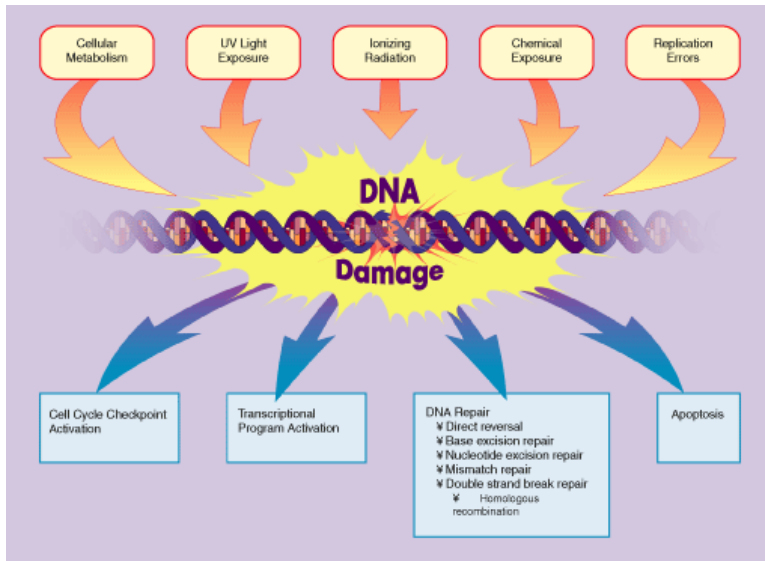


# DNA damage & Repair



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.

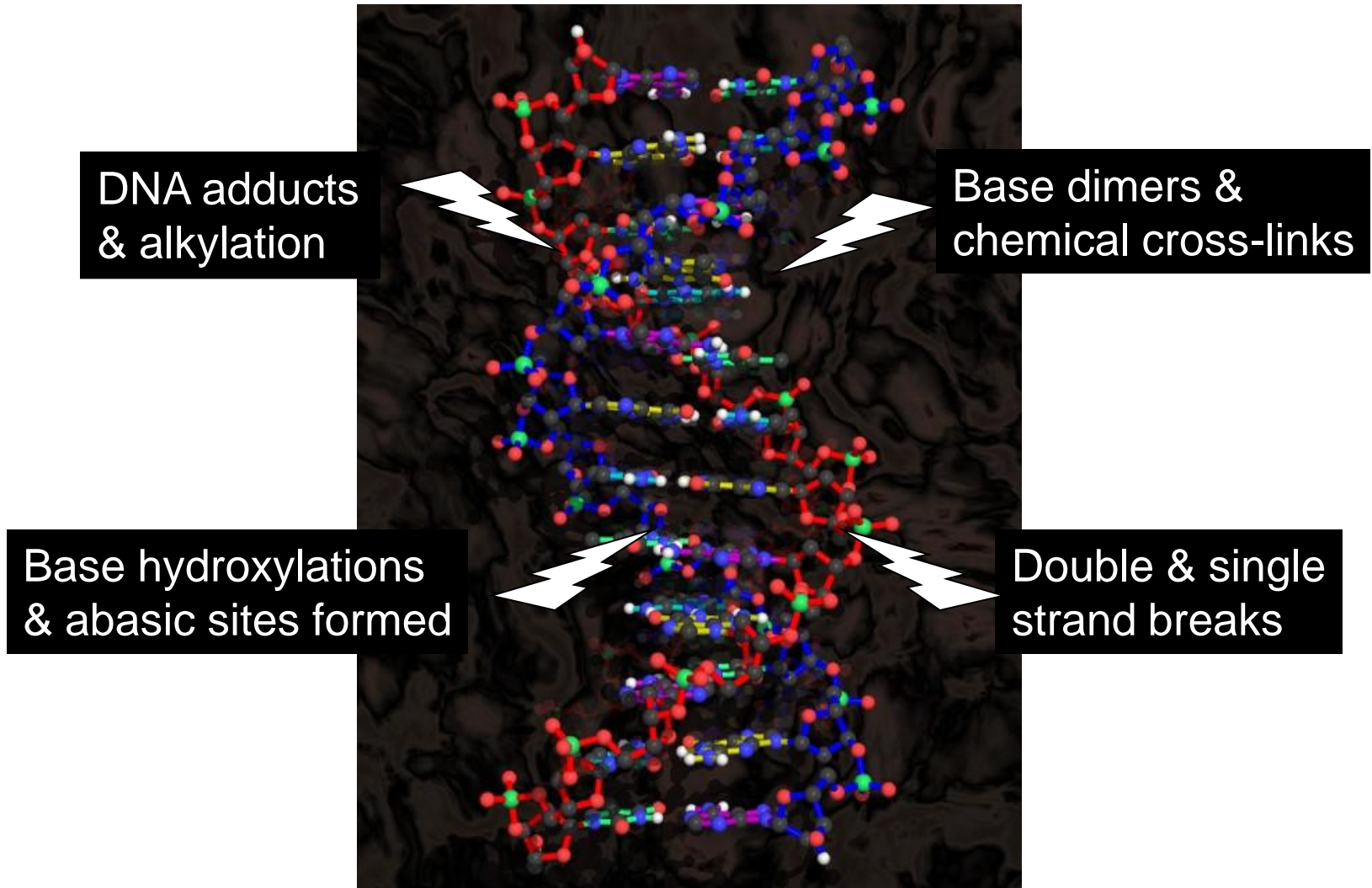
# What can damage DNA?

