MUTATIONS

Dr Neda M Bogari
CONTACTS

• www.bogari.net
• http://web.me.com/bogari/bogari.net/
From DNA to Mutations
A *mutation* is defined as a heritable alteration or change in the genetic material. Mutations drive evolution but can also be pathogenic. Mutations can arise through exposure to mutagenic agents (p. 27), but the vast majority occur spontaneously through errors in DNA replication and repair. Sequence variants with no obvious effect upon phenotype may be termed polymorphisms.
CHROMOSOMAL LEVEL MUTATION

- Numerical abnormalities:
  - **Aneuploidy**
    - Monosomy (45)
    - Trisomy (47)
    - Tetrasomy (48)
  - **Polyploidy**
    - Triploidy \((2n + 1\sigma)\)
    - Tetraploidy \((2n + 2\sigma)\)

The occurrence of one or more extra or missing chromosomes leading to an unbalanced chromosome complement, or, any chromosome number that is not an exact multiple of the haploid number.

An increase in the number of haploid sets \((23)\) of chromosomes in a cell. Triploidy refers to three whole sets of chromosomes in a single cell (in humans, a total of 69 chromosomes per cell); tetraploidy refers to four whole sets of chromosomes in a single cell (in humans, a total of 92 chromosomes per cell).

Mosaic = F only e.g. Liver
bar-body X inactivation OOO
Mongolian is Trisomy in chromosome 21
Chimaera (in Transfugne or pregnancy)
Overview of chromosomal mutations

Relocation of genetic material
- Translocation
  - From another chromosome
- Inversion

Deletion
- Wild-type sequence

Loss of genetic material
- Missing chromosome(s)
- Extra chromosome(s)

Gain of genetic material
- Duplication

changes in structure
changes in number
CHROMOSOMAL LEVEL MUTATION

- Structural abnormalities:
  - Translocations
  - Deletion
  - Insertion
  - Inversions
  - Rings formation

mosaic = F only e.g. Liver
bar-body X inactivation OOO
Mongolian is Trisomy in chromosome 2 21
Chimaera (in Transfugne or pregnancy)
Human cells normally contain 23 pairs of chromosomes, for a total of 46 chromosomes in each cell.

[Image: Normal Human Karyotype]

Monosomy is the loss of one chromosome in cells. Turner syndrome is an example of a condition caused by monosomy.

Trisomy is the presence of an extra chromosome in cells. Down syndrome is an example of a condition caused by trisomy.

People with tetrasomy 18p have this normal pair plus an extra chromosome that consists of two identical short arms (18p) and a centromere (this type of construction is called an isochromosome).

http://www.tetrasomy18p.com/
Cells with one additional set of chromosomes, for a total of 69 chromosomes, are called triploid.


http://www.whitneyjill.com/triploidy.html
KLINEFELTER’S SYNDROME

Three sex chromosomes are associated with Klinefelter rather than the expected 2 - XXY. These individuals are males with some development of breast tissue normally seen in females. Little body hair is present, and such person are typically tall, with or without evidence of mental retardation. Males with XXXY, XXXXY, and XXXXXY karyotypes have a more severe presentation, and mental retardation is expected.

http://homepage.smc.edu/hgp/history.htm

Cause: Males with Klinefelter’s Syndrome receive an extra X chromosome, thus giving them 47 rather than the normal 46 chromosomes (an extra sex chromosome).

Primarily Affects: Males. It is the most common sex chromosome disorder in males.

Discovered By: Dr. Harry Klinefelter

Symptoms: Reduced fertility, neurophysiological impairments in terms of execution, smaller testicles, gynecomastia (increased breast tissue), rounded body, etc.

Key Statistics:
- 47XXY, the most common of the sex chromosome variations, is said to occur in 1 out of 500 males.
- Thousands of 47XXY individuals in the United States alone. Many remain undiagnosed.
TURNER'S SYNDROME

Only 1 sex chromosome is present -X0, or X_. The expected Y chromosome is missing. Turner syndrome is associated with underdeveloped ovaries, short stature, webbed neck, and broad chest. Individuals are sterile, and lack expected secondary sexual characteristics. Mental retardation typically not evident.

http://homepage.smc.edu/hgp/history.htm

**Cause:** Turner's Syndrome is caused by a condition known as Monosomy (absence of entire chromosome) of X chromosome for females.

**Primarily Affects:** Females

**Discovered By:** Dr. Henry H. Turner

**Symptoms:** Low-set ears, webbed neck, broad chest, non-working ovaries, hypothyroidism, diabetes, vision problems, neuro/cognitive deficiency, congenital heart disease, etc.

