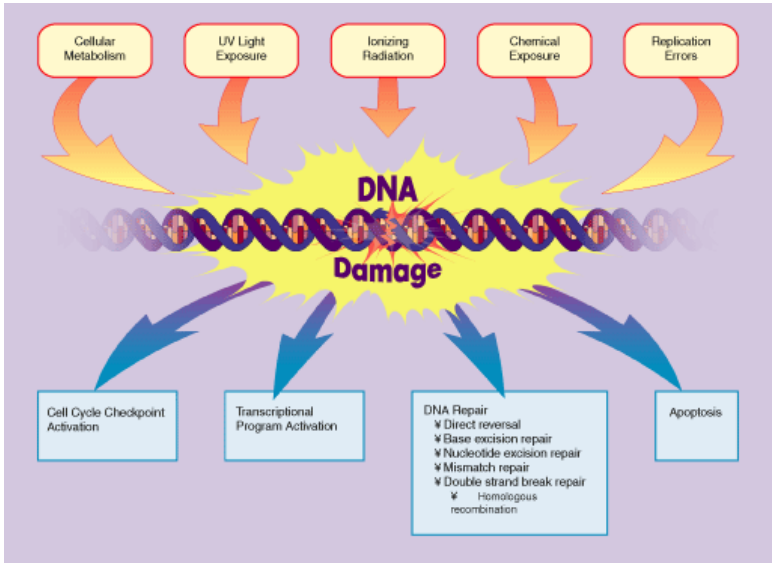
Imperial College London

DNA damage & Repair



Nigel J Gooderham
Biomolecular Medicine

DNA Damage and Repair

Nigel Gooderham

Learning objectives

- Describe how DNA can be damaged by radiation or chemicals (carcinogens) and the role metabolism can play in these reactions.
- Outline in general terms the role of p53 in the detection of, and response to, DNA damage.
- Summarise the natural repair mechanisms for damaged DNA.
- Explain how unrepaired or misrepaired DNA damage can become “fixed” as a mutation.
- Summarise how the potential of a chemical/agent to damage DNA can be assessed.

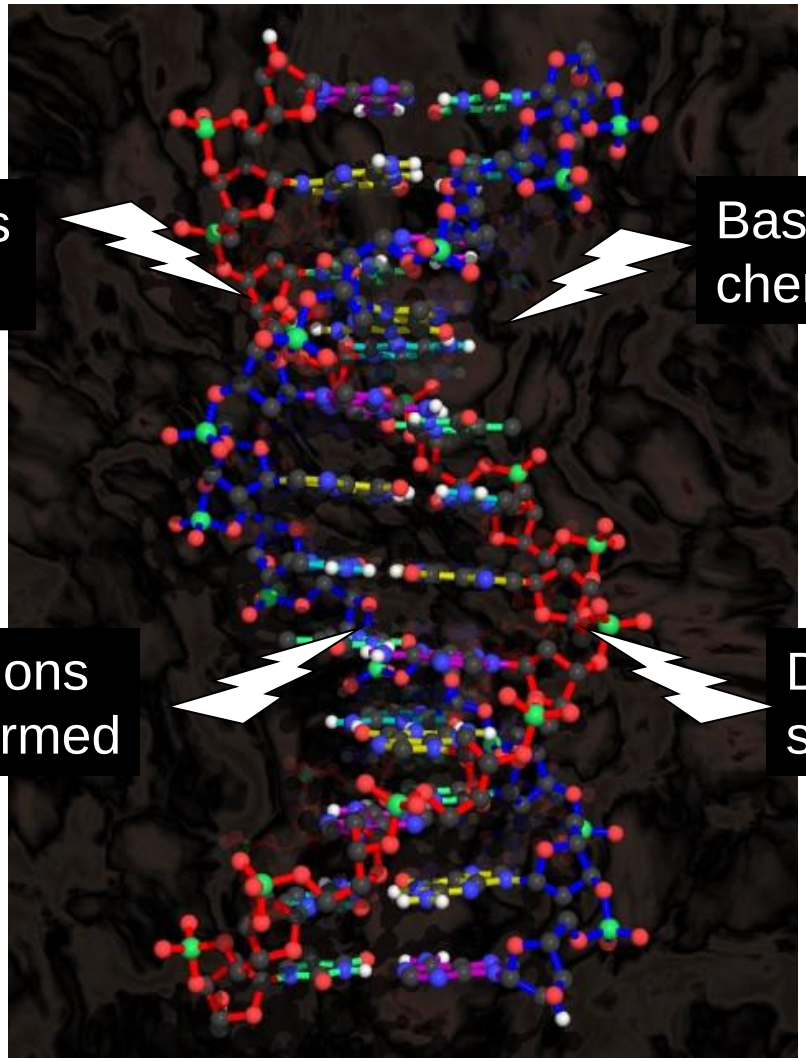
What can damage DNA?

- Chemicals (carcinogens)
  - dietary
  - lifestyle
  - environmental
  - occupational
  - medical
  - endogenous
- Radiation
  - ionizing
  - solar
  - cosmic

Why do we care?

- DNA damage can lead to mutation
- Mutation may lead to cancer

DNA damage by carcinogens



DNA adducts & alkylation

Base dimers & chemical cross-links

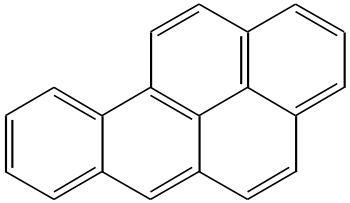
Base hydroxylations & abasic sites formed

Double & single strand breaks

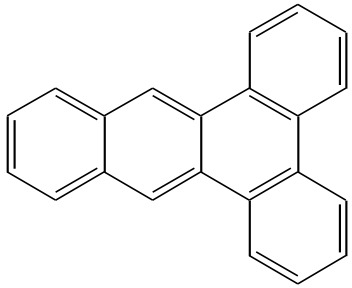
Mammalian metabolism

- Phase I
  - addition of functional groups
    - e.g. oxidations, reductions, hydrolysis
  - mainly cytochrome p450-mediated
- Phase II
  - conjugation of Phase I functional groups
    - e.g. sulphation, glucuronidation, acetylation, methylation, amino acid and glutathione conjugation
  - Generates polar (water soluble) metabolites.

