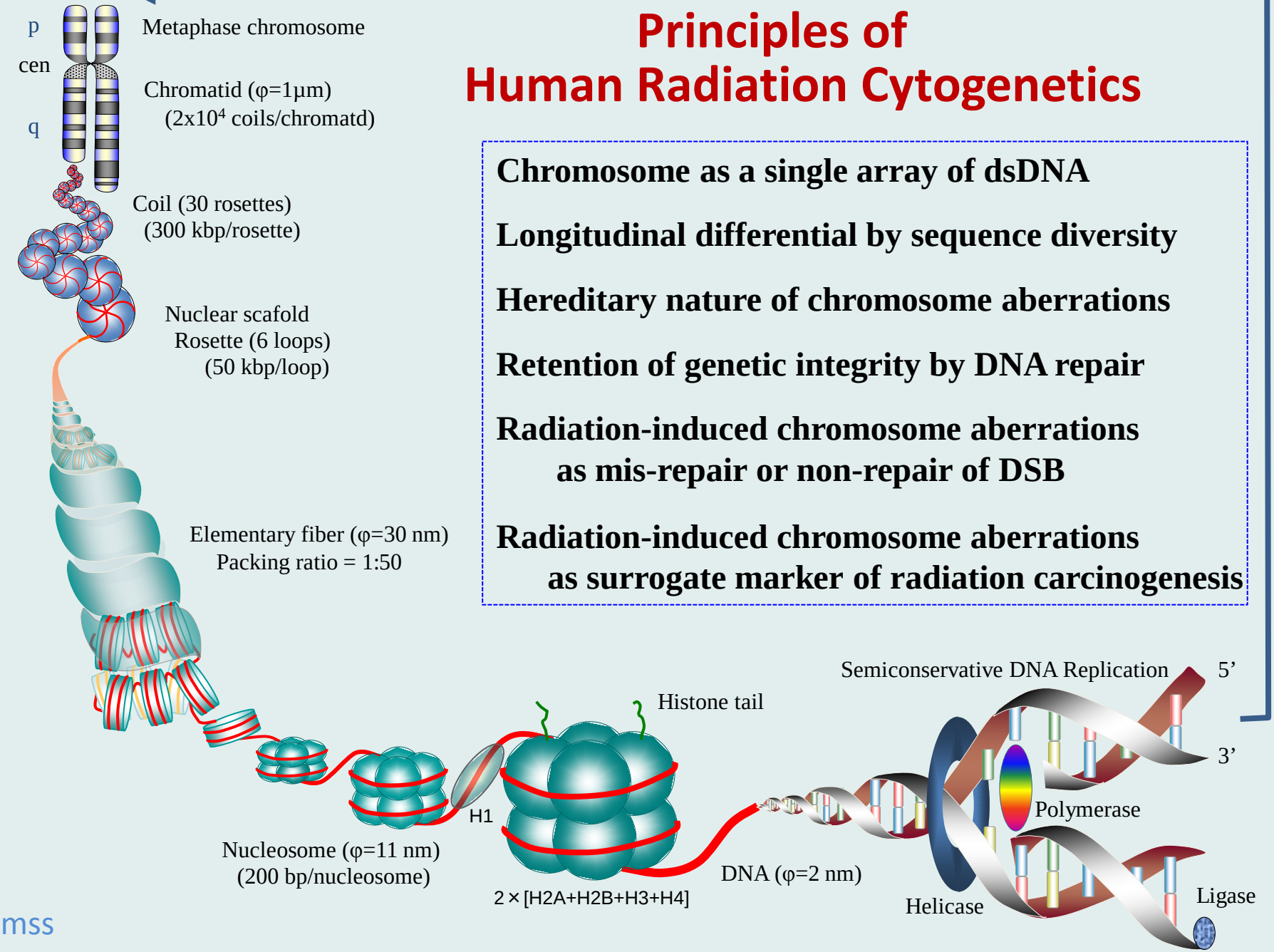
Principles of Human Radiation Cytogenetics



Chromosome as a single array of dsDNA

Longitudinal differential by sequence diversity

Hereditary nature of chromosome aberrations

Retention of genetic integrity by DNA repair

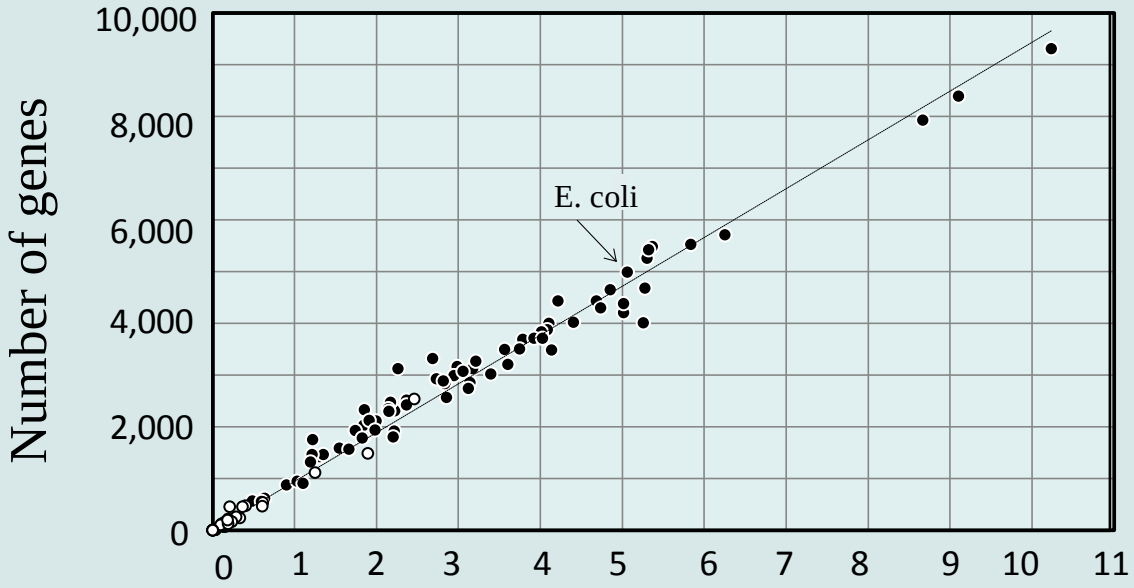
Radiation-induced chromosome aberrations as mis-repair or non-repair of DSB

Radiation-induced chromosome aberrations as surrogate marker of radiation carcinogenesis

mss

Genome size and gene number

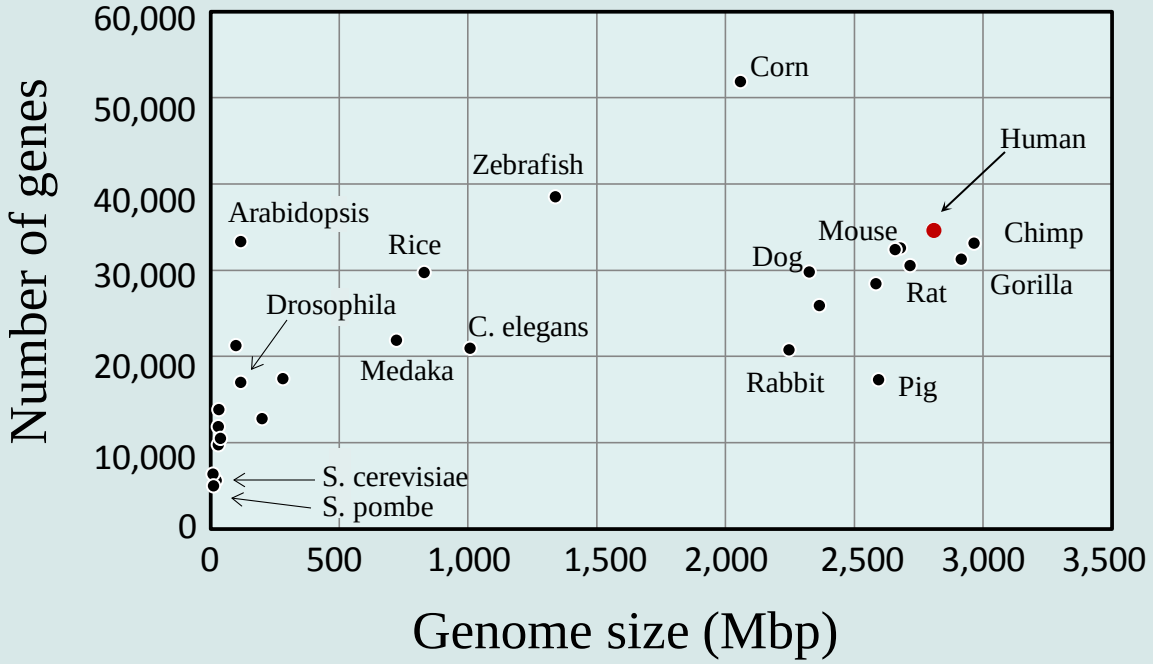
Data compiled from the NCBI/NIH database
"http://www.ncbi.nlm.nih.gov/genome"



Prokaryotes

- ○ dsDNA virus
- ● Bacteria (Arcaea and eubacteria)

$y = 942.5 \cdot x, \quad or\ 1.06 kbp / gene$



Eukaryotes

[I] Proportionality is lost because of

(1) Gain of noncoding sequences
(2) Gain of introns
(3) Copy number variation (CNV)
(4) Gain of repetitive sequences
(5) Gain of noncoding sequences
(6) Amphidiploidy or allopolyploidy

during evolution.

[II] Functional diversity of a gene:
by alternative splicing.

Physical map and gene number of human chromosomes

Chrom	Arm	Size (Mbp)	Gene number
[1]	p	128	3,380
	q	135	
[2]	p	99	2,204
	q	156	
[3]	p	99	1,760
	q	115	
[4]	p	56	1,361
	q	147	
[5]	p	52	1,536
	q	142	
[6]	p	65	1,959
	q	118	
[7]	p	65	1,764
	q	106	
[8]	p	50	1,247
	q	105	
[9]	p	51	1,435
	q	94	
[10]	p	44	1,305
	q	100	

Chrom	Arm	Size (Mbp)	Gene number
[11]	p	58	2,051
	q	86	
[12]	p	39	1,629
	q	104	
[13]	p	16	649
	q	98	
[14]	p	16	1,453
	q	93	
[15]	p	17	1,202
	q	89	
[16]	p	39	1,318
	q	59	
[17]	p	28	1,714
	q	64	
[18]	p	20	517
	q	65	
[19]	p	30	1,992
	q	37	
[20]	p	31	857
	q	41	

Chrom	Arm	Size (Mbp)	Gene number
[21]	p	11	425
	q	39	
[22]	p	13	835
	q	43	
[X]	p	62	1,606
	q	102	
[Y]	p	13	393
	q	46	
Total (♂)		3,286	34,592

Coding sequence=about 3 %

Compiled from:

Morton NE. Parameters of human genome. Proc. Natl. Acad. Sci. USA, 88:7474-7476, 1991.

Lander ES et al. Initial sequencing and analysis of the human genome. Nature, 409:860-921, 2001.

Venter JC et al. The sequence of the human genome. Science, 291:1304-1351, 2001.

