# Chapter 3
# Mutational Effects

**Edward L. Croom**

**Abstract** Mutagens are agents that cause permanent genetic changes (i.e., mutations). These mutations can be limited to a single cell or may become part of an entire population of organisms. Chemicals as well radiation can cause mutations. Mutagens can be divided into direct and indirect acting mutagens. Direct acting chemical mutagens are often electrophilic and chemically reactive. Indirect acting chemical mutagens often require metabolic activation. Some agents (e.g., ionizing radiation) can act as both. Radiation induced mutational damage is related to the size and speed of the particles with larger particles causing more damage but having less penetrating power. Mutations can be limited to a single base or can involve multiple chromosomes. Mutations can occur in coding and non-coding regions of the genome. Mutations in the coding regions of the genome range in severity from generally harmless silent mutations to lethal vital protein destroying mutations. Mutations in the non-coding regions can sometimes cause functional changes (e.g., splice enhancer mutations). The earlier during development a mutation occurs the greater the potential for it to spread and impact the entire organism. Mutations that occur in the germ cells can be passed on to future generations and are of the greatest concern. Mutation frequencies differ depending on the bases being substituted and the mutagens involved. Translational toxicology includes efforts to limit exposures to potent chemical mutagens (e.g., aflatoxin) and mutagenic radiation (e.g., radon gas, UV light).

**Keywords** Mutagens • Mode of action • Radiation • DNA repair

## 3.1 Introduction

Deoxyribonucleic acid (DNA) is the molecule that allows biological information to be passed down from generation to generation. DNA provides the instructions on how many and what types of proteins and ribonucleic acids (RNAs) are made in

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each cell. It is these proteins and RNAs that perform most of the functions of the cell and by extension the functions of the entire organism.

RNAs are short-lived molecules as are most proteins. These molecules are designed to be replaced over time and generally will last in the range of seconds to months. In contrast the DNA that makes up chromosomes is designed to last the lifetime of the cell. In the case of nerve cells those chromosomes have to last the lifetime of the organism which for humans is decades. Although many cells are short lived and are replaced after only a couple of days (e.g., mouth epithelial cells) the underlying cells that form a constant supply of replacement cells are longer lived and are designed to be able to divide regularly as needed.

It is the overwhelmingly successful ability to faithfully copy DNA from one cell to another that allows cells and organisms to function successfully over time. In general mutations are likely to be destructive at worst or silent at best. A small percentage of mutations will convey some advantage. These advantages may even be passed down through generations and maintained if they are sufficiently beneficial to convey some survival advantage. Even mutations that convey some survival advantage tend to have harmful effects. Examples of this are show with mutations in the G6PDH and hemoglobin genes that confer increased resistance to malaria but which are also associated with favism and sickle cell disease respectively.

This chapter is organized into four parts. The first part describes the chemistry of DNA and basic mechanisms of how it is first transcribed into RNA and then next translated into protein. The second part covers the types of mutagens that exist. The third part describes the types of mutations that can occur. The forth part describes repair mechanisms. Throughout the chapter case studies will be provided that highlight clinically relevant mutagens, mutations, and mechanisms.

Because of ethical and practical restrictions on the studies of how mutagens cause human mutations much of the available information is derived from studies involving *in vitro* model systems (e.g., bacteria, blood cells) and *in vivo* models (e.g., repair deficient mice). For this reason case studies may have mechanistic data based on models even when they are based on known clinically relevant human mutations. When possible, examples of translational toxicology efforts to limit exposures to known or anticipated human mutagens will be provided.

## 3.2 Normal Replication

DNA is the molecule that allows biological information to be passed down from generation to generation. This occurs through a process called DNA replication. DNA is normally a doubled stranded molecule that is found in the form of a double helix. For DNA to be copied this helix must first be unwound. This unwinding action is performed by enzymes called helicases (Fig. 3.1).

Although both DNA strands can be copied at the same time they are copied at different rates. This is because DNA is only formed in one direction from 5′ to 3′ because only the 3′ end has an open hydroxyl group open to link to another phosphate and base. Because DNA can only be formed from 5′ to 3′, DNA is only read

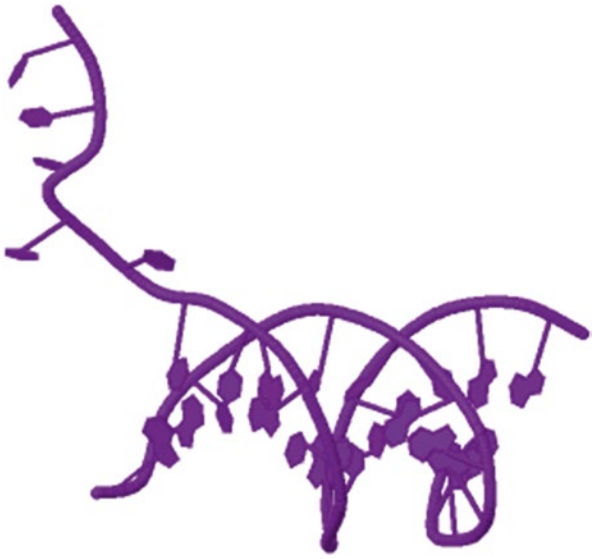

**Fig. 3.1** DNA unwound by a helicase. Image modified from RCSB PDB file 4CGZ deposited by Newman et al. (2014)

from the 3′ to 5′ end (Fig. 3.2. The two strands are identified as the lagging strand (5′ to 3′) and the leading strand (3′ to 5′). Although both strands can be read once that section of DNA is unwound the leading strand can be read continuously while the lagging strand is read in sections.

In humans DNA is organized in 23 pairs of chromosomes. DNA is read and copied by enzymes called DNA polymerases. These DNA polymerases are extremely efficient and have an error rate of less than 1 in 100,000 bases. Proofreading and repair mechanisms further reduce that rate so the overall error rate is only around 1 in a billion. This is a very high degree of fidelity and to continue the text analogy would be the equivalent of having to type roughly 1000 books worth of text but only be allowed a single typo (Wang 2008). These repair mechanisms will be examined later in Sect. 3.1.5.

DNA is made up of four bases; cytosine, guanine, thymine and adenine (Fig. 3.3). These bases differ in terms of their chemical reactivity and likelihood to cause mutations when modified. DNA is normally double stranded with cytosines pairing with guanines and adenines pairing with thymines. These DNA bases are transcribed (i.e., copied) into an RNA format. Human chromosomal DNAs are extremely long molecules, too long and too large to ever fit through the nuclear membrane. Even if the DNA could fit through nuclear membrane even the smallest chromosomes contain many genes and most of the information would need to be removed before a single gene could be transcribed.

