Genetic, Cytogenetic and Epigenetic Effects

A. Some Background Information and General Principles:

1) Unrepaired damage to DNA caused by ionizing radiation can have genetic consequences; this damage is often manifest as gene mutations and chromosome changes

a. the consequence of such genetic and/or cytogenetic changes is usually one of two things:

Cell Death
and/or
(Heritable) Genetic or Epigenetic Change

b. if this damage occurs in gametes, the individual at risk is an unborn offspring, and this is termed "genetic effects of radiation"; if the damage occurs in any other cells of the body, the individual at risk is the person him- or herself, and this is termed "somatic effects of radiation"

1] "Genetic effects" usually refer to:

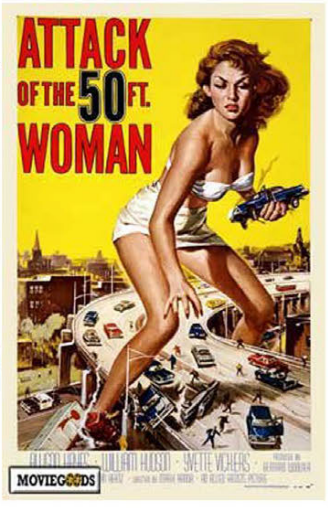
genetic disorders
embryonic death
carcinogenesis

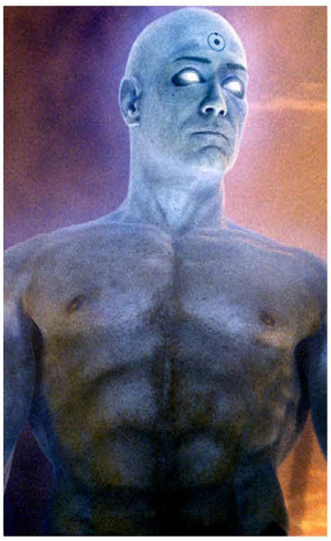
2] "Somatic effects" usually refer to:

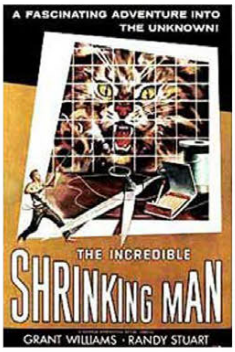
acute radiation syndromes
cataractogenesis
infertility
teratogenesis
carcinogenesis

There is nothing unique or different about effects caused by ionizing radiation exposure in terms of mutant phenotypes...radiation only increases the frequency of those that already occur naturally. (In other words, if there's no evidence for naturally occurring incredible hulks, radiation isn't going to cause any either.)

However, that is not to say that genotypically, there couldn't be molecular damage signatures in genes unique to ionizing radiation. (Plenty of research in this area vis-a-vis potential biomarkers of radiation exposure.)







Not. Gonna. Happen.

a] ***In fact, the spontaneous incidence of most of these mutations is MUCH higher than any increase attributable to radiation exposure*** (in other words, in the grand scheme of things, radiation is *not* a particularly potent mutagen)

Current Estimates of Genetic Risks from Continuing Exposure to Low–Linear Energy Transfer, Low-Dose, or Chronic Irradiation (from UNSCEAR 2001) (Assumes worst case annual dose of ~10mSv)

DISEASE CLASS	BASED FREQUENCY PER 10^6 LIVE BIRTHS	RISK PER Gy PER 10^6 PROGENY FIRST GENERATION	RISK PER Gy PER 10^6 PROGENY UP TO SECOND GENERATION
Mendelian			
Autosomal dominant and X-linked	16,500	750–1,500	1,300–2,500
Autosomal recessive	7,500	0	0
Chromosomal	4,000	[a]	[a]
Multifactorial			
Chronic	650,000	~250–1,200	~250–1,200
Congenital abnormalities	60,000	2,000	2,400–3,000
Total	738,000	~3,000–4,700	3,950–6,700
Total risk per Gy expressed as percent of baseline	—	~0.41–0.64	~0.53–0.91

[a]Assumed to be subsumed in part under the risk of autosomal dominant and X-linked diseases and in part under congenital abnormalities.

From United Nations Scientific Committee on the Effects of Atomic Radiation. *Hereditary Effects of Radiation: UNSCEAR 2001 Report to the General Assembly, with Scientific Annex*. New York, NY: United Nations; 2001.

Estimated frequency of chromosome abnormalities in unselected newborns

Autosomal trisomy	0.14%
Sex chromosome aneuploidy	0.19%
Unbalanced structural rearrangements	0.06%
Triploidy	0.002%
Structurally balanced rearrangements	0.52%
Total	~0.9%

Numerical abnormalities are by far the most common:

- 20% of oocytes and 10% of sperm are aneuploid
- Up to 1 in 3 conceptuses are trisomic or monosomic
- Triploidy affects 1-3% of recognised conceptions

2) ***If they're so hard to quantify, especially at low radiation doses, and especially in human populations, why study radiation-induced mutations at all?***

a] **first, it is clear that one of, if not *the*, earliest event that starts off the process of carcinogenesis is mutations in cellular DNA...so it follows that if we study radiation-induced mutations, we can also learn something about radiation-induced cancers**

b} **a second reason to study mutations caused by radiation is for risk assessment and radiation protection of the public; especially important is an understanding of the shape of the dose response curve for mutation induction, and also, what happens at the very low doses that everybody is exposed to** (and the somewhat higher doses radiation workers are exposed to)

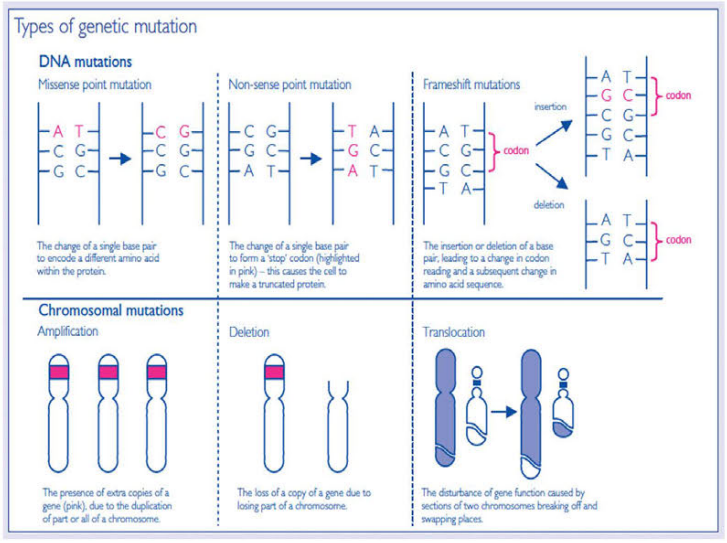
B. Gene Mutations Caused by Radiation

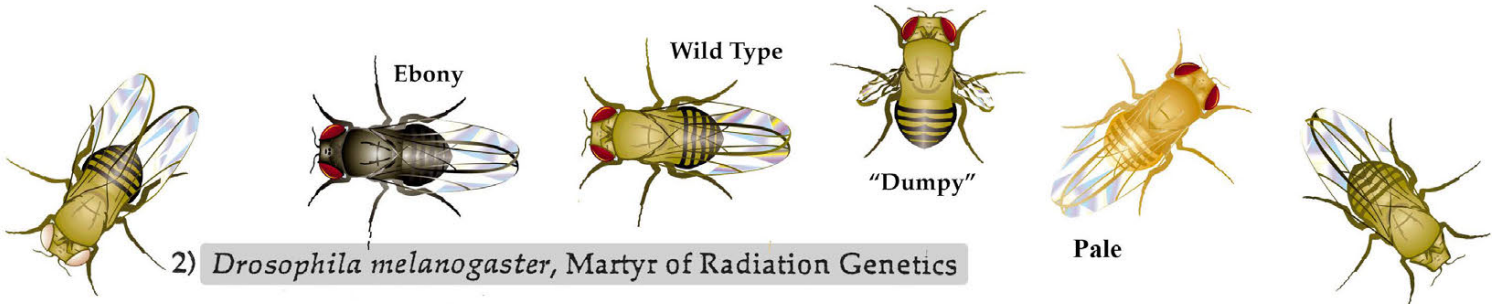
1) What do we mean by "mutation" anyway????

Comparatively speaking, DNA mutations are "small" compared to "large" chromosomal mutations.

Radiation exposure causes both, although the larger, chromosome-level mutations are its forté.

Large mutations typically involve the gain/loss or rearrangment of multiple genes and their regulatory elements.





2) *Drosophila melanogaster*, Martyr of Radiation Genetics

a. fruit flies served as an excellent system for the study of genetics in general, and radiation genetics in particular for a number of reasons:

readily observable mutations
huge numbers of "subjects" for study
short overall lifespans
ability to control breeding patterns

http://www.ergito.com

Normal *Legs instead of Antennae* *Eyeball on Leg* *Double Thorax*

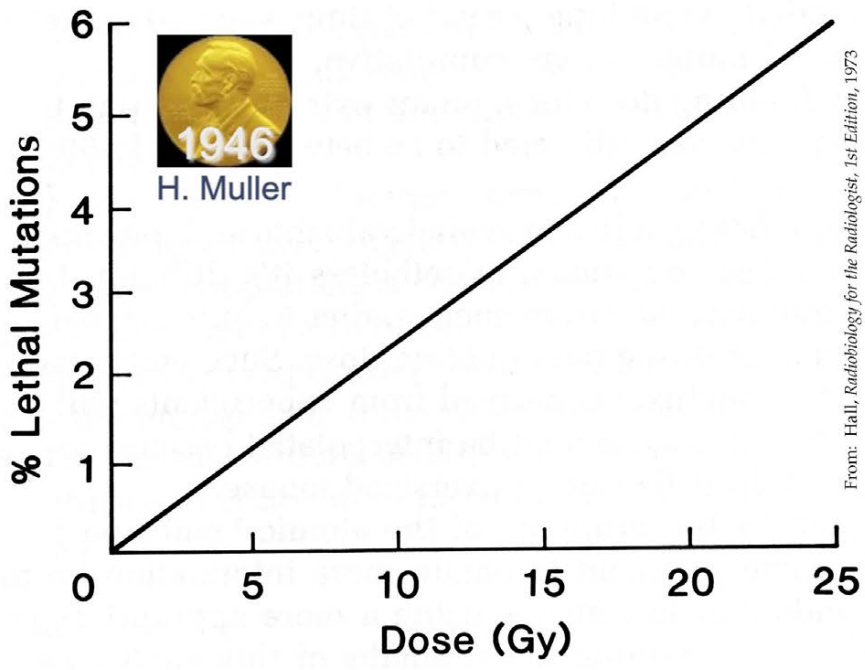
b. What did we learn from studies of mutations in fruit flies (most work done in the 1920's and 1930's)?

the dose it took to double the spontaneous incidence of a particular mutation was estimated to be between about 0.05 - 2.0 Sv

this is called the *genetic doubling dose*

there was NO difference in mutation rate if the dose was delivered all at once versus fractionated over time, that is, there was *NO DOSE RATE EFFECT*

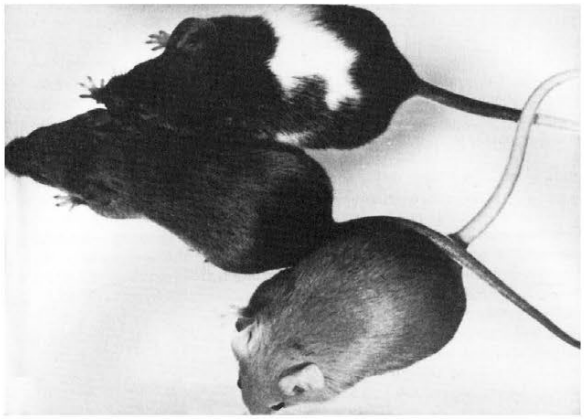
(That 0.05 Sv - 50 mSv - is where our maximum exposure limit as radiation workers originally came from)



All well and good...BUT...to what extent were data derived from insects applicable to the human situation?

