CHEMICAL CARCINOGENESIS

Michael Lea

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CHEMICAL CARCINOGENESIS - LECTURE OUTLINE

1. History of chemical carcinogenesis

2. The diversity in the types of molecules that have been associated with chemical carcinogenesis.

3. Initiation, promotion and progression in chemical carcinogenesis. Genetic and epigenetic mechanisms

4. Factors that influence organ and species specific chemical carcinogenesis including the metabolism of chemical carcinogens and DNA repair.

1. History of Chemical Carcinogenesis

In 1567 Paracelsus suggested that the "wasting disease of miners" might be attributed to exposure to realgar (arsenic sulfide).

In 1761, John Hill noted that nasal cancer occurred in some people who used snuff excessively and in 1859 Bouisson described oral cancer in tobacco smokers.

The London surgeon Percival Pott in reported in 1775 that cancer of the scrotum sometimes developed in men after being exposed in childhood when they worked as chimney sweeps.

Epidemiological evidence has been important in detecting carcinogenic substances. Rehn (1895) reported an increased incidence of bladder cancer in aniline dye workers in Germany. The major carcinogen involved is now believed to be 2-naphthylamine.

Work with radium suggested the induction of skin cancer by repeated X-ray burns and in 1910 to 1912, Marie, Clunet and Raulot-Lapointe reported the induction of sarcoma in rats by the application of X-irradiation.

The first chemical induction of cancer in laboratory animals was achieved by Yamagiwa and Ichikawa (1915) by painting coal tar on the ears of rabbits every 2-3 days for more than a year. The first pure carcinogen, 1,2,5,6-dibenzanthracene, was synthesized in 1929 and in the 1930s Kenneway and Cook and their associates isolated carcinogenic polycyclic aromatic hydrocarbons including benzo(a)pyrene from coal tar.

In the early 1900s, Boveri proposed a mutation theory of carcinogenesis but at that time it was not amenable to chemical investigation.

Reference: A. Luch. Nature and nurture - lessons from chemical carcinogenesis. Nature Reviews Cancer 5; 113-125, 2005.

Figure 2-21a The Biology of Cancer (© Garland Science 2007)

Figure 2-21b The Biology of Cancer (© Garland Science 2007)

Induction of cancer by application of coal tar to the skin of rabbits (Yamagiwa. 1915)

Estimated percentage of cancer deaths attributed to different factors in the United States and the United Kingdom

- Diet   41%
- Tobacco   24%
- Infection   10%
- Ultraviolet light   10%
- Sexual factors   7%
- Occupation   5%
- Alcohol   3%
- Pollution   1%
- Medical procedures   1%
- Ionizing radiation   1%

- The data are taken from a review by Roush et al. And represent the approximate midpoint of ranges derived from the data of Doll and Peto, Higginson and Muir, and Wynder and Gori.

MUTATION AND CARCINOGENESIS

Boveri was the first to suggest that chromosomal changes lead to cancer and in 1916 Tyzzer introduced the term "somatic mutation". Evidence in favor of the somatic mutation theory has been summarized as follows:

1. Most chemical carcinogens are mutagens

2. Most carcinogens and mutagens are strong electrophilic reactants.

3. Ionizing or ultraviolet radiation and most chemical carcinogens cause lesions in DNA.

4. Defects in DNA repair capacity are associated with a high risk of cancer.

5. A high frequency of chromosomal aberration is correlated with an increased risk of malignancy.

6. Cell transformation by oncogenic viruses implies a change in the genetic information.

7. A malignant phenotype is inherited in the cell line.

8. Tumors are mostly monoclonal in origin.

9. Chromosomal changes found in tumors are frequently found to be nonrandom.

Reference: Sorsa: J. Toxicol. Environ. Health 6: 977-982, 1980.

TESTING OF CHEMICAL CARCINOGENS

It has not been economically feasible to test all the compounds to which people may be exposed. Criteria for selection include:

A.   Compounds related to known carcinogens

B.   New compounds that are to be placed in the environment

C.   Compounds that are indicated by epidemiological surveys to be associated with an increased incidence of cancer

TESTING IN LABORATORY ANIMALS

Testing in laboratory animals is the most reliable procedure for detecting carcinogenic activity. There can be metabolic and pharmacokinetic differences between species that make it preferable to examine more than one species.

Pure compounds should be administered to adequate numbers of test animals (not less than 10) and there should be appropriate controls.

The route of administration can influence the numbers of tumors and the tissues affected. The dose level must be high enough to see tumors in a statistically reliable number of animals. Chronic studies over the lifetime of the animal are necessary. Careful pathological examination of all dead animals is essential.

Diet, cage bedding and exposure to insecticides can all influence tumor induction.

Although pure compounds are essential for identification of a carcinogen such a test system will not detect the synergistic action of tumor initiators and promoters.

There is uncertainty on whether threshold levels exist for the detection of carcinogenic compounds.

IN VITRO TESTING OF CHEMICAL CARCINOGENS

The high cost of animal screening has driven the search for short-term in vitro tests. The best known in vitro test is that devised by Bruce Ames which measure mutagenicity in a Salmonella strain that requires histidine for growth. Mutation can result in a reversion to the wild type phenotype that permits growth in the absence of histidine.

Because many carcinogens require metabolic activation, the bacteria are incubated with a rat liver S9 fraction.

