Oncology 520

Carcinogenesis and DNA repair

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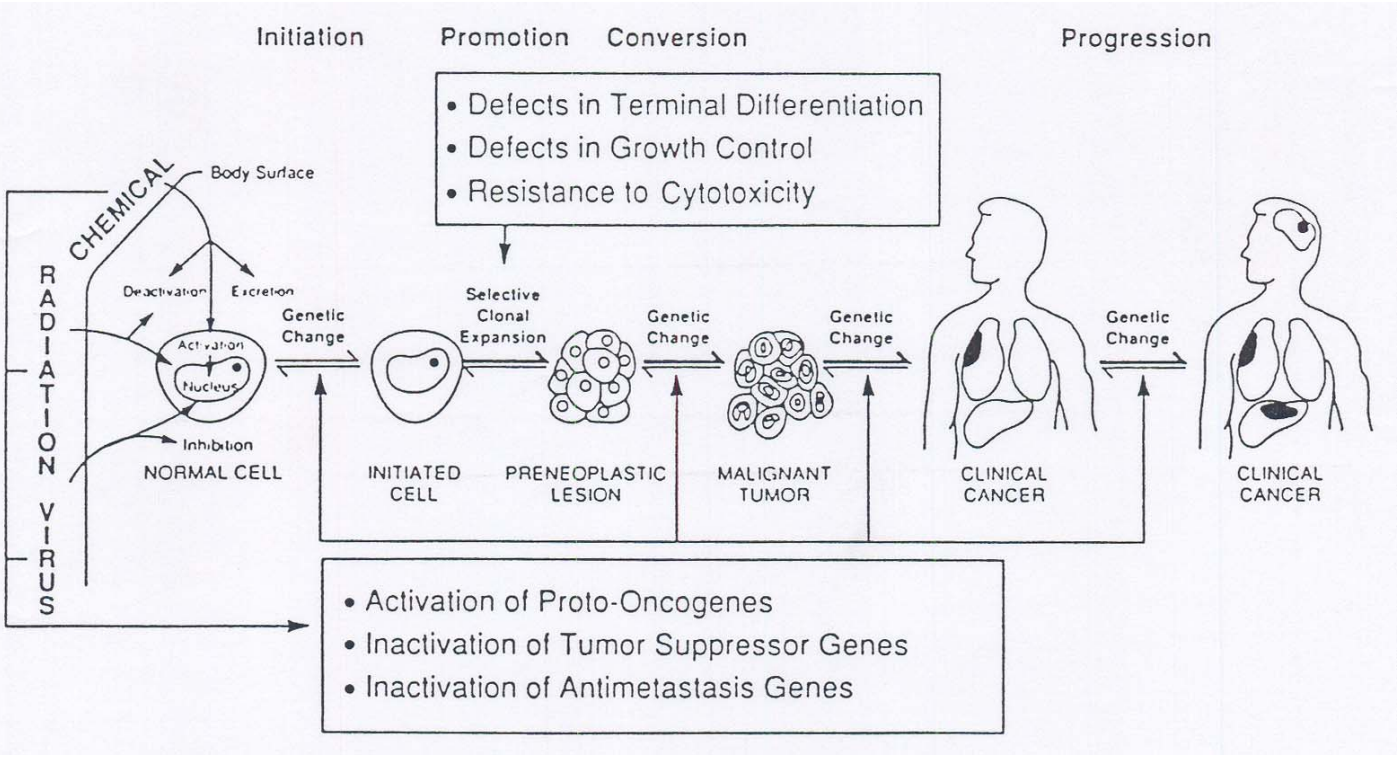
CHEMICAL CARCINOGENESIS

What is carcinogenesis and what role might chemicals play in this process?

Carcinogenesis is the process whereby cells progress from a controlled state to an uncontrolled state. There are two key features of the process:

(a) it is a multistep process

(b) neoplasms are clonal in origin



SOMATIC MUTATION THEORY OF CARCINOGENESIS

The initial step in carcinogenesis induced by a chemical or radiation is a mutation in the DNA of a somatic cell. (T. Boveri, 1914)

EVIDENCE FOR THE ROLE OF MUTATION IN INITIATION OF CANCER

(i) **Most carcinogens cause DNA damage and mutation.**

(ii) **Cancer-prone conditions coupled with DNA repair defects:**

e.g. patients with the syndrome Xeroderma pigmentosum are defective in excision repair of UV-damaged DNA and develop multiple skin tumours when exposed to UV light from the sun.

Hereditary nonpolyposis colon cancer is associated with a defect in the *hMSH2* gene. This gene is involved in the repair of DNA mismatches and maintaining genetic stability.

(iii) **Chromosomal anomalies:**

Cells of certain tumours have consistent chromosomal anomalies e.g. CML cells contain a translocation between the distal ends of chromosome 22 and 9. Similarly, certain childhood tumours are associated with chromosomal abnormalities e.g. Wilm's tumour and retinoblastoma.

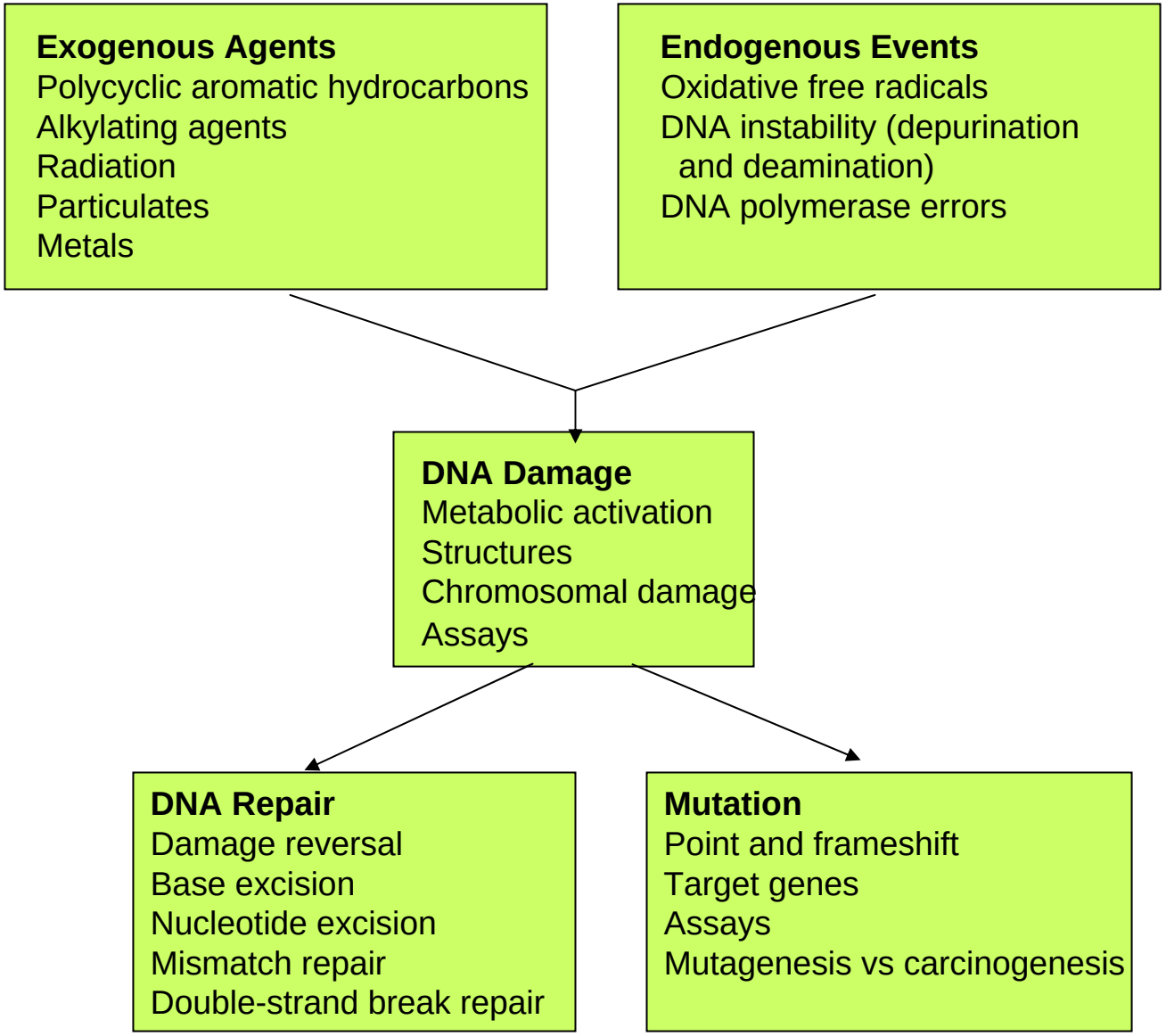
(iv) **Oncogenes and proto-oncogenes**

Some oncogenes differ from the normal cellular proto-oncogenes by virtue of point mutations e.g. codon 12 of c-Ha-ras gene.

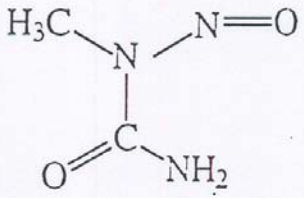
(v) **Clonal origin of tumours:**

If mutation is a critical step in carcinogenesis, it would be expected that tumours would be clonal in origin.

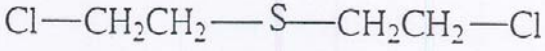
INITIATION


Exogenous Agents
Polycyclic aromatic hydrocarbons
Alkylating agents
Radiation
Particulates
Metals
Endogenous Events
Oxidative free radicals
DNA instability (depurination and deamination)
DNA polymerase errors
DNA Damage
Metabolic activation
Structures
Chromosomal damage
Assays
DNA Repair
Damage reversal
Base excision
Nucleotide excision
Mismatch repair
Double-strand break repair
Mutation
Point and frameshift
Target genes
Assays
Mutagenesis vs carcinogenesis

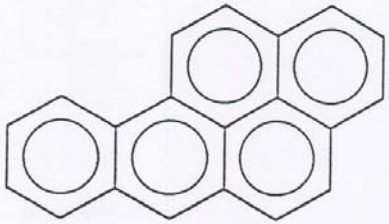
Examples of Chemical Carcinogens



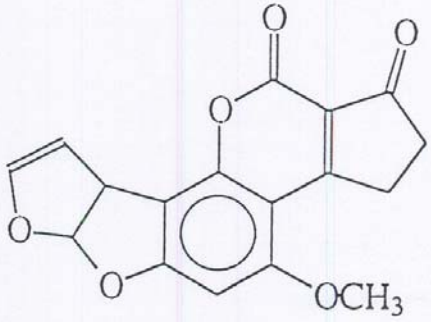
N-methyl-N-nitrosourea



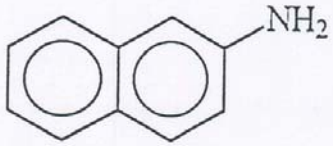
Bis(2-chloroethyl)sulphide



Benzo[a]pyrene



Aflatoxin B_1



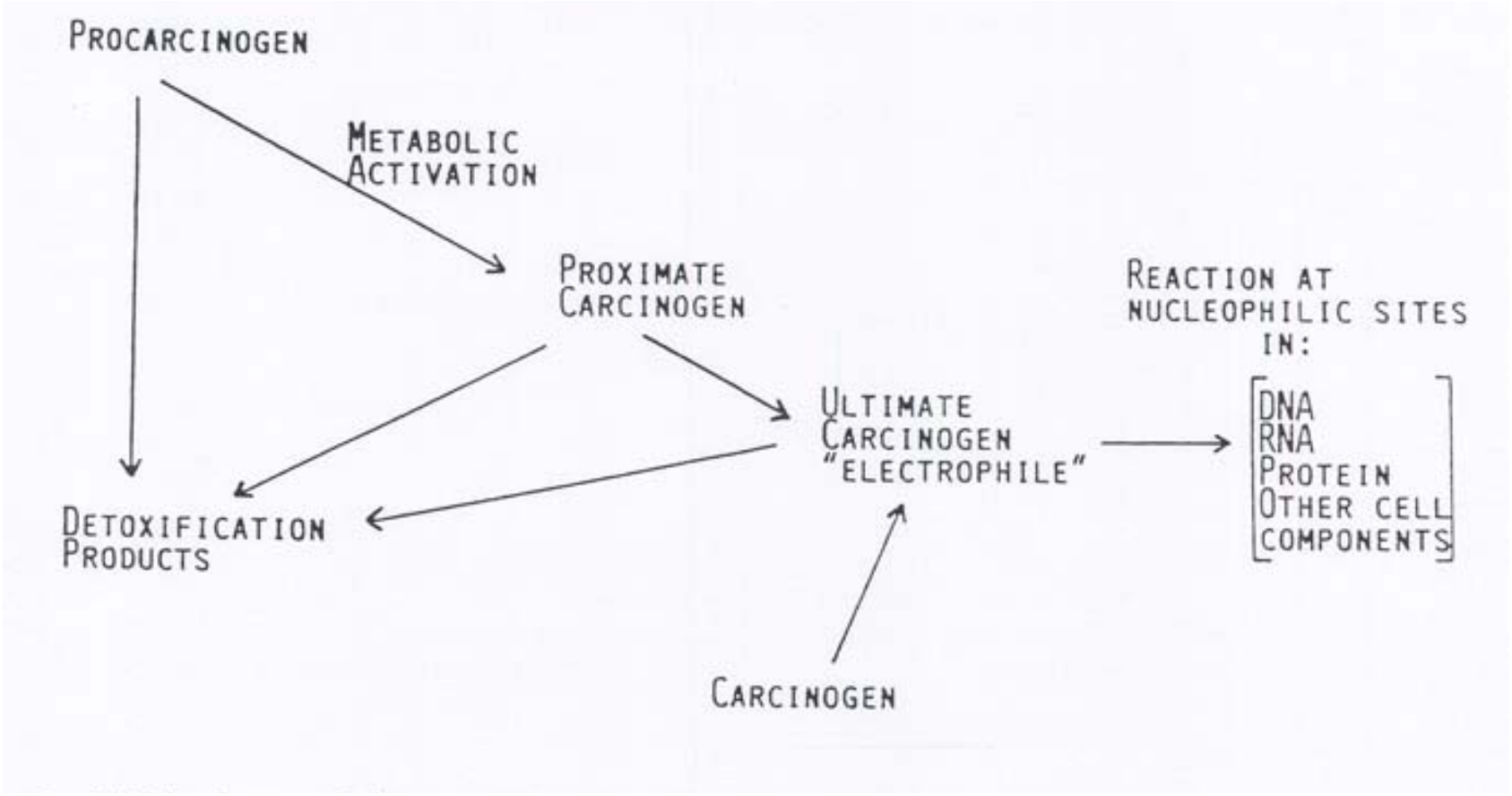
2-Naphthylamine

Ni (CO_4)

Nickel Carbonyl

Metabolic activation

Many chemical carcinogens and mutagens are inactive until metabolically converted into their active forms



