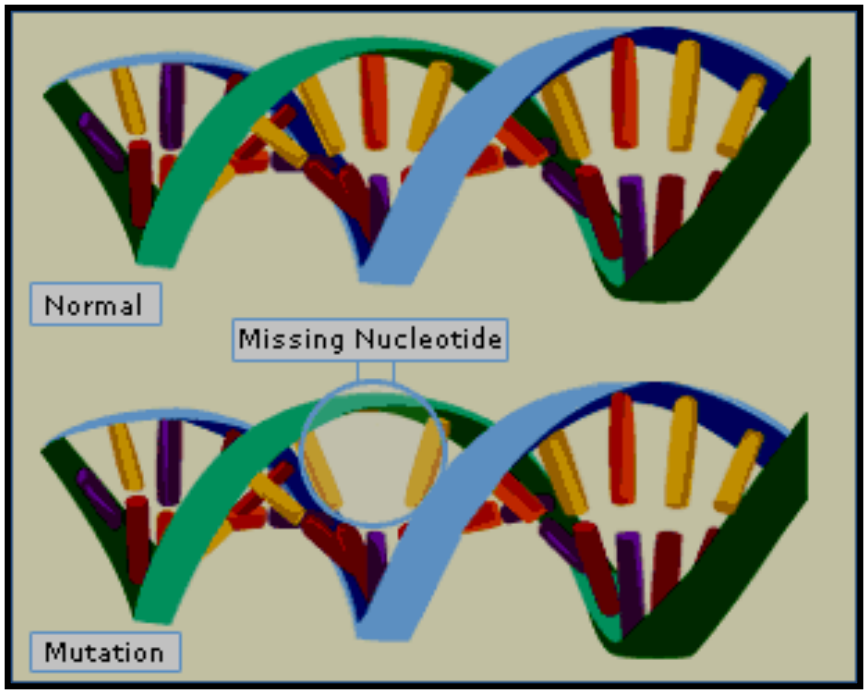
Mutagenicity and carcinogenicity of environmental factors

Mutation

- A change in the DNA molecule
- Process which produces changes in the DNA that may be inherited.

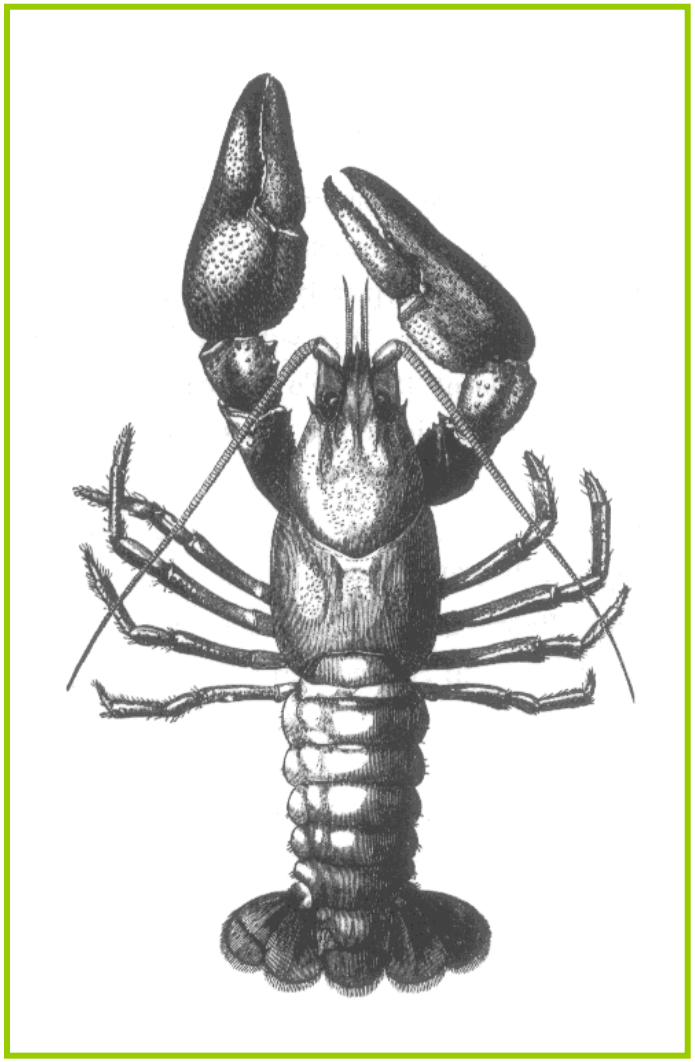


Mutagenicity

- = mutagenic activity
- = genotoxicity (genotoxic activity)
- Mutagenic (genotoxic) compound = a compound that causes mutation
- Mutations in certain genes could lead to malignant transformation of the cell.
- Many mutagens are supposed to be carcinogenic.

Carcinogens

- *Karkinos* (Greek) = crayfish
- Compounds or other factors that induce transformation of a normal cell into a tumor one

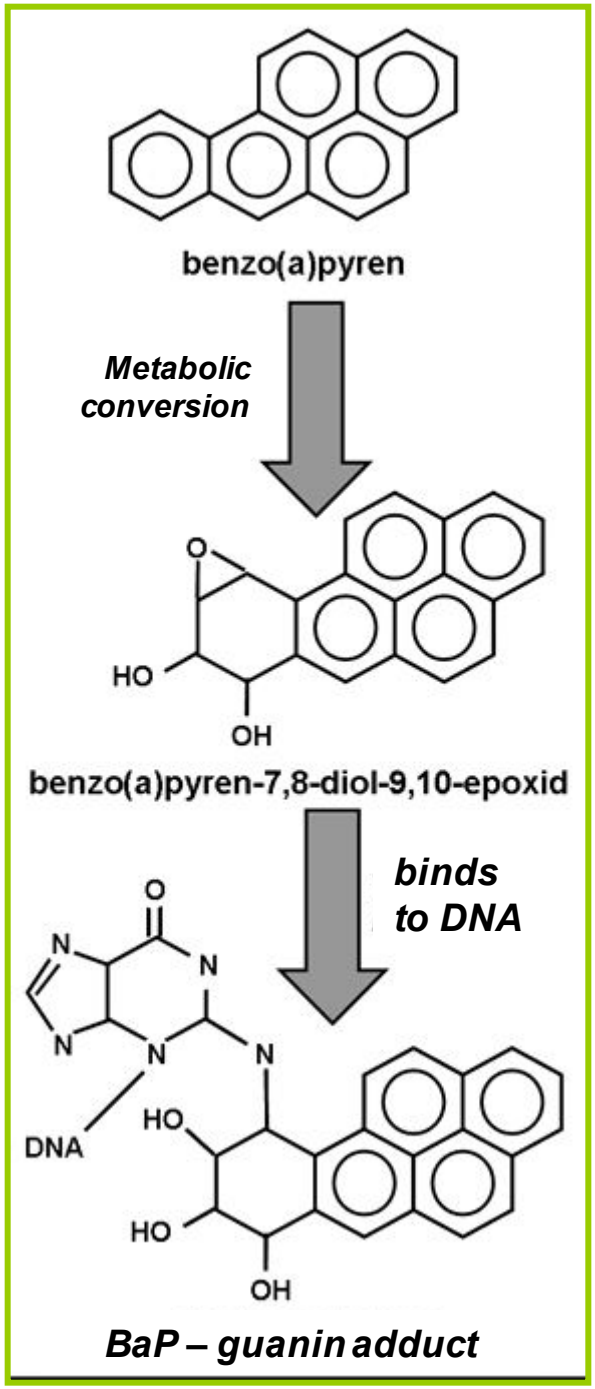


Classification of mutagens

- Chemical (different compounds)
  - Alkylating agents
  - Aromatic hydrocarbons (e.g. benzo(a)pyrene)
  - Intercalating agents (e.g. fluorescent dyes)
  - Artificial derivatives of DNA bases
- Physical (ionizing radiation, UV-radiation)
- Biological (viruses, transposable elements)

According to metabolic conversion we distinguish:

