Relevant Websites

http://www.epa.gov – US Environmental Protection Agency.
http://www.osha.gov – Occupational Safety and Health Administration.
http://www.acgih.org – American Conference of Governmental Industrial Hygienists.
http://www.cdc.gov – National Institute for Occupational Safety and Health.
http://www.iarc.fr – International Agency for Research on Cancer.
http://ntp-server.niehs.nih.gov – National Toxicology Program.

Carcinogen–DNA Adduct Formation and DNA Repair

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Definition

Carcinogen–DNA adducts are addition products formed by covalent binding of all or part of a carcinogen molecule to chemical moieties in DNA; adducts are formed when an activated chemical species (electrophilic, positively charged metabolite) binds covalently to negatively charged moieties in DNA.

Importance of DNA Adduct Formation in the Process of Carcinogenesis

Carcinogen–DNA adducts of exogenous genotoxic chemical carcinogens may induce errors in DNA sequence (mutations). Subsequent transcription on a damaged template may result in the formation of abnormal proteins or the absence of protein. DNA adduct formation and mutagenesis are considered to bring about changes in gene expression that produce clonal expansions of cells lacking in growth control (tumors). A substantial period of time is required for a tumor to become evident, and DNA damage is considered to be necessary but not sufficient for tumorigenesis, since other events must also take place. DNA adduct levels, measured at any point in time, reflect tissue-specific rates of damage processing that include DNA adduct formation and removal (DNA repair), DNA adduct instability, tissue turnover and other events. In experimental model systems dose–response associations have been observed for DNA adduct formation, mutagenesis, and tumorigenesis. Reductions in tumor incidences have been observed when DNA adduct levels have been lowered, either by DNA repair processes or by administration of chemopreventive agents that inhibit DNA adduct formation with no change in dose.

Biotransformation of Carcinogenic Chemicals to Species that Modify DNA

Exogenous carcinogenic chemicals that form DNA adducts can be direct acting if they are highly reactive. Examples are the nitrosoureas, some nitrosamines, ethylene oxide, and ozone. However, most are inert like the polycyclic aromatic hydrocarbons (PAHs) and require biotransformation (metabolic activation). Biotransformation consists of metabolic alteration by families of enzymes that convert a small fraction of the initial dose to highly reactive intermediate metabolites able to 'modify' (become bound to) DNA, thus accomplishing the first essential step (initiation) in the carcinogenic process. Exogenous carcinogens that require metabolic activation include some plant and fungal products (aflatoxins, ochratoxins, hydrazines), pyrolysis products from cooking (heterocyclic amines, PAHs), industrial combustion products (aromatic amines, PAHs, nitro-PAHs, benzene, vinyl chloride, nitrosamines, ethylene oxide), urban pollution contaminants (PAHs, nitro-PAHs, aromatic amines) and components of tobacco (tobacco-specific nitrosamines) and tobacco smoke (PAHs, nitrosamines, and aromatic amines). The metabolic processes that lead to DNA adduct formation for several classes of genotoxic chemical carcinogens, including the PAHs, the aromatic amines, the heterocyclic amines, some fungal products and oxyradical damage, are described briefly.

The PAHs, which include the human carcinogen benzo[*a*]pyrene (BP) (**Figure 1**), are composed of variable numbers of fused benzene rings and are chemically unreactive, as well as insoluble in water. These compounds are ubiquitous environmental contaminants found in cigarette smoke and products of partial combustion, and are produced by many industrial processes. They are metabolized to simple epoxides by cytochrome P-450, hydrated through the action of epoxide hydrolase and subjected again to epoxidation (cytochrome P-450) to form unstable dihydrodiol-epoxides. The unstable metabolites spontaneously convert to positively charged, highly reactive free radicals (carbocations, the ultimate