The theoretical basis for tests of this type is the good but not perfect correlation between mutagenic and carcinogenic activity. For some studies this has been about 90% for large numbers of compounds but other studies have seen a correlation of about 75%.
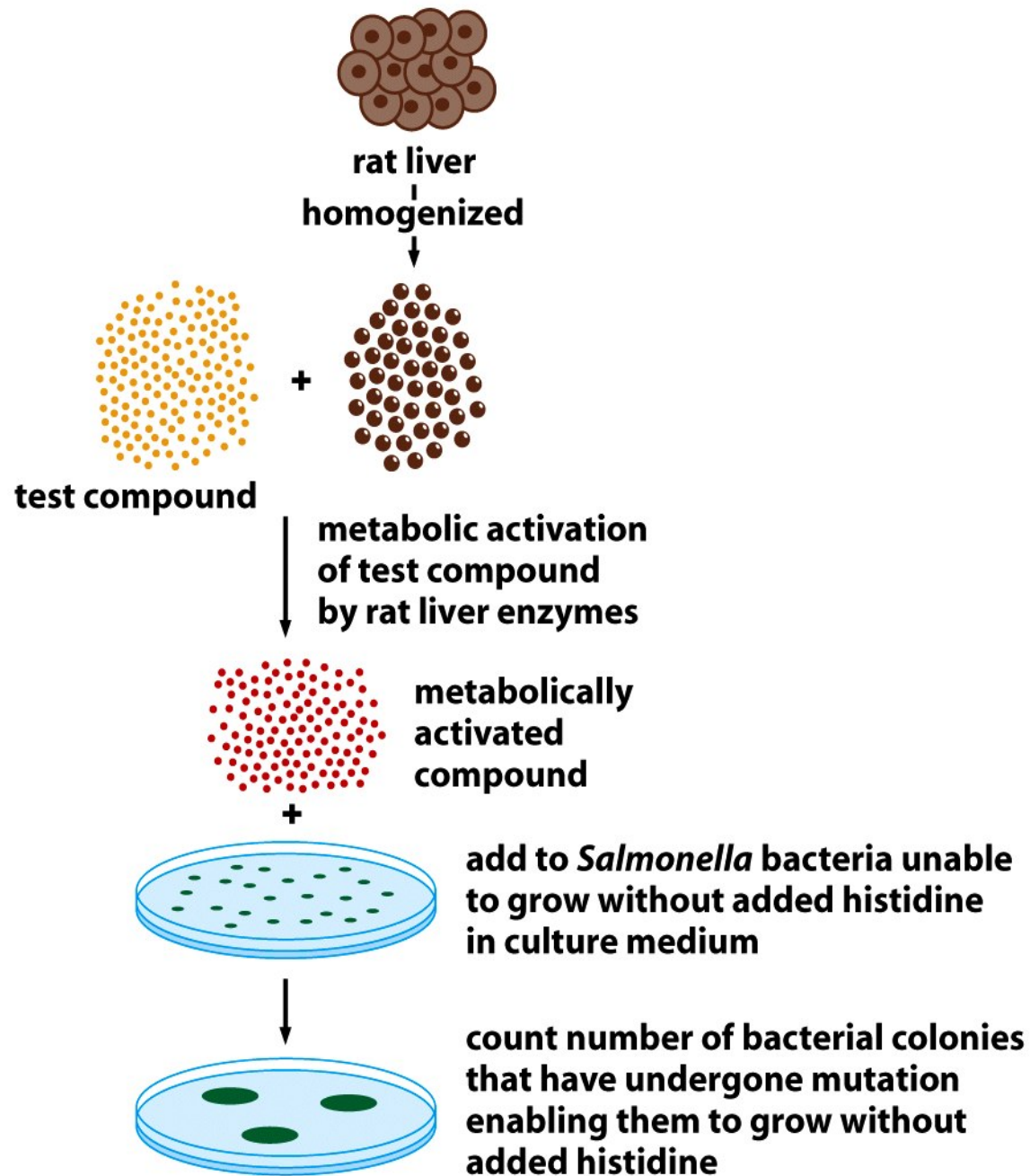
rat liver
homogenized

test compound

+

metabolic activation
of test compound
by rat liver enzymes

metabolically
activated
compound

+

add to *Salmonella* bacteria unable
to grow without added histidine
in culture medium

count number of bacterial colonies
that have undergone mutation
enabling them to grow without
added histidine

Figure 2-24 The Biology of Cancer (© Garland Science 2007)

The Ames Test for mutagenicity
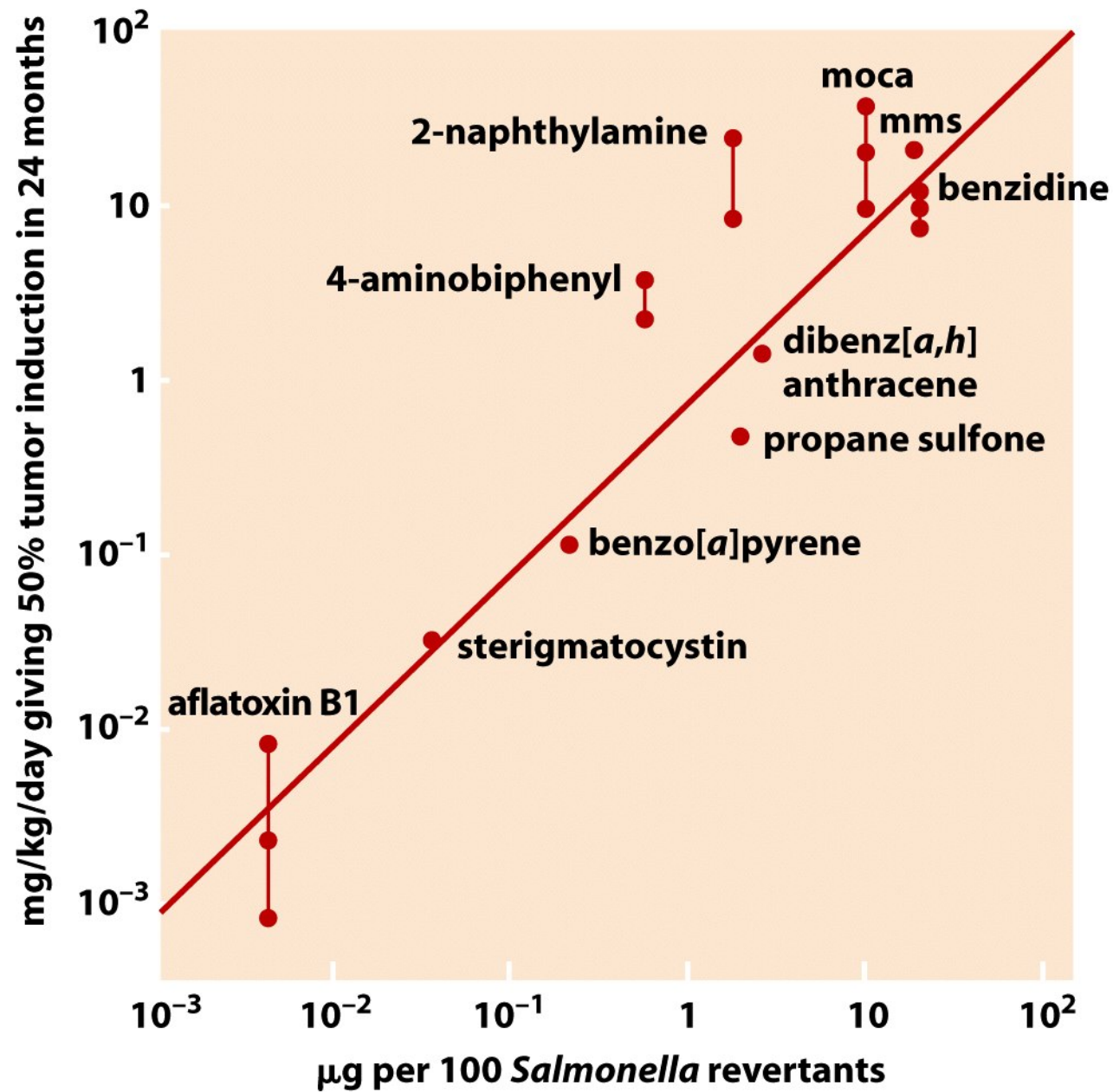
Figure 2-25 The Biology of Cancer (© Garland Science 2007)