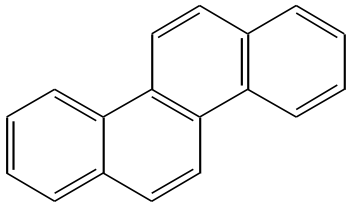
Polycyclic aromatic hydrocarbons



Benzo[a]pyrene

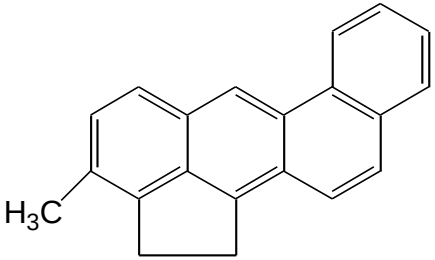


Dibenz[a,c]anthracene

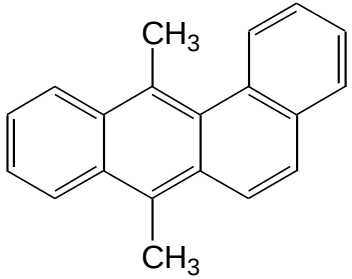


Chrysene

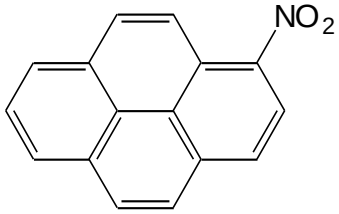
- Common environmental pollutants
- Formed from combustion of fossil fuels
- Formed from combustion of tobacco