To efficiently transfer a gene's worth of information from the nucleus to the cytoplasm where proteins are made a special form of RNA called precursor messenger

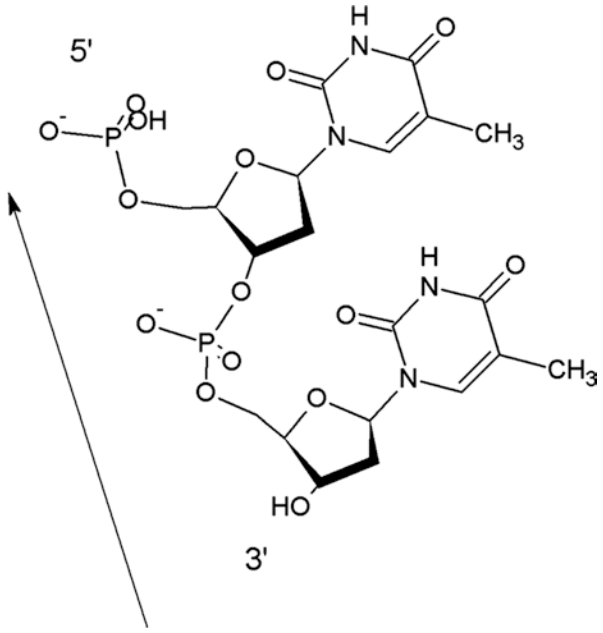


**Fig. 3.2** DNA. The *arrow* next to the DNA molecule shows the direction of the order of which DNA is read

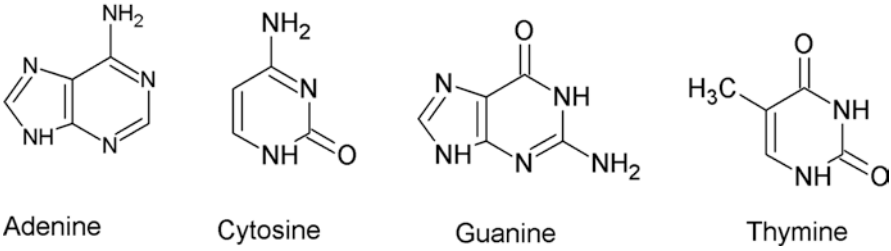


**Fig. 3.3** DNA bases

RNA (pre-mRNA) is created. This pre-mRNA contains codons identical to the opposite DNA sequence except that instead of thymine, RNA has uracil. Both DNA and RNA are read in units of three base pairs called codons. These DNA codons are transcribed into mRNA and then translated into amino acids. DNA can be thought of as an instruction manual where the instructions are always provided using three letter words. For example "USE THE BAR AND PRY THE RED TOP OFF."

There is redundancy in the codon language and there are more codons than amino acids. Typically the first two bases are the same for a given amino acid and only the third base in the codon differs. This is exemplified by proline which is coded for by the four mRNA codons CCU, CCC, CCA, and CCG. An exception to

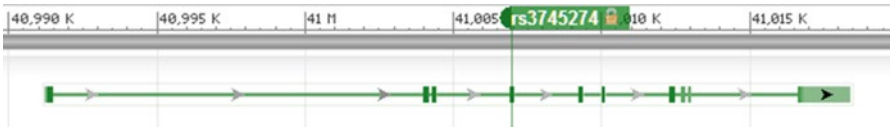


**Fig. 3.4** CYP2B6 exons and introns. CYP2B6 exons are shown as the *vertical green bars*. The introns are shown as the *light green lines* between the exons. The *scale above* shows the position of the gene on the chromosome which ranges roughly 25,000 bp (Source rs3745274, http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=3745274)

redundancy in coding is methionine. Methionine, the start codon, is coded for by just one codon, AUG. Leucine, Arginine and Serine also differ from this rule by having more than four codons each.

DNA is organized into genes that form proteins and surrounding portions of DNA that do not form proteins although they may play a role in regulating gene expression. Genes also contain coding and non-coding portions. Exons are the coding parts and introns are the non-coding parts (Fig. 3.4. Both introns and exons are transcribed into pre-RNA but only exons are taken out of the nucleus as mRNA and translated into protein. Introns generally take up most of the gene sequence while the exons only take up a small amount.

DNA is located inside the nucleus while proteins are created in the cytoplasm. In order to get the instructions on how to make those proteins from the nucleus to the cytoplasm an intermediary, messenger RNA (mRNA), is used. RNA polymerases use a DNA strand as a template the bases forming mRNA. This mRNA contains all of the codons but loses the introns and is further modified with the addition of a cap on the 5′ end and poly A (i.e., adenosine) tail on the 3′ end. This effectively shortens the pre-mRNA from thousands of bases down to just hundreds of bases for mRNA. A special collection of proteins combine to form the spliceosome. The spliceosome is responsible for taking the original transcript, called pre-mRNA, and modifying it by removing the introns and attaching the remaining exons into a much shorter messenger RNA.

Translation occurs in the ribosomes. The ribosomes are where the transfer RNA (tRNA) anticodons are paired with the mRNA codons. This positions the amino acids in the proper order and proximity to allow them to be joined together by the ribosome forming a polypeptide chain. The first amino acid in this polypeptide chain is always methionine however there can be many modifications before the final protein is formed and methionine may not be the first amino acid in the finished protein.

Human DNA is organized into 23 pairs of chromosomes. During most of the cell-cycle these chromosomes are generally in an open state and available for transcription. In this state open state the chromosome is referred to as euchromatin. However the DNA has to be condensed and packed tightly in order for mitosis and meiosis to occur. Proteins called histones function as tiny spools wrapping up the DNA tightly around them. There are many histones per chromosome and in this tightly wrapped form the chromosome is referred to as heterochromatin. There are always small portions of the chromosome that are heterochromatin even during

interphase. These parts are known as constitutive heterochromatin. Even when the X-chromosome is selectively deactivated forming Barr bodies some euchromatin is still accessible and some transcription still occurs. There is very strong evidence of this in the different phenotypes of XO females who exhibit the symptoms of Turner syndrome (e.g., short stature, webbed neck).

Because the enzymes involved in normal replication are not perfect with enough replication events some mistakes will be made even without external exposures. Even without the action of external forces DNA has a slight tendency to change spontaneously. DNA is chemically reactive and can be damaged by ionizing radiation, free radicals and electrophilic chemicals. The next section focuses on the types of chemicals and radiation capable of damaging DNA.

## 3.3 Types of Mutagens

Mutagens can be chemicals or radiation. Chemicals mutagens can be organic (e.g., benzo(a)pyrene) or inorganic (e.g., arsenic, chromium, nickel). Not all inorganic chemical mutagens are metalloids or metals. Nitric oxide is a mutagenic inorganic compound. Mutagenic radiation can be non-ionizing (e.g., UV) or ionizing (e.g., X-rays). There are many different types of mutagens but one of the most important ways of classifying them is as direct and indirect mutagens.

### *3.3.1 Direct Acting Mutagens*

Direct acting mutagens interact directly with DNA. Direct acting mutagens are either electrophilic chemically reactive molecules or are a form of radiation containing enough energy to change DNA directly.

#### 3.3.1.1 Direct Acting Chemical Mutagens

The electrophilic theory of chemical carcinogenesis was developed decades ago to explain the observation that human carcinogens tended to be mutagens that either started out as electrophiles or were activated to become electrophiles (Miller and Miller 1981).

Nitrogen mustards are an example of direct acting chemical mutagens. Nitrogen mustards were originally developed as chemical warfare agents. Their ability to alkylate DNA made them deadly chemicals on the battlefield. Their ability to damage DNA and kill rapidly dividing cells also led to them being used as an early form of chemotherapy.

Alkylating agents remain an important part of chemotherapeutic agents. Melphalan (Fig. 3.5 is an example of a direct acting chemotherapeutic mutagen.

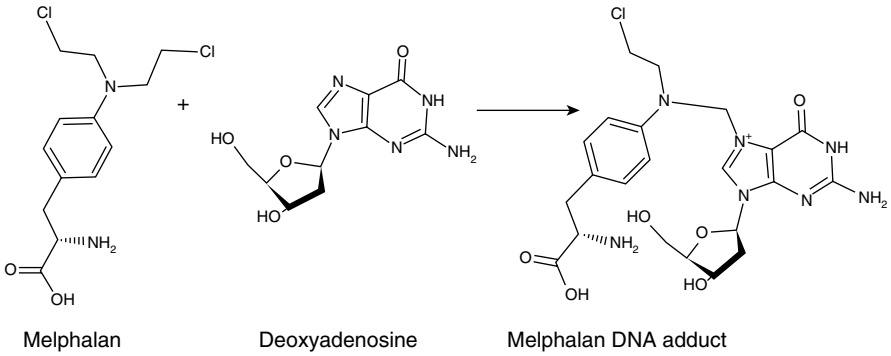


**Fig. 3.5** Melphalan adduct. Melphalan is shown reacting with Deoxyadenosine to form a melphalan DNA adduct

The N7 nitrogens in DNA purine bases are not involved in hydrogen bonding making them good targets for direct acting mutagens.

