

## Chapter 2

# DNA structure and stability: mutations versus repair

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### Introduction

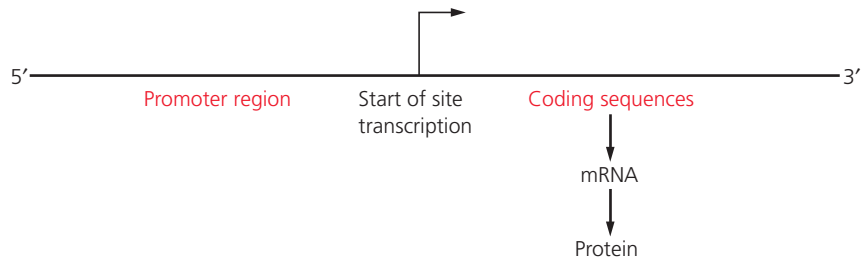
Genetic information, coded within DNA, requires stability. DNA directs the production of proteins needed for the structure and function of cells over a lifetime, through an adaptor molecule, RNA. Unlike RNA and protein, which have a limited existence before they are degraded and/or recycled, DNA must maintain its integrity over that lifetime. However, our genes are subject to a myriad of attacks by both environmental agents and endogenous processes that result in mutation and scission. Changes to the DNA sequence may have severe consequences for the cell and its progeny. Cancer is a disease that involves alterations to gene structure and gene expression at the cellular level. The role of the accumulation of mutations is well established for carcinogenesis. In this chapter we will review the structure of a gene and describe the mutations that occur during carcinogenesis.

When considering the process of carcinogenesis we must be aware that cells are equipped with defense mechanisms against mutations, such as the detection and repair of DNA damage. Detection and repair of DNA damage is particularly crucial in the time before a cell divides since errors existing during replication will be passed on to daughter cells. Pausing the cell cycle is sometimes coupled to the repair of DNA damage. Apoptosis, a more hard-line defense, can be triggered as a last resort; thus, cell suicide is the ultimate price to be paid to prevent perpetuation of DNA damage and to protect the individual from carcinogenesis (see Chapter 7). In this chapter, we will also examine how mutations in DNA occur as a consequence of exposure to carcinogens and, on the other hand, examine the DNA repair systems that are in place to maintain the integrity of the genome and suppress tumorigenesis.

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## 2.1 Gene structure—two parts of a gene: the regulatory region and the coding region

We have 30,000 genes! They are encoded in our DNA, an impressively simplistic double-helical molecule made up of two chains of nucleotides. A nucleotide is made up of a sugar, phosphate, and a nitrogenous base (adenine, guanine, cytosine, or thymine) and it is the sequence of the bases that holds the instructional information of our genes. The central dogma of molecule biology states that DNA is transcribed into RNA and RNA is translated into protein. Gene expression refers to the transcription of a gene. For the purpose of simplicity, keep in mind that there are two distinct functional parts to a **gene** (Figure 2.1). The 5' end of a gene contains nucleotide sequences that make up the **promoter** region, and this region is involved in regulating the expression of the gene. These 5' nucleotide sequences interact with proteins that affect the activity of RNA polymerase and determine when and where a gene is expressed. (Note however, there are exceptions; for many genes, some regulatory regions can be located elsewhere, such as **downstream** of the gene or within introns.) The TATA box (TATAAAA) located near the start site of transcription is one of the most important regulatory elements for most genes. Binding of the TATA box-binding protein (TBP) is crucial for the initiation of transcription. A short sequence of DNA within a promoter that is recognized by a specific protein and contributes to the regulation of the gene is called a **response element** (RE). Common response elements identify genes under a common type of regulation. For example, the sequence CCATATTAGG is referred to as the serum response element (SRE) and is found in genes that are responsive to serum. Also, it is not surprising that the response element for a protein that is essential for the regulation of the cell cycle, the transcription factor E2F, is found in the promoters of the cyclin E and cyclin A genes, the products of which are major players in the cell cycle. Enhancer elements are additional regulatory DNA sequences that are position- and orientation-independent relative to a promoter and are important for tissue-specific and stage-specific expression. Downstream (the direction along the DNA molecule towards the 3' end) of the promoter



**Figure 2.1** A simplistic representation: two functional parts of a gene.

are the nucleotides that will be transcribed into RNA and those coding for exons will be translated into protein. These downstream nucleotide sequences represent the coding region of the gene.

## 2.2 Mutations

As stated previously, most carcinogens are mutagens. These agents induce mutations either by modifying DNA (e.g. forming DNA adducts) or by causing chromosomal damage (e.g. DNA strand breaks). Several types of mutations are illustrated in Figure 2.2: **transitions**, **transversions**, insertions, deletions, and chromosomal translocations. Transitions and transversions are two types of base substitutions. A transition is the substitution for one **purine** for another purine and a transversion is the substitution of a purine for a **pyrimidine** or vice versa. Base substitutions during replication may occur for several reasons. First, DNA polymerase is not always 100% accurate. The enzyme may make an error and insert a wrong nucleotide during DNA synthesis. Also, modifications of bases due to oxidation or covalent additions and alterations of **chromatin** structure can cause misreading of the DNA template by DNA polymerase. Remember that the genetic code is a triplet code read in a sequential but non-overlapping manner. An insertion or deletion of a base can alter the reading frame (marked by a ‘,’ in Figure 2.2) and thus can also be referred to as a frame-shift mutation. In most cases this leads to a non-functional or truncated protein product. A chromosomal translocation is the exchange of one part of one chromosome for another part of a different chromosome and results in changes of the base sequence of DNA. As we will see in later chapters, there are many examples of these types of mutations in genes regulating growth, differentiation, and apoptosis that are involved in carcinogenesis. In theory, initial mutations may occur anywhere across a particular gene but the location will determine whether some of these

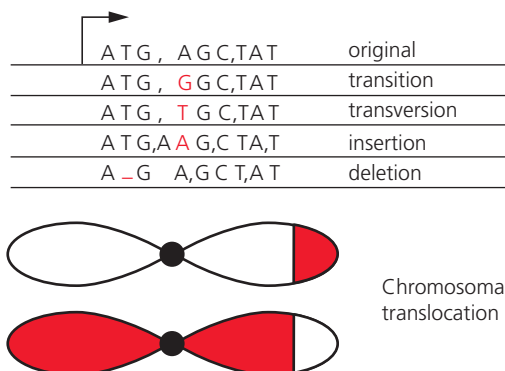


Figure 2.2 Types of mutations.

mutations give rise to a growth advantage and contribute to carcinogenesis. For example, a mutation may alter the conformation of a cyclin protein and result in unregulated progression of the cell cycle, whereas another mutation may have no effect on protein conformation or function.

The consequence of a mutation in a gene is determined by its location with respect to the two functional parts of a gene. Mutations occurring in the promoter region may alter the regulation of the gene and affect the levels or temporal/spatial expression of the gene product. The consequence of such mutations may be over- or under-expression of the protein product or the appearance of the protein product at the wrong time or in the wrong place (i.e. the wrong cell type), respectively. Alternatively, mutations occurring in the coding region of genes may affect the structure and thus alter the function of the gene product or cause a truncation (e.g. the introduction of a stop codon) that abolishes the protein's function completely.

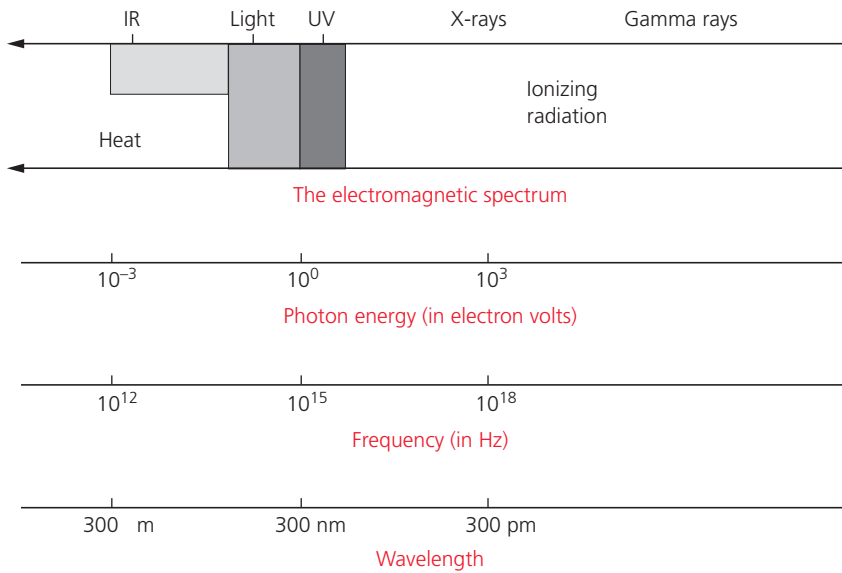
## 2.3 Carcinogenic agents

The backbone of cancer biology has been the identification of carcinogens responsible for cancer-causing mutations, and the identification of specific mutations as causative factors of carcinogenesis along with the elucidation of the pathways they affect. Several classes of carcinogens will now be described, including radiation, chemicals, infectious pathogens, and particular endogenous reactions.