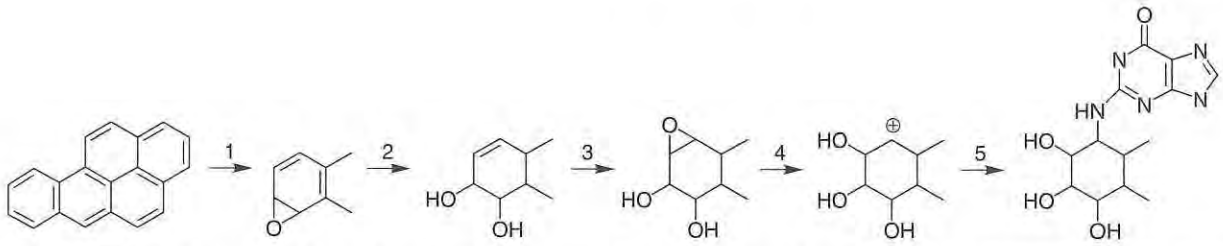

Figure 1 Metabolic activation of benzo[*a*]pyrene (a representative PAH). The parent hydrocarbons are chemically inert and require metabolic activation before they can exert their biological effects. Cytochrome P-450 enzymes (principally CYP1A1) catalyze the formation of simple arene oxides from the parent hydrocarbons (1). The arene oxides are converted to dihydrodiols by the action of epoxide hydrolase (2). The resulting dihydrodiols are further oxidized by cytochrome P-450 enzymes (principally CYP3A4) at the site of the olefinic double bond (3). Vicinal diol-epoxides are high unstable and the arene-ring opening is spontaneous yielding a highly reactive carbocation (4). The electrophilic carbocationic species can form a covalent bond with the exocyclic amino group of deoxyguanosine (5). The resulting polycyclic aromatic hydrocarbon–DNA adduct lies in the minor groove of the double helix.

carcinogenic forms), which bind covalently to DNA and protein. The metabolic scheme for BP is shown in **Figure 1** and the structure of the major DNA adduct, between BP and deoxyguanosine is shown in **Figure 2a**.

Aromatic amines are characterized by the presence of benzene rings and an exocyclic nitrogen. A prototypical aromatic amine, 4-aminobiphenyl (4-ABP), found in tobacco smoke and industrial exhaust, has been implicated in human bladder cancer. The presence of the amino group, that can be either acetylated or nonacetylated, contributes to the complexity of aromatic amine metabolism. Activation of aromatic amines proceeds by *N*-oxidation with sulfotransferase catalysis, resulting in the formation of acetylated (**Figure 2b**), and nonacetylated (**Figure 2c**), guanine adducts. **Figure 2b** and **c** shows guanine adducts of the carcinogen *N*-2-acetylaminofluorene.

Heterocyclic amines, for example, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), are formed from the pyrolysis ($>150°C$) of amino acids, creatinine, and glucose that occurs during cooking of meat and fish. They are known as food mutagens and their metabolism, largely influenced by the amine moiety, is similar to that of the aromatic amines. They undergo cytochrome P-450-induced *N*-hydroxylation (CYP1A2). *N*-hydroxylation metabolites of some heterocyclic amines (2-amino-3-methyl-imidazole-[4,5-*f*]quinoline; IQ) can react directly with DNA, while others require further enzymic *O*-esterification. The major guanine adduct of PhIP is shown in **Figure 2d**.

Fungal mycotoxins, including aflatoxin B_1 derived from *Aspergillus flavus*, contaminate cereals, grains, and nuts, and aflatoxin B_1 ingestion is correlated with a high incidence of liver cancer in animal models and humans. Aflatoxins are heterocyclic and contain several endocyclic oxygen molecules. They are activated by simple epoxidation (cytochrome P-450) across the olefinic double bond at the 8,9-position, giving rise to a carbocation. However, some addition products with DNA are unstable and lead to nonmutagenic depurination. The major aflatoxin-guanine adduct is shown in **Figure 2e**.

Oxyradicals (reactive oxygen species), formed as a result of endogenous processes or exposure to exogenous chemicals, can cause oxidation of DNA. Two common examples of oxyradical damage found in DNA include thymine glycol and 8-hydroxydeoxyguanosine adducts (**Figure 2h**). Probably the most common endogenous oxyradical exposure is to $O_2^{\bullet -}$ (superoxide anion) and H_2O_2 (hydrogen peroxide). This occurs when O_2 is reduced for the production of energy, and although most of the electrons are contained, there is some leakage. Other endogenous sources of oxyradicals include reactions of $O_2^{\bullet -}$ with Fe^{3+} or NO to form unstable intermediates (e.g., ONOOH) that are powerful, direct-acting oxidants or that yield hydroxyl radical. The mechanism involving NO is also the basis for inflammation, and can represent a normal response to infection. Exposure to organic peroxides, catechol, hydroxyquinone and 4-nitroquinoline-*N*-oxide among others, leads to oxyradical damage. Moreover, cells can be stimulated to produce peroxisomes by treatment with certain drugs and plasticizers. The role of oxyradical DNA damage in chemical carcinogenesis is currently unclear, although the mutagenic potential of these adducts has been amply demonstrated in experimental systems.

