Toxicant and carcinogenesis

Unit 1 (part b)

INTRODUCTION

Genetic toxicology is the study of the effects of chemical and physical agents on genetic material. It includes the study of deoxyribonucleic acid (DNA) damage in living cells that leads to cancer, and it also examines changes in DNA that can be inherited from one generation to the next. Thus, genetic toxicology can be defined as a branch of the field of toxicology that assesses the effects of chemical and physical agents on the hereditary material (DNA) and on the genetic processes of living cells. As such, it is important at the outset to distinguish between genotoxicity and mutagenicity. Genotoxicity covers a broader spectrum of endpoints than mutagenicity. For example, unscheduled DNA synthesis, sister chromatid exchanges, and DNA strand breaks are measures of genotoxicity, not mutagenicity, because they, themselves, are not transmissible from cell to cell or generation to generation. Mutagenicity, however, refers to the production of transmissible genetic alterations**.**

GERM CELL MUTATION

These mutations are mainly inherited from previous generations and are expressed when an individual inherits the mutant gene from both parents. New mutations make a larger contribution to the incidence of dominant diseases than to that of recessive diseases because only a single dominant mutation is required for expression. Because genetic mechanisms are well understood, it is possible to evaluate compounds for potential genotoxic effects that are associated with mechanistically with heritable mutation (transmissible germ cell mutation). The potential consequences of somatic and germinal mutations are summarized in Fig. 13.1. Heritable germ cell mutations and cancer are the major concerns when there is exposure to any genotoxic agent, and they provide the rationale for conducting assays to detect potential genotoxicity activity. In addition to being potential germ cell mutagens or carcinogens, there is evidence that the mutagenic events may play an important role in the cause and/or progression of human diseases other than cancer. It is now known that if a compound is genotoxic, then there is a reasonable probability that it will be a carcinogen.

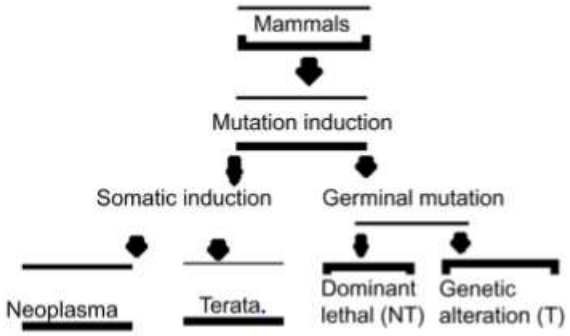

FIGURE 13.1

Potential consequences of somatic and germinal mutation. *NT*, nontransmissible; *T*, transmissible.

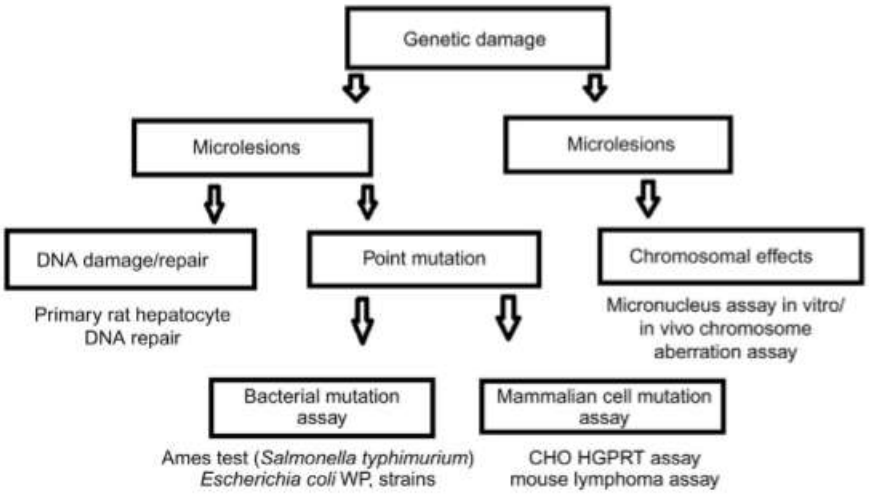

FIGURE 13.2

Types of genetic damage and methods for their detection.