- 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

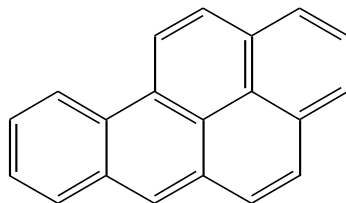
# DNA damage by carcinogens



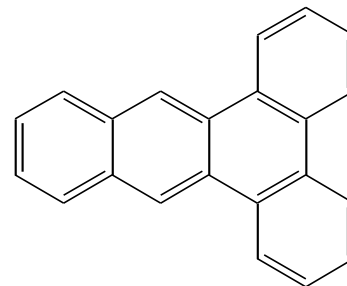
# Mammalian metabolism

- 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.

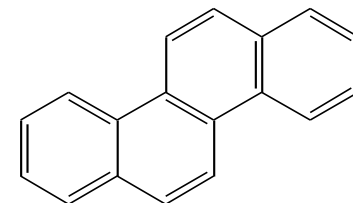
# Polycyclic aromatic hydrocarbons



Benzo[a]pyrene

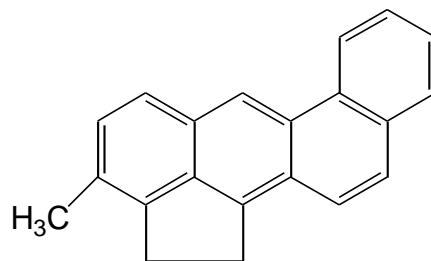


Dibenz[a,c]anthracene

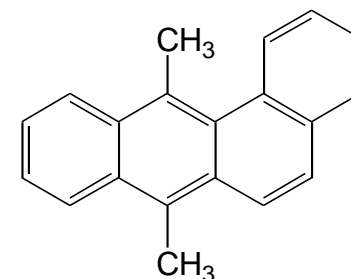


Chrysene

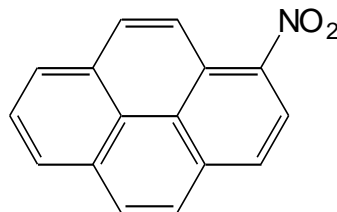
- Common environmental pollutants
- Formed from combustion of fossil fuels
- Formed from combustion of tobacco