Platinum based chemotherapeutic drugs (e.g., cisplatin, carboplatin) are another group of direct acting mutagens that interact with N7 nitrogen atoms of purines (Burnouf et al. 1990). Platinum based chemotherapeutic drugs form intrastrand adducts by linking two adjacent purines (Fig. 3.6).

Another way mutagens can directly interact with DNA is through intercalation. Intercalating chemicals stick inside the double helix disrupting the structure of DNA. DNA contains a minor groove and major groove. Intercalating agents interact with either the major or minor grooves (Fig. 3.7). Intercalating mutagens generally have a ring structure that creates a planar part of the molecule that can slide between the bases.

**Case Study 1: Chemotherapeutic Mutagens** Mutagens can damage rapidly dividing cells which can make them useful as chemotherapeutic agents. However, mutagenic chemotherapeutic drugs are also often quite toxic to normal healthy cells causing significant damage to organs and limiting their use. Translational toxicology includes efforts to either develop chemoprotective drugs or more selective chemotherapeutic mutagens. In the case of doxorubicin, an intercalating agent, the major organ affected is the heart. In the case of cyclophosphamide, an alkylating agent, a major organ affected is the bladder. Melphalan, an alkylating agent, often reduces blood cell counts.

To help overcome the cardiotoxicity of doxorubicin a similar intercalating drug, epirubicin, was created. Epirubicin, can be as damaging to cancer cells as doxorubicin but is more readily metabolized and removed by normal cells. To help overcome the bladder toxicity associated with cyclophosphamide mesna, a chemoprotective drug, is added to scavenge the urotoxic cyclophosphamide metabolite acrolein. There is some evidence that in addition to protecting the bladder, mesna can reduce the mutagenicity of cyclophosphamide. While mesna has been reported to not reduce the mutagenicity of cyclophosphamide *in vitro*, there is

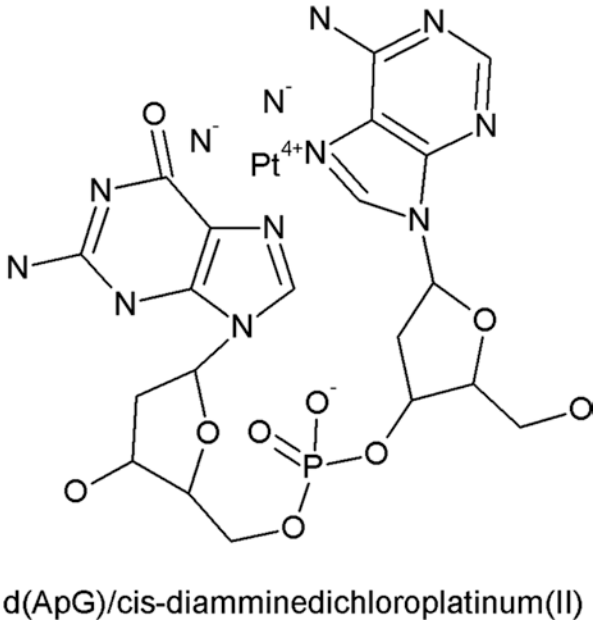


**Fig. 3.6** Cisplatin based DNA adduct (Structure source Burnouf et al. 1990)

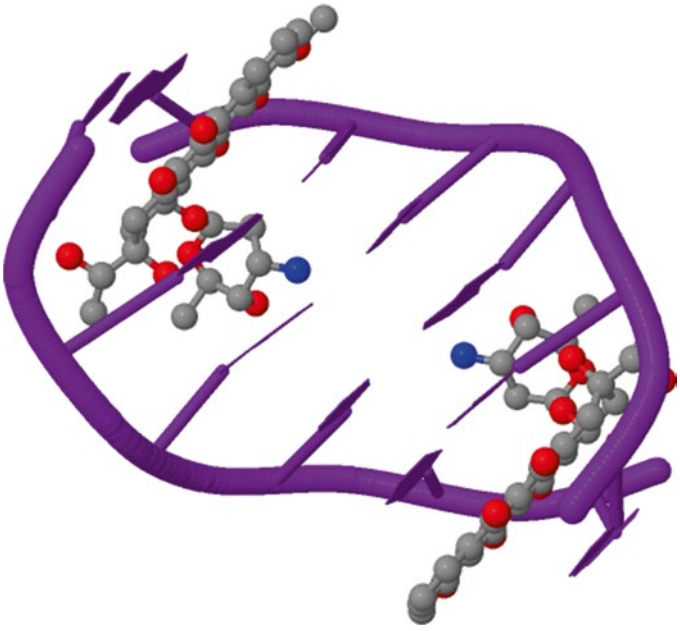

**Fig. 3.7** DNA and intercalating chemotherapeutic agents. Daunorubicin is shown in red, gray and blue. The DNA is shown in purple (Source RCSB PDB file 1DA0 deposited by Wang et al. 1987)

evidence that it can reduce the mutagenicity of cyclophosphamide *in vivo* as it was shown to reduce the amount of urinary mutagens in rodents treated with cyclophosphamide (Pool et al. 1988).

A third method of reducing the mutagenicity of chemotherapeutic drugs has been to develop prodrugs that are selectively activated by cancer cells. In effect this turns direct acting mutagens into targeted indirect acting mutagens. A recent example of this is the investigational drug melphalan-flufenamide, a pro-drug version of the alkylating chemotherapeutic agent melphalan. Melphalan-flufenamide has a peptide bond connecting melphalan to an amino acid which cleaved by a peptidase that is commonly overexpressed in cancer cells (Chauhan et al. 2013).

#### 3.3.1.2 Direct Acting Radiological Mutagens

Radiation can be directly mutagenic. Most electromagnetic radiation is not mutagenic. Visible light lacks sufficient energy to mutate cells as do the types of electromagnetic radiation with even longer wavelengths (e.g., infrared, microwaves). As wavelengths shorten the available energy increases. Only the shortest waves on the end of the electromagnetic spectrum found in ultraviolet (UV) radiation, x-rays, gamma rays and cosmic rays contain enough energy to directly mutate DNA.

UV radiation exposure primarily comes from sunlight. UV radiation is an important source of vitamin D in small exposures for many people but UV radiation causes DNA damage that can result in mutations. UV radiation is associated with cyclobutane lesions. The most common of these is a thymine dimer (Fig. 3.8).

Thymine dimers are one form of cyclobutane lesions. Cyclobutane lesions are more stable when the bonds holding the thymines together are shorter (Fig. 3.9). While the double helix structure of DNA is normally very symmetrical, cyclobutane lesions interferes with structure and reshape the molecule causing it to bend around them (Fig. 3.10).

**Case Study 2: UV Radiation** Exposure to UV radiation can be limited through the use of protective equipment, clothing and sunscreens. UV radiation is divided into UVA and UVB. Sunscreens contain chemicals designed to absorb most of the energy in UV radiation. Early sunscreens were designed primarily to absorb UVB radiation but increasingly they contain multiple active ingredients that when combined are designed to absorb both UVA and UVB radiation. The presence of at least one benzene ring is a common characteristic of many UV absorbing chemicals (Fig. 3.11).

### *3.3.2 Indirect Acting Mutagens*

Indirect acting mutagens interact first with another molecule and then a product of that interaction interacts with DNA. Indirect acting mutations can be chemical or radiological.

