Radiation induced chromosomal aberrations

Outline

- Biological consequences of radiation exposure
- History of chromosome preparation
- Detection of chromosomal aberrations
- Mechanism of origin
- Scoring methods
- Dose effect curves
- Biological dosimetry in lymphocytes of space flight crewmembers

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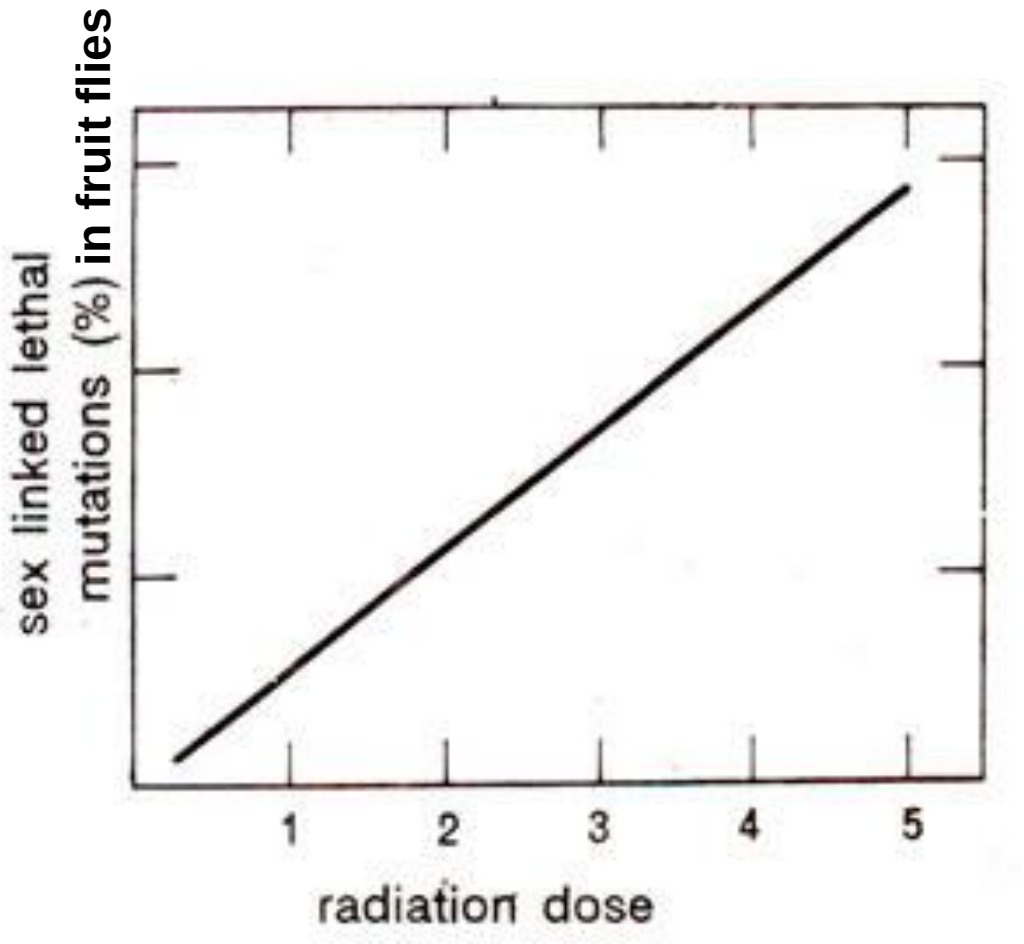
Ionizing radiation induces a variety of biological consequences

- Molecule damage including DNA lesions
- Gene mutations
- **Chromosomal aberrations**
- Cell death (in interphase and mitosis)
- Oncogenic transformation
- Cataracts
- Coronary diseases?

Hermann Joseph Muller observed the mutagenic potential of X-rays in 1927



Chromosomal aberration

- A chromosomal aberration is a large-scale rearrangement of the genetic information in a genome. It includes numerical changes of the chromosomes and changes of the chromosome morphology.
- Using different staining procedures chromosomal aberrations become visible in the microscope.
  Smaller non-visible changes in the genetic material are usually affecting only single genes and are termed gene mutations.
- Cytogenetics is the scientific discipline investigating chromosome morphology and their changes.

History

In the early days of cytogenetic research most chromosome studies - including the analyses of aberrations following radiation exposure - were performed in plant and insect cells, while the quality of the chromosome preparations of vertebrate cells was poor. Squash preparations and sliced specimens were performed from different tissues like root tip meristems in plants or testis from men.

Only in 1956 the diploid chromosome number of the human karyotype was corrected from 48 (47) to 46.

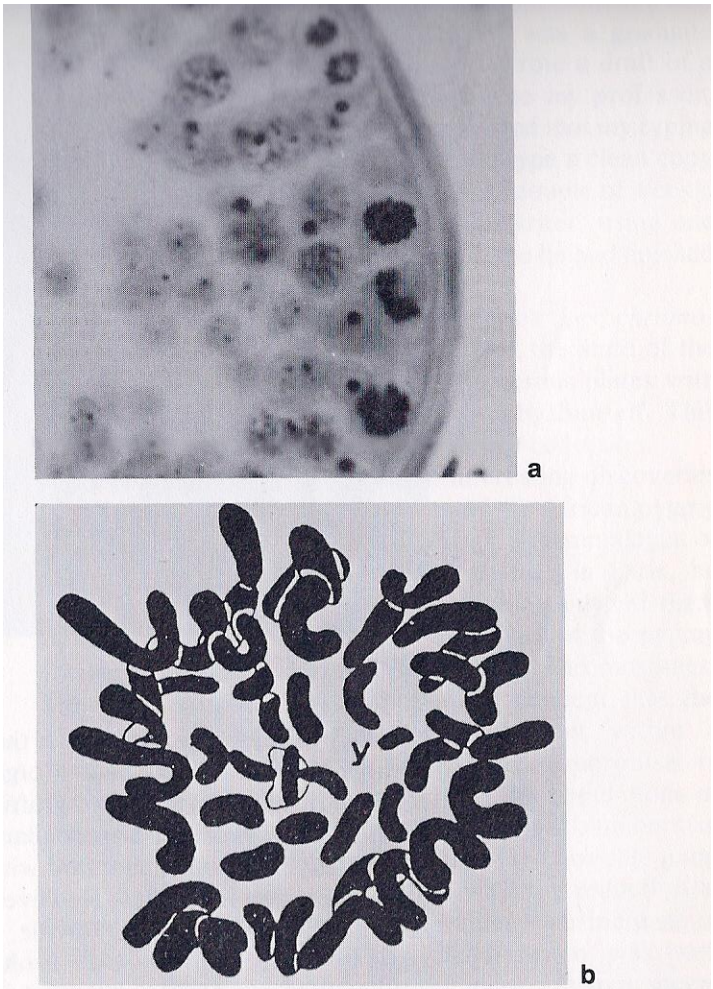


Figure 2.3. a, a section of a human testis with spermatogonial mitoses prepared by T. S. Painter; **b,** a camera lucida drawing of a human spermatogonial metaphase made by Painter.

From the fifties to the eighties of the last century technical improvements have been made to get metaphase preparations of high quality from mammalian including human cells. The main inventions of these years were:

Colchicum autumnale

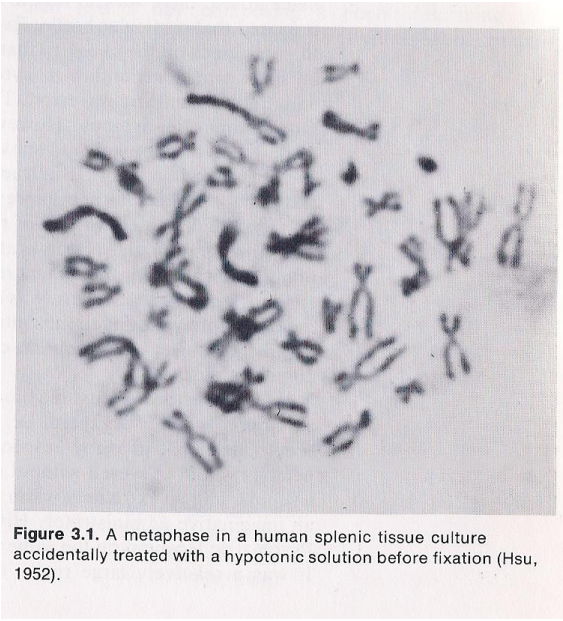


Figure 3.1. A metaphase in a human splenic tissue culture accidentally treated with a hypotonic solution before fixation (Hsu, 1952).

- Cell culturing (to avoid repeated biopsies, but maintaining cells for a long duration under *in vitro* conditions)
- Metaphase arrest by the use of colchicine to disrupt the mitotic spindle in order to get a higher yield of metaphase like stages and a better distribution of the chromosomes
- Hypotonic treatment to swell cells prior to fixation
- Squashing the cells onto a microscopic slide to get the chromosomes into a single plane
- Chromosome banding techniques (beginning of seventies)
- Fluorescence in situ hybridization (FISH) methods (beginning of the eighties)

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Effect of hypotonic solution pretreatment

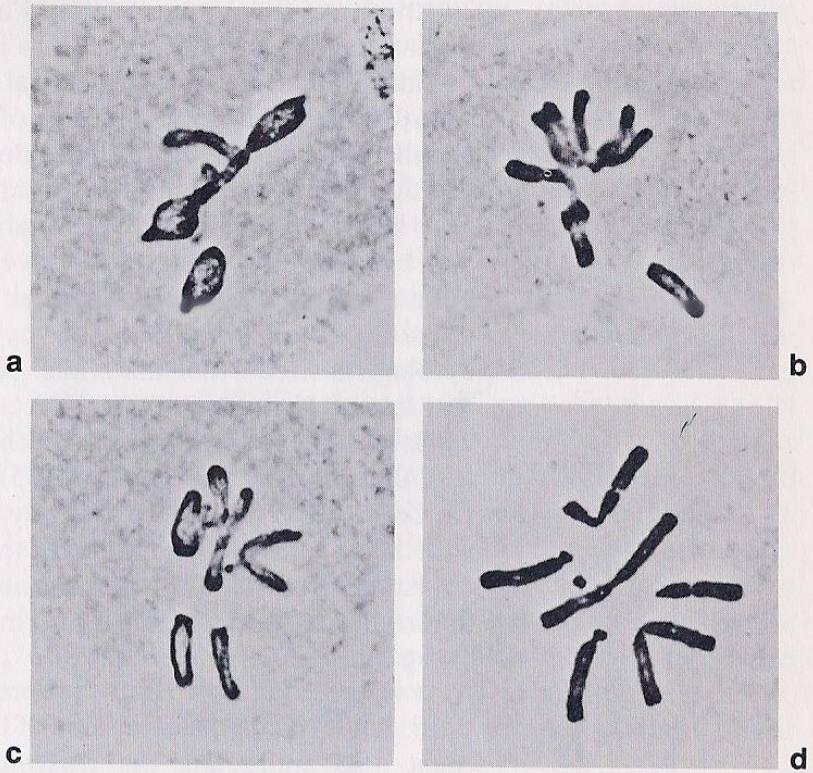
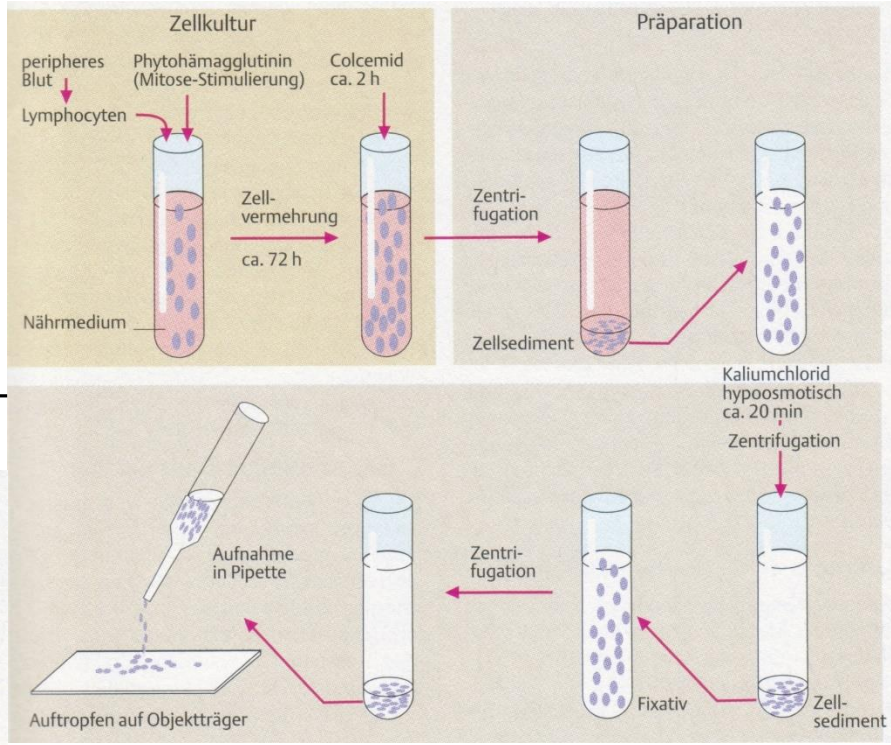
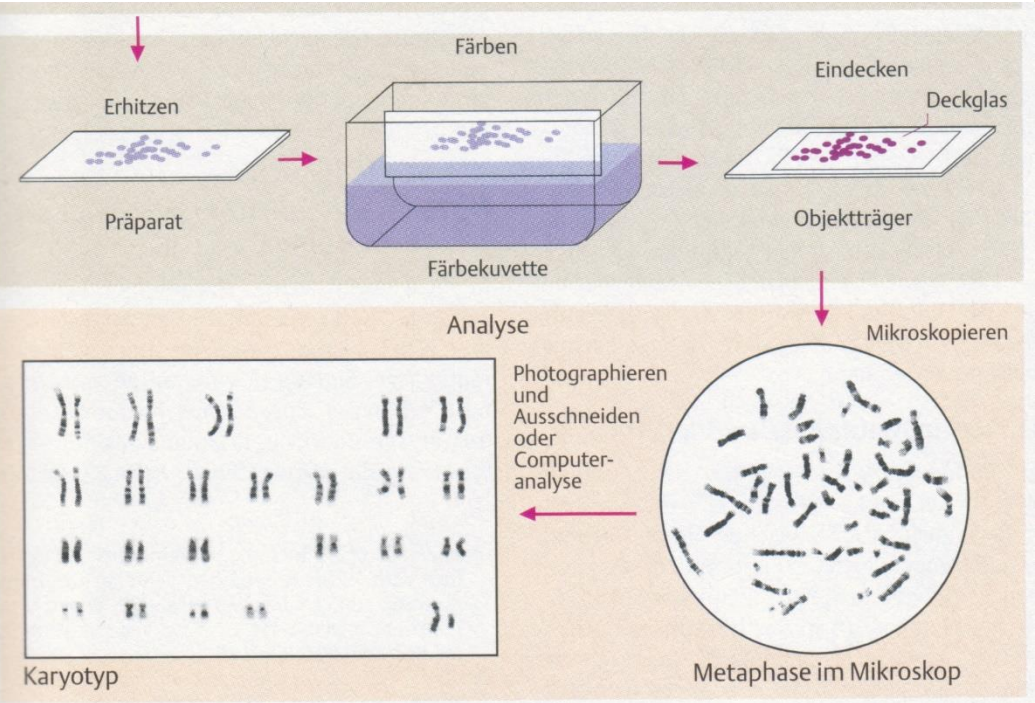


Figure 2.1. Metaphases of a male Indian muntjac ($2n = 7$). **a–c,** acetic orcein squash preparation without a hypotonic solution pretreatment. Note poor chromosome morphology. **d,** same material with a hypotonic solution treatment before fixation. Note distinct number and discrete chromosome morphology.