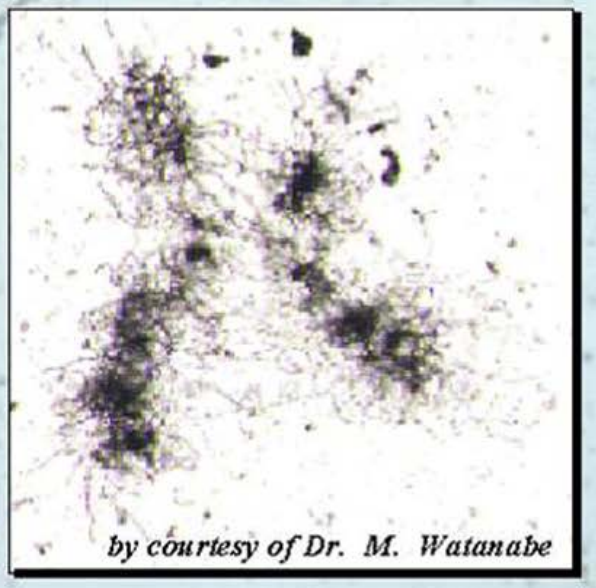
Pre-replication chromosome:

Single array of dsDNA (uninemy)

DNA fiber autoradiography

1 mm

Electron microscopy

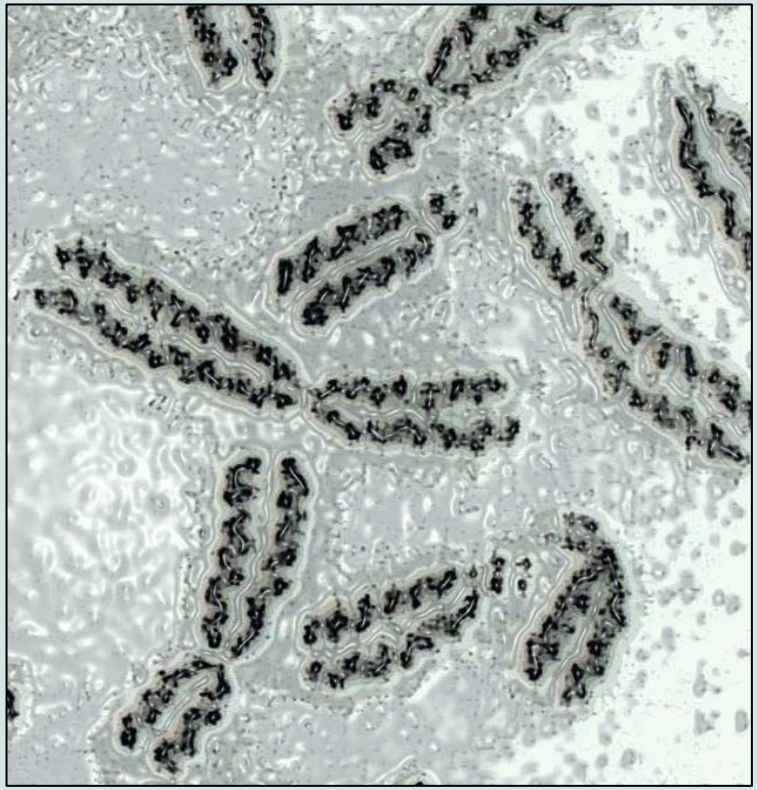


by courtesy of Dr. M. Watanabe

Post-replication chromosome:

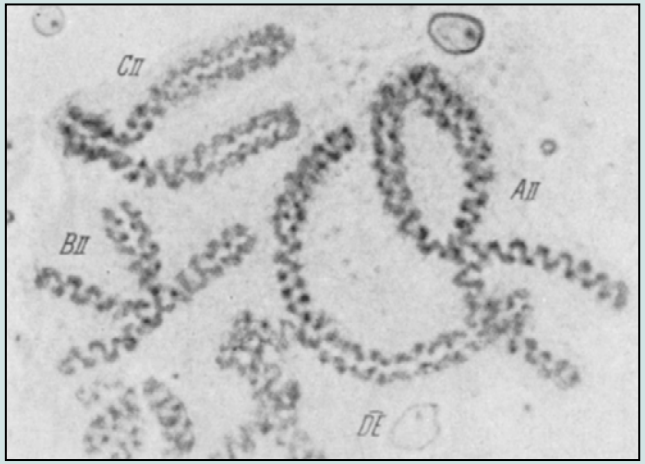
High order structure at methaphase (coiled-coil)

High order structure at metaphase chromosomes (packing)



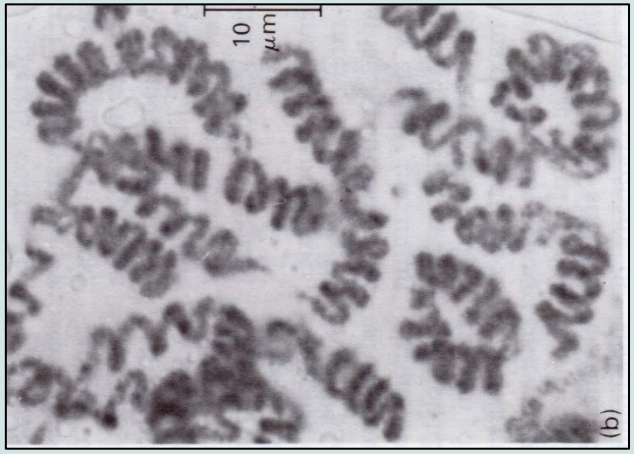
Human chromosomes (somatic)

(by the Courtesy of Dr. Y. Ohnuki)



Trillium (meiosis I)

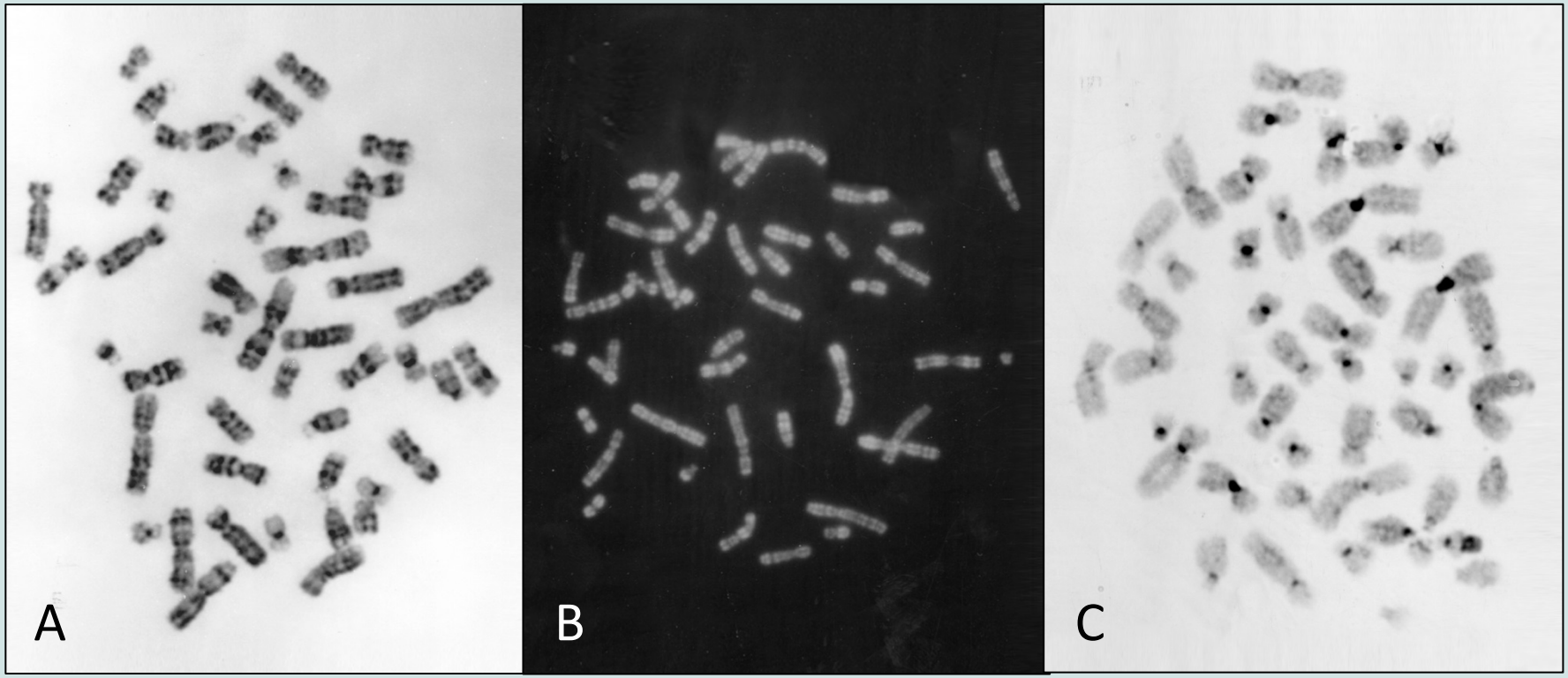
Matsuura H. Chromosoma 4:S273, 1951



Tradescantia (meiosis I)

Dyer, AF. “Investigating Chromosomes”, Wiley, New York, 1979.

Longitudinal differentiation of human chromosomes



G-band

Giemsa (G) banding: Giemsa staining after hot saline treatment.

R-band

Reverse (R) banding: Quinacrine (Q) staining, or Acridine staining for early replication segments.

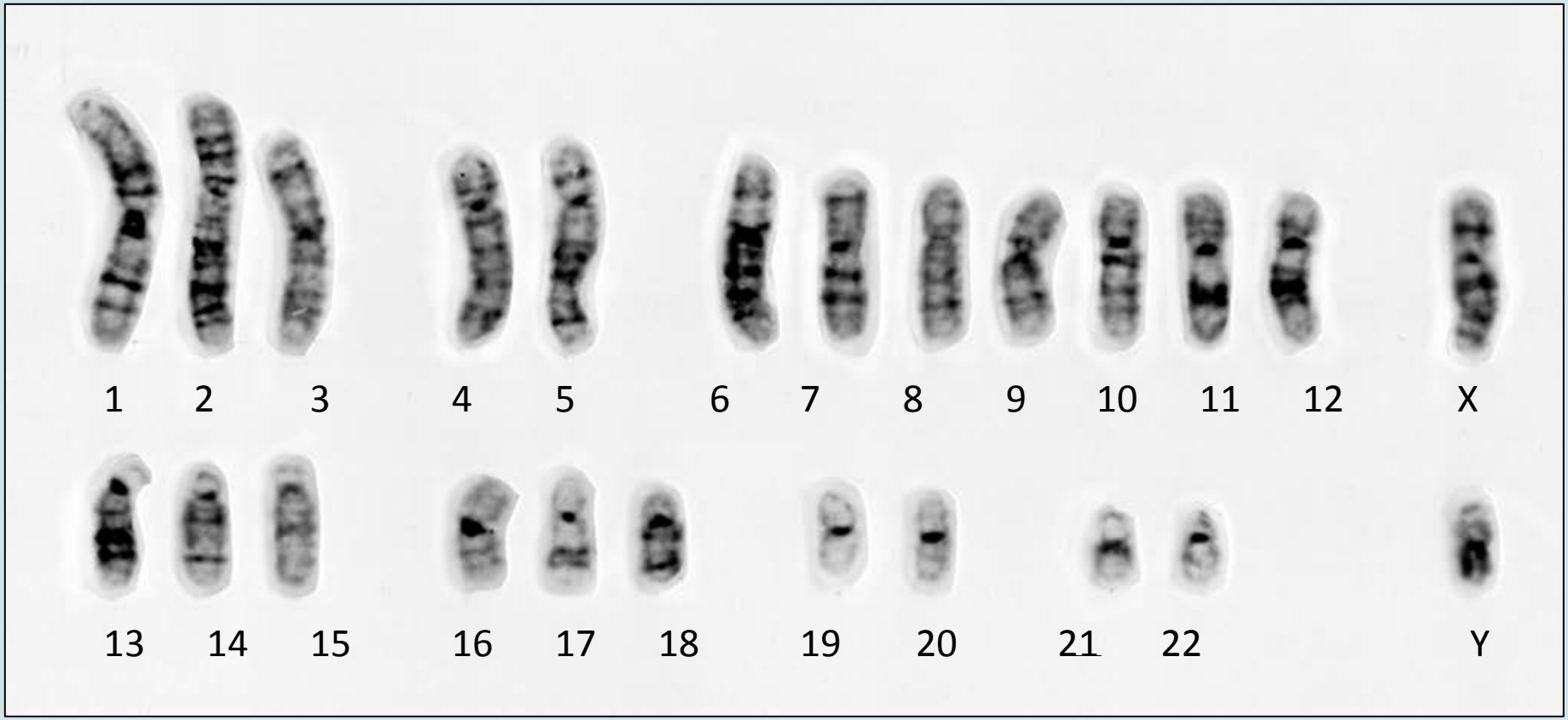
C-band

Centromeric (C) staining: Giemsa staining after denaturation-renaturation treatment.