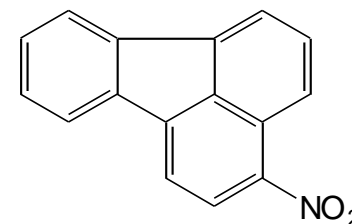
3-Methylcholanthrene



7,12-Dimethylbenz[a]anthracene

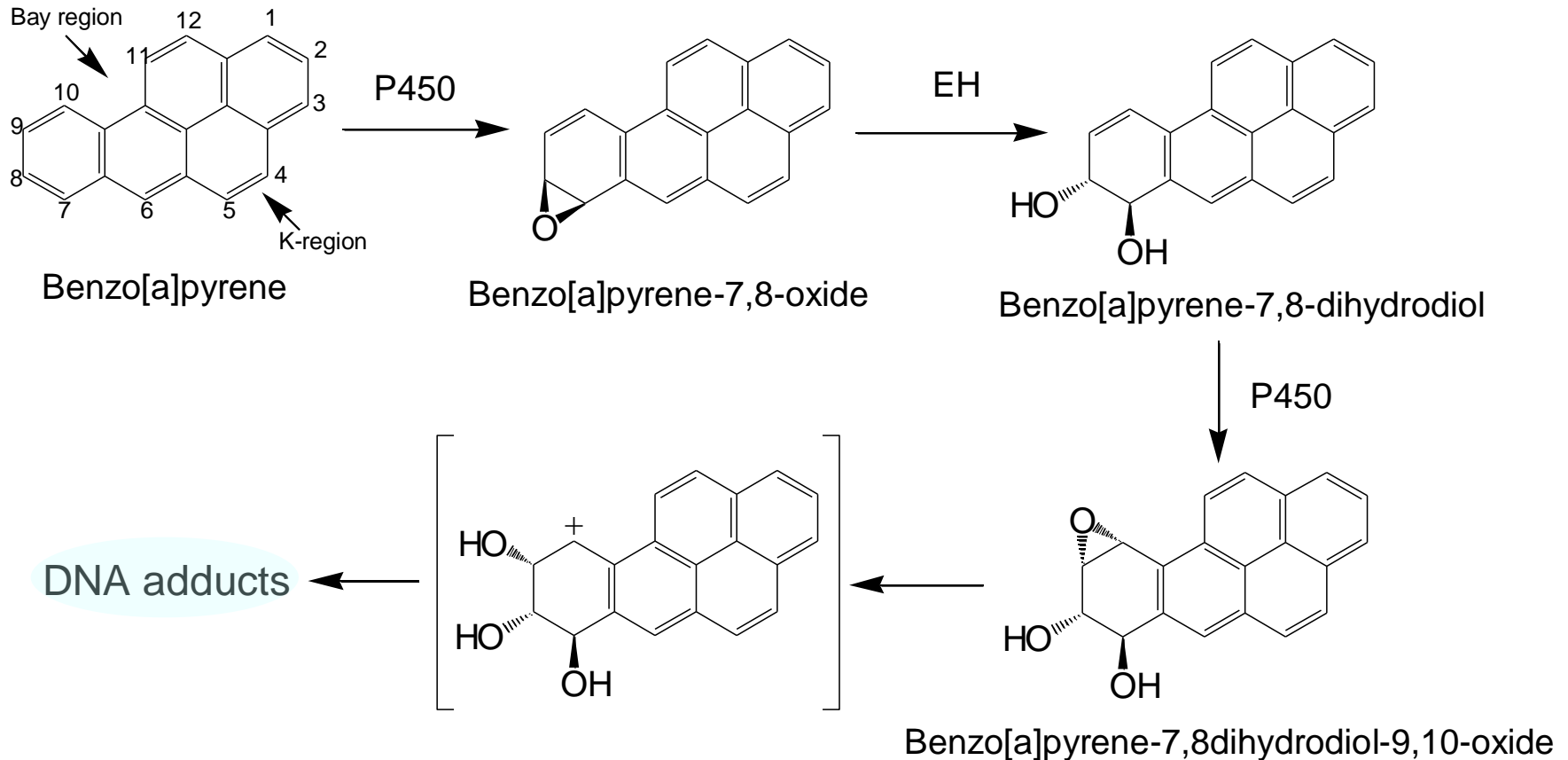


1-Nitropyrene

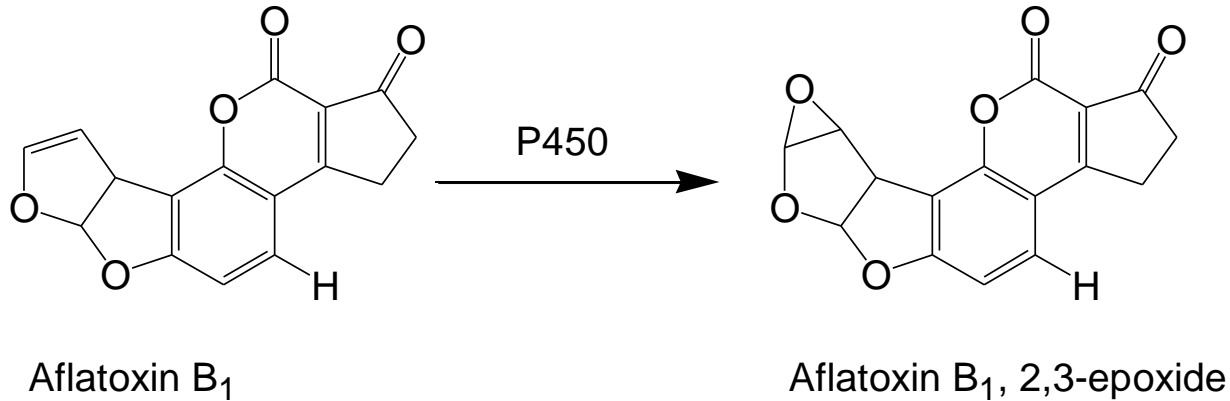


3-Nitrofluoranthene

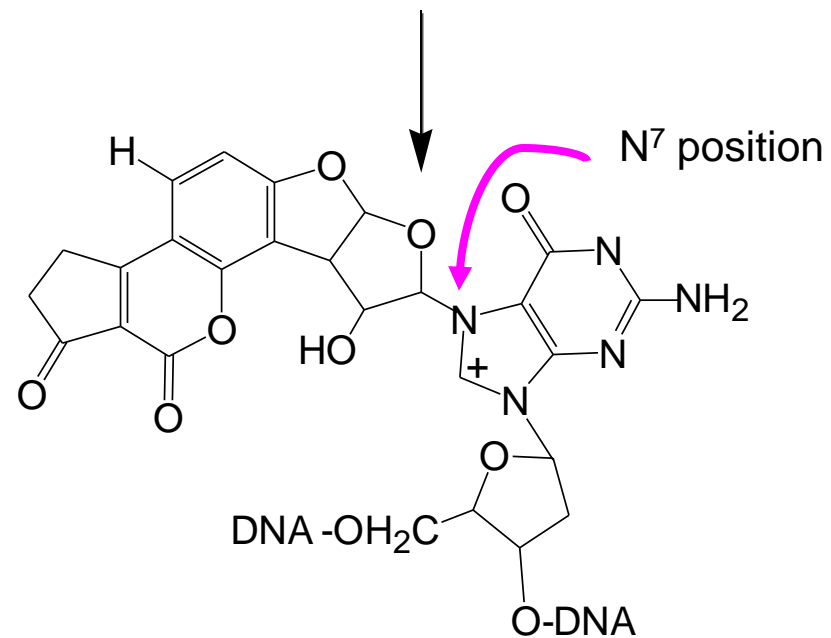
# Two step epoxidation of B[a]P



# Epoxidation of aflatoxin B<sub>1</sub>



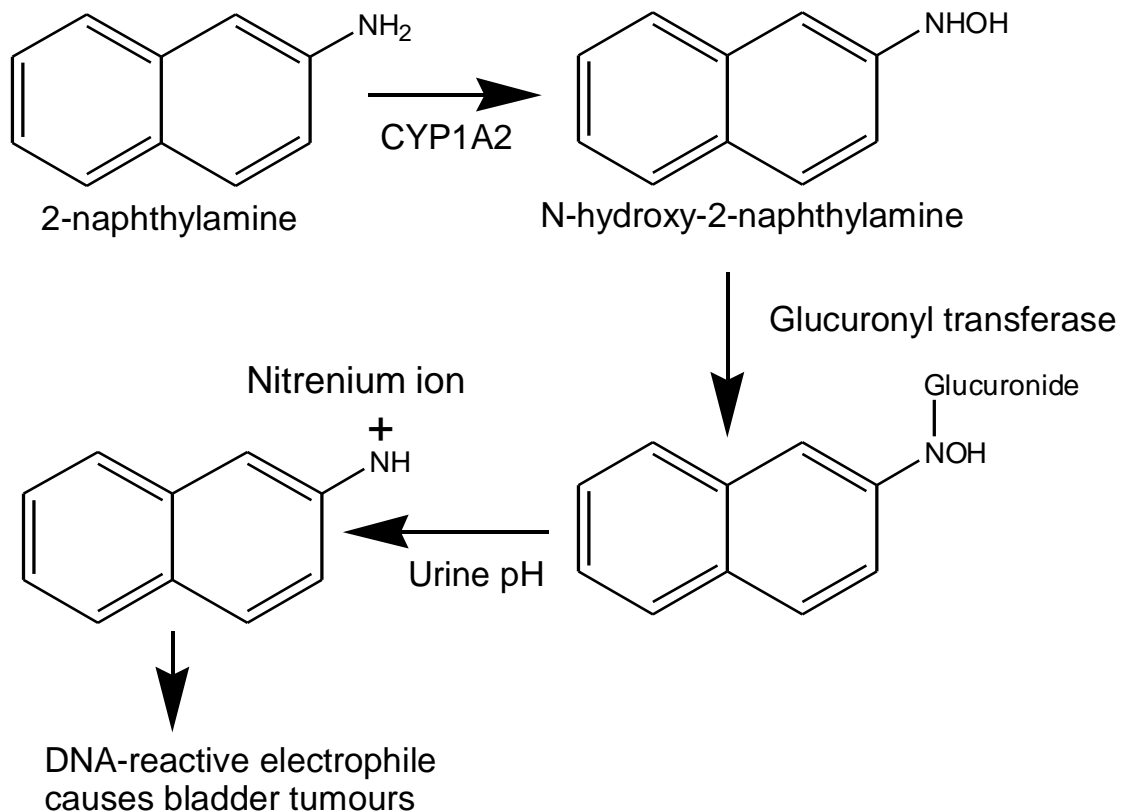