3) **The Megamouse Project**: conducted by Russell and Russell of the Oak Ridge National Lab during the 1950's and 1960's (7 million mice evaluated!)

a. In the mouse studies, several different autosomal traits (e.g., coat color, ear and tail structure) were tracked as indicative of one or more mutational events



1] endpoints assessed:

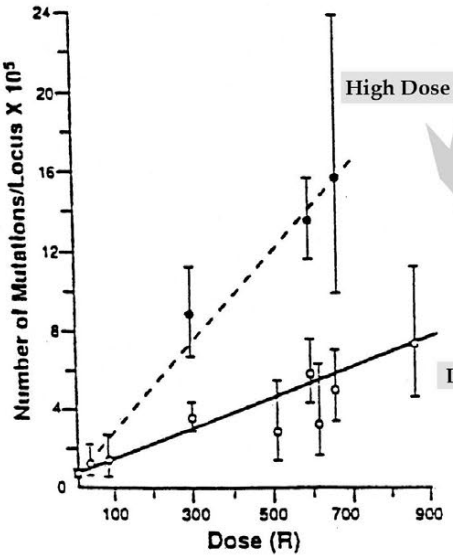
- mutation frequencies for the different traits
- differences between mutation induction in spermatogonia vs. oocytes
- differences in mutation induction as a function of the radiation dose rate
- differences in mutation induction as a function of the radiation quality, i.e., neutrons vs. X-rays

b. the important conclusions of these studies were:

1] there was a 20-fold difference in the mutation frequencies for the 7 different traits studied, implying that there is no absolute radiation-induced mutation frequency

2] unlike the case of the fruit fly, there WAS a large dose rate effect; on average, **the frequency of radiation-induced mutations was at least a factor of 3 lower when the dose was fractionated or protracted rather than given as a large single dose...**

GOOD NEWS for (human) radiation protection purposes!



Redrawn from Hall and Giaccia, Radiobiology for the Radiologist, 6th Edition, 2006.

Mutation frequency in mouse spermatogonia as a function of X-ray dose given at either a high or low dose rate.

Genetic doubling dose for mice?
0.5 – 2.0 Sv

3] **spermatogonia were much more sensitive than oocytes**

4] **there was evidence for the repair of, or selection against, mutated gametes, since allowing time between irradiation and conception greatly reduced the mutation frequencies**

5] **neutrons were about 20x more effective than X-rays at producing these mutations**

4) **The Human Experience**: *children born to irradiated parents*

RERF A-Bomb Cohorts

Cohort	Size
Life Span Study	**120,000** Allows an estimate of cancer incidence and mortality
In-Utero Cohort	**3,600** Allows estimates of mental retardation, microcephaly, etc.
Children of exposed individuals	**77,000** Allows estimate of heritable effects

a. these children were assessed for four indicators of genetic damage:

Miscarriage Frequency
Presence of Gross Malformations
Presence of Variant Proteins
Presence of Chromosome Aberrations

b. *although no one indicator showed a statistically significant elevation, the overall frequency of all the indicators was elevated compared to a matched set of offspring of parents who weren't irradiated during the bombings*

5) based on all of these studies, estimates (on average) of the doubling dose for human genetic mutations have been made by a variety of scholarly organizations (BEIR and UNSCEAR Reports):

Doubling Dose (Gametic) in the Offspring of Survivors of the Atomic Bomb Attacks on Hiroshima and Nagasaki

GENETIC INDICATOR	DOUBLING DOSE, Sv
Untoward pregnancy outcome	0.69
Childhood mortality	1.47
Sex chromosome aneuploidy	2.52
Simple average	1.56

Adapted from Schull WJ, Otake M, Neel JV. Genetic effects of the atomic bomb: a reappraisal. *Science*. 1981;213:1220–1227 with permission.

Assumed to be the approximate genetic doubling dose for humans = ~1.5 Sv.

(Boards!)

C. Mutagenesis in Mammalian Cells in Culture: a better way to get at possible mechanisms of action

1. Arguably, the most important thing we learned from mutagenesis studies in mammalian cells was the existence of what was then-called the "**Mutator Phenotype**", which we now know is a manifestation of *genomic instability*, one of the enablers of the acquisition of the hallmarks of cancer

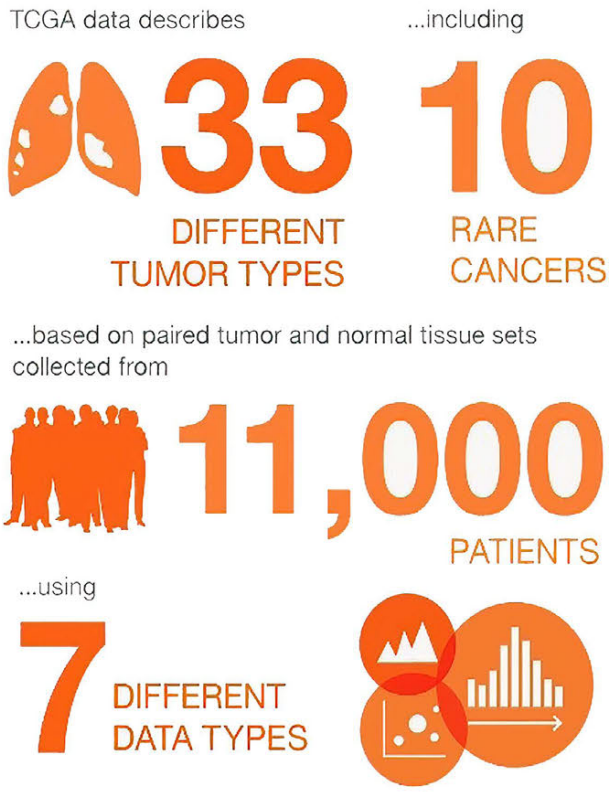
a) **Modern cancer biology tells us that, in most cases, at least 5-6 different mutations need to accrue in order to transform a normal cell into a malignant one**

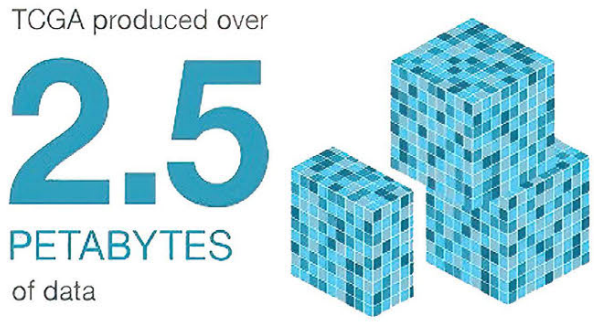
1] But...if this occurred based on a spontaneous mutation rate of 10^{-6} - 10^{-7} mutations/gene/cell division, it would mean that each cell would have to have divided about 5 million times before accumulating that many mutations!

2] Therefore, **we are forced to conclude that tumor cells must be hypermutable, with mutation frequencies in the range of 10^{-2} to 10^{-1} mutations/gene/cell division.** ***This genomic instability is the only way to account for the large number of mutations that tumor cells typically bear, and why no two tumor cells are ever identical.***

What about the mutational landscape of human cancers?

The Cancer Genome Atlas (TCGA) has been instrumental in making all of this possible!





To say the least, TCGA generated a whole lot of Big Data!

RESULTS & FINDINGS

MOLECULAR BASIS OF CANCER	Improved our understanding of the genomic underpinnings of cancer	For example, a TCGA study found the basal-like subtype of breast cancer to be similar to the serous subtype of ovarian cancer on a molecular level, suggesting that despite arising from different tissues in the body, these subtypes may share a common path of development and respond to similar therapeutic strategies.
TUMOR SUBTYPES	Revolutionized how cancer is classified	TCGA revolutionized how cancer is classified by identifying tumor subtypes with distinct sets of genomic alterations.
THERAPEUTIC TARGETS	Identified genomic characteristics of tumors that can be targeted with currently available therapies or used to help with drug development	TCGA's identification of targetable genomic alterations in lung squamous cell carcinoma led to NCI's Lung-MAP Trial, which will treat patients based on the specific genomic changes in their tumor.

D. **Mutagenesis Studies *In Vivo*** - less "mechanistic", however more relevant to what happens to the entire organism when mutations occur

1) these studies are aided by the use of model organisms that:

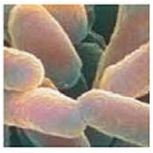
a] already have their entire genomes sequenced (so that it will be clear what, exactly, got mutated and what the ultimate effect was); and

b] can have select mutations deliberately introduced using transgenic or gene editing technologies

Model Organisms Commonly Used in Radiation/Cancer Research

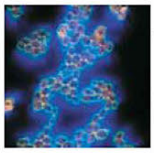
Viruses

Proteins involved in DNA, RNA, protein synthesis
Gene regulation
Cancer and control of cell proliferation
Transport of proteins and organelles inside cells
Infection and immunity
Possible gene therapy approaches



Bacteria

Proteins involved in DNA, RNA, protein synthesis, metabolism
Gene regulation
Targets for new antibiotics
Cell cycle
Signaling



Yeast (*Saccharomyces cerevisiae*)

Control of cell cycle and cell division
Protein secretion and membrane biogenesis
Function of the cytoskeleton
Cell differentiation
Aging
Gene regulation and chromosome structure

Roundworm (*Caenorhabditis elegans*)

Development of the body plan
Cell lineage
Formation and function of the nervous system
Control of programmed cell death
Cell proliferation and cancer genes
Aging
Behavior
Gene regulation and chromosome structure

Fruit fly (*Drosophila melanogaster*)

Development of the body plan
Generation of differentiated cell lineages
Formation of the nervous system, heart, and musculature
Programmed cell death
Genetic control of behavior
Cancer genes and control of cell proliferation
Control of cell polarization
Effects of mutagens/carcinogens

Zebrafish

Development of vertebrate body tissues
Formation and function of brain and nervous system
Birth defects
Cancer

Mice, including cultured cells

Development of body tissues
Function of mammalian immune system
Formation and function of brain and nervous system
Models of cancers and other human diseases
Gene regulation and inheritance
Infectious disease

Plant (*Arabidopsis thaliana*)

Development and patterning of tissues
Genetics of cell biology
Agricultural applications
Physiology
Gene regulation
Immunity
Infectious disease

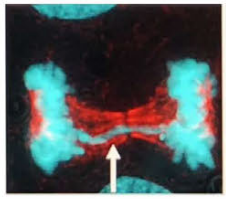
E. Cytogenetic Effects of Radiation

1] What is it about most chromosome aberrations that usually make them fatal to the cell?