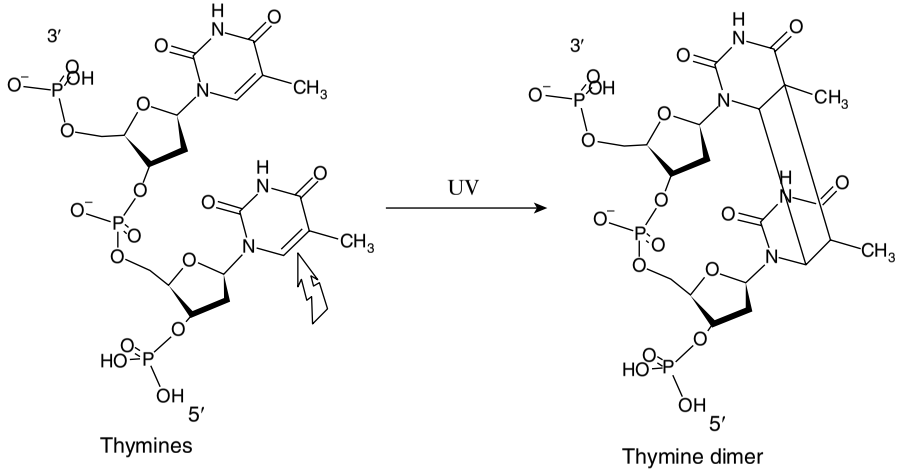


**Fig. 3.8** Thymine dimers. Thymine dimers form when UV light excites the double bonds in a thymine. When there is an adjacent thymine this extra energy makes it possible for the two double bonds to open up forming instead two new covalent bonds linking the thymines together

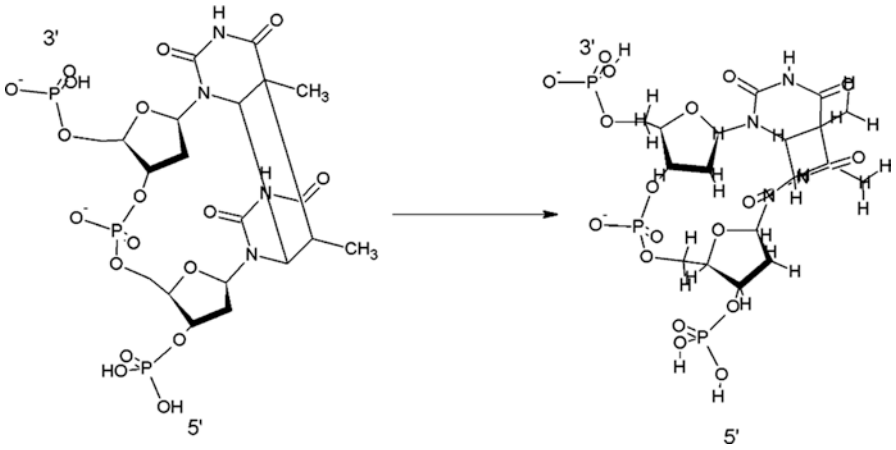


**Fig. 3.9** Cyclobutane lesions. When cyclobutane rings are created as in the formation of the thymine dimer shown tension is created. It takes a great deal of energy to have a cyclobutane ring stretched out and the ring will contract pulling the bases together

**Fig. 3.10** DNA with a thymine dimer is shown above. Note in the ends of the figure how the bases are neatly stacked upon one another and are directly across from their partner on the opposing DNA strand. In the middle of the strand is a thymine dimer where the two thymines have been pulled together after a cyclobutane ring forms. On the opposite strand one of the adenines is shown having been released. Figure created using a structure published on RCSB PDB by Vassylyev et al. (1995)

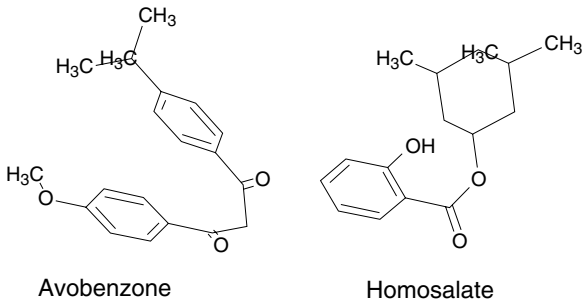


**Fig. 3.11** UV absorbing chemicals

### 3.3.2.1 Indirect Acting Chemical Mutagens

An example of an indirect acting mutagen is the promutagen benzo(a)pyrene. Benzo(a)pyrene is an aromatic hydrocarbon commonly found in combustion byproducts. In the liver it is bioactivated by the cytochromes P450 (CYPs) forming the mutagenic metabolite Benzo(a)pyrene-trans-7,8-dihydrodiol-10,11-epoxide (BPDE). BPDE forms adducts with DNA.

CYPs are commonly involved in bioactivation but other phase I and even phase II enzymes can play a role. In some cases phase I and phase II xenobiotic metabolizing enzymes can work together in sequence to make the ultimate mutagen. For example, sulfotransferases have been implicated in the further bioactivation of hydroxylated polycyclic aromatic hydrocarbons and some amines. The classic carcinogenic activity 2-acetylaminofluorine (2-AAF) has been shown to be the result of first hydroxylation by CYPs and then conjugation by sulfotransferases to form a reactive, electrophilic sulfuric acyl ester metabolite.

**Case Study 3: Aflatoxin** Aflatoxin is a naturally occurring indirect acting chemical mutagen. Aflatoxin is commonly produced by the fungi *Aspergillus flavus*. Aflatoxin $B_1$is metabolized in the liver by CYPs to form the reactive compound Aflatoxin $B_1$8,9-epoxide. High doses of aflatoxin $B_1$ are associated with DNA adduct formation and liver cancer development. To limit exposures to aflatoxin $B_1$ two main strategies are deployed. The first is to alter the storage and handling of the grains and peanuts that can become contaminated with *Aspergillus flavus* under favorable growth conditions (e.g., drought then damp). The second strategy is to screen food products for aflatoxin $B_1$ levels. The availability of relatively inexpensive and rapid testing methods (e.g., ELISAs) has made testing easier and in some cases field ready.

### 3.3.2.2 Indirect Acting Radiological Mutagens

Ionizing radiation is also an indirect acting mutation. Ionizing radiation can create free radicals in molecules near the DNA and those free radicals can then bind to the DNA. This can happen when the energy of the particles that make up ionizing radiation is enough to remove an electron on the outside of an atom. Water is the most

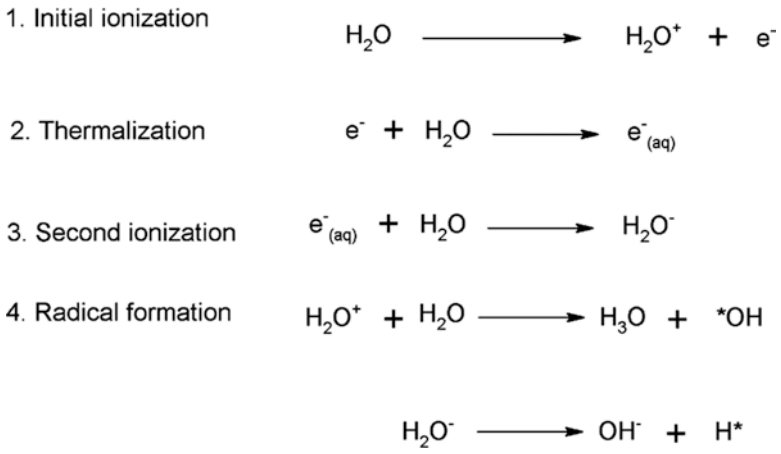


**Fig. 3.12** The first steps involved in the formation of free radicals in water by ionizing radiation

commonly molecule in the human body and not surprisingly is the most frequently ionized molecule as a result of exposure to ionizing radiation (Fig. 3.12).

The amount of energy contained in each particle depends on both the size and the speed of the particle. The larger the particle the less penetrating ability it possesses. Linear energy transfer is the covalent energy force related to the amount of energy transferred by the ionizing particle as it travels through a material by unit of length. Below a certain linear energy transfer level the body will not be damaged and mutations will not form. Linear energy transfer is closely related to stopping power.