### Radiation as a carcinogen

Radiation is energy. There are two forms of radiation: energy traveling in waves or as a stream of atomic particles. Energy waves include gamma ( $\gamma$ ) rays, high-energy **electromagnetic radiation** that is similar to X-rays. Atomic particles include alpha ( $\alpha$ ) and beta ( $\beta$ ) particles that are emitted by radioactive atoms. (Alpha particles comprise two protons and two neutrons, while beta particles comprise electrons.)

Electromagnetic radiation is naturally occurring radiation which possesses a broad range of energies. Electromagnetic radiation moves as waves of energy, which have peaks and troughs (in a manner analogous to waves at sea). The distance between successive peaks (or troughs) is termed the **wavelength**. High-energy electromagnetic radiation such as cosmic radiation has a short wavelength, while low-energy radiation such as radio waves has a long wavelength. The **electromagnetic spectrum** spans electromagnetic radiation of varying wavelengths, as shown in Figure 2.3. The electromagnetic spectrum extends from long-wavelength radiation (not shown) to extremely short-wavelength radiation, such as



**Figure 2.3** The electromagnetic spectrum and corresponding characteristics.

X-rays and gamma radiation. The visible spectrum spans those wavelengths that we can detect with our eyes as visible light. Ultraviolet (UV) radiation is emitted from the sun and has a higher energy (and so a shorter wavelength) than visible light.

Several types of radiation (including both energy waves and atomic particles) can damage DNA and act as carcinogens. The amount of energy released by a particular radiation source affects the mechanism and extent of damage to DNA. The amount of energy released by a particular radiation source and absorbed by the body tissue is measured in grays (Gy). One gray is the release to the body tissue of 1 joule (J) of energy per 1 kg of tissue. The real issue is not how much radiation is absorbed by the body tissue, however, but how much damage is done when the radiation has been absorbed. The amount of damage caused depends on the rate at which a particular radiation source releases energy. If a radiation source releases energy at a high rate, then it causes more damage than a source that releases energy more slowly.

**Linear energy transfer (LET)** is used to help describe the rate at which energy is released. Specifically, it describes the amount of energy released by a radiation source as it travels a fixed distance. High-LET radiation emits more energy than low-LET radiation over the same distance. Therefore, high-LET radiation (such as alpha particles) causes more biological damage than low-LET radiation (such as X-rays). The quantity and type of DNA damage caused by a particular radiation source depends on whether it is high- or low-LET radiation. Double-stranded DNA breaks are more commonly caused by high-LET radiation, and lead to chromosomal translocations and deletions.

The amount of biological damage caused by a particular source of radiation is measured in sieverts (Sv). (The numerical value of these units is determined by multiplying the gray units by a factor relating to the LET value of a particular type of radiation.)

Two classes of radiation, ionizing radiation and UV radiation, have been demonstrated to act as carcinogens and damage DNA. Let us examine both types of radiation below.

### Ionizing radiation

Ionizing radiation includes both alpha and beta particles (atomic particles) and gamma rays (energy waves). When high-energy radiation, such as gamma rays, strikes molecules in its path, electrons may be displaced from atoms within the molecule. The loss of one or more electrons converts the molecule from being electrically neutral to carrying an electrical charge. The charged molecule is called an ion, and hence the radiation causing the formation of an ion is called ionizing radiation.

Ionizing radiation can damage DNA directly by causing ionization of the atoms comprising DNA, or indirectly by the interaction with water molecules (a process known as **radiolysis**) to generate dangerous intermediates called **reactive oxygen species** (ROS) (see Box ‘A little lesson about ROS . . .’). These reactive oxygen species may react with DNA, or with other biomolecules, to cause damage within the cell.

#### A little lesson about ROS . . .

Some radiation exerts its biological effect by the generation of damaging intermediates through the interaction of radiation with water, or radiolysis. Since our body comprises between 55–60% water, radiation is most likely to strike water than any other matter. The striking of water by radiation causes it to lose an electron and become highly reactive. This sets off a chain reaction in which water is converted to oxygen, O<sub>2</sub>, through a three-step process. Radiation interacts with a single molecule of water and thus it cannot split directly into the diatomic gases H<sub>2</sub> and O<sub>2</sub>. Equation (1) below is **not** possible because this equation is not balanced.



The balanced equation (Equation 2) does not apply to radiation since radiation interacts with only a single molecule of water, and not the two molecules required in Equation (2):



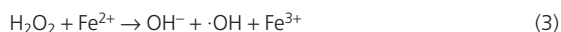
Instead, radiolysis results in the sequential generation of three dangerous reactive oxygen species (ROS) as an electron (e<sup>-</sup>) is lost at each step. The three ROS, formed in sequence, are the hydroxyl radical (·OH), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and the superoxide radical (O<sub>2</sub><sup>-·</sup>):



→ The hydroxyl radical is an extremely reactive molecule; in fact, it is one of the most reactive (and therefore dangerous) molecules known! It immediately removes electrons from any molecule in its path, turning that molecule into a free radical and so propagating a chain reaction. (A free radical is a highly unstable, reactive molecule that possesses an unpaired electron. Both the hydroxyl radical and the superoxide radical shown above are free radicals.)

Neither hydrogen peroxide nor the superoxide radical are as reactive as the hydroxyl radical. Hydrogen peroxide is actually more dangerous to DNA than the hydroxyl radical however. The slower reactivity of hydrogen peroxide (compared with the hydroxyl radical) gives the hydrogen peroxide molecule time to travel into the nucleus of a cell, where it is free to interact with and wreak havoc upon DNA.

Oxidation of DNA (the removal of electrons, by species such as the free radicals mentioned here) is one of the main causes of mutation, and explains why free radicals are such potent carcinogens. Oxidation can produce several types of DNA damage, including oxidized bases. Among the variety of oxidized nitrogenous bases observed, 8-oxoguanine is the most abundant. DNA polymerase mispairs 8-oxoguanine with adenine during DNA replication leading to a G→T transversion mutation. The presence of iron can exacerbate the consequences of H<sub>2</sub>O<sub>2</sub> production. If it encounters iron and receives an electron from it, hydrogen peroxide can be reconverted into the hydroxyl radical that may attack DNA. The Fenton reaction (Equation 3) illustrates this:



The superoxide radical is the third intermediate before the formation of oxygen. It is not very reactive but acts more as a catalyst for the generation of the other two intermediates mentioned because it helps regenerate iron (Equation 4) in the form needed for the above-mentioned Fenton reaction. Thus, the ROS intermediates affect one another.

(Lane, 2002)

People are exposed to varying amounts of ionizing radiation. Exposure to gamma rays from cosmic radiation depends on the altitude at which you live or travel. The average exposure for high-altitude flights is about 0.005–0.01 mSv h<sup>-1</sup>. A chest X-ray required for medical diagnosis of some conditions exposes patients to 0.1 mSv. The contribution of the accumulation of these varying daily exposures towards cancer risk is relatively unknown.

Studies of the victims of the atomic bombing in Japan continue to contribute to our knowledge of ionizing radiation as a carcinogen. Evidence suggests that the most important damage associated with ionizing radiation-induced carcinogenesis is double-strand DNA breaks. The Radiation Effects Research Foundation publishes periodic reports on the mortality of The Life Span Study cohort of 80,000 atomic bomb survivors (latest report Preston *et al.*, 2003). These studies have revealed three important points: (1) leukemia is the most frequent ionizing radiation-induced cancer, (2) age is an important risk factor, whereby those exposed as children are most affected, and (3) the risks of solid cancer increase with dose in

## PAUSE AND THINK

Marie Curie, who worked with radioactivity all of her adult life, died of leukemia at the age of 67. The multi-stage process of carcinogenesis is manifested by the fairly long incubation periods needed to develop cancer after exposure to carcinogens.

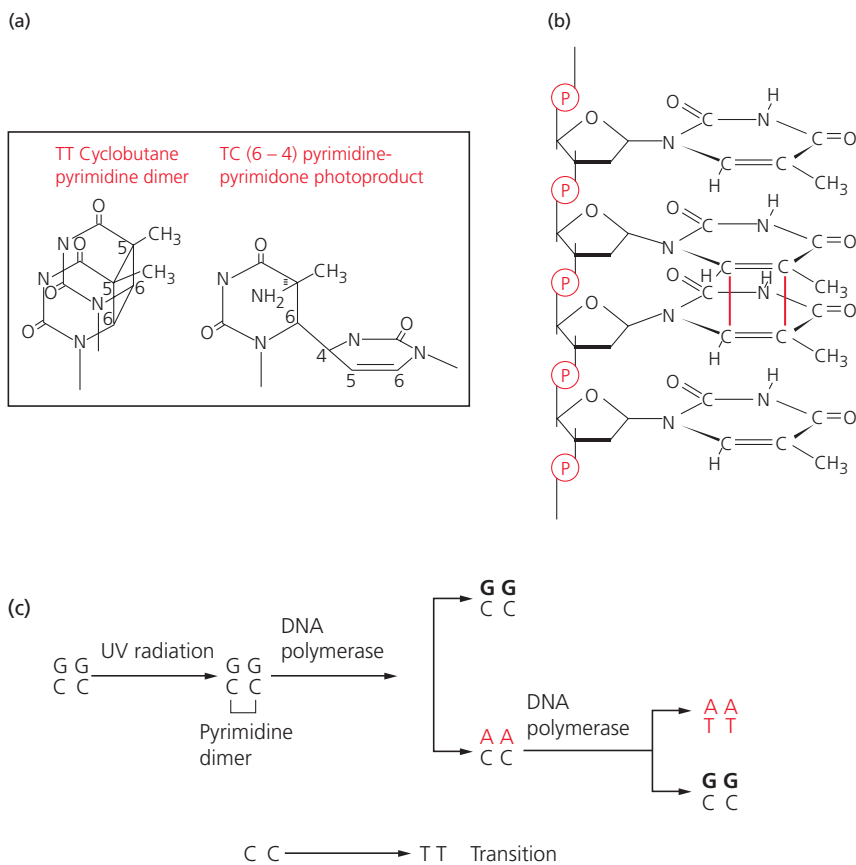
## LIFESTYLE TIP

Short-term tanning salon exposure (10 treatments in 2 weeks) results in the formation of cyclobutane pyrimidine dimers. Look at the data in Whitmore *et al.* (2001). A natural look is less taxing for your DNA!

a linear fashion. People exposed at 30 years of age have a risk of solid cancer that is elevated by 47% per Sv at the age of 70.