Protocol for the preparation of a karyotype from a human lymphocyte culture





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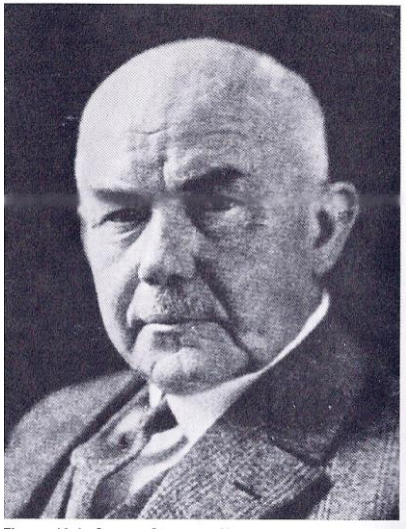
Before staining them chromosomes are not visible in a transmission light microscope



Gustav Giemsa

Solid (uniform) staining with acetic orcein or Giemsa solution

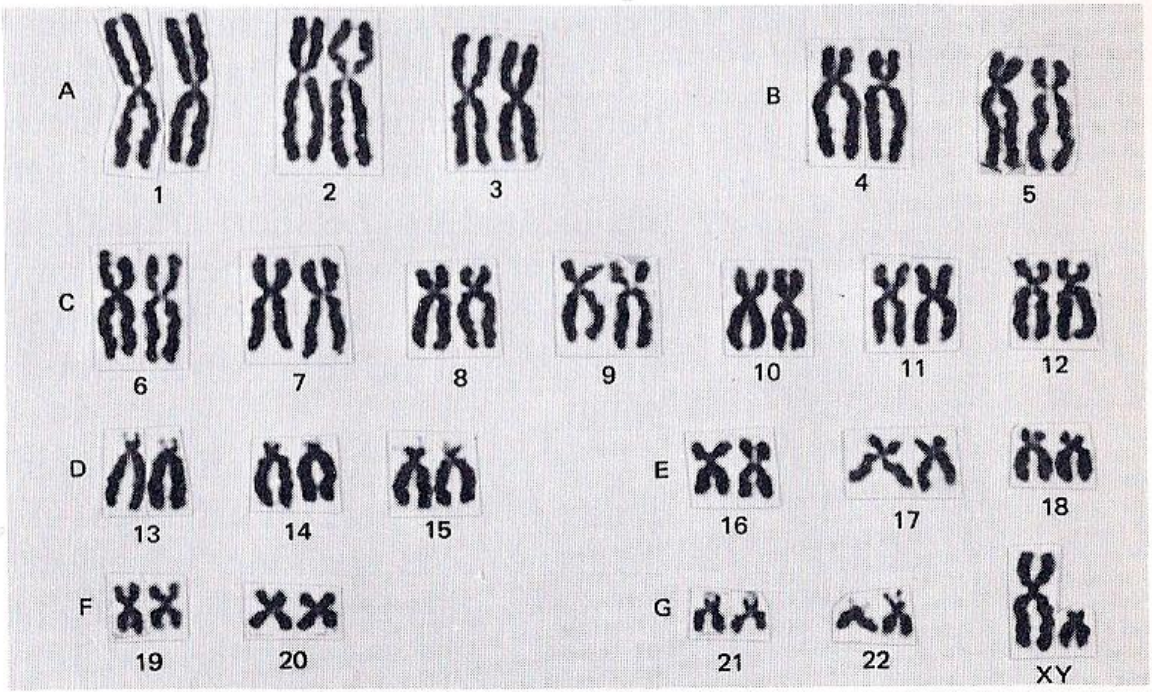
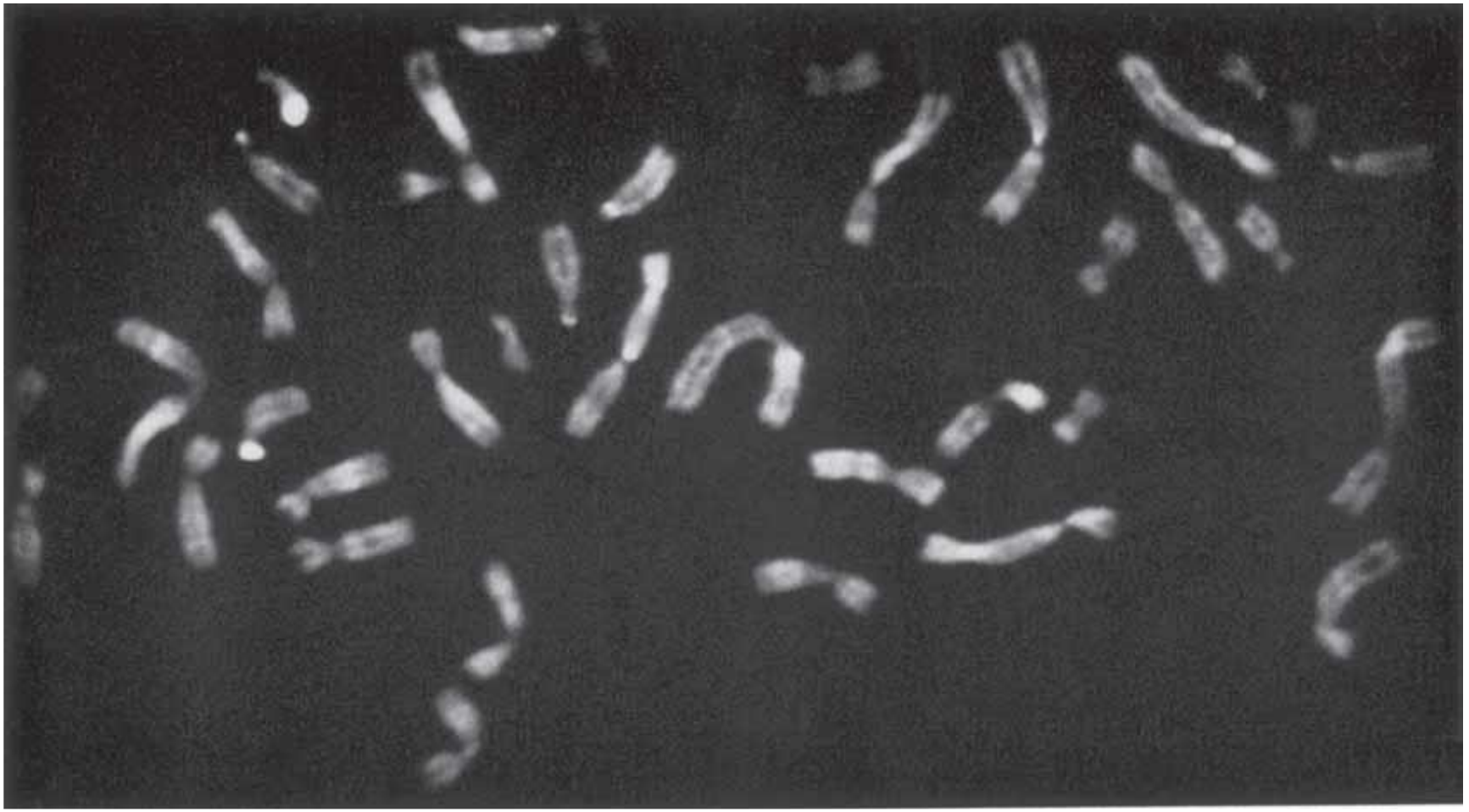
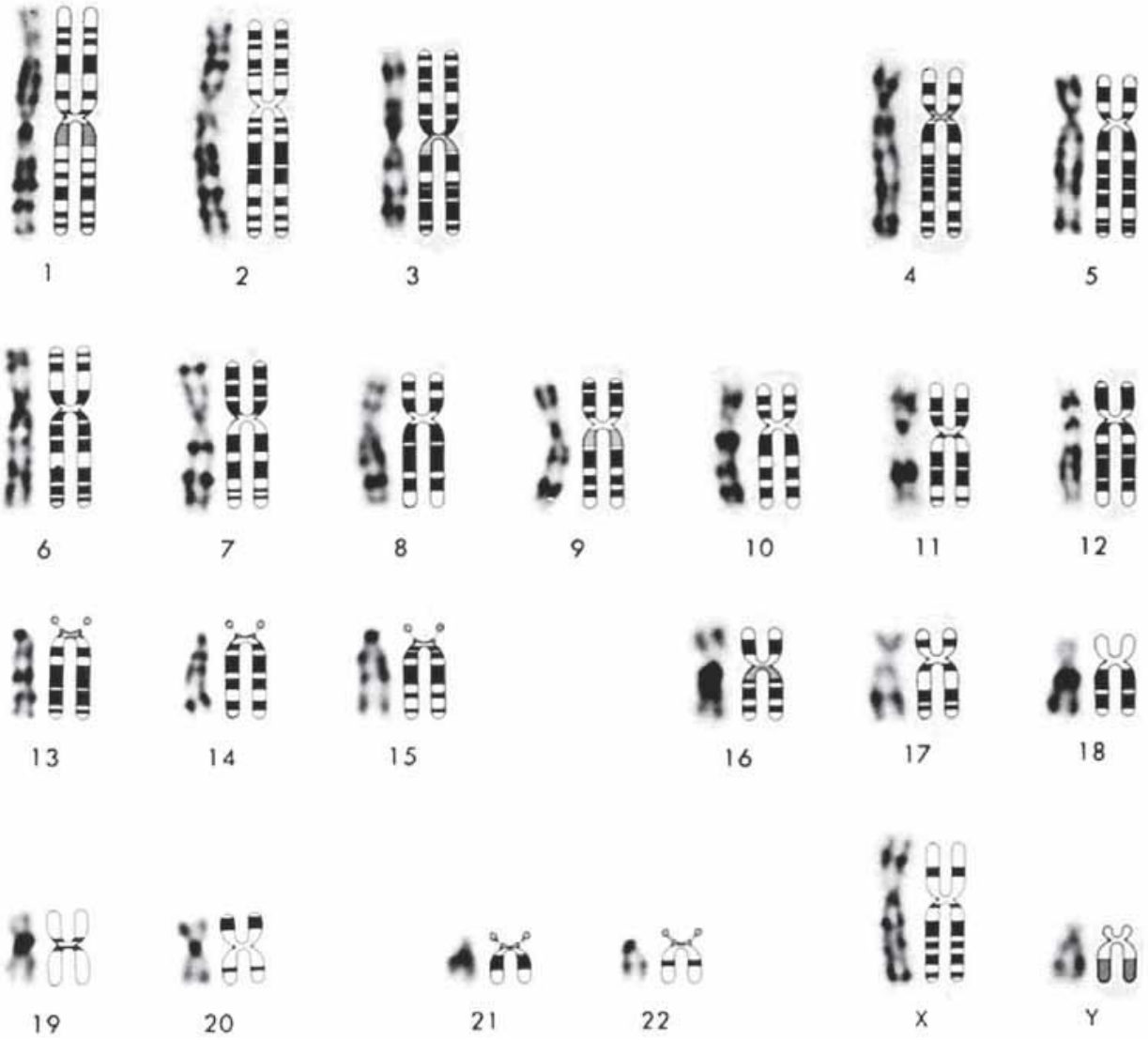


Figure 9.1. A human karyotype arranged according to the Denver nomenclature system.

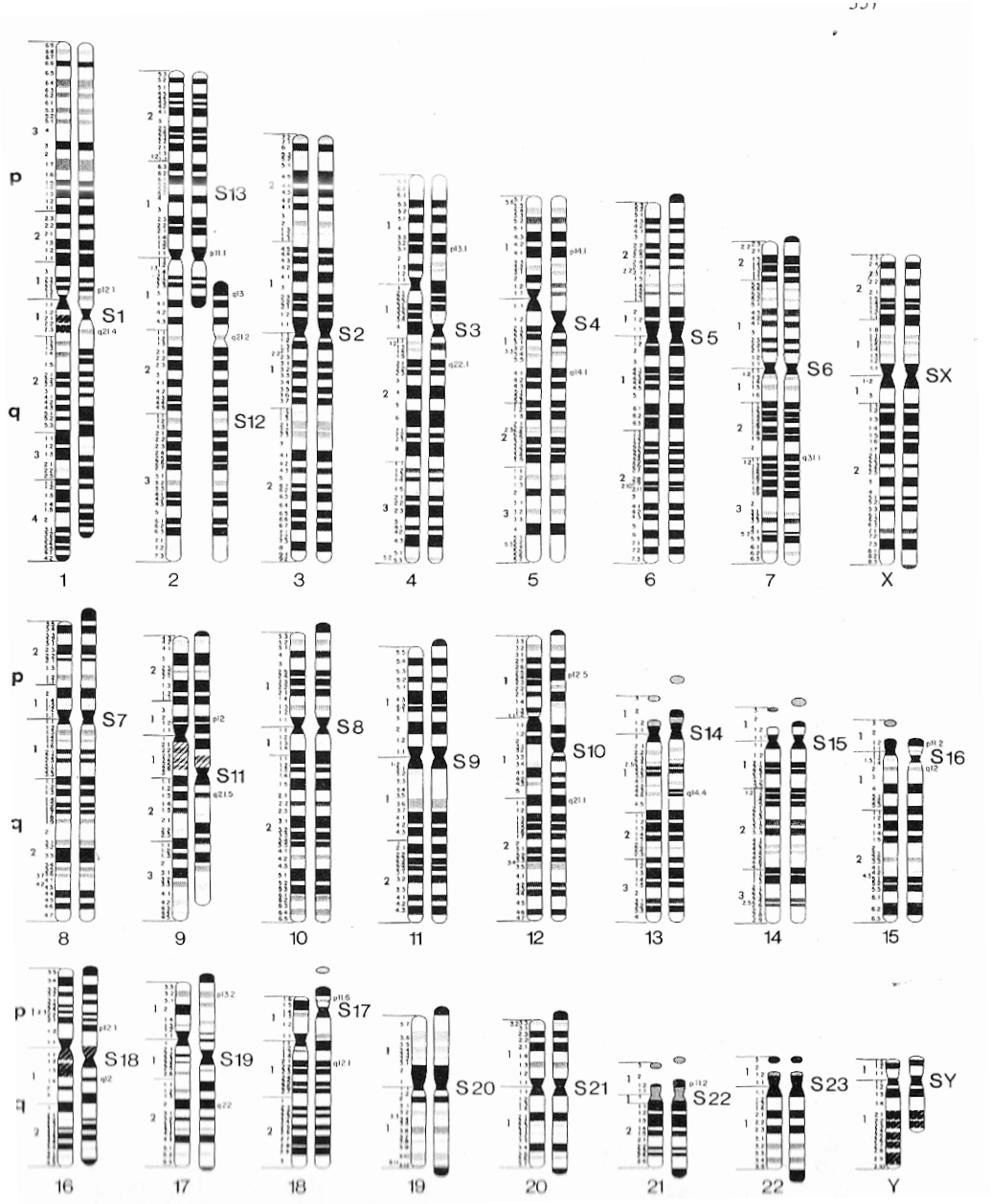
The first photograph of a Q-banded cell published by Caspersson and co-workers in 1970. The figure was originally labeled “Quinacrine mustard treated human metaphase chromosomes (male) from leukocyte culture“.



A composite karyotype of G-banded chromosomes **(left)**
and the corresponding 1971 Paris Conference idiograms **(right)**



Chromosome evolution in primates (left human, right chimpanzee chromosomes)