Alpha particles are the largest particle emitted. They each contain two protons and two neutrons. This size significantly limits their ability to penetrate the body. However, when ingested or inhaled they can cause significant damage. Alpha emitters may be used medicinally but exposures may also be environmental. The high energy and low penetrance of alpha emitters make them useful for targeting tumors. The high energy and size of alpha-particles means that only a few alpha particles crossing the nucleus of a cell are enough to cause cell death. Alpha particles cause a high degree of double strand breaks.

Beta-emitters release beta particles which are electrons. Beta particles have limited linear electron transfer. It can take thousands of beta particle hits to kill a cell. The path length of a beta particle can range from ~50 to 1000 cell diameters. There a naturally occurring beta emitters (e.g., Carbon-14). Strontium-90 is a beta emitter produced when nuclear bombs are detonated. Levels of strontium-90 have declined significantly with implementation of the nuclear test ban treaty.

Gamma emitters lack the mass to directly ionize. Gamma rays are made up of photons and have significant penetrance but limited linear electron transfer. Instead they indirectly ionize by exciting nearby electrons. A single excited electron can transfer its kinetic energy in several ways. It can heat the surrounding tissue, excite nearby electrons or ionize. The ionization is what leads to mutation. A single high energy electron that is the result of absorbing a gamma or x-ray photon can produce over 1,000 low energy secondary electrons (i.e., delta rays). Eventually the excitation

energy becomes less than excitation level of water (7.4 eV), and the final extra energy is transferred through vibrational, rotational and collisional energy exchanges. This can lead to chromosomal breakage and other forms of DNA damage.

X-rays have even lower energy than gamma rays and are the last form of electromagnetic radiation that can ionize. Ultraviolet radiation, the next lowest down has ~100 fold less energy than X-rays and although UV radiation is mutagenic it lacks the ability to ionize water and other molecules. In contrast X-rays can penetrate soft tissues although the lack the ability to penetrate bone.

**Case Study 4: Alpha Particles** The size of alpha particles limits their pathway to only ~2–10 cell diameters. This makes them ideal candidates for being conjugated to tumor selective antibodies (e.g., Bi-213). When alpha particles conjugated with tumor selective antibodies are used against small tumors they have the potential to cause significant damage to the tumors but limited damage to the surrounding tissues.

Radon is an important alpha emitter that forms during the natural radioactive decay of uranium and thorium in the earth. Because radon is a gas it can concentrate inside homes build above uranium containing rock. Radon exposure is associated with increased lung cancer rates. While alpha particles have limited penetrance the thin lung alveoli walls make them easily damaged.

**Case Study 5: Beta Particles** One of the properties of radioactive chemicals is that they have a tendency to accumulate in bones and teeth. Strontium-90 levels have been studied non-invasively for years by collecting baby teeth. One of the more widely used beta emitters is Iodine-131. Iodine collects in the thyroid. To prevent the accumulation of radioactive iodine in the thyroid people at risk of being exposed (e.g., living near nuclear power plant) are often supplied with potassium iodide tables). These tablets can be taken preemptively to saturate the thyroid limiting the ability of radioactive iodide to accumulate. This tendency to accumulate in the thyroid has also made Iodine-131 useful to treat thyroid diseases (e.g., Graves' disease). In order to protect others from their temporarily radioactive bodies, patients receiving Iodine-131 have to follow strict procedures to protect their family members. They particularly have to stay away from very young children.

**Case Study 6: Gamma Knife** Gamma radiation has significant penetrating power. Gamma rays generated from gamma emitters (e.g., Cobalt 60) have become an important therapy for tumors that would otherwise be inoperable. Stereotactic radiosurgery uses externally generated radiation that is aimed at a target to destroy diseased tissue while preserving surrounding healthy tissue. With this technique tumor tissues inside the skull can be destroyed without having to create an incision.

### *3.3.3 Types of Human Mutagens*

Global Harmonization System of Classification and Labeling of Chemicals (GHS) is the international standard used to classify chemicals with respect to toxicity. Chemicals are classified into categories based on the weight of evidence. Mutagens

are classified as Category 1, 2 or not classified based on whether or not they are regarded as human germ cell mutagens.

#### 3.3.3.1 Category 1 Mutagens

Category 1 mutagens are human germ mutagens. They are further divided into Category 1A and Category1B. To date no chemicals have been classified as Category 1A as none have had adequate human epidemiological evidence. Category 1B human germ cell mutagens have some positive evidence towards mammalian germ cells. This evidence may be supported by evidence of mutagenicity towards somatic cells. With mammalian germ cells there may be evidence of generational inheritance. With human germ cells the evidence is limited to one generation. For example aneuploidy of the sperm cells is not going pass down to the next generation as changing the number of chromosomes will typically be lethal.

#### 3.3.3.2 Category 2 Mutagens

Category 2 mutagens are chemicals of concern as they are considered potential human germ cell mutagens. Category 2 chemicals are positive in animals and/or in vitro assays. The in vivo assays include sister chromatid exchange assays and assays of DNA damage (e.g., comet assay). The in vitro assays include chromosomal aberration tests and salmonella reverse mutagenicity tests (Hansel et al. 1997).

## 3.4 Types of Mutations

### *3.4.1 Base Substitutions*

#### 3.4.1.1 Introduction

Base substitutions are a point mutation where a single DNA base is mutated and changed from one base into another. Transition mutations occur when a purine is mutated into another purine (e.g., A → G). Transversion mutations occur when a pyrimidine is converted into a purine or a purine is converted into a pyrimidine (e.g., A → T). Mutation frequencies differ depending on the genes, tissues and the mutagens involved. Human lung tumors have been examined for mutation frequencies. With human tumors there is no controlling the exposures unlike with animal models. However, when tumor tissues collected from the same organs from hundreds of human donors were examined trends were seen. Roughly 40 % of the tumors had TP53 mutations while <20 % of the tumors had Kras2 mutations and <2 % of the tumors had Braf mutations (Jackson et al. 2006).

For example with the Kras2 gene exposures to 2-amino-3-methylimidazo[4,5-f] quinolone and 7H-dibenzo[c,g]carbazole were associated primarily with AT>TA

transversions (80 % and 92 % respectively) (Jackson et al. 2006). In contrast exposures to benzidine resulted primarily in GC>TA transversions and NNK caused Kras2 mutations in mouse lungs that were predominantly (96 %) GC>AT (Jackson et al. 2006).

#### 3.4.1.2 Silent Mutations

Silent mutations are mutations that change the base pair but do not change the amino acid (Fig. 3.13). These mutations most often occur in the third base of a codon. For example CGA codes for Arginine but so does AGA. With a silent mutation the original message "USE BAR AND PRY THE TOP OFF END" would stay the same "USE BAR AND PRY THE TOP OFF END."

Silent mutations in coding regions can still have an effect of protein formation. This is because coding regions also can contain regulatory elements such as splice enhancers. An example of this is the silent mutation (c.321C>T, p.D107D) in the rennin receptor gene (*ATP6AP2*) that nonetheless results in functional changes due to its function as a splice enhancer mutation. Ramser et al. reported a loss of exon 4 in 50 % of the mRNA and confirmed that truncated proteins were produced that had reduced function towards ERK1/2 activation (Ramser et al. 2005).