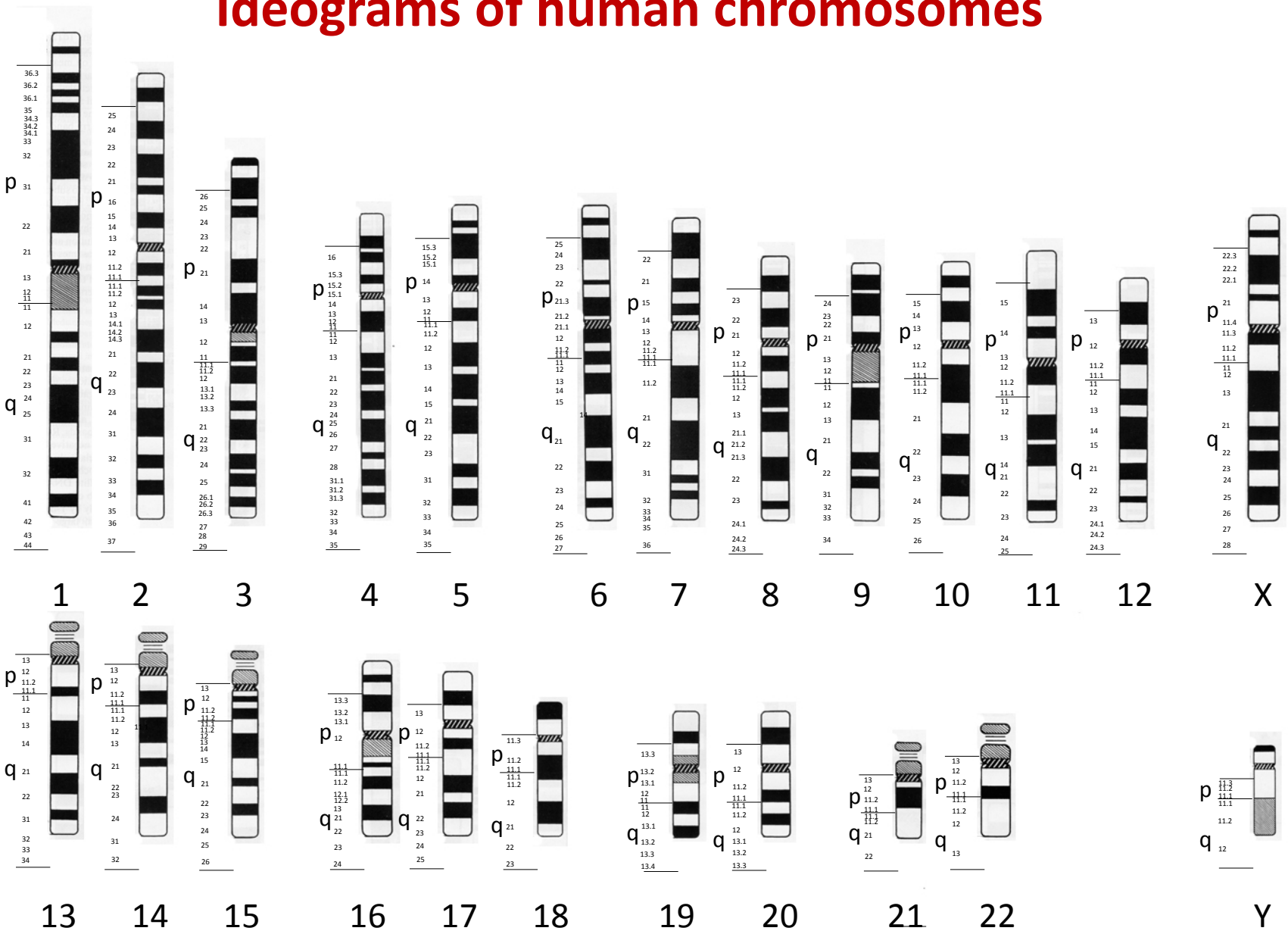
G-band and R-band are in opposite images. G-positive regions are A-T rich and gene poor regions, and G-negative (R-positive) regions are G-C rich and gene rich regions. DNAs in G-positive (R-negative) regions replicate in early stages of S-phase. C-positive regions are abundant of highly repeated sequences.

Banding patterns of human mitotic chromosomes after staining with Giemsa (G-bang)

(400 bands level)

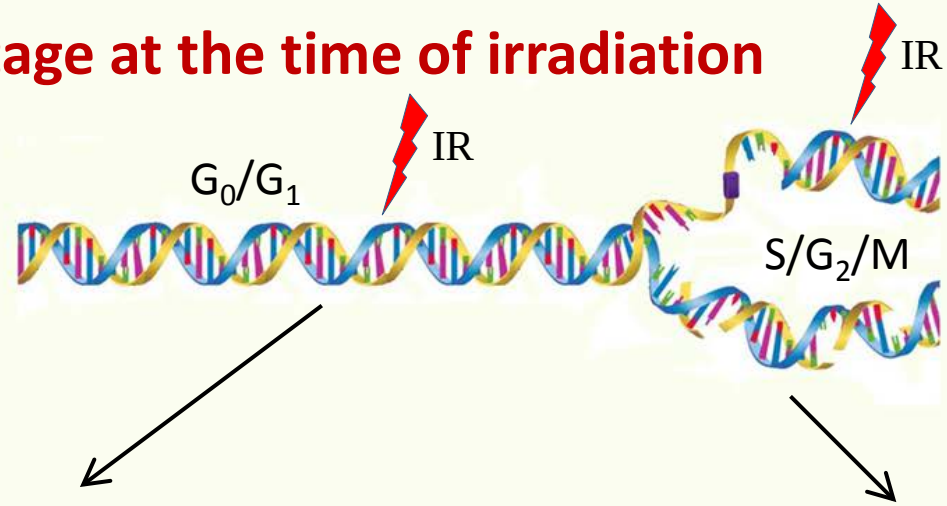


Ideograms of human chromosomes

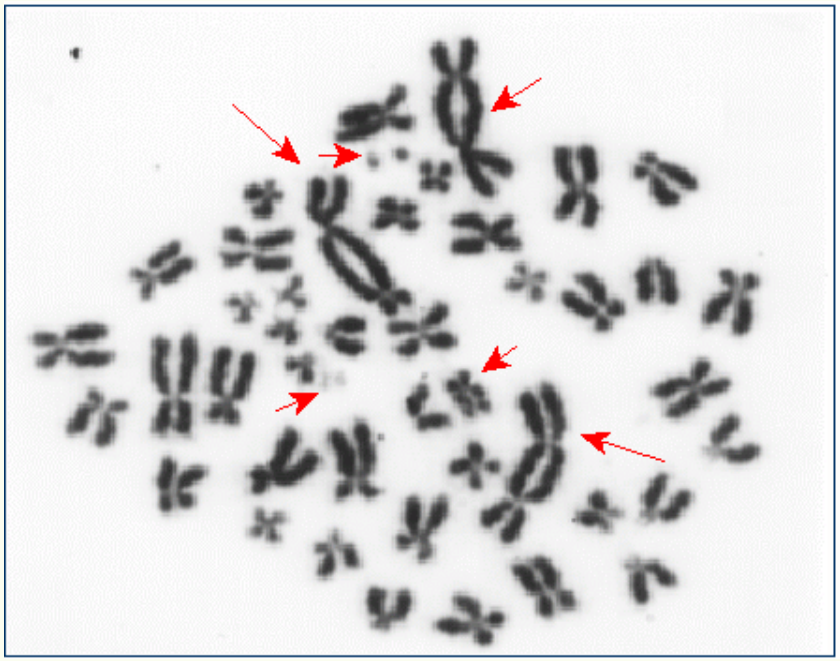


Modified from: ISCN2005; International System for Human Cytogenetic Nomenclature (2005)

Types of chromosome aberration and cell-cycle stage at the time of irradiation

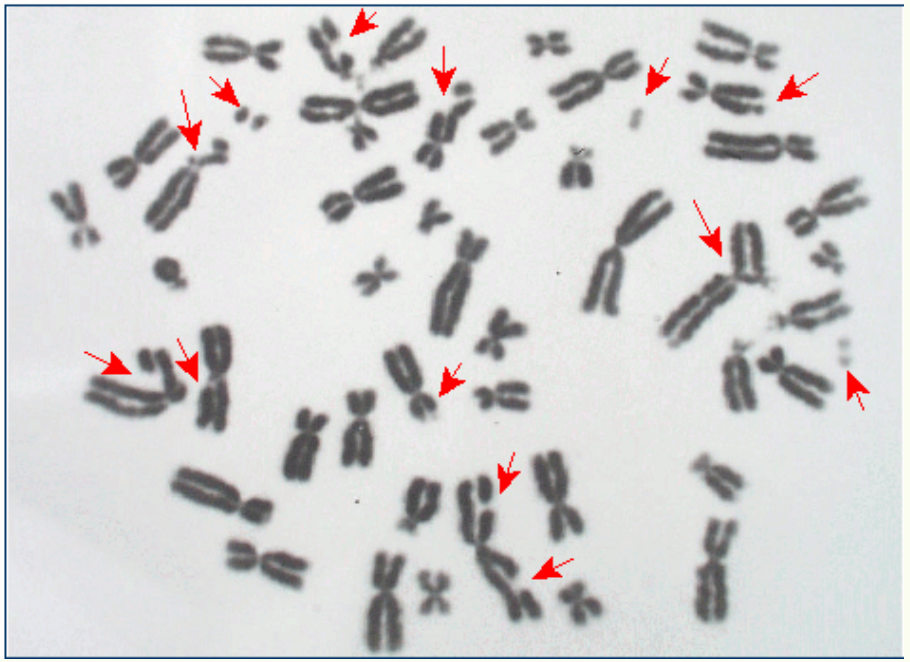


Chromosome-type aberrations



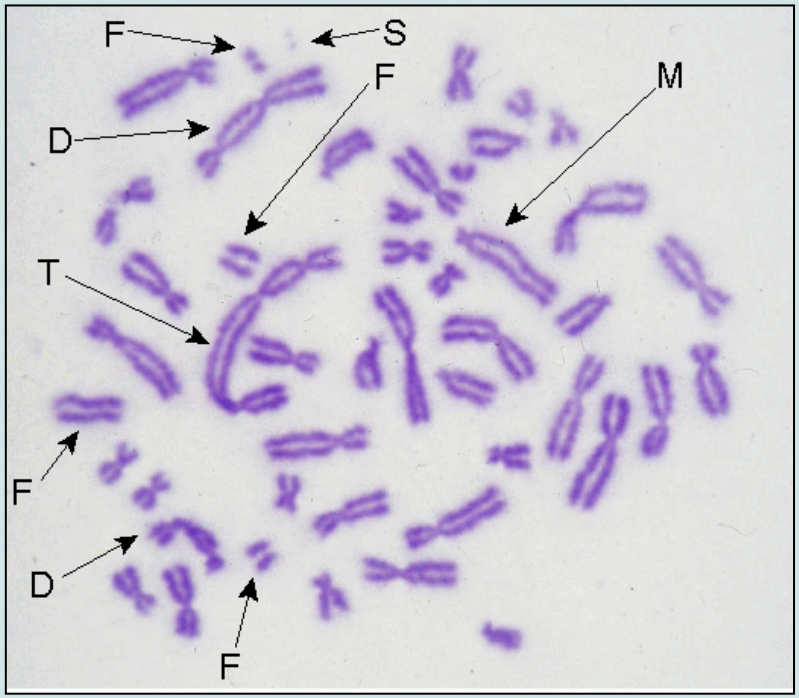
Unit of aberration is chromosome

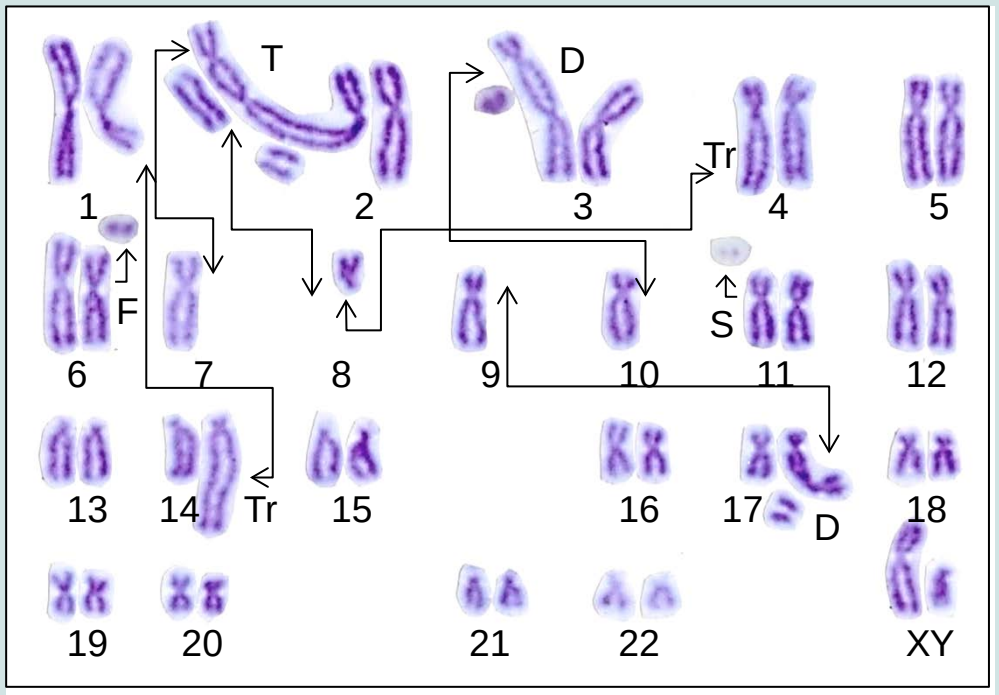
Chromatid-type aberrations



Unit of aberration is chromatid

Origin of chromosome aberrations by ionizing radiation





Hexacentric (H): *n*=6, associated max (*n*-1) fragments (F or S)
Pentacentric (P): *n*=5, associated max (*n*-1) fragments (F or S)
Tetracentric (Q): *n*=4, associated max (*n*-1) fragments (F or S)
Tricentric (T): *n*=3, associated max (*n*-1) fragments (F or S)
Dicentric (D): *n*=2, associated max 1 fragment (F or S)
Centric ring (R): *n*=1, associated max 1 fragment (F or S)
Acentric ring (A): *n*=0

n: number of centromeres.