**Key Statistics:**
- Turner's Syndrome occurs in approximately 1 of every 2,000 female births and in as many as 10% of all miscarriages.
DOWN'S SYNDROME

Normally associated with 3 copies of chromosome number 21 (trisomy of chromosome 21), rather than the 2 found normally. Down syndrome is characterized by differing degrees of mental retardation, a skin fold over the eye, typically short stature, and short hands with a deep crease in the palm. Down is also known as mongolism (mongoloid).

http://homepage.smc.edu/hgp/history.htm
**Cause:** In most cases, Down syndrome occurs when there is an extra copy of chromosome 21. This form of Down syndrome is called Trisomy 21.

**Primarily Affects:** Equal frequency for males and females.

**Discovered By:** Dr. John Langdon Down

**Symptoms:** Symptoms of Down Syndrome include small ears, small mouth, upward slanting eyes, flattened nose, decreased muscle tone at birth, wide hands, delayed mental development, eye problems, hearing problems, hypothyroidism, hip problems, sleep apnea, etc.

**Key Statistics:**
- There are more than 400,000 people living with Down syndrome in the United States.
- Life expectancy for people with Down syndrome has increased dramatically in recent decades - from 25 in 1983 to 60 today.
- The incidence of births of children with Down syndrome increases with the age of the mother.
Somatic mutations may cause adult-onset disease such as cancer but cannot be transmitted to offspring. A mutation in gonadal tissue or a gamete can be transmitted to future generations unless it affects fertility or survival into adulthood. It is estimated that each individual carries up to six lethal or semilethal recessive mutant alleles that in the homozygous state would have very serious effects. These are conservative estimates and the actual figure could be many times greater. Harmful alleles of all kinds constitute the so-called genetic load of the population.

There are also rare examples of 'back mutation' in patients with recessive disorders. For example, reversion of inherited deleterious mutations has been demonstrated in phenotypically normal cells present in a small number of patients with Fanconi anemia.
SPLICE SITE MUTATION

- **Inserts or Deletes** in a number of nucleotides in the specific site at which splicing of an intron takes place during the processing of precursor mRNA into mature mRNA.

- The abolishment of the splicing site results in one or more introns remaining in mature mRNA and may lead to the production of aberrant proteins.

- Several genetic diseases may be the result of splice site mutations. For example, mutations that cause the incorrect splicing of β-globin mRNA are responsible of some cases of β-thalassemia.
Polymorphism is change with **no effect in the phenotype**.
MUTATION & POLYMORPHISM

Mutation

Rare Genetic change (Less than 1%) Sever and alter the function of the protein or the Enzyme.

Polymorphism

Common variation (greater than 1%) no change in function or small effect and occur on average one every 200-1000 base Pairs.

Very useful for genetics linkage analysis
Mutations can be considered in two main classes according to how they are transmitted from generation to another.

- **Fixed/Stable mutations**: mutation which is transmitted unchanged (unaltered).
- **Dynamic Or Unstable Mutations**: This is new class of mutation which undergo alteration as they are transmitted in families.
Fixed/stable point mutations can be classified according to the specific molecular changes at the DNA level.

These include single base pair

- Substitutions,
- Insertions,
- Deletions, or
- Duplications
Substitutions

A substitution is the replacement of a single nucleotide by another. These are the most common type of mutation. If the substitution involves replacement by the same type of nucleotide, i.e. a pyrimidine for a pyrimidine (C for T or vice versa) or a purine for a purine (A for G or vice versa), this is termed a transition. Substitution of a pyrimidine by a purine or vice versa is termed a transversion. Transitions occur more frequently than transversions. This may be due to the relatively high frequency of C to T transitions, which is likely to be the result of the nucleotides cytosine and guanine occurring together, or what are known as CpG dinucleotides (p represents the phosphate) frequently being methylated in genomic DNA with spontaneous deamination of methylcytosine converting them to thymine. CpG dinucleotides have been termed 'hot spots' for mutation.
An insertion involves the addition of one or more nucleotides into a gene. Again, if an insertion occurs in a coding sequence and involves one, two or more nucleotides that are not a multiple of three, it will disrupt the reading frame (p. 20). Large insertions can also result from unequal crossover (e.g. HMSN type 1a, p. 297) or the insertion of transposable elements (p. 18).
In this example, one nucleotide (adenine) is added in the DNA code, changing the amino acid sequence that follows.
Deletions

A deletion involves the loss of one or more nucleotides. If it occurs in coding sequences and involves one, two or more nucleotides that are not a multiple of three, it will disrupt the reading frame (p. 18). Larger deletions may result in partial or whole gene deletions and may arise through unequal crossover between repeat sequences (e.g. HNPP, p. 298).
DELETION MUTATION

In this example, one nucleotide (adenine) is deleted from the DNA code, changing the amino acid sequence that follows.
FIXED/STABLE MUTATION

Wild-type

Substitution

Deletion

Insertion
Fixed / stable point mutations
Unstable or dynamic mutations consist of \textbf{triplet repeat sequences} which, in affected persons, occur in \textbf{increased copy number} when compared to the general population.

Triplet amplification or expansion has been identified as the \textbf{mutational basis for a number of different single gene disorders}.