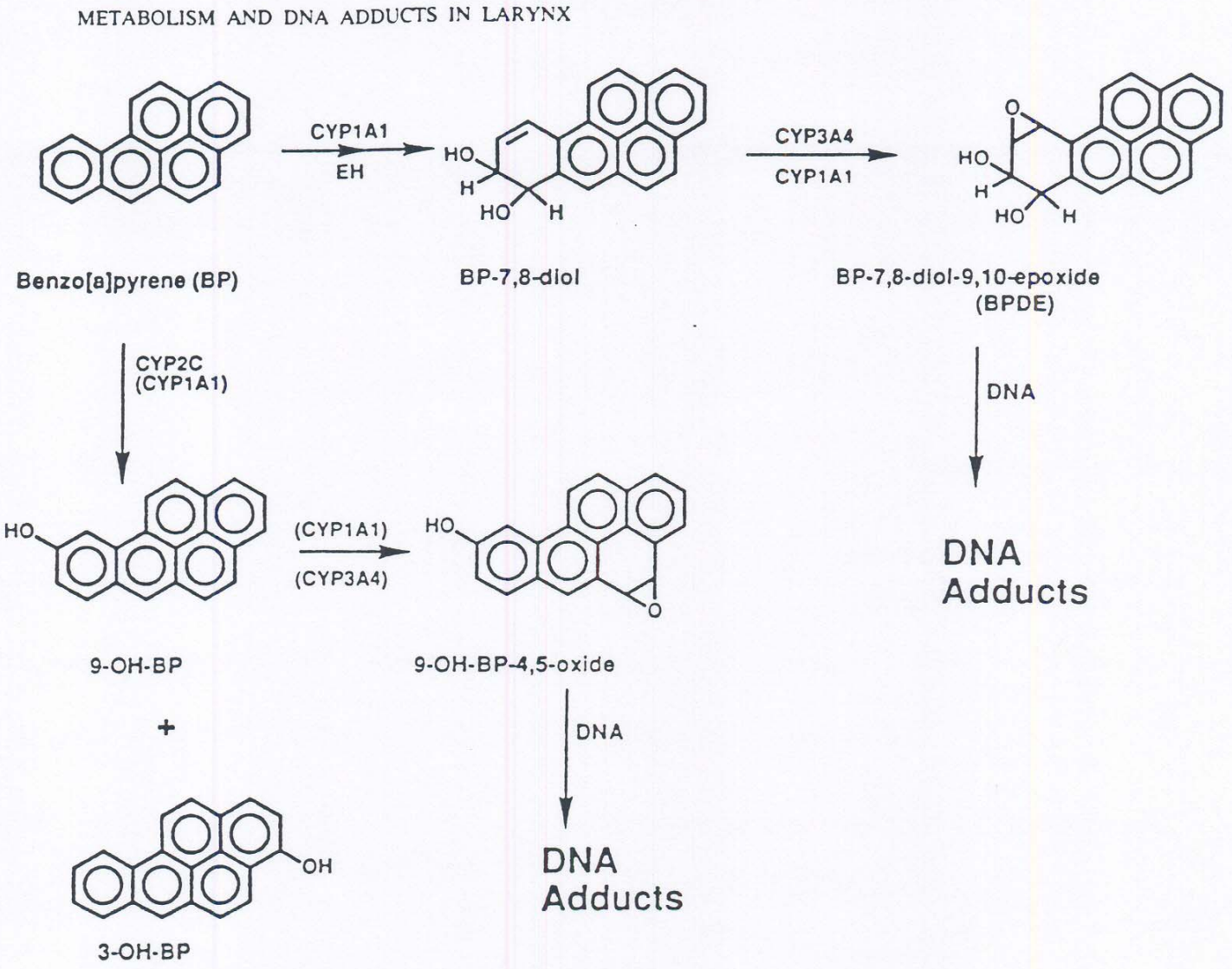
Most ultimate carcinogens are electrophiles seeking the electron rich (nucleophilic) centres in their macromolecular targets

Polycyclic aromatic hydrocarbon metabolism

Phase I enzymes (activators)
- **Aryl hydrocarbon hydroxylase (cytochrome P450)**
- **Epoxide hydrolase**

Phase II enzymes (detoxifiers)
- **Glutathione S-transferases**
- **NAD(P)H quinone reductase (QR)**
- **UDP-glucuronosyl-transferases**

Cancer risks may be related to metabolic imbalance between activating and detoxifying pathways for carcinogens.



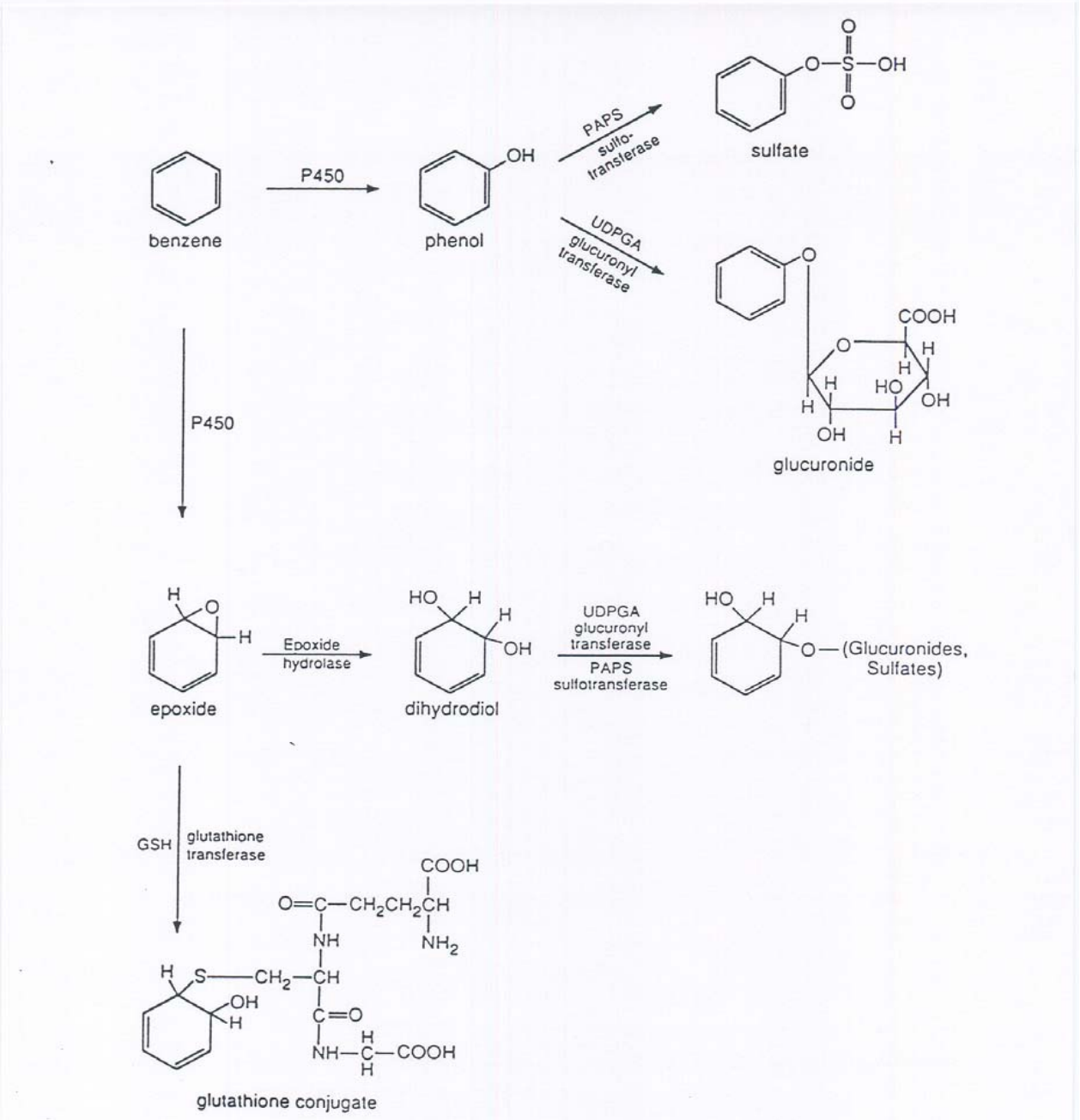
Hypothesis for the role of specific cytochromes P450 in the metabolic activation of B[a]P and other PAHs in cigarette smoke by human larynx tissues. CYP = cytochrome P450; EH = epoxide hydrolase

Polycyclic aromatic hydrocarbon metabolism

Phase I enzymes (activators)
Aryl hydrocarbon hydroxylase (cytochrome P450)
Epoxide hydrolase

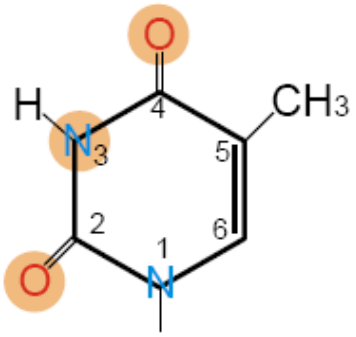
Distribution of AHH Inducibility in Mitogen-stimulated Lymphocytes from Healthy Control Individuals and Patients with Bronchogenic Carcinoma

	No.	*Low AHH*	*Inter-mediate AHH*	*High AHH*
Healthy control group	230	42.2% (97)	46.9% (108)	10.9% (25)
Patients with bronchogenic carcinoma	121	5.0% (6)	64.4% (78)	30.6% (37)

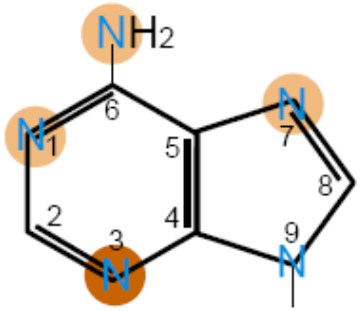


Summary of some of the phase II routes of metabolism of benzene including epoxide hydrolase, sulfotransferase, glutathione transferase and glucuronidation.

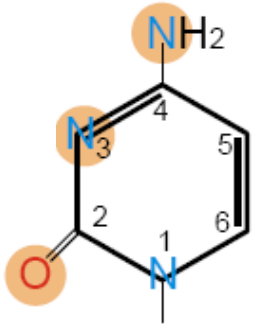
Nucleophilic sites in DNA



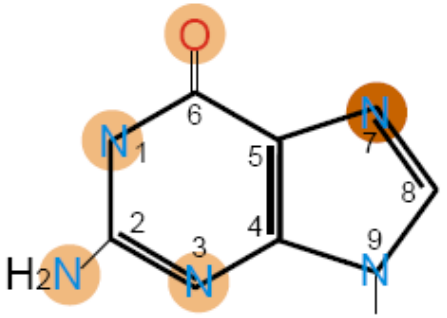
Thymine



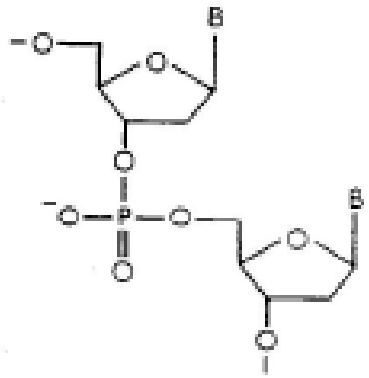
Adenine



Cytosine



Guanine

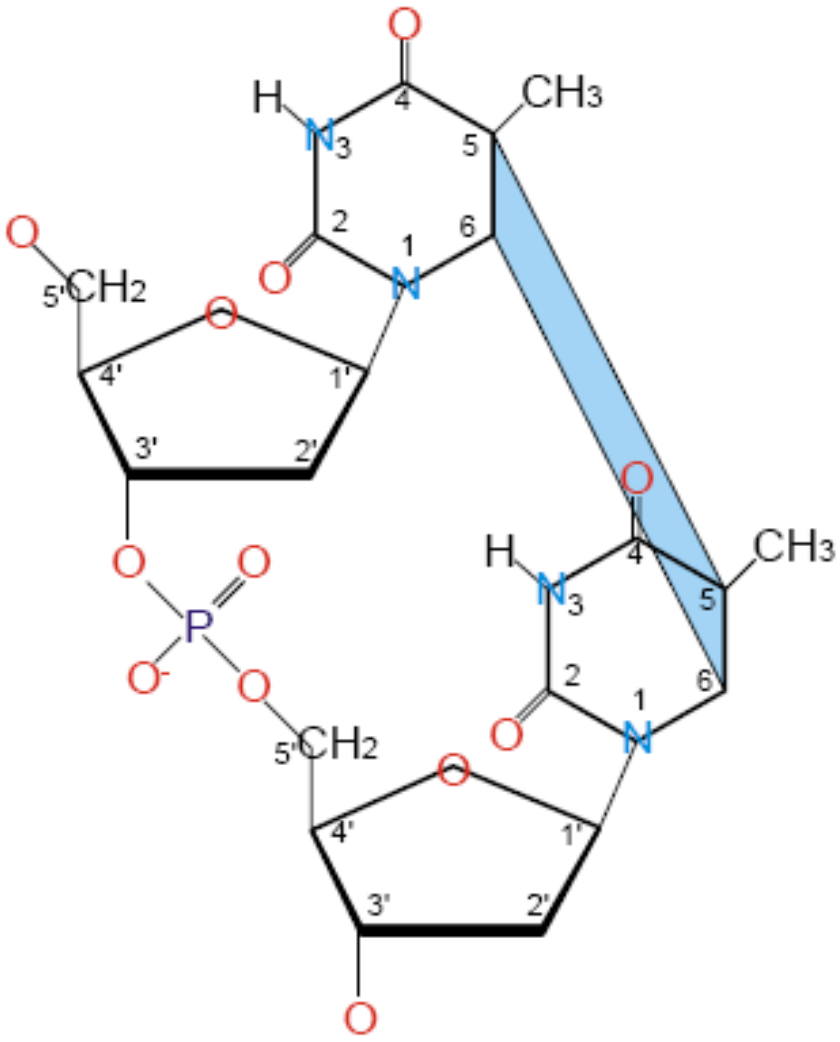


Phosphodiester

Effects of Sunlight:

Cyclobutane pyrimidine dimers (CPDs)