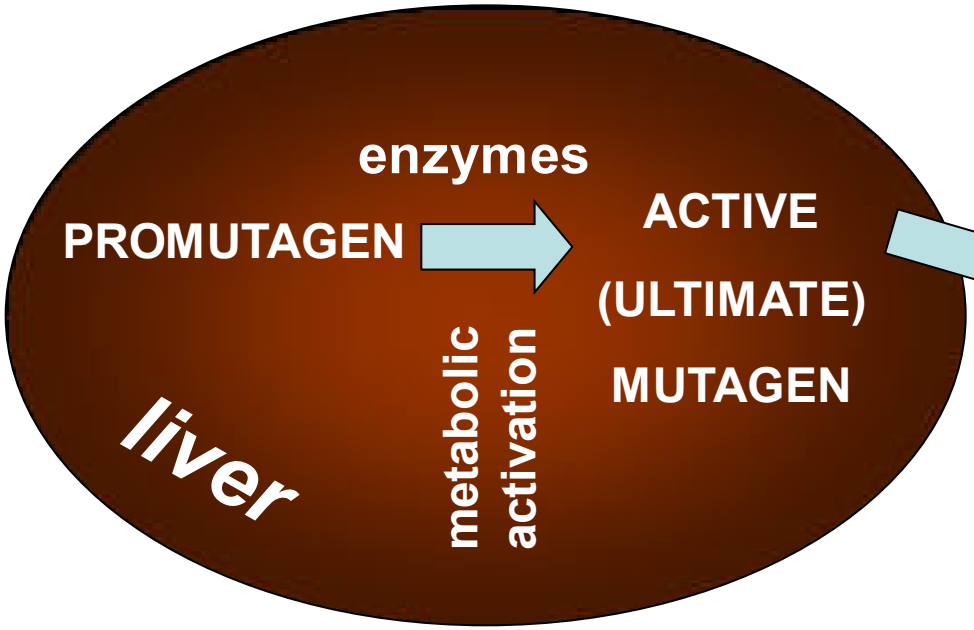
- Direct acting mutagens (active without metabolic conversion)
- Promutagens (require metabolic activation)

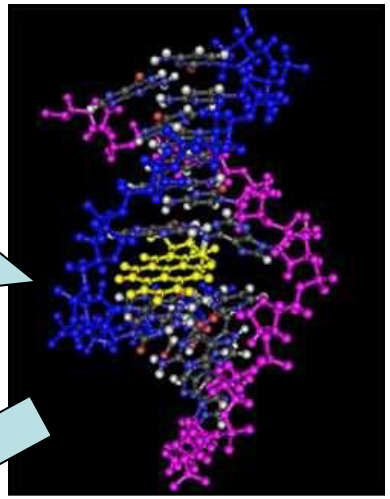


Benzo(a)pyrene

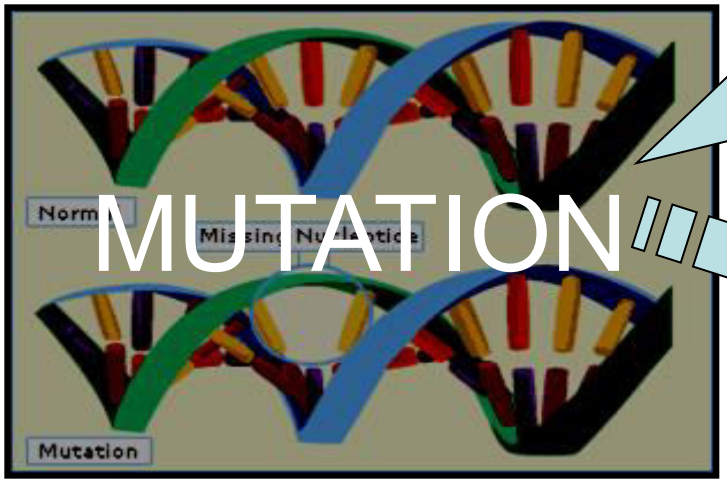
- Polycyclic aromatic hydrocarbon
- Carcinogenic activity confirmed
- A product of combustion processes (e.g. tobacco smoking)
- A typical promutagenic agent
- Metabolic conversion (addition of epoxide group and two OH-groups).
- A metabolic product binds to DNA → adduct (a compound that results from addition).
- Presence of adduct can cause mutation.

Metabolic activation of mutagens

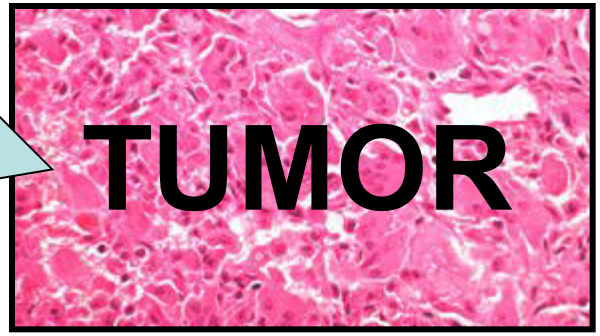




DNA ADDUCT (OR OTHER CHEMICAL MODIFICATION OF DNA BASES)



MUTATION



Genotoxicity screening tests

- On molecular level
- On gene level
- On chromosomal level

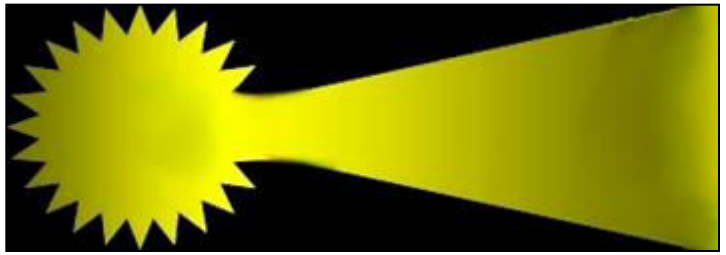
Tests on molecular level

- Identification of adducts
- Unscheduled DNA synthesis – measure of DNA repair using radioactively labeled nucleotide.
- Comet assay

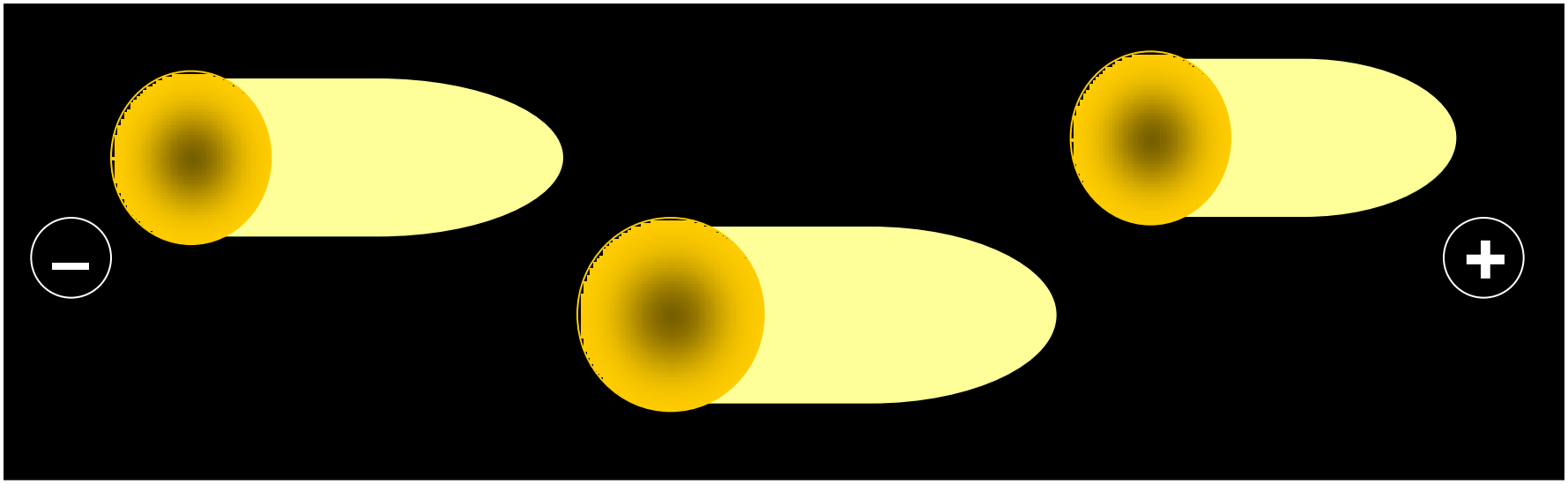
Comet assay

- Single Cell Gel Electrophoresis (SCGE)
- Identification of small DNA fragments inside nuclei of affected cells
- The fragments result from mutagenic affect.