The mechanism by which amplification or expansion of the triplet repeat sequence occurs is \textbf{not clear at present}.

A number of single-gene disorders have subsequently been shown to be associated with triplet repeat expansions (Table 2.5). These are described as \textit{dynamic mutations} because the \textit{repeat sequence becomes more unstable as it expands in size}. The mechanism by which amplification or expansion of the triplet repeat sequence occurs is not clear at present. Triplet repeats below a certain length for each disorder are faithfully transmitted in mitosis and meiosis. Above a certain repeat number for each disorder they are more likely to be transmitted unstably, usually with an increase or decrease in repeat number. A variety of possible explanations have been offered as to how the increase in triplet repeat number occurs. These include unequal cross-over or unequal sister chromatid exchange (pp. 44, 288) in non-replicating DNA, and slipped strand mispairing and polymerase slippage in replicating DNA.
In this example, a repeated trinucleotide sequence (CAG) adds a series of the amino acid glutamine to the resulting protein.
DYNAMIC/UNSTABLE MUTATION

1. Vary large expansion of repeats outside coding sequences

2. Moderate expansion of CAG repeats within coding sequences
   A. Different types of trinucleotide repeat expansions

B. Unstable trinucleotide repeats in different diseases
### Diseases Associated with Triplet Repeat Expansion

<table>
<thead>
<tr>
<th>Disease</th>
<th>Repeat sequence</th>
<th>Repeat number</th>
<th>Mutation number</th>
<th>Repeat location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huntington’s disease (HD)</td>
<td>CAG</td>
<td>9-35</td>
<td>37-100</td>
<td>Coding</td>
</tr>
<tr>
<td>Myotonic dystrophy (DM)</td>
<td>CTG</td>
<td>5-35</td>
<td>50-4000</td>
<td>3' UTR</td>
</tr>
<tr>
<td>Fragile X site A (FRAXA)</td>
<td>CCG</td>
<td>10-50</td>
<td>200-2000</td>
<td>5' UTR</td>
</tr>
<tr>
<td>Machado-Joseph disease (MJD)</td>
<td>CAG</td>
<td>12-36</td>
<td>67-&gt;79</td>
<td>Coding</td>
</tr>
<tr>
<td>Spino-oaebellar ataxia 6 (SCA6)</td>
<td>CAG</td>
<td>4-16</td>
<td>21-27</td>
<td>Coding</td>
</tr>
<tr>
<td>Spino-oaebellar ataxia 7 (SCAT)</td>
<td>CAG</td>
<td>7-35</td>
<td>37-200</td>
<td>Coding</td>
</tr>
<tr>
<td>Spino-oaebellar ataxia 8 (SCA8)</td>
<td>CTG</td>
<td>16-37</td>
<td>100-&gt;500</td>
<td>UTR</td>
</tr>
<tr>
<td>Tatorubral-pallidoluysian atrophy (DRPLA)</td>
<td>CAG</td>
<td>7-23</td>
<td>49-&gt;75</td>
<td>Coding</td>
</tr>
<tr>
<td>Friedreich’s ataxia (FA)</td>
<td>GAA</td>
<td>17-22</td>
<td>200-900</td>
<td>Intronic</td>
</tr>
<tr>
<td>Fragile X site E (FRAXE)</td>
<td>CCG</td>
<td>6-25</td>
<td>&gt;200</td>
<td>Promoter</td>
</tr>
<tr>
<td>Fragile X site F (FRAXF)</td>
<td>GCC</td>
<td>6-29</td>
<td>&gt;500</td>
<td>?</td>
</tr>
<tr>
<td>Fragile 16 site A (FRA16A)</td>
<td>CCG</td>
<td>16-49</td>
<td>1000-2000</td>
<td>?</td>
</tr>
</tbody>
</table>

UTR = untranslated region.
Structural Effects of Mutations on the Protein

- Mutations can also be subdivided into two main groups according to the effect on the polypeptide sequence of the encoded protein, being either synonymous or non-synonymous.
If a mutation does not alter the polypeptide product of the gene, this is termed a synonymous or silent mutation. A single base pair substitution, particularly if it occurs in the third position of a codon because of the degeneracy of the genetic code, will often result in another triplet which codes for the same amino acid with no alteration in the properties of the resulting protein.

**Synonymous/silent mutations**

If a mutation does not alter the polypeptide product of the gene, this is termed a *synonymous* or *silent mutation*. A single base pair substitution, particularly if it occurs in the third position of a codon because of the degeneracy of the genetic code, will often result in another triplet that codes for the same amino acid with no alteration in the properties of the resulting protein.
Non-synonymous mutations

If a mutation leads to an alteration in the encoded polypeptide, it is known as a non-synonymous mutation. Non-synonymous mutations are observed to occur less frequently than synonymous mutations. Synonymous mutations will be selectively neutral, whereas alteration of the amino acid sequence of the protein product of a gene is likely to result in abnormal function that is usually associated with disease or lethality that has an obvious selective disadvantage.
In this example, the nucleotide adenine is replaced by cytosine in the genetic code, introducing an incorrect amino acid into the protein sequence.
NONSENSE MUTATION

In this example, the nucleotide cytosine is replaced by thymine in the DNA code, signaling the cell to shorten the protein.