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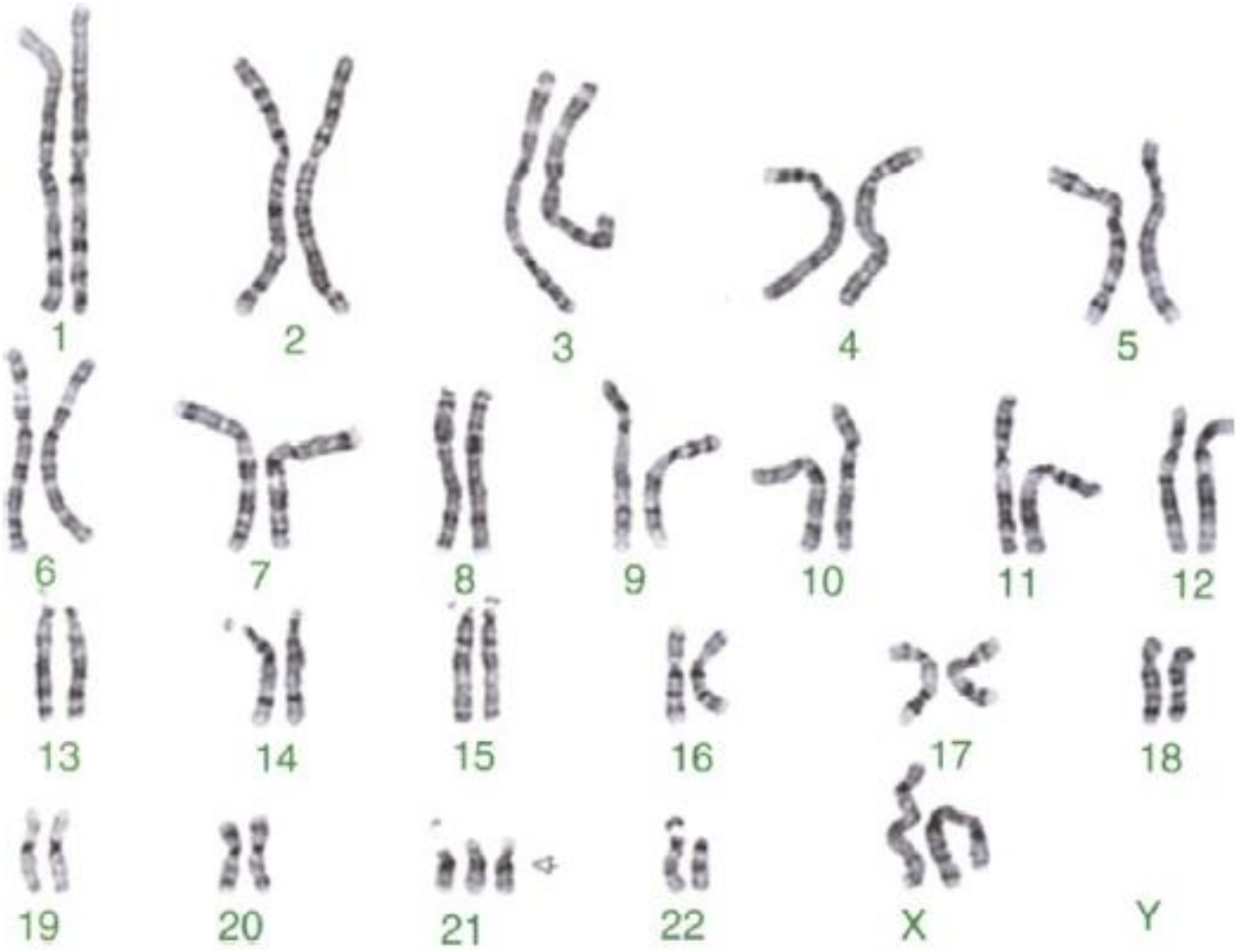
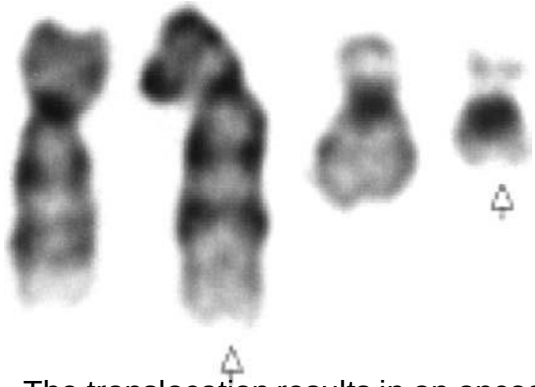
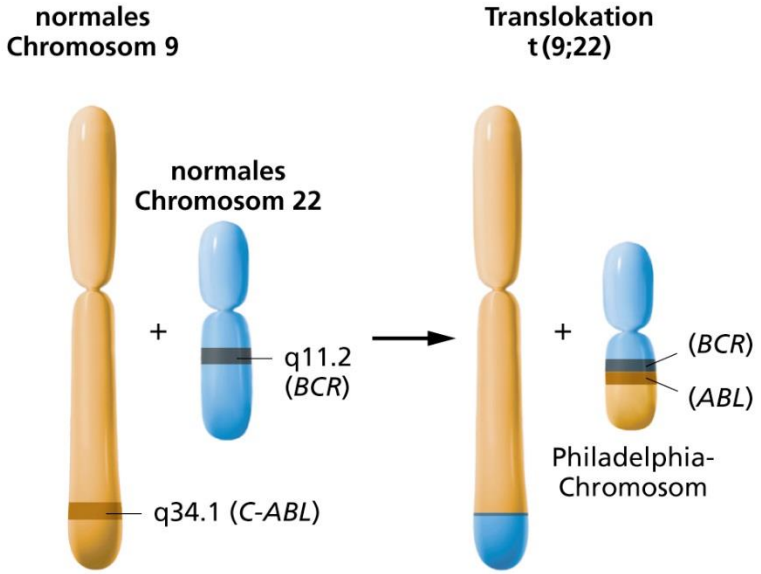
Giemsa-banded (G-banded) female karyotype with trisomy 21



The Philadelphia (Ph) chromosome is detected in over 90% of marrow cells from patients with chronic myelogenous leukemia (CML).
The Ph is the consequence of the balanced translocation between chromosomes 9 (left) and 22 (right).

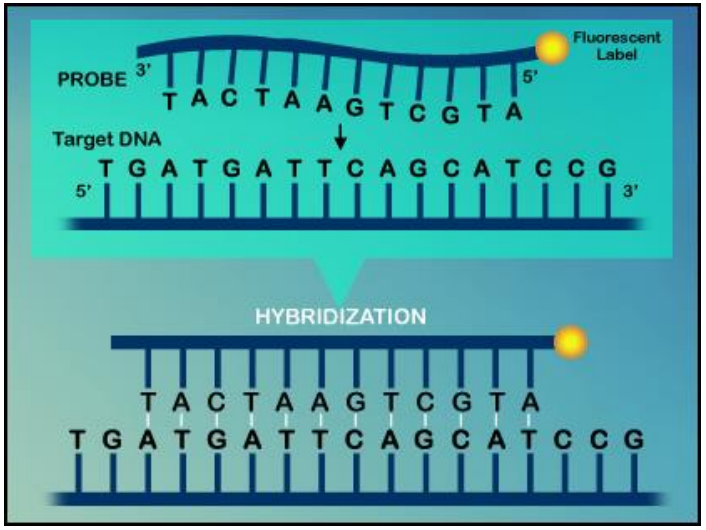


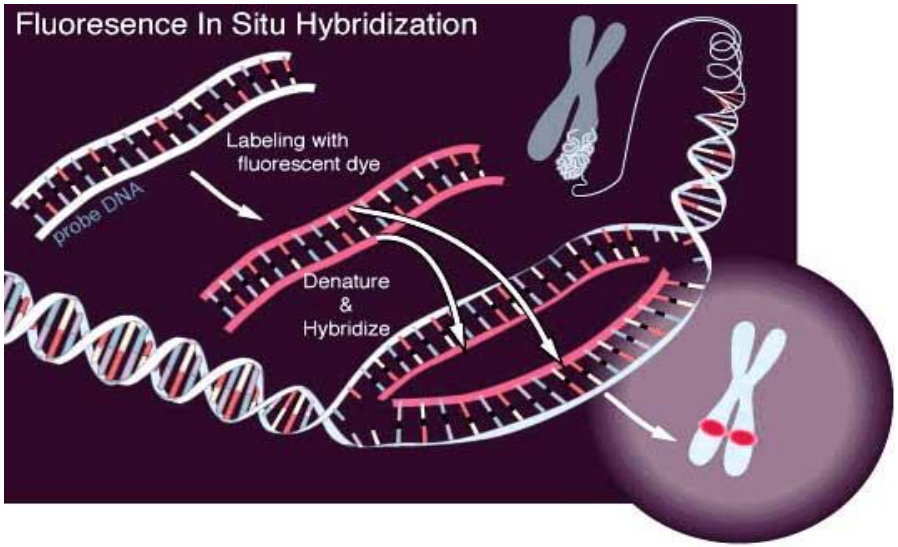
The translocation results in an oncogenic BCR-ABL gene fusion that can be found on the shorter derivative 22 chromosome. This gene encodes for a Bcr-abl fusion protein.
Depending on the precise location of fusion, the molecular weight of this protein can range from 185 to 210 kDa.



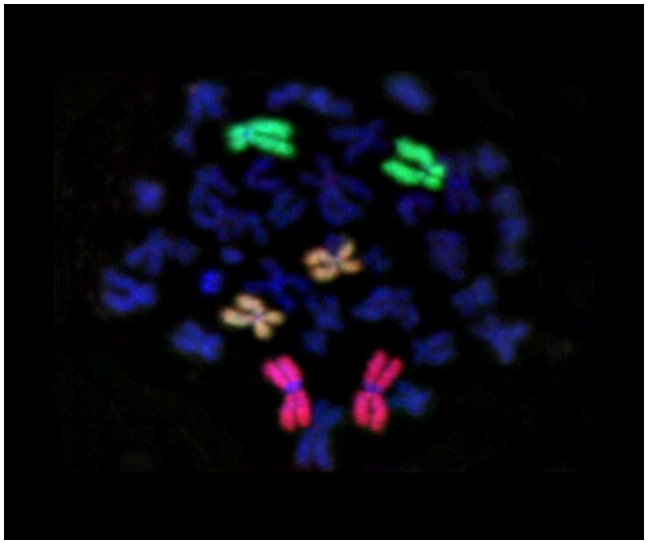
The Abl gene expresses a membrane-associated protein, a tyrosine kinase, and the BCR-Abl transcript is also translated into a tyrosine kinase, but the mutant tyrosine kinase of the BCR-Abl transcript codes for a protein that is "always on" or continuously activated, which results in unregulated cell division (i.e. cancer)

Fluorescence in situ hybridization (FISH)





Three-colour chromosome painting‘



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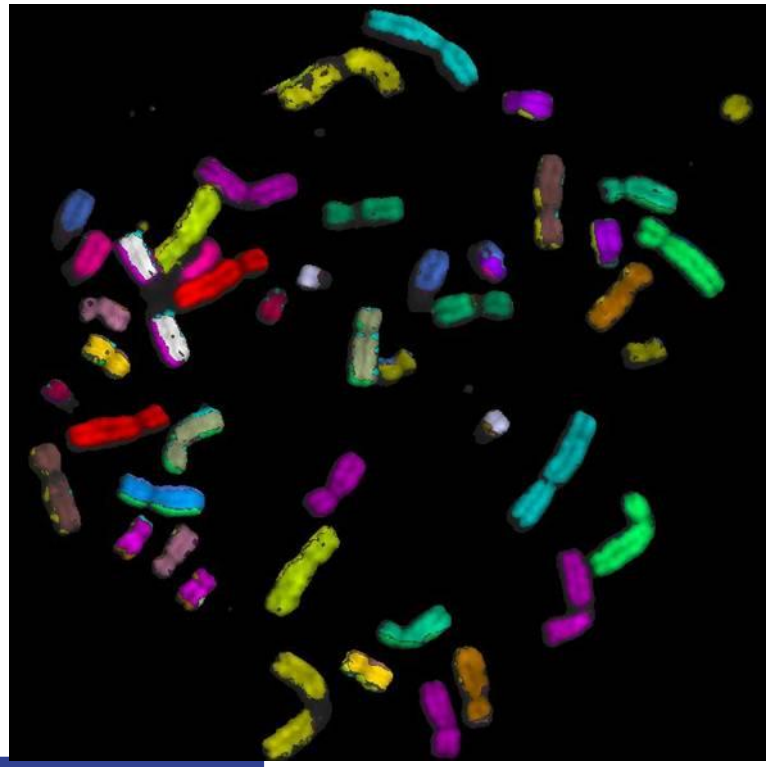
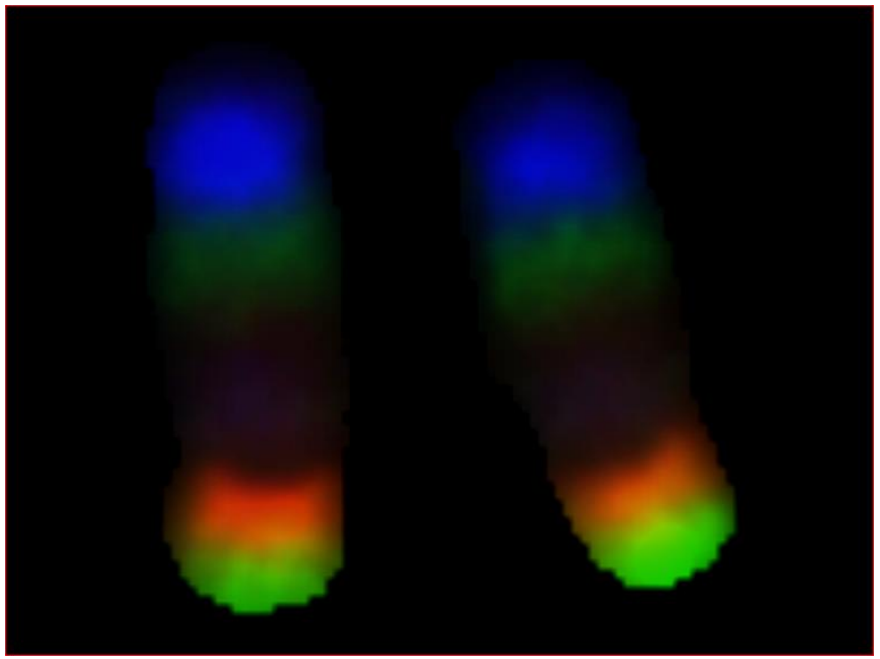
Multicolour FISH

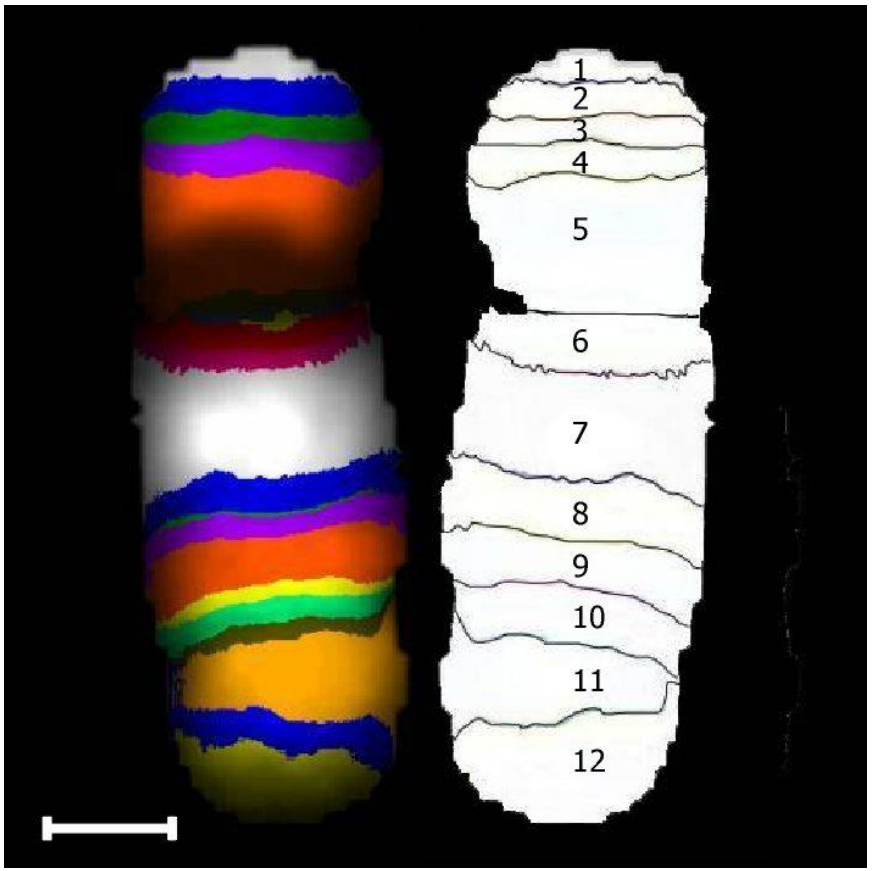
#	FITC	SpeOra	TexRed	Cy5	DEAC
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
X					
Y					

The 24 different human chromosomes are labeled with a single fluorochrome or with a unique combination of two or three fluorochromes, thus making it possible to distinguish them properly using an image analysis software.



