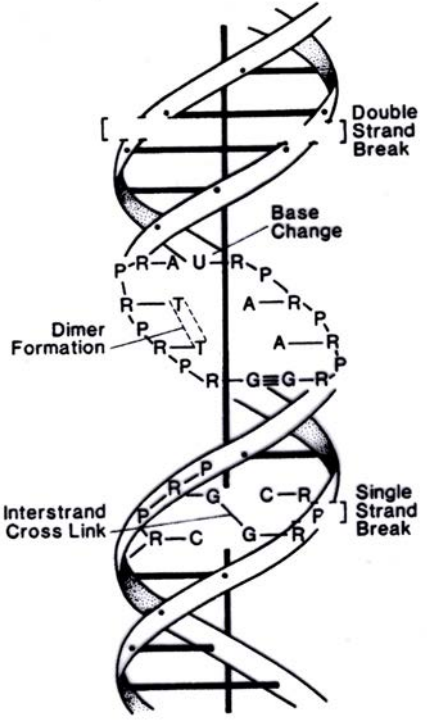
Question 5

Following irradiation with 3 Gy of 250 kVp x-rays, the number of single strand DNA breaks vs. double strand DNA breaks vs. altered DNA bases

A. altered DNA bases > single strand DNA breaks > double strand breaks
B. double strand DNA breaks > altered DNA bases > single strand DNA breaks
C. altered DNA bases > double strand DNA breaks > single strand DNA breaks
D. single strand DNA breaks > altered DNA bases > double strand DNA breaks

Yield of DNA Damage

- For mammalian cells, the # of DNA lesions per cell by **1 Gy** of x-ray is approximately
  - Base damage – > 1,000
  - SSB – 1,000
  - DSB – 40
- The yield of DSB is ~ 0.04x of SSB



Question 6

Which of the following statements concerning chromosome aberrations produced in cells after whole body X-irradiation is TRUE?

A. The formation of terminal deletions follows an exponential dose response

B. Translocations are an unstable type of chromosome aberration

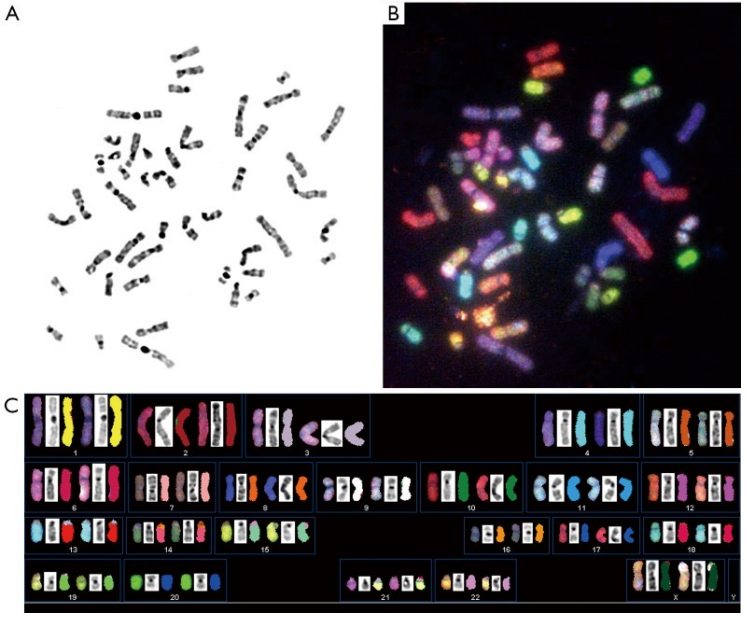
C. The number of dicentric chromosomes detected in peripheral blood lymphocytes remains relatively constant with time

D. SKY (spectral karyotyping) is a useful method for detection of stable aberrations decades following irradiation

E. The minimum dose that can be estimated by scoring dicentric chromosomes is 2 Gy

SKY (Spectral Karyotyping)

- SKY are FISH based methods allowing for the simultaneous display of **all chromosomes** in different colors *without a priori knowledge of any abnormalities involved*
- SKY can discern the aberrations that can't be detected very well by conventional banding technique and FISH
- SKY cannot identify chromosomal changes that do not lead to a discernable color change: small deletions, duplications and intrachromosomal inversions



Spectral Karyotyping (SKY) of a normal female in SKY image, from left to right: (A) DAPI image; (B) SKY karyotype; (C) Karyotype analysis image of SKY.

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Scoring Lymphocyte Aberrations

Lymphocytes in the blood sample may be **stimulated to divide** *in vitro* by adding phytohemagglutinin (PHA)

⇩

Stopped at their 1st metaphase by addition of Colcemid after about 45 hrs of culture

⇩

Slides containing metaphase spreads stained with Giesma or FISH probes and scored

In suspected exposure case, typically 500 cells are scored

Can detect a recent total-body exposure of as low as **0.25 Gy**

Useful in distinguishing “real” vs. “suspected” exposures