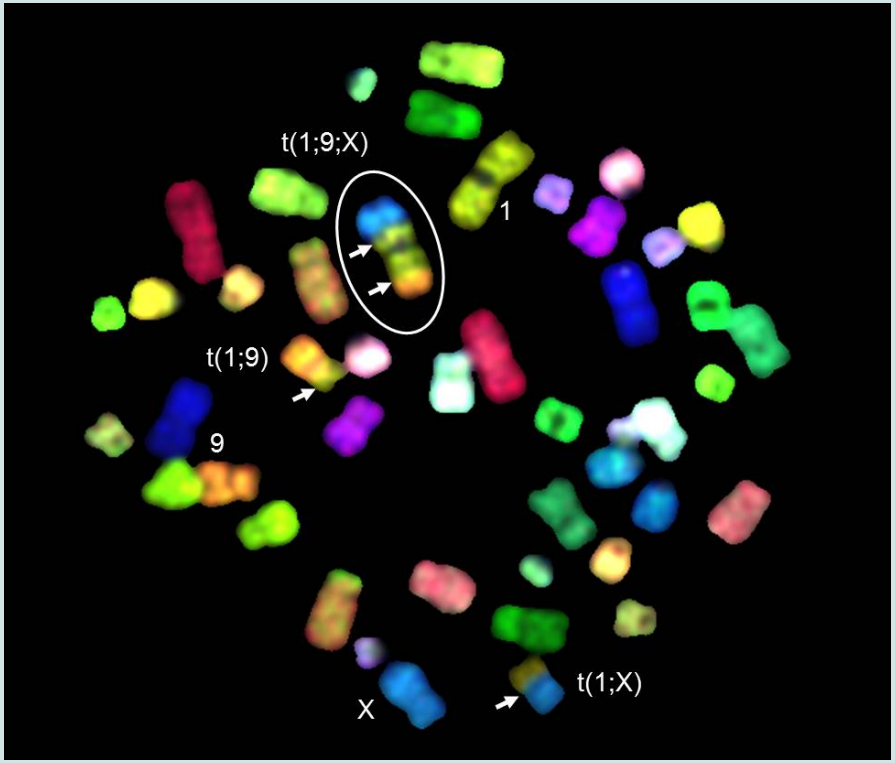
Aberration is expressed by a single digit for computer use;
upper case for chromosome-type aberrations and lower case for chromatid-type aberration.

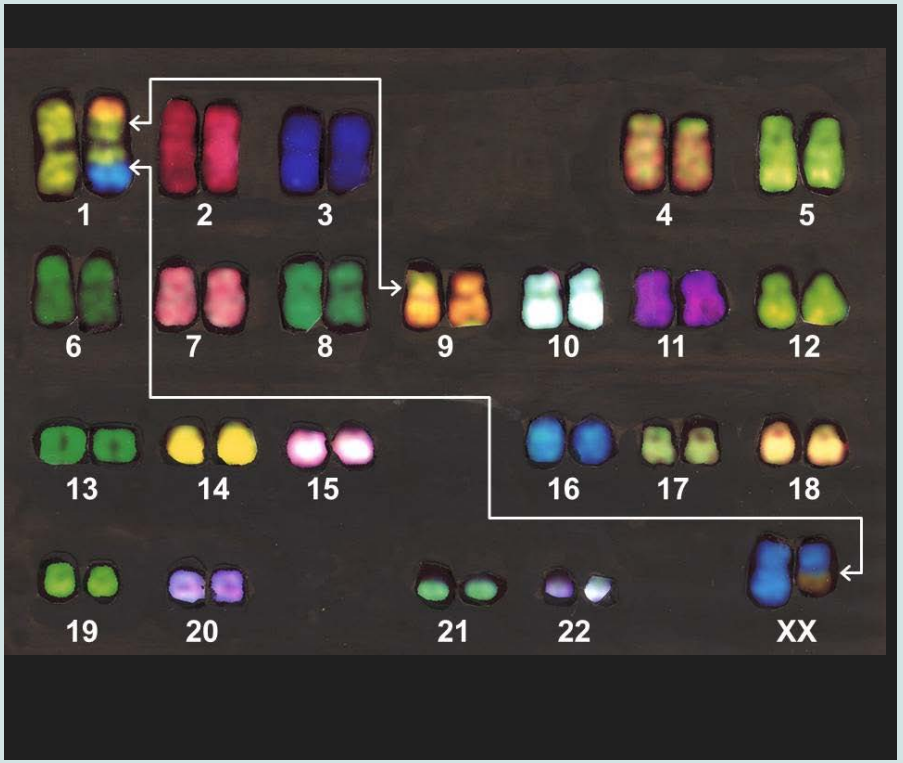
Minute dots (S): *n*=0, small-sized dots with their diameter less than the width of chromatid.

Fragment (F): *n*=0, chromosome fragment with no centromere. They are either terminal deletions (F_{del}) or those associated with multicentric aberrations.

Abnormal monocentric (M):
n=1, formed as a consequence of either Translocation (Tr), interstitial deletion (A or S), or terminal deletion (F).

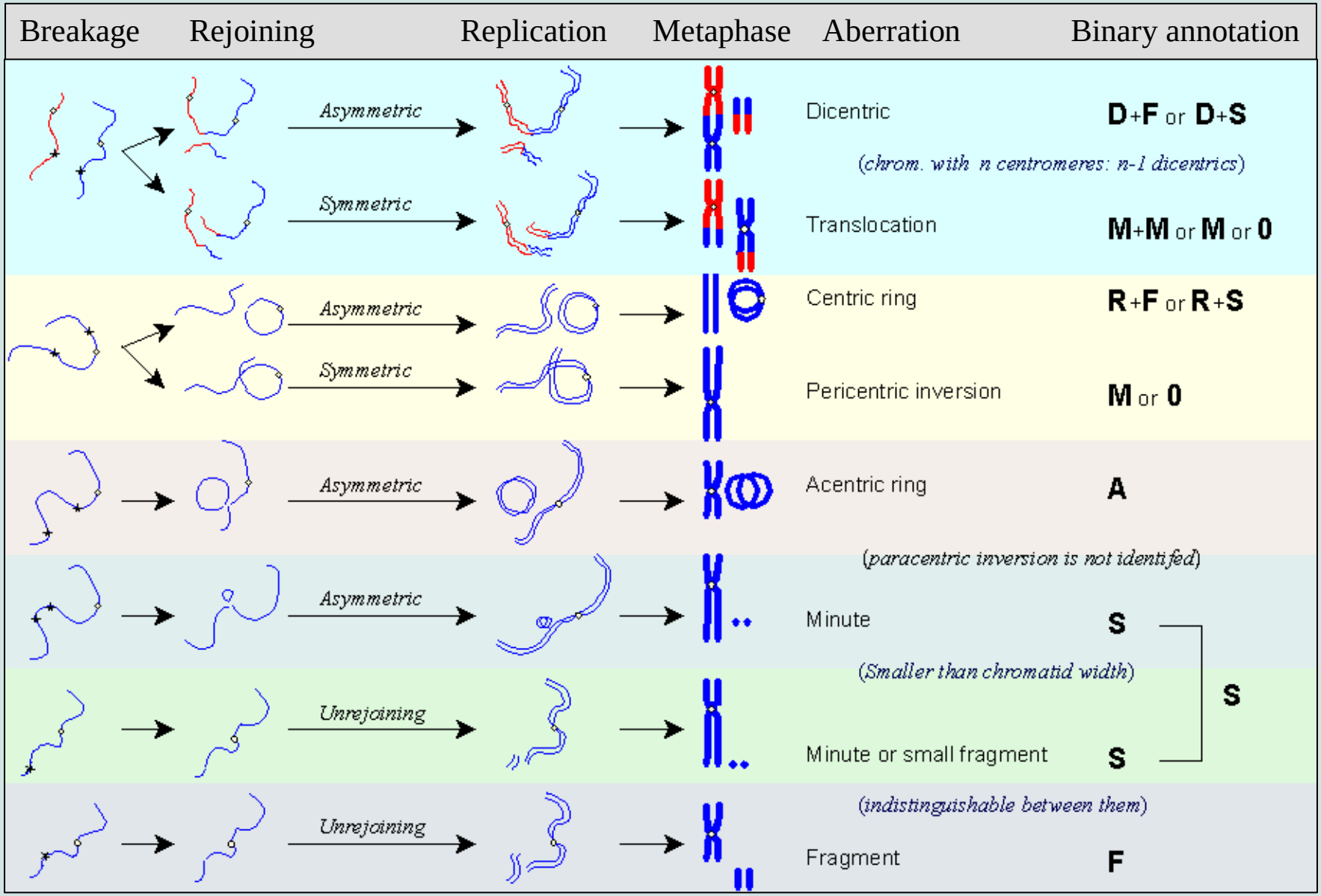
Multiple translocations



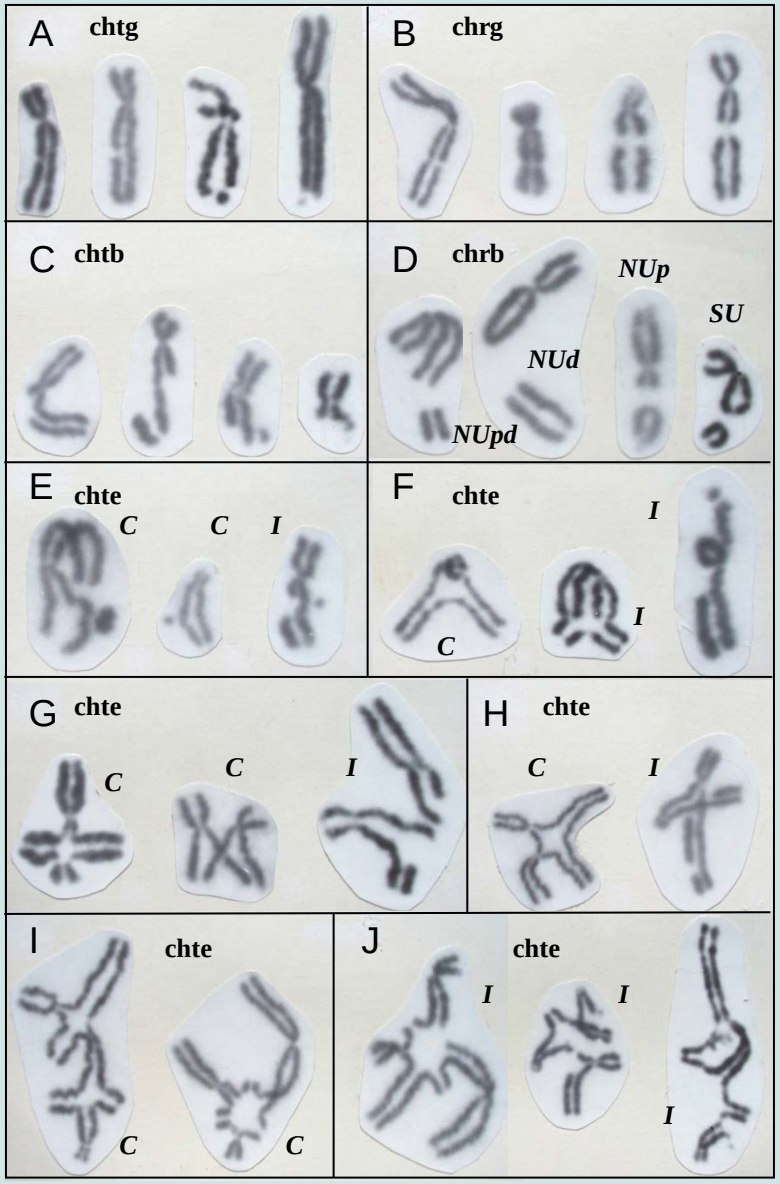


Reproduced from Sasaki, M. S., Int. J. Radiat. Biol., 85:26-47, 2009, in which mFISH image is by the courtesy of Dr. Y. Kodama.