FRAMESHIFT MUTATION

A frameshift mutation changes the amino acid sequence from the site of the mutation.
## SUBSTITUTION MUTATION

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Codon</th>
<th>Amino acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Missense</td>
<td>GAG</td>
<td>Glu</td>
</tr>
<tr>
<td></td>
<td>AAG</td>
<td>Lys</td>
</tr>
<tr>
<td>Nonsense</td>
<td>GAG</td>
<td>Glu</td>
</tr>
<tr>
<td></td>
<td>UAG</td>
<td>Stop</td>
</tr>
<tr>
<td>silent</td>
<td>GAG</td>
<td>Glu</td>
</tr>
<tr>
<td></td>
<td>GAA</td>
<td>Glu</td>
</tr>
</tbody>
</table>
A single base pair substitution can result in coding for a different amino acid and the synthesis of an altered protein, a so-called 
missense mutation.

- **Non-conservative substitution**: If mutation coding for an amino acid which is chemically dissimilar such different charge of protein or structure of protein will be altered.

**Missense**

A single base pair substitution can result in coding for a different amino acid and the synthesis of an altered protein, a so-called missense mutation. If the mutation codes for an amino acid that is chemically dissimilar, e.g. a different charge, the structure of the protein will be altered. This is termed a non-conservative substitution and can lead to a gross reduction, or even a complete loss, of biological activity. Single base pair mutations can lead to qualitative rather than quantitative changes in the function of a protein, such that it retains its normal biological activity (e.g. enzyme activity) but differs in characteristics such as its mobility on electrophoresis, its pH optimum, or its stability so that it is more rapidly broken down in vivo. Many of the abnormal hemoglobins (p. 152) are the result of missense mutations.

Some single base pair substitutions, although resulting in the replacement by a different amino acid if it is chemically similar, will often have no functional effect. These are termed conservative substitutions.
NON-SYNONYMOUS MUTATIONS: **MISSENSE**

- **Conservative substitution**: If mutation coding for an amino acid which is chemically similar, have no functional effect.

- Non-conservative substitution will result in complete loss or gross reduction of biological activity of the resulting protein.
NON-SYNONYMOUS MUTATIONS: NONSENSE

- A substitution of base pair which leads to the generation of one of the stop codons will result in premature termination of translation of a peptide chain.
- Nonsense mutation result in reduce the biological activity of the protein

**Nonsense**

A substitution that leads to the generation of one of the stop codons (Table 2.2) will result in premature termination of translation of a peptide chain or what is termed a *nonsense* mutation. In most cases the shortened chain is unlikely to retain normal biological activity, particularly if the termination codon results in the loss of an important functional domain(s) of the protein. Messenger RNA transcripts containing premature termination codons are frequently degraded by a process known as *nonsense-mediated decay*. This is a form of RNA surveillance that is believed to have evolved to protect the body from the possible consequences of truncated proteins interfering with normal function.
NON-SYNONYMOUS MUTATIONS : FRAMESHIFT

- If a mutation involves the insertion or deletion of nucleotides which are not a multiple of three, it will disrupt the reading frame and constitute what is known as a frameshift mutation.

- The amino acid sequence resulting from such mutation is not the same sequence of the normal amino acid.

- This mutation may have an adverse effect in its protein function.

- Most of these mutation result in premature stop codon.

Frameshift

If a mutation involves the insertion or deletion of nucleotides that are not a multiple of three, it will disrupt the reading frame and constitute what is known as a frameshift mutation. The amino acid sequence of the protein subsequent to the mutation bears no resemblance to the normal sequence and may have an adverse effect upon its function. Most frameshift mutations result in a premature stop codon downstream to the mutation. This may lead to expression of a truncated protein, unless the mRNA is degraded by nonsense-mediated decay.
NON-SYNONYMOUS MUTATIONS: FRAMESHIFT

- A frameshift mutation causes the reading of codons to be different, so all codons after the mutation will code for different amino acids. Furthermore, the stop codon "UAA, UGA, or UAG" will not be read, or a stop codon could be created at an earlier or later site.

- The protein being created could be abnormally short, abnormally long, and/or contain the wrong amino acids. It will most likely not be functional.

- Frameshift mutations frequently result in severe genetic diseases such as Tay-Sachs disease.

- A frameshift mutation is responsible for some types of familial hypercholesterolemia.