Multi-colour banding FISH





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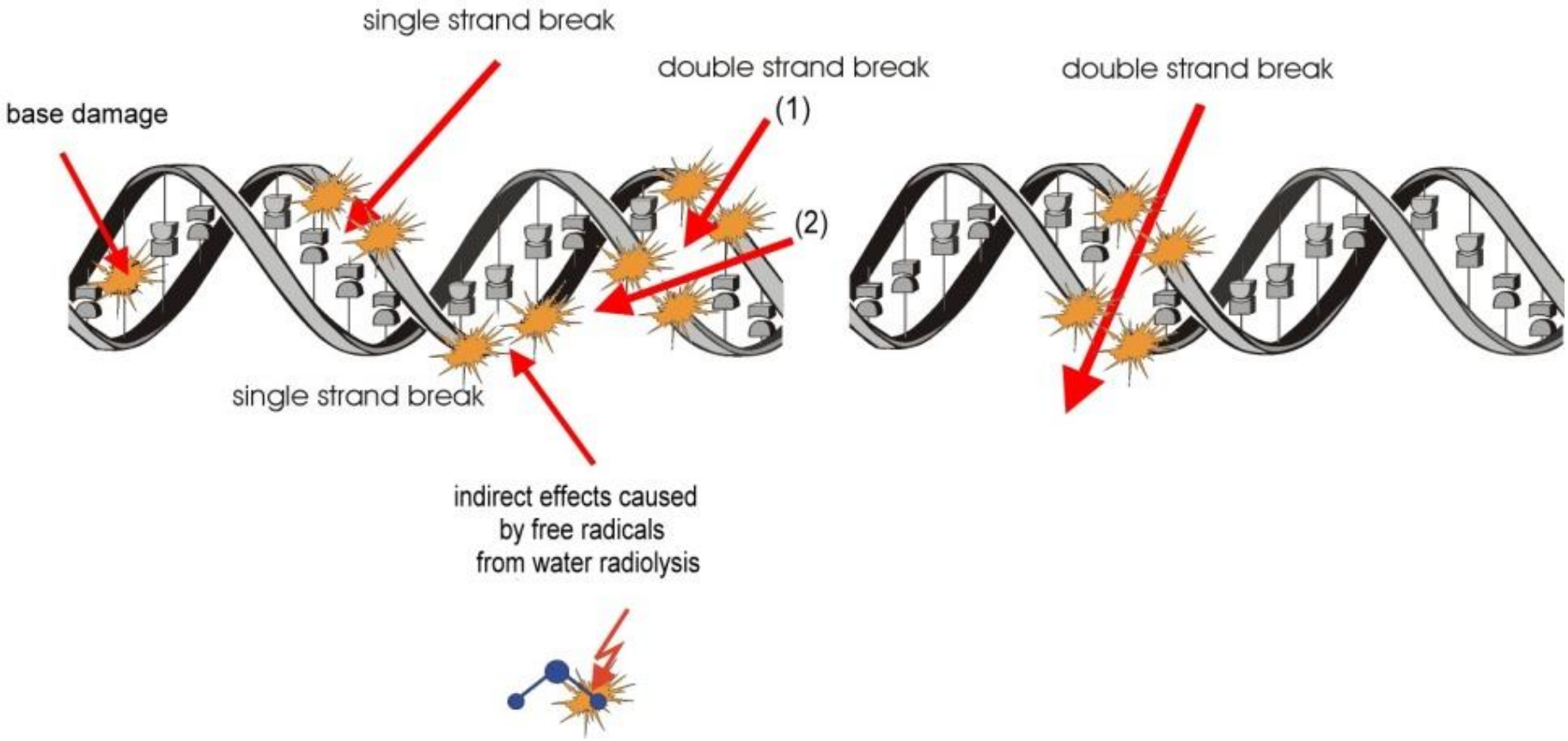
DNA damage after radiation exposure



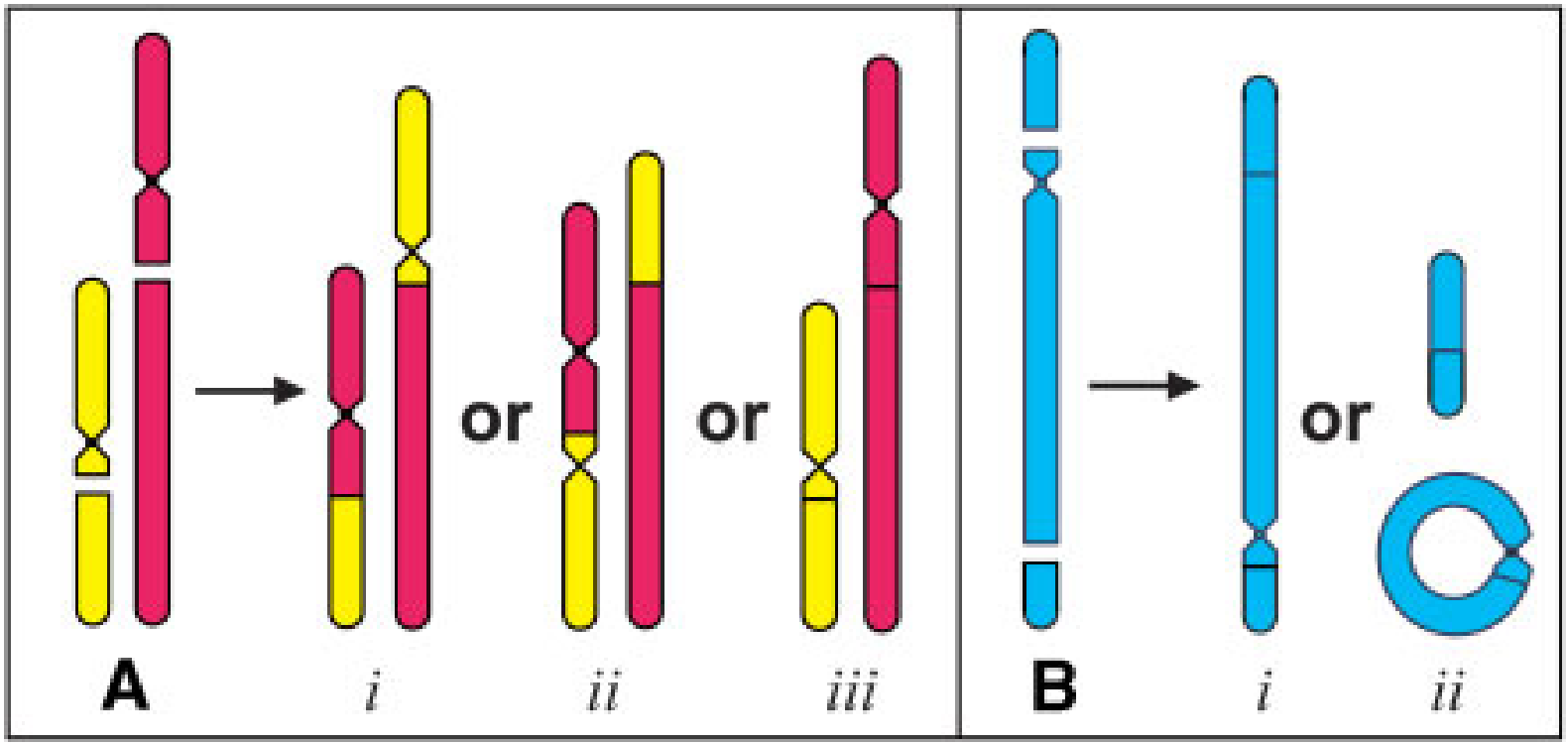
DNA double strand breaks (DSB) are major lesions for the immediate (**S-phase-independent**) induction of chromosomal aberrations by radiation

- The energy of ionizing radiations is high enough to directly or indirectly induce DSB (apart from other damage in the DNA). Such DSB are causative for the immediate formation of chromosomal aberrations independent from the cells passing through an S-phase (S-independent mutagen).
- Other mutagens (e.g. UV-irradiation, most chemical mutagens) depend on a passage of the affected cell through the S-phase to transform a primary DNA lesion (e.g. alkylations, base damages, base losses, pyrimidine dimerization, or adduct formation) into a cytogenetic damage (S-dependent mutagens).
- Apart from ionizing irradiations only few other agents are capable to induce DSB, thus leading to chromosomal aberrations S-independently. Examples are the cancer therapeutic drugs Bleomycin and Neokarzinostatin, and endonucleases. These agents are therefor termed radiomimetica.

Mechanism of **chromosome-type aberration** origin in G0/G1 phase cells within unreplicated chromosomes

Interchromosomal

Intrachromosomal



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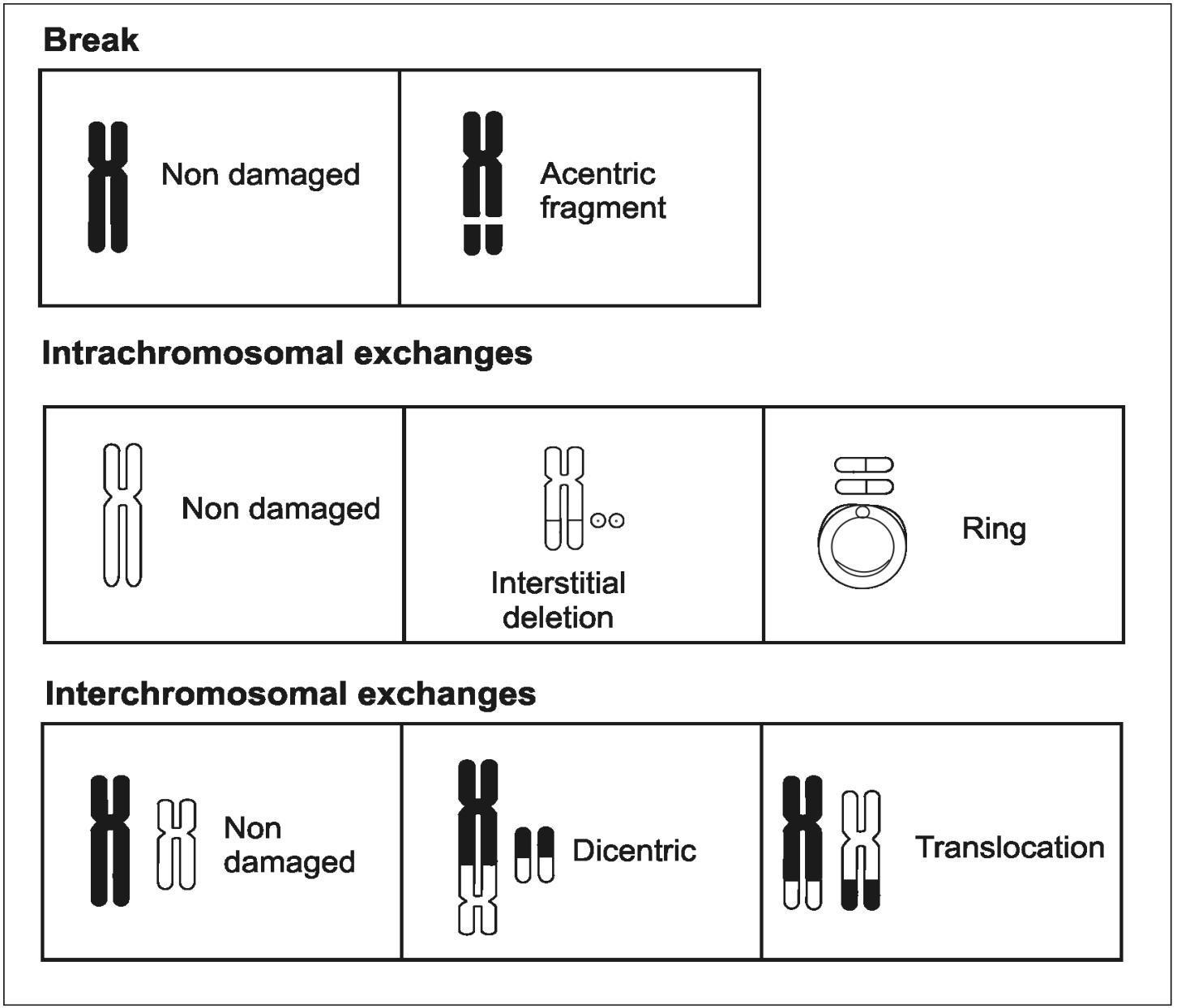
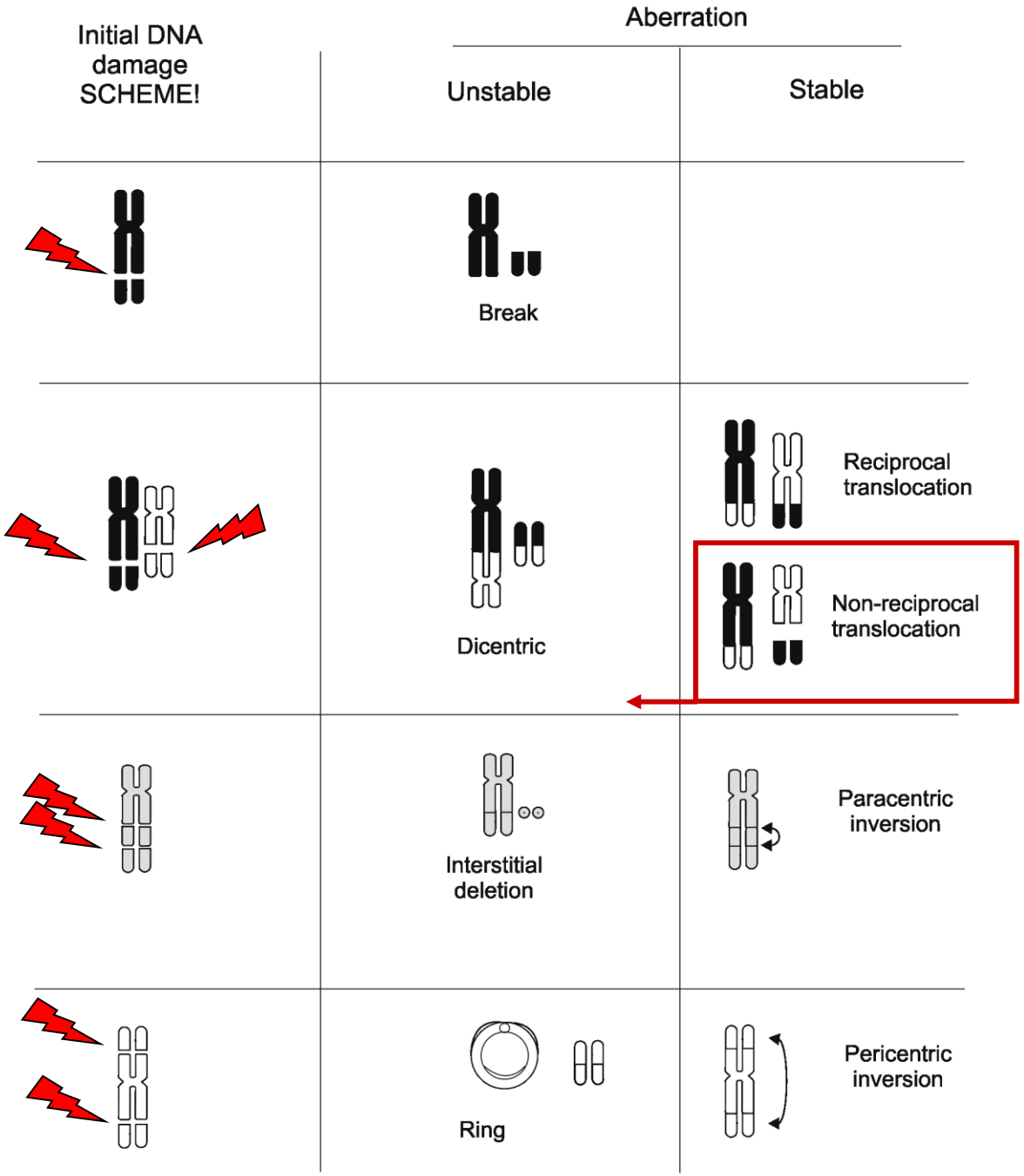
Chromosome-type aberrations as they appear in metaphase preparations

(Figure from Andrzej)