T-T > T-C, C-T > C-C



DNA helix bends 7-9°

http://saturn.roswellpark.org/cmb/huberman/DNA_Repair/DNA_Repair.htm

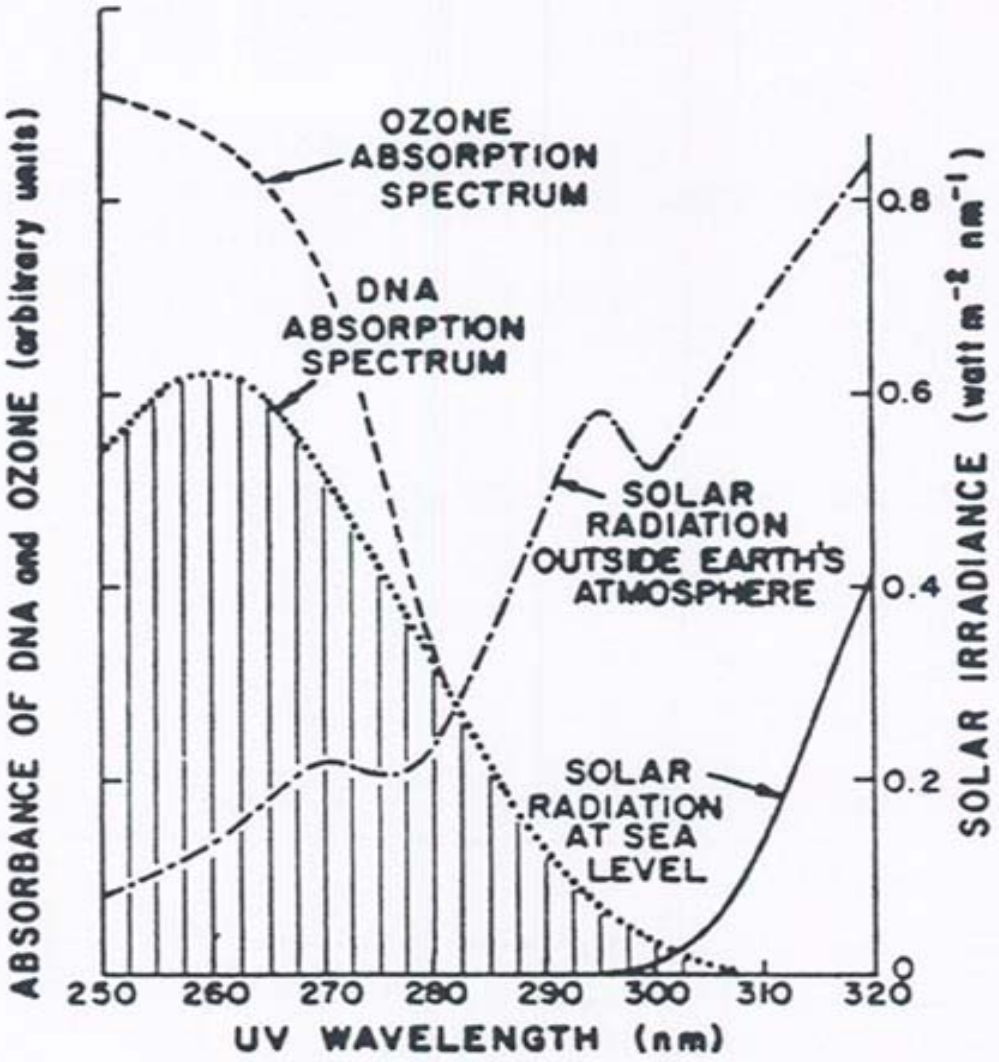
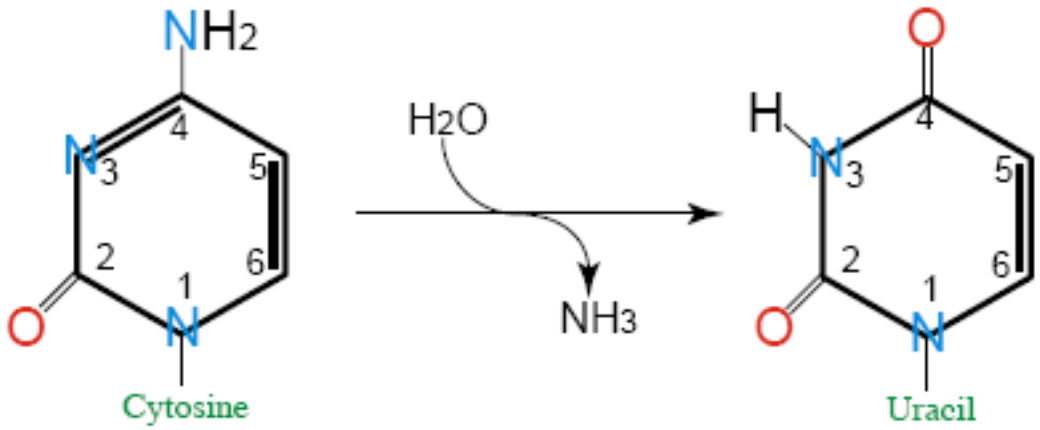


Fig. 2. Absorption spectra of DNA and ozone. For comparative purposes, the two spectra have been normalized to the same absorbance at 290 nm. The solar irradiation spectra outside the earth's atmosphere and at sea level are also shown. Note that because of absorption by stratospheric ozone, only a small fraction of solar mid-UV reaches the earth's surface and that the solar UV wavelengths which overlap with the absorption spectrum of DNA have been drastically reduced. [From Gentner and Myers (1980) with permission of Atomic Energy of Canada Limited.]



Spontaneous deamination:

~100 uracils per haploid genome per day.

Also:

Adenine→hypoxanthine
Guanine→xanthine
5-Methyl cytosine→thymine

http://saturn.roswellpark.org/cmb/huberman/DNA_Repair/DNA_Repair.htm

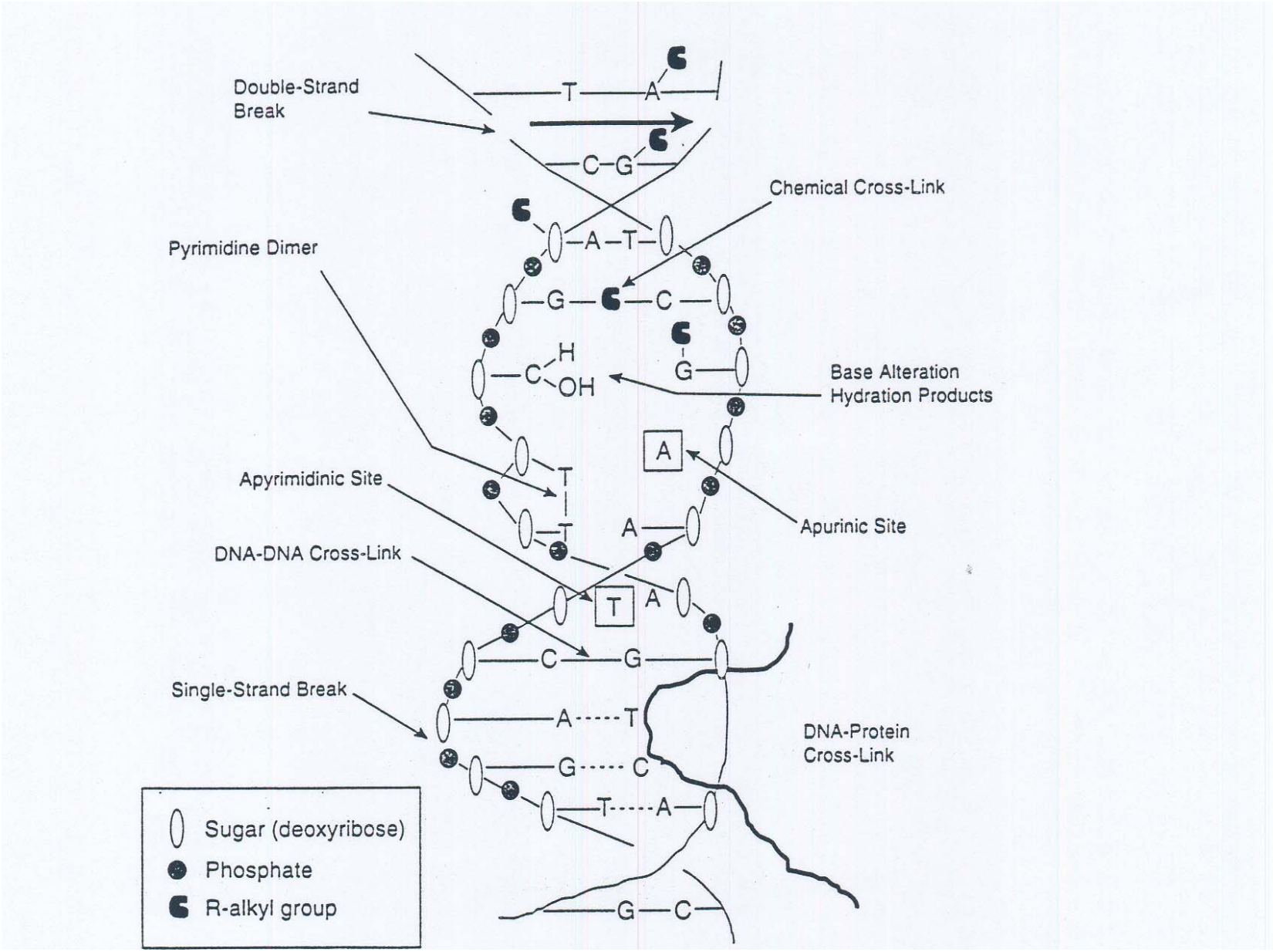
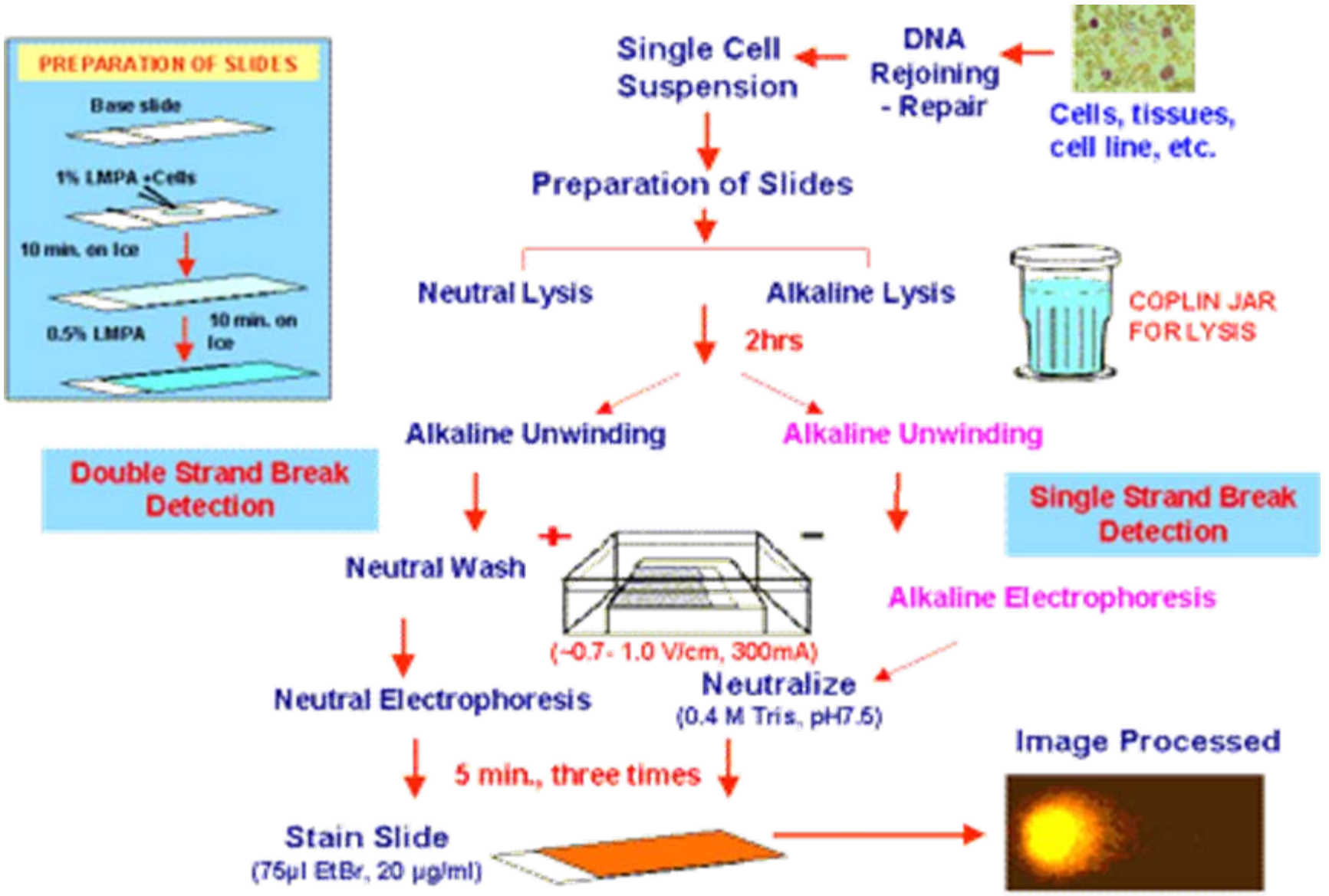
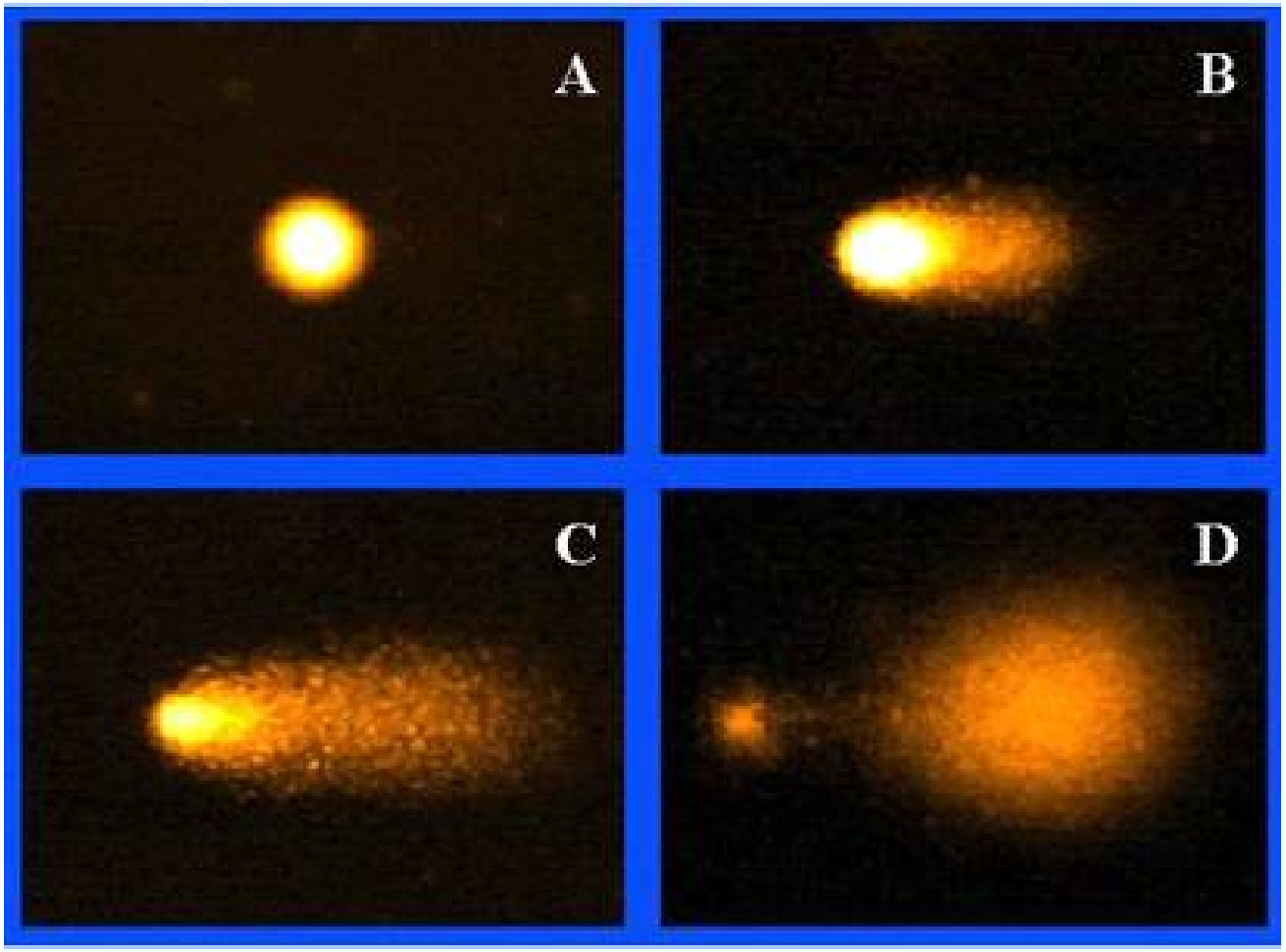


Figure 8–12. Schematic representation of chemical and radiation-induced lesions in DNA. [Adapted from Fry et al. (1982), with permission of the authors and publisher.]