Formation of chromosome-type aberrations by ionizing radiation



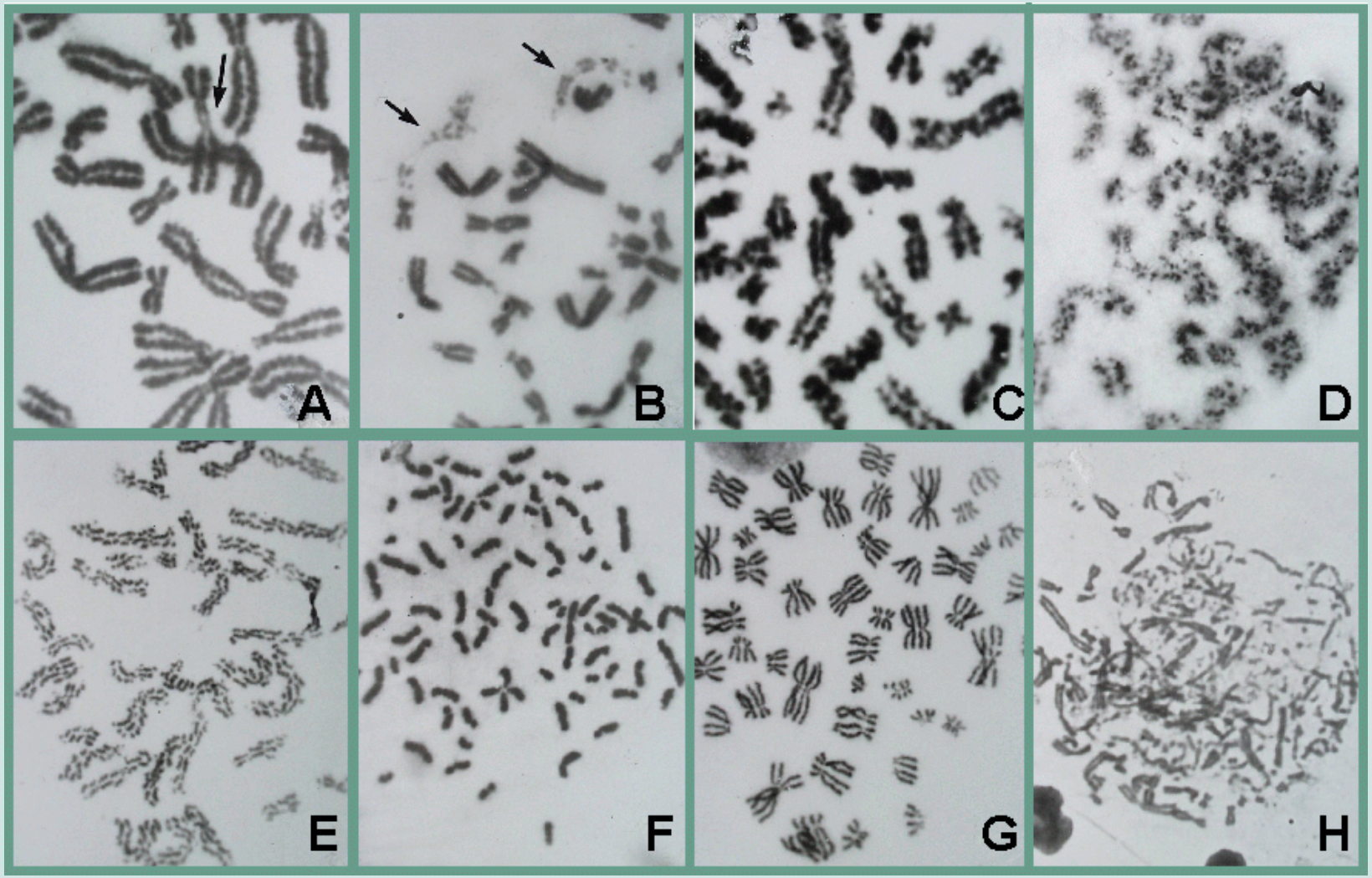
Chromatid-type aberrations



ISCN 2005	Binary annotation
A: chromatid gap (*chtg*)	[g]
B: chromosome gap (*chrg*)	[i]
C: chromatid break (*chtb*)	
D: chromosome break (*chrb*)	
E-J: chromatid exchange (*cte*)	[e]
NUp: nonunion proximal	[e]
NUd: nonunion distal	[e]
NUpd: nonunion proximal and distal	[F]
SU: sister union	[e]
C: complete exchange	
I: incomplete exchange	

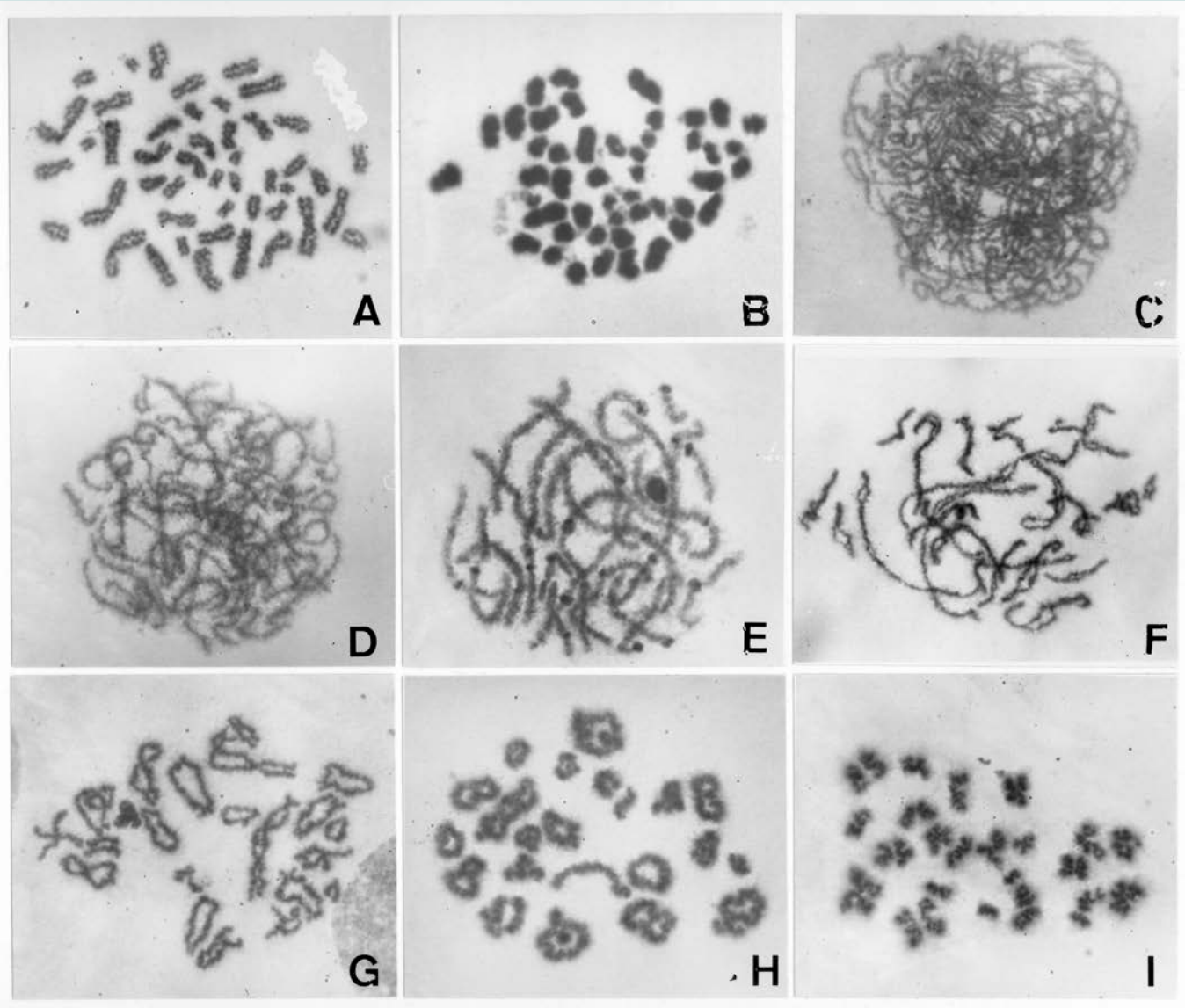
ISCN2005: International System for Human Cytogenetic Nomenclature (2005)

Chromosome abnormalities not directly relevant to radiation



A: attenuation, B: erosion, C: ruffling or fuzziness, D: stickiness or haziness, E: shattering, F: centromere dissociation, G: endoreduplication, H: pulverization

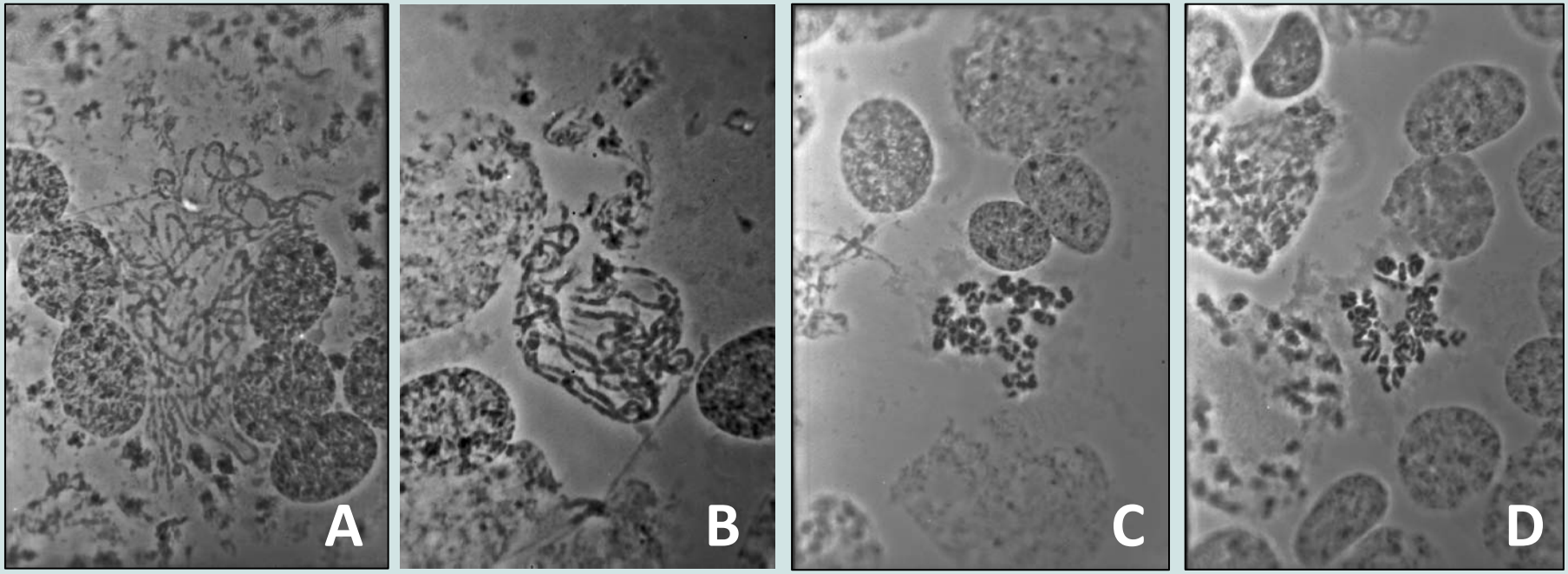
Chromosomes in meiotic process (male spermatogenesis)



A,B: spermatogonia C: leptotene D: zygotene E,F: diakinesis G,H: metaphase I I: metaphase II

Chromosomes in meiotic process (female)

In oocytes, meiotic processes proceed to pachitene/diakinesis stages (dictyotene) during late stages of gestation.
The 1st and 2nd maturation divisions (meiosis I and meiosis II) takes place before ovulations in puberty and thereafter.