Mutagenic versus carcinogenic potency

The Diversity of Chemical Carcinogens

Before considering the mechanism(s) of chemical carcinogenesis it is appropriate to review the variety of substances associated with the induction of cancer. The number of known carcinogens in experimental animals is large. it is suspected that most of these are potentially carcinogenic in humans but documentation is lacking in most cases. The following list includes substances for which there is good evidence of carcinogenicity in humans. The list is adapted from one given by Miller and Miller in "The Molecular Biology of Cancer" edited by H. Busch, Academic press: New York, (1974)

_____

Carcinogen	Target
2-Naphthylamine	Urinary bladder
Benzidine	Urinary bladder
4-Aminobiphenyl	Urinary bladder
Bis(2-chloroethyl)sulfide	Lung
Nickel compounds	Lung, nasal sinuses
Chromium compounds	Lung
Asbestos	Lung, pleura
Soots, tars	Skin, lungs
Cigarette smoke	Lung, other sites
Betel nut	Buccal mucosa
Arsenite	Skin, liver

Table 6-3. OCCUPATIONAL CANCERS

Agents or Groups of Agents	Human Cancer Site for Which Reasonable Evidence Is Available	Typical Use or Occurrence
Arsenic and arsenic compounds	Lung, skin, hemangiosarcoma	Byproduct of metal smelting. Component of alloys, electrical and semiconductor devices, medications and herbicides, fungicides, and animal dips.
Asbestos	Lung, mesothelioma; gastrointestinal tract (esophagus, stomach, large intestine)	Formerly used for many applications because of fire, heat, and friction resistance; still found in existing construction and fire-resistant textiles, friction materials (i.e., brake linings), underlayment and roofing papers, and floor tiles.
Benzene	Leukemia, Hodgkin lymphoma	Principal component of light oil. Although use as solvent is discouraged, many applications exist in printing and lithography, paint rubber, dry cleaning, adhesives and coatings, and detergents. Formerly widely used as solvent and fumigant.
Beryllium and beryllium compounds	Lung	Missile fuel and space vehicles. Hardener for lightweight metal alloys, particularly in aerospace applications and nuclear reactors.
Cadmium and cadmium compounds	Prostate	Uses include yellow pigments and phosphors. Found in solders. Used in batteries and as alloy and in metal platings and coatings.
Chromium compounds	Lung	Component of metal alloys, paints, pigments, and preservatives.
Ethylene oxide	Leukemia	Ripening agent for fruits and nuts. Used in rocket propellant and chemical synthesis, in fumigants for foodstuffs and textiles, and in sterilants for hospital equipment.
Nickel compounds	Nose, lung	Nickel plating. Component of ferrous alloys, ceramics, and batteries. Byproduct of stainless steel arc welding.
Radon and its decay products	Lung	From decay of minerals containing uranium. Can be serious hazard in quarries and underground mines.
Vinyl chloride	Liver, angiosarcoma	Refrigerant. Monomer for vinyl polymers. Adhesive for plastics. Formerly inert aerosol propellant in pressurized containers.

Modified from Stellman JM, Stellman SD: Cancer and workplace. CA Cancer J Clin 46:70, 1996.