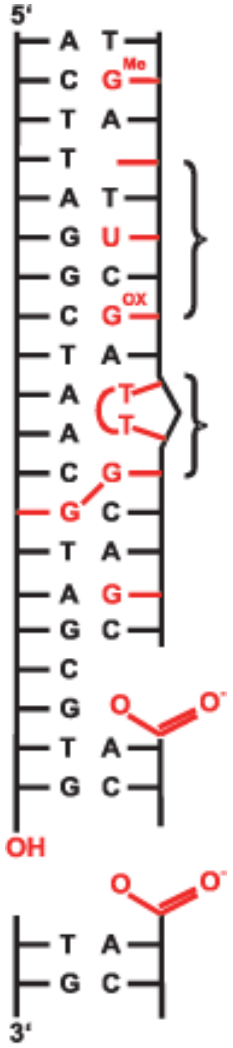
Comet assay to measure DNA damage




A
B
C
D

DNA damage and associated repair pathways

Origin	Repair pathway	DNA damage
Alkylating agents	Direct damage removal	O^6-methylguanine
Spontaneous reactions Oxygen radicals Alkylating agents Ionizing radiation	Base excision repair	Abasic sites, deaminated bases Oxidized bases Alkylated bases Oxidized bases
UV light Polycyclic aromatic hydrocarbons Cis-platin	Nucleotide Excision repair	Pyrimidine dimers Bulky adducts Intrastrand crosslinks
Replication errors	Mismatch repair	Base mismatches
Ionizing radiation DNA repair pathways	Single strand break repair	Single strand breaks
	Double strand break repair	Double strand breaks



(Alexis Corp)

Nucleotide Excision Repair

- Extremely flexible
- Corrects any damage that distorts the DNA molecule
- In all organisms, NER involves the following steps:

1. Damage recognition
2. Binding of a multi-protein complex at the damaged site
3. Double incision of the damaged strand several nucleotides away from the damaged site, on both the 5′ and 3′ sides
4. Removal of the damage-containing oligonucleotide from between the two nicks
5. Filling in of the resulting gap by a DNA polymerase
6. Ligation

http://saturn.roswellpark.org/cmb/huberman/DNA_Repair/DNA_Repair.htm

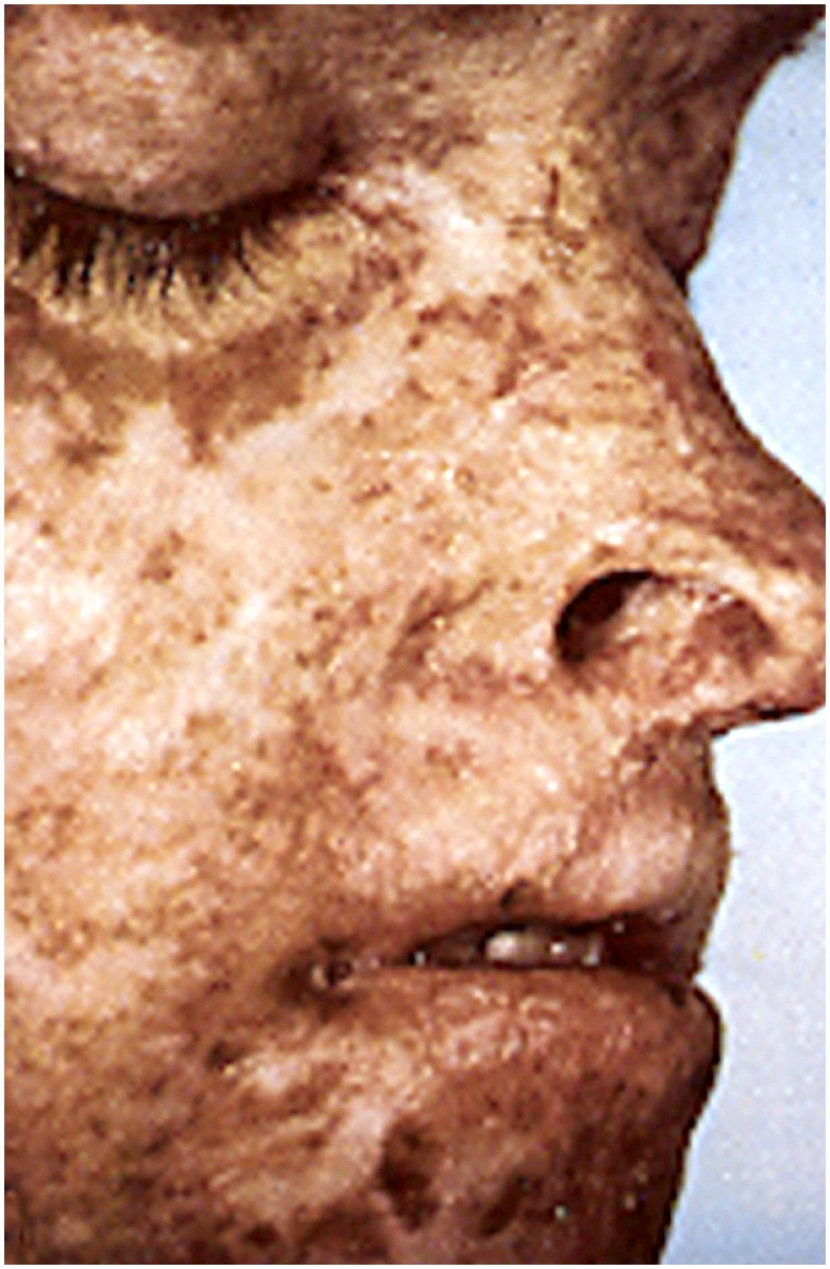
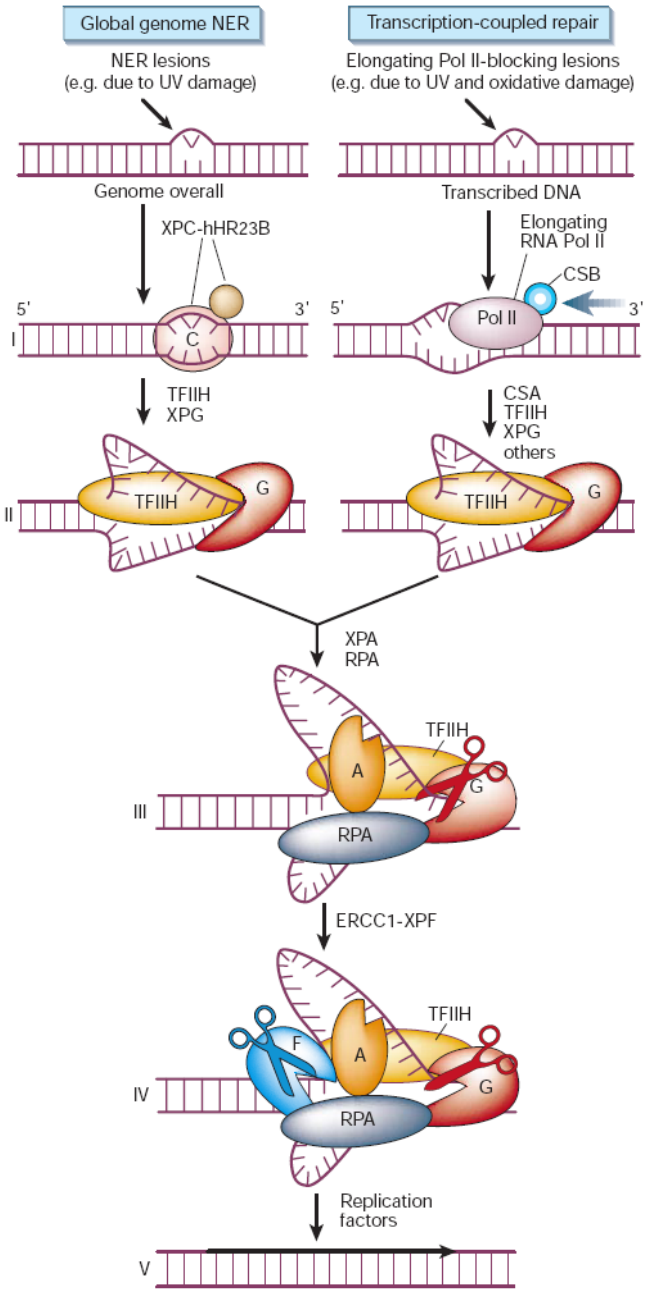
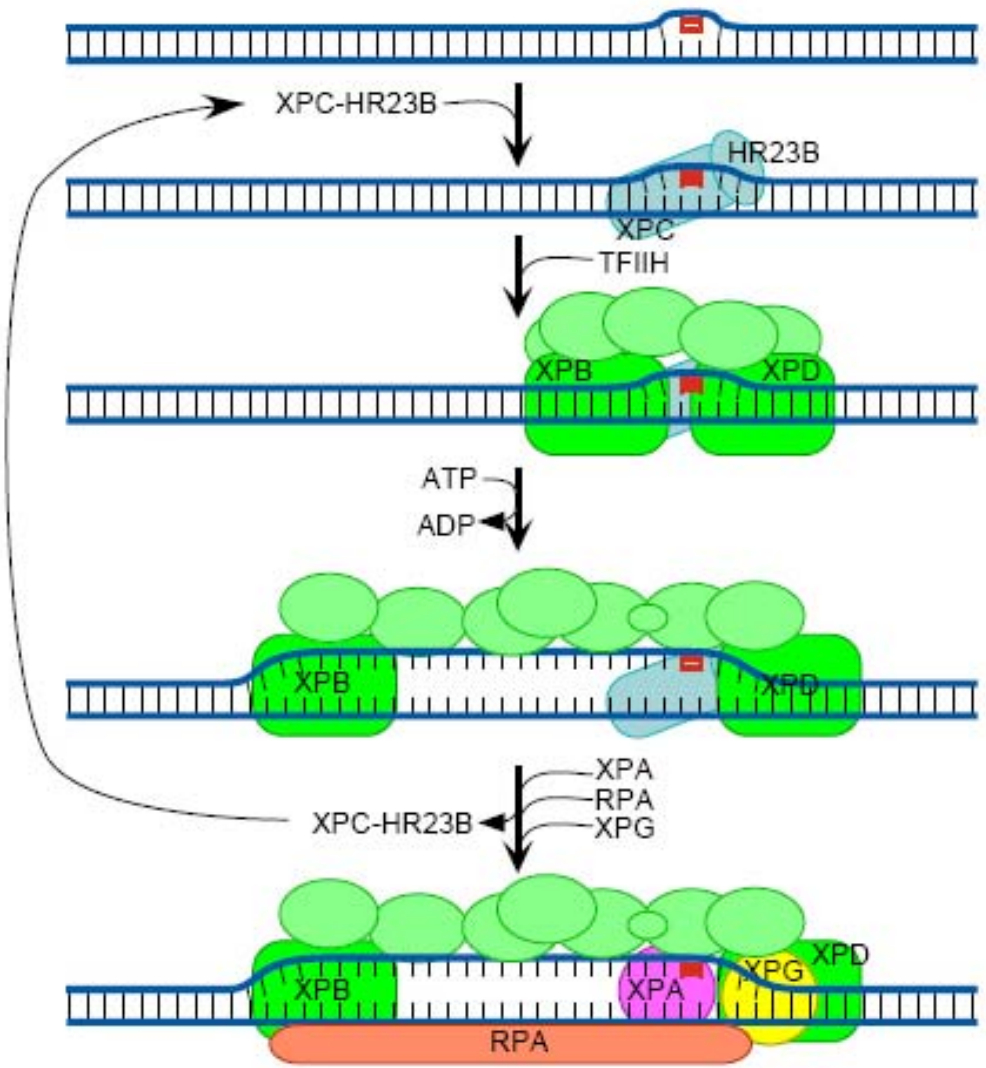


Figure 12.25 *The Biology of Cancer* (© Garland Science 2007)



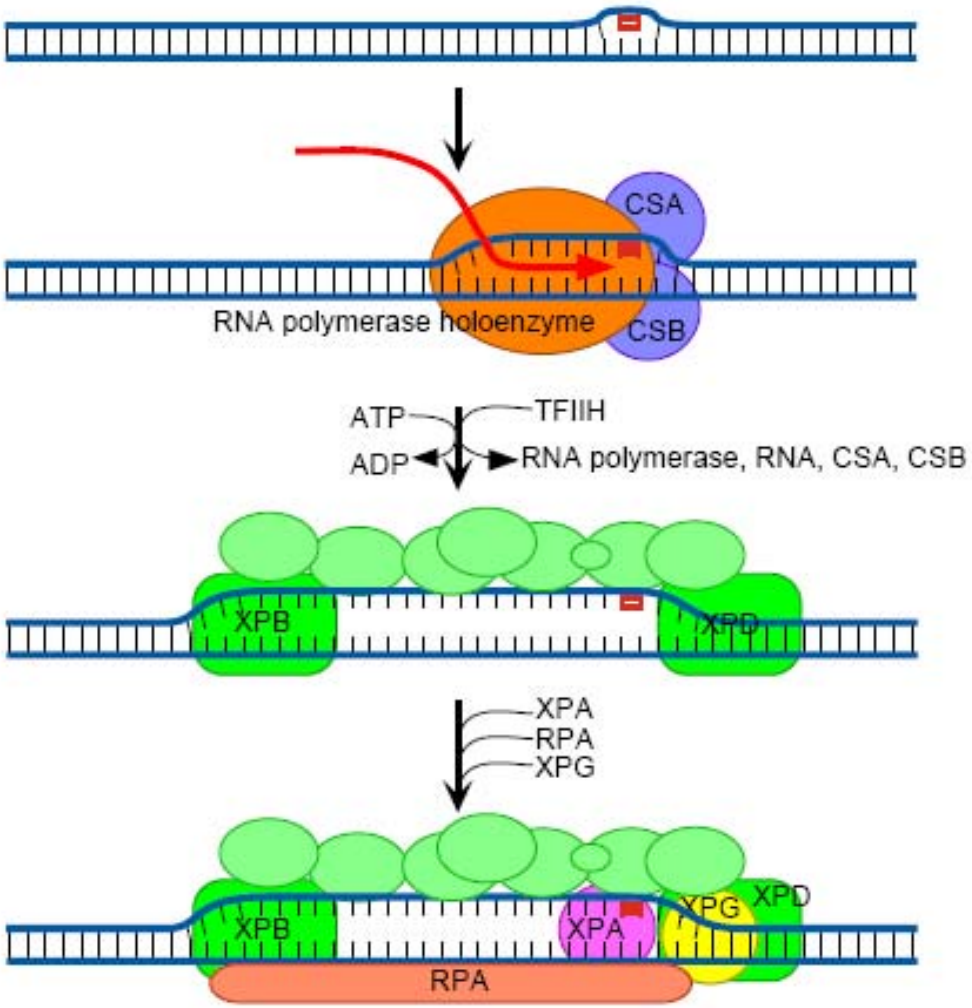
Hoeijmakers, Nature 2001.