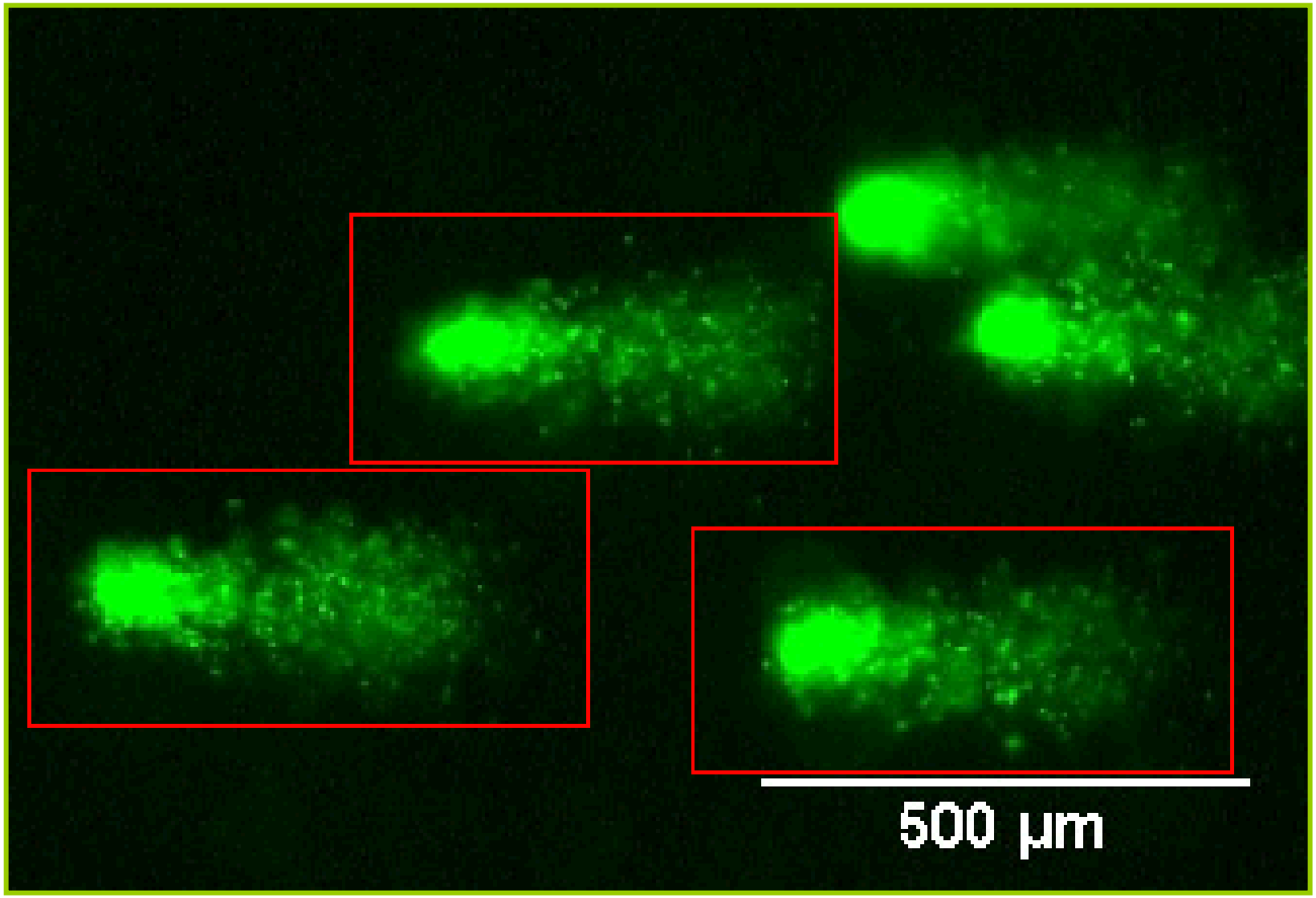
Comet assay



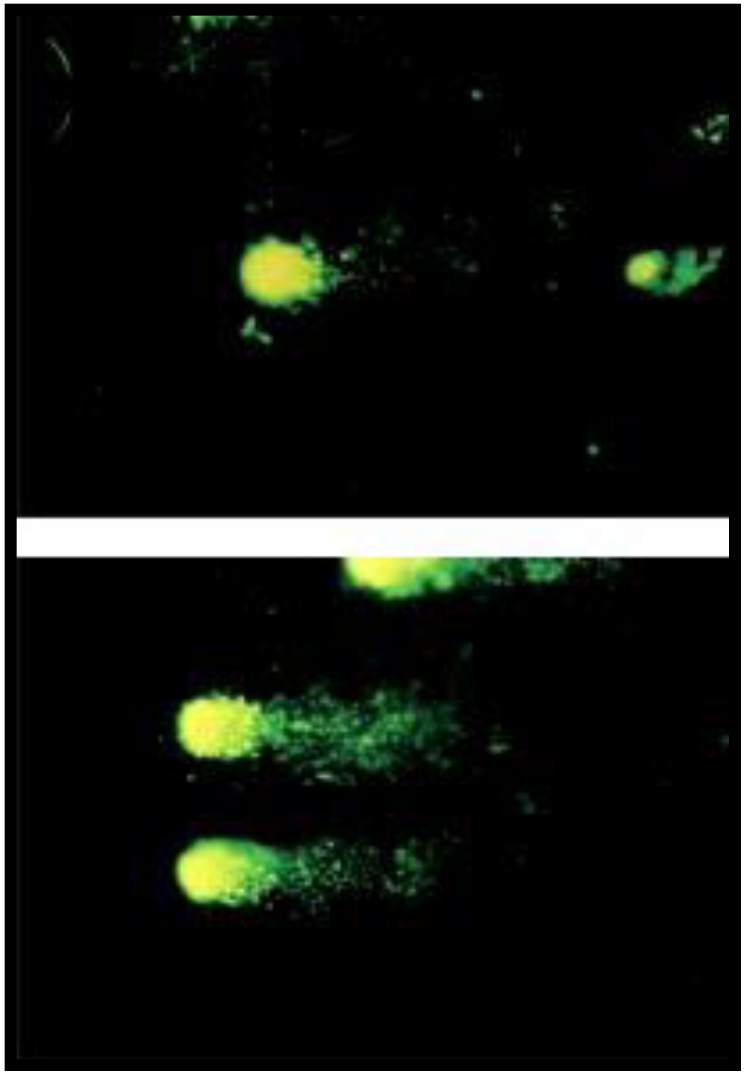
- DNA fragments are released from nuclei using electrophoresis
- Isolated nuclei are mounted into electrophoretic gel – after electrophoresis are stained with fluorescent dye.
- If DNA fragments are present a „comet tail“ is present observed in the vicinity of the nuclei.