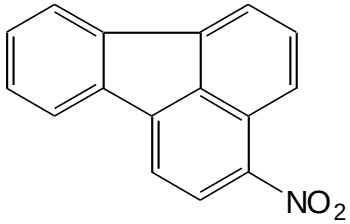
3-Methylcholanthrene



7,12-Dimethylbenz[a]anthracene

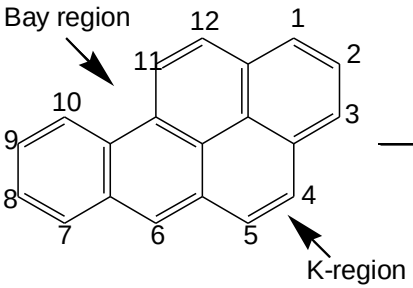


1-Nitropyrene



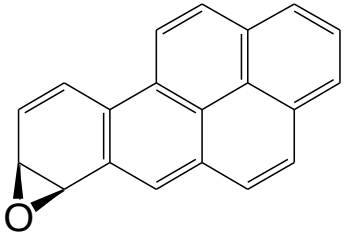
3-Nitrofluoranthene

Two step epoxidation of B[a]P



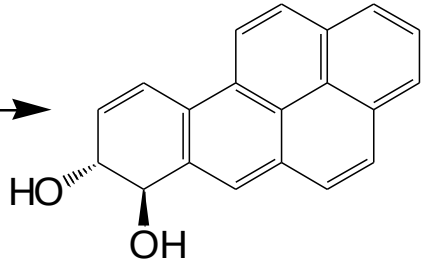
Benzo[a]pyrene

P450



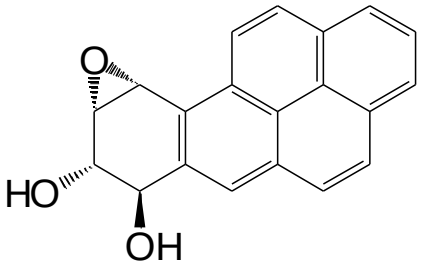
Benzo[a]pyrene-7,8-oxide

EH

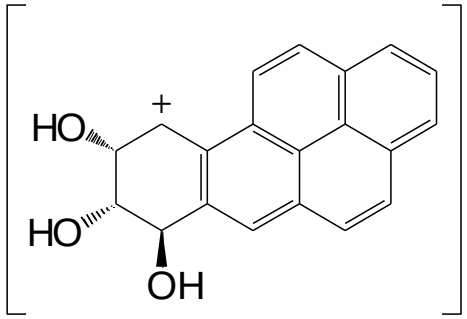


Benzo[a]pyrene-7,8-dihydrodiol

P450

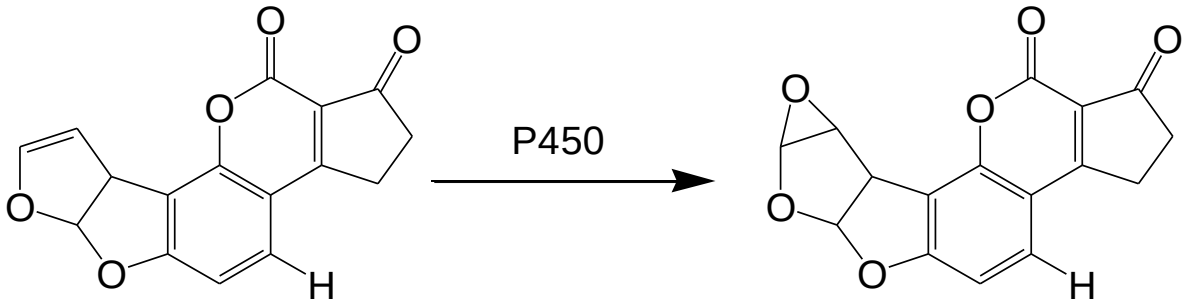


Benzo[a]pyrene-7,8dihydrodiol-9,10-oxide



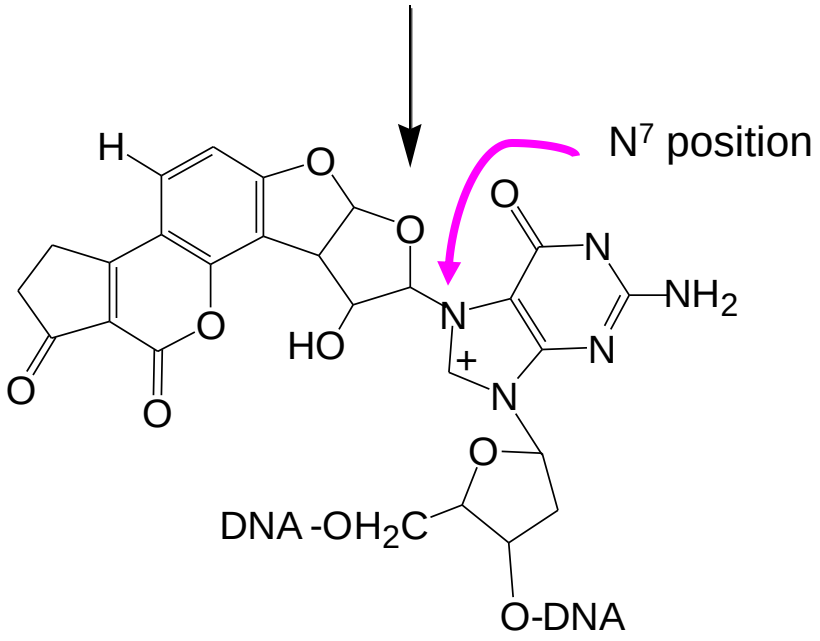
DNA adducts

Epoxidation of aflatoxin B_1



Aflatoxin B_1

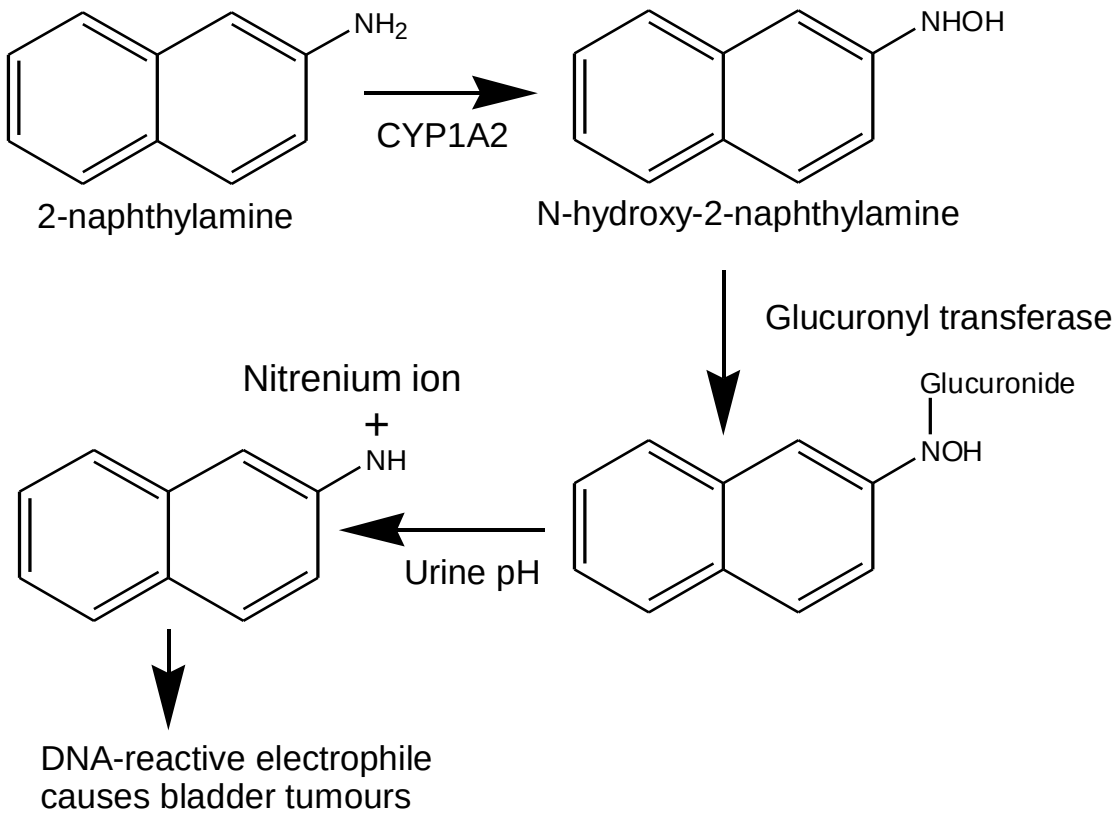
Aflatoxin B_1, 2,3-epoxide



- Formed by *Aspergillus flavus* mould
- Common on poorly stored grains and peanuts
- Aflatoxin B_1 is a potent human liver carcinogen, especially in Africa and Far-East

Metabolism of 2-naphthylamine

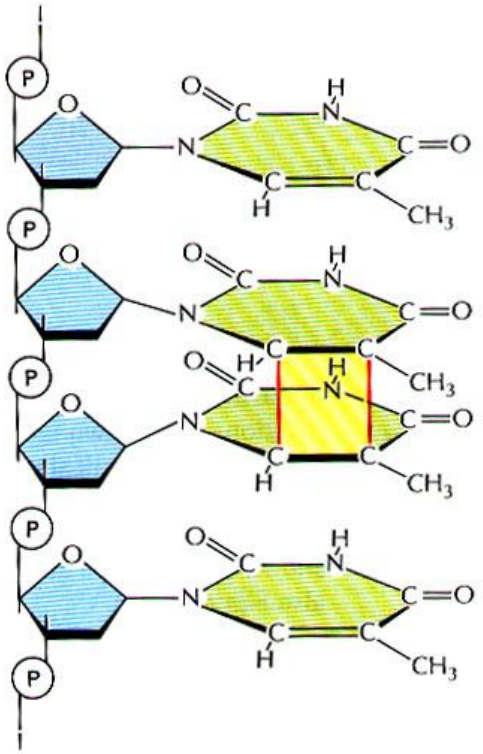
- Past components of dye-stuffs
- Include 2-naphthylamine and benzidine
- Potent human bladder carcinogens
- German dye industry epidemiology (1895 Rehn)