Measurement of Carcinogen–DNA Adducts as Human Exposure Dosimeters

The promise of human DNA adduct biomonitoring is the application of a human biomarker that is directly correlated with cancer risk for cancer prevention or

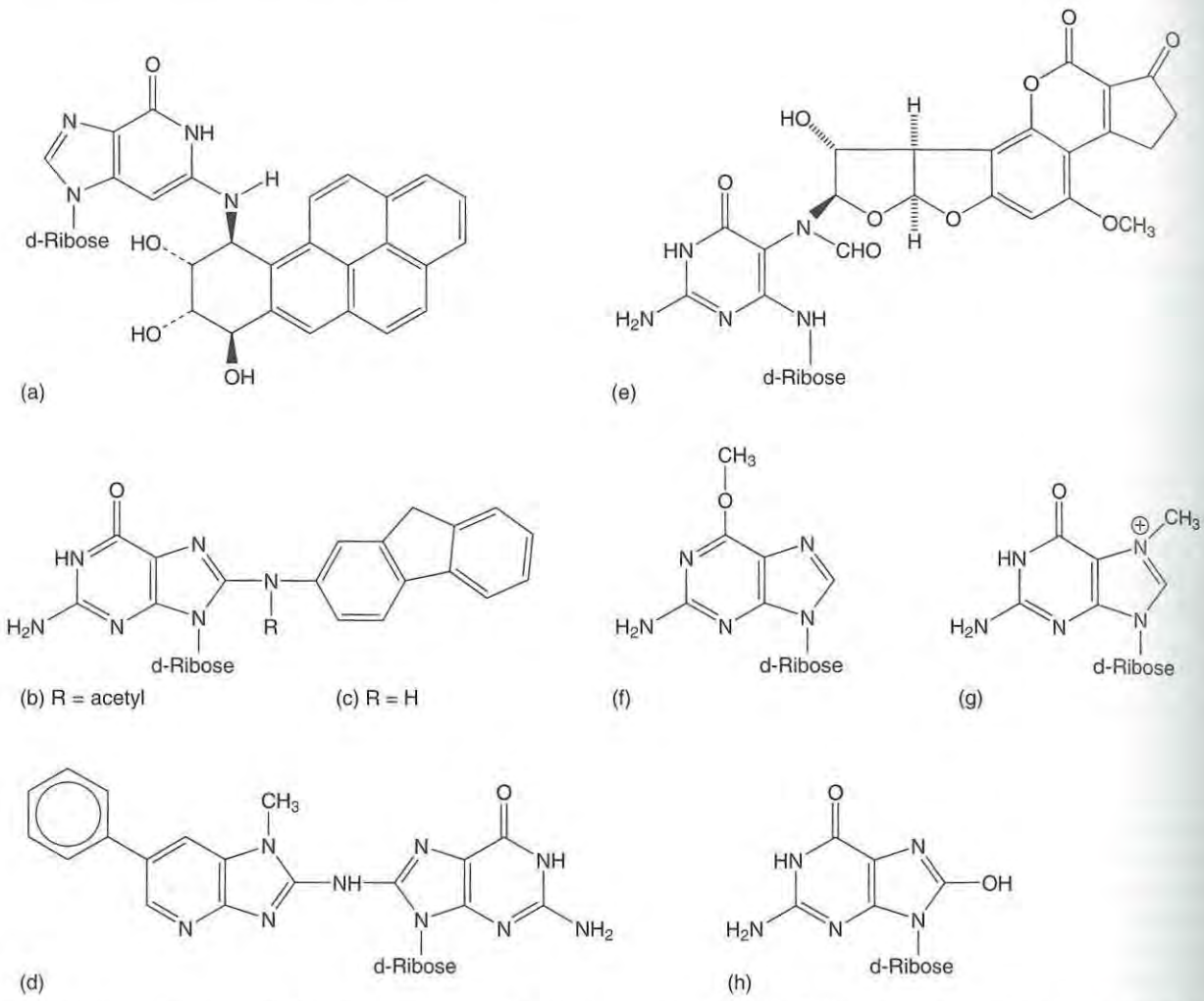

Figure 2 Molecular structures of carcinogen adducts of deoxyguanosine: (a) (7R)-N^2-(10-{7β,8α,9α-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene}-yl)-deoxyguanosine, formed when benzo[*a*]pyrene-7,8-diol 9,10-epoxide reacts with the exocyclic amino group of deoxyguanosine; (b) *N*-(deoxyguanosin-8-yl)-2-(acetylamino)fluorene, formed when *N*-hydroxylacetylaminofluorene reacts with the C8 position of the imidazole ring; (c) *N*-(deoxyguanosin-8-yl)-2-(amino)fluorene, formed when *N*-hydroxylaminofluorene reacts with the C8 position of the pyrimidine ring structure; (d) *N*-(deoxyguanosin-8-yl)-2-amino-1-methyl-6-phenylimidazo-[4,5*b*]-pyridine, formed when the *N*-hydroxylamine metabolite of 2-amino-1-methyl-6-phenylimidazo-[4,5*b*]-pyridine (PHIP), a glutamic acid pyrolysate, reacts with deoxyguanosine; (e) ring-opened form of *N*-(deoxyguanosin-7-yl)-9-hydroxyaflatoxin B_1, formed following the reaction of the 8,9-epoxide metabolite of aflatoxin B_1 at the N7 position of deoxyguanosine; (f) O^6-methyldeoxyguanosine, formed when an alkyl radical (CH_3^+), derived from an alkylating agent, reacts at the O^6-position of deoxyguanosine; (g) N7-methyldeoxyguanosine, formed when an alkyl radical (CH_3^+), derived from an alkylating agent, reacts at the N7-position of deoxyguanosine; (h) 8-hydroxydeoxyguanosine, formed through exogenous or endogenous oxy-radical damage (H_2O_2, $^{\bullet}OH$, $O_2^{\bullet -}$) at the C8 position of deoxyguanosine.

intervention. This field has expanded exponentially since the 1980s, a progress made possible by the development of highly sensitive methods for the detection of DNA adducts in human tissue. The most widely used methods include immunoassays and immunohistochemistry, ^{32}P-postlabeling, fluorescence and phosphorescence spectroscopy, and gas chromatography/mass spectrometry. Detection limits for quantitative assays are typically in the range of 1 adduct in 10^9 nucleotides. However, accelerator mass spectrometry, a highly sophisticated but less accessible method, has a detection limit of ~1 adduct in 10^{12} nucleotides.