#### 3.4.1.3 Missense Mutations

Missense mutations are mutations that change the base pair and change the amino acid (Fig. 3.14). Clinically important missense mutations tend to change the amino acid property. Changing a large amino acid into a small amino acid or a polar amino acid into a non-polar amino acid are major changes that can disrupt the structure of the protein. Missense mutations can also result in a loss in protein expression if they also disrupt regulatory elements. An example of this is the CYP2B6*6 allele which

**a**

```
DNA:     5' - ATG ACT CAC CGA GCG CGA AGC TGA - 3'
         3' - TAC TGA GTG GCT CGC GCT TCG ACT - 5'
mRNA:    5' - AUG ACU CAC CGA GCG CGA AGC UGA - 3'
Protein:      Met Thr His Arg Ala Arg Ser Stop
```

**b**

```
DNA:     5' - ATG ACT CAC CGG GCG CGA AGC TGA - 3'
         3' - TAC TGA GTG GCC CGC GCT TCG ACT - 5'
mRNA:    5' - AUG ACU CAC CGG GCG CGA AGC UGA - 3'
Protein:      Met Thr His Arg Ala Arg Ser Stop
```

**Fig. 3.13** Silent mutations. Both the normal strand (**a**) and the mutant strand (**b**) form Arginine in the fourth position shown in *bold*

**a**

```
DNA:     5' - ATG ACT CAC CGA GCG CGA AGC TGA - 3'
         3' - TAC TGA GTG GCT CGC GCT TCG ACT - 5'
mRNA:    5' - AUG ACU CAC CGA GCG CGA AGC UGA - 3'
Protein:      Met Thr His Arg Ala Arg Ser Stop
```

**b**

```
DNA:     5' - ATG ACT CAC GGA GCG CGA AGC TGA - 3'
         3' - TAC TGA GTG CCT CGC GCT TCG ACT - 5'
mRNA:    5' - AUG ACU CAC GGA GCG CGA AGC UGA - 3'
Protein:      Met Thr His Gly Ala Arg Ser Stop
```

**Fig. 3.14** Missence mutations. Normal strand (**a**) forms Arginine while mutant strand (**b**) forms Glycine instead due to a missence mutation changing Cytosine to Guanine. Affected amino acids and nucleotides are *bolded*

involves the 516G>T, Q172H missense mutation removes a splice enhancer (Hoffman 2008). These types of missense mutations can be common and may play a major role in human variablitiy such as the common CYP2B6 polymorphisms and their impact on drug metabolism (Lang et al. 2001).

#### 3.4.1.4 Nonsense Mutations

Nonsense mutations are mutations that create a premature stop codon (Fig. 3.15). There are three stop codons and they have been named for colors UAA (Ochre), UAG (Amber), UGA (Opal). With a nonsense mutation the instructions to make a functioning protein are stopped prematurely. In text it would be as if instead of the sentence "USE THE BAR AND PRY THE RED TOP OFF END" you were only given "USE THE BAR AND END." It is not surprising that premature stop codons often result in non-functioning proteins.

### *3.4.2 Small Insertions and Deletions (INDELS)*

Small insertions (Fig. 3.16) and deletions (Fig. 3.17) (i.e., INDELS) are defined as insertions or deletions of a size between 1 and 10,000 bases. While INDELS are less common than base pair changes and SNPs they are still widespread. Several million INDELS have been identified in the human genome (Mulaney et al. 2010). Continuing the text example "USE THE BAR AND PRY THE RED TOP OFF END" could change to "USE THE BAR PRY THE RED TOP OFF END" with a small deletion or "USE THE BAR TOP OFF END" with a larger deletion. Similarly an insertion could change "USE THE BAR AND PRY THE RED TOP OFF END" to "USE THE BAR AND CAR FOR GET TEN PRY THE RED TOP OFF END."

**a**

```
DNA:    5' - ATG ACT CAC CGA GCG CGA AGC TGA - 3'
        3' - TAC TGA GTG GCT CGC GCT TCG ACT - 5'
mRNA:   5' - AUG ACU CAC CGA GCG CGA AGC UGA - 3'
Protein:     Met Thr His Arg Ala Arg Ser Stop
```

**b**

```
DNA:    5' - ATG ACT CAC TGA GCG CGA AGC TGA - 3'
        3' - TAC TGA GTG ACT CGC GCT TCG ACT - 5'
mRNA:   5' - AUG ACU CAC UGA - 3'
Protein:     Met Thr His Stop
```

**Fig. 3.15** Nonsense mutations. Normal strand (**a**) is four amino acids longer than the shorter mutant strand (**b**) that forms as a result of a nonsense mutation when a cytosine is replaced with a thymine resulting a premature stop codon

**a**

```
DNA:     5' - ATG ACT CAC CGA GCG CGA AGC TGA - 3'
         3' - TAC TGA GTG GCT CGC GCT TCG ACT - 5'
mRNA:    5' - AUG ACU CAC CGA GCG CGA AGC UGA - 3'
Protein:      Met Thr His Arg Ala Arg Ser Stop
```

**b**

```
DNA:     5' - ATG ACT CAC CGA GGA GCG CGA AGC TGA - 3'
         3' - TAC TGA GTG GCT CCT CGC GCT TCG ACT - 5'
mRNA:    5' - AUG ACU CAC CGA GGA GCG CGA AGC UGA - 3'
Protein:      Met Thr His Arg Gly Ala Arg Ser Stop
```

**Fig. 3.16** Insertion mutations. Normal strand (**a**) forms Arginine while mutant strand (**b**) forms Arginine and inserted Glycine. Affected amino acids and nucleotides are *bolded*

With insertions or deletions that occur within a gene the amount of damage caused depends on the number of bases inserted or deleted and whether or not the reading frame changes. Often insertions and deletions destroy the function of a gene but they can at times create different functions.

Frameshifts occur as a result of deletions or insertions. DNA is read three bases at a time. If a single base is deleted or inserted it shifts the entire reading frame (Fig. 3.18). This is a major mutation that generally destroys the gene. Insertions and deletions are equally deleterious. In a text example a deletion of the letter e in the first word would change the reading from frame shifting the instructions from "USE THE BAR AND PRY OFF THE RED TOP OFF END" to "UST HEB ARA NDP RYT HER EDT OPO FFE NDN OWT…"

**a**

```
DNA:     5' - ATG ACT CAC CGA GCG CGA AGC TGA - 3'
         3' - TAC TGA GTG GCT CGC GCT TCG ACT - 5'
mRNA:    5' - AUG ACU CAC CGA GCG CGA AGC UGA - 3'
Protein:      Met Thr His Arg Ala Arg Ser Stop
```

**b**

```
DNA:     5' - ATG ACT CAC GCG CGA AGC TGA - 3'
         3' - TAC TGA GTG CGC GCT TCG ACT - 5'
mRNA:    5' - AUG ACU CAC GCG CGA AGC UGA - 3'
Protein:      Met Thr His Ala Arg Ser Stop
```

**Fig. 3.17** Deletion mutations. Normal DNA strand (**a**) has its last four amino acids as Arginine, Alanine, Arginine, and Serine while the mutated DNA strand (**b**) has lost the first Arginine so its last four amino acids are Histidine, Alanine, Arginine and Serine

**a.**

```
DNA:     5' - ATG ACT CAC CGA GCG CGA AGC TGA - 3'
         3' - TAC TGA GTG GCT CGC GCT TCG ACT - 5'
mRNA:    5' - AUG ACU CAC CGA GCG CGA AGC UGA - 3'
Protein:      Met Thr His Arg Ala Arg Ser Stop
```

**b.**

```
DNA:     5' - ATG ACT CAC CGG CGC GAA GCT GAT - 3'
         3' - TAC TGA GTG GCT GCG CTT CGA CTA - 5'
mRNA:    5' - AUG ACU CAC CGG CGC GAA GCU GAU - 3'
Protein:      Met Thr His Arg Glu Gly Asp
```

**Fig. 3.18** Frameshift mutations. Normal DNA strand (**a**) has its last three amino acids as Alanine, Arginine, and Serine while the mutated DNA strand (**b**) has had its reading frame shifted due to the deletion of an adenine. This replaces the stop codon with Aspartic Acid and the other three previous amino acids with Arginine, Glutamic acid and Glycine

### *3.4.3 Chromosomal Alterations*

Chromosomal changes are another type of mutation. Chromosomes alterations come in two major forms; numerical and structural. Numerical changes involve the loss or gain of chromosomes. Polyploidy is when there are more than two copies of each chromosome. In humans polyploidy is not survivable at the organism level. Roughly 1–3 % of pregnancies are triploid but these pregnancies rarely result in term. Some infants are born triploid with a very limited survival time (Boghossian et al. 2012).