Other carcinogens

Solar (UV) radiation

- Pyrimidine (thymine) dimers
- Skin cancer



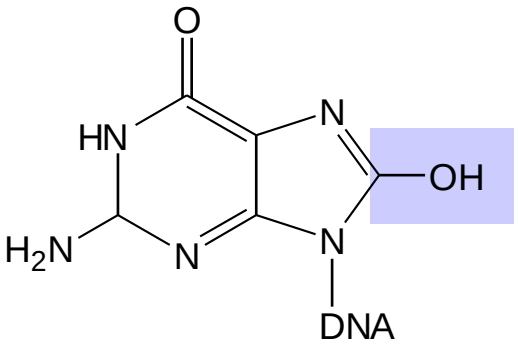
Other carcinogens

Ionising radiation

- Generates free radicals in cells
- Includes oxygen free radicals
  - super oxide radical: $O_2^{\bullet}$
  - hydroxyl radical: $HO^{\bullet}$
- Possess unpaired electrons
  - electrophilic and therefore seek out electron-rich DNA

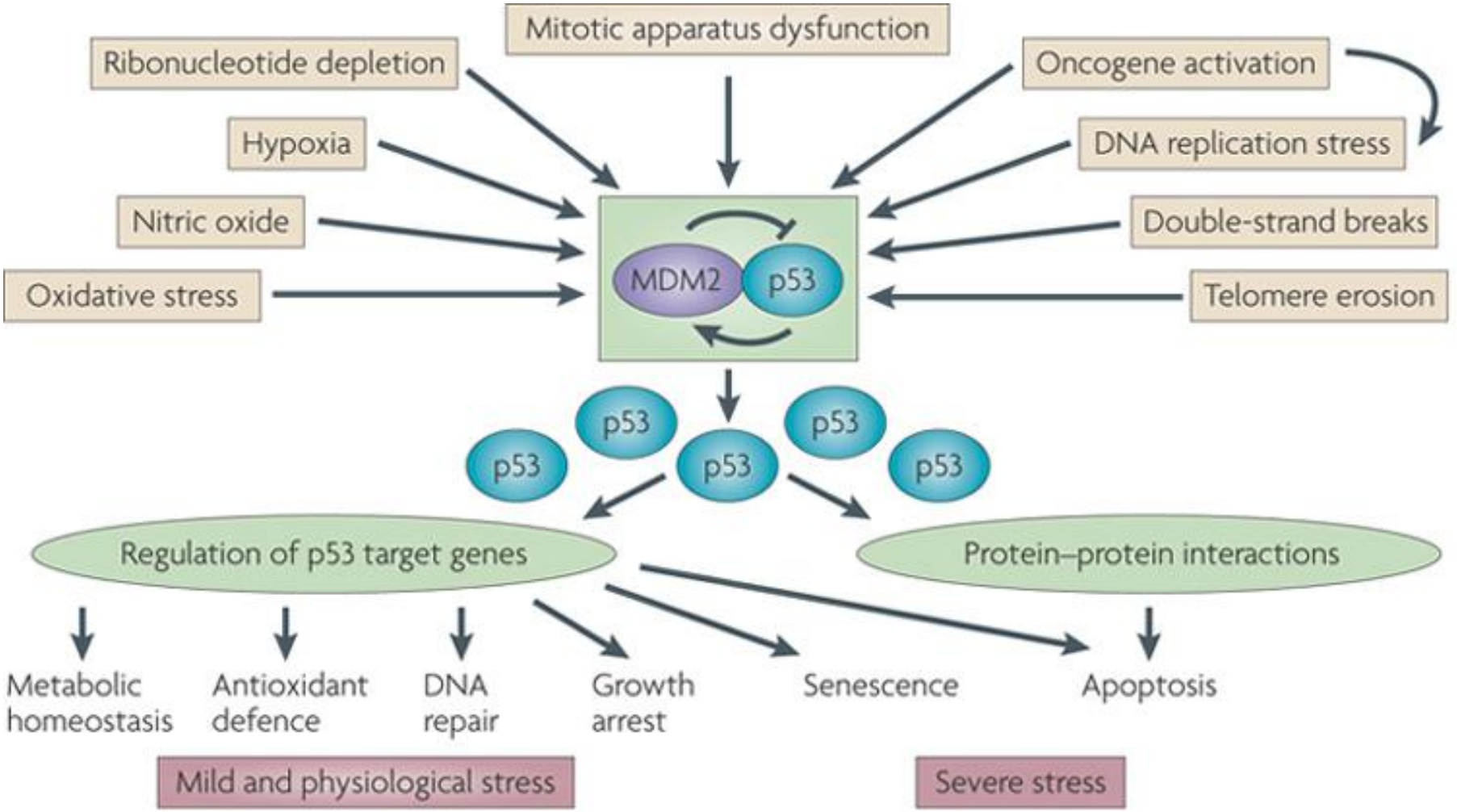
Oxygen free radical attack on DNA

- Double and single strand breaks
- Apurinic & apyrimidinic sites
- Base modifications
  - ring-opened guanine & adenine
  - thymine & cytosine glycols
  - 8-hydroxyadenine & 8-hydroxyguanine (mutagenic)