Genetic damage can be broadly classified as either microlesions or macrolesions (Fig. 13.3). Microlesions (not visible microscopically) are detected by measuring a cell's response to DNA damage (ie, DNA repair) or by measuring a subsequent change in the phenotype of the cell (ie, point mutation assay). Macrolesions are detected in cytogenetic assays as microscopically visible alterations in chromosomal structure/number (ie, in vitro/in vivo cytogenetics or chromosome aberration assays) or as micronuclei remaining in erythrocytes following expulsion of the nucleus (micronucleus assay).

Mutagenesis can occur as a result of interaction between mutagenic agents (mutagens) and genetic materials present in organisms. Although spontaneous mutations and natural selection are the major means of evolution, in recent decades, a number of toxicants have been found to induce mutagenic effects in a variety of organisms. Mutations can be produced by external factors (e.g., high temperatures), toxic chemicals (most carcinogens, etc.), radiation (ionizing and nonionizing), as well as internal factors (e.g., cellular metabolism and respiration, reactive oxygen species, and reactive nitrogen species), and DNA replication/transcription error. Electromagnetic fields and free radicals generated from electronic equipment/devices, medical devices (e.g., x-ray, MRI, etc.), electricity, microwaves, radar, and cellular phones could be sources of mutations. Mutations are classified into microlesions (e.g., gene mutation: frame shift and base substitution) and macro lesions (e.g., chromosomal abnormalities: gap, fragments, deletion, ring, translocation, and numerical change). Some individuals exhibit mutations in skin cells or other tissues, termed somatic mutations. In contrast, germ mutations occur only in the sex cells and are more threatening because they are transmitted to subsequent generations.

GENERAL ASPECT OF MUTAGENICITY

Mutagens are chemical and physical agents that are capable of producing a mutation. Mutagens include agents such as radiation, chemotherapeutic agents, and many carcinogens. A mutation is a permanent alteration in the genetic information (DNA) of the cell. DNA-damaging agents/mutagens can produce (1) point mutations involving single base pair substitutions that can result in amino acid substitutions in the encoded protein and frame-shift mutations involving the loss or gain of one or two base pairs, resulting in an altered reading frame and gross alterations in the encoded protein, (2) chromosome aberrations including gross chromosomal rearrangement such as deletions, duplications, inversions, and translocations, and (3) aneuploidy and polyploidy, which involve the gain or loss of one or more chromosomes. Point mutations are classified as missense or nonsense mutations. A missense mutation produces an altered protein in which an incorrect amino acid has been substituted for the correct amino acid. A nonsense mutation is an alteration that produces a stop codon and

results in a truncated protein. A point mutation can also be characterized based on the mutageninduced substitution of one base for another within the DNA. When a point mutation produces a substitution of a purine for another purine (i.e., guanine for adenine) or a pyrimidine for another pyrimidine (i.e., thymine for cytosine), the mutation is referred as a transition. If a purine is substituted for a pyrimidine, and vice versa (i.e., thymine for adenine or guanine for cytosine), the mutation is referred to as a transversion

MUTAGENS ARE CARCINOGENS

Chemicals that induce mutations in the DNA are called mutagens; when these changes lead to cancer, the chemical is called a carcinogen. Not all mutagens are carcinogens, and not all carcinogens are mutagens. In 1946, it was shown that nitrogen mustards (derived from mustard gas first used by the military in 1917 during WWI) could induce mutations in the fruit fly and reduce tumor growth in mice. Genetic toxicology developed ways to test chemical and physical agents for their mutagenic potential. In the 1970s, Bruce Ames and others developed a cellular-based test for genetic mutations. This test became known as the Ames assay. Sophisticated variations of these tests are now required by many government regulatory agencies to test chemicals for mutagenicity. Often, it is a metabolite (breakdown product) of the compound that causes cancer, not the original compound. Ideally, a foreign chemical is made less toxic when metabolized, but sometimes a chemical can be made more toxic. This more toxic chemical can then interact with cellular DNA or proteins and produce malignant cells. This process is called bioactivation. It is also possible for a chemical to encourage bioactivation or to accelerate the development of a cancer. Efforts to understand the underlying biology of cancer are ongoing. The genomic sciences are helping to explain why some people are more susceptible to cancer than others.