## Ultraviolet radiation

Ultraviolet radiation (UV) from the sun is also carcinogenic and is a principal cause of skin cancer. Of the three types of UV light—UVA (wavelength 320–380 nm), UVB (wavelength 290–320 nm), and UVC (wavelength 200–290 nm)—UVB is the most effective carcinogen. The conjugated double bonds in the rings of the nitrogenous bases of DNA absorb UV radiation. UVB directly and uniquely causes characteristic UV photoproducts: cyclobutane pyrimidine dimers and pyrimidine–pyrimidone photoproducts (Figure 2.4a,b). Cyclobutane pyrimidine dimers are most prevalent, formed at least 20–40 times more frequently than other UV photoproducts. The formation of a pyrimidone (6–4) photoproduct mimics an abasic site (a nucleotide minus a base) and is more efficiently repaired than cyclobutane pyrimidine dimers. The formation of a pyrimidine dimer causes a bend in the DNA helix and, as a result, DNA polymerase cannot read the DNA template. Under these conditions DNA polymerase preferentially incorporates an ‘A’ residue. Consequently, TT dimers are



**Figure 2.4** (a) UV photoproducts. (b) A pyrimidine dimer in the context of a polynucleotide chain. (c) Steps involved in UV-induced transitions.

often restored but TC and CC dimers result in transitions (TC→TT and CC→TT) (Figure 2.4c). Results from a mammalian cell system showed that cyclobutane pyrimidine dimers are responsible for at least 80% of UVB-induced mutations. The precise class of mutations resulting from pyrimidine dimers is a unique molecular signature of skin cancer (see Box ‘Skin cancer’)—they are not found in any other types of cancer.

UVA indirectly damages DNA via free radical-mediated damage. Water is fragmented by UVA, generating electron-seeking ROS (such the hydroxyl radical as mentioned above) that cause DNA damage (e.g. oxidation of bases). G→T transversions are characteristic of UVA damage.

### Skin cancer

UV light is specifically carcinogenic to the skin because it does not penetrate the body any deeper than the skin. The skin is made up of squamous cells, basal cells, and melanocytes and skin cancers are classified by the cell type they affect: squamous cell carcinoma (SCC), basal cell carcinoma (BCC), and melanoma, respectively. The depth of transmission of each type of UV light is dependent on the wavelength: UVC only penetrates into the superficial layer of the skin, UVB penetrates into the basal level of the epidermis, and UVA penetrates into the more acellular dermis level. Sunscreens work on the basis of including UV-absorbing organic chemicals (e.g. cinnamates), inorganic zinc-containing pigments, or titanium oxides in their ingredients to minimize UV absorption by the skin. (Note that melanin formation, known to most people as tanning, is a natural defense mechanism against UV absorption.) Additional ingredients in sunscreens must be used with care as we have learned that some compounds may be photosensitized carcinogens, chemicals that can be activated by UV to become carcinogenic. Ironically, some early sunscreens included bergamot oil which contains 5-methoxy psoralen, a photosensitized carcinogen! Some drugs such as fluoroquinolone antibiotics are also photosensitized carcinogens, which explains the reasons for the precautions from doctors to stay out of the sun during their administration.

A cellular mechanism for the elimination of UV-damaged skin cells is to initiate apoptosis. This phenomenon is familiar to us as the peeling of the skin after a sunburn. The tumor suppressor p53 protein (introduced in Chapter 1 and discussed in detail in Chapter 6) is an important regulator of apoptosis. Mutation of the *p53* gene is important for the initiation of squamous cell and basal cell carcinoma, but not melanoma. The characteristic mutations (CC→TT transitions) caused only by UV and no other carcinogen were identified in the *p53* gene. Mutations in the *p53* gene which disrupt normal p53 function and provide cells with a growth advantage, may induce the formation of tumor cells. The pattern of mutation is not random but rather tends to be localized to nine places, called hot spots. This suggests that *p53* mutations are causal for skin cancer. Further investigation of why there are so few hotspots within the context of hundreds of sites with adjacent pyrimidine dimers in the *p53* gene yielded an explanation. The hot spots in *p53* are not repaired efficiently. Removal of cyclobutane pyrimidine dimers is particularly slow at these sites. The resulting loss of p53 function causes a block in apoptosis and consequently allows the proliferation of mutated *p53* cells. Thus UV radiation not only induces *p53* mutations but also selects for the clonal expansion of the *p53* mutated cells, by inducing apoptosis in normal cells with wild-type *p53*. →

→ Different pathways seem to be central for melanoma. The elucidation of one of these pathways was one of the first successes of the Cancer Genome Project. It identified mutations in the *BRAF* gene in 66% of malignant melanomas (Davies *et al.*, 2002). *BRAF* is a serine/threonine kinase that functions in the signal transduction pathway downstream of a melanocyte-stimulating hormone and may explain why there is a high frequency of *BRAF* mutations in melanoma relative to other cancers. Surprisingly, the major mutation identified (T→A) in the kinase domain is not characteristic of UV-induced mutations (CC→TT).

## Chemical carcinogens

Many chemicals in our environment and in our diet play a role in human carcinogenesis. The common mechanism of action of carcinogens is that an **electrophilic** (electron-deficient) form reacts with nucleophilic sites (sites that can donate electrons) in the purine and pyrimidine rings of nucleic acids. Some chemical carcinogens can act directly on DNA but others become active only after they are metabolized in the body, forming what are called ultimate carcinogens, the molecules that execute the damage. A family of enzymes called the cytochrome P450 enzymes is involved in the metabolism of chemicals in the liver and is important in the activation of carcinogens to ultimate carcinogens. Genetic polymorphisms and variable expression account for differences in responses to chemical carcinogens among individuals. For example, the expression of one of the P450 enzymes called CYP1A1 (aryl hydrocarbon hydroxylase) can vary 50-fold in human lung tissue and may be responsible for the delivery of varying doses of ultimate carcinogens among smokers (Alexandrov *et al.*, 2002).

Carcinogens can be segregated into 10 groups:

- (i) polycyclic aromatic hydrocarbons
- (ii) aromatic amines
- (iii) azo dyes
- (iv) nitrosamines and nitrosamides
- (v) hyrazo and azoxy compounds
- (vi) carbamates
- (vii) halogenated compounds
- (viii) natural products
- (ix) inorganic carcinogens
- (x) miscellaneous compounds (**alkylating agents**, aldehydes, phenolics).

Four major classes of carcinogens are described below: polycyclic aromatic hydrocarbons (PAHs), aromatic amines, nitrosamines, and alkylating agents. These carcinogens exert their effects by adding functional groups covalently to DNA. Chemically modified bases, called DNA

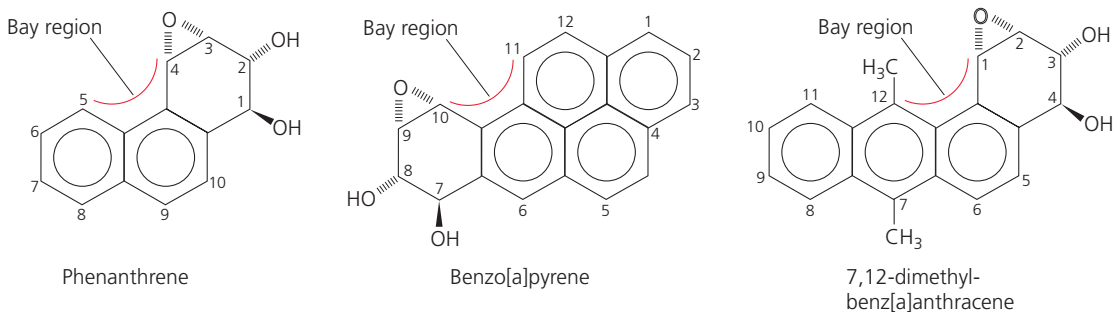
adducts, distort the DNA helix causing errors to be made during replication. The resulting mutations initiate cell carcinogenesis.