Stable vs unstable aberrations



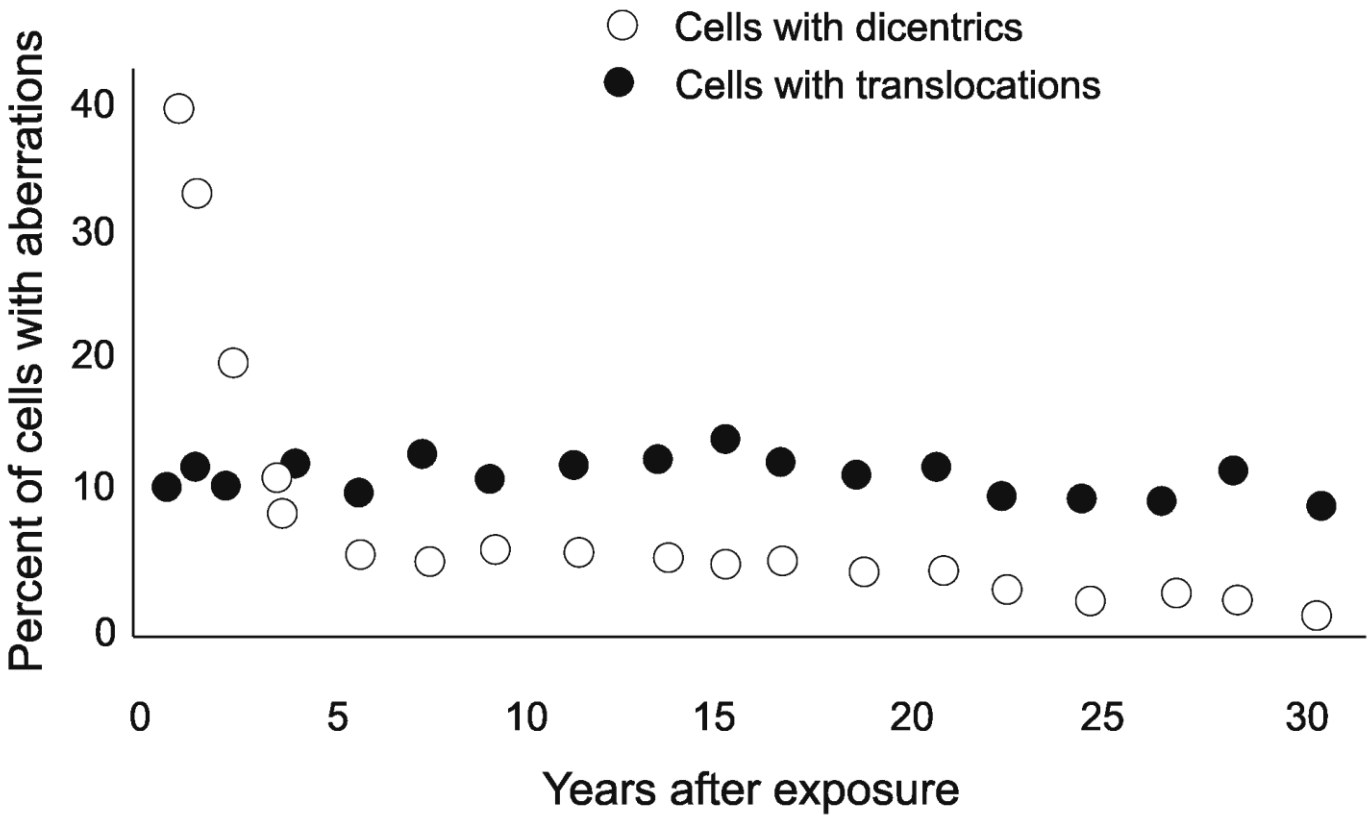
complete

incomplete

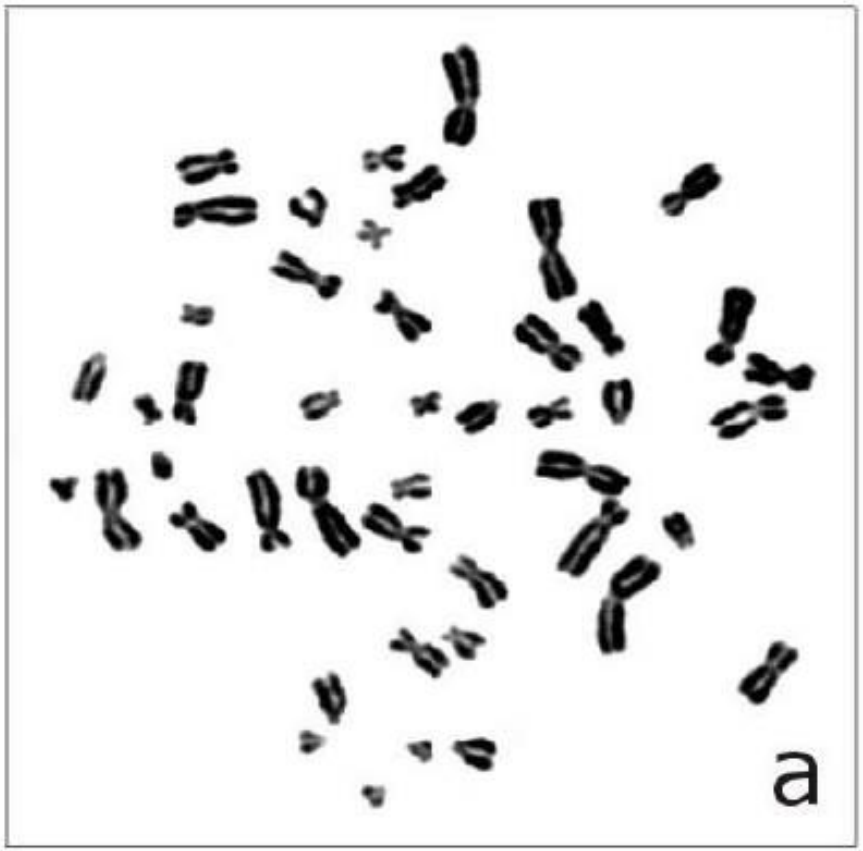
Why are aberrations reffered to as stable?

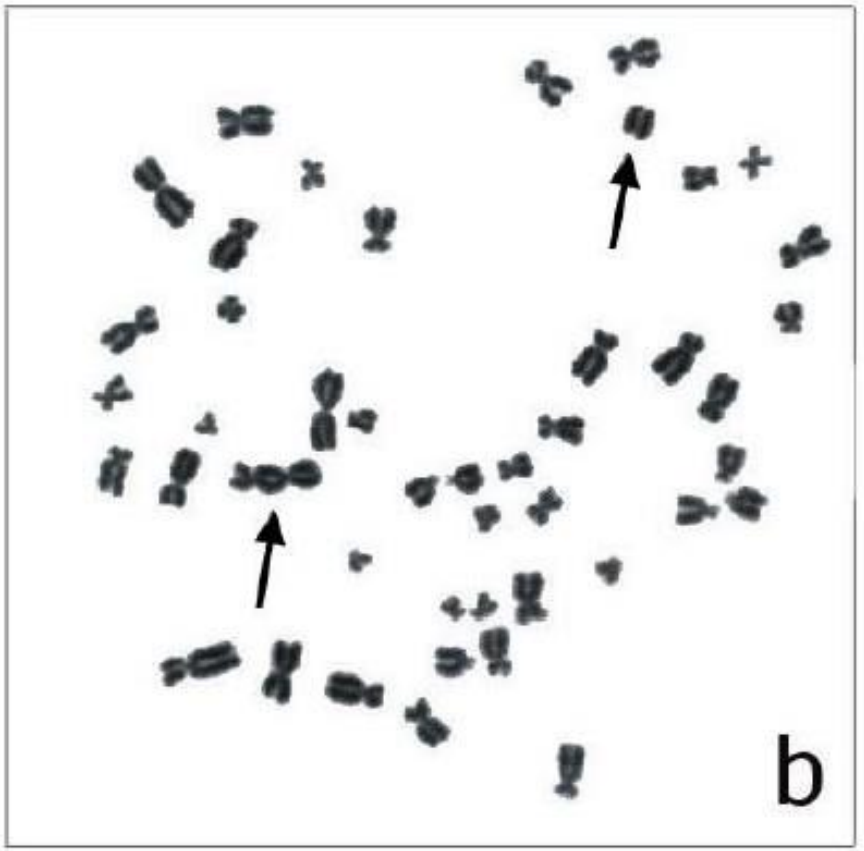
Analysis of dicentrics (unstable) and translocations (stable) in peripheral blood lymphocytes of patients treated by radiotherapy for ankylosing spondylitis (morbus Bechterew)

Buckton et al. 1983



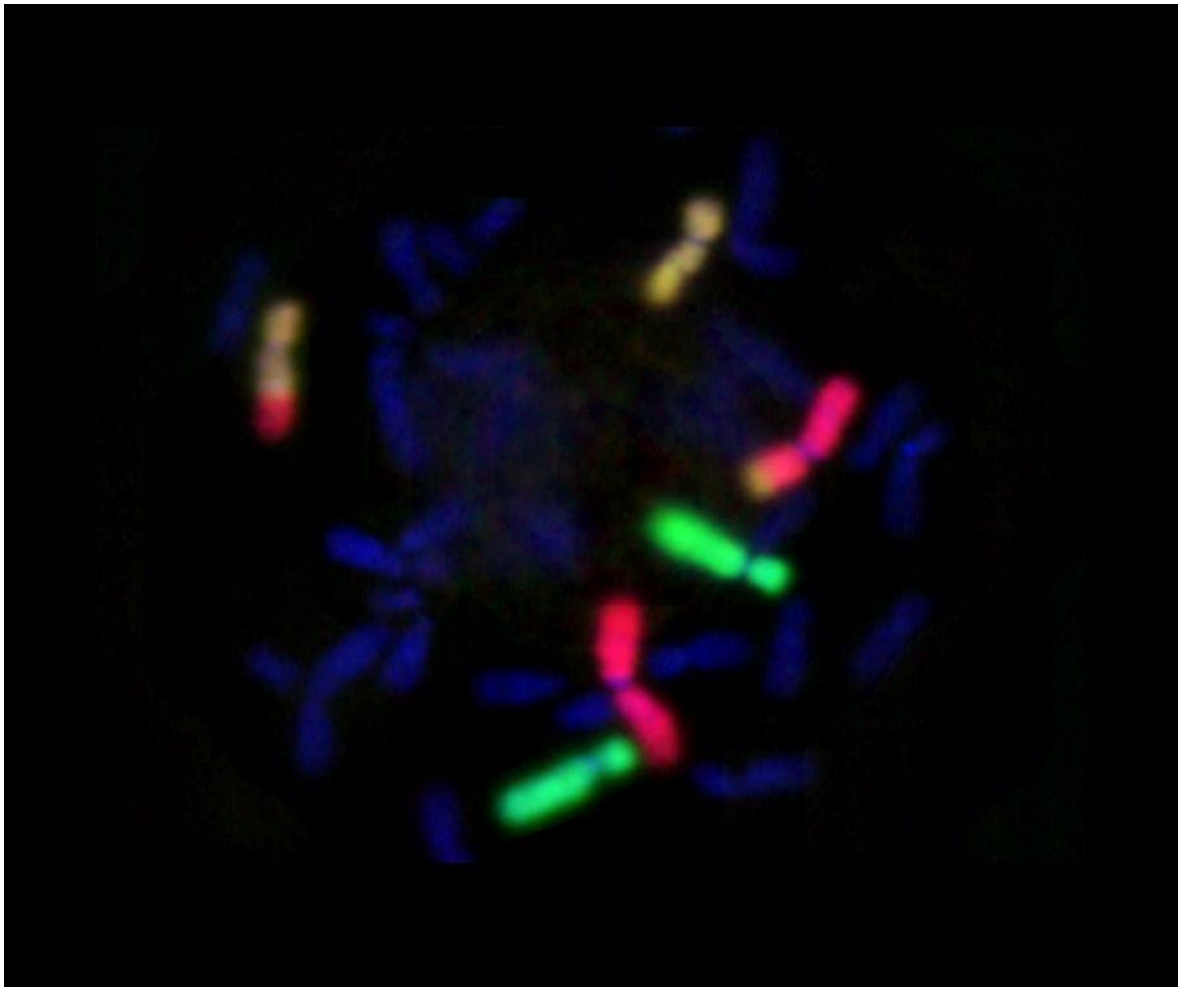
Example in a Giemsa stained preparation





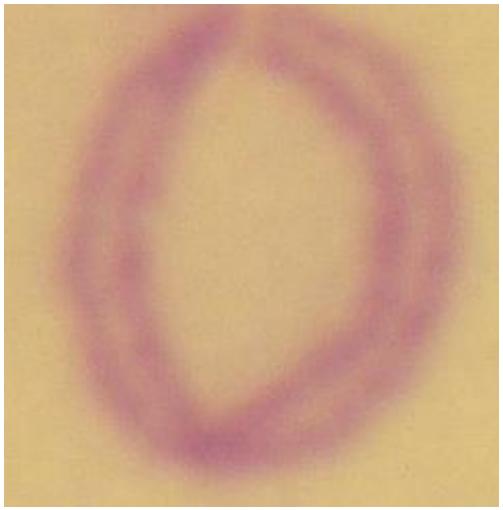
Example of a reciprocal translocation in a three colour FISH

#1
#4
#7

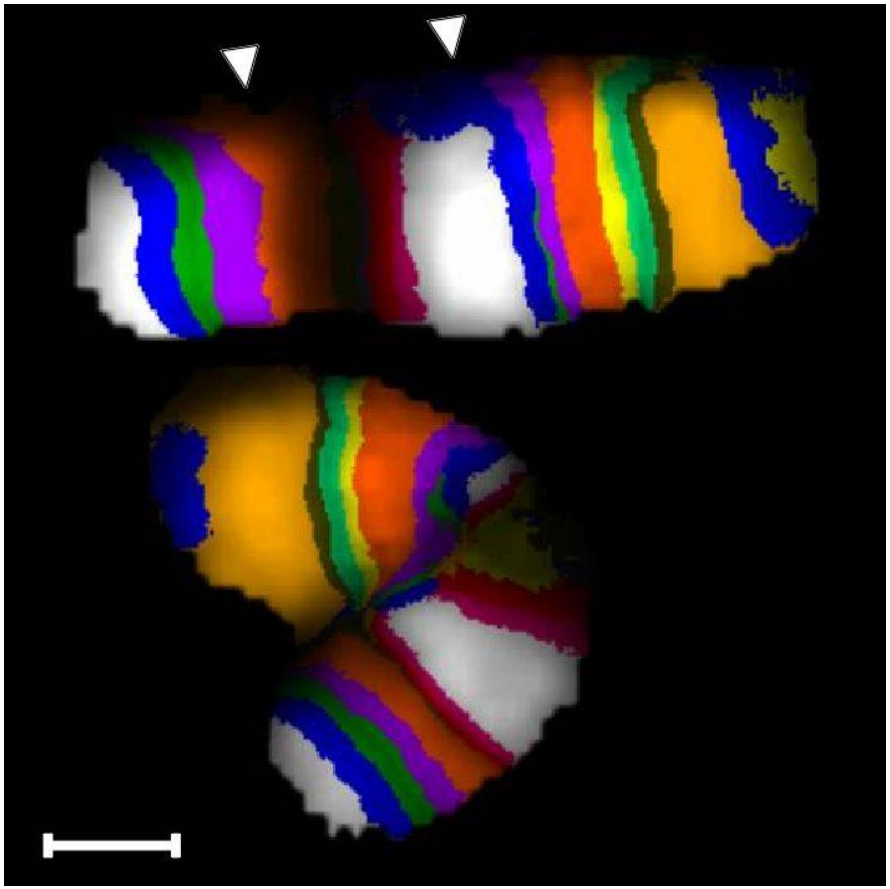


Unstable and stable intrachanges

Ring chromosome

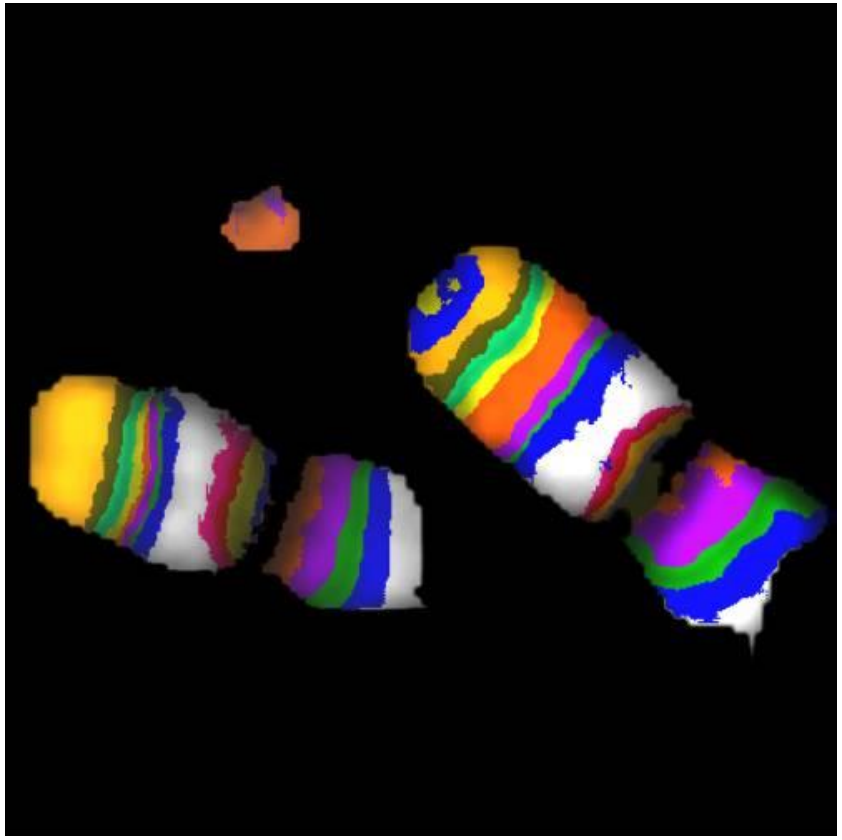


Pericentric Inversion

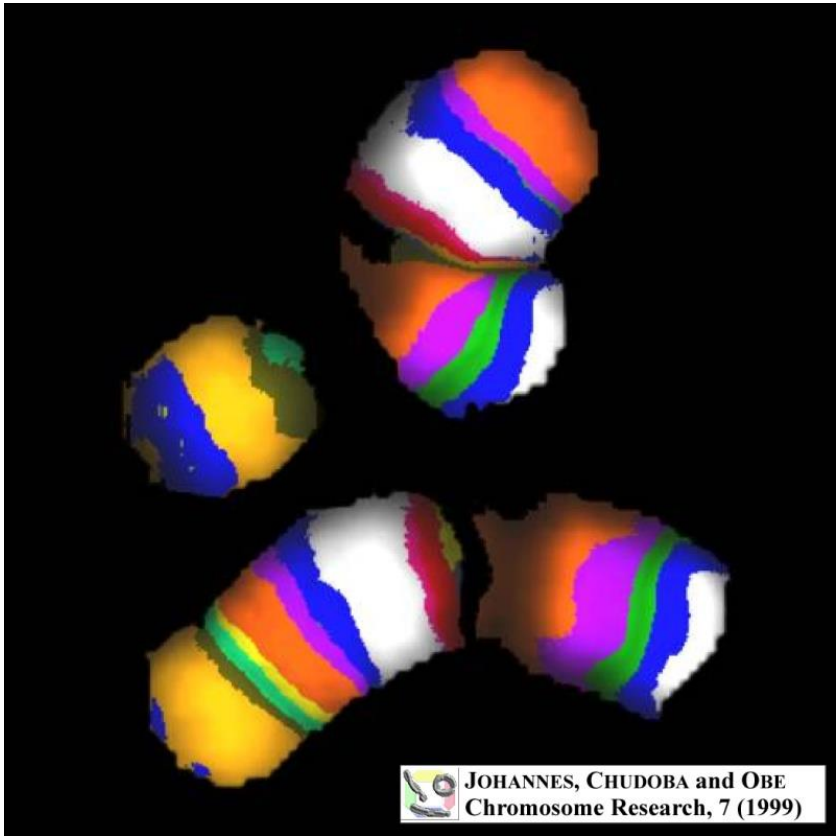


Deletions

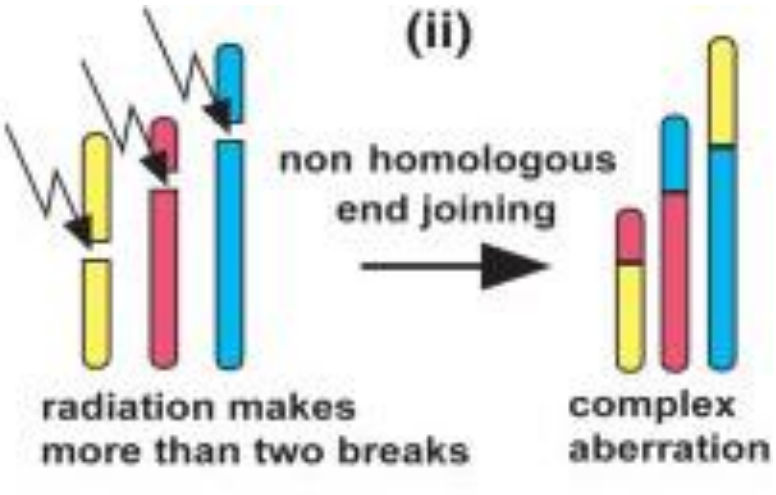
Interstitial



Terminal



Complex aberrations



A complex aberration is one involving at least 3 breaks in at least 2 chromosomes