Consequences of Mutagenesis There are multiple ways in which mutations can alter the structure of DNA, and thus potentially initiate carcinogenesis. Mutations that affect only a single pair of bases in the DNA chain are known as gene or point mutations . Point mutations generally affect one single codon (the group of three bases that code for a single amino acid in protein synthesis), potentially causing substitution of one amino acid for another in the resulting protein. This change may or may not affect protein function, depending on the location of the substituted amino acid in the molecule and on the similarity of the substituted amino acid for the original amino acid (Figure 6.2). Mutations that lead to the insertion or deletion of bases, however, disrupt the triplet code and can affect all downstream codons. Such an event is known as a frame-shift mutation and is likely to block production of functional proteins by that gene (Figure 6.2). Are these single-gene changes sufficient to induce carcinogenesis? Again, there is significant debate over this issue. However, mutations can also lead to events that are much larger on the molecular scale. These include changes in chromosome structure, e.g., breakage and

rearrangement, or loss of genetic material (potentially leading to aneuploidy). Radiation is an example of a potent genetic carcinogen that can produce changes in chromosome structure.

DNA MUTATIONS

To understand cancer, it is necessary to know the cellular changes that turn a normal cell into a malignant cell that repeatedly and uncontrollably divides. This transformation occurs when there is genetic damage or an alteration in the structure of a cell's DNA, the coding machinery of life. The DNA is a double helix made of the compounds adenine (A), guanine (G), thymine (T), and cytosine (C). These chemicals are bound in long stretches as AT and CG pairs and wrapped in sugar molecules that hold them together (Fig. 13.3). Long stretches of these AT and CG combinations form genes that, when "read," produce the proteins that drive our cells. Ideally, the DNA sequence would not change except in the recombining that occurs during reproduction. However, DNA damage occurs regularly as part of the cell process and from interaction with both normal cellular chemicals and with toxic chemicals. A very robust repair mechanism rapidly and very accurately repairs the DNA damage, but if for some reason the DNA is repaired incorrectly, then a mutation occurs. The mutation is a subtle or not so subtle change in the A, G, C, or T that comprise the DNA. Many of the mutations have no effect, some have minor effects, and a small number have life-threatening effects. If a mutation occurs in the wrong place, then a cell can start to divide uncontrollably, becoming a malignant cell and causing cancer. If a mutation occurs in our germ line cells, then the mutation can be passed to our offspring. The common forms of DNA damage are summarized in Fig. 13.

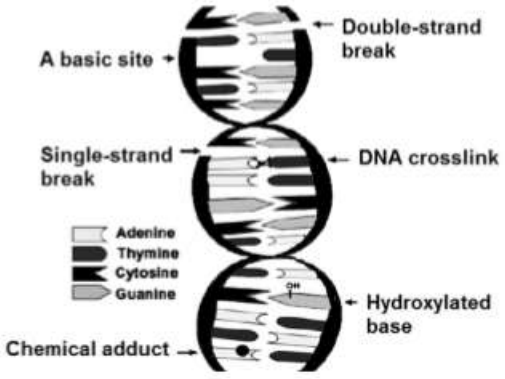

FIGURE 13.3

Schematic diagram showing common forms of DNA damage.

Gene mutations involve additions or deletions of base pairs or substitution of a wrong base pair in DNA molecules. Substitutions consist of transitions and transversions. The former involve the replacement of a purine (adenine, guanine) by another or a pyrimidine (cytosine, thymine) by another. With transversion, a purine is replaced by a pyrimidine, or vice versa. When the number of base pairs added or deleted is not a multiple of three, the amino acid sequence of the protein coded distal to the addition or deletion will be altered. This phenomenon is called frameshift mutation and is likely to affect the biological property of the protein. Figure 9.1 clearly illustrates the effects of a deletion and an addition of a nucleotide base. In addition, a mutagenic chemical, or a part of it, may be incorporated into the DNA molecule. For example, a number of electrophilic compounds react with DNA forming covalent addition products, known as "DNA adducts." Thus acetyl aminofluorene (AAF) binds specifically to the carbon at the eight position of guanine. For a partial list of such chemicals, see discussions on procarcinogens in "Categories of Carcinogens" in Chapter 8. Alkylating agents, such as ethylnitrosourea and diethyl sulfate, donate an alkyl group to DNA. Further, each mutagenic agent shows a predilection for damaging specific nucleotides, which produce recognizable patterns of DNA base pairs. DNA substitution mutations are of two types. Transitions are interchanges of two-ring purines (A<->G) or of one-ring pyrimidines (C<->T): they therefore involve bases of similar shape. Transversions are interchanges of purine for pyrimidine bases which therefore involve the exchange of one-ring and two-ring structures. The imino tautomer of adenine can pair with cytosine, eventually leading to a transition from A-T to G-C. These various changes in the DNA molecule may cause the substitution of a new amino acid in the subsequently coded protein molecule or result in a different sequence of amino acids in the protein synthesized. Further, a protein synthesis termination codon may be formed, yielding a shortened protein. While the first type of effect may or may not result in a modification of the biological property of the protein molecule, the latter two types almost invariably do.