### Polycyclic aromatic hydrocarbons (PAHs)

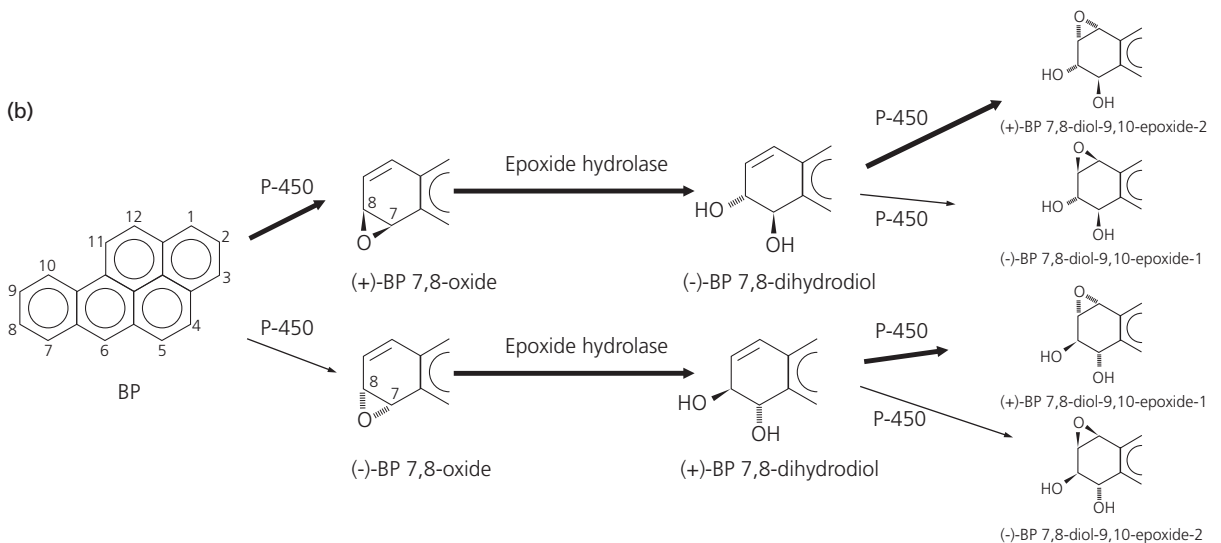
The first demonstration that chemicals could be used to induce cancer in animals was carried out in 1915. Coal tar, containing carcinogenic PAHs, induced skin carcinomas on the ears of rabbits. Carcinogenic PAHs are derived from phenanthrene (Figure 2.5a). Additional rings and/or methyl groups in the bay region of the three aromatic rings can convert inactive phenanthrene into an active carcinogen. Benzo[a]pyrene (BP), the most well known carcinogen in cigarette smoke, and 7,12-dimethyl benz[a]anthracene (DMBA), one of the most potent carcinogens, are examples of PAHs. PAHs must be metabolized further in order to give the ultimate carcinogen that will form adducts with purine bases of DNA. The P450 enzyme, CYP1A1, is the predominant enzyme that metabolizes BP to the highly reactive mutagenic BP diol epoxides (Figure 2.5b). BP results mainly in G→T transversions.

**Figure 2.5** (a) Examples of polycyclic aromatic amines. (b) Metabolic activation of BP.

(a)



(b)



**LIFESTYLE TIP**

The International Agency for Research on Cancer (IARC) has classified 81 compounds in cigarette mainstream smoke as carcinogens (Smith *et al.*, 2003). Benzo[a]pyrene (BP) ranks high in the measure of lipophilicity (a feature that allows easy entry into cells) and associated carcinogenicity. The presence of nicotine makes smoking addictive. Smoking is a cause of cancer; not smoking prevents illness. Don't choose to smoke.

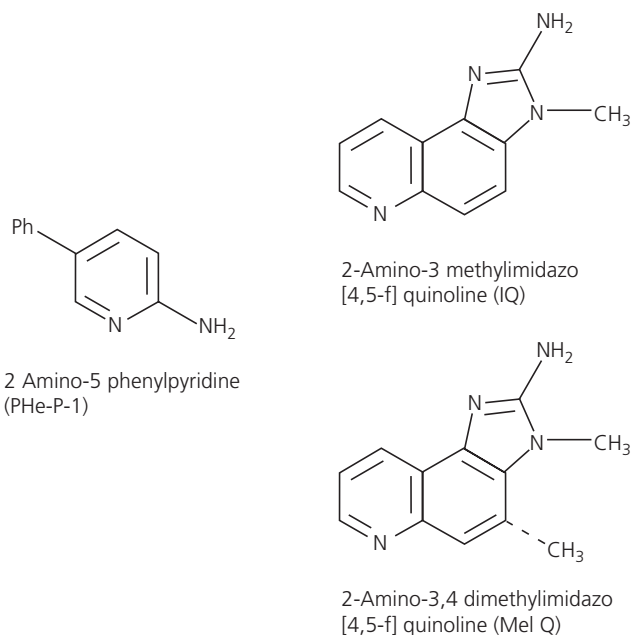
**A LEADER IN THE FIELD . . . of molecular carcinogenesis: Gerd Pfeifer**

Gerd Pfeifer has made important contributions to determining the molecular mechanisms of cancer. Investigations into skin and lung cancer provided strong evidence that UV radiation and carcinogens in cigarette smoke are causative agents for each cancer, respectively. Gerd and his colleagues demonstrated that the mutational hotspots of the *p53* gene observed in skin cancer cells are due to low-efficiency repair of DNA at these sites as discussed in the Box 'Skin cancer' above. By mapping DNA adducts of the *p53* gene that are formed after exposure to benzo[a]pyrene diol epoxide (a potent cigarette carcinogen), Gerd and colleagues showed that the locations of these adducts matched the distribution of *p53* gene mutations in lung tumors from smokers. This seminal work, reported in *Science* in 1996, provided a direct causal link between a defined carcinogen and lung cancer.

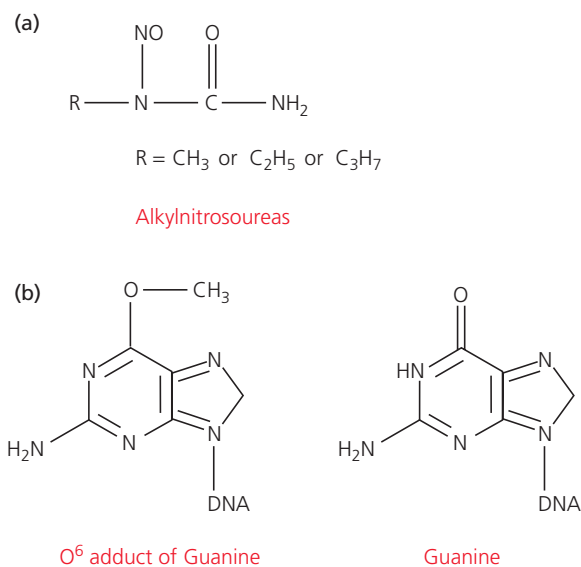
Gerd Pfeifer received his PhD from the University of Frankfurt, Germany. He has crossed the Atlantic and is currently a Professor and Chair at the City of Hope, Beckman Research Institute in California. His research group is continuing to study the mechanisms of mutagenesis in cancer and is also currently investigating **epigenetic** mechanisms of gene regulation in cancer (discussed in Chapter 3).

**Aromatic amines**

Heterocyclic amines (HCAs) are carcinogens produced by cooking meat, formed from heating amino acids and proteins. About 20 HCAs have been identified. Three examples, Phe-P-1, IQ, and Mel Q, are shown in Figure 2.6. It is important to be aware of these since they illustrate an example of carcinogens to which we may be exposed daily and which are produced in our own kitchens.



**Figure 2.6** Heterocyclic amines.



**Figure 2.7** (a) An example of nitrosamines: alkylnitrosoureas. (b) A potential carcinogenic product of nitrosamines: O<sup>6</sup> adduct of guanine. Guanine is shown for comparison.

### Nitrosamines and nitrosamides

Many nitrosamines and nitrosamides are found in tobacco or are formed when preservative nitrites react with amines in fish and meats during smoking. The structure of alkylnitrosoureas, examples of nitrosamines, is shown in Figure 2.7(a). Their principal carcinogenic product is alkylated O<sup>6</sup> guanine derivatives, as shown in Figure 2.7(b) (guanine is depicted next to it for comparison).

### Alkylating agents

Mustard gas (sulfur mustard, Figure 2.8) is the most well-known example of an alkylating agent because of its use and consequences observed during World War I. It is a bi-functional (having two reactive groups) carcinogen that is able to form intra-chain and inter-chain cross-links on DNA directly.

### Fibrous minerals: asbestos and erionite

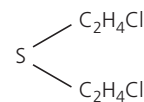
Asbestos and erionite are naturally occurring fibrous minerals that act as chemical carcinogens and mutagens. Asbestos is a group of fibrous silicate minerals that was used extensively in building materials because of its insulating properties but is now prohibited in several countries (but is still used in the developing world) due to association with several diseases of the lung, including lung cancer and mesothelioma. Erionite is a fibrous zeolite mineral formed from volcanic rock. Mechanisms of carcinogenesis include generation of ROS and induction of a chronic inflammatory response (see Chapter 10). Genetics may predispose some people to the carcinogenic effects of fibrous materials.

### PAUSE AND THINK

What is the structural difference between IQ and MeI Q?

### LIFESTYLE TIP

It has been suggested that changes in the way we prepare food can reduce the amounts of HCAs produced. Oven-roasting, marinating, and coating food with breadcrumbs before frying are modifications that may reduce the formation of HCAs.