- Formed by *Aspergillus flavus* mould
- Common on poorly stored grains and peanuts
- Aflatoxin B<sub>1</sub> is a potent human liver carcinogen, especially in Africa and Far-East





# Metabolism of 2-naphthylamine

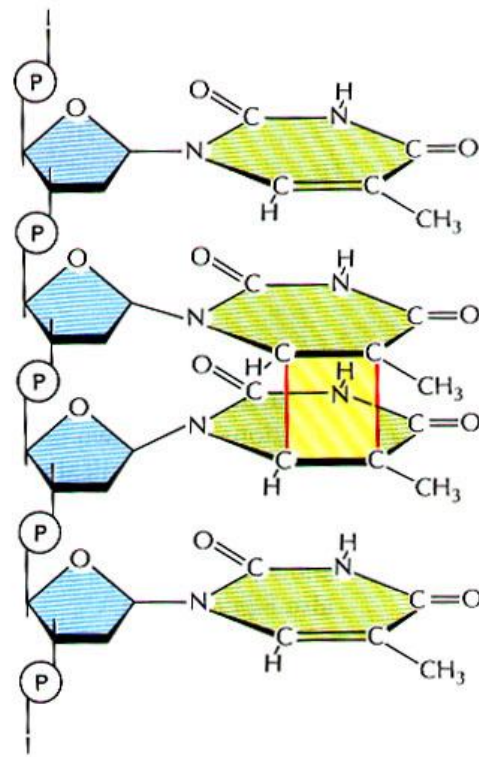
- Past components of dye-stuffs
- Include 2-naphthylamine and benzidine
- Potent human bladder carcinogens
- German dye industry epidemiology (1895 Rehn)