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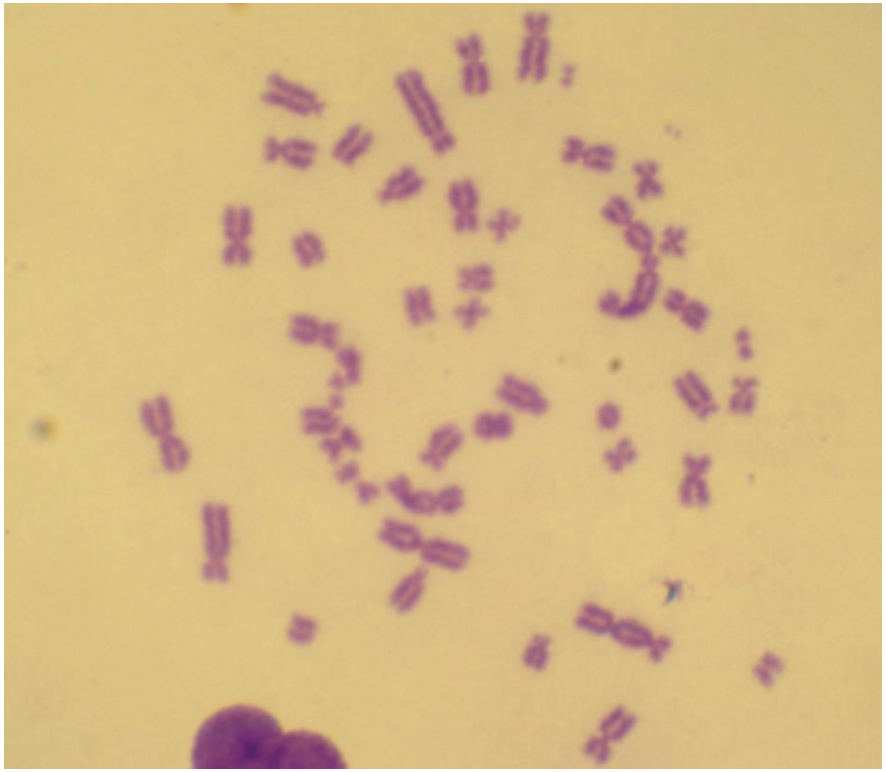
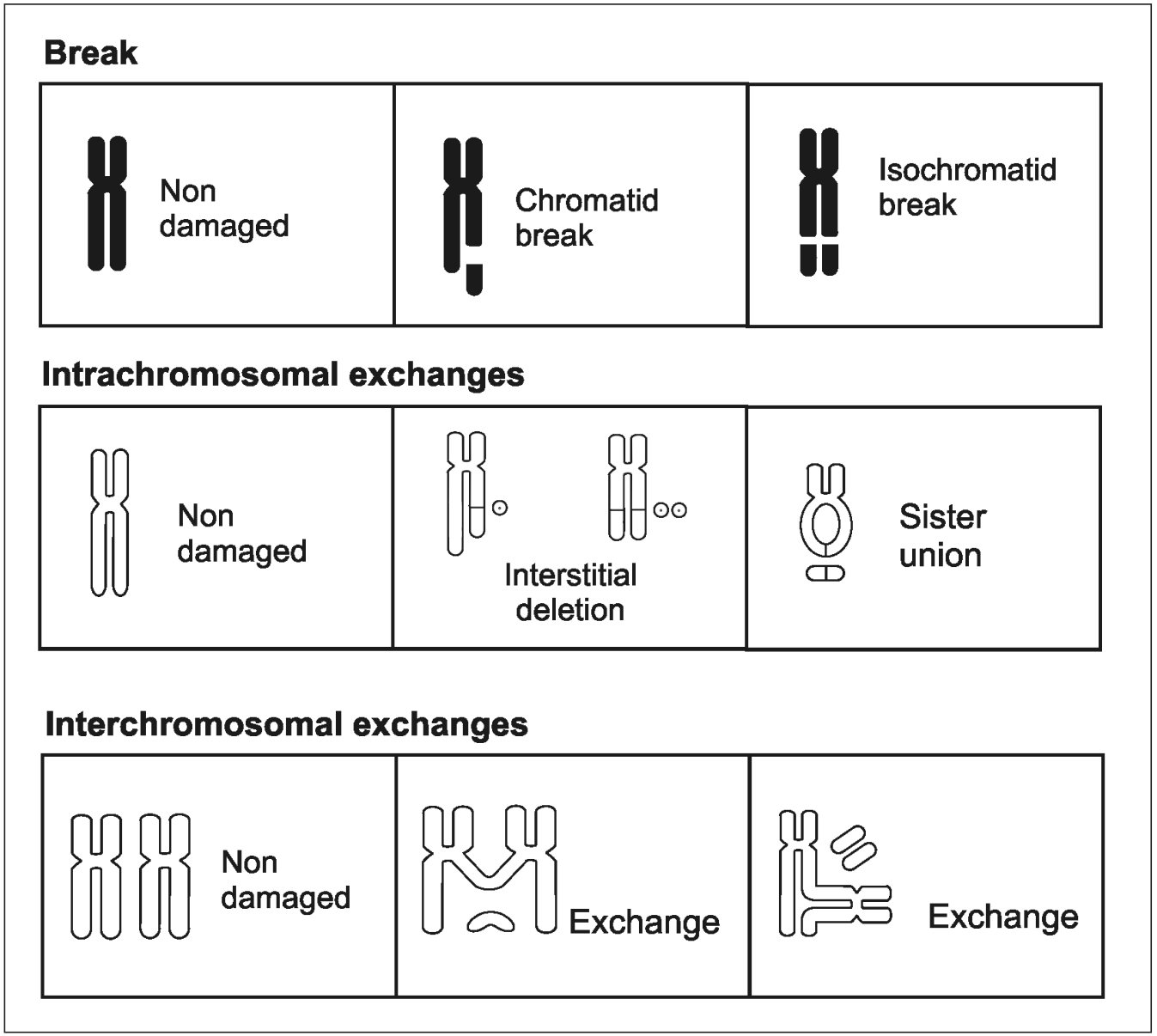
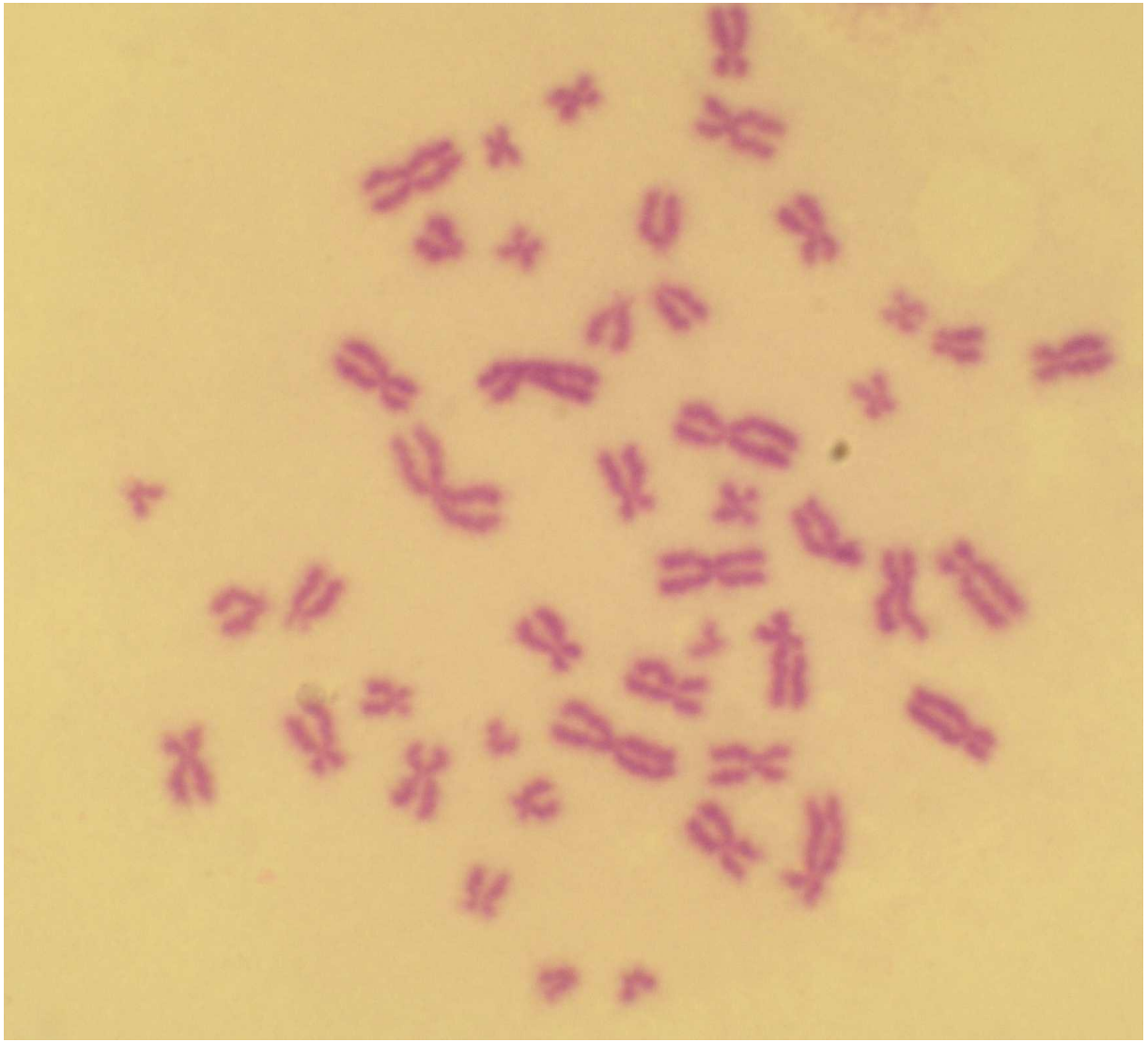
Complex aberrations and multiple damaged cells



Chromatid-type aberrations arising in or after S-phase as they appear in metaphase
(drawings from Andrzej)



Chromatid type break



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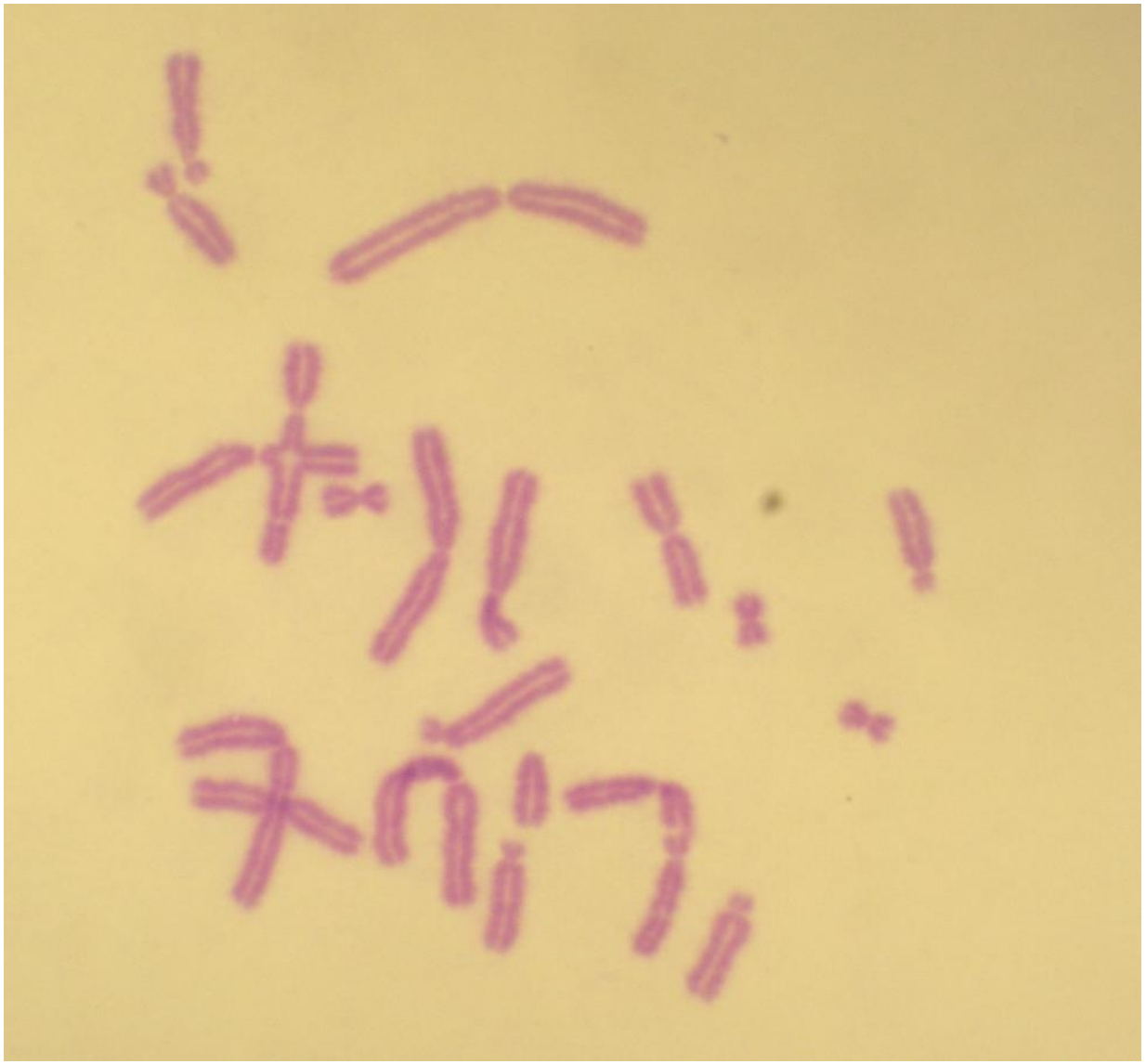
Quadriradial chromosome (Chromatid-type interchange)