TYPES OF GENETIC DAMAGE

Chromosomal damage is defined as microscopically visible modification of the number or structure of chromosomes. Variations in the number of chromosomes may involve the complete complement (polyploidy) or only some of the chromosomes (aneuploidy). The loss of a chromosome is a lethal event, but the gain of a chromosome can be viable and create significant genetic imbalances. Structural changes are mainly the result of breaks in the chromatid arms. Some of these are unstable and are not transmitted through successive cellular generations, e.g. achromatic gaps, breaks of one or both chromatid arms, chromatid interchanges, acentric fragments, ring and dicentric chromosomes. Stable structural modifications that are transmissible are inversions, translocations and some small deletions. These genetic factors clearly play important roles in many human diseases. Great toxicological importance attaches to the alkylation of DNA (commonly by methylating and ethylating agents) since this can cause many base modifications. The target bases are primarily purines (although phosphate

oxygen is also a target). While N7 is frequently the quantitatively dominant alkylated product, O6-alkylguanine is the most mutagenic because this interferes with the normal hydrogen bonding of G with C and there is a very high probability of G with T pairing when the modified strand replicates. Should this occur, there is said to have been a GC → AT transition mutation. Many of these alkylations (particularly methyl, but also ethyl and hydroxyethyl) can be removed by the protein O6-methylguanine-DNA methyltransferase (MGMT), which is capable of functioning as an 'enzyme'only once, since it is inactivated when the alkyl group is transferred from the guanine to a cysteine on the protein. Haloethylation can be particularly damaging to cells unless removed by MGMT, since inter-strand crosslinks can be formed which prevent strand separation during DNA replication. Adducts with the bases of DNA can also be formed by much larger molecules, including polycyclic aromatic hydrocarbons, aromatic amines and heterocyclic aromatic amines (which may be produced from certain amino acids during cooking). Usually, these molecules are not immediately reactive with DNA but must first be metabolised, frequently by oxidative systems, to electrophilic intermediates. This metabolism provides a large number of products, but, as in the case of benzo [a] pyrene, which is a carcinogenic and mutagenic constituent of any burnt carbonecous material. There may be a high degree of specificity in the metabolite that seems to be responsible for the carcinogenic/mutagenic effects in vivo. Damage to DNA is also a common result of oxidative metabolism of xenobiotics during which molecular oxygen is reduced in a series of four one-electron steps through superoxide anion and hydrogen peroxide to the hydroxyl radical and hydroxyl ion. The hydroxyl radical in particular is highly reactive and this is also the most active mutagen generated by ionising radiation. A particularly well-studied mutagen is UV radiation of about 260 nm. Such UV radiation produces a number of DNA photoproducts, prominent among which are intra-strand dimers joined by a cyclobutane structure involving carbons 5 and 6 of thymidine. These can be completely removed by a photoreactivating enzyme that, in the presence of light (especially with a wavelength of about 370 nm), binds to the cyclobutane region of DNA. However, another intra-strand dimer, the 6-4 photoproduct, is not repaired in this way and is the cause of mutations when the DNA replicates

REPAIR OF DAMAGED DNA

Processes in addition to those already mentioned are frequently involved in the repair of DNA that has been damaged. These processes have been largely studied in prokaryotes and while excision repair is also important in mammalian cells, the status of SOS repair (see below) and error-prone repair in mammalian cells is not yet clearly defined.