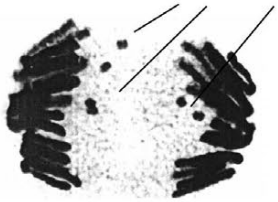
Anaphase Bridge

they physically interfere with the division process, in which case, the cell would die right then and there while trying to divide

OR

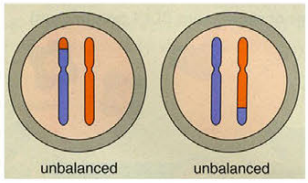


Acentric Fragments



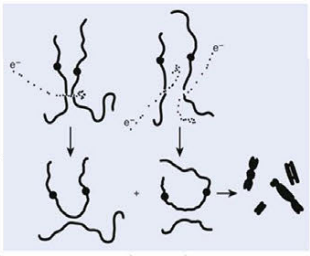
broken parts of chromosomes float away during the division process (because the pieces have no centromeres), and then the cell later finds itself missing hundreds, if not thousands, of genes, so it dies a gradual, lingering death over several days..and it might even divide a few more times before finally dying

AND/OR



a gene dosage effect occurs such that a cell receives either too many or two few copies of certain genes (mostly occurs when there is a chromosomal translocation - see below)

2] how are chromosome aberrations classified?



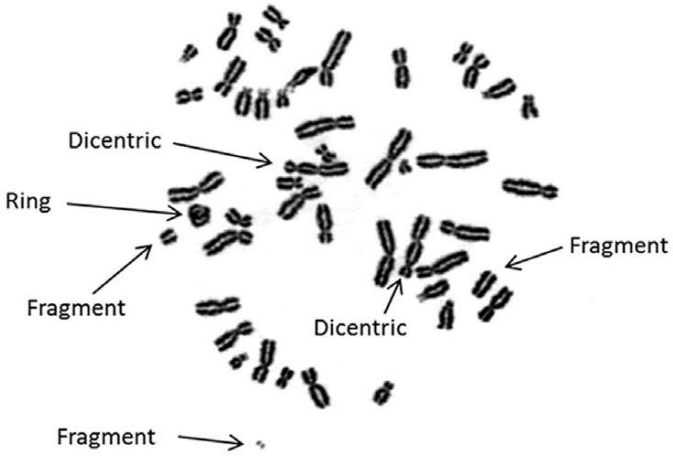
1. **"single-hit" versus "two-hit"**: refers to whether the particular chromosome aberration was formed by the passage of a single charged particle track, or whether two (or more) tracks/damage sites were involved

2. **chromosome-type versus chromatid-type aberrations**: *chromosome-type aberrations are formed when cells are initially irradiated in the G_1 or G_0 phase of the cell cycle, whereas chromatid-type aberrations are formed in cells irradiated in the S, G_2 or M phases of the cell cycle*

3] **symmetrical versus asymmetrical aberrations**: refers to whether the aberration produced leaves behind acentric fragments (asymmetrical) or not (symmetrical)

a. ***this can be an important consideration because aberrations that lead to the loss of large chunks of chromosomal real estate (such as would be contained in an acentric fragment) are very likely to be lethal to the cell, whereas those that only involve rearrangement of the genetic material with nothing lost are less likely to be fatal and more likely to be carcinogenic***

Know your chromosome aberrations!

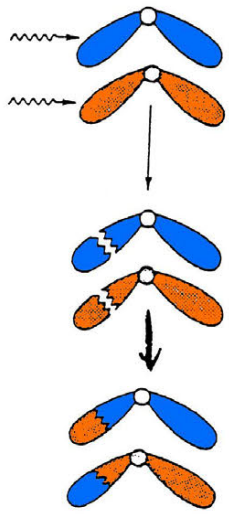


"Classical" chromosome aberrations include dicentrics, rings and acentric fragments.

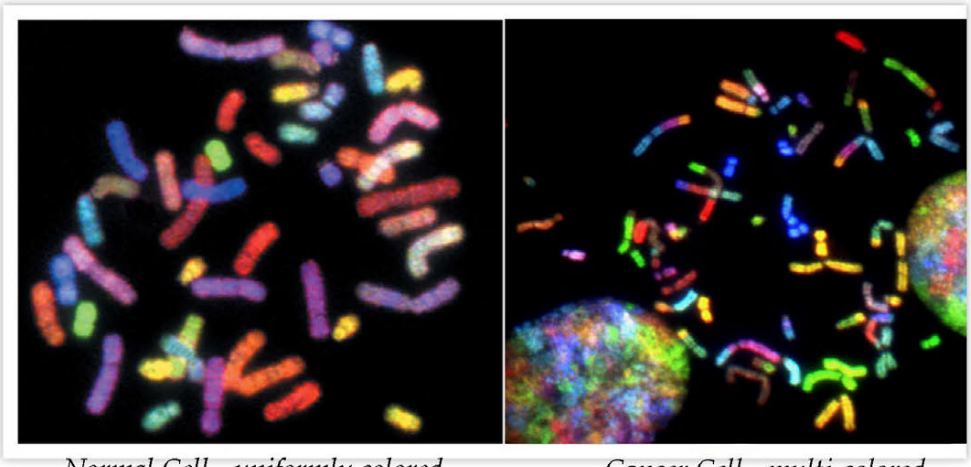
These are "two-hit" types of aberrations that are lethal to the cell either immediately, or else within a few cell divisions.

Other types of chromosome aberrations involve a reciprocal exchange of genetic material, i.e., with no acentric fragments produced, and as such may not be lethal and are potentially long-lived

inversions, translocations and symmetrical exchanges (two hit types):

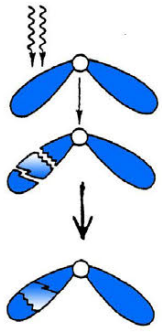


Translocation
(exchage between two different chromsomes)

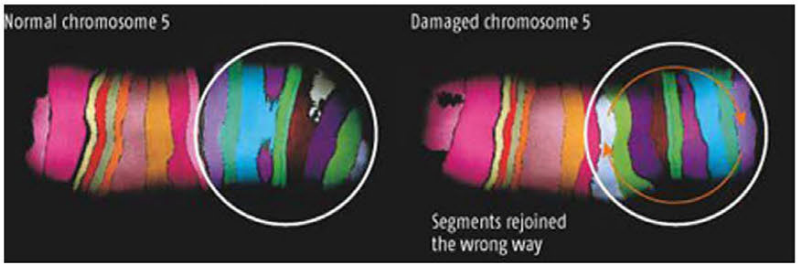


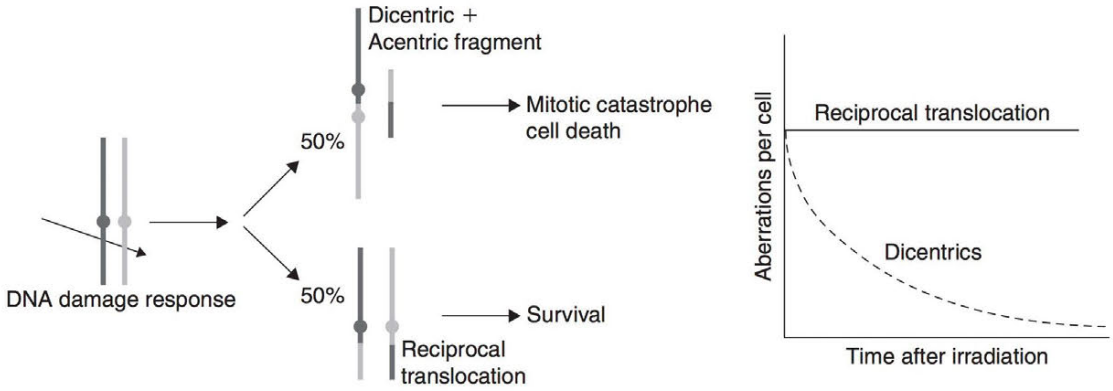
Normal Cell - uniformly colored chromosomes

Cancer Cell - multi-colored chromosomes indicative of multiple translocations



Inversion
(two breaks in the same chromosome, but that rejoin "backwards")





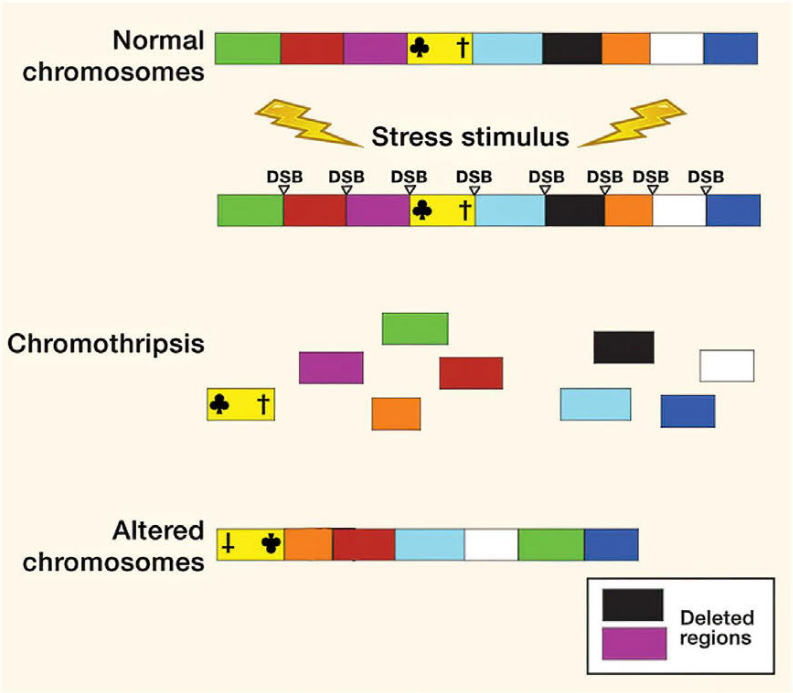
Boards Question: Which types of chromosome aberrations are potentially long-lived (and therefore could be used for dosimetric purposes)?