A,B: pachytene

C,D: diakinesis

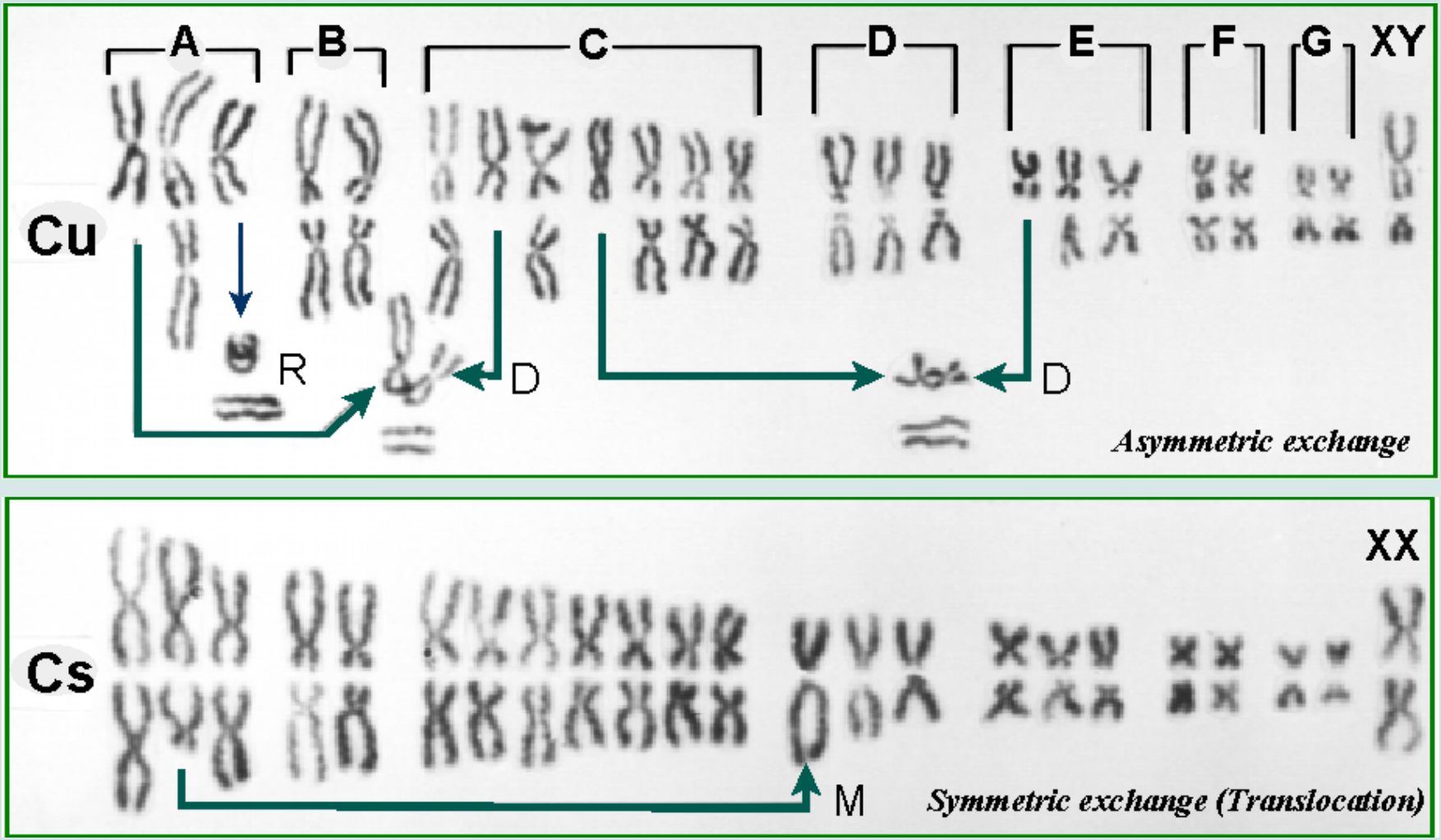
Dictyotene stage (special name in oogenesis)

Survival of chromosome aberrations depends on the cell type

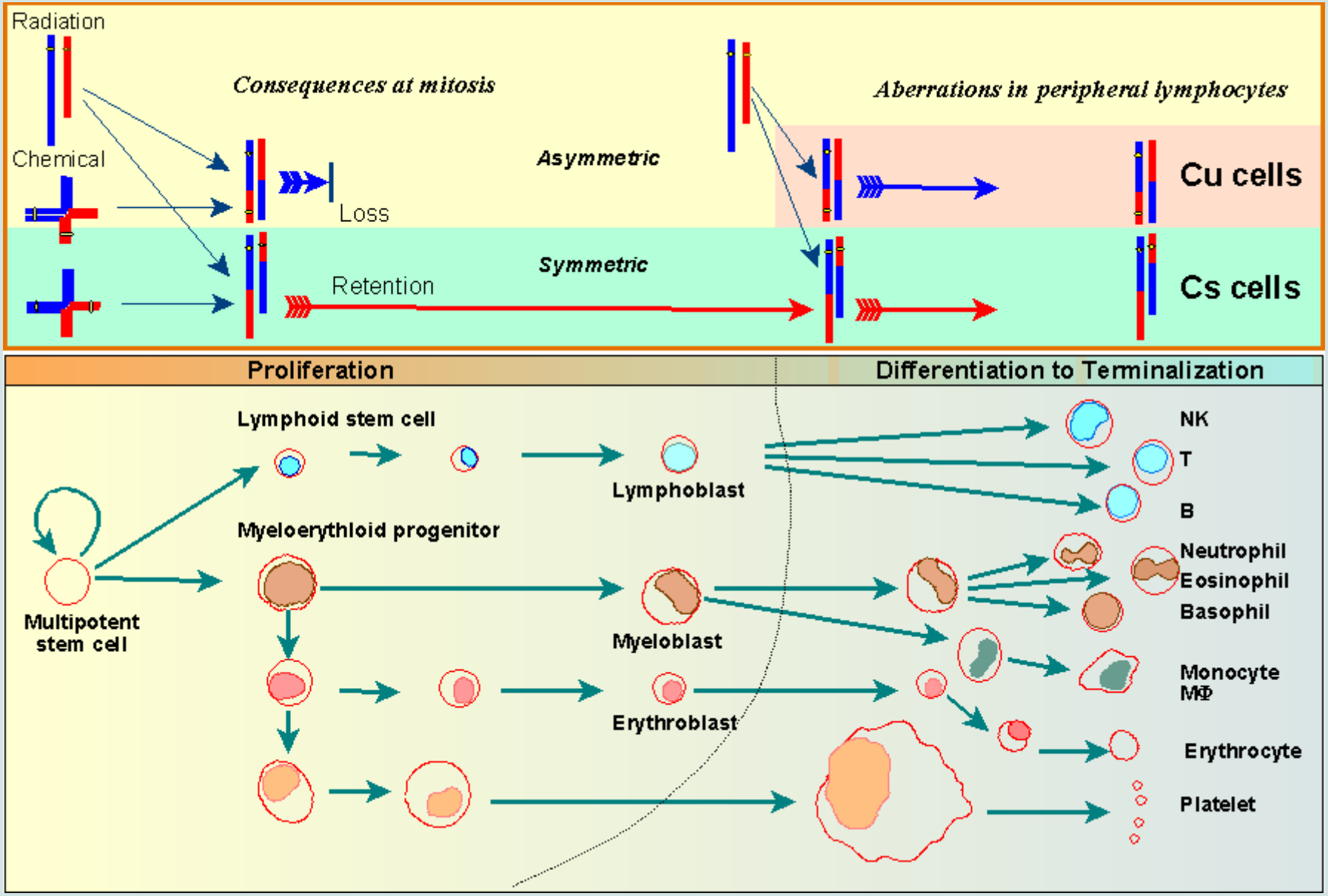
Cu cells: cells with unstable-type aberrations.
Aberrations will be lost when the cells attempt at mitosis.

Cs-cells: cells with only stable-type aberration (s).
Aberrations will survive cell divisions.

(Nomenclature defined by K. E. Buckton *et al*. Lancet ii:176-682, 1962)

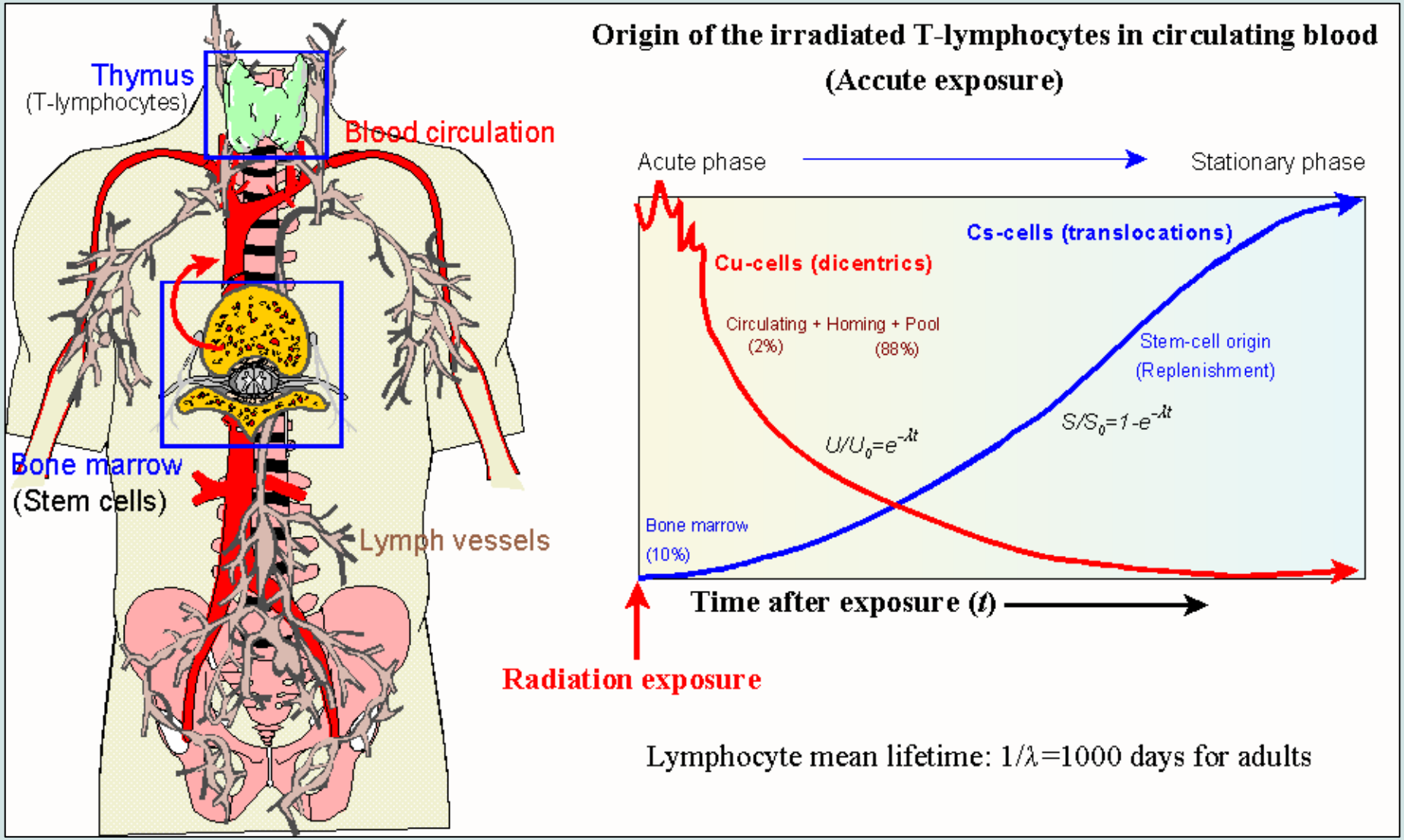


Survival of chromosome aberrations and fate of cells during proliferation and differentiation of blood cells