**Figure 2.8** Structure of mustard gas.

## HOW DO WE KNOW THAT?

### Family pedigrees

Malignant mesothelioma is a rare cancer that is linked to fibrous minerals. It is rare in the USA and the UK; however, there is an epidemic of malignant mesothelioma in several small villages of Turkey that has been linked to exposure to erionite. Fifty per cent of all deaths in these regions are due to malignant mesothelioma! Pedigree studies of families that live in these villages uncovered a link between genetics and fiber carcinogenesis. Chemical and physical analysis, including scanning electron microscopy, mass spectrometry, and x-ray

diffraction, showed that there was no difference between the type of erionite in villages that exhibited the epidemic and those that did not. However, malignant mesothelioma was prevalent in some families and absent in others and marriages between these two groups led to some offspring developing the disease. Thus the data demonstrate that a genetic predisposition can influence mineral fiber carcinogenesis.

Dogan *et al.* (2006) and family pedigree data within: available online at <http://cancerres.aacrjournals.org/cgi/reprint/66/10/5063>

## Infectious pathogens as carcinogens

Early in the 20th century, viruses were shown to cause tumors in animals. As we will see in Chapter 4, they have been invaluable tools for investigating the molecular events of cell transformation. Viruses that are oncogenic can be classified as DNA tumor viruses or RNA tumor viruses (also called retroviruses), depending on the nucleic acid that defines their genome. The mechanisms of carcinogenesis for these two classes of virus differ. DNA tumor viruses encode viral proteins that block tumor suppressor genes, often by protein–protein interactions (discussed in Chapter 6). Many retroviruses cause cancers in animals by encoding mutated forms of normal genes (i.e. oncogenes) that have a dominant effect in host cells (discussed in Chapter 4). Mechanisms of replication between DNA and RNA viruses also differ. Some DNA viruses, such as human papilloma and Epstein–Barr viruses, replicate strictly as episomes within host cells. Retroviruses replicate by integration of the viral genome into the host DNA and utilize the host’s translational machinery to produce viral proteins. Integration may lead to deregulated gene expression.

Direct causation of cancer by specific viruses and bacteria has been demonstrated and will be discussed in detail in Chapter 10. However, a few named examples are given below. The International Agency for Research on Cancer (IARC) has classified human papillomavirus (type 16 and 18) as a human carcinogen and a causative agent of cervical cancer. In addition, Kaposi’s sarcoma-associated herpesvirus (KSHV) causes Kaposi’s sarcoma, hepatitis B virus is associated with liver cancer, and Epstein–Barr virus (EBV) with nasopharyngeal carcinoma. The human T-cell leukemia virus type 1 (HTLV-1) is the only retrovirus known to cause cancer in humans. It causes acute T-cell leukemia (ATL). *Helicobacter pylori*, a Gram-negative spiral bacterium, establishes chronic infection

and ulcers in the stomach and alters host cell function, which is associated with carcinogenesis. The International Agency for Research on Cancer has classified *H. pylori* as a human carcinogen and one of the causative agents of gastric cancer. The typhoid pathogen, *Salmonella enterica* serovar Typhi (*S. typhi*), establishes chronic infection in the gallbladder and has been linked to hepatobiliary and gallbladder carcinoma. The molecular events behind the mechanism of bacteria-induced transformation are the subject of current studies. The promotion of host cell proliferation, the generation of oxygen free radicals and subsequent DNA damage, and the activation of oncogenes are areas of investigation (Lax and Thomas, 2002).

### Endogenous carcinogenic reactions

In addition to carcinogens, endogenous cellular reactions generate mutations. Oxidative respiration and lipid peroxidation, two processes of normal cell metabolism, produce ROS that can react with DNA and lipids to produce oxidized products (e.g. 8-oxoguanine) also seen by exposure to radiation (see above). During respiration, the initiating radical, superoxide anion ( $O_2^-$ ) is produced upon reduction of NADH and formation of ubiquinone during oxidative phosphorylation. Therefore, breathing generates the same ROS intermediates as those generated by radiation! However, the dose of these intermediates differs between the two sources: radiation produces extremely reactive hydroxy radicals immediately and randomly within a cell, while respiration produces the less reactive superoxide radical immediately and only at specific locations within the cell.

Spontaneous chemical reactions (e.g. hydrolysis of the glycosidic bond between a base and deoxyribose producing an abasic site) also contribute to the formation of mutations. Deamination of cytosine to form uracil is the most common. Errors during DNA replication and DNA recombination contribute to the formation of mutations, although the DNA polymerases used possess proofreading ability to help minimize the number of mutations caused in this way. The proofreading function is dependent on the 3'-5' exonuclease activity of the polymerase. If an incorrect nucleotide is added to the growing 3' end of the newly synthesized strand the DNA double helix exhibits melting; that is, the strands remain separated at this point. Melting causes the polymerase to pause and the strand is transferred to the exonuclease site. Here the incorrect nucleotide is removed, the strand is transferred back to the original polymerase binding site, and DNA synthesis reoccurs. Overall, it is estimated that  $10^4$  to  $10^6$  mutations occur in a single human cell per day. By and large, under normal circumstances, this immense error burden is successfully dealt with by the highly efficient cellular DNA repair mechanisms.

## 2.4 DNA repair and predispositions to cancer

DNA repair is an important line of defense against mutations caused by carcinogens and by endogenous mechanisms. If DNA lesions are not repaired before a cell replicates, they may contribute to carcinogenesis. Repair of the various types of mutation is accomplished by several different DNA repair mechanisms. Five types of DNA repair systems are described below: one-step repair, nucleotide excision repair, base excision repair, mismatch repair, and recombinational repair. Defects in most of these pathways result in a predisposition to cancer.

### One-step repair

One-step repair involves the direct reversal of DNA damage. The repair enzyme alkyltransferase directly removes an alkyl group from the O<sup>6</sup> atom of guanine after exposure of DNA to alkylating carcinogens such as *N*-methylnitrosourea. In this case, a methyl group is transferred to a cysteine residue on the alkyltransferase and the alkyltransferase becomes inactive.

### Nucleotide excision repair (NER)

Nucleotide excision repair is specific for helix-distorting lesions such as pyrimidine dimers and bulky DNA adducts induced by environmental agents (UVB and PAHs, respectively). This damage interferes with transcription and replication as described above. Two subpathways exist: global genome NER surveys the genome for helix distortion and transcription-coupled repair identifies damage that interferes with transcription. The lesion, along with some (24–32) adjacent nucleotides, is excised out by endonucleases, and DNA polymerase  $\delta/\epsilon$  is used to fill in the gap using the opposite strand as a template. Proliferating cell nuclear factor is part of the polymerase holoenzyme and physically forms a ring that encircles and binds the damaged region. Xeroderma pigmentosum (XP) is an inherited disorder characterized by a defect in NER. Affected individuals are hypersensitive to the sun and have a 1000-fold increased risk of skin cancer. Seven XP gene products (XPA–XPG) have been identified out of the 25 proteins involved in NER.

### Base excision repair

Base excision repair targets chemically altered bases (e.g. 8-oxoguanine) induced mostly by endogenous mechanisms; in the absence of such repair the damage will cause a point mutation. The chemically altered bases may

be small enough not to interfere with replication or transcription. For example, failure to remove a 8-oxoguanine:A base pair before replication results in a G→T transversion mutation. The first step of base excision repair is carried out by a family of DNA-damage-specific glycosylases which scan millions of base pairs per second for lesions. Glycosylases then flip the lesion outside of the helix and cleave the base from the DNA backbone, creating an abasic site. Subsequently an endonuclease cleaves the DNA strand at the abasic site and DNA polymerase  $\beta$  replaces the nucleotide and ligase fills the gap. Mutations in the *OGG1* gene that codes for the principal glycosylase responsible for the repair of 8-oxoguanine have not been identified in tumors to date. No inherited defects in BER had been identified in humans until recently—mutations in the *MYH* gene that encodes a DNA glycosylase responsible for the removal of mismatched adenines paired with 8-oxoguanine may be the principal cause of multiple colorectal adenoma syndrome (Al-Tassan *et al.*, 2002).