Aneuploidy occurs when there is a loss or gain in one or more chromosomes. Monosomy occurs when there is only one chromosome instead of two. Monosomy of the X-chromosome results in Turner's syndrome. Monosomies of the other

chromosomes are lethal at the organismal level with the exception of the y-chromosome that is normally paired with the much larger x-chromosome. Trisomy occurs when there are three chromosomes instead of two. Trisomy 21 results in Down Syndrome. Trisomy in humans at the organism level is rarely survivable and survivability is inversely proportional to the size of the chromosomes. The most common trisomies that make it to term are trisomy 13, 18 and 21 and their hospital mortality rates have been reported as 92 %, 89 % and 33 % respectively (Boghossian et al. 2012).

Aneuploidy is different for the sex chromosomes. Males develop normally with only one X-chromosomes and females develop normally without at y-chromosome. This is because the y-chromosome has few genes, none that are needed by females, while the x-chromosome is selectively deactivated so that only one X-chromosome per cell is open during most of the cell-cycle while the other X-chromosome is largely deactivated through the process of lionization. These chromosomes are also smaller than most allowing them to be added or deleted with far less clinical impact than larger chromosomes. This is also true with XXY males who have Klinefelter syndrome (Strachan and Read 1999).

Chemicals and energy particles that break chromosomes are called clastogens. Benzene and arsenic represent organic and inorganic chemical clastogens respectively. Non homologous end joining (NHEJ) is a repair mechanism for chromosomal damage. When multiple chromosomes are broken it is possible to have different parts of different chromosomes joined together. This can result in the creation of acentric (i.e., without centromeres) chromosomes and dicentric (i.e., with two centromeres) chromosomes. Small acentric chromosomes are also known as micronuclei and are tested for as a sign of chromosome damage.

Chromosome breaks can alter the expression of a gene even if they occur outside of the gene. This is because a chromosome break can move a gene away from a regulatory element, toward a regulatory element or can move the DNA from normally active euchromatin that is available for transcription into condensed heterochromatin that is protein bound and inaccessible for transcription.

Chromosomal abnormalities are considered unbalanced if there is a net loss of chromosomal material and balanced if there is no net loss of chromosomal material. Inversions occur when a piece of a chromosome breaks off and is reattached in the opposite direction. Abnormal chromosomes can be passed down through cell division if they have the proper number of centromeres. Chromosomes without a centromere or with two centromeres cannot be properly moved to one of the new daughter cells during division and will be lost. The exception to this is with Robertsonian translocations where only a small portion of DNA is lost and the two centromeres are so close together that they can function as one centromere.

Chromosome specific dyes can be used to identify which parts of which chromosomes have been joined together. These breakages and joinings can result in pathological changes. A type of trisomy 21 is the result of the large arm of chromosome 21 being added to the large arm of chromosome 14. The Philadelphia chromosome is an example of what happens when a gene that normally has low expression is combined with a promoter that is normally associated with a gene that has high expression.

Mutations that occur in somatic cells are limited to that individual. Mutations may be limited to a single cell or they may be spread throughout the body. Some

common mutations occur throughout the body because of extensive damage. Thymine dimers caused by UV exposure are examples of this type of widespread mutation. Other mutations may spread throughout the body because they occur early in development.

This is most significant during the embryonic stage when a mutation can be passed on to many different cells. Mosaics are examples of this where they have been seen. This is most apparent with mutations in skin color. A striking example of this involves a reported case of identical twins who shared mostly identical genetics but differed in terms of the neurofibromatosis-1 (NF-1) gene (Kaplan et al. 2010). These twins had their monozygosity confirmed. The mutation status of the NF-1 gene was tested the fibroblasts, lymphocytes and buccal cells of the twins and their relatives. The twins shared a non-sense mutation in the NF-1 gene but only one twin had the phenotype of neurofibromatosis.

Neurofibromatosis type 1 is a progressive condition that affects the skin and nerves. Only one twin had the café-au-lait macules. Every cell tested in the affected twin possessed the NF-1 nonsense mutation. In contrast many of the cells tested in the unaffected twin lacked the NF-1 nonsense mutation. The unaffected twin was identified as a mosaic. It is hypothesized that the NF1 mutation occurred after fertilization but before the zygote split into two separate zygotes. The affected twin developed from part of the zygote that only possessed mutated cells while the unaffected twin developed from a part of the zygote that possessed both mutated cells and cells lacking the NF-1 mutation.

Constitutional mutations are present in all of the cells of the body. Constitutional mutations must occur in the sperm, egg or fertilized egg to be spread throughout the entire organism as it develops. However, constitutional mutations that are only present in one copy may not be passed on to the next generation as during meiosis the diploid cells divide to form haploid gametes that pass down only one copy of each chromosome. For example an X-linked constitutional mutation present in the father would not be passed down to a son but would be passed down to a daughter.

Another way mutations can spread is through the growth and metastasis of cancer cells. The clonal theory of expansion for cancers describes how a single cell is mutated and that cell grows forming clones. Genomic instability is a common characteristic of cancer cells and cancer cells generally lack adequate repair mechanisms. Over time these cells may be exposed to other mutagens and will be involved in new cell divisions. Genomic analysis of late stage cancers reveals that it can take decades for mutations to accumulate and a single patient can develop over a dozen different clones. Analysis of pancreatic cancer metastatic tumors demonstrated that different clones from a primary tumor can form different metastases. Those metastatic cells can continue to evolve developing new clones and those clones can even form their own metastatic cells and produce new metastatic tumors (Campbell et al. 2010).

Comparisons between the germline DNA of the patient can be compared to the DNA collected from the primary tumor and metastastic tumors to determine when and where a mutation develops. These analysis reveal that many of the mutations that appear to provide a selective advantage to clones include mutations that damage the cellular repair mechanisms. One of the most consistently mutated genes in tumor samples is P53 (Table 3.1).

**Table 3.1** TP53 mutations by cancer cell type

| Type | Mutation (%) |
|---|---|
| Renal cell carcinoma | 2 |
| AML | 9 |
| Breast adenocarcinoma luminal | 24 |
| Endometrial carcinoma | 28 |
| Glioblastoma multiforme | 30 |
| ALL | 41 |
| Bladder adenocarcinoma | 51 |
| Lung adenocarcinoma | 52 |
| Colorectal adenocarcinoma | 58 |
| Lung squamous-cell | 72 |
| Breast adenocarcinoma basal | 80 |
| Ovarian carcinoma | 94 |

Source. Hoadley et al. (2014)

Mutations that occur in germ cells may be passed on to the next generation. An example of this is with retinoblastoma related rb mutations where roughly half of the mutations are inherited.

Most mutagens are associated with environmental exposures. When these exposures are limited or eliminated it may be possible to eliminate or limit the mutations associated with these exposures. There are some mutagens that are unavoidable. For example, iron and oxygen are two vital elements that are also involved in the creation of reactive oxygen species which can damage DNA causing mutations.