Comet assay



Possible results of a comet assay

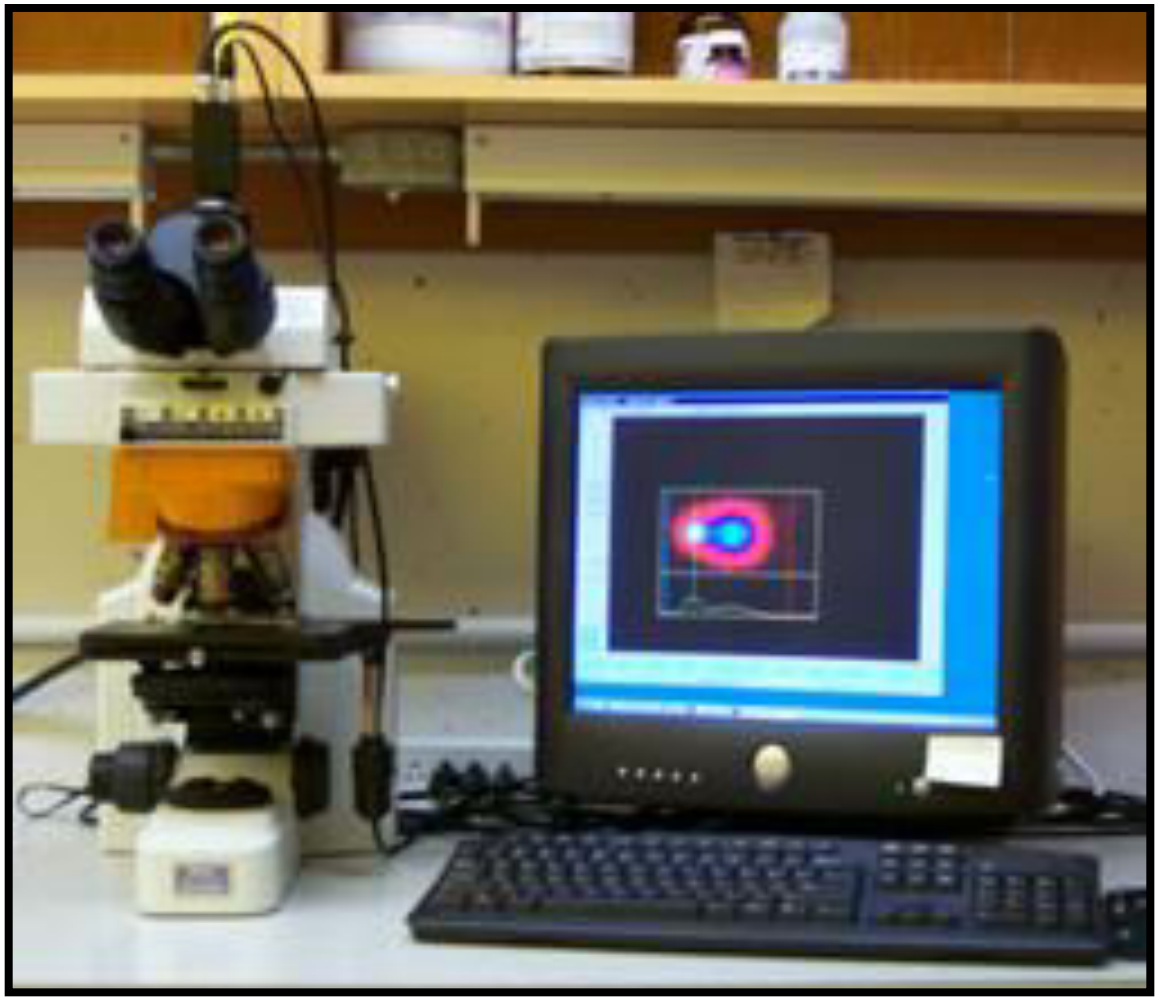


Normal nucleus without fragments

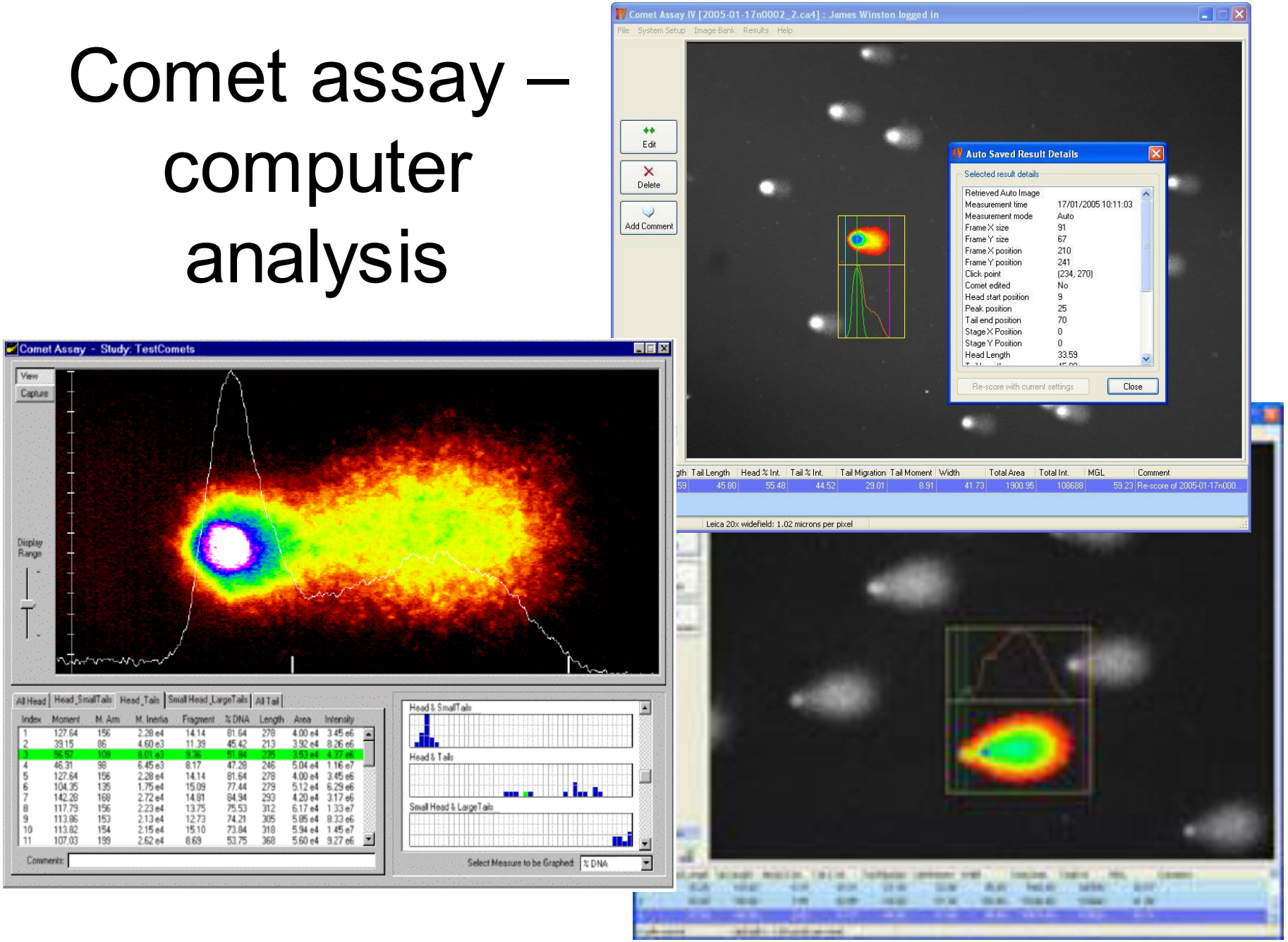
(DNA is not damaged – mutagenicity excluded)