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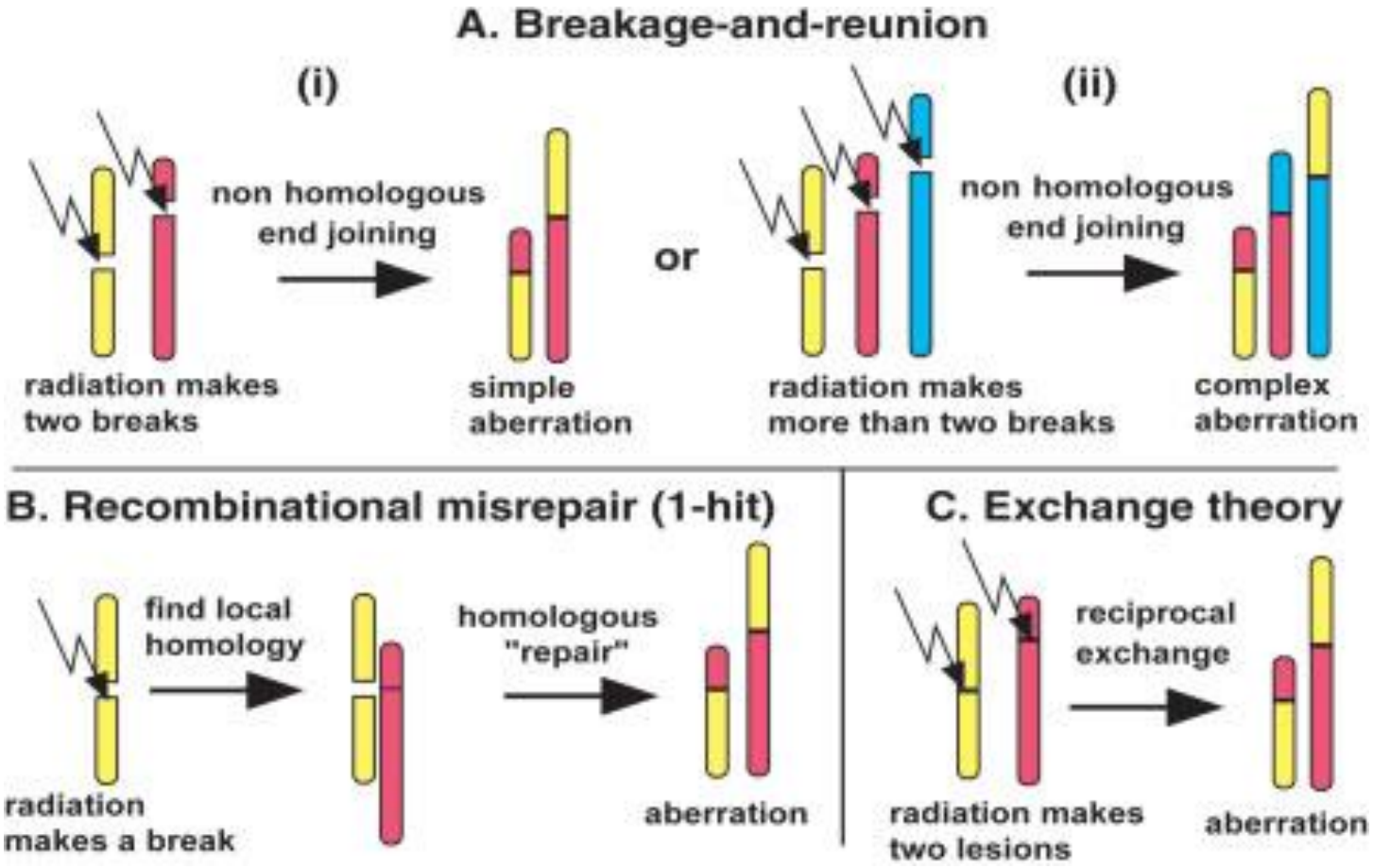
Mechanism of origin



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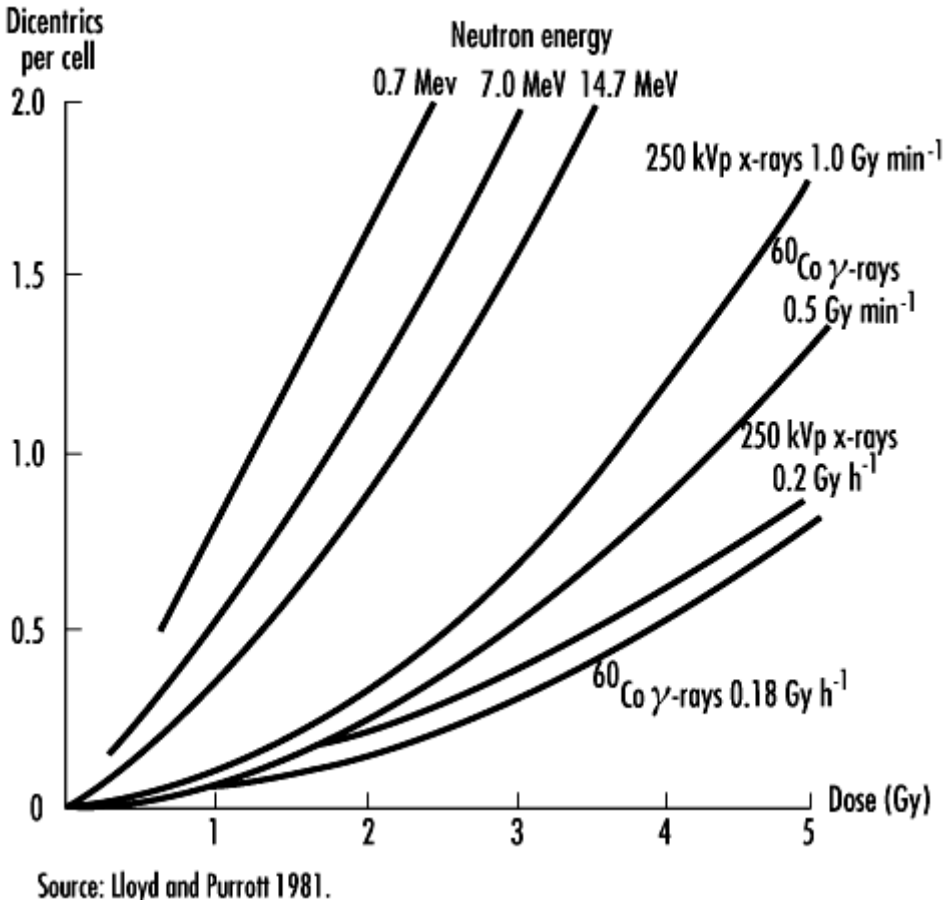
Dose response-curves



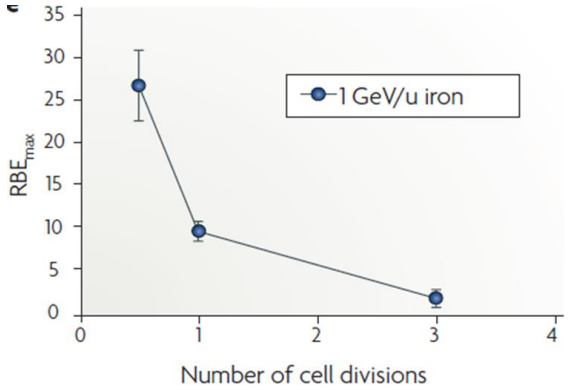
Source: Lloyd and Purrott 1981.

The shape of the dose-response curves varies with the aberration type and the radiation quality:

High LET radiation can induce more than one DSB in close proximity and therefore result in linear curves for all types of aberrations

Which post-irradiation mitosis do I look at?

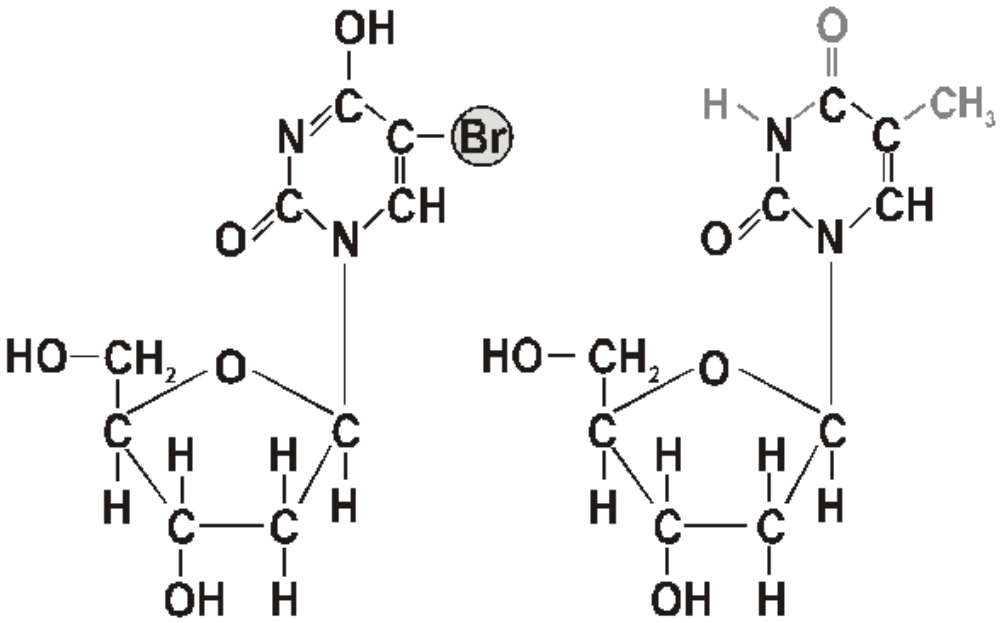
The control of the cell cycle progress is necessary in order to get a realistic picture of the chromosomal damage.



Usually the BrdU-FPG method is used to determine the number of cell cycles after irradiation

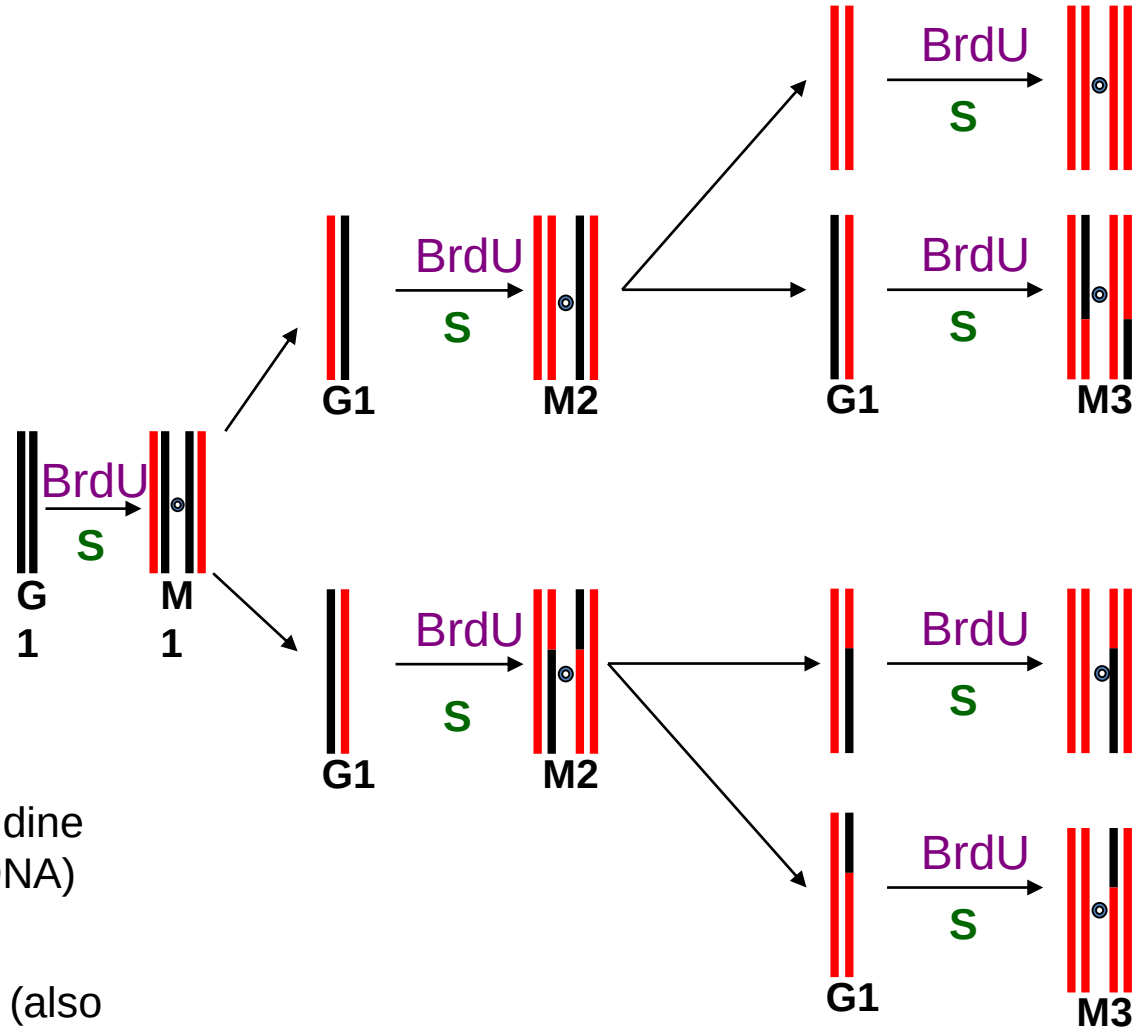
Control of the cell cycle progression

First step: Incorporation of the base analogon bromo-deoxyuridine (BrdU) into the replicating DNA substituting for thymidine during cell cultivation. BrdU pairs with adenine like thymidine does.



5-Bromo-2'deoxy-Uridin Thymidin

Incorporation of BrdU during three rounds of replication

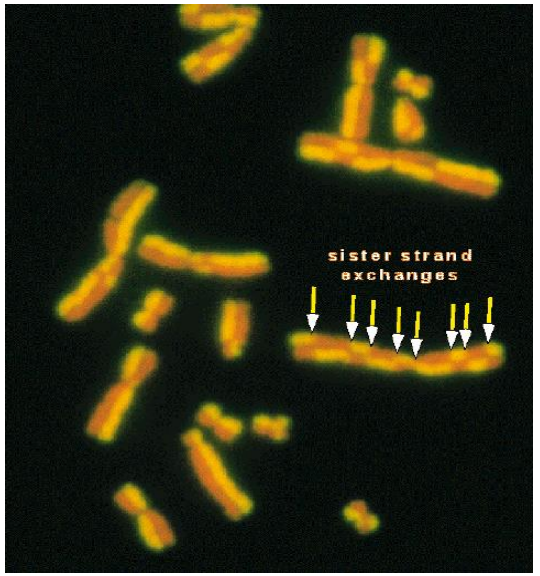


Single strand with thymidine (naturally occurring in DNA)

Single strand with BrdU (also pairing with adenine in dsDNA)

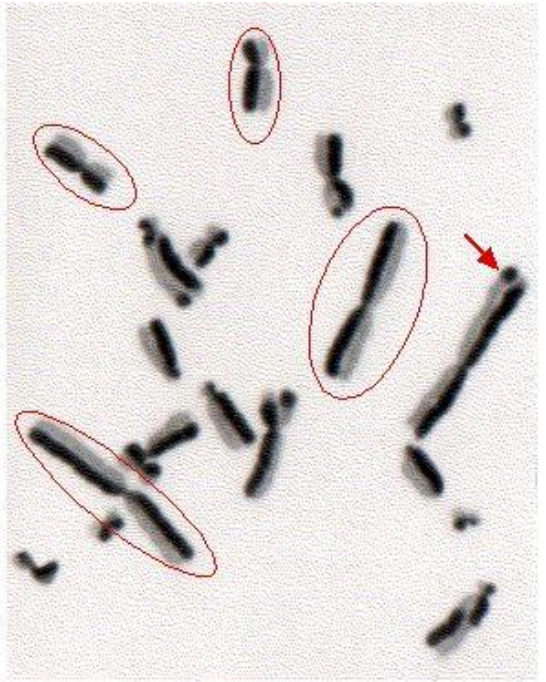
Visualization of the chemical difference

Second step: Visualization of differentially labeled chromatids



Staining of chromosomes with the fluorochrome HOECHST 33258 leads to a quenching of the fluorescence signal in the BrdU labeled DNA. Unlabeled chromatids or chromatids containing less BrdU then the sister chromatid show a brighter signal in the fluorescence microscope.

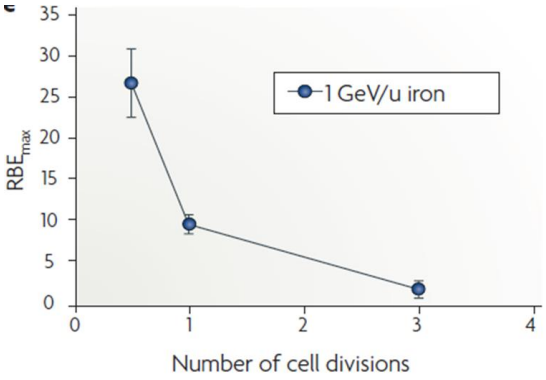
With fluorescently labeled Anti-BrdU antibodies detection of BrdU is possible at very low concentrations of substitution.



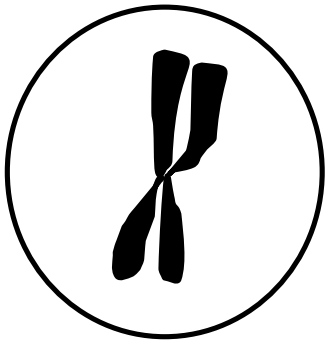
Chromatin containing BrdU and stained with HOECHST 33258 is very photo-sensible. Following irradiation with UV-light at 60°C the DNA degrades and can be washed out of the chromatids. The differential loss of stainable material is finally visualized by a simple Giemsa staining. It results in a darker and a lighter stained chromatid and exchanges are detectable.

Cell cycle control

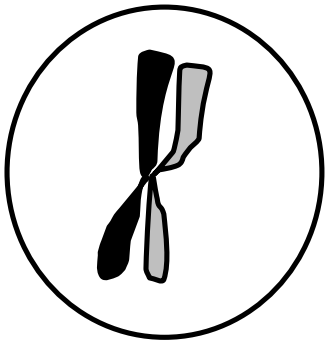
If BrdU is added to the culture medium in adequate amounts cells will incorporate the base analogue during each round of replication.
Following metaphase preparation and subsequent appropriate staining the results will be different for first (M1), second (M2), and third (M3) mitosis. Thus, the staining pattern of the chromosomes gives information on the type of mitosis you look at.