8-hydroxy guanine

Role of p53 in dealing with cellular stress



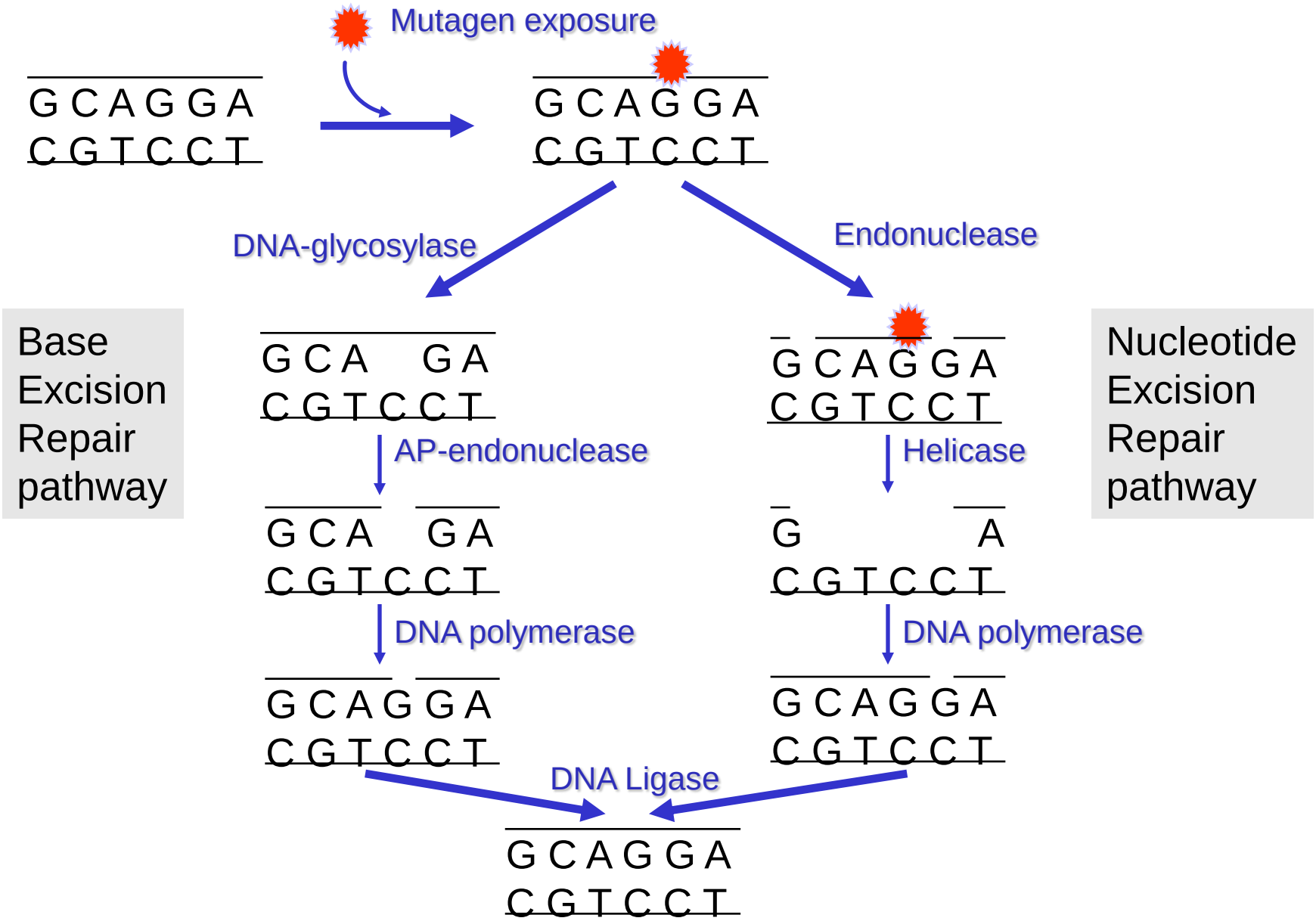
Nature Reviews | Cancer

Repair is a key event

Types of DNA repair

- **Direct reversal of DNA damage**
  - photolyase splits cyclobutane pyrimidine-dimers
  - methyltransferases & alkyltransferases remove alkyl groups from bases
- **Base excision repair** (mainly for apurinic/apyridinic damage)
  - DNA glycosylases & apurinic/apyrimidinic endonucleases + other enzyme partners
  - A repair polymerase (e.g. Polβ) fills the gap and DNA ligase completes the repair.
- **Nucleotide excision repair** (mainly for bulky DNA adducts)
  - Xeroderma pigmentosum proteins (XP proteins) assemble at the damage. A stretch of nucleotides either side of the damage are excised.
  - Repair polymerases (e.g. Polδ/β) fill the gap and DNA ligase completes the repair.
- **During- or post-replication repair**
  - mismatch repair
  - recombinational repair