Two nuclei with DNA damage

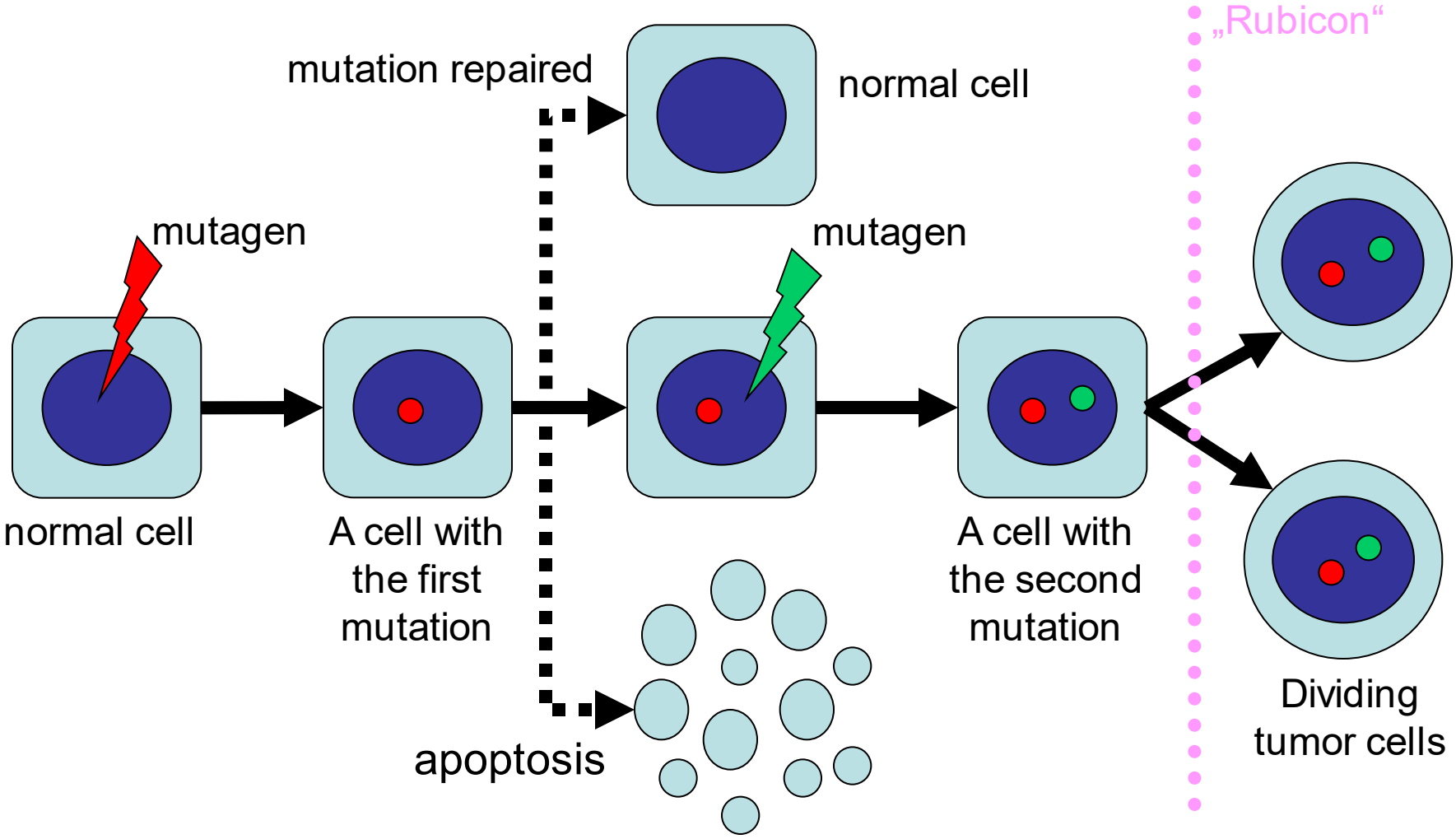
Evaluation of a comet assay



Comet assay – computer analysis



Carcinogenesis (simplified scheme)



Mutagenic X carcinogenic agent

- All mutagens are supposed to be carcinogenic but in some of them the carcinogenicity was not detected.
- Some carcinogenic agents are not mutagenic – they belong to the group of epigenetic carcinogens.
- Evaluation of carcinogenicity to humans is difficult and requires combination of various tests.

Carcinogenic agents

- Chemical (mostly mutagenic compounds)
- Physical (radiation, asbestos)
- Biological (oncogenic viruses – e.g. HTLV = human T-leukemia virus).

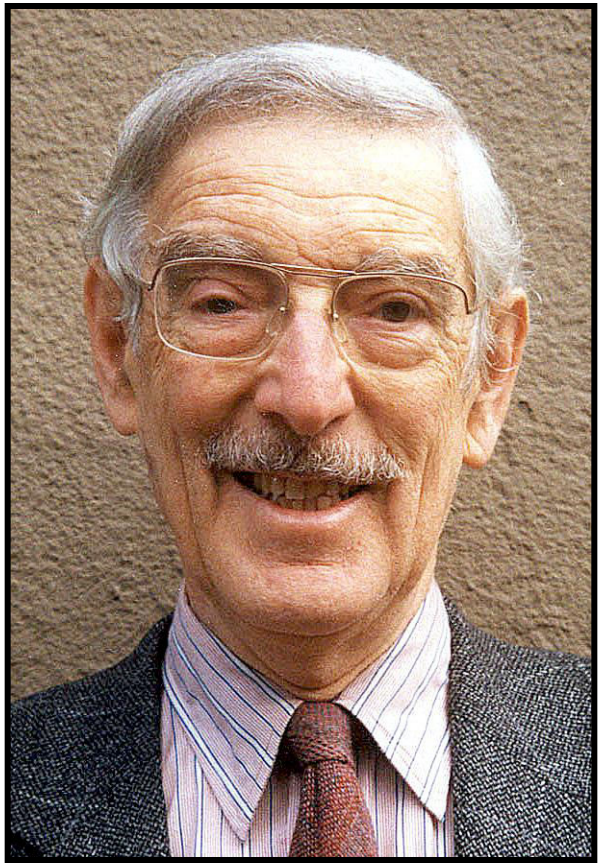
Direct evaluation of carcinogenicity

- Laboratory tests
  - In animals
  - *In vitro* transformation of cultivated cells
- Epidemiologic studies of exposed human population – mostly reliable

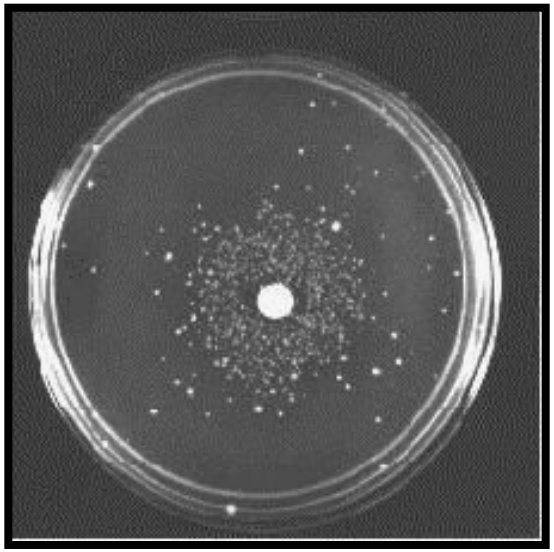
Tests on gene level

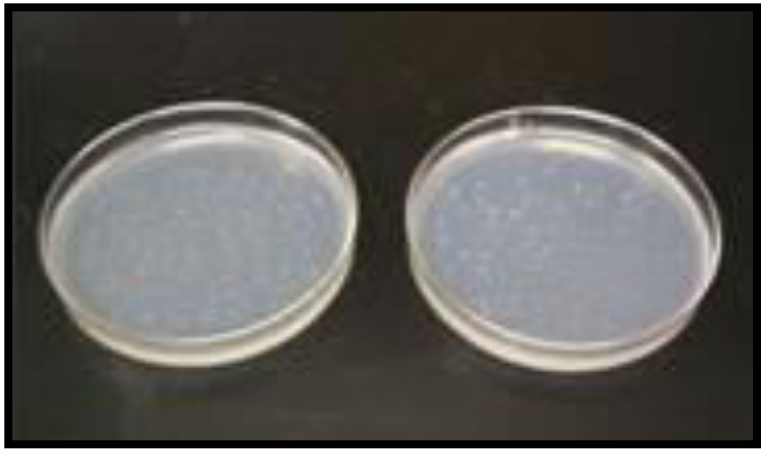
- Induction of *in vitro* mammalian cells resistant to certain chemical compound
  - (e.g. 8-azaguanine, 6-thioguanin, ouabain)
- SOS/umu test – measurement of DNA repair in bacteria
  - The bacteria is transformed by plasmide with mutator (repair) genes umuC and umuD fused with the gene for galactosidase. If the DNA is damaged by mutagen both umu mutator genes and gal gene are transcribed and galactosidase turns a specific substrate into a color product.
- Ames test

Ames test



Bruce Ames (born 1928)

