Mutations & Diseases

1. Overview of Topic

We have, thus far, learnt about the processes of DNA replication and cell division. These processes are fundamental in an organism's life cycle as they ensure that normal cellular activities can be continually carried out by new cells which originate from existing ones. However, as with most systems, perfection is hard to come by. Gene mutations and chromosomal mutations occur in cells during DNA replication and cell division. Though there are repair mechanisms which can correct such errors in eukaryotes, in some cases, cells could still end up with such mutations. These mutations could manifest in the phenotypes of organisms and lead to diseases. In this chapter, we will be taking a closer look at the types of mutations which can occur in cells and examples of diseases which could be brought about due to these mutations.

2. Learning Outcomes

a. Explain what is meant by the terms gene mutation and chromosome aberration. For gene mutation, knowledge of how substitution, addition, deletion could change the amino acid sequence (e.g. frameshift) is required. For chromosomal aberration, knowledge of numeral (e.g. aneuploidy, as in the case of trisomy 21, i.e. Down syndrome) and structure (e.g. translocation, duplication inversion, deletion) aberration is required.
b. Explain how gene mutations can result in diseases (including sickle cell anaemia).

3. References

Campbell, N.A. and Reece, J.B. (2008). Biology, 9th edition. Pearson.
Jones & Jones. (1997). Advanced Biology.
Hoh Yin Kiong (2003). Longman A-Level Course in Biology, Vol. 1. Longman
Green, Stout and Soper (1990). Biological Sciences, Vol 1. Cambridge
Raven, Johnson, Losos, Mason & Singer (2008). Biology, 8th edition. McGraw-Hill.

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4. Gene mutations

Notes to self

Definition: A gene mutation arises as a result of a **change in the sequence of nucleotides in the DNA of a gene.**

A mutation could be caused by a **mutagen** – a chemical or physical agent that interacts with DNA causing a mutation. E.g. UV, gamma radiation, carcinogens (cancer causing chemicals).

An **alteration in the sequence of nucleotides** may change **the sequence of amino acids** in a polypeptide chain. This may change the 3D shape of the protein, affecting the protein function and subsequently **affect the characteristics (phenotype) of the organism.** Thus, this could manifest as a disease in the organism as well.

Gene mutations can result in **inheritable diseases.**

Q. In which cells must the mutation occur for it to be inheritable?
Germline cells, gametes, sex cells, cells of the gonads.

Gene mutation may involve a change in **one or a few bases**. If it involves a change in just a **single base**, it is called a **point mutation.** (Note: gene mutation ≠ point mutation).

Types of gene mutations:

1) **Substitution** – occurs when one nucleotide is replaced by another.

ATGGCCA → A**C**GGCCA

2) **Insertion/addition** – occurs when one or several nucleotides are added/inserted in a sequence.

ATGGCCA → AT**CT**GGCCA

3) **Deletion** – occurs when one or several nucleotides are removed from a sequence.

A**TGG**CCA → ACCA

4) **Inversion** – A segment of nucleotide sequences separates from the allele and rejoins at the original position but it is inverted and the sequence is now reversed.

ATGGCCA → AT**CCGG**A

Deletion or insertion (of 1 or 2 nucleotides) often results in the production of a **non-functional protein** as ribosomes begin to read incorrect triplets from the point of insertion or deletion. The original codons downstream of the point of mutation are not read correctly.

Such mutations are known as **frame-shift mutations**, and are more severe in their consequences.

An analogy:

Notes to self

Original message (in-frame):
THE **CAT BIT THE RAT**

Insertion of one letter (frame shifted):
THE **CXA TBI TTH ERA T**

Deletion of one letter (frame shifted):
THE **CTB ITT HER AT**
↳ A

} 'Meaning' is lost beyond point of mutation

Q. What is the effect of adding 3 consecutive letters to the reading frame here?
THE **M**CA TBI TTH ERA T
THE **MA**C ATB ITT HER AT
THE **MAD** CAT BIT THE RAT

Addition of 3 consecutive nucleotides will result in the restoration of the reading frame

However, the addition will result in changes to the primary sequence of the polypeptide chain and this could result in a loss or modification of function of the polypeptide. It is usually less severe than a frameshift mutation.

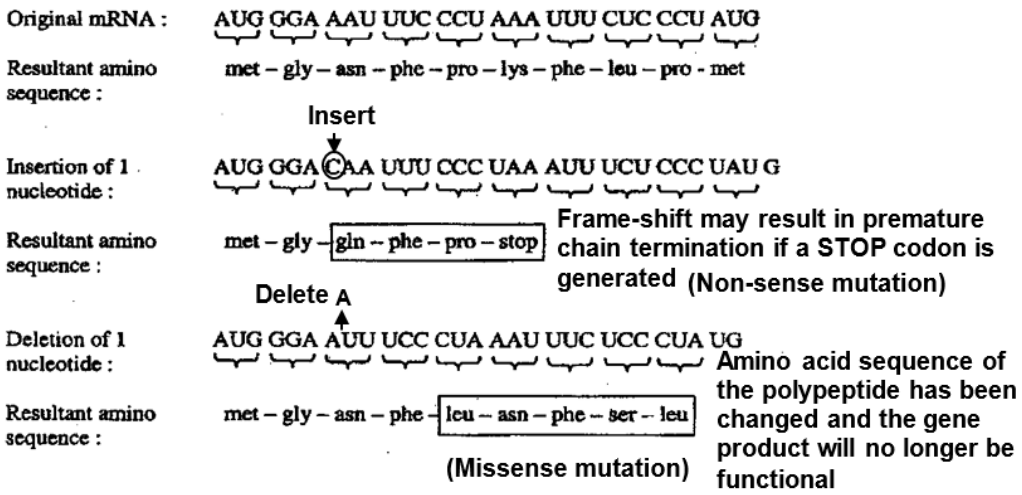


Fig. 1: Possible results of frameshift mutations

Substitution is the most common type of gene mutation. This type of mutation is usually not as serious as deletion or addition as the replacement of a nucleotide may not necessarily affect the function of the protein as much.