# Other carcinogens

## Solar (UV) radiation

- Pyrimidine (thymine) dimers
- Skin cancer



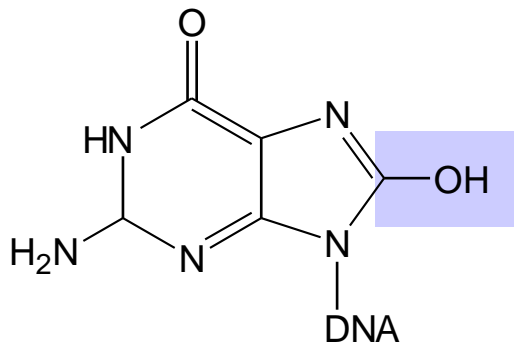
# Other carcinogens

## 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

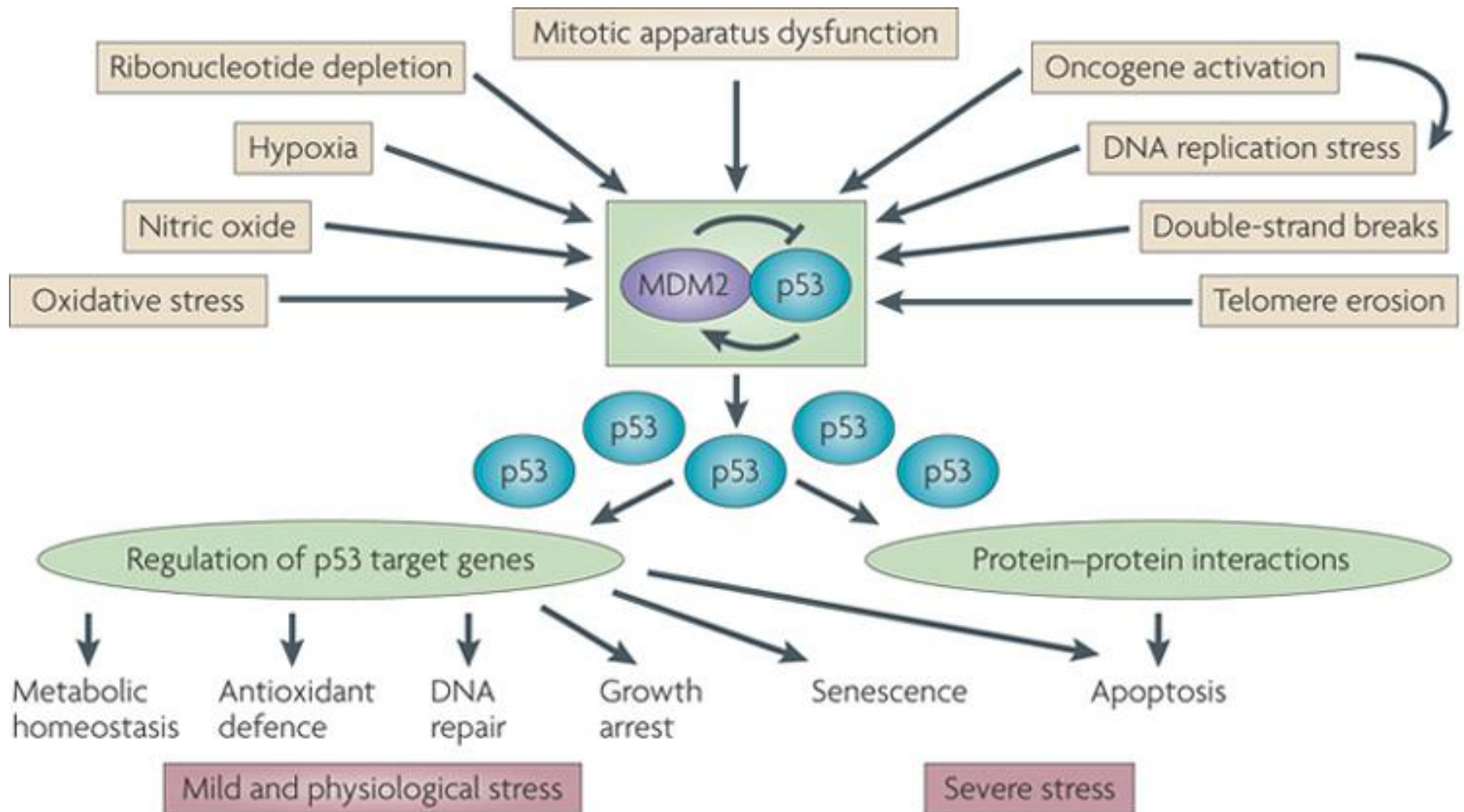
# Oxygen free radical attack on DNA

- 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



# 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. Pol $\beta$ ) 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. Pol $\delta/\beta$ ) fill the gap and DNA ligase completes the repair.
- **During- or post-replication repair**
  - mismatch repair
  - recombinational repair