### Mismatch repair

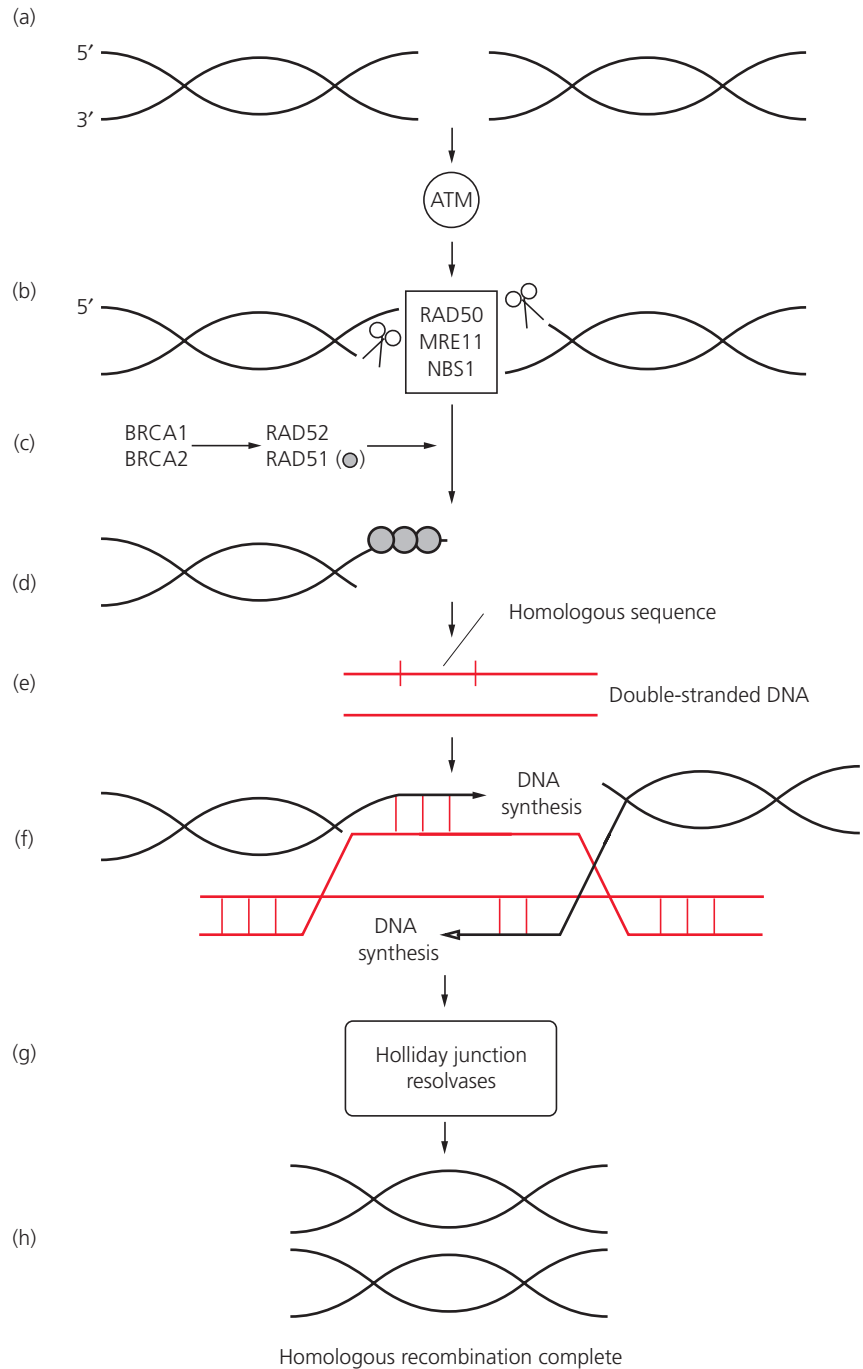
Mismatch repair corrects replication errors that have escaped editing by polymerases. It includes repair of insertions and deletions produced as a result of slippage during the replication of repetitive sequences as well as nucleotide mismatches. The molecular events can be described in brief as follows:

- recognition of the mismatch is carried out by proteins HMSH2/6 and hMSH2/3
- hMLH1/hPMS2 and hMHL1/hPMS1 are recruited
- the newly synthesized strand is identified (flagged by the replication machinery)
- endonucleases and exonucleases remove the nucleotides around and including the mismatch
- DNA polymerases resynthesize a newly replicated strand.

Hereditary non-polyposis colorectal cancer (HNPCC) is one of the most common cancer syndromes in humans. Half of all patients with HNPCC carry a **germline mutation** in *bMLH1* or *bMSH2*. Loss of function of the protein products encoded by these genes is responsible for complete loss of mismatch repair. Thus, cells are vulnerable to mutations.

### Recombinational repair

Homologous recombination and non-homologous end-joining are two types of recombinational repair that mend double-strand DNA breaks. Homologous recombination depends on the presence of sister chromatids



**Figure 2.9** Recombinational repair.

formed during DNA synthesis as a template for recombining severed ends. Many members of the same protein family make up a complex that performs what has been nicknamed DNA gymnastics. The molecular events shown in Figure 2.9 are described in brief:

- (a) A double-strand break activates the ataxia telangiectasia mutated (ATM) kinase.
- (b) The RAD50/MRE11/NBS1 complex (a substrate of ATM) uses its 5'–3' exonuclease activity (depicted by scissors in Figure 2.9) to create single-stranded 3' ends.
- (c) BRCA1/2 aids in the nuclear transport of RAD51 (shown as gray circles).
- (d) RAD52 facilitates RAD51 binding to these exposed ends to form a nucleoprotein filament.
- (e) RAD51 can exchange a homologous sequence from a single strand within a double-stranded molecule (shown in red; e.g. a sister chromatid), with a single-stranded sequence.
- (f) The sequences from the double-stranded molecule are then used as a template sequence for repair.
- (g) Resolvases restore the junctions formed as a result of homologous recombination, called Holliday junctions.
- (h) Two copies of intact DNA molecules are produced with rarely any errors.

Ataxia telangiectasia is an inherited syndrome whereby patients have a mutation in the ataxia telangiectasia mutated (ATM) kinase. Patients are sensitive to X-rays and have an increased risk of lymphoma.

Suffice it to say that the other type of recombinational repair, end-joining, links non-homologous ends and is therefore error prone, and can possibly result in chromosomal translocations.

One of the main molecular players involved in carcinogenesis is p53 and should be mentioned here. p53, 'the guardian of the genome', is a protein that plays an important role in the molecular events that protect the integrity of DNA; it is central in the orchestration of DNA repair. The details of this important tumor suppressor protein will be discussed in Chapter 6.

## Therapeutic strategies

### 2.5 Conventional therapies: chemotherapy and radiation therapy

Conventional therapies continue to extend and save lives. It is important to understand their rationale before moving to more molecular approaches discussed later in the text. Several conventional therapies aim to induce extensive DNA damage in order to trigger apoptosis and paradoxically

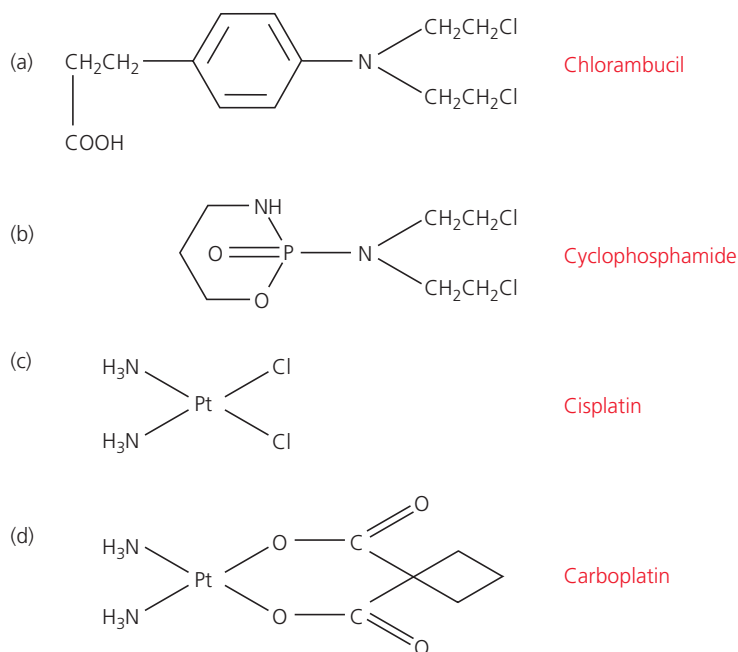
include agents classified as carcinogens. Other conventional therapies inhibit DNA metabolism in order to block DNA synthesis in the rapidly dividing cancer cells. DNA synthesis is essential to produce a new set of chromosomes for the daughter cells produced by cell division. Still other drugs interfere with the mechanics of cell division. Both chemotherapies and radiotherapy will be discussed.

## Chemotherapy

A brief description and examples of the three main types of classical chemotherapy are given below.

### Alkylating agents and platinum-based drugs

Alkylating agents and platinum-based drugs work by a similar mode of action. Alkylating agents have the ability to form DNA adducts by covalent bonds via an alkyl group. They may act during all phases of the cell cycle. Chlorambucil (Figure 2.10a) is one example of a member of the nitrogen mustard family of drugs. Its usual target is the N7 position of guanine residues. Bi-functional alkylating agents (compounds with two reactive groups) form intra-strand and inter-strand cross-links in DNA that alter the conformation of the double helix or prevent separation of the DNA strands and interfere with DNA replication. They are much more potent than monofunctional analogs, indicating that cross-linking is the basis of their function since monofunctional analogs cannot cross-link.



**Figure 2.10** Examples of alkylating agents and platinum-based drugs.

Some drugs require metabolic activation within the body. The alkylating agent cyclophosphamide (Figure 2.10b) is one example. Oxidases in the liver produce an aldehyde form that decomposes to yield phosphoramidate mustard, the biologically active molecule.