Table 6-5. MAJOR CHEMICAL CARCINOGENS

Direct-Acting Carcinogens

Alkylating agents
  Anticancer drugs (cyclophosphamide, chlorambucil, nitrosoureas, and
    others)
Acylating agents
  1-Acetyl-imidazole
  Dimethylcarbamyl chloride

Procarcinogens That Require Metabolic Activation

Polycyclic and heterocyclic aromatic hydrocarbons
  Benz[a]anthracene
  Benzo[a]pyrene
  Dibenz[a,h] anthracene
  3-Methylcholanthrene
  7,12-Dimethylbenz[a]anthracene
Aromatic amines, amides, azo dyes
  2-Naphthylamine (β-naphthylamine)
  2-Acetylaminofluorene
  Dimethylaminoazobenzene (butter yellow)
Natural plant and microbial products
  Aflatoxin B1
  Griseofulvin
  Betel nuts
Others
  Nitrosamine and amides
  Vinyl chloride, nickel, chromium
  Insecticides, fungicides
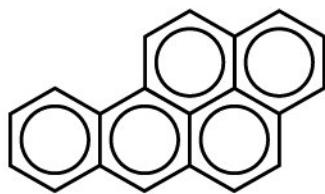 Polychlorinated biphenyls (PCBs)
  Arsenic
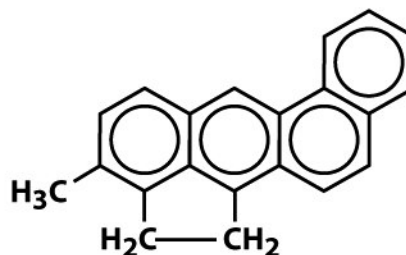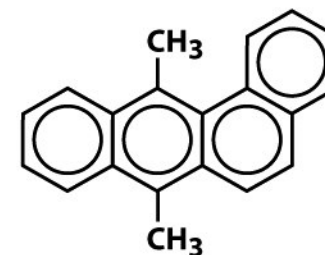 Asbestos
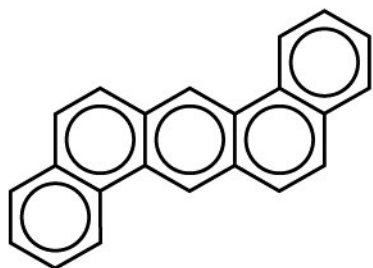
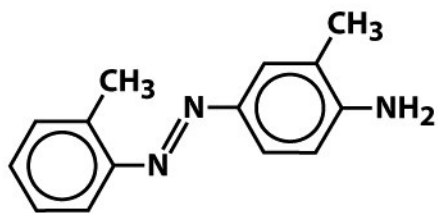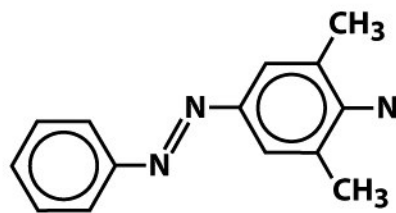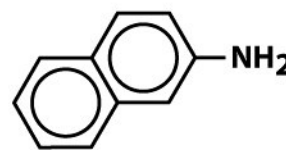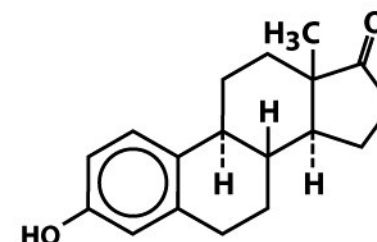dibenz[a,h]anthracene benzo[a]pyrene 3-methylcholanthrene 7,12-dimethylbenz[a]-anthracene

2·,3-dimethyl-4-amino-azobenzene N,N-dimethyl-4-amino-azobenzene 2-naphthylamine estrone

Figure 2-22 The Biology of Cancer (© Garland Science 2007)

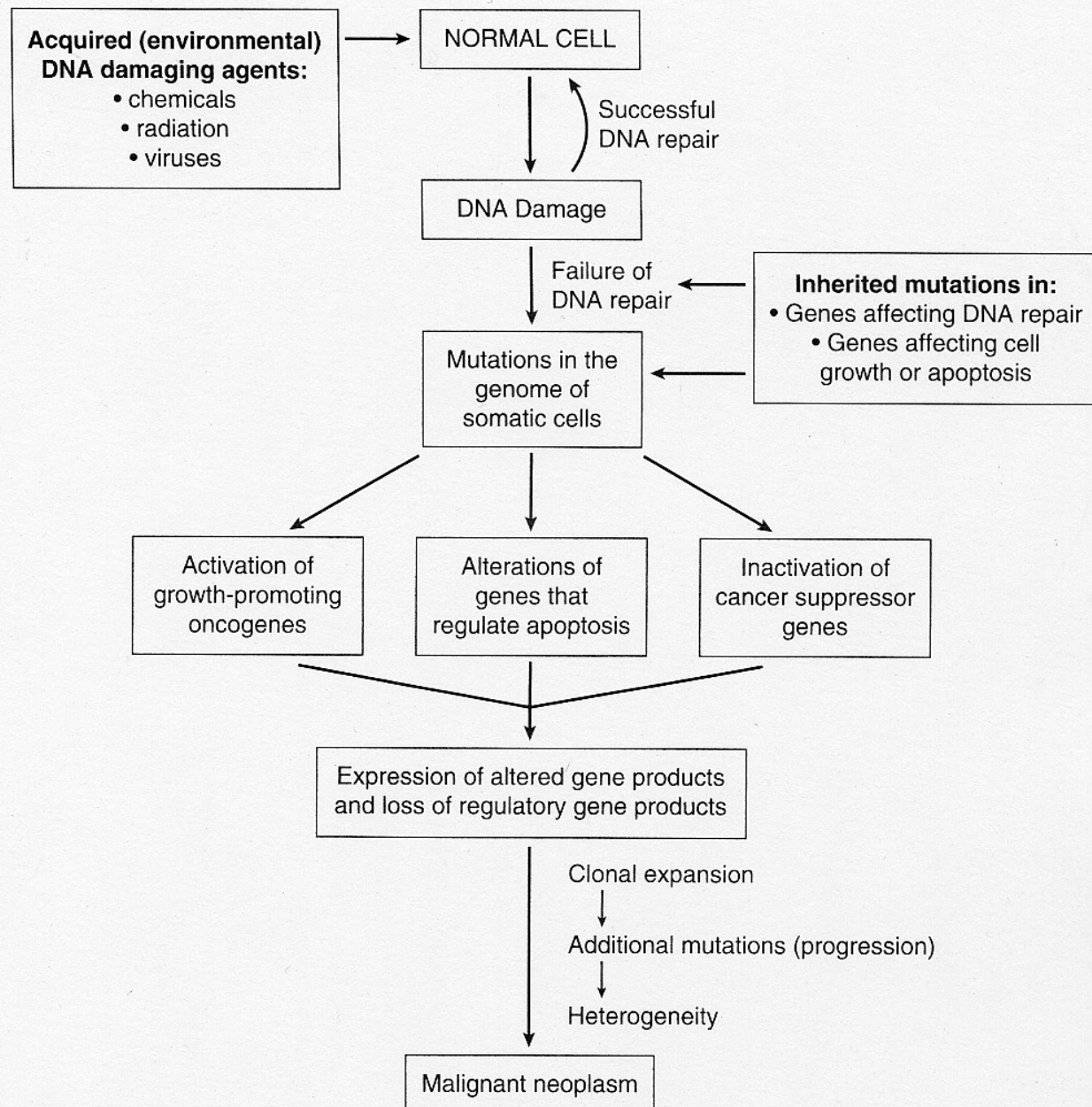Structure of carcinogenic hydrocarbons (Weinberg, The Biology of Cancer, Fig.2.22)