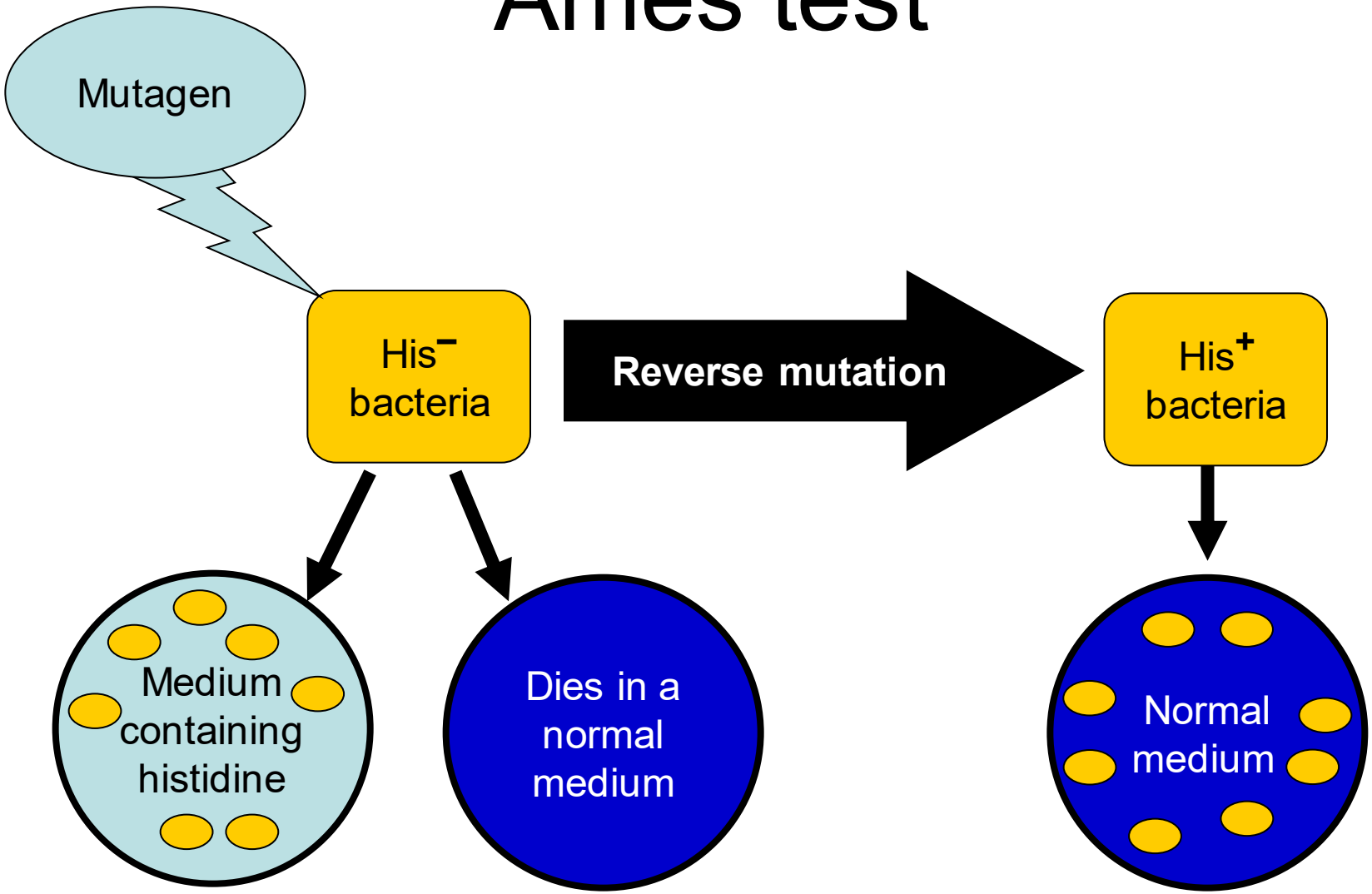




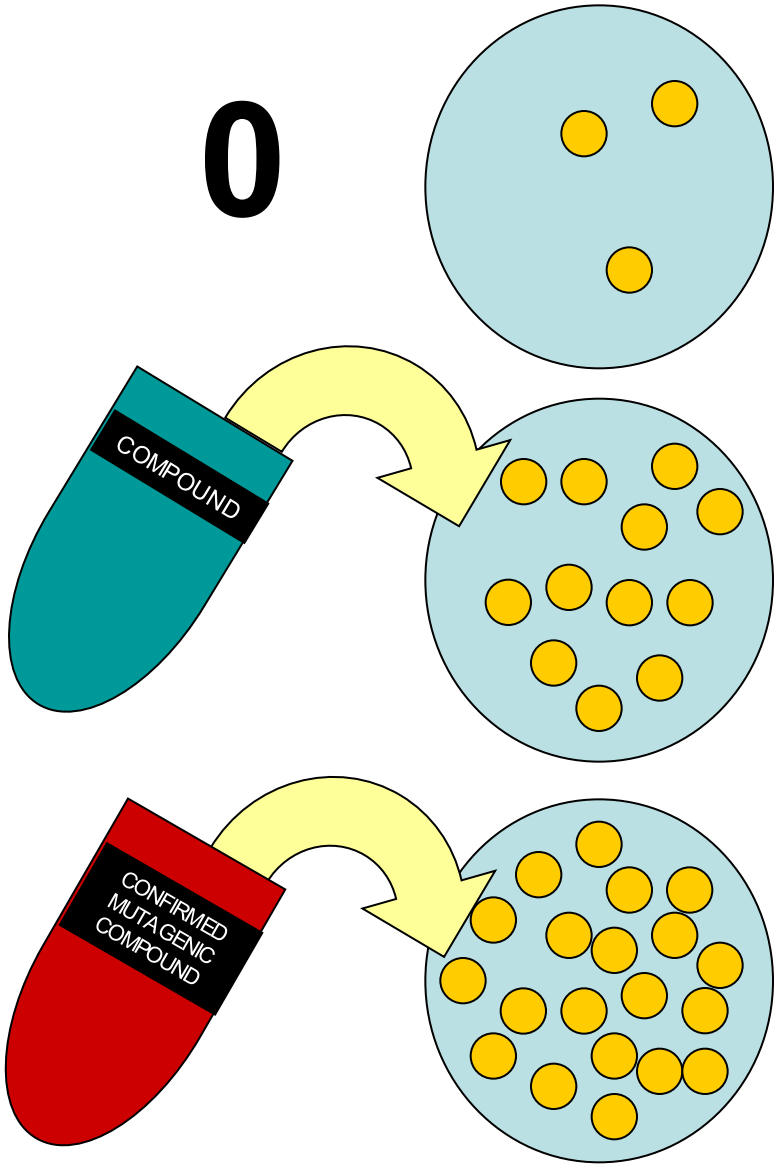
Ames test

- Test system – auxotrophic strain of *Salmonella typhimurium* – survives only in medium with histidine (dies in normal medium without histidine)
- After treatment with mutagen some auxotrophic cells are turned into normal ones that synthesize histidine and survive in a normal medium.
- These cells are called revertants (due to reverse mutation).

Ames test


Mutagen
His⁻ bacteria
Reverse mutation
His⁺ bacteria
Medium containing histidine
Dies in a normal medium
Normal medium