When used without preparative procedures, the most commonly used techniques are typically unable to provide quantitation of individual adducts and chemical characterization of a specific adduct. This is because humans are exposed to complex mixtures of chemical carcinogens, and human DNA will contain multiple DNA adducts induced by different xenobiotic agents. The development of preparative strategies for sample purification that can be applied

prior to the ultimate DNA adduct quantitation has made possible chemical characterization of specific DNA adducts in human tissues. The combination of preparative methods (immunoaffinity chromatography, high-performance liquid chromatography or other chromatography) with immunoassays, ^{32}P-postlabeling or synchronous fluorescence spectrometry has made possible the identification of specific DNA adduct structures. In addition, chemical derivatization approaches have facilitated the various novel permutations of gas chromatography/mass spectrometry that have become widely applied for the determination of specific human DNA adducts.

The majority of studies designed to monitor DNA adducts in human tissues fall into the category of exposure documentation and have concentrated on environmental and occupational exposures to agents for which precise dosimetry is difficult or impossible. Many studies have shown decreases in DNA adduct levels (qualitative dosimetry) in groups of subjects removed from exposure by virtue of location or season. Quantitative dosimetry for human DNA adduct formation has been established with medicinal (cisplatin, procarbazine, dacarbazine, and 8-methoxypsoralen) and dietary (aflatoxins) exposures where dosimetry can be established accurately. A major goal of carcinogen dosimetry is the application of human DNA adduct formation data within epidemiologic study designs to predict human cancer risk. This goal has been achieved in two prospective nested case–control studies and several case–control studies. In the prospective studies which involved lung cancers in smokers and liver cancers in individuals exposed to aflatoxins, relative risks for cancer were increased three- to sevenfold in individuals with elevated DNA adduct levels. In the case–control studies elevated DNA adduct levels (odds ratios 2.3–16.2) were found in the cases compared to the controls. Whereas the epidemiologic studies investigating the relationship between human DNA adduct levels and cancer risk will take many years, these early studies appear to support the data from experimental models that has shown that DNA adduct formation is necessary but not sufficient for tumorigenesis.