Acquired (environmental)
DNA damaging agents:
• chemicals
• radiation
• viruses

NORMAL CELL

Successful
DNA repair

DNA Damage

Failure of
DNA repair

Inherited mutations in:
• Genes affecting DNA repair
• Genes affecting cell
growth or apoptosis

Mutations in the
genome of
somatic cells

Activation of
growth-promoting
oncogenes

Alterations of
genes that
regulate apoptosis

Inactivation of
cancer suppressor
genes

Expression of altered gene products
and loss of regulatory gene products

Clonal expansion

Additional mutations (progression)

Heterogeneity

Malignant neoplasm

Figure 6–16   ■

Flow chart depicting a simplified scheme
of the molecular basis of cancer.

Initiation, promotion and progression

Carcinogenesis appears to involve several events and in some systems 3 phases may be distinguished: initiation, promotion and progression.

Initiation seems to be a mutational event involving one or more genes. It may not be sufficient for cancer to develop but it is irreversible or very prolonged.

Promotion can be reversible in the early stages. For the induction of cancer it must occur after initiation.

Progression is seen in many malignancies and may reflect genomic instability and clonal selection of cells with additional mutations.

Initiation and promotion were first reported for the induction of skin cancer in mice by application of carcinogenic polycyclic aromatic hydrocarbons. Application of a low dose did not result in skin cancer but subsequent treatment with croton oil resulted in tumors. Croton oil alone did not cause tumors. The active components of croton oil were found to be phorbol esters such as tetradecanoyl phorbol acetate (TPA).

Factors that increase cell proliferation can function as promoters in some systems.

Carcinogen Metabolism and Activation

Chemical carcinogenesis appears to be associated with reaction with cellular nucleophiles. Alkylating agents can act directly in this manner but many carcinogens must be metabolized to form electrophilic species. Some of the most significant targets of alkylating agents, such as mustard gas, are probably guanine bases in DNA. N7 is the most nucleophilic center in guanine.
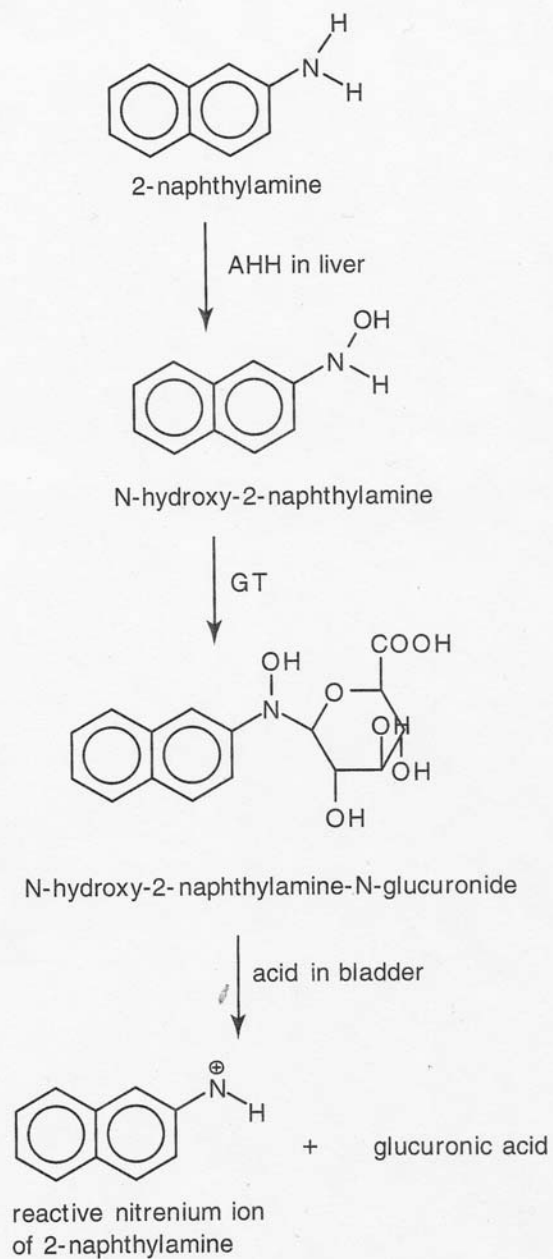
Organic compounds with double bonds may be metabolized to form reactive epoxides e.g. with benzo(a)pyrene, vinyl chloride and aflatoxin.

Nitrosamines can be metabolized to form carbonium ions that react with guanine to give an O^6-methyl derivative.