Answer: Reciprocal translocations

HOT TOPIC

3] **Chromothripsis** - the most extreme kind of chromosome aberration

a. Definition: ***A catastrophic mutational process characterized by up to thousands of clustered chromosomal rearrangements (and usually, small deletions as well) that occur in a single event in localized genomic regions in one or a few chromosomes***



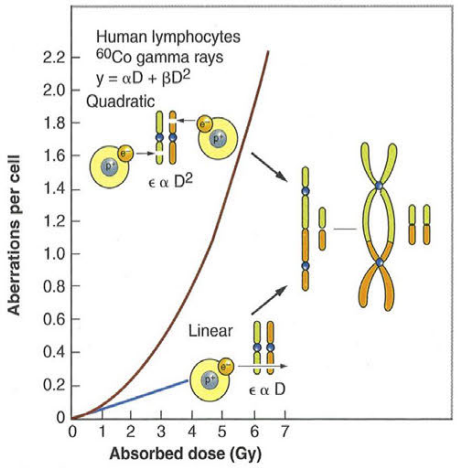
b. Esoteric? For sure, but also relevant in two respects:

1) **radiation can cause it**, with *some* evidence that it is more likely to occur after high LET than low LET radiation exposure

b. if chromothripsis were to occur, **it could transform a cell in a single step**, i.e., that the "*5 or 6 mutations minimally required to initiate carcinogenesis*" would occur all at once

4] Radiation Cytogenetics with Humans and Human Cells: Radiation Dose Response

a. *dose response curves have been generated for human cells (usually lymphocytes) irradiated in vitro and for lymphocytes taken from individuals irradiated in vivo*

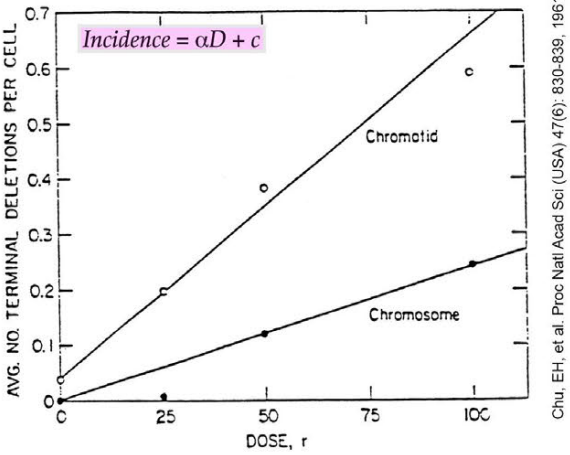


1] for aberrations that only require a "single-hit" chromosome break, the incidence is linear and can be expressed by an equation such as $I = \alpha D + c$

2] for "two-hit" aberrations that require breaks in two separate chromosomes or two parts of a single chromosome, such as rings or dicentrics, the incidence follows a linear-quadratic relationship such as $I = \alpha D + \beta D^2$

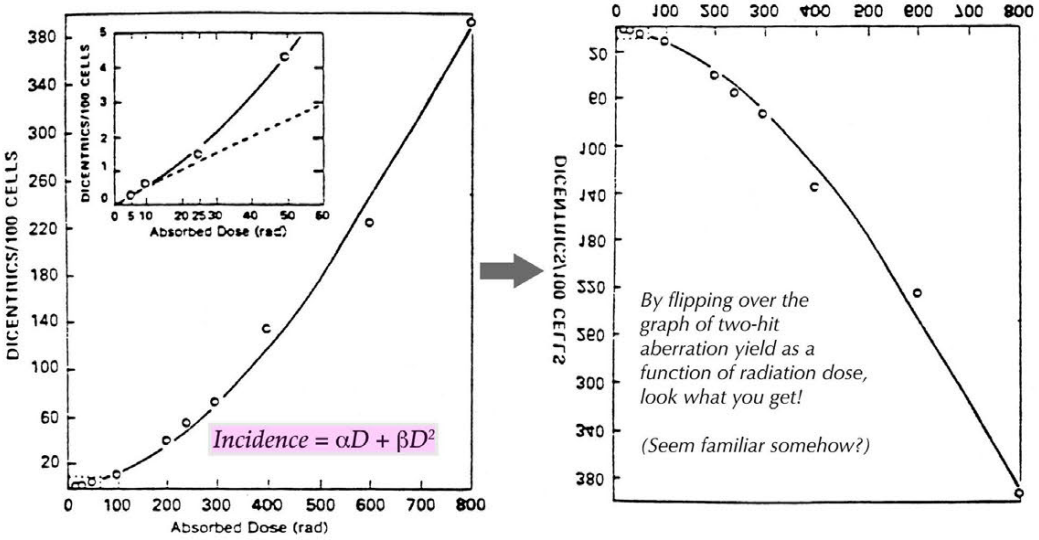
Dose Response for Single-Hit Aberrations

Average number of "single hit" chromosome or chromatid aberrations in human cells irradiated with 250 kv X-rays.



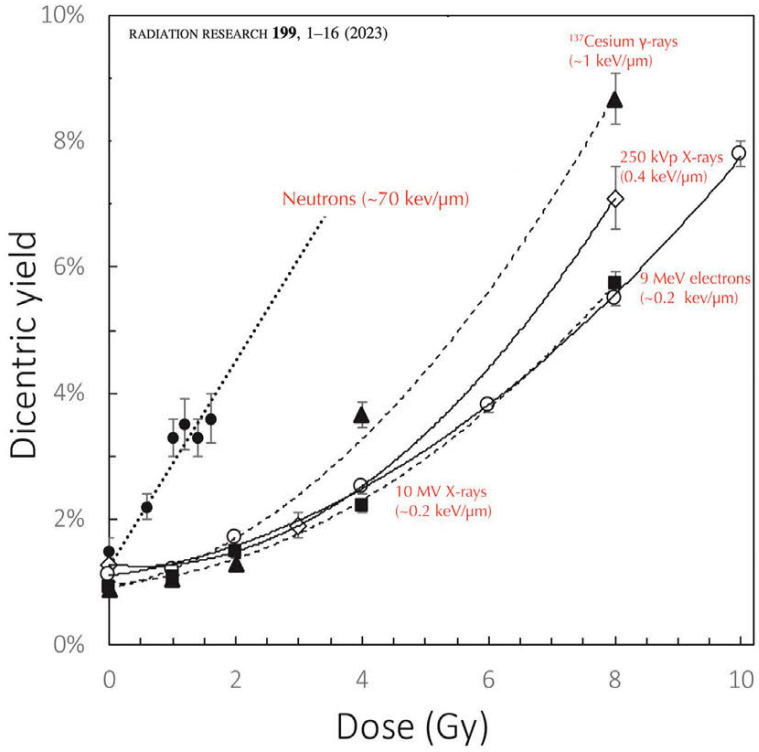
Chu, EH, et al. Proc Natl Acad Sci (USA) 47(6): 830-839, 1961

Dose Response for Two-Hit Aberrations



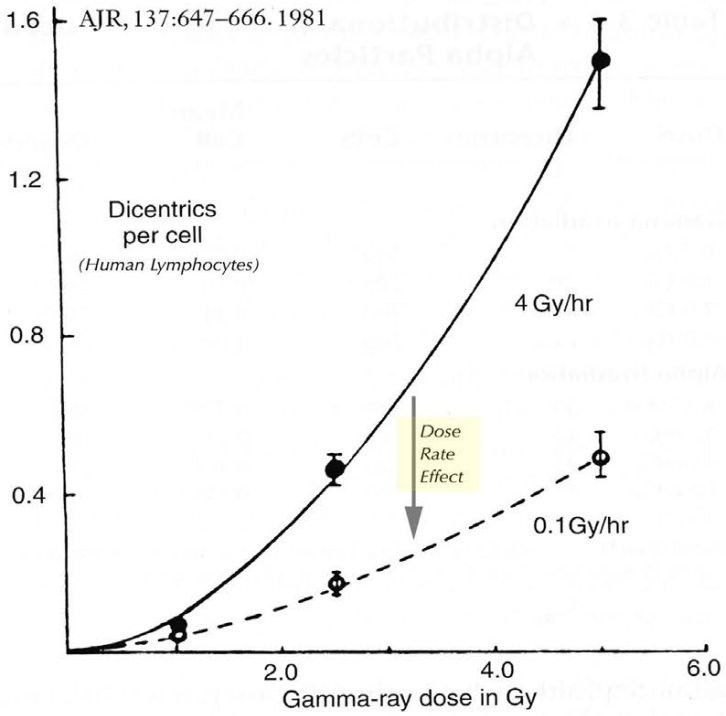
Frequencies of dicentrics in human lymphocytes exposed to x-radiation doses ranging from 5–800 rads (250 kVp x rays, 100 R min^{-1}). Over 14,000 metaphases were scored to obtain data for the 5, 10, 25, and 50 rad points. Insert is an expanded graph showing data at low-dose points and the slope of the α coefficient (Redrawn from data from Lloyd *et al.*, 1975).

b. influence of radiation quality on aberration induction: **the higher the LET of the radiation, the more linear the induction curves for two-hit aberrations, and the steeper the induction curves for all types of aberrations**



c. influence of dose rate on aberration induction:

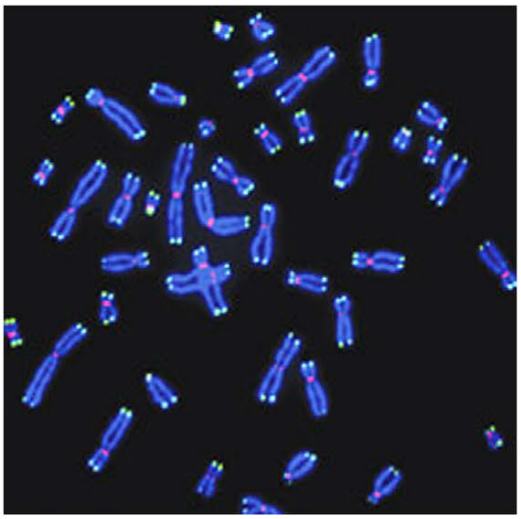
the lower the dose rate, the shallower and more linear the induction curve for two-hit aberrations becomes (β term becomes small)



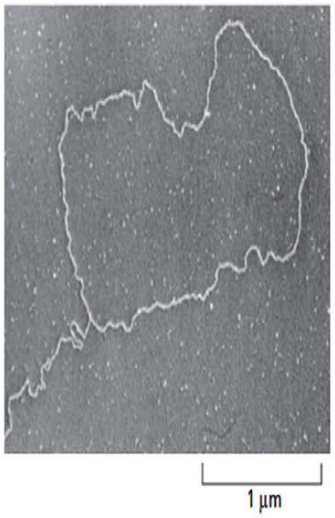
5] Telomeres and Telomerase

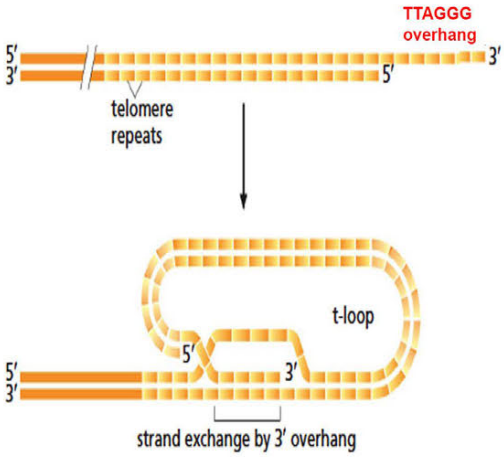
a) *chromosomes normally end in structures called telomeres, which are composed of repeated sequences of 5-8 nucleotides in length; without telomeres, chromsomes become unstable and "sticky", i.e., prone to being lost, auto-digested, or fused with other chromosomes*