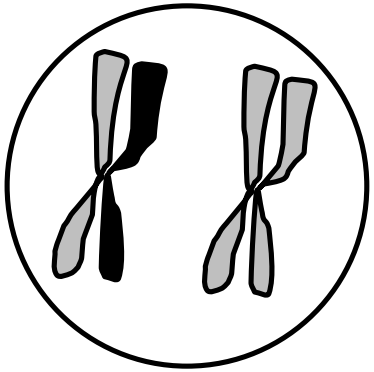
Uniformly dark stained chromosomes of a M1 cell



Differentially stained chromosomes of a M2 cell



Uniformly light and differentially stained chromosomes of a M3 cell



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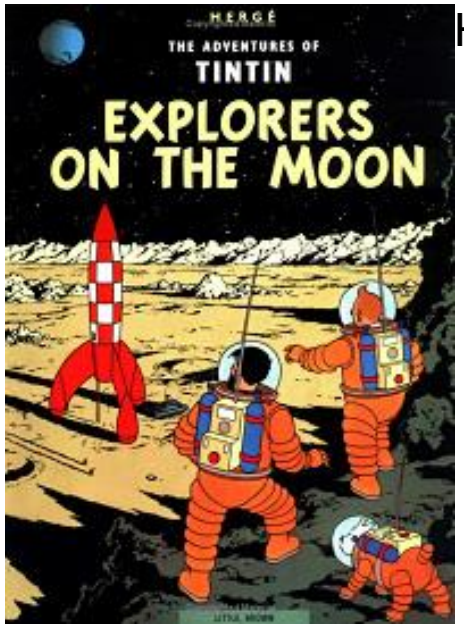
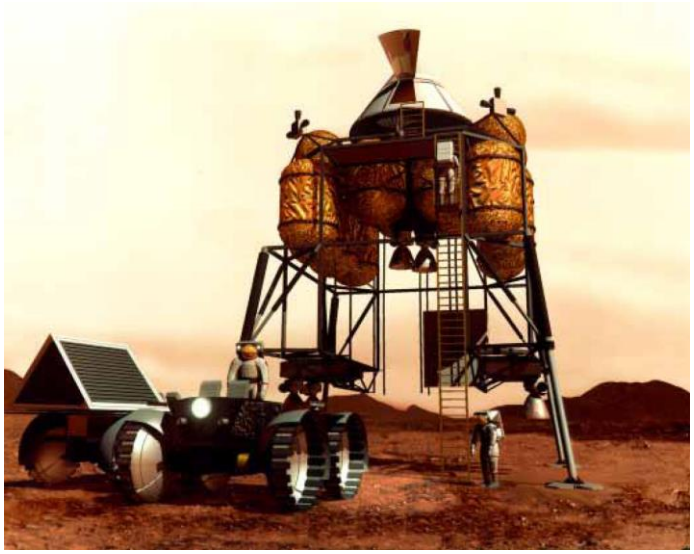
Biological dosimetry in space flight crew members

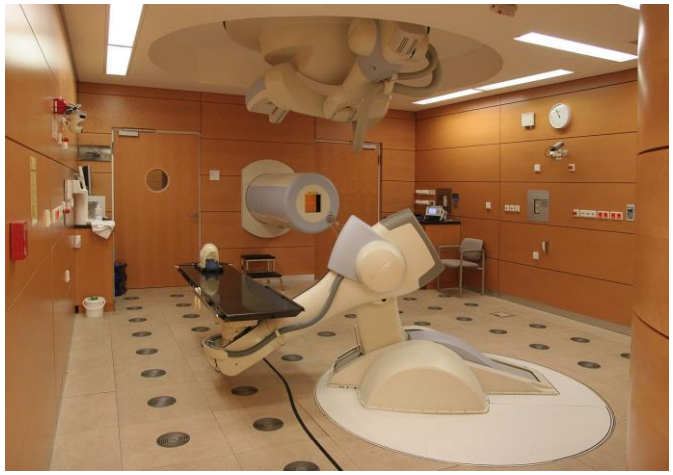
DLR



Herge

NASA

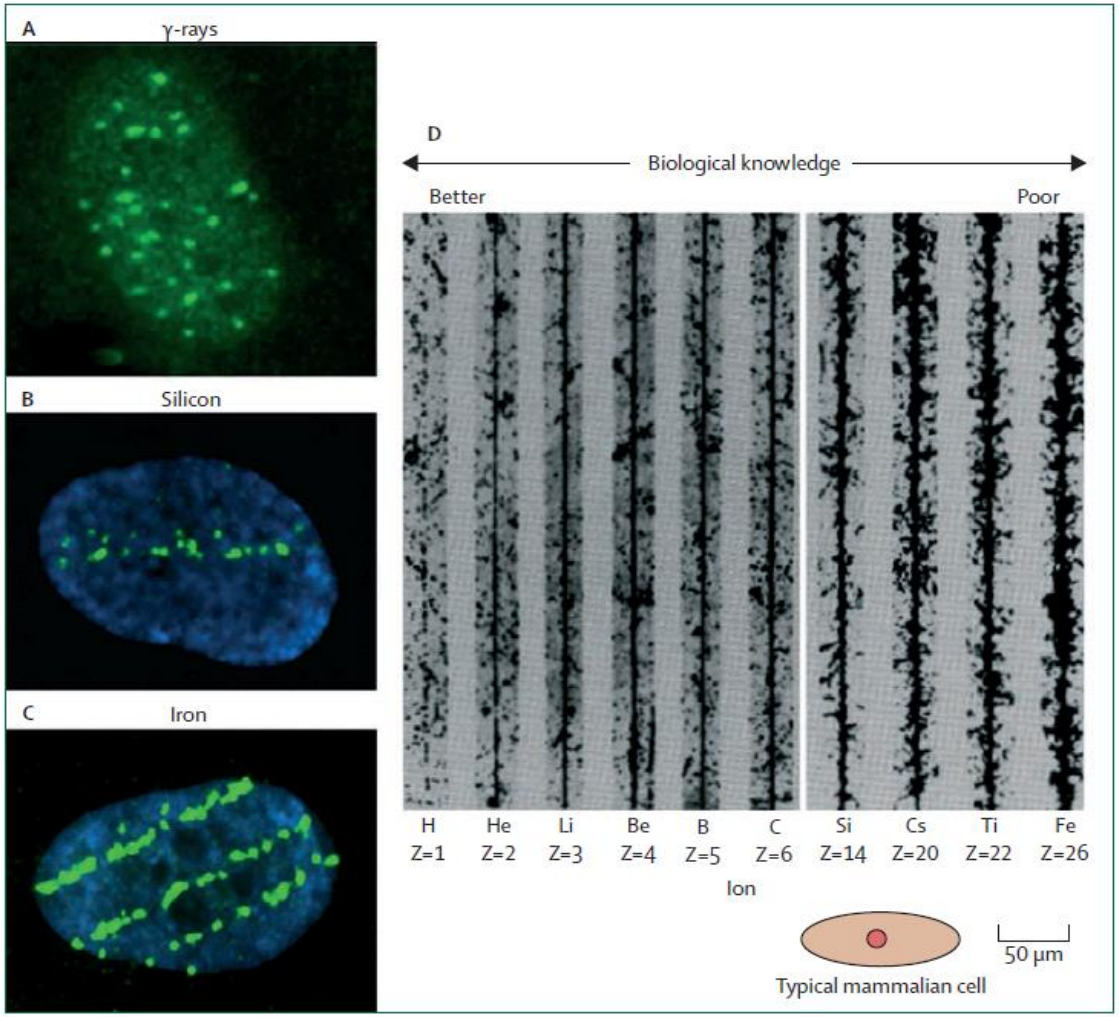




DKFZ

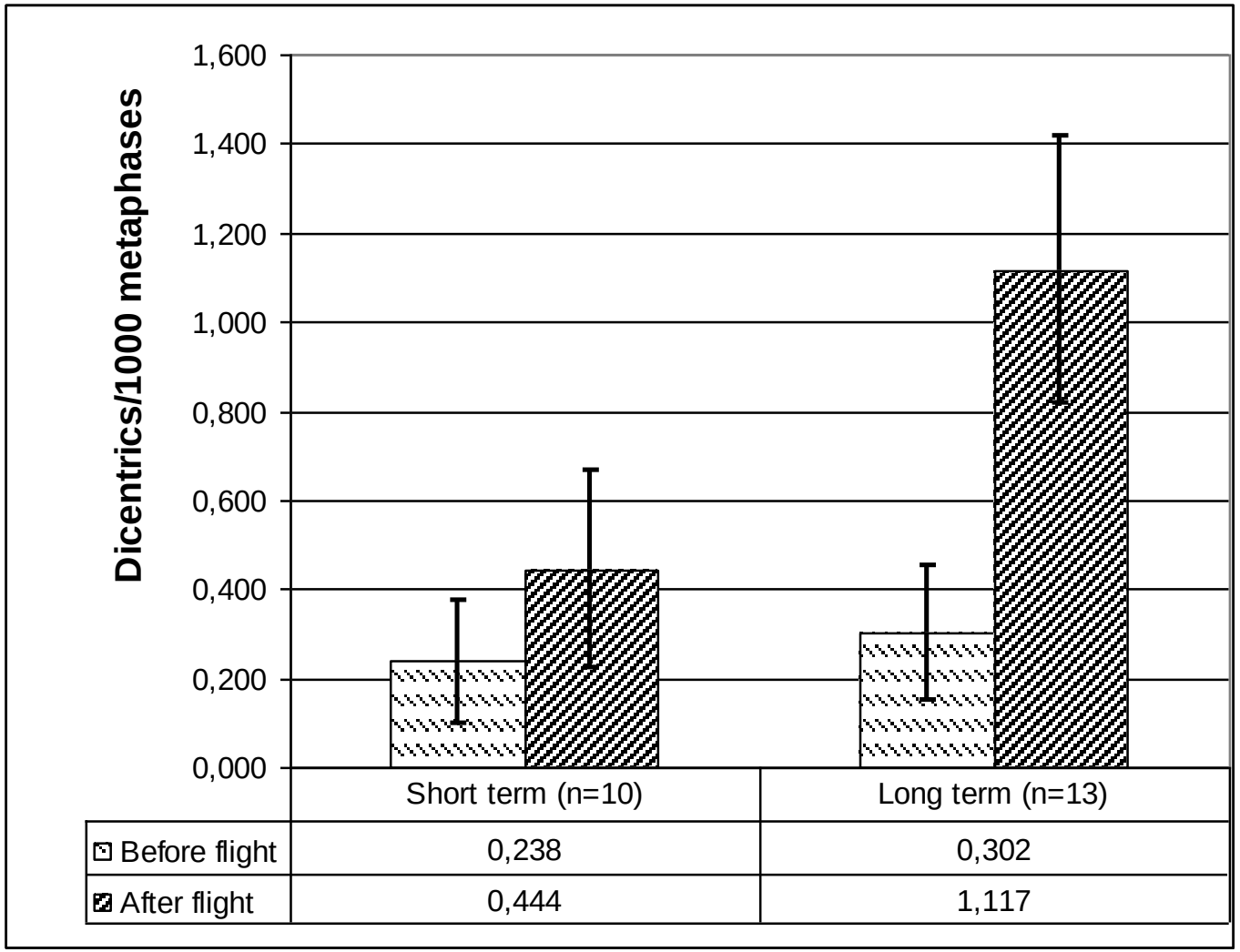
Cosmic rays - absorbed dose in mSv

- Sea level (per year): 0,3
- transatlantic flight at 10 km altitude (per year): 40
- ISS at 350 km altitude (per year): 400
- Inside the ISS (per year): 150
- Dose during a short duration mission (10 days): 4
- Dose during a long duration mission (190 days): 80
- Dose during a Mars exploration (1100 days): ~1000
- Total life time dose in Germany (80 years): ~200



Cucinotta, Durante, 2006

Increase of dicentrics following space flights on the ISS



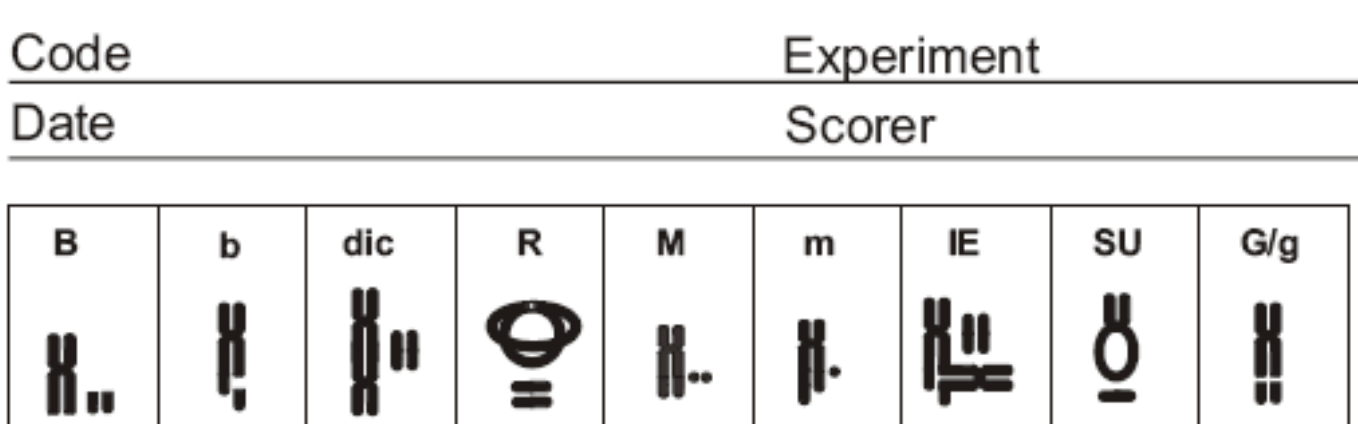
	Short term (n=10)	Long term (n=13)
Before flight	0,238	0,302
After flight	0,444	1,117

Aberration score sheet

Code Experiment

Date Scorer

	B	b	dic	R	M	m	IE	SU	G/g
1									
10									



B chromosome break
b chromatid break
dic dicentric (1 tric = 2 dics, etc)
R ring
M chromosome minute
m chromatid minute
IE interchromosomal exchange
SU sister union
G chromosome gap
g chromatid gap

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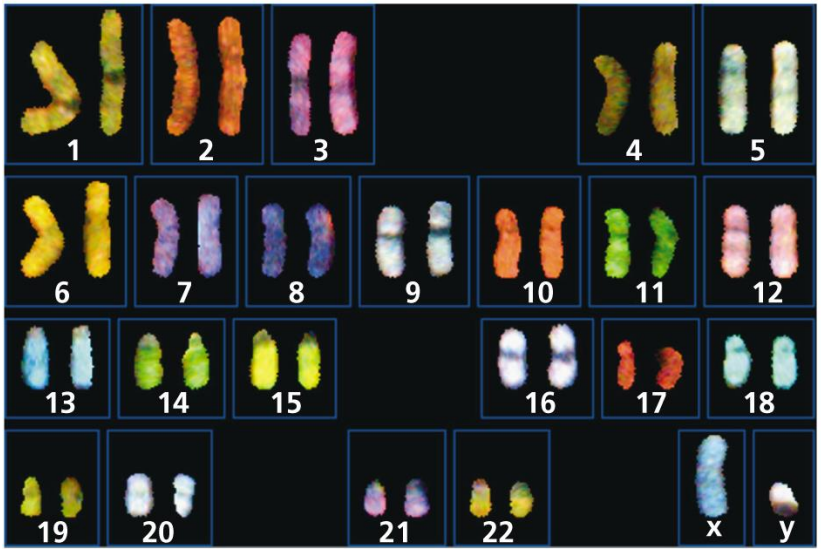
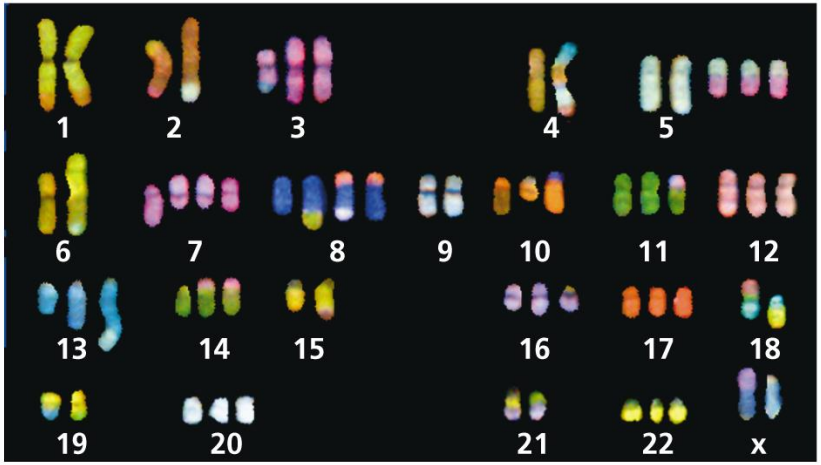
Normal karyotype (a) and multiple damaged karyotype of a tumor cell (b)



(a)



(b)