Differences in organ and species specificity for carcinogens may reflect differences in phase I and phase II drug metabolism together with differences in pharmacokinetics and DNA repair.
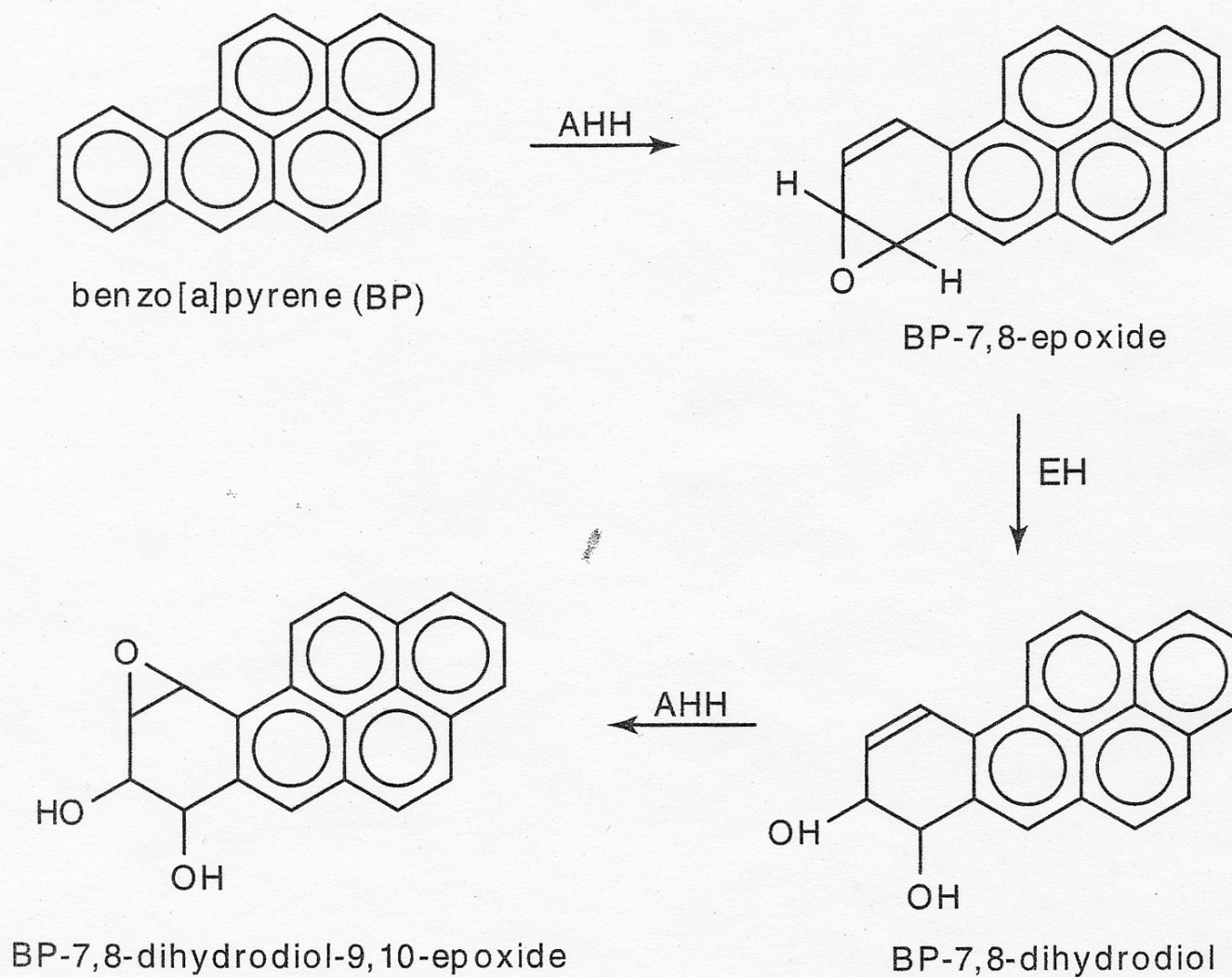
Figure 3–4. Metabolic activation of the aralkylating agent benzo[a]pyrene (BP). Activation is mediated by the P-450 microsomal enzymes and specifically arylhydrocarbon hydroxylase (AHH), which oxidizes the carbon-carbon double bond to form an epoxide. Another enzyme, epoxide hydratase (EH), destroys the epoxide, and AHH further metabolizes the BP to form the presumed reactive intermediate.