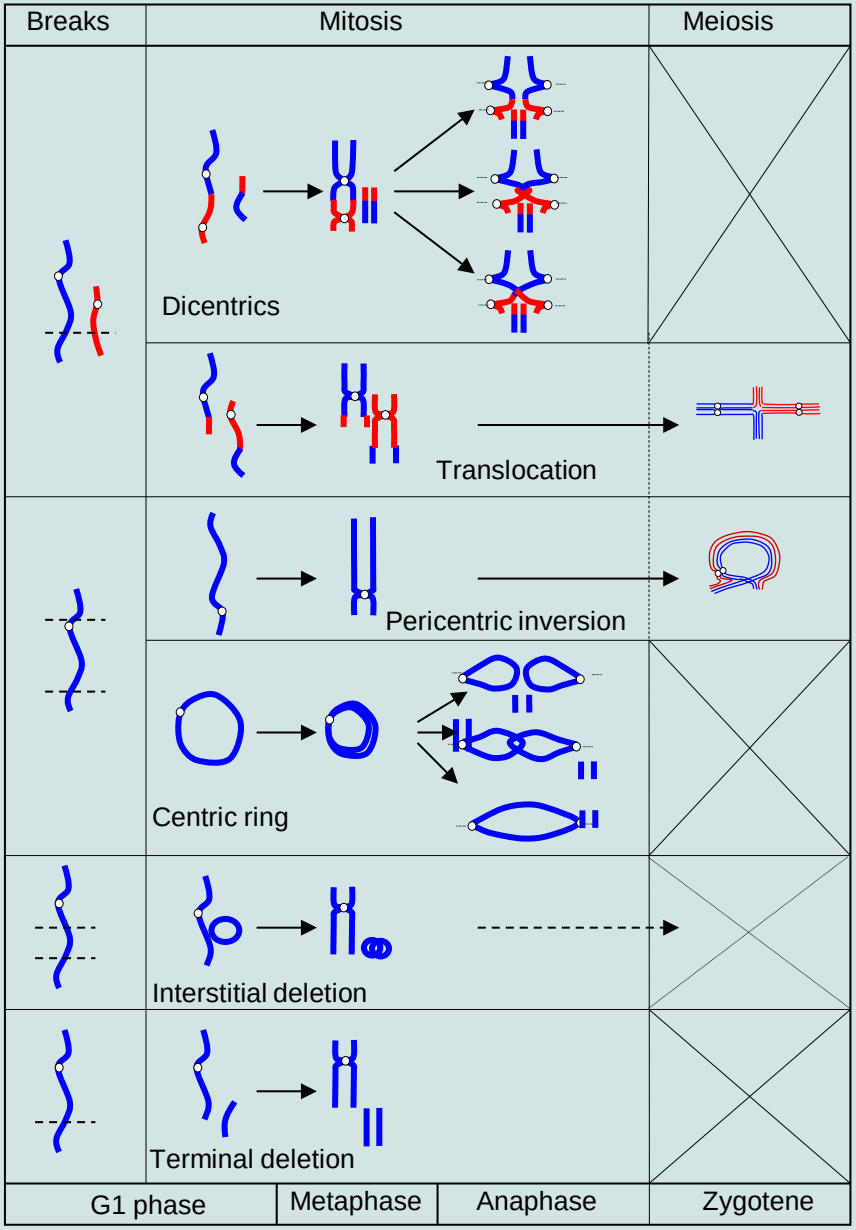


Kinetics of T-lymphocytes with aberrations in peripheral blood after exposure to ionizing radiation

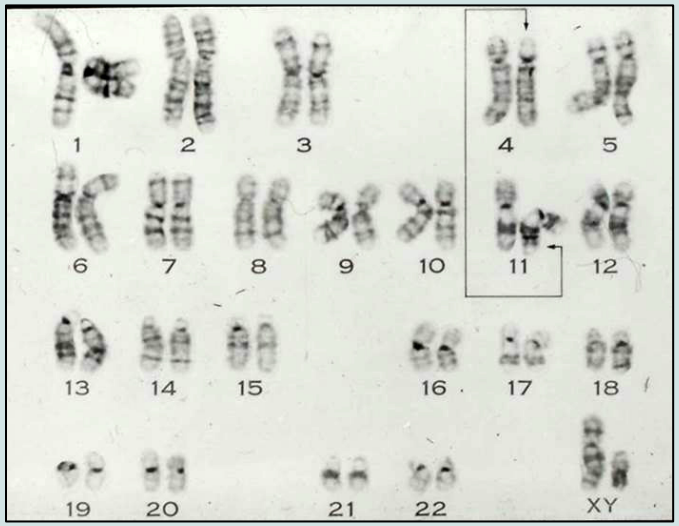
(Cu vs Cs cells)

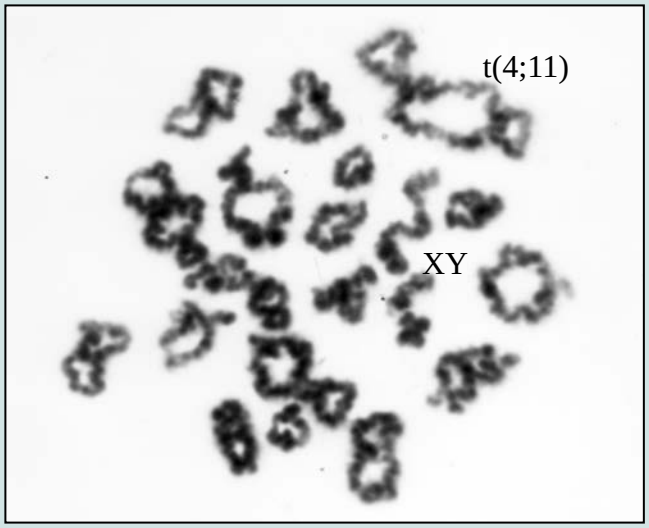


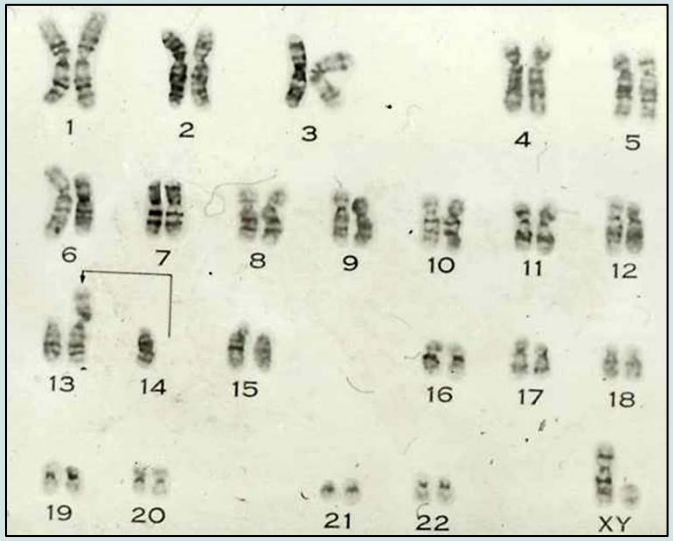
Fate of chromosome aberration during meiotic process

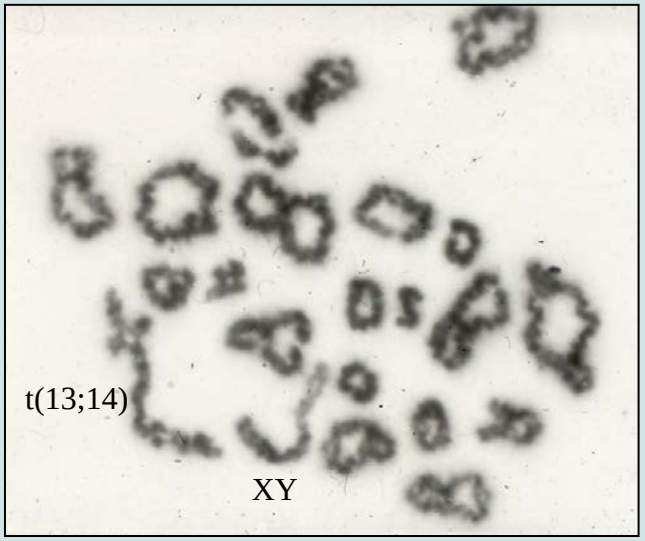


Stable aberrations and its consequence in meiotic process

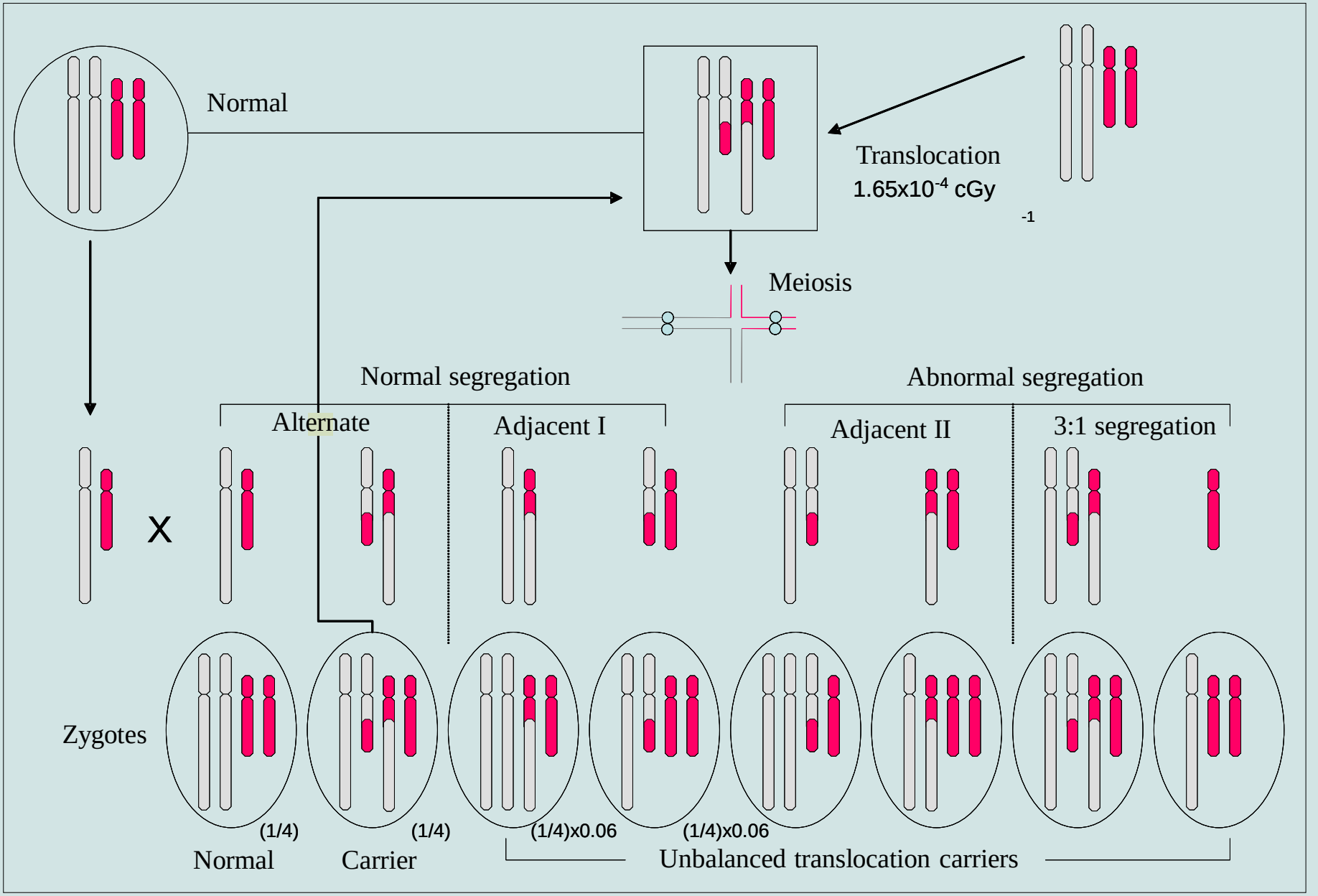








Hereditary effects of radiation-induced translocations



Hereditary risk of chromosome aberrations after radiation exposure

Consequences of 1 cGy exposure to 10^6 population

Persons with	Equilibrium	Generations			
		1	2	3	4
Unbalanced translocation	6	5	1		
Balanced translocation	55	41	10	3	1