Excision Repair

Excision repair can be initiated by DNA alkylation, arylation, the production of pyrimidine dimers by UV radiation or the production of apurinic or pyrimidinic sites. Apurinic sites can arise as a result of glycosylase activity removing abnormal bases or by non-enzymatic depurination following labilisation of the glycosyl linkage due to alkylation of N 3 or N 7 positions. The excision repair enzyme system can also

repair cross-linking damage. There are two main modes of DNA excision repair: 'short-patch' or apurinic repair, and 'long-patch' or nucleotide excision repair. Short-patch repair involves the removal and replacement of only a few (perhaps three or four) nucleotides. Long-patch repair results in the removal of the damaged DNA site and up to about 100 adjacent nucleotides. It is initiated by large distortions in the double helix, e.g. pyrimidine dimers or adducts with large ring systems (polycyclic aromatic amines or hydrocarbons). Excision repair can occur throughout the cell cycle. In the bacterium Escherichia coli, the uvrA protein detects a distortion due to a dimer or some bulky adduct and binds to DNA distant from the damaged site. The uvrB protein binds to the DNA–uvrAprotein complex and, by DNAgyrase activity, unwinds the DNAstrands down to the damaged area. UvrC, with endonuclease activity, cleaves the damaged DNA strand on either side of the lesion. The damaged segment is unwound from the undamaged strand by helicase II (uvrD gene product), after which DNApolymerase 1 fills the gap and DNA ligase seals the remaining nick.

Post-Replication Repair

Post-replication DNArepair is an error-prone mechanism that occurs only during the DNA synthetic phase (S-phase) of the cell cycle. During DNA replication, if the polymerase enzyme encounters a large site of damage on the template strand, then that portion of DNA cannot be used as a template. The result is a newly synthesised DNA that contains gaps of up to 1000 nucleotides, as well as the possibility of shorter gaps. These gaps and nicks are eventually filled by chain elongation (i.e. post-replication repair) during S-phase and are ligated. Because the repair polymerase must use a damaged DNA template, it is probable that the newly synthesised DNA strand will contain errors. If repair is not error-free and the cell survives to replicate, the genetic information may be altered. Although these changes are essential to the evolutionary process leading to the diversity of life, the great majority of them are harmful to the cell. The severity of the harm depends, to some extent, upon whether the affected cell is a unicellular organism or one of many cells in a multi-cellular organism. If the affected cell of the latter is a germ cell that is involved in the reproductive process, then the resulting offspring will carry in their

genetic material the potentially harmful information (although it is not necessarily expressed). If the affected cell in a multicellular organism is a somatic cell (i.e. one which does not give rise to gametes, spermatozoa, or ova), then it may experience impaired function or impaired susceptibility to homeostatic, regulatory controls. In this case, the cell may have taken a step along the path leading to the emergence of a cancer.

Base Replacement

Replacement of one base by another has several possible consequences. There may be no effect at all, either because the base change is in an intron or because it results in a new codon that codes for the

same amino acid, a result of the redundancy in the DNA triplet code mentioned above. Alternatively, base change may result in the coding of a different amino acid. This type of change is a miss-sense mutation. Occasionally, the codon for an amino acid residue within the original polypeptide will be changed to a stop codon. This is a nonsense mutation that results in a truncated polypeptide. A mutation in a pre-existing stop codon may code for another amino acid and the continuation of translation into a polypeptide that is elongated up to the next stop codon.

Deletions and Insertions

Deletions or insertions of bases in a gene may be large or small. If large, and in a codon region, the almost inevitable result is the prevention of the production of a useful polypeptide. The effects of short deletions or insertions depend upon whether or not they involve multiples of three bases. If whole codons are involved, then the consequence is the deletion or insertion of the corresponding number of amino acid residues. The deletion or insertion of any number of bases other than a multiple of three causes a shift in the reading frame during translation. Such a frameshift mutation results in a complete change in the amino acid sequence in the C-terminal direction from the point of mutation. Nonsense and frameshift mutations almost always result in the destruction of protein function. If the protein function lost is essential for cell survival, the cell will die. In addition to the base changes that can result from the interaction of a mutagenic agent with DNA, the development of a discontinuity in the DNA imposes an extra strain on the structure of the chromosome. This can result in lesions that are microscopically visible (e.g. at 400 magnification or greater) after appropriate staining of the chromosomes. Such lesions are particularly prone to develop if the DNAdamage involves DNA inter-strand cross-linking or DNA–protein cross-linking agents. Numerical changes in the chromosomes can also occur if there has been interference with their movements during cell division by agents that have formed adducts with cytoskeletal proteins, particularly (but not only) tubulin. What a cytogeneticist refers to as a chromosome consists of two chromatids, one having been derived from each germ-line cell when they come together during sexual reproduction to form the zygote. Each pair of chromatids is joined by the centromere, which is also the point of attachment of the cytoskeletal proteins that push and pull the chromosomes during cell division. Each of these chromatids is what has been referred to as a chromosome elsewhere in this and other texts.