Biological Repair of Adduct Damage in DNA (DNA Repair)

Toxicological damage to DNA can alter its chemical structure in many different ways. Covalent addition products may be formed with activated, bulky aromatic compounds or smaller alkyl-species (**Figure 2f** and **g**). Oxyradical formation, often the by-product of normal metabolic processes, dimerization and deamination also modifies the chemical structure of DNA, and single- and double-strand breaks (DSBs) can occur. All of these types of DNA damage may lead to permanent changes in DNA sequence, and some have been associated with the development of disease (e.g., cancer, progeria, Cockayne's syndrome, retinal dystrophy, thalassemia, xeroderma pigmentosum, and birth defects) and normal aging.

A series of metabolic pathways has evolved to counteract DNA damage through removal of the lesions. Mechanisms of DNA repair are complex, generally requiring the products of several genes to act in concert to accomplish restoration of DNA structure. Cell cycle restriction point genes are responsible for conducting the whole DNA repair process. These complexes usually comprise a damage sensor, a damage eliminator, a polymerase or patch synthesizer, and a ligase. However, more than 150 genes are known to participate in DNA repair and some contribute to more than one pathway. For example, genes from multiple pathways are assembled in the BRCA-1-associated genome surveillance complex that constitutes a sensory apparatus for detection and binding to damaged DNA.

There are six general mechanisms of DNA repair: direct repair (DR), nucleotide excision repair (NER), base excision repair (BER), mismatch repair (MMR), homologous recombination repair (HRR), and nonhomologous end joining (NHEJ).

Direct DNA Repair

In contrast to the general scheme outlined above, DR employs only a single suicide enzyme. An alkyltransferase commutes an alkyl group from an alkylated base (O^6-methyldeoxyguanosine) to a cysteine residue in its own active site. Because there is no strand scission, there is also no need for patch synthesis or ligation. In this suicidal process one adduct consumes one molecule of enzyme.

Nucleotide Excision Repair and Base Excision Repair

Both of these processes require multiple enzymes acting either sequentially or, as indicated above, as molecular complexes. The hallmarks of both NER and BER are strand scission, removal of a segment of DNA containing the adducted base, 5′ to 3′-oriented DNA patch synthesis through the action of a polymerase, using the intact strand as a template, and ligation of the free ends. The distinctions between these two mechanisms are the proteins involved and the types of adducts that are repaired. Bulky adducts, like those of aromatic compounds or

malondialdehydes (the result of lipid metabolism), cause DNA distortions that are relatively large and are repaired by NER. In NER the structural distortion is recognized by products of sensory genes (e.g., *XPA*, *XPC*, or *XPE*) and excised by endonucleases (e.g., XPF, XPG, or FEN), the patch is synthesized by a polymerase (polΔ or polε) and ligated (DNA ligase I or DNA ligase III). Further, there are two types of NER, global genomic repair (GGR) and transcription-coupled repair (TCR), which are characterized by different sets of genes. The repair patch generated for TCR is limited to the transcribed strand of transcriptionally active genes and involves multiple (15–30) nucleotides. The repair patch for GGR is limited to a single nucleotide, but GGR can occur on either strand in both transcriptionally active and inactive regions of the genome.

Small adducts, like 3-methyladeneine, 5-hydroxuracil and 5-hydroxymethlyuracil, are repaired by BER. In BER a lesion is detected and removed by a glycosylase (e.g., hOgg1 or UDG) creating an apurinic site (AP). Subsequently an endonuclease degrades the damaged strand (e.g., APEI, FEN1) a patch is synthesized by a polymerase (polβ) and ligation occurs (DNA ligase I or DNA ligase III). The patch size in BER can either be short (one nucleotide) or long (two to 10 nucleotides). Short BER is polβ-dependent and ligation is accomplished by DNA ligase III, whereas long BER is associated with proliferating cell nuclear antigen (PCNA) and ligation is accomplished by DNA ligase I. Oxidative damage may be repaired by either NER or BER.