Ames test



Negative control

SPONTANEOUS REVERTANTS

A dish with a compound to be tested

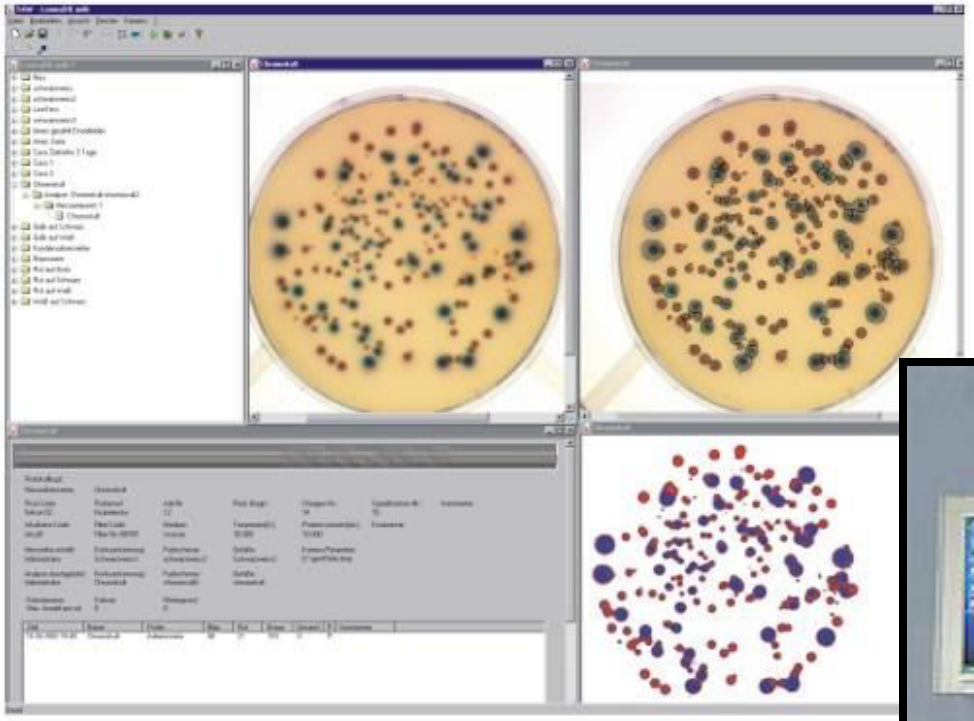
GENOTOXICITY CONFIRMED

Positive control

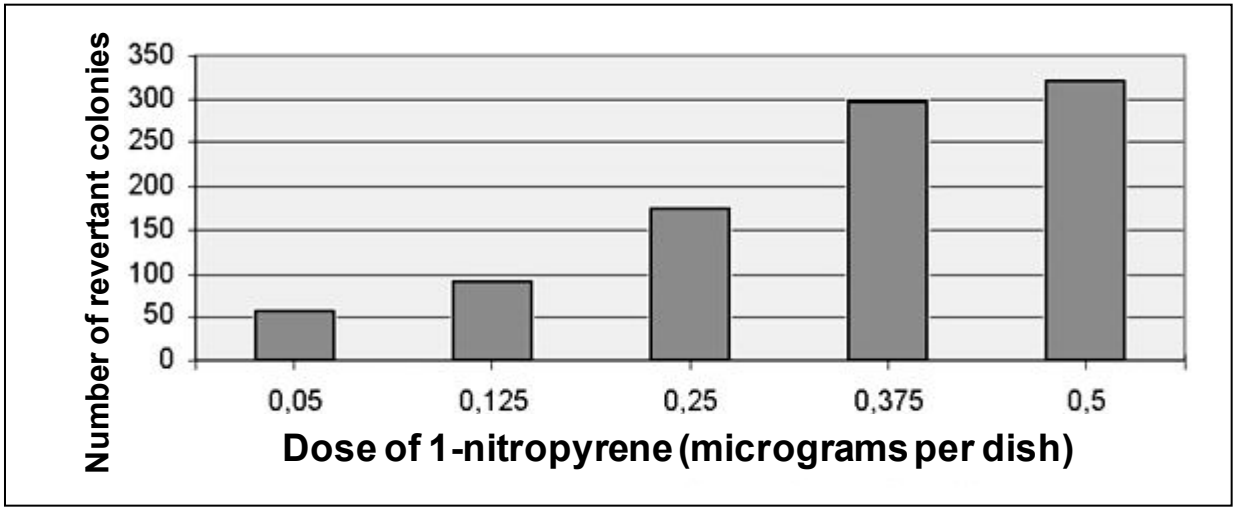
IS USED FOR CONTROL OF THE TEST

Result of the Ames test

Ames test – automatic evaluation of results

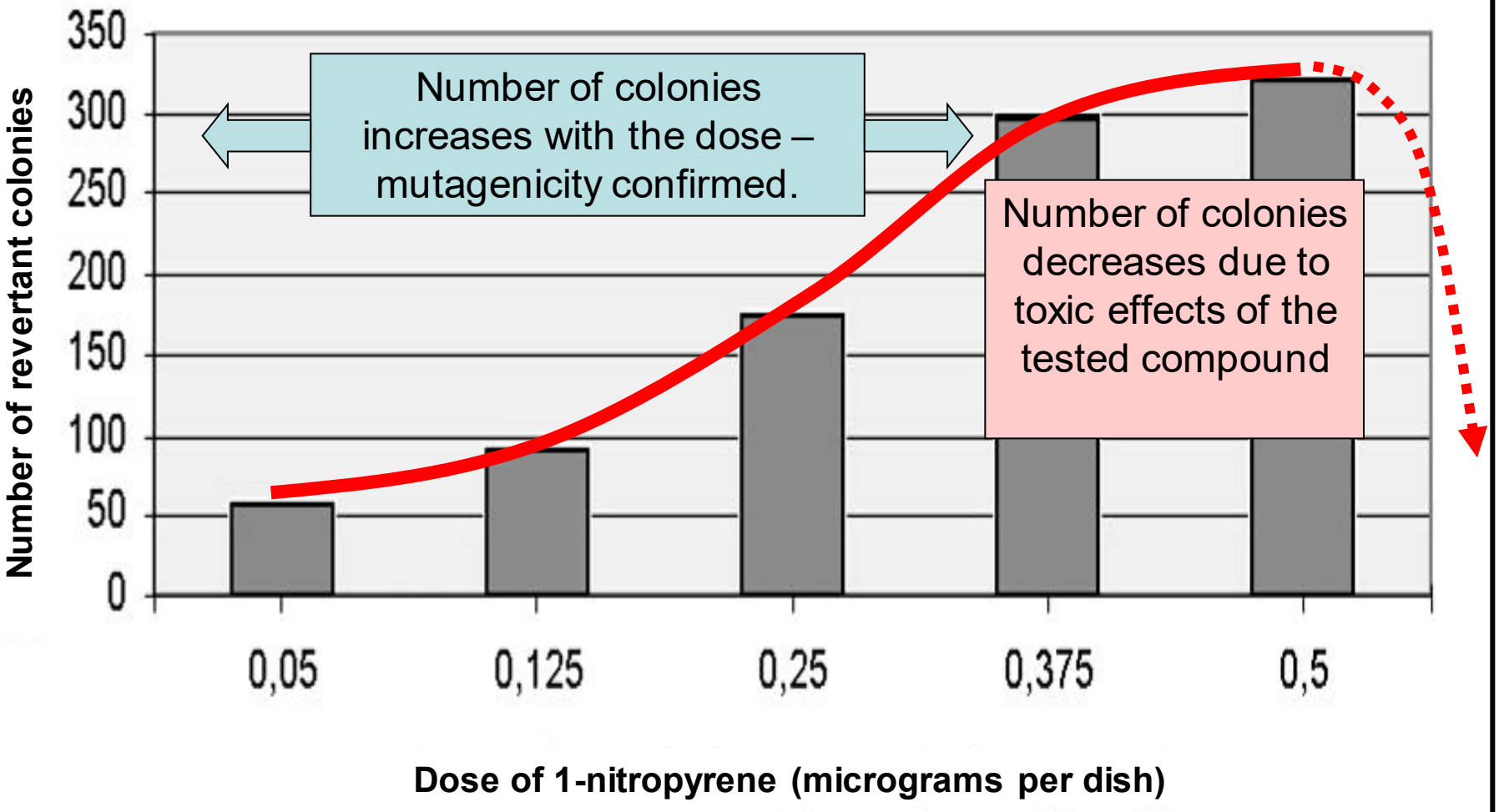


Evaluation of mutagenic activity of 1-nitropyrene by means of the Ames test



- The graph describes results of the Ames test of various 1-nitropyrene doses.
- Describe the relationship between the dose and number of revertant colonies. Is it possible to confirm the mutagenic activity?
- Why we test the compound in various concentrations?