1. **once this happens, it ultimately leads to cell death by a process known as senescence**

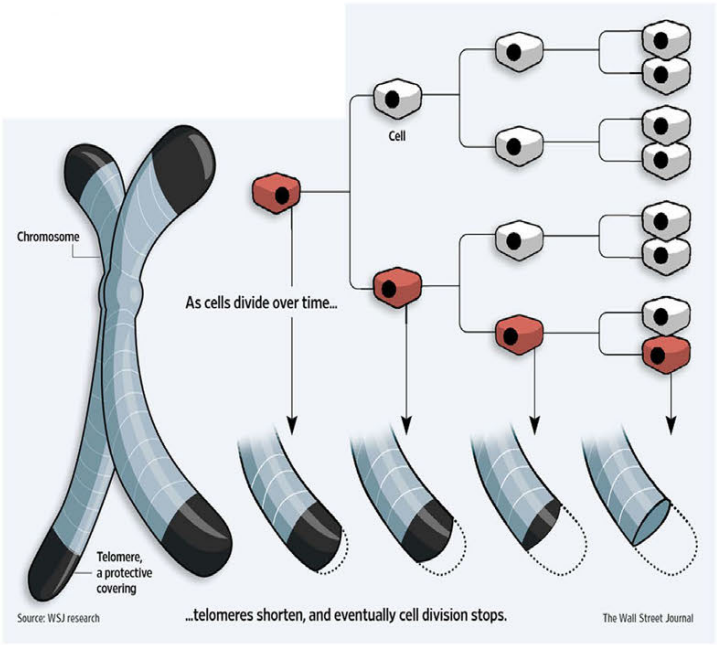


Use of fluorescently-labeled *in situ* hybridization probes to identify specific chromosomal regions. In this example, telomeres fluoresce greenish-yellow and centromeres fluoresce pink.





Telomere Structure



b) in normal cells, each round of DNA replication causes telomeres to shorten slightly; this is thought to be responsible for aging, i.e., that the shortening of telomeres eventually becomes incompatible with the viability of the cell, and ultimately, the organism

c) a few normal cell types (germ and stem cells in particular) avoid aging thanks to the enzyme **telomerase**, which can regenerate telomeres before they become too short

1] Telomerase is encoded by the telomerase reverse transcriptase (***TERT***) gene; besides in germ and stem cells, this gene is turned off in virtually all other somatic cells

d) unfortunately, **malignant cells have learned how to reactivate telomerase causes them to become immortalized**, one of cancer's hallmarks

F. Epigenetic Effects in the Context of Cancer and Its Treatment

1] ***the term "epigenetics" refers to stable, heritable changes in gene expression NOT associated with changes in the DNA coding sequence***

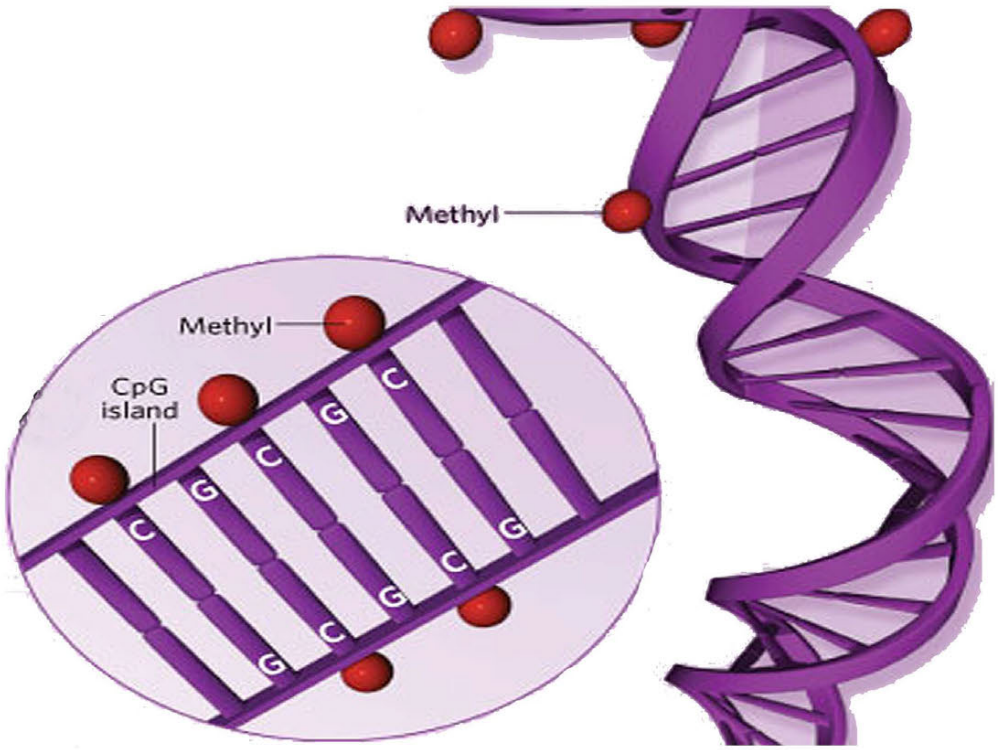
a) a classic example of an epigenetic effect is "**Genomic Imprinting**", a heritable pattern of which allele and its asscoiated genes – maternal or paternal – is expressed in a newly-conceived embryo and which is silent

2] ***there are both transcriptional and post-transcriptional mechanisms for the epigenetic regulation of genes***, and these help explain why different tissues in the same organism express very different genes, even though the underlying DNA sequence in all cells is the same

a) ***mamamalian cells use three main mechanisms to regulate their own genes: DNA methylation, histone modification and RNA interference***

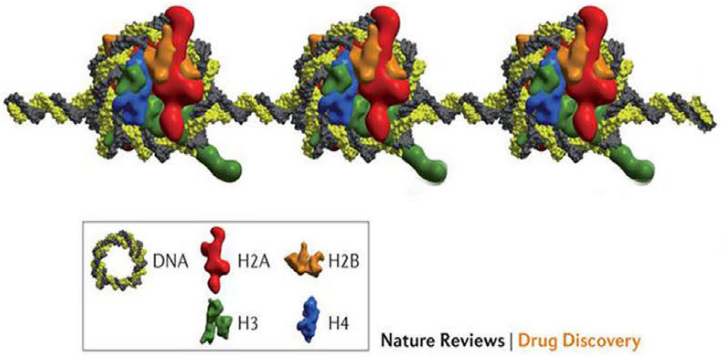
DNA Methylation - *hypermethylation of genes, especially in their promoter regions, has the net effect of silencing genes at the transcriptional level*

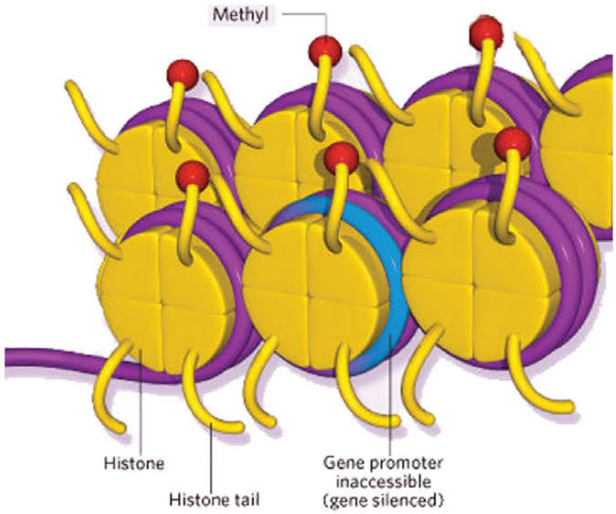
1. ***many human tumors*** ***(both hematological and solid) show substantial changes in epigenetic markers, including*** ***hypermethylation of one or more tumor suppressor genes***



c) **Histone Modification** - *the histone proteins that make up the nucleosome are subject to chemical modifications that affect the "accessibility" of the associated DNA; when inaccessible, genes encoded by that DNA cannot be transcribed, and are thus silenced*

1. when histones are methylated (by histone methylases), transcription is inhibited, and when demethylated, the asscociated genes become active again





Clinical Correlate: IDH1/2 Mutant versus Wild-Type Gliomas

1. IDH = isocitrate dehydrogenase, best-known as an enzyme involved in the Krebs cycle, but that also is involved in the regulation of histone methylation

a) Normally, DNA methylases turn genes off and demethylases turn them back on, but ***if cells harbor IDH1 (or its homolog, IDH2) mutations, their demethylases – including one called TET2, a member of the "ten-eleven translocation" family – are inactive, resulting in many genes ending up hypermethylated***

b) ***IDH1/2 mutations are implicated in the etiology of diffuse gliomas and are a frequent finding on genome sequencing; tumor cells exploit these mutations as a way to hypermethylate, and thereby turn off, tumor suppressor genes*** (among others)

1. however, hypermethylated genes can also represent an Achilles' heel for diffuse gliomas in that one gene that can be turned off is MGMT, and this has two implications for these tumors compared to tumors with wild-type IDH1/2 :

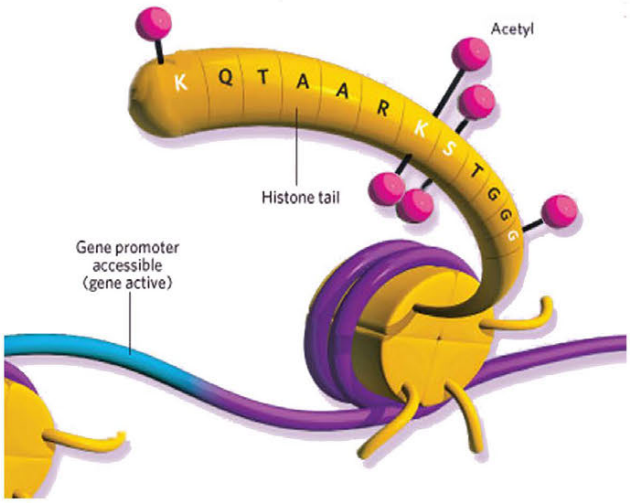
• patients whose tumors harbor IDH1/2 mutations are sensitive to treatment with temozolomide, whereas wild-type tumors are not

• these tumors also have a better prognosis overall than wild-type tumors

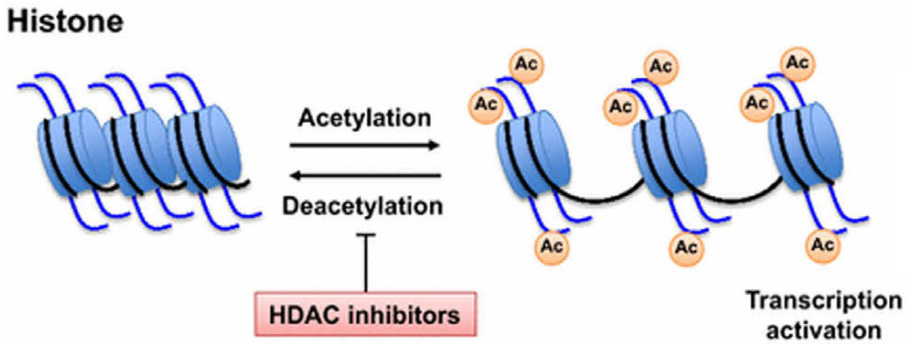
2. a second type of histone modification that alters DNA accessibility is acetylation; when histones are acetylated (by histone acetylase, "HAT"), transcription can occur, and when de-acetylated (by histone deacetylase, "HDAC"), transcription is silenced

Acetylation/deacetylation represents another way to epigenetically control gene expression, and defects in the enzymes that control this can result in oncogenes being activated inappropriately and/or tumor suppressor genes silenced

Luckily, tumors with hyperactive deacetylases (i.e., that would result in too many genes being silenced) can be targeted to clinical advantage...