Mismatch Repair

Nucleotide mismatches occur when DNA repair processes insert an inappropriate but unmodified, conventional base opposite a noncomplementary partner. These may be transitions (purine to purine or pyrimidine to pyrimidine: G–T or A–C) or transversions (purine to pyrimidine or pyrimidine to purine: C–C, T–T, C–T; A–A, G–G, A–G). For example, in post-replication 'repair', a DNA damage tolerance mechanism that leaves a gap in response to replication on a damaged template, the polymerase always inserts an adenine in the gap. In addition, deamination of cytosine results in thymidine. Both NER and MMR feature degradation of a relatively large portion of the damaged strand, followed by 5′ to 3′ patch synthesis using the undamaged strand as a template, and ligation to complete the repair. The DNA mismatch is recognized by a repair protein complex (either MSH1–MSH2–MSH6–PMS1 or MSH1–MSH2–MSH6–PMS2) that simultaneously anchors to both the mismatch and the closest unmethylated adenine in the GATC recognition sequence. The entire sequence between the mismatch and the GATC recognition sequence is eroded, and PCNA is recruited to act as a sliding clamp and support the action of a DNA polymerase (polΔ or polε) in replication of the repair patch. A ligase (DNA ligase I) subsequently complexes with polymerase (polΔ) to complete the repair function.

Mismatch DNA repair is critical in the maintenance of a stable genome. Inheritance of mutations in genes involved in mismatch DNA repair (*MSH1, MSH2, MSH6, PMS1, PMS2*) can predispose to cancers of the brain, endometrium, ovaries, and bowel. Somatic mutations in these genes may also contribute to the mutator phenotype.

Homologous Recombination Repair

Double-strand breaks can be caused by ionizing radiation, oxidative stress and mechanical stress (e.g., when a topoisomerase encounters a bulky adduct during DNA replication). There are two distinct mechanisms for repairing DSBs, HRR and NHEJ. Several sensors of HRR that trigger the process include ATM, RAD3-related ATM, and DNA-protein kinases (Chk2). Homologous recombination involves a number of DNA repair proteins (RAD51, RAD51B, RAD51C, RAD51D, RAD52, RAD54, XRCC2, XRCC3, BRCA1, and BRCA2). First, a nibrin (NBS1) complex with RAD50/MRE11 (also known as MRN complex) brings about simultaneous recision of both strands, which is thought to be 5′ to 3′. RAD51 and its paralogs (accessory proteins – RAD51B, C, D) prepare the single-stranded DNA segments for sister chromatid exchange and invasion of the homologous duplex. Polymerization occurs using the undamaged homologous duplex DNA sequences as a template. The process is completed by either through resolution of Holliday junctions by the action of an endonuclease and strand sealing by a ligase, or disengagement of the Holliday junctions, DNA pairing and gap filling in the damaged homolog. The first scenario would give an equal opportunity for crossover events and noncrossover events to occur. However, there is now evidence to suggest that HRR is more often accomplished without a crossover event, a mechanism that is more conservative in reducing genomic alterations.

Nonhomologous End-Joining

The other major repair pathway involved in DNA DSB repair is NHEJ. While this pathway brings about DSB repair, it is also involved in immunological diversification by re-ligating the products of recombinase (RAG1 and RAG2) cleavage. Unlike

HRR, NHEJ is independent of a genetic DNA sequence homolog because repair occurs without copying an undamaged template. Components of the NHEJ pathway include XRCC4, ligase IV, KU70, and DNA protein kinase. In addition, if ligation of two blunt ends cannot restore the original sequence, a deletion mutation will result. Therefore, this mechanism is sometimes referred to as 'illegitimate'. In the presence of single-stranded over-hanging segments, some degradation may occur to create a blunt end before ligation occurs (ligase IV). Alternatively, recruitment of polμ or polλ by the XRCC4-ligase IV complex may result in gap filling.

See also: Cytochrome P-450; Polycyclic Aromatic Amines; Polycyclic Aromatic Hydrocarbons (PAHs).

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Carcinogenesis

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Overview

Cancer, or neoplasia, which occurs in one of every four individuals and results in the death of one of every five individuals in the United States, is a complex disease with multiple causes. Many intrinsic and extrinsic factors influence the development of cancer. Intrinsic or host factors include age, sex, genetic constitution, immune system function, metabolism, hormone levels, and nutritional status. Extrinsic factors include substances eaten, drunk, or smoked; workplace and environmental (air, water, and soil) exposures; natural and medical radiation exposure; sexual behavior; and elements of lifestyle such as social and cultural environment, personal behavior, and habits. Intrinsic and extrinsic factors can interact with one another to influence the development of cancer. Because of the physical and emotional suffering associated with cancer and the immense cost to the nation in lost production and income and medical and research expenditures, considerable effort continues to be exerted to understand this complex disease so that strategies can be developed to decrease or prevent its occurrence. Current regulatory guidelines have been crafted to reduce the probability of developing cancer by lowering human exposure to agents identified as potentially capable of causing cancer.

During the past 40 years of cancer research, much information has been generated indicating that