- Frameshifting may also occur during protein translation, producing different proteins from overlapping open reading frames.
<table>
<thead>
<tr>
<th>Type of Mutation</th>
<th>Designation</th>
<th>Description</th>
<th>Consequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Missense</td>
<td>482 G&gt;A</td>
<td>R117H</td>
<td>Arginine to histidine</td>
</tr>
<tr>
<td>Nonsense</td>
<td>1756 G&gt;T</td>
<td>G542X</td>
<td>Glycine to Stop</td>
</tr>
</tbody>
</table>
Missense
R4K

Nonsense
Stop

Silent
FUNCTIONAL EFFECTS OF MUTATIONS ON THE PROTEIN

The mutations effect can appear either through loss- or gain-of-function.
Loss-of-function

*Loss-of-function* mutations can result in either reduced activity or complete loss of the gene product. The former can be the result of either reduced activity or of decreased stability of the gene product and is known as a *hypomorph*, the latter being known as a *null allele* or *amorph*. Loss-of-function mutations in the heterozygous state would, at worst, be associated with half normal levels of the protein product. Loss-of-function mutations involving enzymes will usually be inherited in an autosomal or X-linked recessive manner, because the catalytic activity of the product of the normal allele is more than adequate to carry out the reactions of most metabolic pathways.
Loss-of-function mutations in the heterozygous state would be associated with half normal levels of the protein product (haploinsufficiency mutation).

haploinsufficiency occurs when a diploid organism only has a single functional copy of a gene (with the other copy inactivated by mutation) and the single functional copy of the gene does not produce enough of a gene product (typically a protein) to bring about a wild-type condition, leading to an abnormal or diseased state.

haploinsufficiency

Loss-of-function mutations in the heterozygous state in which half normal levels of the gene product result in phenotypic effects are termed haploinsufficiency mutations. The phenotypic manifestations sensitive to gene dosage are a result of mutations occurring in genes that code for either receptors, or more rarely enzymes, the functions of which are rate limiting, e.g. familial hypercholesterolemia (p. 170) and acute intermittent porphyria (p. 175).

There are a number of autosomal dominant disorders where the mutational basis of the functional abnormality is the result of haploinsufficiency, in which, not surprisingly, homozygous mutations result in more severe phenotypic effects, e.g. angioneurotic edema (p. 195) and familial hypercholesterolemia (p. 170).
Gain-of-function mutations

*Gain-of-function* mutations, as the name suggests, result in either increased levels of gene expression or the development of a new function(s) of the gene product. Increased expression levels due to activating point mutations or increased gene dosage are responsible for one type of Charcot-Marie-Tooth disease, hereditary motor and sensory neuropathy type I ([p. 277](#)). The expanded triplet repeat mutations in the Huntington gene cause qualitative changes in the gene product, which result in its aggregation in the central nervous system leading to the classic clinical features of the disorder ([p. 293](#)).

Mutations that alter the timing or tissue specificity of the expression of a gene can also be considered to be gain-of-function mutations. Examples include the chromosomal rearrangements that result in the combination of sequences from two different genes seen with specific tumors ([p. 206](#)). The novel function of the resulting chimeric gene causes the neoplastic process.

Gain-of-function mutations are dominantly inherited and the rare instances of gain-of-function mutations occurring in the homozygous state are associated with a much more severe phenotype, which is often a prenatally lethal disorder, e.g. homozygous achondroplasia ([p. 92](#)) or Waardenburg syndrome type I ([p. 90](#)).
Charcot-Marie-Tooth disease (CMT), known also as Hereditary Motor and Sensory Neuropathy (HMSN), Hereditary Sensorimotor Neuropathy (HSMN), or Peroneal Muscular Atrophy, is a heterogeneous inherited disorder of nerves (neuropathy) that is characterized by loss of muscle tissue and touch sensation in the feet and legs but also in the hands and arms in the advanced stages of disease.

Presently permanent, this disease is one of the most common inherited neurological disorders, with 37 in 100,000 affected.
DOMINANT-NEGATIVE MUTATIONS

- A mutation whose gene product adversely affects the normal, wild-type gene product within the same cell.
- Usually by dimerize (combining) with it. In cases of polymeric molecules, such as collagen, dominant negative mutations are often more harmful than mutations causing the production of no gene product (null mutations or null alleles).

Dominant-negative mutations

A dominant-negative mutation is one in which a mutant gene in the heterozygous state results in the loss of protein activity / function, as a consequence of the mutant gene product interfering with the function of the normal gene product of the corresponding allele. Dominant-negative mutations are particularly common in proteins that are dimers or multimers, e.g. structural proteins such as the collagens, mutations in which can lead to osteogenesis imperfecta (p. 117).
PHARMACOGENETICS
IMPORTANCE OF MUTATION

★ Affect the metabolism of the drugs
  • Enhanced sensitivity of drug or low activity

★ Drug resistance e.g.
  • HIV drug resistance mutations e.g. nucleoside reverse transcriptase inhibitor resistance including M184V
  • Codeine
MUTAGENS AND MUTAGENESIS
Naturally occurring mutations are referred to as spontaneous mutations and are thought to arise through chance errors in chromosomal division or DNA replication. Environmental agents which cause mutations are known as mutagens. These include natural or artificial ionizing radiation and chemical or physical mutagens.
Mutation can be ...