# Excision repair of DNA damage

Mutagen exposure

G C A G G A  
C G T C C T



G C A G G A  
C G T C C T

DNA-glycosylase

Endonuclease

Base  
Excision  
Repair  
pathway

G C A GA  
C G T C C T

AP-endonuclease

G C A GA  
C G T C C T

DNA polymerase

G C A G G A  
C G T C C T

G C A G G A  
C G T C C T

Helicase

G A  
C G T C C T

DNA polymerase

G C A G G A  
C G T C C T

Nucleotide  
Excision  
Repair  
pathway

DNA Ligase

G C A G G A  
C G T C C T

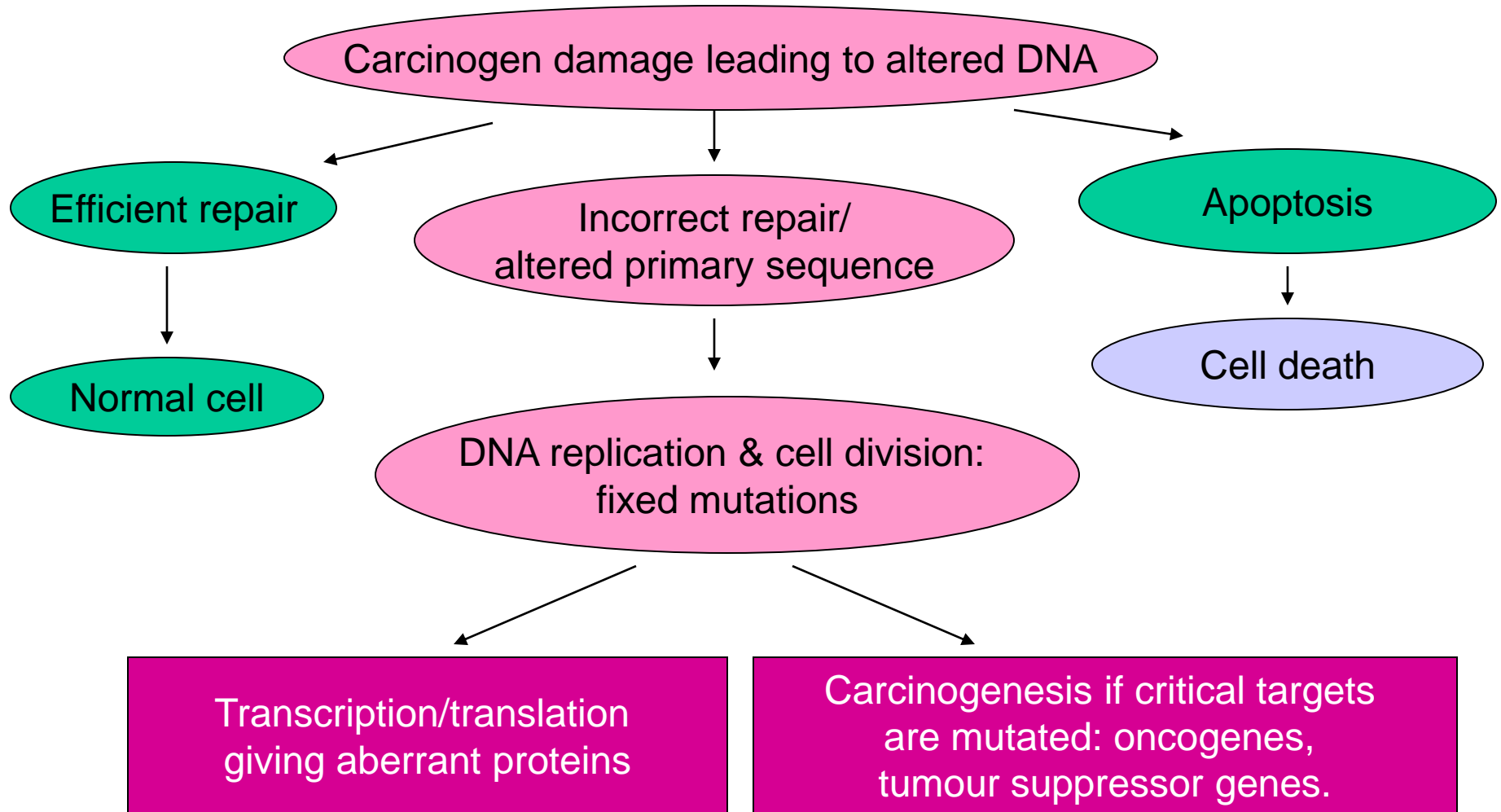
## Estimated rates of endogenous damage and repair

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 <sup>6</sup> -methylguanine)	130	10,000
Free radical base oxidations	120	100,000