Early Stages of Global Genome Repair



http://saturn.roswellpark.org/cmb/huberman/DNA_Repair/DNA_Repair.htm

Initial Steps of Transcription-Coupled NER



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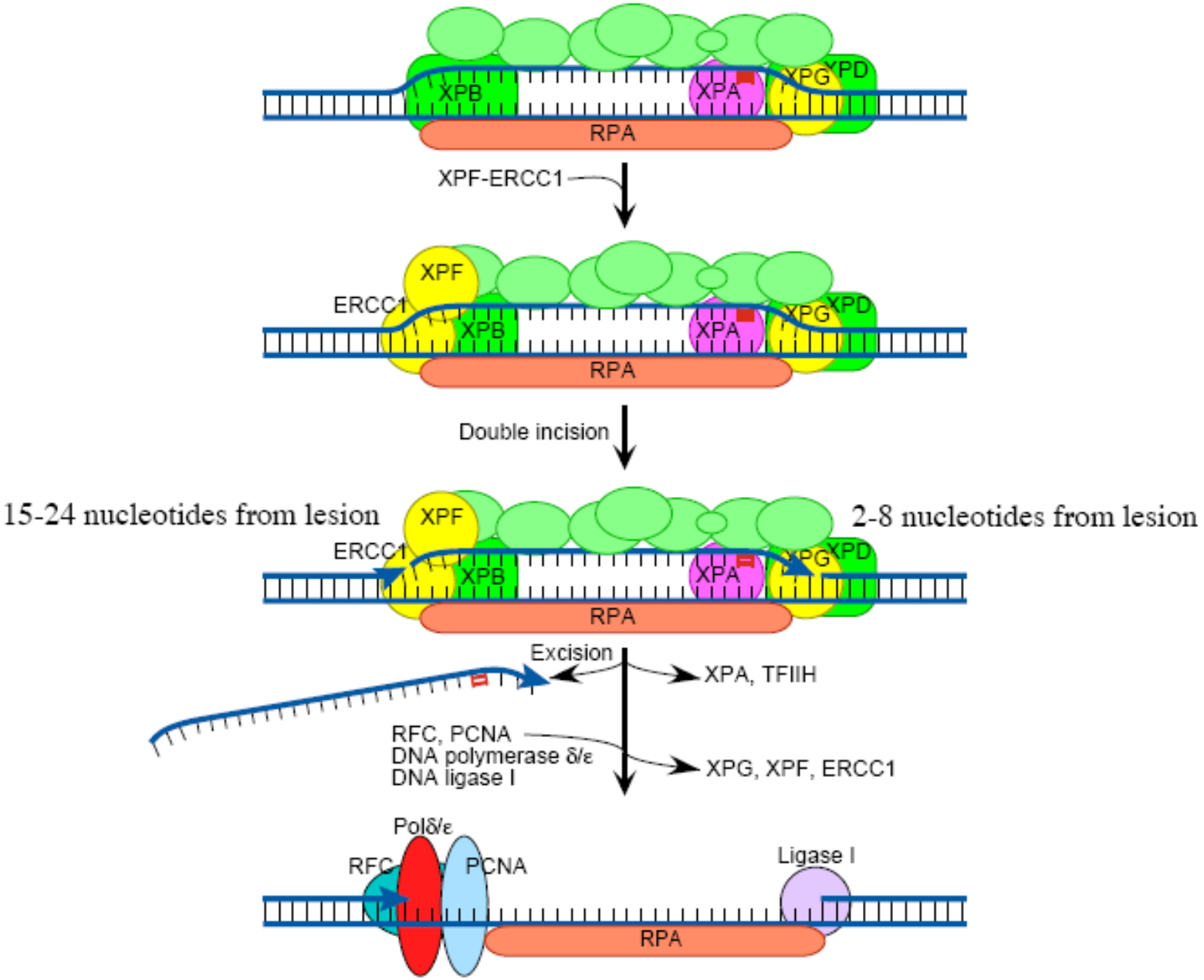
Final Steps of Eukaryotic NER



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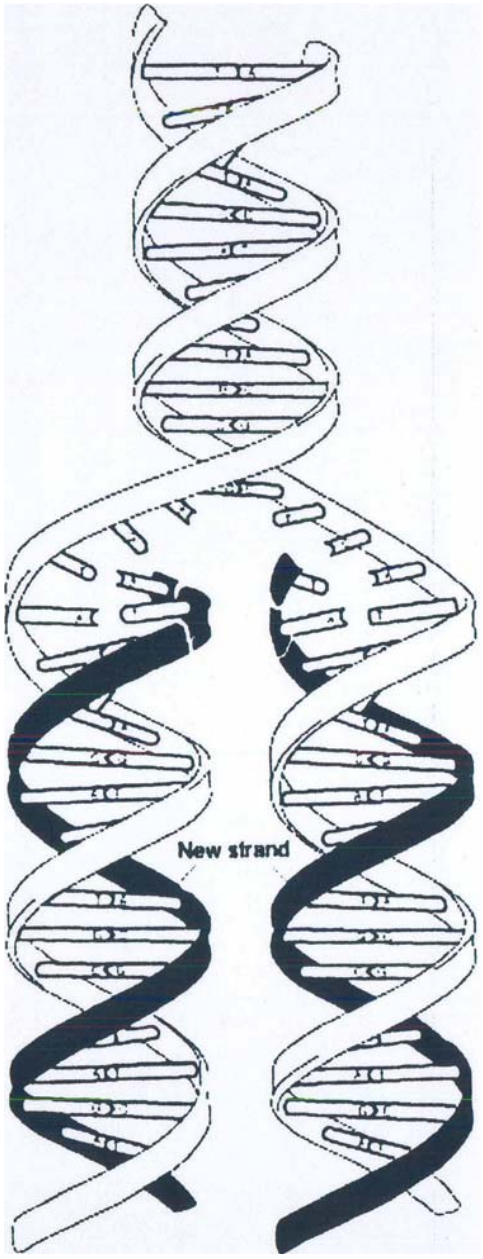
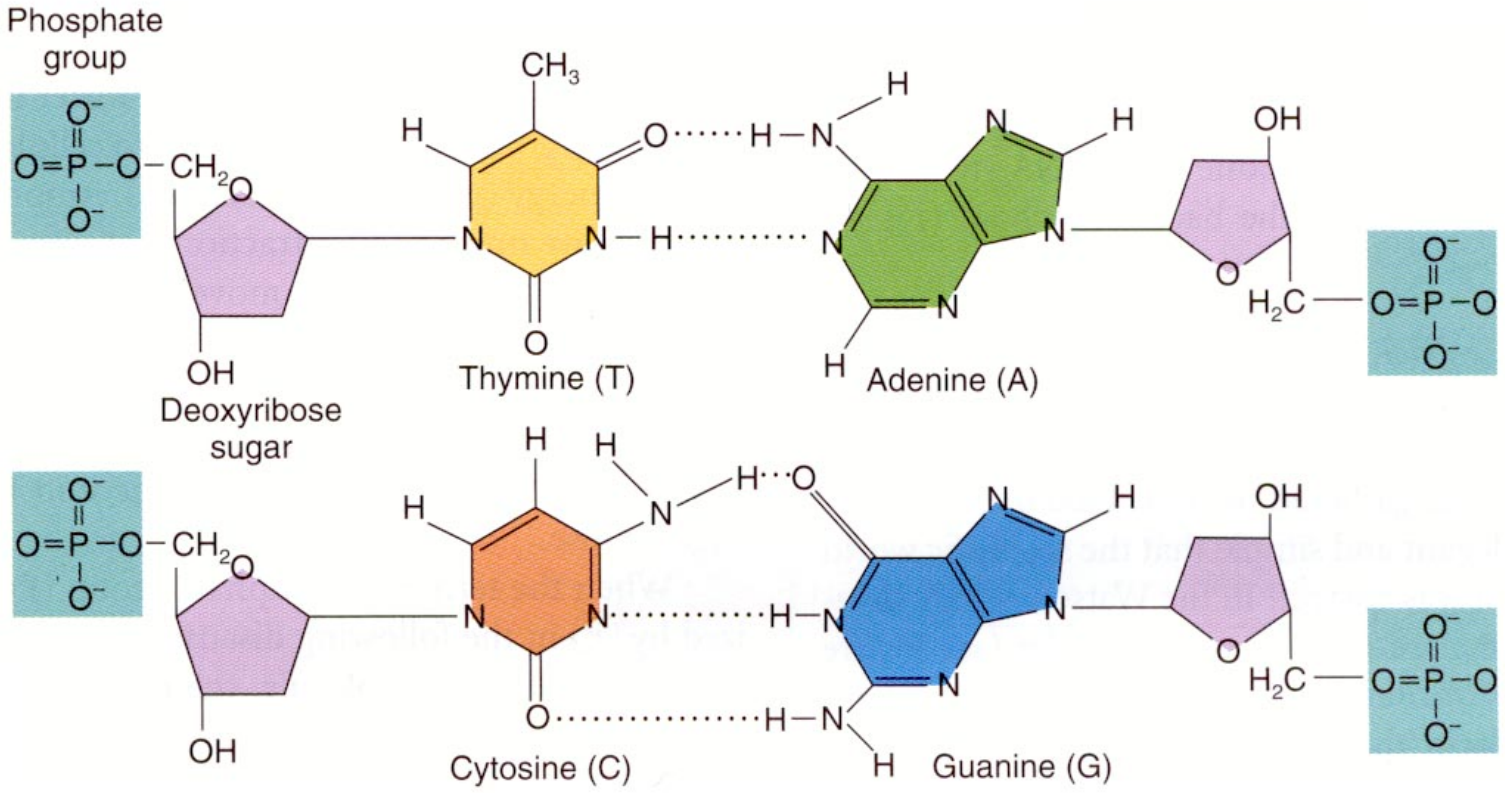
DNA Replication




Phosphate group
Deoxyribose sugar
Thymine (T)
Adenine (A)
Cytosine (C)
Guanine (G)

TYPES OF DNA MUTATION

(i) Point mutations

(a) Base substitution
(b) Frameshift - deletion or addition of one or more bases.

Missense mutations are point mutations which lead to a codon change resulting in the insertion of the wrong amino acid into a polypeptide.
Nonsense mutations are point mutations that convert a codon normally specifying an amino acid to one which is a terminator codon.

(ii) Chromosomal mutations

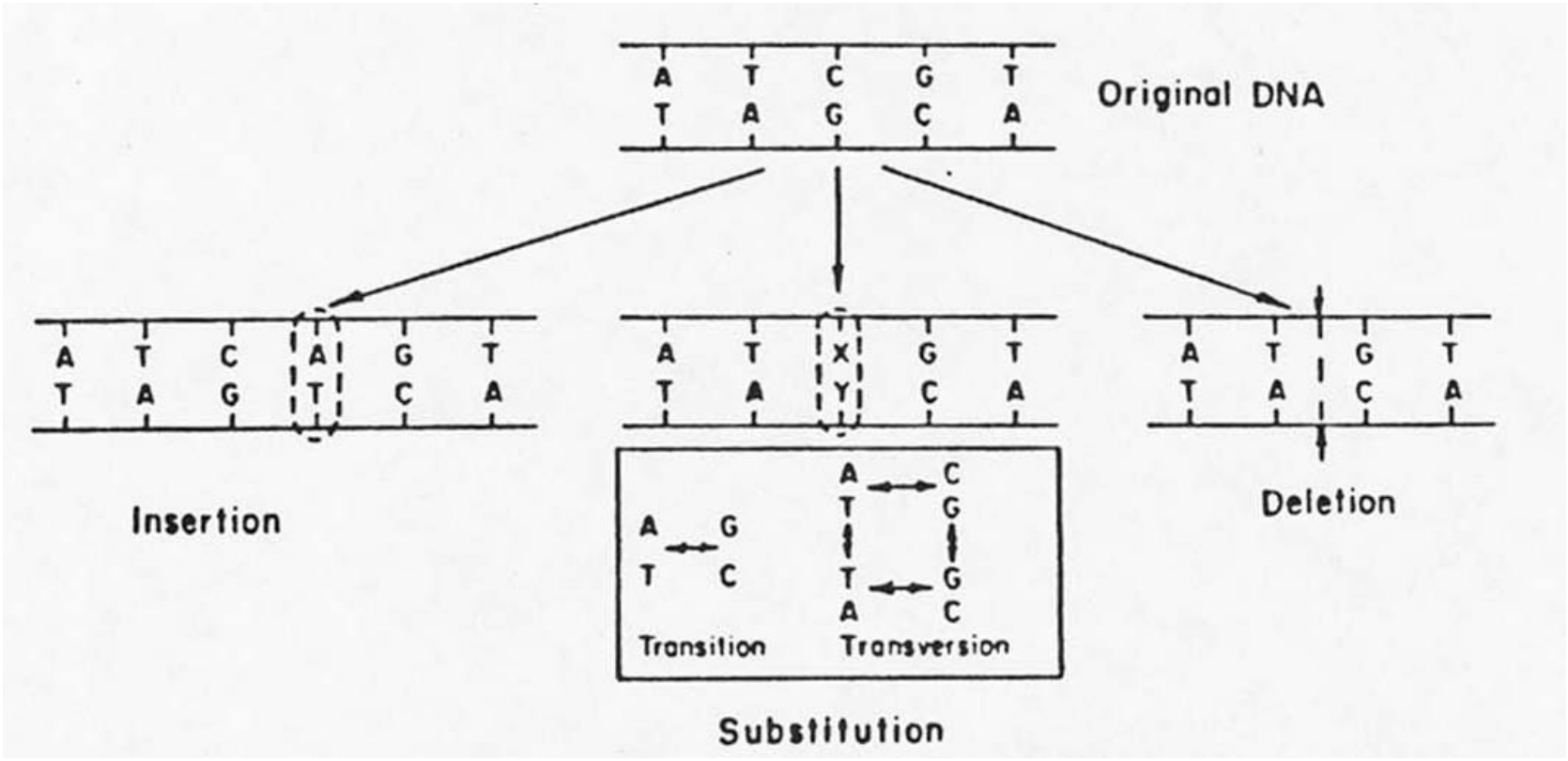
These result from breakage and reunion of chromosomal material during the cell cycle and include:

(a) Inversions - a length of chromosome is inserted back to front.
(b) Translocations - one section of chromosome becomes attached to another.
(c) Sister Chromatid Exchanges (SCE) - a consequence of the interchange of replicating DNA between chromatids at apparently homologous loci.

(iii) Genomic mutations

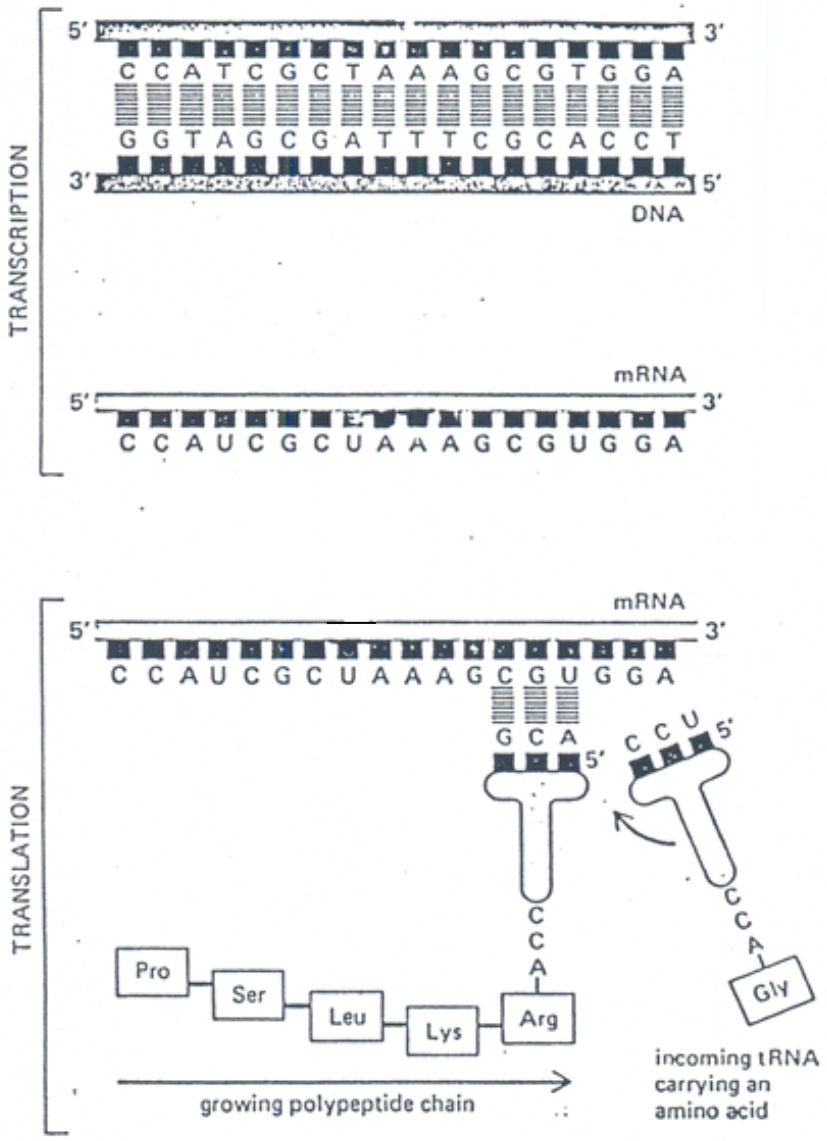
These are changes in the number of chromosomes in the genome.