- Inherited.
- Induced.
  - Increase rate of Mutation
    - Chemical
    - Physical
    - Biological
- Naturally Occurring (Spontaneous)
  - Errors in chromosomal division
  - Errors in DNA replication
  - Faulty in DNA repair
CHEMICAL MUTAGENS

Mutagenic chemicals in food contribute to 35% of cancers

1. Base analogues (A to T & C)
2. Intercalating agents (VG & ethidium bromide) lead to deletions and additions
3. Agents altering bases (nitrous oxide)
4. Agents altering DNA structure (Free radical) DNA strand breakage

In humans, chemical mutagenesis may be more important than radiation in producing genetic damage. Experiments have shown that certain chemicals, such as mustard gas, formaldehyde, benzene, some basic dyes and food additives, are mutagenic in animals. Exposure to environmental chemicals may result in the formation of DNA adducts, chromosome breaks or aneuploidy. Consequently all new pharmaceutical products are subject to a battery of mutagenicity tests which include both in vitro and in vivo studies in animals.

1 Base analogues. 2-amino-purine is incorporated into DNA in place of adenine, but pairs as cytosine, causing a substitution of thymine by guanine in the partner strand. 5-bromouracil (5-BU) is incorporated as thymine, but can undergo a tautomeric shift to resemble cytosine, resulting in a transition from T-A to C-G.

2 Chemical modifiers. Nitrous acid converts cytosine to uracil, and adenine to hypoxanthine, a precursor of guanine. Alkylating agents modify bases by donating alkyl groups.

3 Intercalating agents. The antiseptic proflavine and the acridine dyes become inserted between adjacent base pairs, producing distortions in the DNA that lead to deletions and additions.

4 Other. A wide range of other chemicals cause DNA strand breakage and cross-linking.
<table>
<thead>
<tr>
<th>Agent</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcinogen</td>
<td>Causes Cancer</td>
</tr>
<tr>
<td>Clastogen</td>
<td>Causes fragmentation of chromosomes</td>
</tr>
<tr>
<td>Mutagen</td>
<td>Causes mutations</td>
</tr>
<tr>
<td>Oncogen</td>
<td>Induces tumor formation</td>
</tr>
<tr>
<td>Teratogen</td>
<td>Results in developmental abnormalities</td>
</tr>
</tbody>
</table>
Measures of radiation

The amount of radiation received by irradiated tissues is often referred to as the 'dose', which is measured in terms of the radiation absorbed dose or rad. The rad is a measure of the amount of any ionizing radiation that is actually absorbed by the tissues, 1 rad being equivalent to 100 ergs of energy absorbed per gram of tissue. The biological effects of ionizing radiation depend on the volume of tissue exposed. In humans, irradiation of the whole body with a dose of 300-500 rads is usually fatal, but as much as 10,000 rads can be given to a small volume of tissue in the treatment of malignant tumors without serious effects.

Humans can be exposed to a mixture of radiation, and the rem (roentgen equivalent for man) is a convenient unit as it is a measure of any radiation in terms of X-rays. A rem of radiation is that absorbed dose that produces in a given tissue the same biological effect as 1 rad of X-rays. Expressing doses of radiation in terms of rems permits us to compare the amounts of different types of radiation to which humans are exposed. A millirem (mrem) is one-thousandth of a rem. 100 rem is equivalent to 1 sievert (Sv), and 100 rad is equivalent to 1 gray (Gy) in SI units. For all practical purposes, sieverts and millisieverts (mSv) will be used as the units of measure.
UV light is the non-visible, short wavelength fraction of sunlight. It exerts a mutagenic effect by causing **dimerization** (linking) of adjacent pyrimidine residues, mainly T-T (but also T-C and C-C). It does not cause germline mutations, but is a major cause of skin cancer, especially in homozygotes for the red hair allele (see Chapter 16), who have white, freckled skin.

Most UV radiation from the sun is blocked by a 4-mm layer of ozone in the upper atmosphere, but this is currently under destruction by industrial **fluorocarbons**.
Ionizing radiation includes electromagnetic waves of very short wavelength (X-rays and gamma rays), and high energy particles (α particles, β particles and neutrons).