Another Clinical Correlate!



1. There is ongoing research in the use of **histone deactylase inhibitors ("HDACi")** as a strategy for keeping key genes acetylated and active

a. **tumor cells treated with HDACi can experience cell cycle blocks and delays, undergo apoptosis, change their differentiation status and/or reverse drug resistant phenotypes...in theory due to the reactivation of previously silenced genes (e.g., TP53)**

HDAC inhibitors in clinical use, either alone or in combination with other cancer therapies

Cancers **2021**, *13*, 6280 — Properties of HDAC inhibitors in clinical trials.

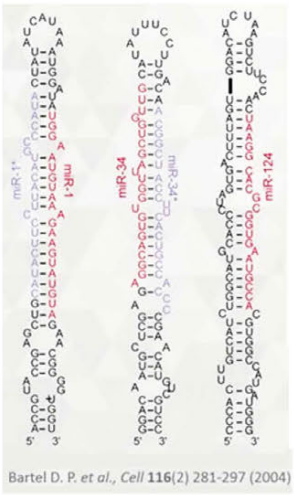
Compound	Target	Source	Chemical Class	Study Phase - Clinical trial
Vorinostat (SAHA) Belinostat (PXD-101) Panobinostat (LBH-589) Trichostatin A (TSA) Quisinostat (JNJ-16241199) WW437 Dacinostat (LAQ824)	Pan-HDAC	Synthetic Synthetic Synthetic Natural Synthetic Synthetic Synthetic	Hydroxamic acid	FDA approval for Cutaneous T-Cell Lymphoma FDA approval for Peripheral T Cell lymphoma (PTCL) FDA approval (PTCL and multiple myelomas) Toxic–Phase I Phase II Cutaneous T-Cell Lymphoma Pre-clinical Phase I
Pivaloyloxmethylbutyrate (AN-9) Sodium Butyrate (NaB) Sodium Phenylbutyrate (4-PB) Valproate (valproic acid)	Pan-HDAC	Synthetic Natural Synthetic Synthetic	Short chain fatty acids	Phase II Melanoma and Phase I Chronic lymphocytic leukemia (CLL) Phase I Colorectal cancer FDA approval (urea cycle disorders) Phase I (Brain and Central Nervous System Tumors)
Romidepsin (FK228)	Pan-HDAC	Natural	Cyclic tetrapeptides	Phase II
Entinostat (MS-275) Tacedinaline (CI-994) Mocetinostat (MG-0103)	Pan-HDAC	Synthetic Synthetic Synthetic	Benzamides	Phase II (Hodgkin's Lymphoma) Phase II (Myeloma) Phase I (Hodgkin's Lymphoma)
Trapoxins (TPX) α-ketoamides Heterocyclic ketones	Pan-HDAC	Natural Synthetic Synthetic	Ketones	NA (Not approved) NA NA
Cambinol EX-527 Sirtinol Nicotinamide	Sirtuin inhibitors	Synthetic Synthetic Synthetic Synthetic		Pre-clinical Pre-clinical Pre-clinical Phase III (laryngeal cancer)
Azelaic Bishydroxamic Acid (ABHA) m-carboxycinnamic acid bis-hydroxamide (CBHA) Ricolinosta (ACY-1215) Tubacin	Selective HDAC	Synthetic Synthetic Synthetic Synthetic	Hydroxamate Derivatives Benzamides	NA Pre-clinical Phase II Pre-clinical Acute lymphocytic leukemia (ALL)

Regulatory RNAs - engineers of the cell's complex transcriptional and translational landscape

A. A lot has changed since back in the days when there was only mRNA and tRNA...

1) ***it is now clear that RNA is not only the intermediary between DNA and protein, but also plays major roles in the organization of the genome, and the epigenetic regulation of gene expression***

RNA Interference - *a post-transcriptional gene silencing strategy, in which the cell uses its own genetic and enzymatic machinery to block the translation of its own mRNA's*



Bartel D. P. *et al.*, *Cell* **116**(2) 281-297 (2004)

MicroRNAs (miRNAs)

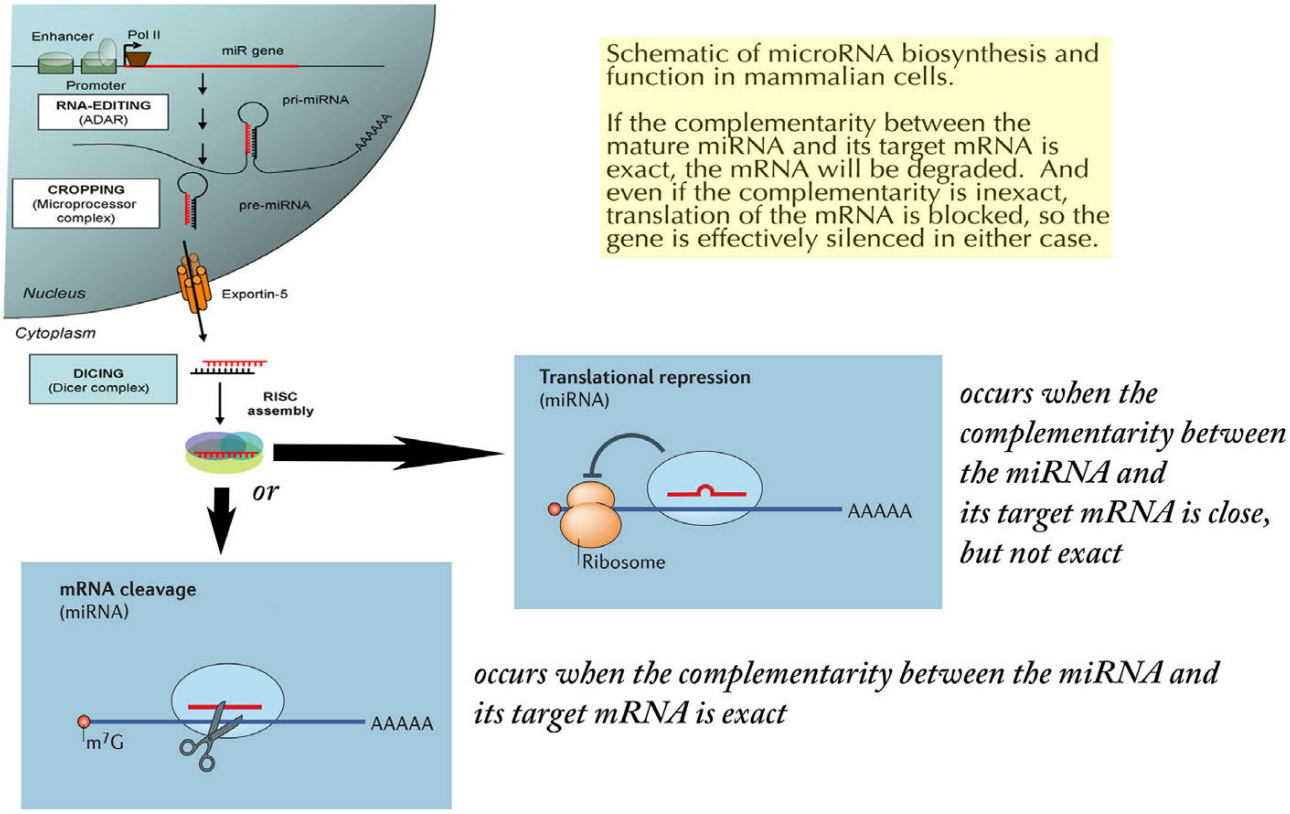
miRNAs are endogenous 18–23 nucleotide non-coding RNAs that refine gene expression

miRNAs control gene expression by targeting mRNAs based on sequence complementarity

Functional studies have confirmed that miRNA dysregulation is causal in many cases of cancer

The widespread deregulation of miRNAs in disease conditions can be harnessed as biomarkers and potential therapeutics

miRNA's are transcribed from cellular DNA, and go on to silence *other* genes



Schematic of microRNA biosynthesis and function in mammalian cells.

If the complementarity between the mature miRNA and its target mRNA is exact, the mRNA will be degraded. And even if the complementarity is inexact, translation of the mRNA is blocked, so the gene is effectively silenced in either case.

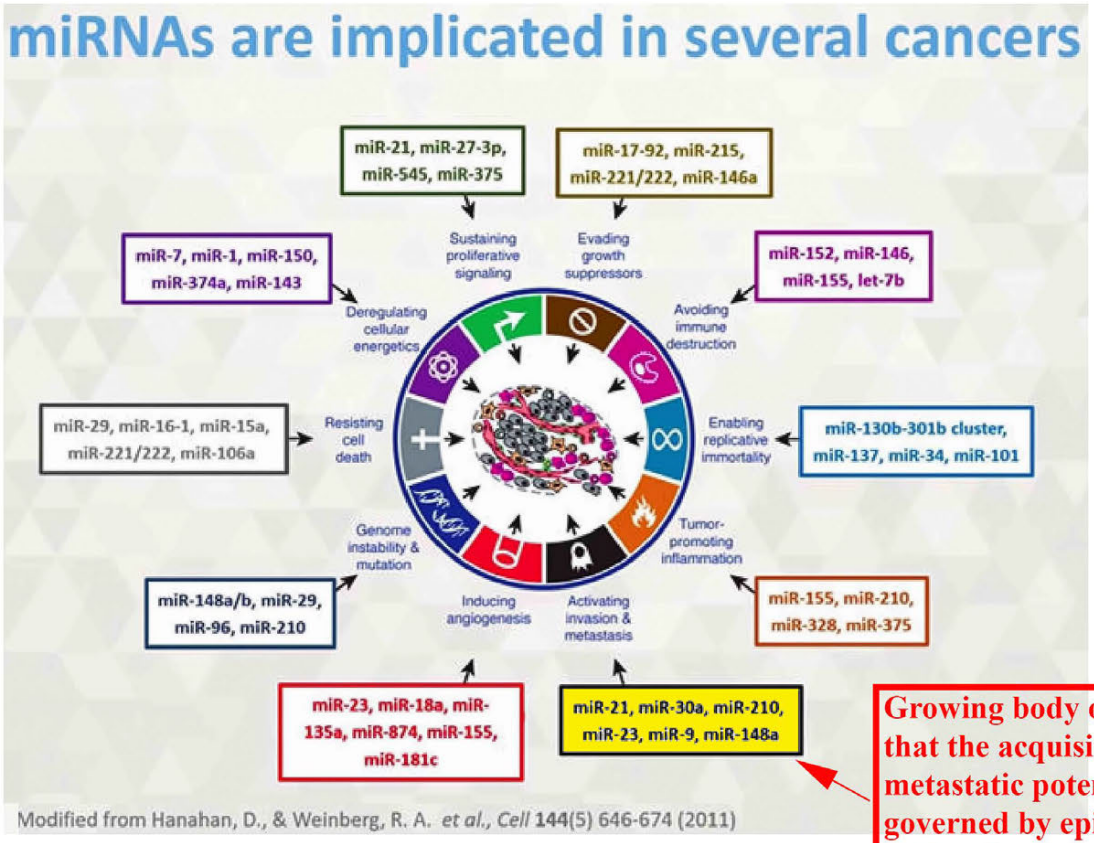
occurs when the complementarity between the miRNA and its target mRNA is close, but not exact

occurs when the complementarity between the miRNA and its target mRNA is exact

C. Kanellopoulou, S. Monticelli / *Seminars in Cancer Biology* 18 (2008) 79–88

1. Because the miRNA's work to block translation even when they are not exactly complementary to their target, some of them can regulate the expression of many genes, rather than just one