In humans trisomy is only survivable in some cases. When survivable it is limited to the smaller chromosomes (e.g., 21). Tetraploidy is even more limited in humans and is predominantly limited to the X-chromosome. In individual cells the number of chromosomes can increase dramatically. Similarly loss of chromosomes on an organism wide scale is almost always fatal with the notable exception of the sex chromosomes. This is a condition known as aneuploidy. Cancer cells can have anywhere from over 100 chromosomes to under 38 chromosomes. Monosomy is limited at the organism level to the X-chromosome. This is because in humans the X-chromosome is selectively inactivated so that only one copy is active in both women and men. All of the other chromosomes have evolved to produce the gene products from both parents.

There are many instances of haplo-insuficiency where both copies of the gene are needed to produce an adequate amount of the gene product. Examples of haploinsufficiency are seen with Williams and Marfan syndromes. With Marfan syndrome a single mutated copy of fibrillin-1 is enough to cause widespread tissue weakening (e.g., aortic dilation). With Williams syndrome a deletion of ~26 genes from chromosome 7 results in cognitive, physical and metabolic defects.

### *3.4.4 Mutation Penetrance*

Mutations can be highly penetrant or of limited penetrance. For many syndromes with known genetic causes it is common to for individuals to have wildly different phenotypes even when they share the same mutation. These cases highlight the complexity

of the human body and environment and how genes interact with other genes and the environment. An example of this can be seen with syndromes known to reduce height. Among the affected individuals there is often still a wide range of heights as many other factors impact body height including health, diet, sex, and parental height.

What area of the genome is mutated is critical. Only some areas of the genome are expressed. Some areas of the genome are hypervariable and mutations in these regions may have limited impact. At the other end of the spectrum some areas are highly conserved. Mutations in some regions are more likely to result in cancers. Tumor suppressor genes (e.g., TP53) and oncogenes (e.g., RAS) are two major examples of genes whose mutation is often involved in carcinogenesis. Mutations involving large chromosomal changes alter many genes at once. When expressed over the entire organism they are often lethal.

What part of the body is mutated is critical. Cells in the lining of the digestive tract and the outer layers of the skin are routinely sloughed off. Genetic damage to these cells may be of limited impact if they are removed. In contrast the cells that are below these layers and are frequently dividing to form new replacement cells are potentially dangerous cells to mutate. Many of the most common primary cancer sites involve these kinds of linings (e.g., glandular epithelial tissue).

The period of development is also important. Mutations are more problematic earlier in development. At the earliest stage they are more likely to be carried throughout the body during cell divisions. However even with younger adults mutations are likely to be more problematic than a similar mutation occurring with an elderly adult. This is because it often takes decades for an initiating mutation to lead to tumor formation. Growth during development also lends itself towards susceptibility towards mutagens because of the widespread cell divisions. Regrowth is also an issue as the same genes that are turned on for cellular repair can become involved in tumorogenesis.

## 3.5 Repair Mechanisms

### *3.5.1 Overview of Repair*

The fidelity in DNA replication is significantly improved by the presence of multiple DNA repair mechanisms. In general the smaller the lesion the easier and more likely is the repair. Repair can restore the fidelity of the DNA or can result in a mutation but still allow replication to occur. Repair enzymes may be at low levels until significant DNA damage is detected. If DNA damage is detected then a global response known as the SOS response can be initiated. During the SOS response cells block replication until repair is completed. Lower levels of damage can result in repair genes increases in response to the damage.

Similar to the immune system which has multiple ways of responding to different types of infections and injuries, humans have multiple ways of responding to DNA damage. Some pathways (e.g., homologous repair) require replication to be occurring. Other pathways (e.g., global genomic nucleotide excision repair) are not dependent on replication. Humans have a range of enzymes and other proteins

involved in DNA repair and their importance in health can be seen with their association with mutation related diseases (e.g., XPA and xeroderma pigmentosa).

### 3.5.2 Base Repair

Damaged DNA bases can be excised and replaced through the use of DNA polymerases through a process known as nucleotide excision repair. Because DNA is double stranded in the case of an abasic site the opposing strand can be used as the template (Fig. 3.19). This type of repair restores the original DNA copy. Humans have multiple DNA polymerase types only some of which have significant DNA repair ability.

Cyclobutane lesions (Fig. 3.11) which are caused by UV radiation are repaired by photolyase enzymes. Photolyases use photon energy to drive photochemical reactions. This process is known as photoreactivation (Cooper 2000).

$O^6$-methylguanine methyltransferase is another DNA repair enzyme that specializes in the repair of alkylated DNA that has been modified at the $O^6$ residue of guanine.

### 3.5.3 Chromosomal Repair

Ligases can reassemble broken chromosomes. Repair can involve either joining the ends together or by capping the end of the chromosome with a telomere. With homologous recombination ends are joined together that have been matched up with an extensive homologous sequence to guide the repair. With non-homologous

**Fig. 3.19** Abasic site. DNA is shown missing a base. This figure was created using the structure published by Serre et al. (2002) on RCSB PDB

recombination the repair is not guided by an extensive homologous sequence. Small sequences (i.e., microhomologies) that are only a few bases long can guide non-homologous end joining. This can lead to accurate repair but often results in the loss of a few bases as the pieces are not perfectly lined up.

### *3.5.4 Cell Death*

The last resort is cell death which can be viewed on an organismal scale as a form of repair as it can allow for the replacement of damaged cells with new healthy ones. This cell death can be uncontrolled death where the cell contents spill out and can damage surrounding cells and tissues or it can be programmed and controlled through the process of apoptosis. Apoptosis limits the damage to surrounding cells and tissues. Apoptosis is the primary mechanism used to remove normal but unneeded tissue during development such as the webbing between fingers in the developing embryo. Apoptosis is largely directed by checkpoint regulator p53 which has been nicknamed "guardian of the genome." When extensive DNA damage is detected p53 can interact with other proteins to stop cell division and initiate repair. If the genetic damage is too severe to allow for repair then apoptosis may be initiated. A common cause of this is double stand breaks.

**Case Study 6: Xeroderma Pigmentosa** Xeroderma Pigmentosa is a condition where the normal repair mechanism for UV radiation caused pyrimidine dimers is broken. As a consequence the patients are unable to repair the damage to their skins cells and can develop skin lesions and skin cancers at an early age. Some patients, if diagnosed early and extensively shielded from UV radiation avoid this profound skin damage. These efforts include use of protective clothing and sunscreen as well as a nocturnal lifestyle. Efforts continue to develop effective therapies to limit the development of skin cancers which remain a leading cause of death for patients with Xeroderma Pigmentosa. Recently there have been preclinical studies to develop methods to provide gene therapy. The goal of these gene therapy studies is to insert repair genes into skin cells so they can begin expressing repair enzymes (e.g., photolyases).

## 3.6 Conclusion

In order to alter DNA mutagens must have the ability to interact with electrons on the DNA either directly or through an intermediary. Mutagens can be chemicals or radiation. Chemicals are generally either direct or indirect mutagens. Indirect mutagens need to be activated before they can react with DNA. Radiation can be both indirect and direct mutagens. When acting as direct mutagens the radiation particles ionize the DNA. When acting as indirect mutagens the radiation ionizes a neighboring molecule that then reacts with the DNA.

Mutagens can damage a small part of a single base or can break apart an entire chromosome. The larger the radiation particle the more damage it can cause. However, larger particles have limited penetrating ability. This makes alpha emitters less able to cause damage with dermal exposures but they can still be extremely dangerous if inhaled or ingested. Mutagens are most damaging at the earliest stages of development. At the organism level minor mutations are more survivable. Mutagens that activate proto-oncogenes, inactivate tumor suppressor genes or damage DNA repair genes can lead to the development of uncontrollable cell-growth, new mutations and cancers.

Mutagens are further classified based on their likelihood of causing mutations towards germ cells. Mutagens that affect the germ cells are the most troubling as they can be passed on to future generations. For this reason the Global Harmonizing System classifies mutagens based on their ability or expected ability to cause mutations in human germ cells.

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