- X-rays, cosmic rays, gamma rays
- More powerful than UV
- Penetrates glass
- Water ionizes: mutagenic effect
  - Free radicals e.g. OH-
  - Inactivate DNA
  - Cell death

**Ionizing radiation**

Ionizing radiation includes electromagnetic waves of very short wavelength (X-rays and γ rays), and high-energy particles (α particles, β particles and neutrons). X-rays, γ rays and neutrons have great penetrating power, but α particles can penetrate soft tissues to a depth of only a fraction of a millimeter and β particles only up to a few millimeters.
BIOLOGICAL MUTAGENS

- Insertion of a transposon within a gene
  - Disrupts the reading frame
  - Loss of function
  - Jumping genes - transposable elements move to different positions in the chromosome. Transposon carries other genes with it.
  - Antibiotic resistance
MUTAGENESIS

A process by which the genetic information of an organism is changed in a stable manner, either in nature or experimentally by the use of chemicals or radiation.
CAUSES OF SPONTANEOUS MUTATIONS

- Error in DNA replication
- Faulty DNA repair e.g. mismatch
- Natural mutagens
DNA-REPAIR MECHANISMS

- DNA-repair mechanisms exist to correct DNA damage due to:

1. Environmental mutagens.
2. Accidental base misincorporation at the time of DNA replication.

DNA repair
Apart from direct repair, all the DNA repair systems in human cells require exonucleases, endonucleases (defective in Cocayne syndrome), polymerases and ligases. Ataxia telangiectasia and Fanconi anaemia patients have defects in DNA damage detection and are extremely sensitive to X-rays.

1 Direct repair. A specific enzyme reverses the damage, e.g. by de-alkylation of alkylated guanine.
2 Base-excision repair. The damaged base, plus a few others on either side, are removed by cutting the sugar-phosphate backbone and the gap filled by re-synthesis directed by the intact strand.
3 Nucleotide excision repair. This removes thymine dimers and chemically modified bases over a longer stretch of DNA. Defects can cause xeroderma pigmentosum.
4 Mismatch repair. This corrects base mismatches due to errors at DNA replication and involves nicking the faulty strand at the nearest GATC base sequence. Defects occur in hereditary non-polyposis colon cancer.
5 Postreplication repair. This corrects double-strand breaks either unguided, or by using the homologous chromosome as a template. Defects occur in Bloom syndrome (a 'chromosome breakage syndrome') and breast cancer.

Mutagenesis
There are four different types of DNA-repair mechanisms:

- Excision repair
- Mismatch repair
- Recombination repair system
- Double-strand repair

The occurrence of mutations in DNA, if left unrepaired, would have serious consequences for both the individual and subsequent generations. The stability of DNA is dependent upon continuous DNA repair by a number of different mechanisms (Table 2.7). Some types of DNA damage can be repaired directly. Examples include the dealkylation of O-alkyl guanine or the removal of thymine dimers by photoreactivation in bacteria. The majority of DNA repair mechanisms involve cleavage of the DNA strand by an endonuclease, removal of the damaged region by an exonuclease, insertion of new bases by the enzyme DNA polymerase, and sealing of the break by DNA ligase.

Nucleotide excision repair (NER) removes thymine dimers and large chemical adducts. It is a complex process involving over 30 proteins which remove fragments of approximately 30 nucleotides. Mutations in at least eight of the genes encoding these proteins can cause Xeroderma pigmentosum (p. 288), which is characterized by extreme sensitivity to UV light and a high frequency of skin cancer. A different set of repair enzymes is utilized to excise single abnormal bases (base excision repair or BER), with mutations in the gene encoding the DNA glycosylase MYH having recently been shown to cause an autosomal recessive form of colorectal cancer (p. 216).

Naturally occurring reactive oxygen species and ionizing radiation induce breakage of DNA strands. Double-strand breaks result in chromosome breaks that can be lethal if not repaired. Post-replication repair is required to correct double-strand breaks and usually involves homologous recombination with a sister DNA molecule. Human genes involved in this pathway include NBS, BLM and BRCA1/2 (mutated in Nijmegen breakage syndrome Bloom syndrome p. 287 and hereditary breast cancer p. 217, respectively). Alternatively, the broken ends may be rejoined by non-homologous end-joining which is an error-prone pathway.

Mismatch repair (MMR) corrects mismatched bases introduced during DNA replication. Cells defective in MMR have very high mutation rates (up to 1000x higher than normal). Mutations in at least six different MMR genes cause hereditary non-polyposis colorectal cancer (HNPPCC, p. 215).

Although DNA repair pathways have evolved to correct DNA damage and hence protect the cell from the deleterious consequences of mutations, some mutations arise from the cell's attempts to tolerate damage. One example is translesion DNA synthesis, where the DNA replication machinery bypasses sites of DNA damage, allowing normal DNA replication and gene expression to proceed downstream. Human disease may also be caused by defective cellular response to DNA damage. Cells have complex signaling pathways which allow cell cycle arrest to provide increased time for DNA repair. If the DNA damage is irreparable, the cell may initiate programmed cell death (apoptosis). The ATM protein is involved in sensing DNA damage and has been described as the 'guardian of the genome'. Mutations in the ATM gene cause ataxia telangiectasia (AT, p. 287), characterized by hypersensitivity to radiation and a high risk of cancer.
**EXCISION REPAIR SYSTEM**

- Excision repair system: removing one strand with damage DNA site.
- Excision repair can be:
  - Base-excision repair
  - Nucleotide-excision repair
- The system recognizes the damaged site which caused by UV and radiation.
- Three proteins are important in this process (XPA, XPB, and XPC).
- These proteins detect the damaged site and form which is known as repair protein complex.
EXCISION REPAIR SYSTEM

- The process starts by the action of exonuclease which cleaves the damage strand at two sites (27 nucleotides before the damaged site and 29 nucleotides after the damaged site) and removed.