The platinum-based drugs, such as cisplatin [cis Pt(II)(NH<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>] and carboplatin (Figure 2.10c and 2.10d, respectively), form covalent bonds via the platinum atom. Cisplatin is a water-soluble molecule that contains a Pt atom bound to four functional groups. The Pt–N bond has covalent character and is essentially irreversible, whereas that with Cl is more labile. Cl is replaced with water in the plasma and cytosol before the molecule binds to the N7 position of guanine and adenine in its DNA target. The GG, AG, and GXG (where X can be any base) adducts comprise over 90% of the total. The resulting DNA damage triggers apoptosis. Although cisplatin had a major impact on some cancers, such as ovarian cancer, it was associated with irreversible kidney damage. Later, carboplatin was identified as a less toxic platinum analog.

#### PAUSE AND THINK

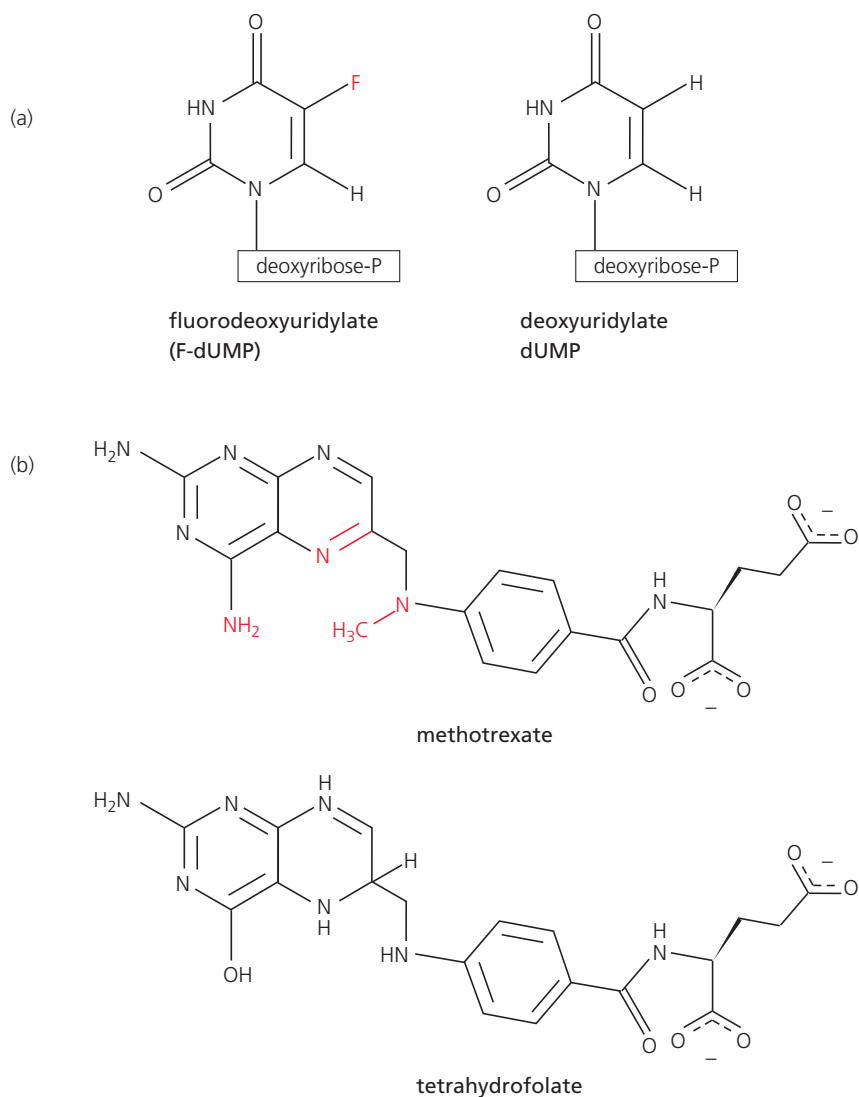
Do you recall a similar mechanism of action for any carcinogens?

### Antimetabolites

**Antimetabolites** are compounds that are structurally similar to endogenous molecules (e.g. nitrogenous bases of DNA) and therefore can mimic their role and inhibit nucleic acid synthesis. Two examples, fluorodeoxyuridylate (F-dUMP) and methotrexate, are shown alongside similar endogenous molecules, deoxyuridylate and tetrahydrofolate, respectively, in Figure 2.11. 5-Fluorouracil (5-FU) is a derivative of uracil and is converted into F-dUMP. F-dUMP competes with the natural substrate dUMP for the catalytic site of thymidylate synthase, the enzyme that produces thymidylate (dTMP) (Figure 2.12). F-dUMP forms a covalent complex with the enzyme and acts as a suicide inhibitor, generating an intermediate that inactivates the thymidylate synthase through covalent modification. As a result, the dTMP and dTTP pools are depleted, dUMP and dUTP accumulate, and DNA synthesis in rapidly dividing cells is severely compromised. Another important antimetabolite, methotrexate, targets an accessory enzyme of the same reaction. As an analog of dihydrofolate, methotrexate is a competitive inhibitor of dihydrofolate reductase, the enzyme used to regenerate tetrahydrofolate that is required in the thymidylate synthase reaction (Figure 2.12; see Chapter 11 for further discussion of tetrahydrofolate).

### Organic drugs

Doxorubicin is a fungal anthracycline antibiotic that inhibits topoisomerase II, an enzyme that releases torsional stress during DNA replication, by trapping single-strand and double-strand DNA intermediates. Doxorubicin diffuses across cell membranes and accumulates in most cell types. Cardiac damage is its most severe side-effect, but new compounds (e.g.



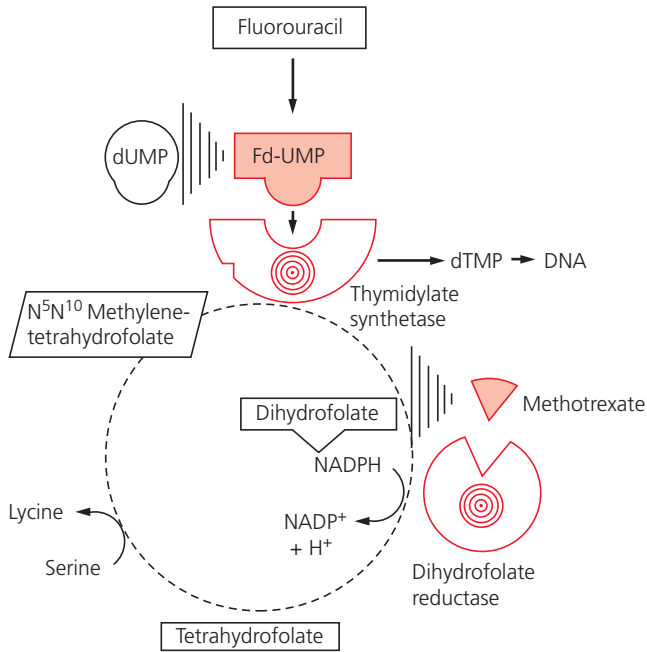
**Figure 2.11** Antimetabolites: (a) fluorodeoxyuridylylate (F-dUMP) and (b) methotrexate. Structural differences between the antimetabolite and endogenous molecule are shown in red.

ICRF-187) that can block the cardiac toxicity are being investigated. These drugs are primarily used to treat solid tumors (e.g. of the breast or lung).

The plant alkaloids vincristine and vinblastine (from the Madagascar periwinkle plant) bind to tubulin and prevent microtubule assembly in contrast to the drug paclitaxel (taxol) which binds to the  $\beta$ -tubulin subunit in polymers and stabilizes the microtubules against depolymerization. Thus two opposing strategies can be used to disrupt the mitotic spindle.

## Radiation therapy

Radiation therapy, either alone or in combination with other therapies, is received by approximately 60% of cancer patients in the USA. Ionizing

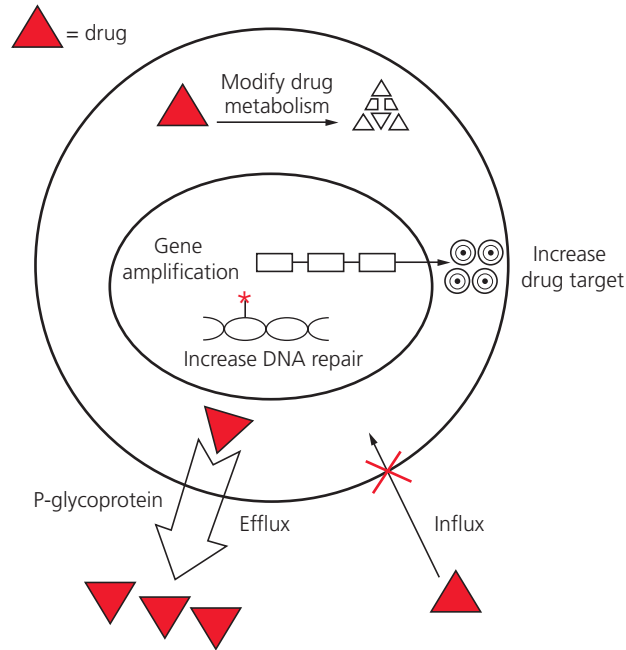


**Figure 2.12** Action of antimetabolites fluorodeoxyuridylate (F-dUMP) and methotrexate (both shaded red). The enzyme thymidylate synthetase uses N<sup>5</sup>N<sup>10</sup> methylenetetrahydrofolate as a methyl donor and catalyzes the methylation of dUMP to form dTMP. The cancer drug fluorouracil is converted into the antimetabolite F-dUMP (red rectangular shape), which competes (////) with dUMP and targets thymidylate synthetase (target symbol, ⊗, shown). Methotrexate (red triangle) is an antimetabolite that competes (////) with dihydrofolate and methotrexate targets the enzyme dihydrofolate reductase (target, ⊗, symbol).

radiation is usually delivered to the tumor by electron linear accelerators. Radiation reacts with water inside cells to generate reactive oxygen species that damage DNA. Apoptosis will be induced in cells that contain large amounts of DNA damage. The supply of oxygen affects the potency of ionizing radiation and is thought to be due to the generation of ROS. Oxygen can assist in making radiation-induced damage permanent. More double-strand breaks occur in cells irradiated in the presence of oxygen than in cells irradiated in the absence of oxygen. Therefore, the number of zones of hypoxia within a solid tumor influences the outcome of radiation treatment. Targeting of the tumor has been made more precise by modern techniques such as magnetic resonance imaging (MRI) and computed tomography (CT) which produce three-dimensional images of the tumor within the body.