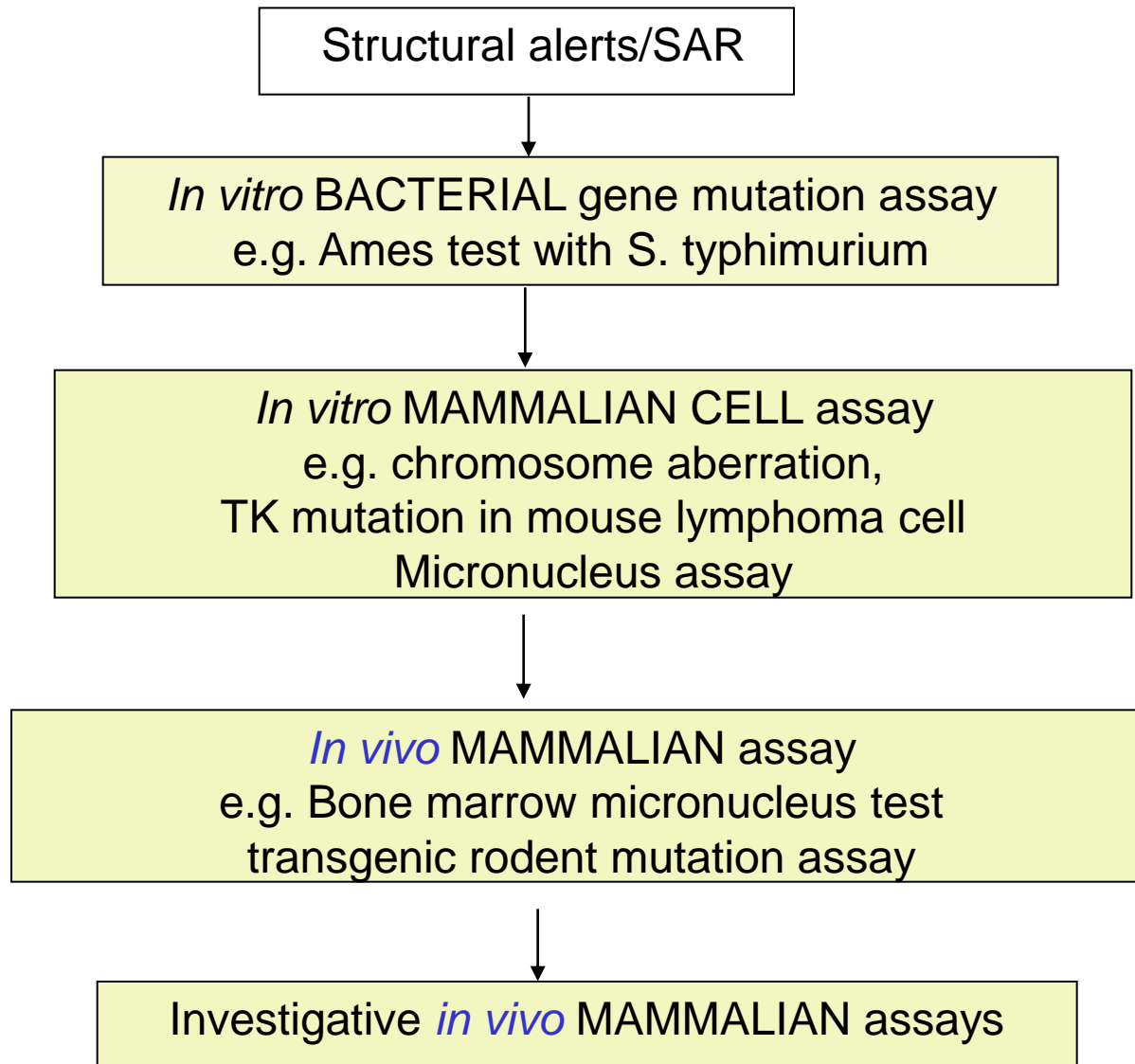
The greater the persistence of damage then the greater the chance of a mutagenic event



# Fate of carcinogen-DNA damage



# Testing for DNA damage

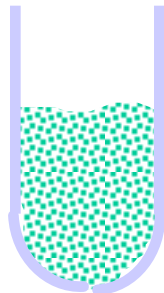
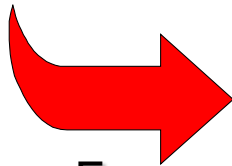


# Bacterial (Ames) test for mutagenicity of chemicals

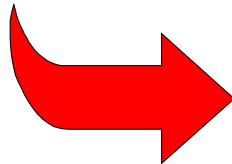
Chemical to be tested



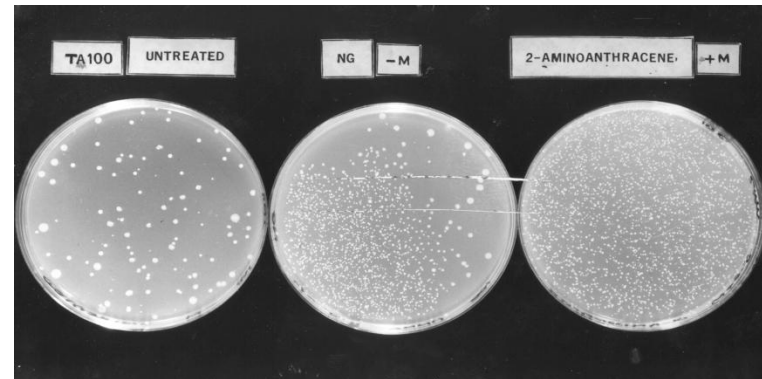
+ rat liver Enzyme preparation (S9)



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

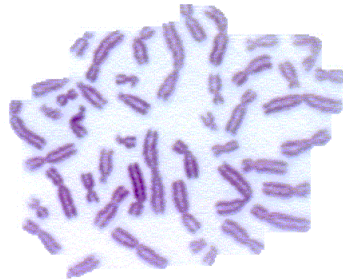
# Detecting DNA damage in mammalian cells

## Chromosomal aberrations

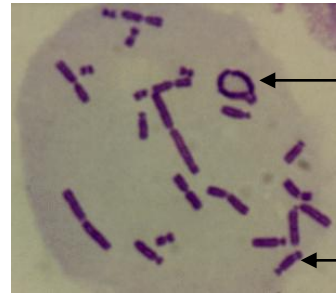
Treat mammalian cells with chemical in presence of liver S9. Look for chromosomal damage