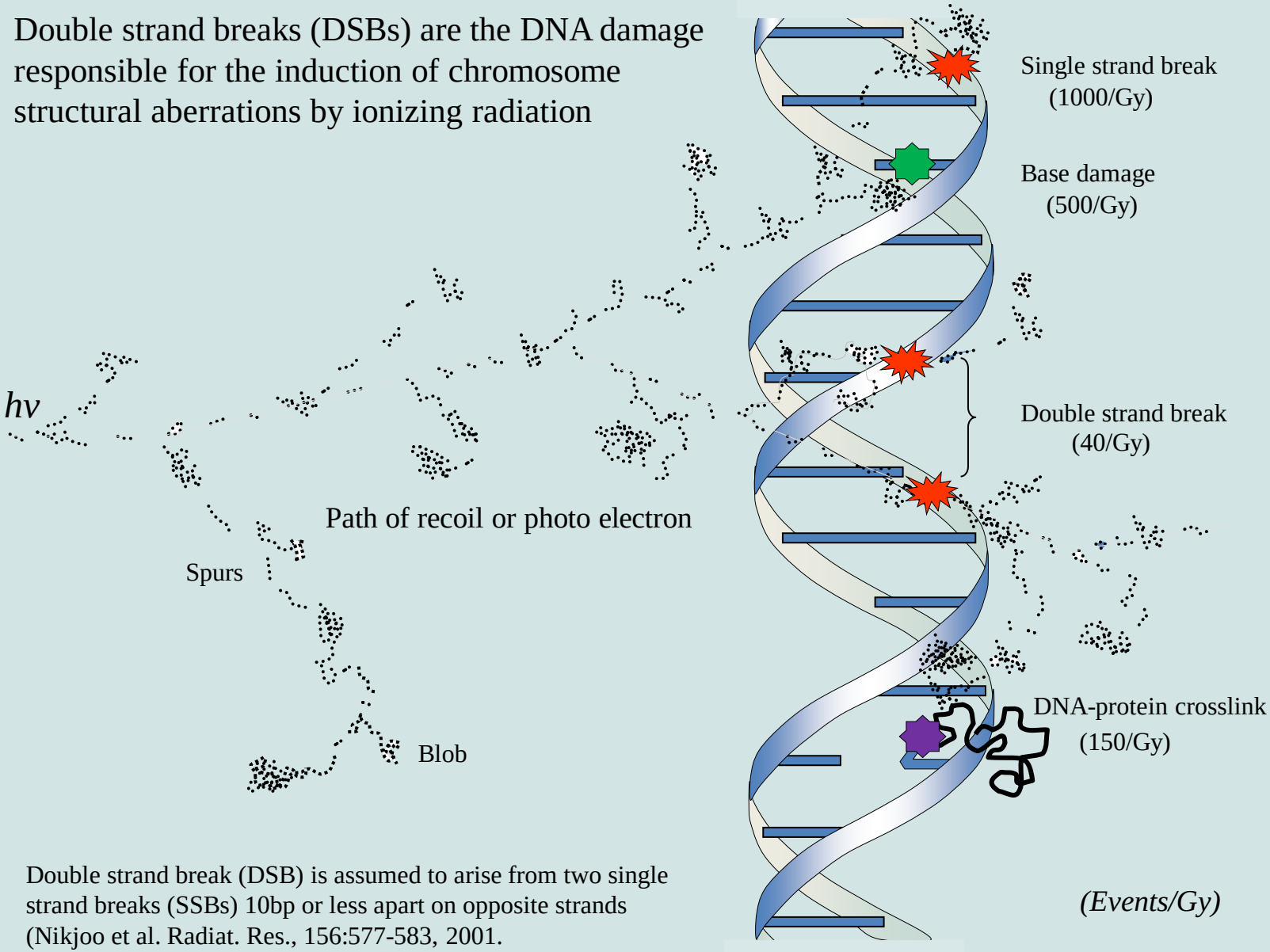
Assuming $4x10^{-5}$/cGy for the appearance of balanced translocation carries in the 1 st post-exposure generation.

New mutation of balanced translocations in F1 generation of A-bomb survivors (mean dose: 60cGy) (Awa et al. 1987)

$1/8322/60cGy=2x10^{-6}/cGy$

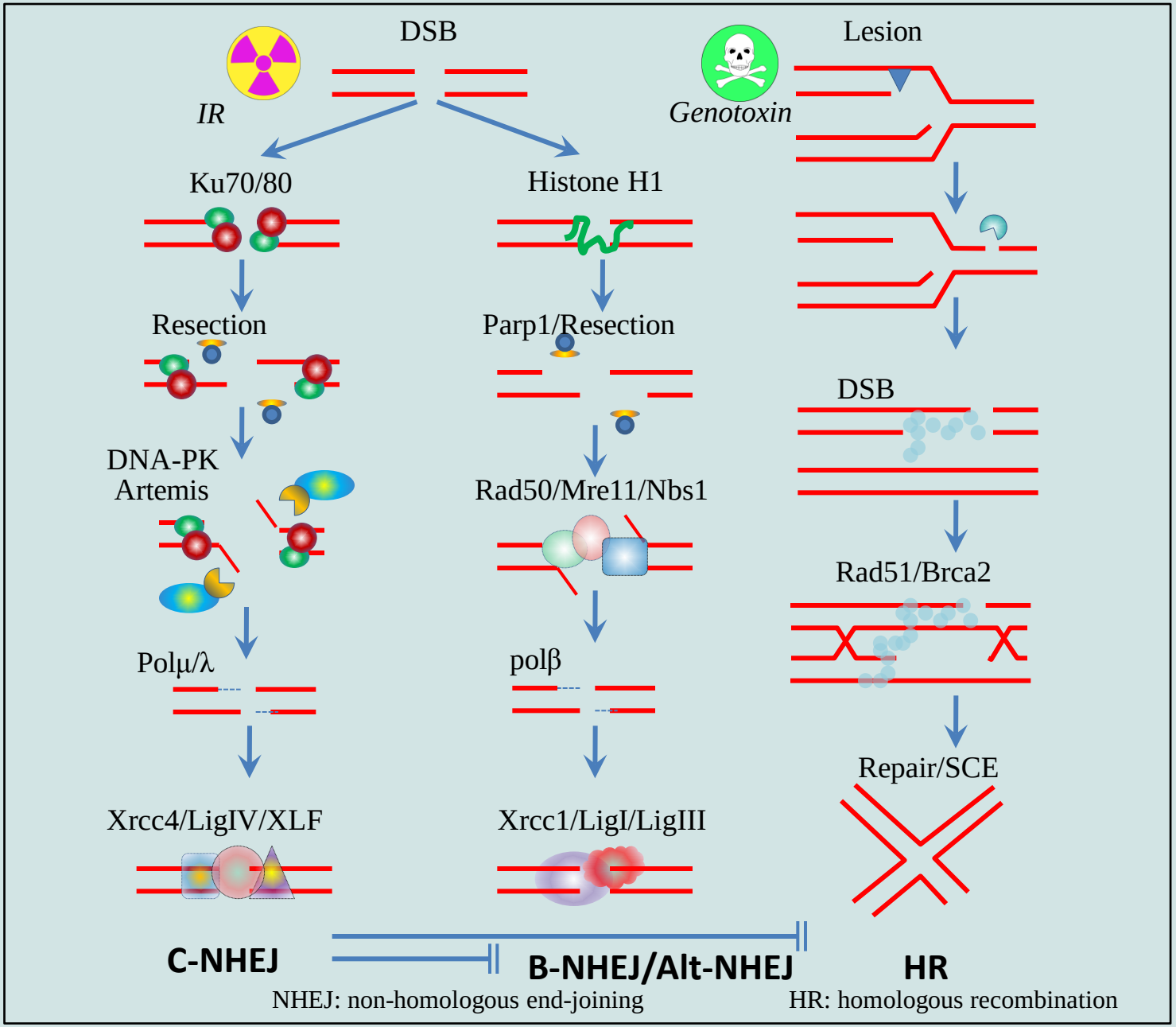
Mechanisms of genome (chromosomes) maintenance and DNA repair

Double strand breaks (DSBs) are the DNA damage responsible for the induction of chromosome structural aberrations by ionizing radiation



Double strand break (DSB) is assumed to arise from two single strand breaks (SSBs) 10bp or less apart on opposite strands (Nikjoo et al. Radiat. Res., 156:577-583, 2001.

Induction of double strand breaks and their repair pathways

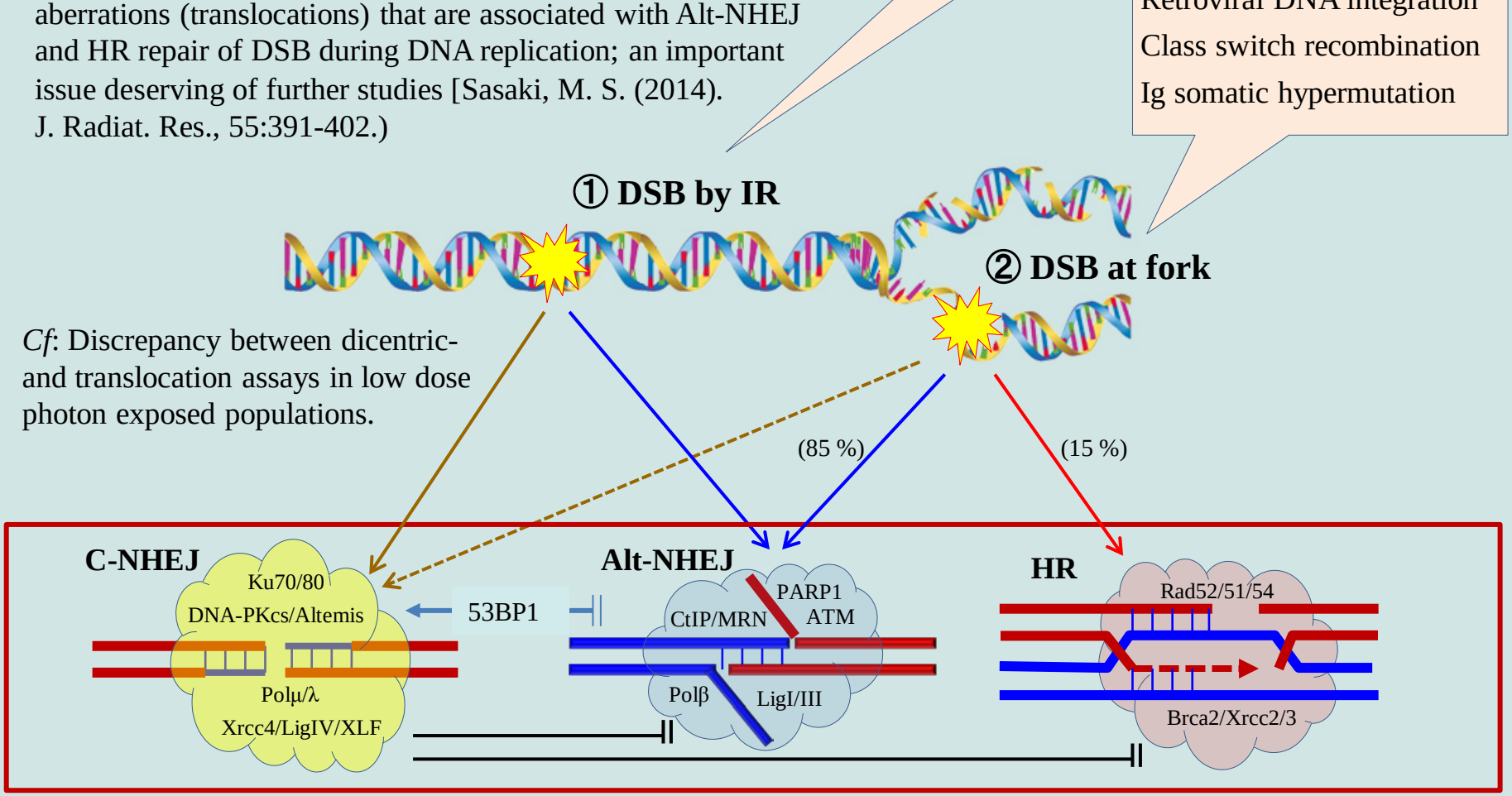


Dose specificity of DNA DSB repair pathways and their cross-talk

Impact on radiation-induced chromosome aberrations:

Activation of C-NHEJ pathway by low dose and/or low dose-rate could suppress spontaneously arising chromosome aberrations (translocations) that are associated with Alt-NHEJ and HR repair of DSB during DNA replication; an important issue deserving of further studies [Sasaki, M. S. (2014). J. Radiat. Res., 55:391-402.)

Cf: Discrepancy between dicentric- and translocation assays in low dose photon exposed populations.



Cross-talk between DSB repair pathways

Conclusion

- Chromosome is a single array of double strand DNA (dsDNA) and a carrier of a series of genes (linkage group)
- Its end is protected against digestion by exonuclease or chemical hydrolysis by sealing with special hairpin structure called "*telomere*" in eukaryotes or by forming a circle with no ends in prokaryotes.
- Chromosomal integrity is retained by semiconservative DNA replication and DNA repair.
- Double strand DNA breaks (DSB) are major causes of radiation-induced chromosome aberrations, cell death, and possibly of cancer as well.
- Chromosome structural aberrations are the cytological manifestation of mis-repair or non-repair of DNA.
- Clear quantitative response to dose and quality of radiation provides a basis of the use of chromosome aberration analysis as biological dosimetry and risk assessment of radiation carcinogenesis