2. Different cancers show some common and some unique patterns when it comes to the expression of miRNAs, and it is hoped that in the future, miRNAs could serve either as cancer biomarkers and/or as therapeutic targets



Growing body of evidence that the acquisition of metastatic potential is largely governed by epigenetic changes rather than genetic ones

3. Not only is the activity of miRNA's associated with carcinogenesis, there is also an association between the up- or down-regulation of certain miRNA's and radiation sensitivity

RADIATION RESEARCH 185, 668–677 (2016)

Effect of Up-/Downregulation of Different miRNAs on Sensitivity to Radiotherapy in *In Vivo*/Xenograft Studies

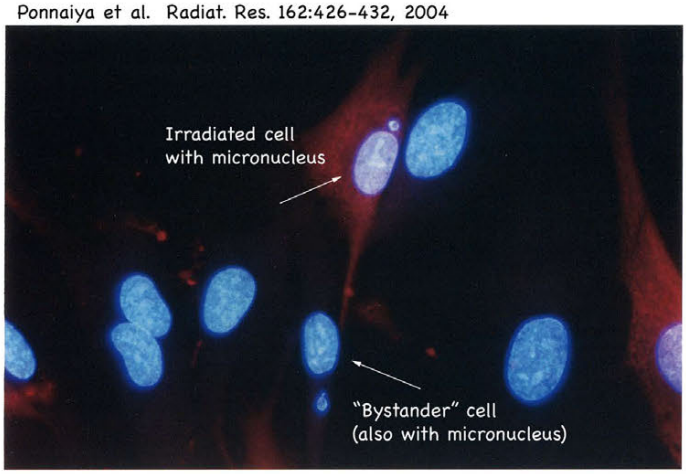
MiRNA	Regulation	Correlation with response	Animal model	Dose	Possible targets
let-7	↑	↑ Sensitivity	*C. elegans*	200/400 Gy	let-60/RAS, DDR
let-7b	↑	↑ Sensitivity	Uveal melanoma (mice)	12/18 Gy	Cyclin D1
miR-181a	↑	↑ Resistance	Cervical (mice)	16 Gy	PRKCD
miR-101	↑	↑ Sensitivity	Glioma/lung cancer (mice)	10 Gy	DNA-PKcs, ATM
miR-145	↑	↑ Sensitivity	Cervical (mice)	24 Gy	HLTF
miR-381	↑	↑ Sensitivity	Esophageal (mice)	5 Gy	CTNNB1, LEF1, CDK1, XIAP, CXCR4
miR-181a	↑	↑ Resistance	Cervical (mice)	16 Gy	PRKCD
miR-185	↑	↑ Sensitivity	Renal cell (mice)	4 Gy	ATR
miR-210	↓	↑ Sensitivity	Hepatoma (mice)	8 Gy	AIFM3

Note. DDR = DNA damage response; PRKCD = protein kinase C delta type; DNA-PKcs = DNA-dependent protein kinase catalytic subunit; ATM = ataxia-telangiectasia mutated; HLTF = helicase-like transcription factor; ATR = ataxia telangiectasia and Rad3-related protein; AIFM3 = apoptosis-inducing factor mitochondrion-associated 3.

The Bystander Effect: an epigenetic phenomenon of particular interest to the radiation biology community

a) **the bystander effect can be defined loosely as the activation of radiation-induced signalling cascades, and the appearance of radiation injury (including cell death), in cells *neighboring* irradiated cells, but that were not themselves irradiated**

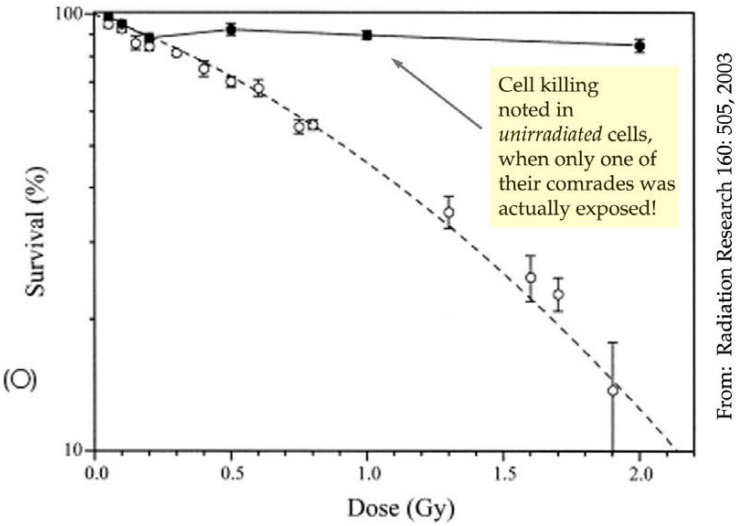
Ponnaiya et al. Radiat. Res. 162:426-432, 2004



Micronucleus formation is an indicator that mitotic catastrophe has occurred, i.e., that a cell has limped through mitosis but with acentric fragments present in the cytoplasm.

These are often noted in cells following their first mitosis after irradiation, and usually means that the cell has or will lose its reproductive integrity and die.

You would **not** expect to see micronuclei in unirradiated cells, least of all in the immediate vicinity of irradiated ones!



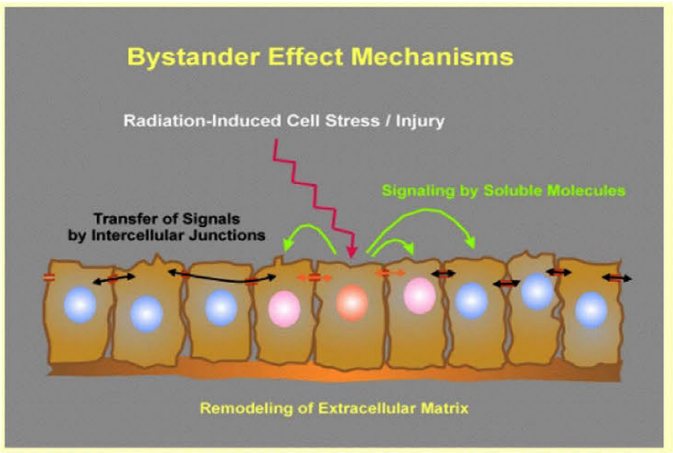
From: Radiation Research 160: 505, 2003

Comparison of surviving fractions after all-cell irradiation (○) and single-cell irradiation (●).

b) there are two known mechanisms of action for the radiation-induced bystander effect

1. one clearly involves diffusible substances (e.g. cytokines, ROS, exosomes, miRNAs, DAMPS, immune molecules, etc.) released by irradiated cells that cause responses in their irradiated neighbors...including at a distance

2. the second mechanism of action requires cell-to-cell contact, and is mediated by "cross-talk" through cellular tight junctions



c) What makes the bystander effect important for radiation biology and oncology?

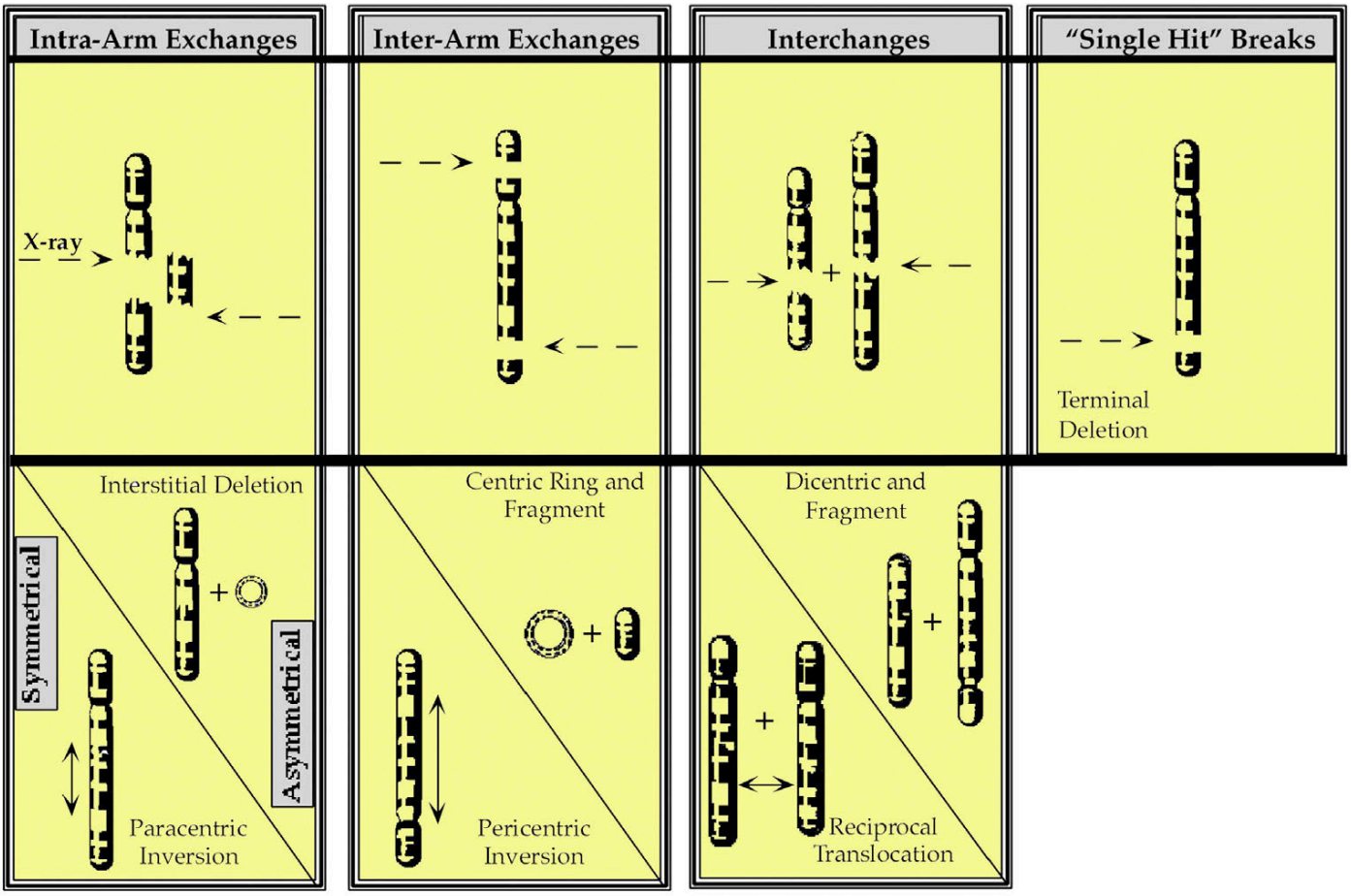
1. for radiation oncology, the infrequently-noted (and still of unclear significance) **abscopal effect** qualifies as a type of bystander effect, as do many of the cellular interactions that can occur in the tumor microenvironment that have the potential to affect radiation sensitivity