Excision repair of DNA damage

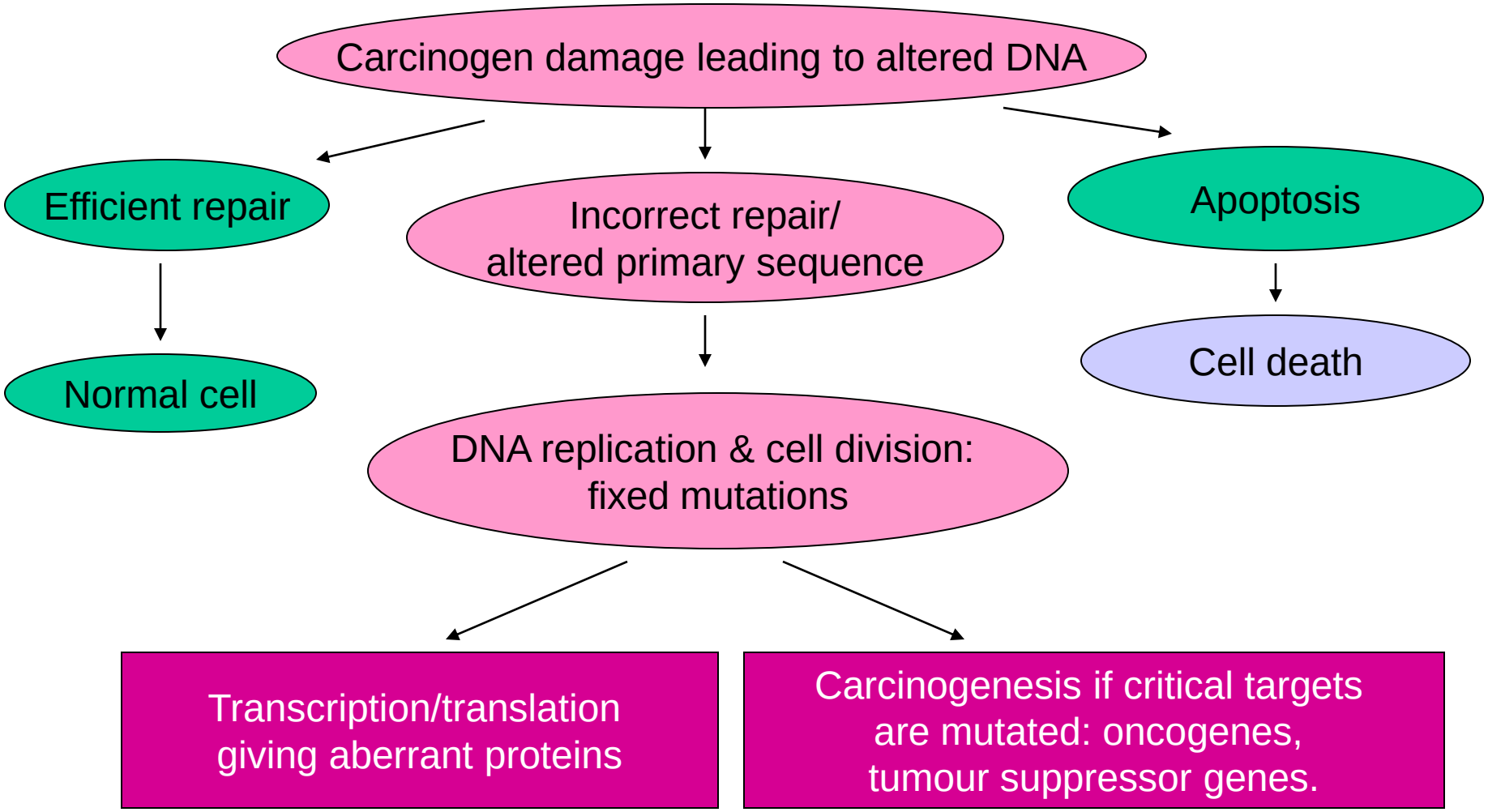


Estimated rates of endogenous damage and repair

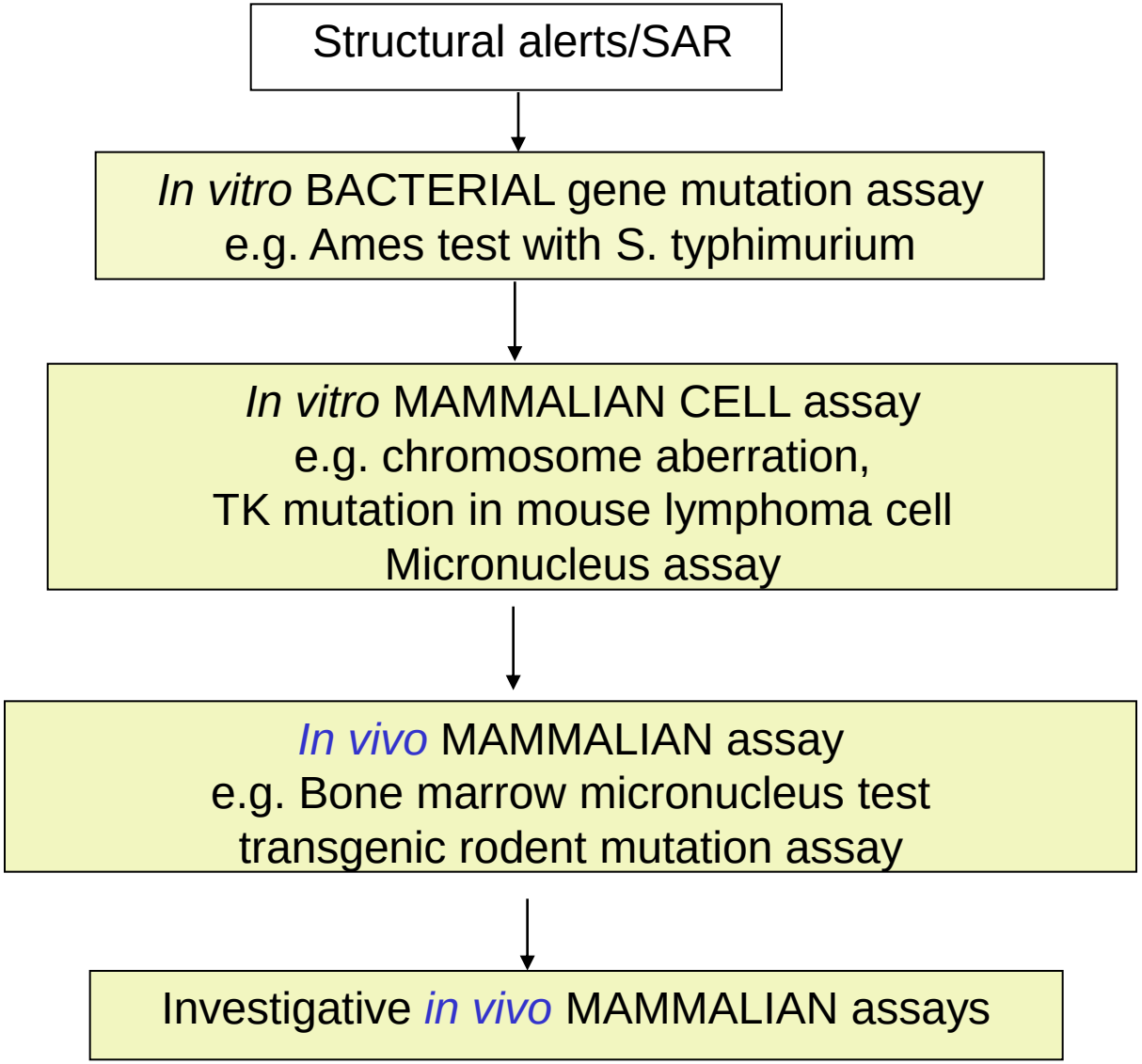
Type of damage	Damage per hour per cell	Max repair rate: BP/hour per cell
Depurination	1000	10,000
Depyrimidination	55	10,000
Single-strand breaks	5000	200,000
Alkylation (O^6-methylguanine)	130	10,000
Free radical base oxidations	120	100,000

The greater the persistence of damage then the greater the chance of a mutagenic event