(a) Polyploidy - occurs where the genome is doubled or tripled.
(b) Aneuploidy - loss or gain of single chromosomes.

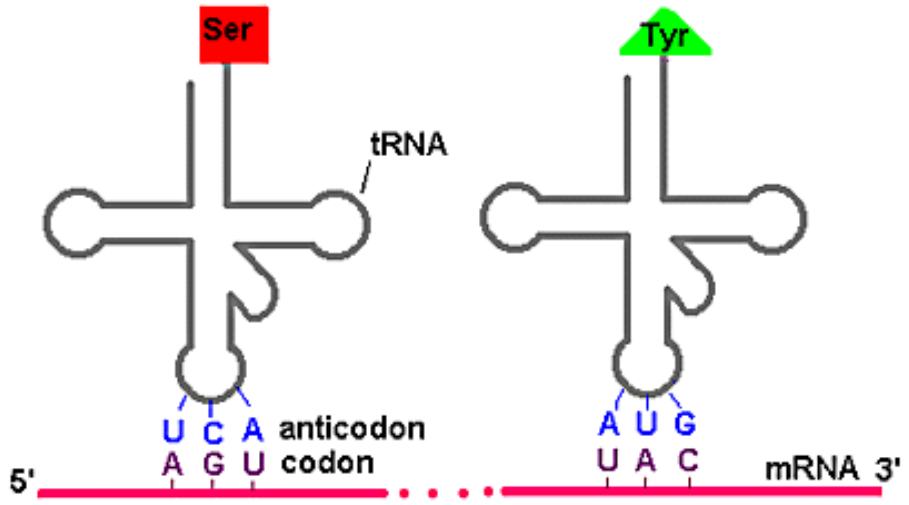


Types of mutations. Note that in the two sub-classes of frameshift mutations, insertions and deletions, one base-pair is inserted and deleted, respectively, and in the two subclasses of base pair substitutions, transitions and transversions, the purine-pyrimidine orientation is preserved and reversed, respectively.

Transcription and Translation



Information flow in protein synthesis. The nucleotides in messenger RNA are formed as a complementary copy of a segment of one strand of DNA. They are then matched three at a time to a complementary set of three nucleotides in the anticodun region of particular tRNA molecules. At the other end of the tRNA molecule, an amino acid is held in a high-energy linkage, and when matching occurs, this amino acid is added to the growing protein chain end. Translation of the mRNA nucleotide sequence into an amino acid sequence depends on complementary base-pairing between a codon of the mRNA and the corresponding anticodon of the appropriate tRNA. The molecular basis of information transfer in translation is therefore closely similar to that in DNA replication and transcription.



2nd base in codon

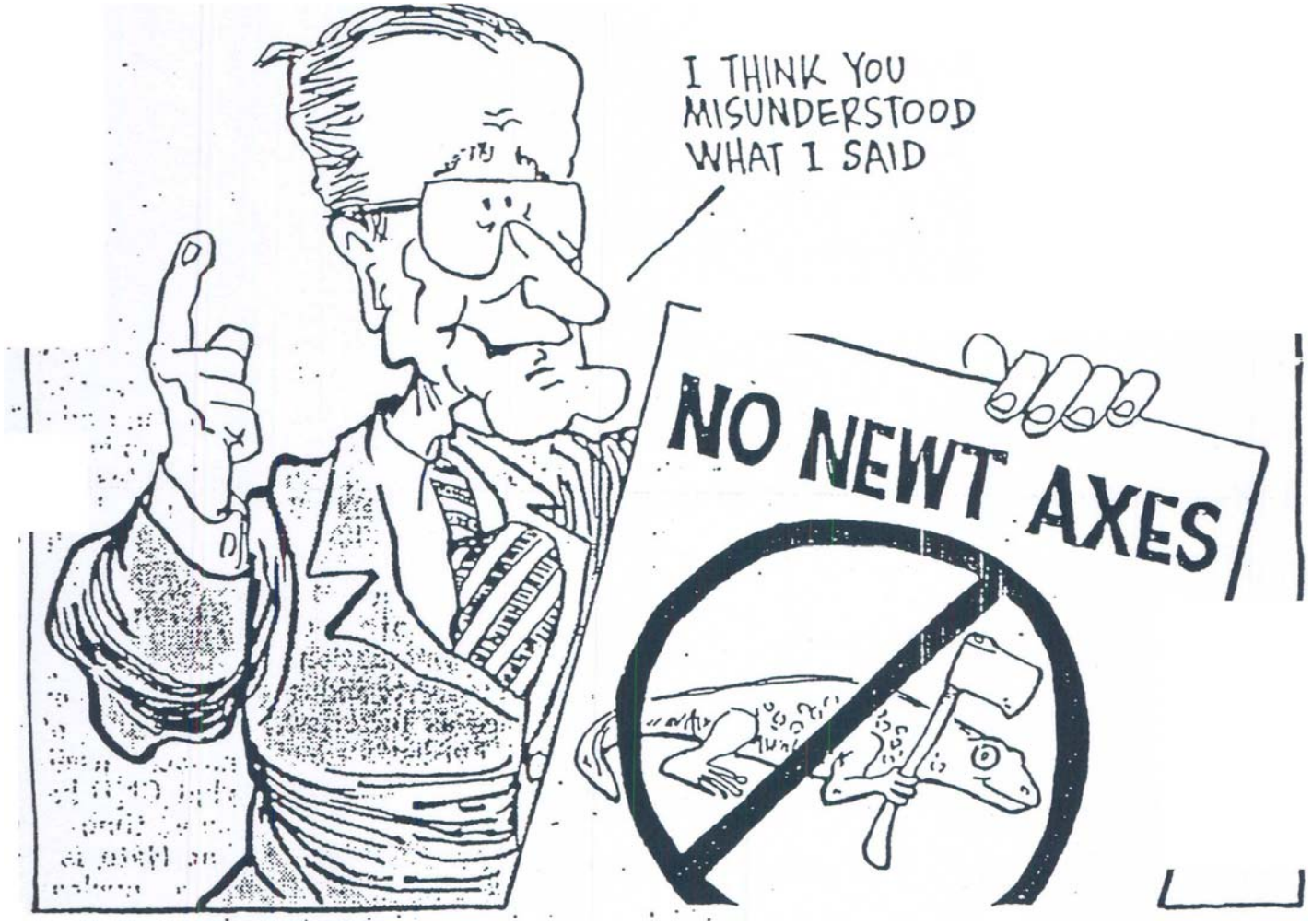
1st base in codon	U	C	A	G	3rd base in codon
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

The Genetic Code

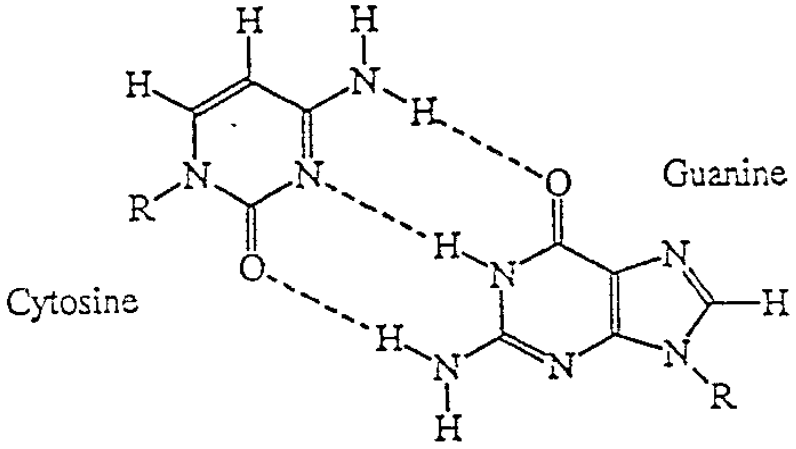
POINT MUTATIONS

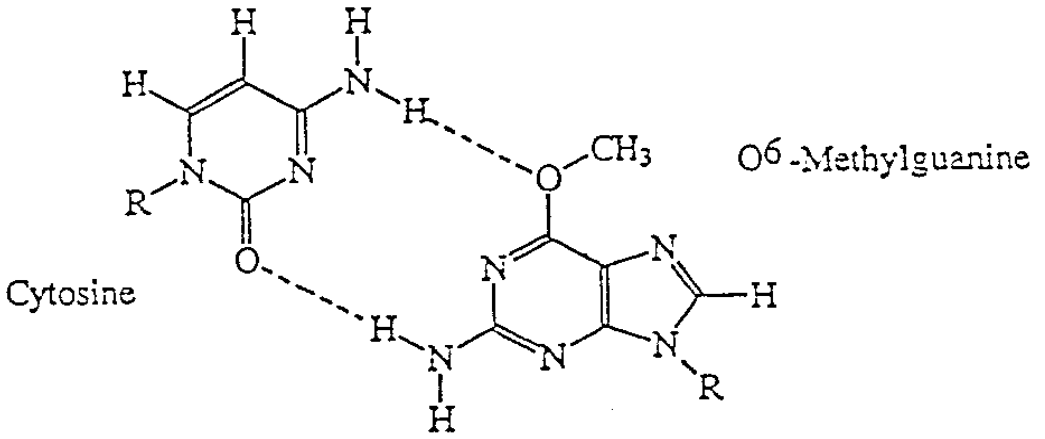
Normal sequence	-CTC-AGC-TGG-GTT-ACC- Leu Ser Trp Val Thr
Base substitution	-CTC-GGC-TGG-GTT-ACC- Leu Gly Trp Val Thr
Nonsense mutation	-CTC-AGC-TAG-GTT-ACC- Leu Ser
Frameshift mutation	-CTC-ATG-CTG-GGT-TAC-C- Leu Met Leu Gly Tyr

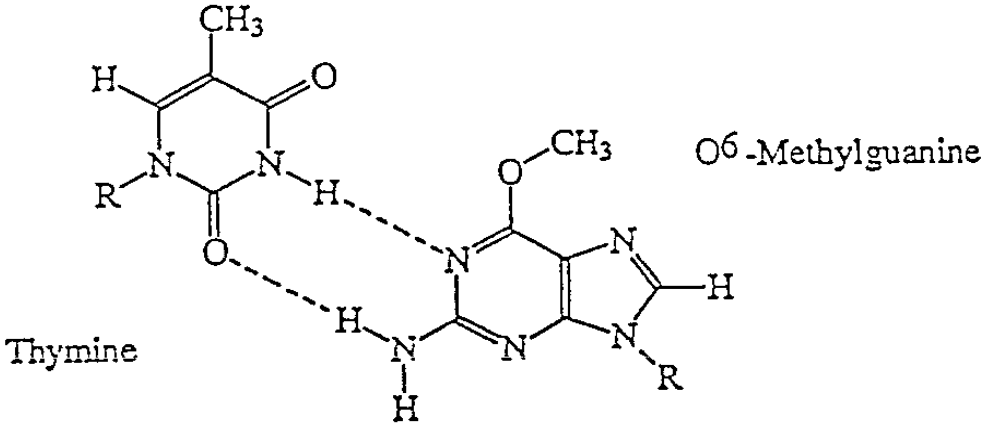
Another frame shift mutation



Base mispairing by O^6-methyl guanine







Benzo[a]pyrene adduct indicating DNA distortion that may lead to frameshift mutation during replication

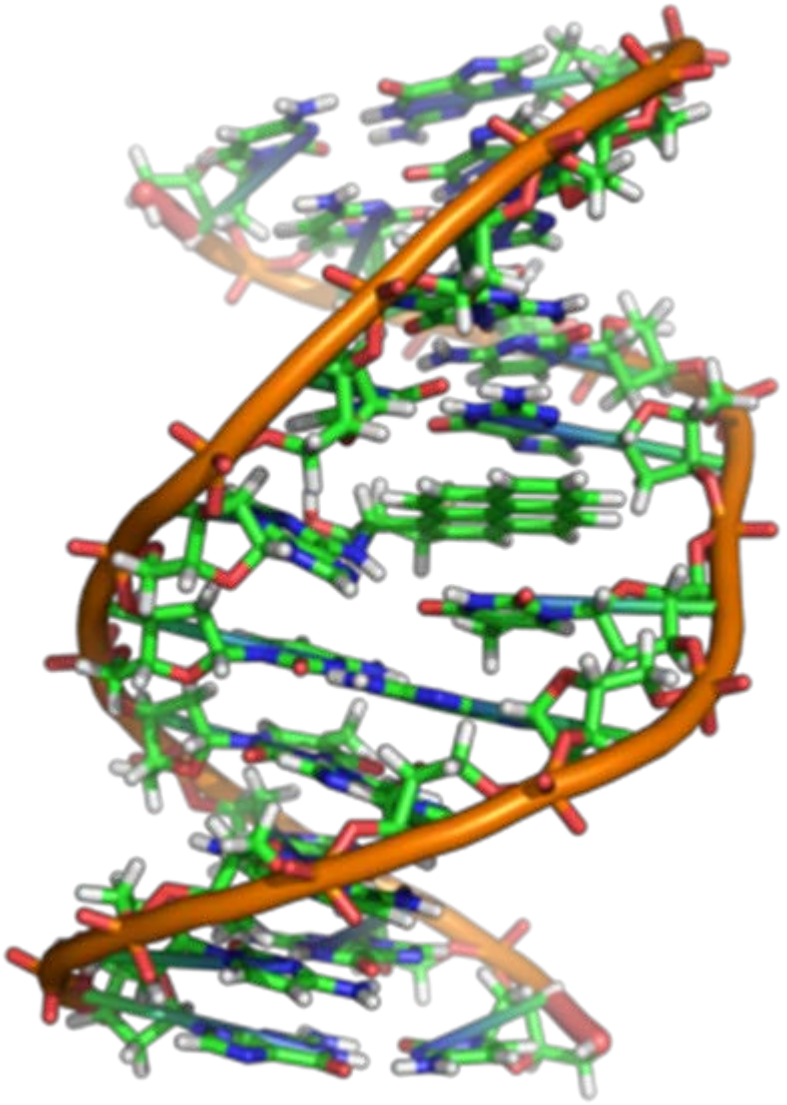


Table 2. Two classes of genes involved in carcinogenesis

Proto-oncogenes	Tumor-suppressor genes
Involved in cellular growth and differentiation	Function unknown but possibly involved in cellular growth and differentiation (negative regulators of cell growth?)
Family of genes exists	Family of genes exists
Activated (quantitatively or qualitatively) in cancers	Inactivated or lost in cancers
Activation by point mutation, chromosome translocation, or gene amplification	Inactivation by chromosome loss, chromosome deletion, point mutation, somatic recombination of gene conversion
Little evidence for involvement in hereditary cancers	Clear evidence for involvement in hereditary and non-hereditary cancers

A third class of genes would be DNA repair genes, since loss of repair function would enhance the chances of further mutations, i.e., cells would have mutator phenotype, as is the case for HNPCC cells.