CHROMOSOMAL CHANGE

Chromosomal damage is defined as a microscopically visible modification of the number or structure of chromosomes. Variations in the number of chromosomes may involve multiples of the complete complement (polyploidy) or the elimination or multiplication of only some of the chromosomes

(aneuploidy). The loss of a chromosome is a lethal event, but the gain of a chromosome can be viable and create significant genetic imbalances. Structural changes are mainly the result of breaks in chromatid arms (Figure 8.3). Some of these are unstable and are not transmitted through successive cellular generations, e.g. achromatic gaps, breaks of one or both chromatid arms, chromatid interchanges, acentric fragments, ring and dicentric chromosomes. Stable structural modifications that are transmissible are inversions, translocations and some small deletions.

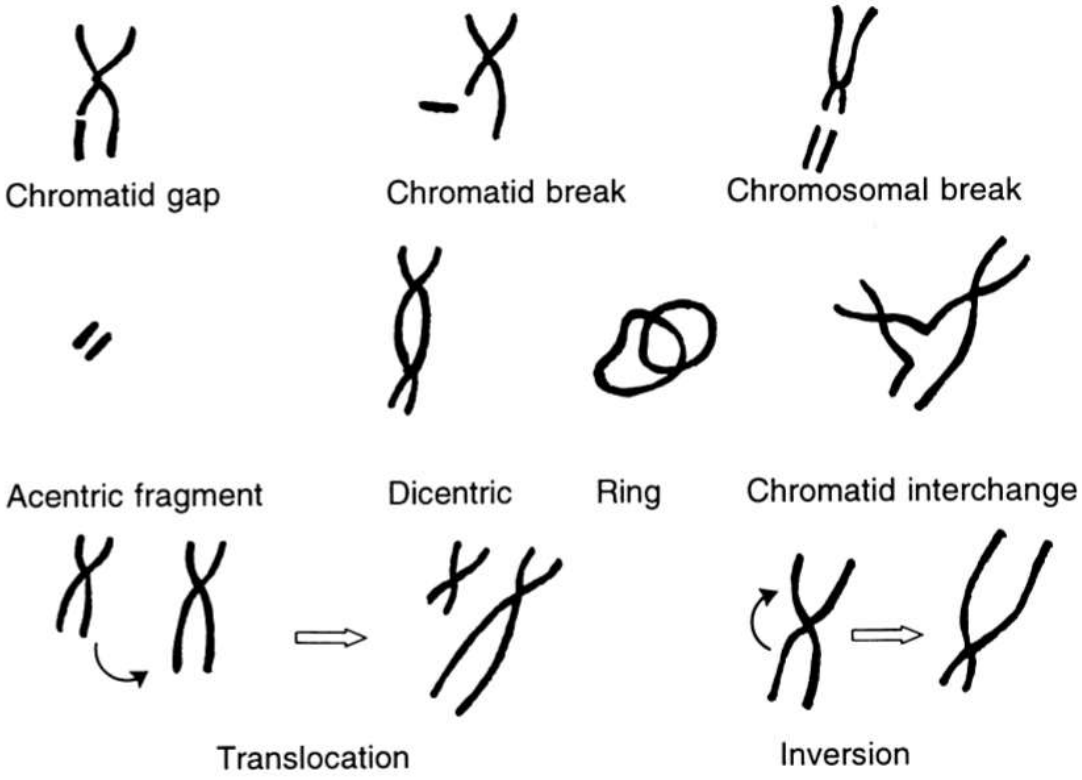

Figure 8.3 *Categories of chromosomal damage*

ONCOGENES

Mutational Activation of Proto-oncogenes

Much evidence has accumulated for a role of covalent binding of reactive electrophilic carcinogens to DNA in chemical carcinogenesis. It is known that chemical mutagens and carcinogens can produce point mutations, frameshift mutations, strand breaks, and chromosome aberrations in mammalian cells. If the interaction of a chemical carcinogen with DNA leading to a permanent alteration in the DNA is a critical event in chemical carcinogenesis, then the identification of these altered genes and the function of their protein products is essential to our understanding of chemical carcinogenesis. While specific DNA-