Appendix Materials

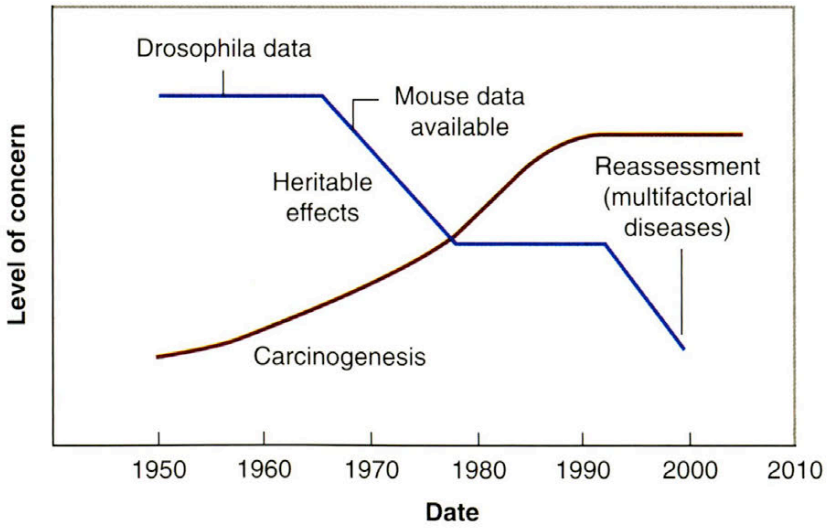
Quantitative Dose-Response Relationships for Radiation-Induced Chromosome Aberrations

Radiobiological Property	Aberration Type	Low LET Radiation	High LET Radiation
Dose Response Curve Shape	Two-Hit	Linear-Quadratic	Linear for both, with higher yields than low LET
	One-Hit	Linear	
Dose Rate Effect	Two-Hit	Yield Drops (to a point) with Dose Rate	No change in yield with dose rate
	One-Hit	Unchanged	
Dose Fractionation Effect	Two-Hit	Yield Drops (to a point) with Decreasing Fraction Size	No change in yield with dose fractionation
	One-Hit	Unchanged	
Oxygen Effect	Two-Hit	OER from 2.0 – 3.0 for both (single doses; a bit lower if fractionated)	OER from 1.0 – 1.5 for both (depending on LET)
	One-Hit		
Hypoxic Cell Sensitizers	Two-Hit	SER's from about 1.2 – 2.2 for both depending on the radiosensitizer	Uncertain, but probably little or no change
	One-Hit		

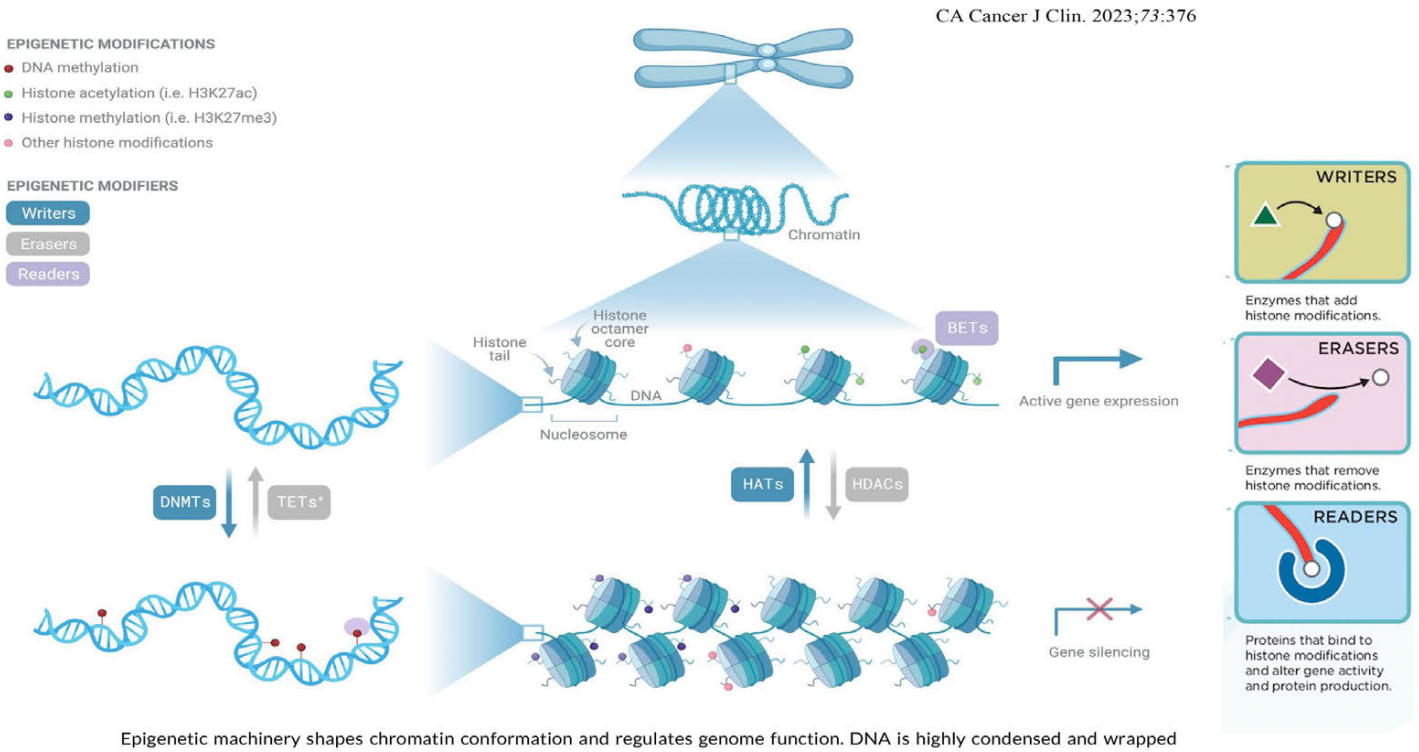
Keeping Track of Different Classes of Chromosome Aberrations



Radiation Risk: Genetic versus Somatic

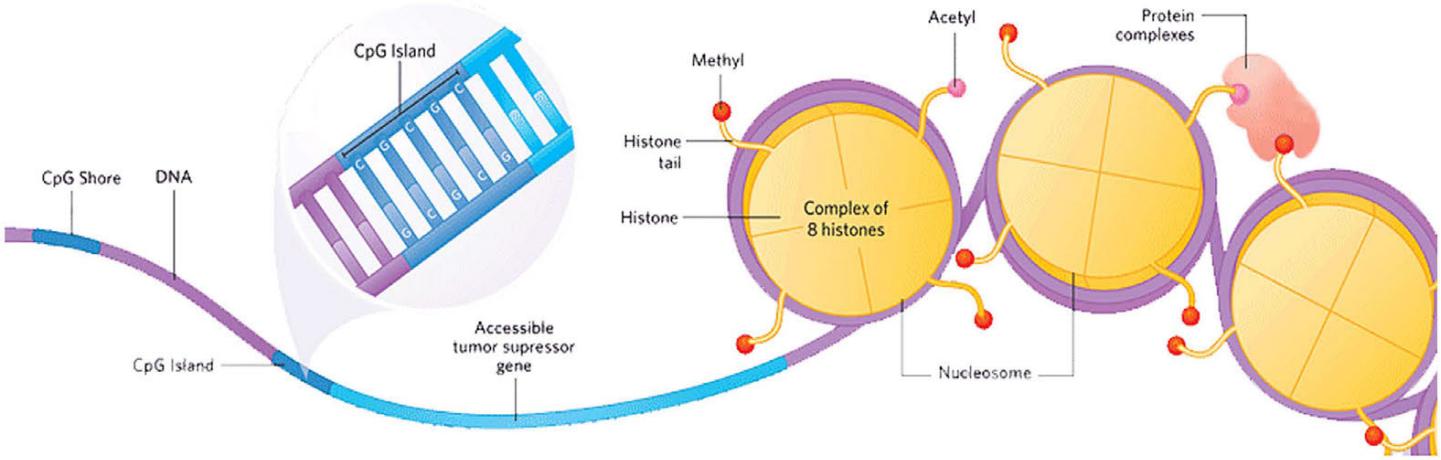


Illustrating how, over the past half a century, the concern regarding exposure to ionizing radiation has changed from heritable (genetic) effects to carcinogenesis.



Epigenetic machinery shapes chromatin conformation and regulates genome function. DNA is highly condensed and wrapped around a histone octamer core to form a nucleosome, which is the fundamental subunit of chromatin. Epigenetic modifications, including DNA methylation and histone marks, form a complex regulatory network that modulates chromatin structure and genome function. Epigenetic players include enzymes that introduce (writers), recognize (readers), and remove (erasers) epigenetic marks to DNA or histone tails. DNA methylation is catalyzed by DNA methyltransferases (DNMTs) and is removed by ten-eleven translocation enzymes (TETs) or passively through sequential cell divisions (*). Several histone modifications have been described; acetylation and methylation are depicted here because they are the most widely studied histone marks. Histone methylation status is determined by the opposing actions of histone methyltransferases (HMTs) and histone demethylases (HDMs). The same interplay occurs between histone acetyltransferases (HATs) and histone deacetylases (HDACs), which add or remove acetyl groups to lysine residues in the histone tails.

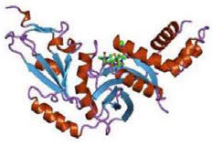
Keeping Your Epigenetic Modifications Straight



Euchromatin = "On" = DNA Hypomethylated; Histones Hyperacetylated and (relatively) Hypomethylated

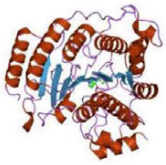
Heterochromatin = "Off" = DNA Hypermethylated; Histones Hypoacetylated and Hypermethylated

How HATs and HDACs Work



HAT1

- Histone acetyltransferases (HAT)
  Transfer the acetyl groups to the histone residues and are usually involved in activation of transcription



HDAC8

- Histone Deacetylases (HDAC)
  Remove acetyl groups and are usually involved in repression of transcription