Transformation of ras proto-oncogene to oncogene in transplacentally induced tumours caused by base change which may be ascribed to chemical modification of the normal gene
(Sukamar and Barbacid, Proc Natl Acad Sci U S A. 1990 87:718-22)

Codon	11	12	13
Normal aa sequence	Ala	Gly	Gly
Normal gene sequence	GCT	GGA CCT	GGC
Modification of DNA by MNU		Me GGA CCT	
DNA synthesis 1		Me GGA CTT	
DNA synthesis 2		CTT	
Oncogene sequence	GCT	GAA	GGC
Transforming protein	Ala	Glu	Gly

Examples of human cancers with base substitution mutations in the p53 tumor suppressor gene

Cancer	No. of mutations detected in tumor cell lines and tumors	Mutational "hotspots" (codon)	Mutations at					
			A:T	G:C	CpG-TpG	G:C-A:T	G:C-T:A	G:C-C:G
Lung	43	273	6	37	7	12	19	6
Breast	31		5	26	4	13	7	6
Colon	39	175,248 273,282	7	32	26	31	0	1
Esophagus	37		12	25	7	16	9	0
Liver	19	249	1	17	0	3	14	1
Bladder	15		3	12	5	7	2	3
Leukemia and lymphomas	53		19	34	25	30	2	2
Sarcomas	12		0	12	7	8	2	2
Brain	20	273	3	17	9	15	1	1

Preferential formation of Benzo(a)pyrene adducts at lung cancer mutational hotspots in *P53* gene
(Denissenko et al Science 274:430-432, 1996)

- About 60 % of human lung cancers contain mutations in the *P53* tumor suppressor gene
- The *P53* mutation database includes more than 500 entries of sequenced *P53* mutations for lung cancer. There is a large percentage of G to T transversion mutations
- Benzo(a)pyrene diol epoxide (BPDE) frequently causes G to T transversions
- Mutational hotspots for p53 in lung cancer are found in codons 248 and 273.
- Denissenko et al used an LMPCR assay to map the distribution of BPDE adducts in the exons of the *P53* gene in BPDE-treated bronchial epithelial cells
- Observed strong and selective adduct formation at guanines in codons 248 and 273.
- Thus targeted adduct formation rather than phenotypic selection appears to shape *P53* mutational spectrum in lung cancer.
- These results provide a direct etiological link between a defined chemical carcinogen and human cancer

Sir Percival Pott

Table 8–22
Exposures to Chemical Carcinogens in the Workplace

Established

AGENT	INDUSTRIES AND TRADES WITH PROVED EXCESS CANCERS AND EXPOSURE	PRIMARY AFFECTED SITE
Para-aminodiphenyl	Chemical manufacturing	Urinary bladder
Asbestos	Construction, asbestos mining and milling, production of friction products and cement	Pleura, peritoneum, bronchus
Arsenic	Copper mining and smelting	Skin, bronchus, liver
Alkylating agents (mechloroethamine hydrochloride and bis[chloromethyl]ether)	Chemical manufacturing	Bronchus
Benzene	Chemical and rubber manufacturing, petroleum refining	Bone marrow
Benzidine, beta-naphthylamine, and derived dyes	Dye and textile production	Urinary bladder
Chromium and chromates	Tanning, pigment making	Nasal sinus, bronchus
Isopropyl alcohol manufacture	Chemical manufacturing	Cancer of paranasal sinuses
Nickel	Nickel refining	Nasal sinus, bronchus
Polynuclear aromatic hydrocarbons from coke, coal tar, shale, mineral oils, and creosote	Steel making, roofing, chimney cleaning	Skin, scrotum, bronchus
Vinyl chloride monomer	Chemical manufacturing	Liver
Wood dust	Cabinetmaking, carpentry	Nasal sinus

Suspected

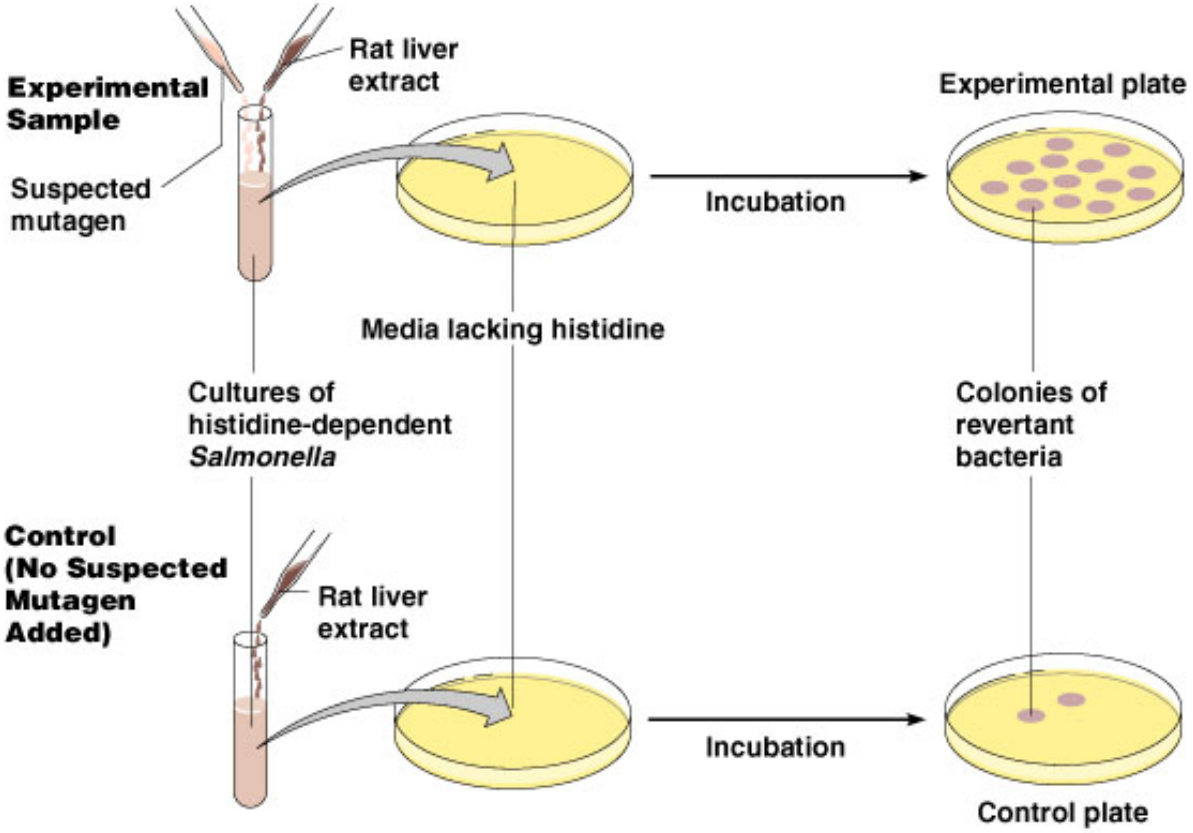
AGENT	INDUSTRIES AND TRADES	SUSPECTED HUMAN SITES
Acrylonitrile	Chemical and plastics	Lung, colon, prostate
Beryllium	Beryllium processing, aircraft manufacturing, electronics, secondary smelting	Bronchus
Cadmium	Smelting, battery making, welding	Bronchus
Ethylene oxide	Hospitals, production of hospital supplies	Bone marrow
Formaldehyde	Plastic, textile, and chemical production; health care	Nasal sinus, bronchus
Synthetic mineral fibers (e.g., fibrous glass)	Manufacturing, insulation	Bronchus
Phenoxyacetic acid	Farming, herbicide application	Soft tissue sarcoma
Polychlorinated biphenyls	Electrical-equipment production and maintenance	Liver
Organochlorine pesticides (e.g., chlordane, dieldrin)	Pesticide manufacture and application, agriculture	Bone marrow
Silica	Casting, mining, refracting	Bronchus

SOURCE: Modified from Cullen et al. (1990).

SHORT-TERM "SCREENING" ASSAYS FOR CARCINOGENICITY AND MUTAGENICITY

TEST	END POINT
PROKARYOTE MUTAGENESIS IN VITRO	BACK OR FORWARD MUTATIONS IN SPECIFIC BACTERIAL STRAINS (WITH ADDED TISSUE PREPARATION)
HOST-MEDIATED PROKARYOTE MUTAGENESIS IN VIVO	BACK OR FORWARD MUTATIONS IN SPECIFIC BACTERIAL STRAINS
DOMINANT LETHAL ASSAY	DEATH OF IMPLANTED EMBRYOS IN MAMMALIAN SPECIES
SPERM ABNORMALITY INDUCTION	MICROSCOPICALLY ABNORMAL SPERM
MUTAGENESIS IN CULTURED CELLS	SCORING OF DOMINANT OR LINKED MUTATIONS
MUTATIONS IN NEUROSPORA	SCORING OF MUTATIONS
MITOTIC RECOMBINATION IN YEAST	CONVERSION OF HETEROZYGOUS ALLELES TO HOMOZYGOUS STATE
DROSOPHILA	RECESSIVE LETHAL TEST, DOMINANT LETHAL TEST
INDUCED CHROMOSOMAL ABERRATIONS	VISIBLE ALTERATIONS IN KARYOTYPE
MICRONUCLEUS TEST	APPEARANCE OF MICRONUCLEI IN BONE MARROW CELLS IN VIVO
SISTER CHROMATID EXCHANGE	VISIBLE EXCHANGE OF DIFFERENTIALLY LABELED SISTER CHROMATIDS
DNA REPAIR IN VIVO OR IN VITRO	UNSCHEDULED DNA SYNTHESIS AND/OR DNA STRAND BREAKS
FIDELITY OF DNA POLYMERASE IN VITRO	ALTERED FIDELITY OF DNA SYNTHESIS IN CELL-FREE SYSTEM

Ames test



1. Two cultures are prepared of *Salmonella* bacteria that have lost the ability to synthesize histidine (histidine-dependent).
2. The suspected mutagen is added to the experimental sample only; rat liver extract (an activator) is added to both samples.
3. Each sample is poured onto a plate of medium lacking histidine. The plates are then incubated at 37°C for two days. Only bacteria whose histidine-dependent phenotype has mutated back (reverted) to histidine-synthesizing will grow into colonies.
4. The numbers of colonies on the experimental and control plates are compared. The control plate may show a few spontaneous histidine-synthesizing revertants. The test plates will show an increase in the number of histidine-synthesizing revertants if the test chemical is indeed a mutagen and potential carcinogen. The higher the concentration of mutagen used, the more revertant colonies will result.

Mutagenicity Testing

Bacterial mutation assays using reverse mutation - Ames test

Bacterial strains are used which carry base substitutions or frameshift mutations in operons coding for synthesis of specific amino acids i.e. the mutants (unlike the wild-types) cannot synthesize all their required amino acids from inorganic sources of nitrogen. The assay determines whether the test chemical can reverse the effect of the preexisting mutation by introducing a second mutation. Because the bacteria used in these assays lack the enzymes which metabolize pro-carcinogens to ultimate carcinogens, a rat liver microsome fraction (59) is added as a surrogate of mammalian metabolism. These assays detect point mutations only.

Assay

Bacteria, test compound, S9mix, molten dilute agar (+ trace of histidine) are mixed and poured onto the surface of minimal-agar plates containing glucose. The top layer sets and the plates are incubated at 37°C for 2-3 days. In the first few hours the whole population of bacteria grow until all the histidine is used up. After this only histidine reverse mutants will grow to form visible colonies, each colony descerx1ing from a single revertant. An increase in revertant colonies with increasing dose of test compound indicates a mutagenic response. Parallel assays without 59 are included to look for direct-acting test compounds.