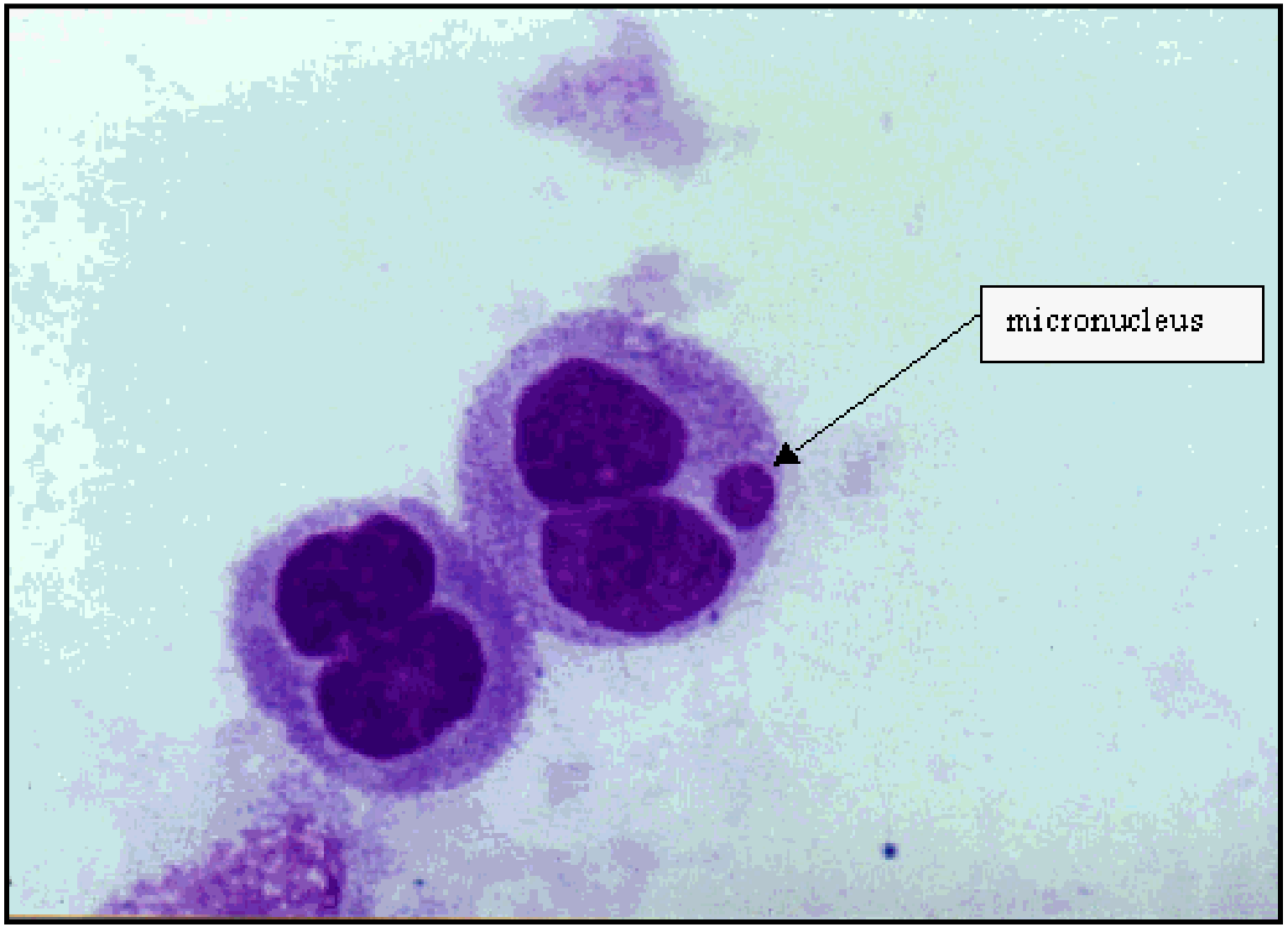
Result of the task



Tests on chromosomal level

- Micronucleus test
  - Micronucleus – a small body in the vicinity of the nucleus (the body usually consists of an acentric chromosomal fragment that originated from deletion)
- Frequency of chromosomal aberrations, mainly breakages
  - Possible in laboratory animals, cultivated mammalian cells and humans (e.g. persons exposed to radiation of other supposed mutagenic factors.
- SCE – sister chromatid exchanges
  - A special cultivation method that allows identification of exchanges between sister chromatids

Result of micronucleus test



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Possible results of chromosomal analysis

Percentage of cells with chromosomal aberrations	Result
Less then 2%	Normal finding, spontaneous level of aberrations
2 – 4 %	A border result – mutagenic effect is neither confirmed nor excluded
More than 4%	Mutagenic effect confirmed with high probability

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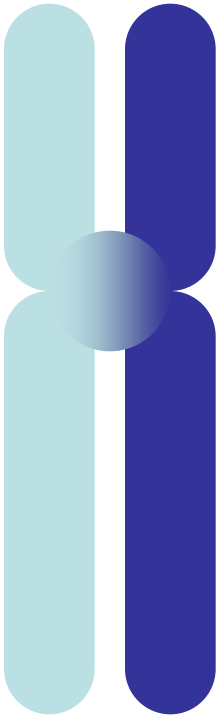
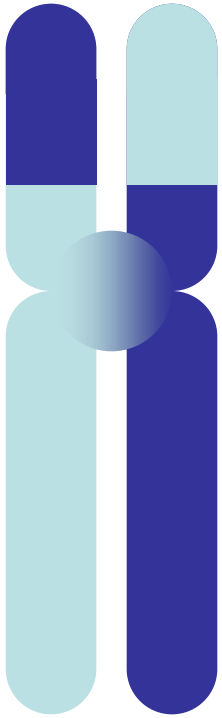
Sister Chromatid Exchanges



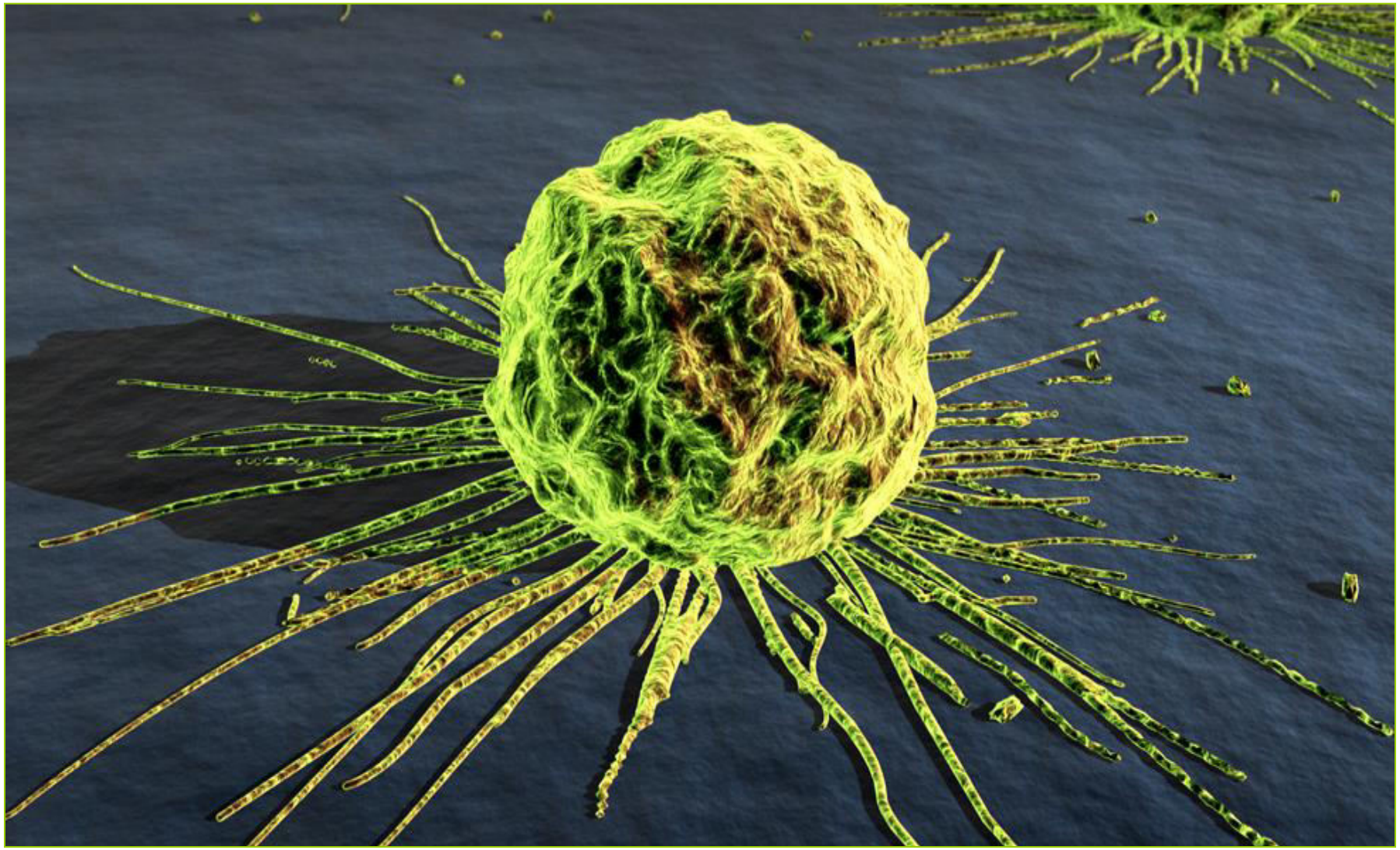
Normal chromosome



A chromosome with SCE

The higher SCE frequency – the higher mutagenic activity.

About the origin of a tumor cell



IARC database

International Agency for Research of Cancer

IARC database
(International Agency for Research of Cancer)

- Direct connection to „Monographs“:

http://monographs.iarc.fr/index.php

IARC classification

- **Group 1:** Compounds (mixtures) carcinogenic to humans
- **Group 2A:** Probable human carcinogens
- **Group 2B:** Possible human carcinogens
- **Group 3:** Carcinogenicity not confirmed
- **Group 4:** Compounds probably not carcinogenic to humans