carcinogen adducts were isolated in the 1970s and 1980s, it was not until the early to mid-1980s that the identification of specific genes that were mutationally altered by chemical carcinogens became known. Certain normal cellular genes, termed proto-oncogenes, can be mutated by chemical carcinogens providing a selective growth advantage to the cell. The mutational activation of proto-oncogenes is strongly associated with tumor formation, carcinogenesis, and cell transformation. Proto-oncogenes are highly conserved in evolution and their expression is tightly regulated. Their protein products function in the control of normal cellular proliferation, differentiation, and apoptosis. However, when these genes are altered by a mutation, chromosome translocation, gene amplification, or promoter insertion, an abnormal protein product or an abnormal amount of product is produced. Under these circumstances these genes have the ability to transform cells in vitro, and they are termed oncogenes. Over a 100 oncogenes have been identified with approximately 30 oncogenes having a major role in human cancer. Most oncogene protein products appear to function in one way or another in cellular signal transduction pathways that are involved in regulating cell growth, differentiation or apoptosis. Signal transduction pathways are used by the cells to receive and process information to ultimately produce a biological cellular response. These pathways are the cellular circuitry conveying specific information from the outside of the cell to the nucleus. In the nucleus, specific genes are expressed, and their encoded proteins produce the evoked biological response. Oncogenes encode proteins that are components of this cellular circuitry (Table 12.7). If a component of the circuit is altered, then the entire cellular circuit of which the component is a part is altered. It is not difficult to imagine how an alteration in a pathway that regulates cellular growth, differentiation, or apoptosis could have very profound effects on cellular homeostasis. Indeed, this is the molecular basis of how oncogenes contribute to the cancer process.

Table 12.7 Oncogene Classification

Families	Genes
Growth factors	*sis, hst-1, int-2, wnt-1*
Growth factor receptor tyrosine kinases	*EGFR, fms, met/HGFR, ErbB2/new/HER2, trk/NGFR*
Nonreceptor tyrosine kinases	*abl, src, fgr, fes, yes, lck*
Guanine nucleotide binding proteins	H-*ras*, K-*ras*, N-*ras*, *TC21*, GA_{12}
Serine/threonine kinases	*mos, raf, bcr, pim-1*
DNA-binding proteins	*myc, fos, myb, jun, E2F1, ets, rel*

Ras genes are frequently mutated in chemically induced animal tumors and are the most frequently detected mutated oncogenes in human tumors. Approximately 20–30% of all human tumors contain mutated ras. The Ras subfamily includes H-ras, K-ras, and N-ras, and all have been found to be mutationally activated in numerous types of tumors from a large variety of species including humans.

Activated ras oncogenes have been detected in a large number of animal tumors induced by diverse agents including physical agents, such as radiation, and a large number of chemical carcinogen.

TUMOR SUPPRESSOR GENES

Inactivation of Tumor Suppressor Genes

Activation of oncogenes results in a gain of function while inactivation of tumor suppressor genes results in a loss of function. Tumor suppressor genes have also been termed anti-oncogenes, recessive oncogenes, and growth suppressor genes. Tumor suppressor genes encode proteins that generally function as negative regulators of cell growth or regulators of cell death. In addition some tumor suppressor genes function in DNA repair and cell adhesion. The majority of tumor suppressor genes were first identified in rare familial cancer syndromes, and some are frequently mutated in sporadic cancers through somatic mutation. There are approximately 18 known tumor suppressor genes (e.g., p53, Rb, APC, p16, and BRCA1) that have been shown to have a role in cancer and another 12 putative tumor suppressors have been identified. When tumor suppressor genes are inactivated by allelic loss, point mutation, or chromosome deletion, they are no longer capable of negatively regulating cellular growth leading to specific forms of cancer predisposition. Generally, if one copy or allele of the tumor suppressor gene is inactivated, the cell is normal, and if both copies or alleles are inactivated, loss of growth control occurs. In some cases a single mutant allele of certain tumor suppressor genes, such as p53, can give rise to an altered intermediate phenotype. However, inactivation of both alleles is required for full loss of function and the transformed phenotype.

p53 encodes a 53 kDa protein. p53 is mutated in 50% of all human cancer and is the most frequently known mutated gene in human cancer.