Fate of carcinogen-DNA damage

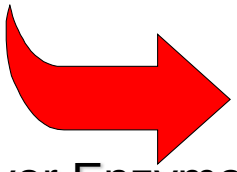


Testing for DNA damage

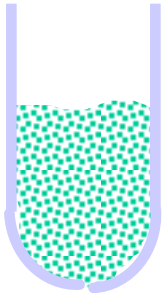


Bacterial (Ames) test for mutagenicity of chemicals

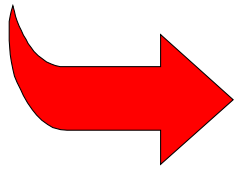
Chemical to be tested



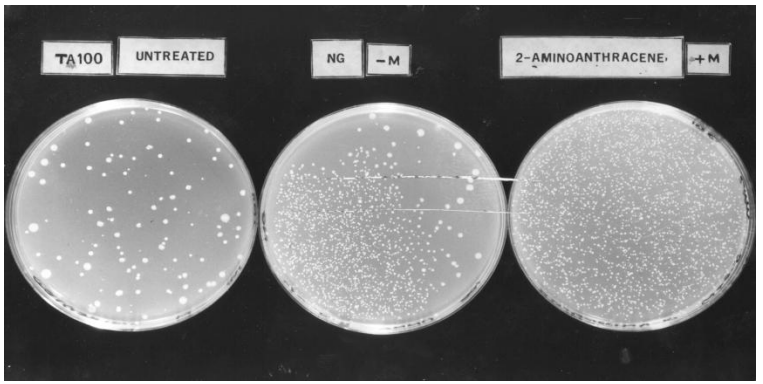
+ rat liver Enzyme preparation (S9)



Bacteria that do not synthesise histidine e.g. *Salmonella* strain



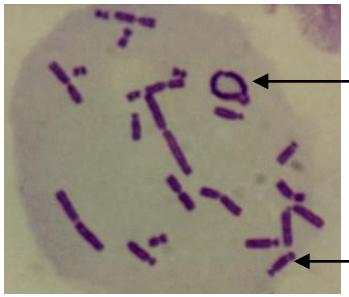
Conversion of chemical to reactive metabolite?

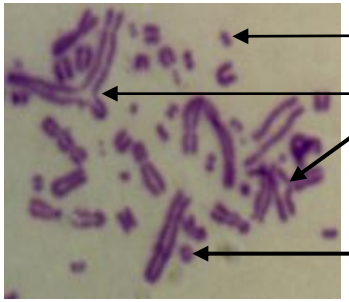


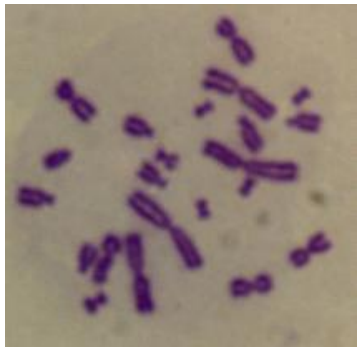
On histidine-free media: if mutations occur in bacterial genome then bacteria acquire ability to synthesise histidine = colonies

Detecting DNA damage in mammalian cells
Chromosomal abberrations

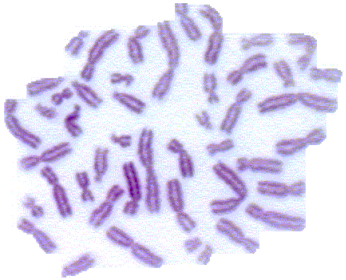
Treat mammalian cells with chemical in presence of liver S9. Look for chromosomal damage