- Then, DNA synthesis will restore the missing strand and DNA ligase close the gap.
DNA repair
Mismatch repair system corrects the errors of replication.

Eight gene loci have been identified:

- **MSH2** (2p16).
- **MSH3** (5q3).
- **MSH4** (6p21.3).
- **MSH6** (2p16).
- **MLH1** (3p21).
- **MLH3** (14q24.3).
- **PMS1** (2q31-33).
- **PMS2** (7p22).
Mismatch repair system

- The process begins by removing the strand which has an error bases.
- DNA synthesis starts by DNA polymerase III, which replace the damages strand.
- With somatic loss of the second normal allele, the cell will accumulate genetic mutations, as a result of this, the pattern of microsatellite repeats at a polymorphic locus can differ from the surrounding normal tissue (also called RER +, replication error positive).

The occurrence of mutations in DNA, if left unrepaired, would have serious consequences for both the individual and subsequent generations. The stability of DNA is dependent upon continuous DNA repair by a number of different mechanisms (Table 2.7).

Some types of DNA damage can be repaired directly. Examples include the dealkylation of O6-alkyl guanine or the removal of thymine dimers by photoreactivation in bacteria. The majority of DNA repair mechanisms involve cleavage of the DNA strand by an endonuclease, removal of the damaged region by an exonuclease, insertion of new bases by the enzyme DNA polymerase, and sealing of the break by DNA ligase.

Nucleotide excision repair (NER) removes thymine dimers and large chemical adducts. It is a complex process involving over 30 proteins which remove fragments of approximately 30 nucleotides. Mutations in at least eight of the genes encoding these proteins can cause Xeroderma pigmentosum (p. 288), which is characterized by extreme sensitivity to UV light and a high frequency of skin cancer. A different set of repair enzymes is utilized to excise single abnormal bases (base excision repair or BER), with mutations in the gene encoding the DNA glycosylase MYH having recently been shown to cause an autosomal recessive form of colorectal cancer (p. 216).

Naturally occurring reactive oxygen species and ionizing radiation induce breakage of DNA strands. Double-strand breaks result in chromosome breaks that can be lethal if not repaired. Post-replication repair is required to correct double-strand breaks and usually involves homologous recombination with a sister DNA molecule. Human genes involved in this pathway include NBS, BLM and BRCA1/2 (mutated in Nijmegen breakage syndrome Bloom syndrome p. 287 and hereditary breast cancer p. 217, respectively). Alternatively, the broken ends may be rejoined by non-homologous end-joining which is an error-prone pathway.

Mismatch repair (MMR) corrects mismatched bases introduced during DNA replication. Cells defective in MMR have very high mutation rates (up to 1000x higher than normal). Mutations in at least six different MMR genes cause hereditary non-polyposis colorectal cancer (HNPPC, p. 215).

Although DNA repair pathways have evolved to correct DNA damage and hence protect the cell from the deleterious consequences of mutations, some mutations arise from the cell's attempts to tolerate damage. One example is translesion DNA synthesis, where the DNA replication machinery bypasses sites of DNA damage, allowing normal DNA replication and gene expression to proceed downstream. Human disease may also be caused by defective cellular response to DNA damage. Cells have complex signaling pathways which allow cell cycle arrest to provide increased time for DNA repair. If the DNA damage is irreparable, the cell may initiate programmed cell death (apoptosis). The ATM protein is involved in sensing DNA damage and has been described as the 'guardian of the genome'. Mutations in the ATM gene cause ataxia telangiectasia (AT, p. 287), characterized by hypersensitivity to radiation and a high risk of cancer.
Mismatch Repair
DNA Repair

Base pair mismatch

Normal DNA repair

Mutation introduced by unrepaired DNA
Mismatch Repair Failure Leads to Microsatellite Instability (MSI)

Normal

Microsatellite instability

Addition of nucleotide repeats
Microsatellite Instability (MSI)

- 10%-15% of sporadic tumors have MSI.
- 95% of HNPCC tumors have MSI at multiple loci.
Replication repair system

- DNA damage interfere with replication and transcription.
- In replication: DNA damaged will affect the leading strand
- In transcription: DNA damage will affect the process due to RNA polymerase cannot use the leading strand as a template.
Double-strand repair system

- Double-strand damage is a common consequence of gamma radiation.
- The process require three important genes:
  - ATM (ataxia telangiectasia)
  - BRCA1
  - BRCA2
HOMOZYGOUS

- 2 allele same
- AA OR aa
HETRTOZYGOTE

- Aa
- Aa
- Aa
THANX