### Heterogeneous cell sensitivity and drug resistance: obstacles to these treatments

In addition to the severe side-effects that result, there are also practical problems with conventional therapies. Cancer cells, as part of a large tumor



**Figure 2.13** Mechanisms of drug resistance. Based on a diagram printed in *Annu. Rev. Med.* 53, Copyright (2002) by Annual Reviews (<http://www.annualreviews.org>).

### PAUSE AND THINK

We are familiar with the concept that overuse of antibiotics can lead to the creation of resistant bacteria. Their rapid division and high frequency of mutation allows for the selection of bacterial strains that can evolve to survive in the presence of the drug. Rapidly dividing human cells that are genetically unstable can acquire resistance in a similar manner.

mass, will receive different doses of treatment depending on the location of individual cells within the mass. Cells deep within the tumor and therefore furthest from the blood supply will receive lower doses than cells on the surface of the tumor. Cells within the same tumor may have acquired different mutations and some cells may have become resistant to the drug.

Anticancer drugs impose a strong force for the selection of cells that can acquire drug resistance. There are several mechanisms that a cancer cell may utilize to become resistant to chemotherapy (Figure 2.13). Cells may become resistant by increasing the efflux of the drug, decreasing the intake of the drug, increasing the number of target molecules within the cell, or altering drug metabolism or DNA repair processes. Increasing the efflux of a drug is regulated at the cell surface. There is a family of ATP-dependent transporters that are involved in the movement of nutrients and other molecules across membranes. The multi-drug resistance gene (*MDR1*) codes for one member of this family called P-glycoprotein (P-gp) or the multi-drug transporter. This protein, normally a chloride ion efflux pump, can bind a variety of chemotherapeutic drugs including doxorubicin, vinblastine, and taxol. Upon binding, ATP is hydrolyzed and causes a conformational change of P-gp. As a result, the drug is released extracellularly. The transporter can be recycled by a second hydrolysis of ATP and continue to increase the efflux of the drug. Some drugs utilize specific transporters to enter cells. Mutations in these receptors may render them non-functional and decrease influx of the drug. Resistance to methotrexate commonly occurs by mutation of the folate transporter. An increase in the number of drug target molecules by gene amplification

is another means of developing resistance against methotrexate. The *DHFR* gene is amplified in some cancer cells. An increase in the efficiency of DNA repair, such as increased alkyltransferase activity, can give rise to resistance from alkylating agents such as doxorubicin. Levels of this enzyme are found to be highly variable in different tumors.

### ■ CHAPTER HIGHLIGHTS—REFRESH YOUR MEMORY

- In simplistic terms, a gene consists of a regulatory region and a coding region. Mutations in the former may alter gene expression while mutations in the latter may affect the gene product.
- Most carcinogens are mutagens.
- Several types of mutations include: base substitutions (transitions and transversions), frameshift mutations (insertions or deletions), and chromosomal translocations.
- Mutations in the promoter region of a gene may alter its regulation.
- Mutations in the coding region of a gene may alter the function of the gene product.
- Carcinogens include radiation, chemicals, and infectious pathogens.
- Radiation can damage DNA directly or indirectly through the formation of reactive oxygen species (ROS).
- Three intermediate ROS formed from the radiolysis of water are the hydroxyl radical, hydrogen peroxide, and the superoxide radical.
- The hydroxyl radical is one of the most reactive substances.
- Many carcinogens need to be metabolized to form an ultimate carcinogen that covalently binds to DNA.
- Many chemical carcinogens add functional groups covalently to DNA.
- Both viruses and bacteria have been classified as carcinogens for specific cancers.
- One-step repair, nucleotide excision repair, base excision repair, mismatch repair, and recombinational repair are five systems for repairing damaged DNA.
- Patients with xeroderma pigmentosum have an inherited defect in NER and have a 1000-fold increased risk of skin cancer.
- Many patients with hereditary non-polyposis colorectal cancer (HNPCC) have an inherited defect in mismatch repair.
- The major types of chemotherapies are:
  - alkylating agents—two examples are clorambucil and cisplatin;
  - antimetabolites—two examples are 5-FU and methotrexate;
  - organic drugs—two examples are vincristine and vinblastine.
- The development of drug resistance is a major problem for chemotherapy.

### ■ ACTIVITY

1. Make a list of five carcinogens and the mutations they cause. Describe the method of DNA repair used to correct each type of mutation.

### ■ FURTHER READING

Chabner, B.A. and Longo, D.L. (2001) *Cancer chemotherapy and biotherapy—principles and practice*, 3rd edn. Lippincott Williams and Wilkins, Philadelphia, PA.

- David, S.S., O'Shea, V.L., and Kundu, S. (2007) Base excision repair of oxidative DNA damage. *Nature* **447**: 941–950.
- Gottesman, M.M. (2002) Mechanisms of cancer drug resistance. *Annu. Rev. Med.* **53**: 615–627.
- Hecht, S.S. (2003) Tobacco carcinogens, their biomarkers and tobacco-induced cancer. *Nature Rev. Cancer* **3**: 733–737.
- Hoeijmakers, J.H.J. (2001) Genome maintenance mechanisms for preventing cancer. *Nature* **411**: 366–374.
- Ichihashi, M., Ueda, M., Budiyanoto, A., Bito, T., Oka, M., Fukunaga, M., Tsuru, K., and Horikawa, T. (2003) UV-induced skin damage. *Toxicology* **189**, 21–39.
- Lane, N. (2002) *Oxygen—the molecule that made the world*. Oxford University Press, Oxford.
- Lax, A.J. and Thomas, W. (2002) How bacteria could cause cancer: one step at a time. *Trends Microbiol.* **10**: 293–299.
- Manning, C.B., Vallyathan, V., and Mossman, B.T. (2002) Diseases caused by asbestos: mechanisms of injury and disease development. *Int. Immunopathol.* **2**: 191–200.
- Pfeifer, G.P., You, Y.-H., and Besaratinia, A. (2005) Mutations induced by ultraviolet light. *Mutation Res./Fund. Mol. Mech. Mutagenesis* **571**: 19–31.
- Williams, G.M. and Jeffrey, A.M. (2000) Oxidative DNA damage: endogenous and chemically induced. *Regulatory Toxicol. Pharmacol.* **32**: 283–292.

### ■ SELECTED SPECIAL TOPICS

- Alexandrov, K., Cascorbi, I., Rojas, M., Bouvier, G., Kriek, E. and Bartsch, H. (2002) CYP1A1 and GSTM1 genotypes affect benzo[a]pyrene DNA adducts in smokers' lung: comparison with aromatic/hydrophobic adduct formation. *Carcinogenesis* **23**: 1969–1977.
- Al-Tassan, N., Chmiel, N.H., Maynard, J., Fleming, N., Livingston, A.L., Williams, G.T., Hodges, A.K., Davies, D.R., David, S.S., Sampson, J.R., and Cheadle, J.P. (2002) Inherited variants of MYH associated with somatic G:C→T:A mutations in colorectal tumors. *Nature Genet.* **30**: 227–232.
- Davies, H., Bignell, G.R., Cox, C., Stephens, P., Edkins, S., Clegg, S., Teague, J., Woffendin, H., Garnett, M.J., and Bottomley, W. (2002) Mutations of the *BRAF* gene in human cancer. *Nature* **417**: 949–954.
- Dogan, A.U., Baris, Y.I., Dogan, M., Emri, S., Steele, I., Elmishad, A.G., and Carbone, M. (2006) Genetic predisposition to fiber carcinogenesis causes a mesothelioma epidemic in Turkey. *Cancer Res.* **66**: 5063–5068.
- Preston, D.L., Shimizu, Y., Pierce, D.A., Suyama, A., and Mabuchi, K. (2003) Studies of mortality of atomic bomb survivors. Report 13: solid cancer and noncancer disease mortality; 1950–1997. *Radiat. Res.* **160**: 381–407.
- Smith, C.J., Perfetti, T.A., Garg, R., and Hansch, C. (2003) IARC carcinogens reported in cigarette mainstream smoke and their calculated log P values. *Food Chem. Toxicol.* **41**: 807–817.
- Whitmore, S.E., Morison, W.L., Potten, C.S., and Chadwick, C. (2001) Tanning salon exposure and molecular alterations. *J. Am. Acad. Dermatol.* **44**: 775–780.