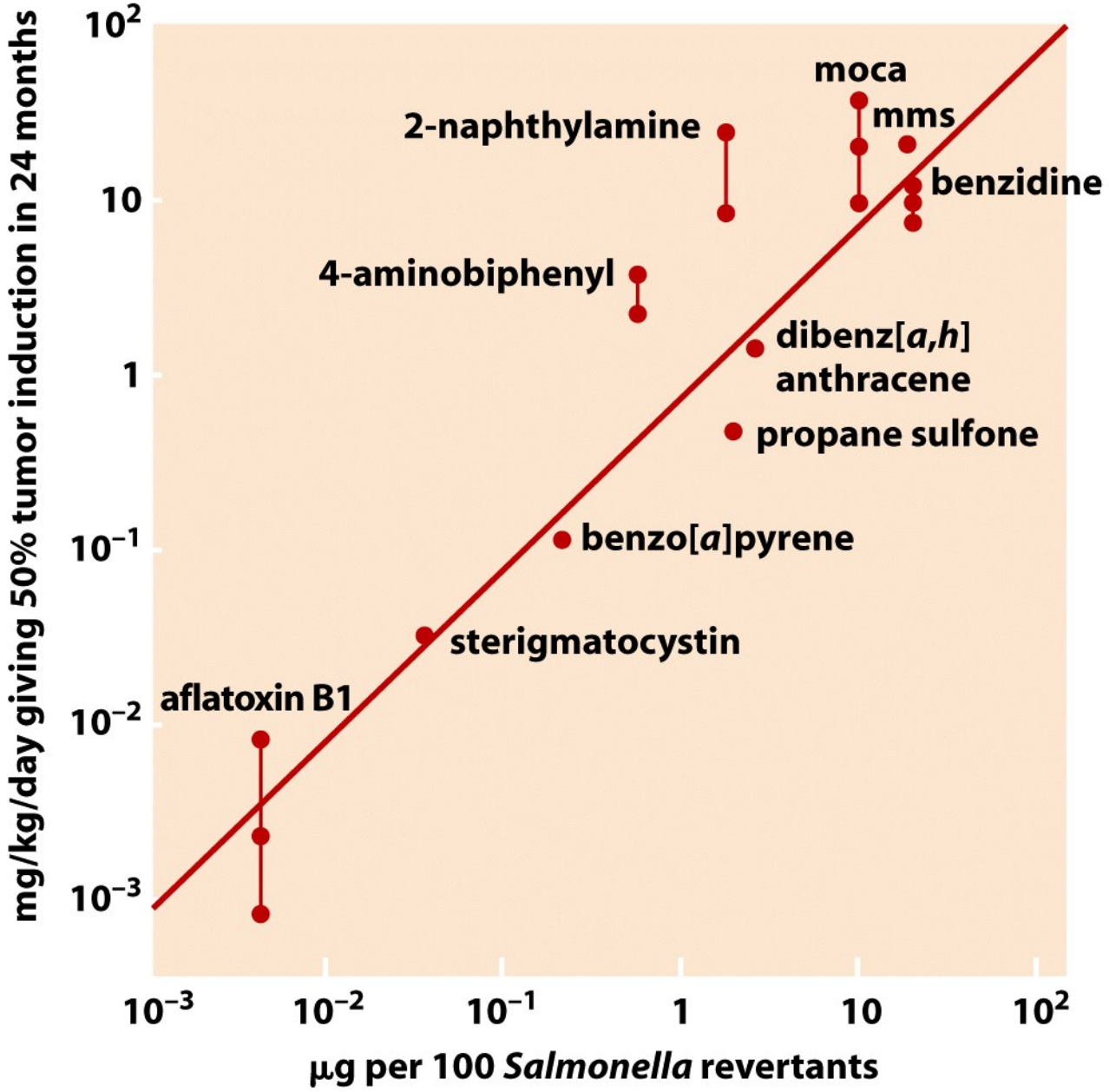
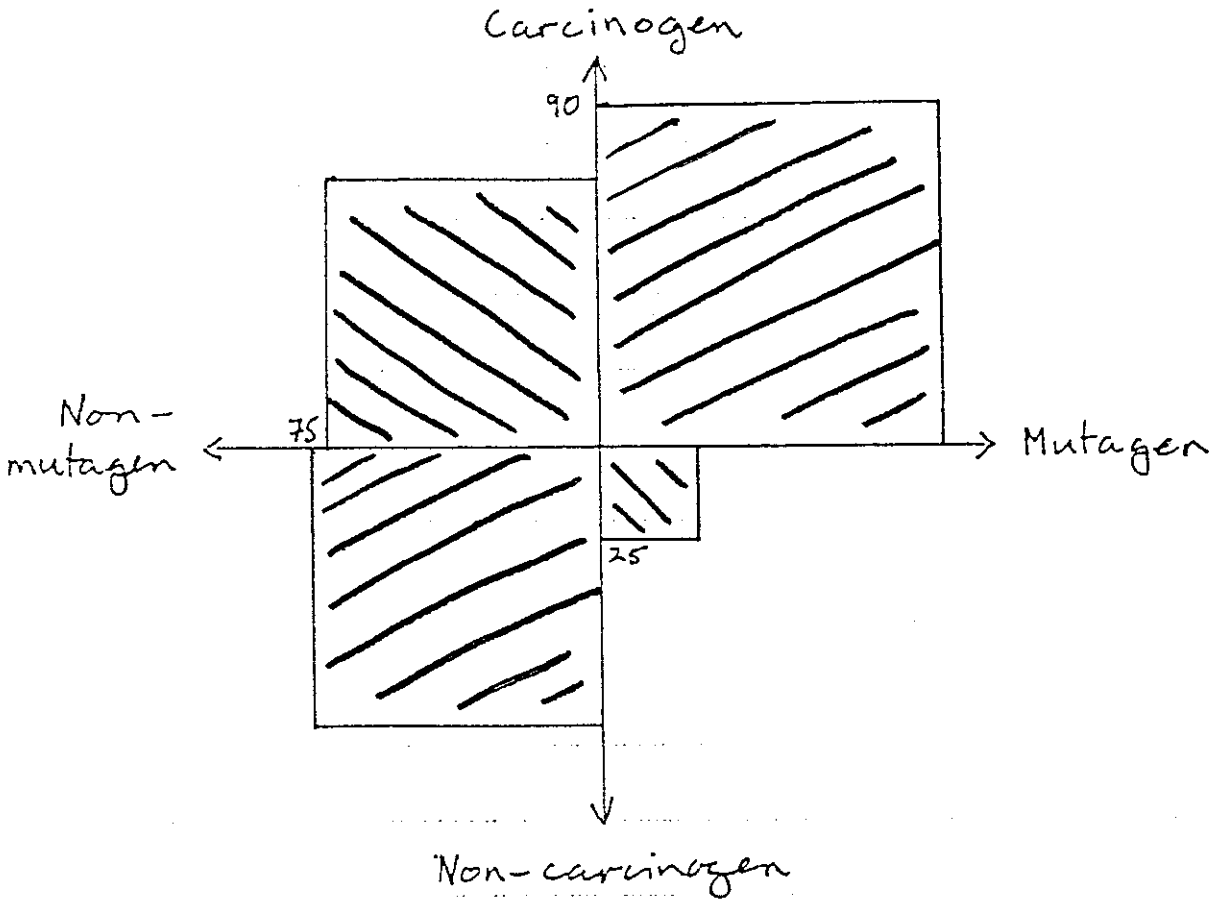


Figure 2.25 *The Biology of Cancer* (© Garland Science 2007)



Correlation between carcinogens and mutagens

Ashby & Tennant (1991)

Possible explanations for nonmutagenic carcinogens and mutagenic noncarcinogens.

Putative nonmutagenic carcinogens

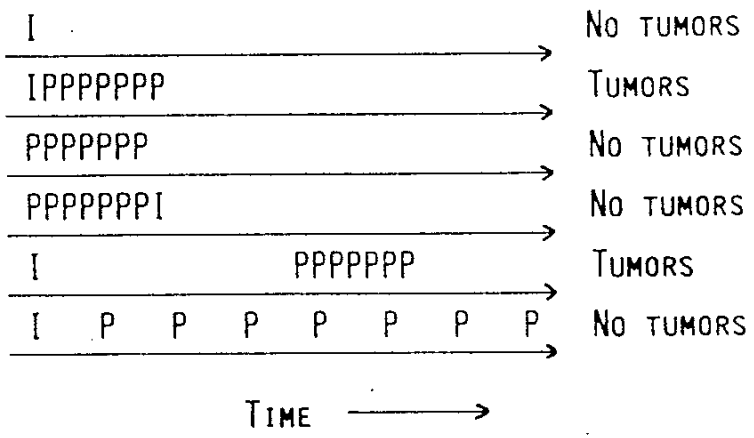
- Unusual metabolic activation is required for activity in mutational assays (examples: amitrole and DES*).
- Mutagenic activity of chemical is limited to chromosomal level, i.e., structural or numerical chromosome changes (examples: benzene, arsenicals, DES, and asbestos).
- Chemicals are inhibitors of DNA methylation (examples: 5-azacytidine and ethionine).
- Chemicals act as tumor promoters (examples: Phenobarbital, 2,3,7,8-tetra-chlorodibenzo-p-dioxin, hormones, and asbestos).

Mutagenic noncarcinogens

- Mutagenicity exhibited in test system may not be exhibited *in vivo* due, for example, to differences in metabolic activation/detoxification or DNA repair.
- Mutagenicity of a chemical may be limited to a particular type of genetic change (e.g., aneuploidy); because carcinogenesis requires multiple genetic events of diverse types, a particular chemical mutagen may affect only a singlestep in the carcinogenesis process.
- Mutagenicity per se is not sufficient for carcinogenicity due to lack of pro-liferation in the target tissue.
- Less-than-lifetime carcinogenicity studies may be too short to detect late-appearing tumors.
- *In vivo* rodent models are insensitive to weak mutagens.

*DES, diethylstilbestrol.

INITIATION AND PROMOTION PHASES OF CARCINOGENESIS ON MOUSE SKIN



I = Application of initiator P = Application of promotor

Promotion is the experimentally defined process by which the initiated cell clonally expands into a visible tumour, often a benign lesion such as a papilloma.

- This process involves at least some epigenetic factors that selectively influence the proliferation of the initiated cell. Whether genetic mechanisms are involved is unclear.
- The process takes place over a prolonged period of time
- It is reversible
- Phorbol ester is the archetypal tumour promotor

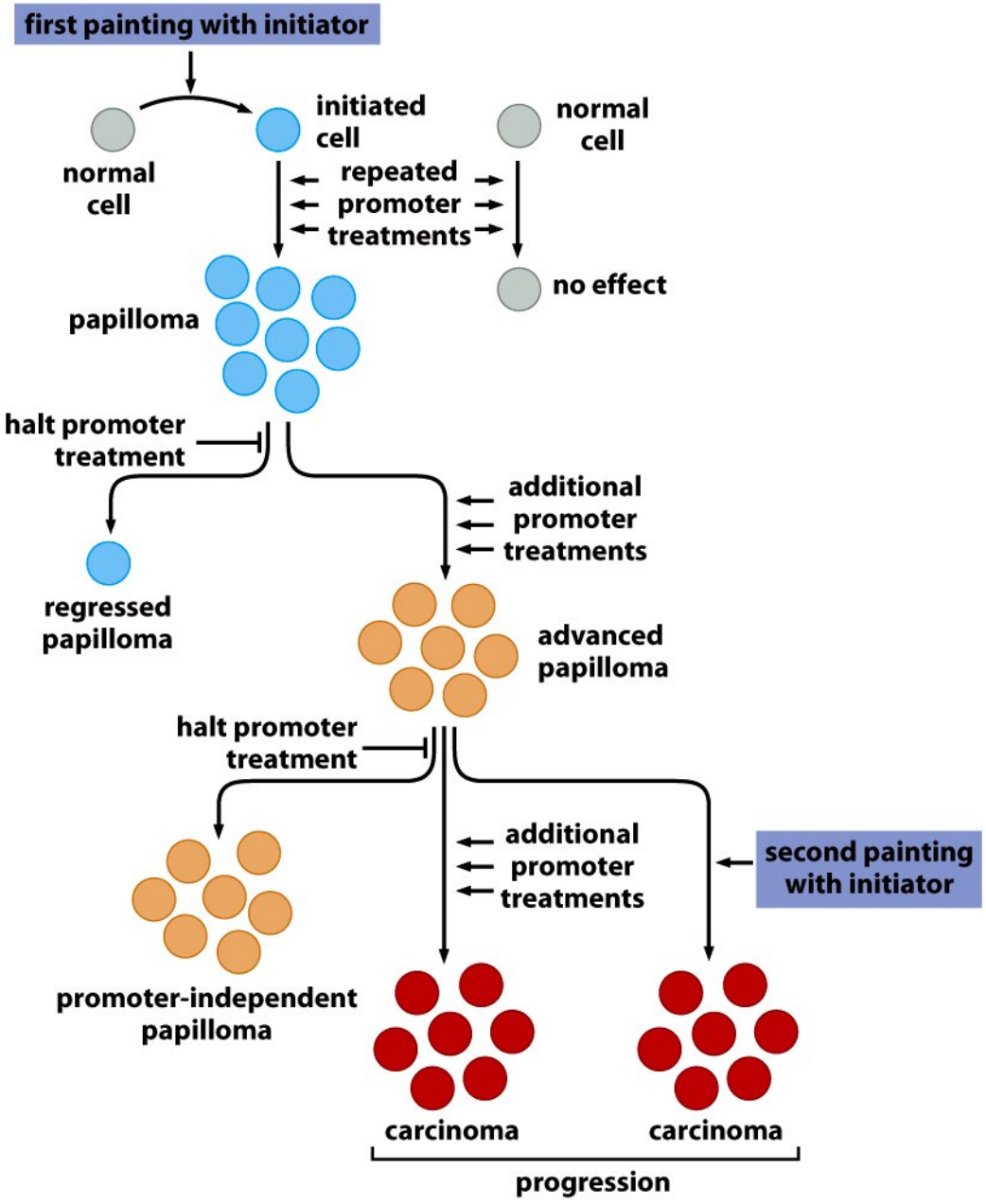


Figure 11.29 *The Biology of Cancer* (© Garland Science 2007)

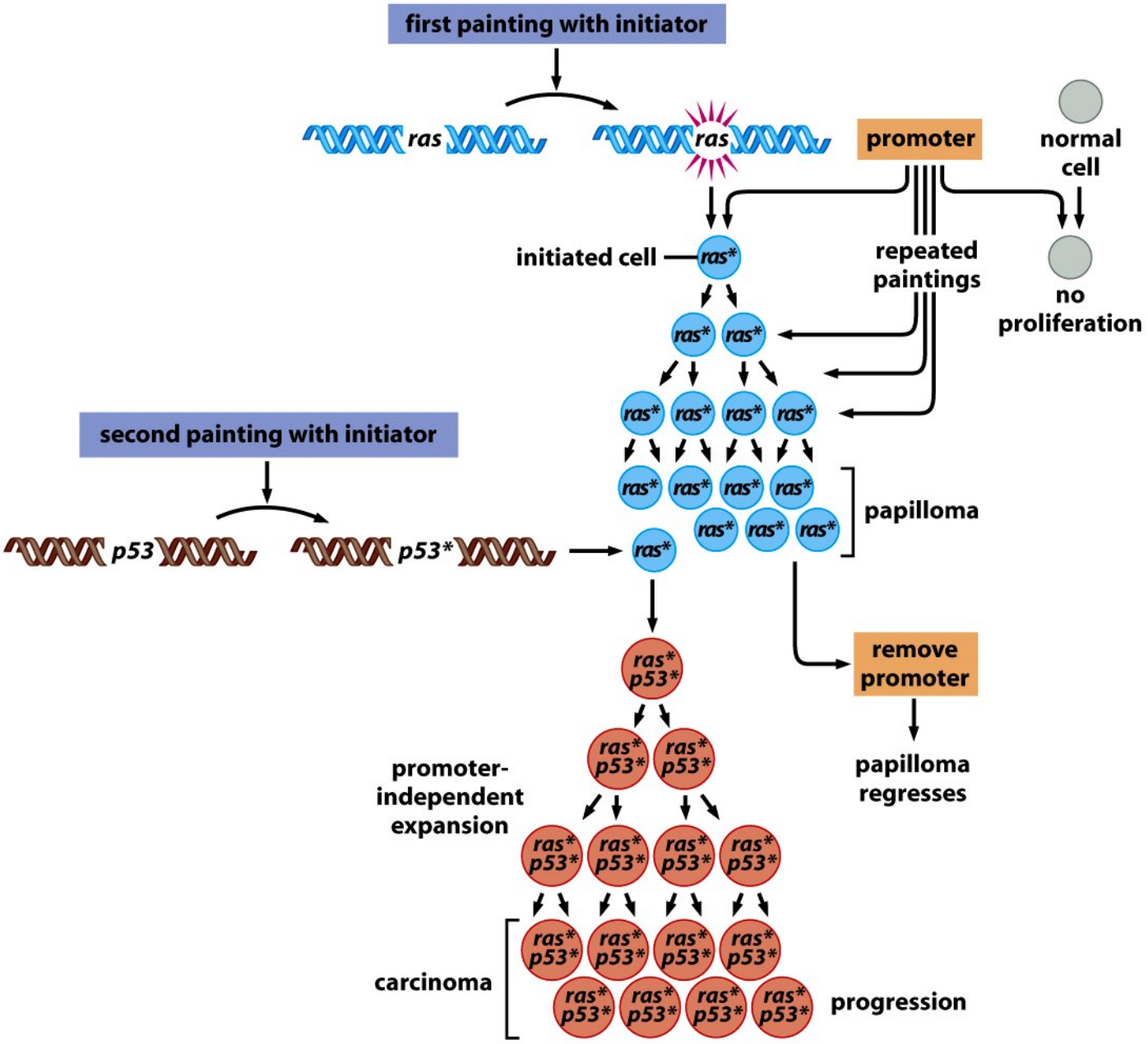


Figure 11.30 *The Biology of Cancer* (© Garland Science 2007)

Summary

- **Somatic mutation theory - Initiating carcinogens cause mutations in the DNA of somatic cells.**
- **Many carcinogens (pro-carcinogens) require metabolic activation to produce ultimate carcinogens.**
- **Damage can lead to base and deoxyribose modifications, inter and intra-nucleotide crosslinks, and single and double-strand breaks.**
- **A variety of multi-enzyme repair pathways exist to cope with DNA damage produced by endogenous and exogenous agents.**
- **Individuals with lower DNA repair capacity are at increased risk to develop cancer.**
- **Carcinogens can be tested in short term mutagenesis assays.**
- **Initiators and promoters**