CHO Cell  
Normal karyotype,  $2n = 22$



Human Lymphocyte  
Normal karyotype  $2n = 46$



chromatid exchange

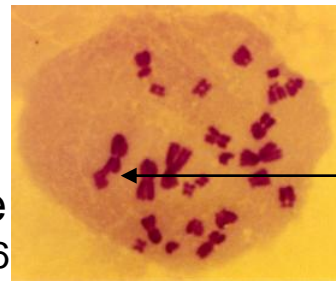
chromosome gap



double minutes

chromosome interchanges

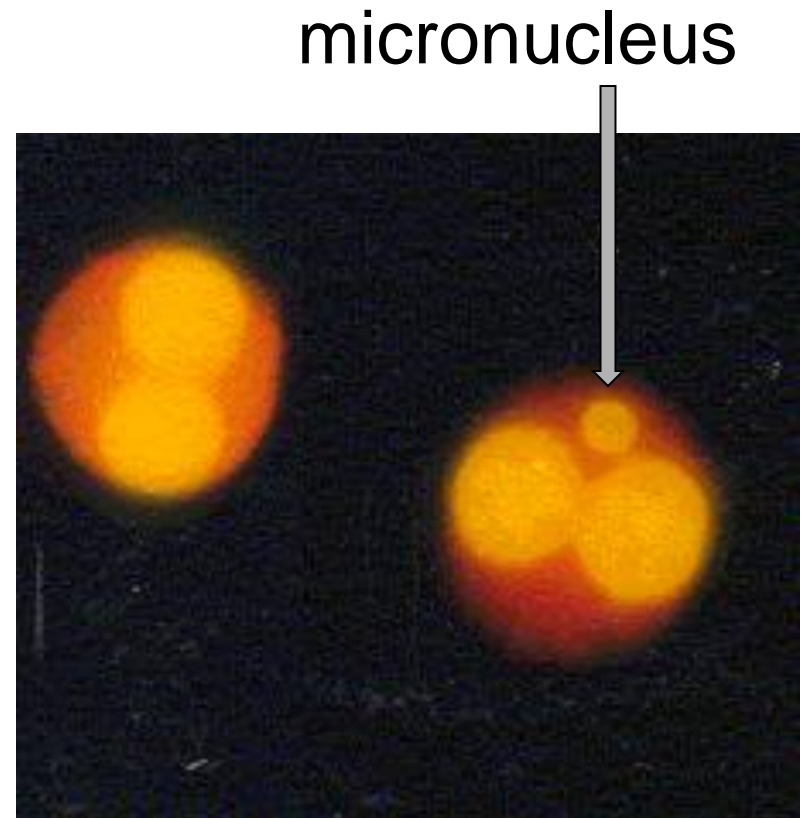
acentric ring



chromosome break

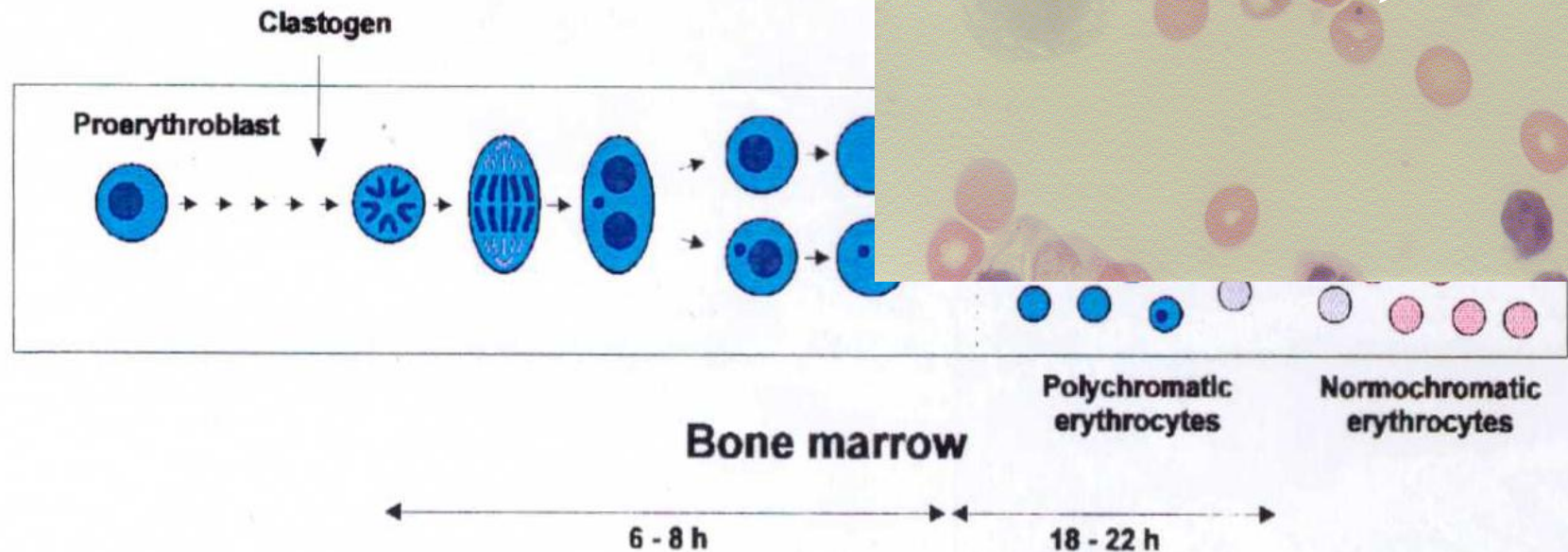
# *In vitro* micronucleus assay

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