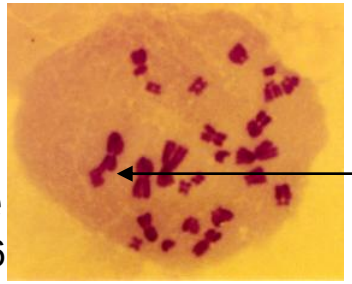




CHO Cell
Normal karyotype, 2n = 22

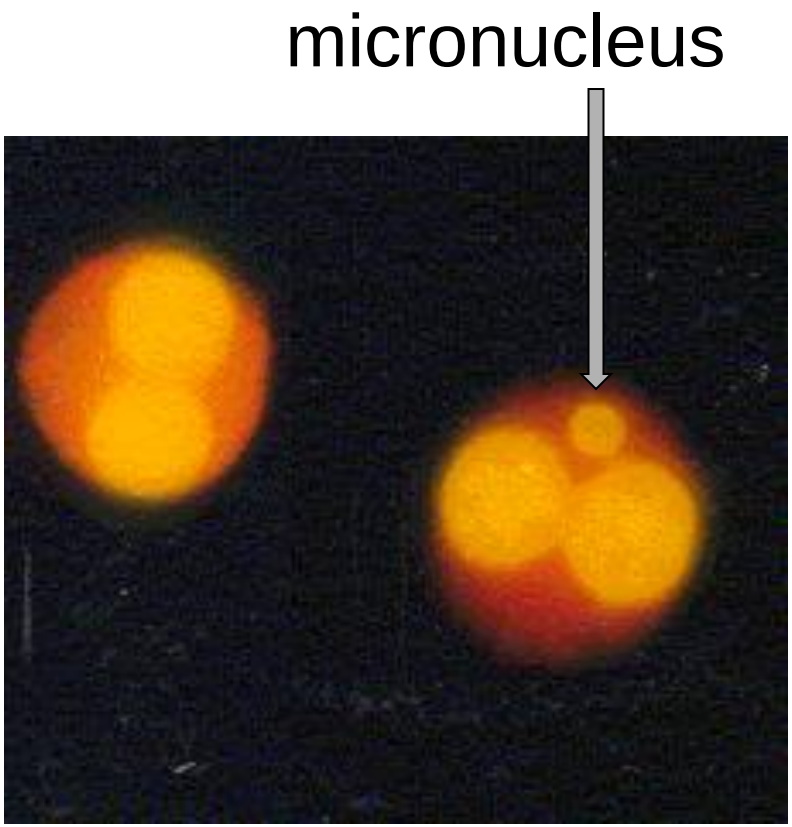


Human Lymphocyte
Normal karyotype 2n = 46



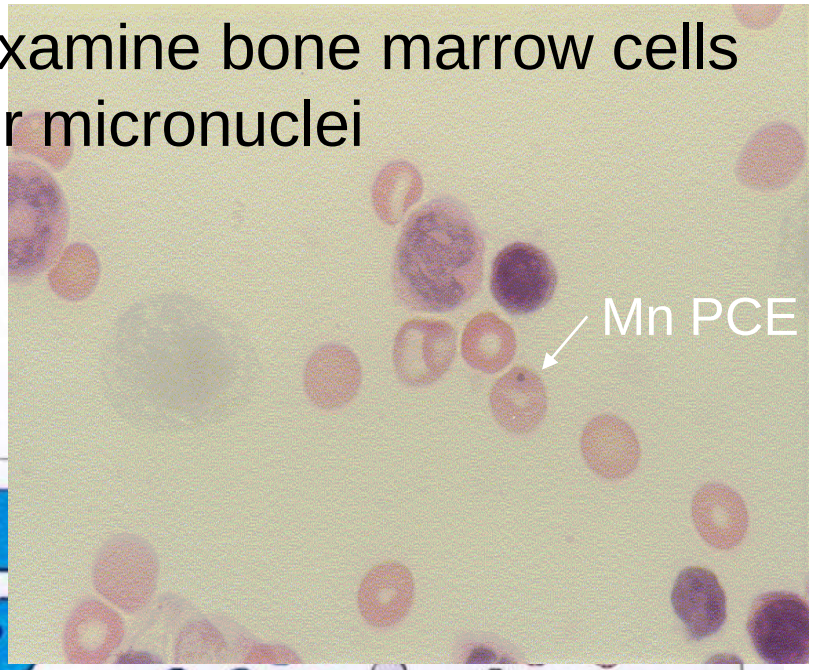
In vitro micronucleus assay

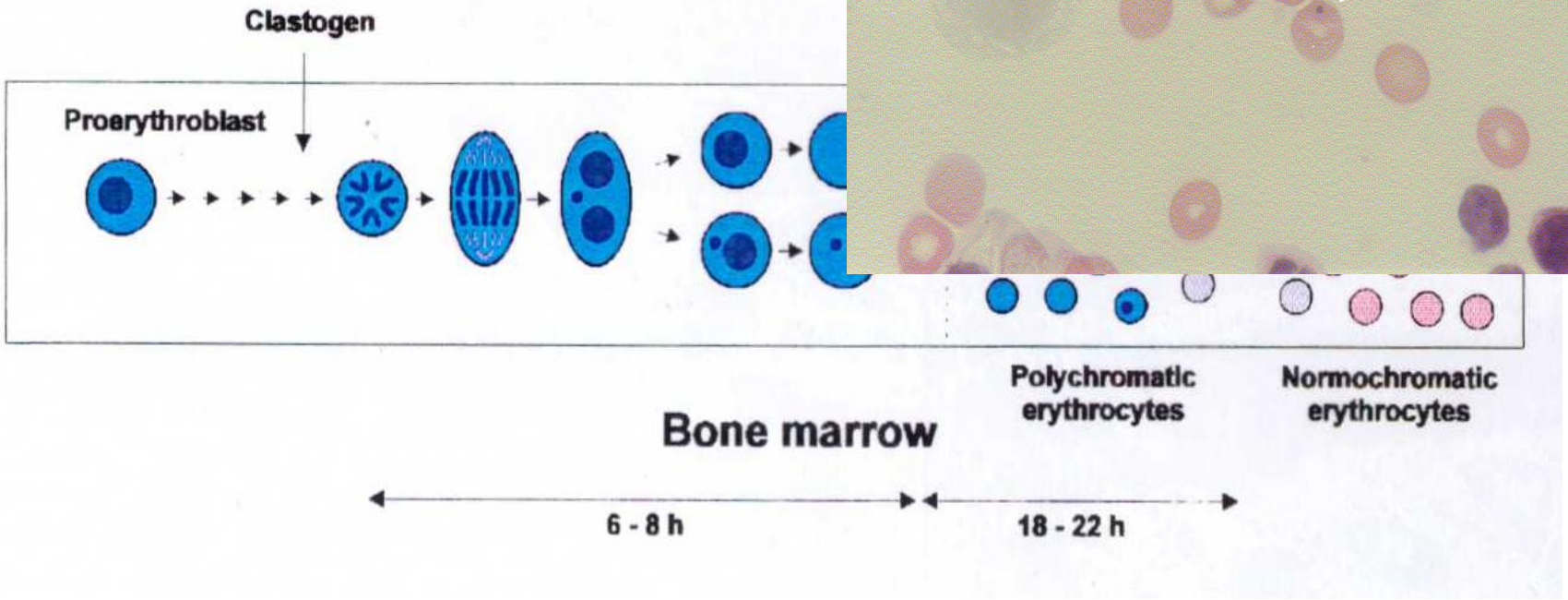
- Cells treated with chemical and allowed to divide
- Cytokinesis blocked using cytochalasin-B
- Binucleate cells assessed for presence of micronuclei
- Can stain the kinetochore proteins to determine if chemical treatment caused clastgenicity (chromosomal breakage) or aneuploidy (chromosomal loss)



Bone marrow micronucleus assay in mice or rats

Treat animals with chemical and examine bone marrow cells or peripheral blood erythrocytes for micronuclei





Summary

- Chemicals and radiation can damage DNA.
- Chemicals often require metabolic activation (e.g. by cytochrome P450) before they are able to damage DNA.
- Radiation induces pyrimidine dimers, strand breaks, abasic sites and modified bases in DNA.
- Damaged DNA can be repaired by direct reversal or excision of damaged bases or nucleotides.
- Incorrect repair can lead to mutation and possibly neoplasia.
- Detecting the ability of agents (chemicals and radiation) to damage DNA is essential.

Reading material

- The Cancer handbook 2nd Ed (*MR Alison, 2007, Wiley pub*).
- Cells *(B. Lewin et al. 2007, Pub Jones and Bartlett)*
- Review articles in journals *Cell* and *Cancer Research.*