2-naphthylamine

AHH in liver

N-hydroxy-2-naphthylamine

GT

COOH

N-hydroxy-2-naphthylamine-N-glucuronide

acid in bladder

reactive nitrenium ion
of 2-naphthylamine

+   glucuronic acid

Figure 3–5. Metabolism of the arylhydroxylamine 2-naphthylamine. AHH activity in the liver oxidizes the nitrogen to generate the N-hydroxy derivative. The conjugating enzyme glucuronide transferase (GT) adds the sugar residue to the carcinogen. This stabilizes the compound until it reaches the bladder, where acid conditions cause the formation of the carcinogenic nitrenium ion.
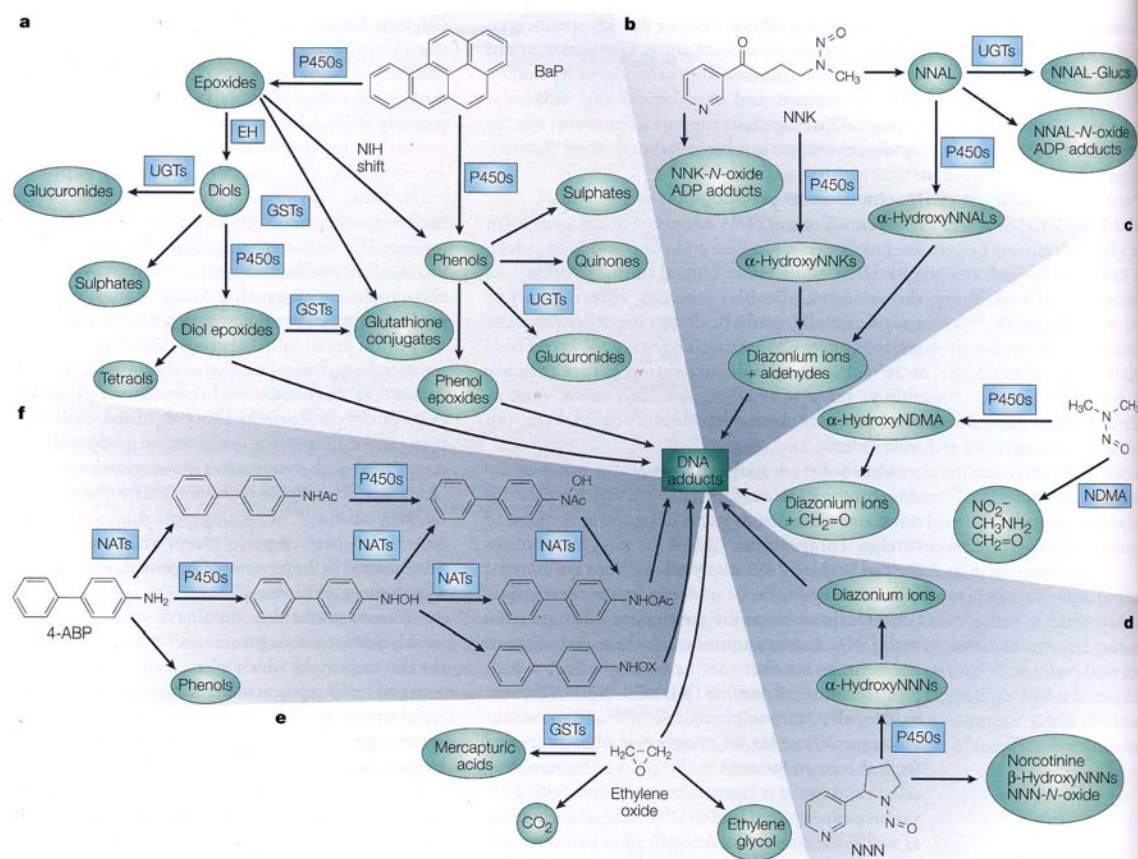
Figure 2 | **Metabolic activation of tobacco-smoke carcinogens to DNA adducts known to be present in human lung.**
Six tobacco-smoke carcinogens that can form DNA adducts are shown. **a** | Benzo[a]pyrene (BaP) is metabolized to epoxides by cytochrome P450s (P450s), which are then converted to diol epoxides by epoxide hydrolases (EHs) and P450s. The diol epoxides react with DNA. The initially formed epoxides can undergo a National Institutes of Health (NIH) shift to produce phenols, most of which are detoxification products. However, some phenols can be further oxidized to epoxides, which react with DNA. The other pathways of BaP metabolism shown here result mainly in detoxification and excretion of metabolites. The enzymes involved include glutathione-S-transferases (GSTs) and UDP-glucuronosyl transferases (UGTs). **b** | 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is metabolized to α-hydroxyNNKs by P450s. These spontaneously decompose to diazonium ions and aldehydes. The diazonium ions react with DNA to form adducts. NNK can also undergo reduction to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), which is converted to NNAL-glucuronides (NNAL-Glucs; detoxification products). α-Hydroxylation of NNAL by P450s results in diazonium ions, which can produce DNA adducts. **c** | N-Nitrosodimethylamine (NDMA) undergoes P450-catalysed α-hydroxylation, producing α-hydroxyNDMA, which spontaneously decomposes to methyldiazonium ions and formaldehyde. The former is a methylating agent and reacts with DNA to produce methyl adducts. Detoxification pathways of NDMA include the production of nitrite and methylamine. **d** | P450s catalyse the α-hydroxylation of N'-nitrosonornicotine (NNN), giving α-hydroxyNNNs. These are unstable and rearrange to give diazonium ions that react with DNA. Detoxification pathways of NNN include the production of norcotinine, β-hydroxyNNNs and NNN-N-oxide. **e** | Ethylene oxide can react directly with DNA. It is also detoxified by GST catalysis to mercapturic acids, hydration to ethylene glycol and oxidation to CO_2. **f** | 4-Aminobiphenyl (4-ABP) undergoes P450 catalysed N-oxidation to a hydroxylamine. O-Acetylation, catalysed by N-acetyl transferases (NATs), produces an O-acetoxy compound that reacts with DNA. Other esterification reactions of the hydroxylamine lead to related intermediates that can react with DNA.

Non-enzyme-catalyzed reactions may contribute to carcinogen formation

Conditions in the stomach can favor the formation of nitrosamines.

The low pH in the stomach favors the conversion of nitrite to the uncharged nitrous acid which reacts with amines to form nitrosamines.

The reaction is inhibited by ascorbic acid (vitamin C).

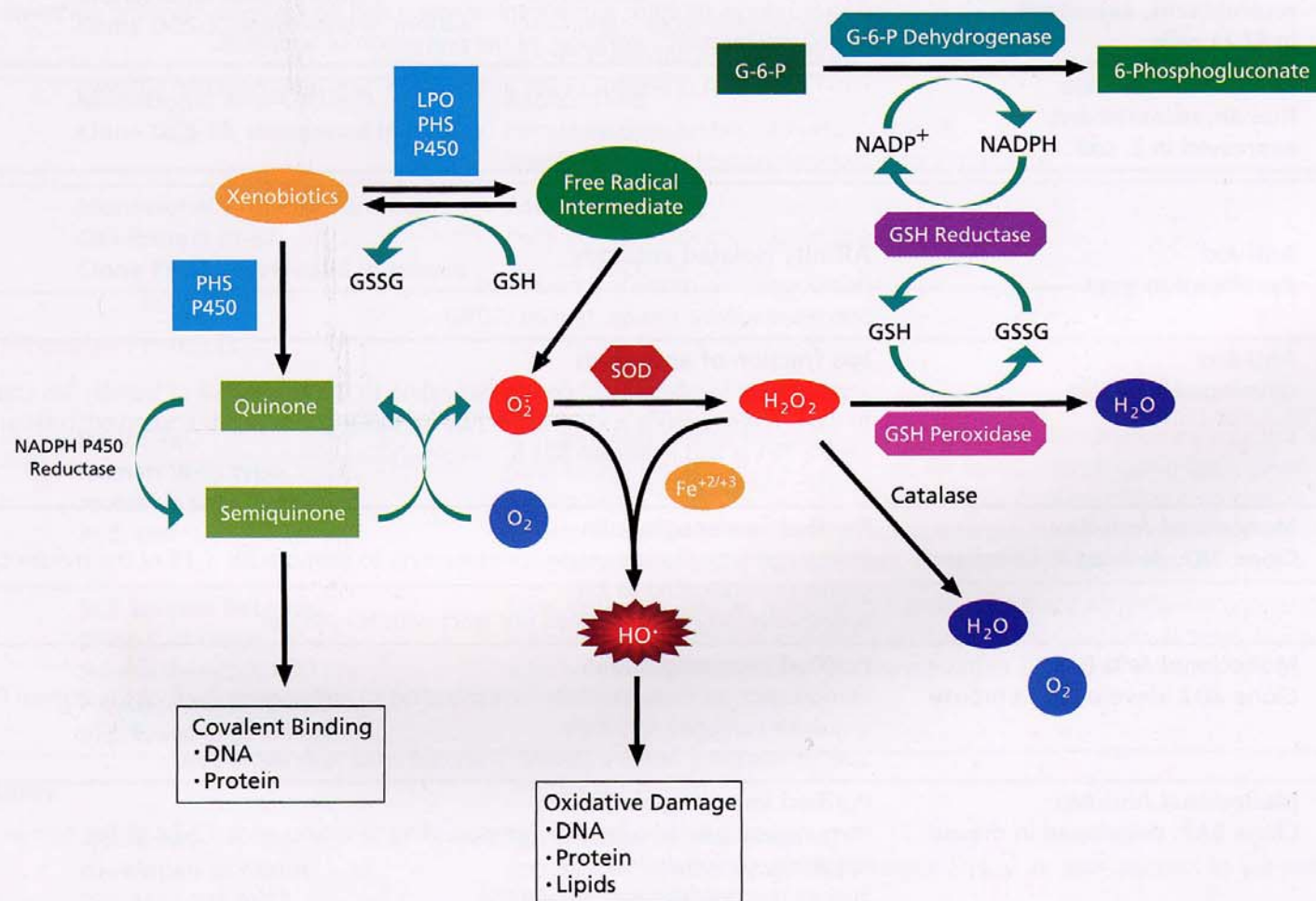# Potentially Carcinogenic Agents or Events which Damage DNA

A. Spontaneous damage

     1. Mispairing of bases during DNA synthesis

     2. Deamination of bases

     3. Loss of bases -> AP sites

     4. Oxidative damage

B. Environmental damage to DNA

     1. Ionizing and ultraviolet radiation

     2. Chemical agents that modify bases or form strand breaks

Oxidative Stress.

Oxidative stress is imposed on cells as a result of one of three factors: 1) the release of reactive oxygen species (ROS), 2) a decrease in antioxidant protection, or 3) a failure to repair oxidative damage. ROS are either free radicals, reactive anions containing oxygen, or molecules containing oxygen atoms that can either produce free radicals or are chemically activated by them. Examples are hydroxyl radical, superoxide, hydrogen peroxide, and peroxynitrite. While the main source of ROS *in vivo* is aerobic respiration, they are also produced by peroxisomal β-oxidation of fatty acids, microsomal cytochrome P450 metabolism of xenobiotics, stimulation of phagocytosis by pathogens or lipopolysaccharides, arginine metabolism, and tissue specific enzymes. Some carcinogens and anti-cancer agents are oxidized by cytochrome P450 enzymes, generating reactive intermediates that alkylate or form adducts with DNA. Other anti-cancer agents, such as bleomycin and doxorubicin, form complexes with DNA and iron ions that generate ROS with changes in the redox state. Under normal conditions, ROS are cleared from the cell by the action of super-oxide dismutase (SOD), catalase, or glutathione (GSH) peroxidase. The main damage to cells results from the ROS-induced alteration of macromolecules. ROS increases the probability of both single-strand and double-strand breaks in DNA with the concomitent loss or translocation of genetic material. If these genetic mutations are not repaired prior to cell division, they may become part of the somatic genome.

DNA REPAIR

A. Repair of O^6-guanine alkylation

A cysteine residue on O^6-guanine-DNA methyltransferase is used as a methyl group acceptor. The enzyme operates by suicide kinetics.

B. Repair of single-stranded breaks

DNA ligase rejoins strand breaks. This requires that there are no missing nucleotides and 3'-OH and 5'-phosphate termini exist at the site of the break.

C. Base excision repair

Modified bases or uracil in DNA are excised as free bases. Several enzymes are required: DNA glycosylase, 5' AP endonuclease, DNA deoxyribophosphodiesterase, DNA polymerase beta, DNA ligase I and II.

D. Nucleotide excision repair

Bulky adducts and thymine dimers are excised from DNA as part of oligonucleotide fragments. The process requires damage recognition factors, local unwinding of DNA, dual incisions, excision of an oligonucleotide fragment with the lesion, synthesis of a new DNA fragment by DNA polymerases delta and epsilon and DNA ligation

E. Double strand break repair

The repair mechanism involves recombination by at least two pathways: homologous recombination and nonhomologous end joining.

F. Mismatch repair

Defects in mismatch repair are associated with tumor progression in hereditary nonpolyposis colorectal cancer and human epithelial cancers.

Suggested reading

A. Weston and C.C. Harris in Holland-Frei Cancer Medicine (6th edition) Part II Scientific Foundations, Section 3 - Cancer Etiology, 17. Chemical Carcinogenesis (2003).

B. Weinberg, R.A. The Biology of Cancer, Garland Science. Page 46-56, 2007