Notes to self

Q. Can you think of two reasons as to why this is the case?
Hint 1: The 3rd nucleotide of the codon….
Hint 2: Type of amino acid….

Reason 1:
This is because the genetic code is degenerate. i.e. more than 1 codon can code for an amino acid. These codons differ at their 3rd nucleotide e.g. UCU, UCC, UCA and UCG and they all code for the amino acid serine. Therefore, the amino acid sequence is not affected as a result of the mutation at the third base of a codon. Such a mutation is also known as a 'silent' mutation as the amino acid being coded for is not changed.

Reason 2:
*Even though the substitution mutation results in a different amino acid being coded for, the 'new' amino acid has an R group which has similar chemical properties to the amino acid it had replaced. E.g. the codon T**T**C codes for lysine whereas T**C**C codes for arginine. Both lysine and arginine have positively charged R-groups. Thus, a mutation from **T → C** may not affect the folding of the polypeptide and the function of the protein as both amino acids have R-groups with similar properties. Such mutations are known as conservative mutations.*

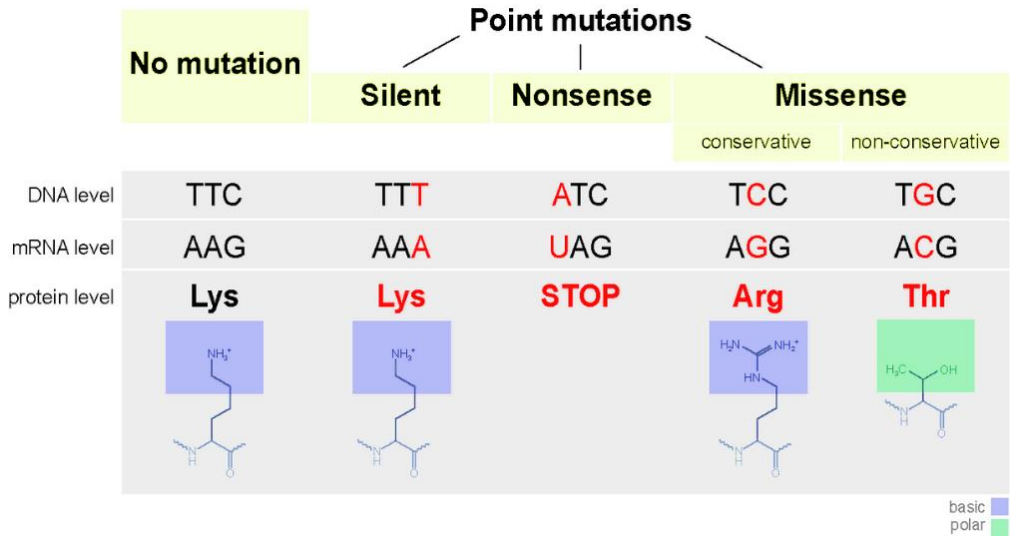


Fig. 2: Types of substitution mutations and their effects

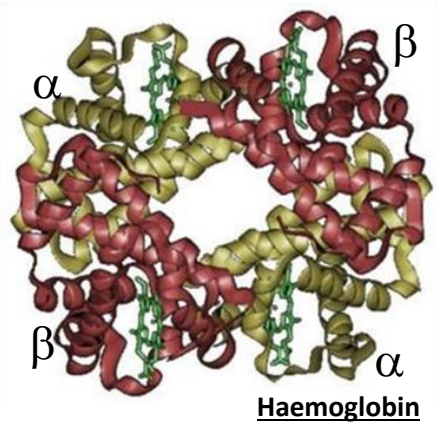
Example of disease: Sickle Cell Anaemia

Notes to self

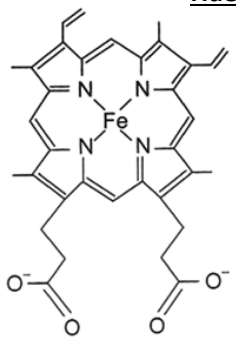
Sickle cell anaemia is an example of a disease caused by a **single nucleotide substitution mutation**.

In a normal adult:

- The normal adult haemoglobin (Hb A) is a quaternary protein which is a **tetramer composed of 2 different types of polypeptide chains.**
  - 2 α-globin chains and 2 β-globin chains
- The α and β chains are coded for by **2 different genes** found on 2 different chromosomes.
- The haemoglobin is abundant in red blood cells (RBC) and serves a role in **transporting oxygen from the lungs to the tissue**.



Haemoglobin



Haem

Fig. 3: Haemoglobin and the haem group with Fe^{3+} that binds oxygen

In sickle cell anaemia, there is a change in sequence of DNA nucleotide:

- Occurs as a result of a **single nucleotide substitution** in the gene which codes for the **β-globin chain**

  C**T**C → C**A**C (i.e. T is substituted by A) on the template strand

This results in change in sequence of amino acids in polypeptide chain:

- In the mRNA produced, the **6th triplet codon** is changed from G**A**G to G**U**G as a result of the point mutation in the DNA.

→ The new codon codes for the amino acid **valine** instead of amino acid **glutamate** at position 6 of the β -globin chain.

→ This causes the formation of a variant **sickle cell haemoglobin (Hb S)**

As a result, there is a change in properties of haemoglobin and phenotype of the red blood cells containing the HbS:

- **The R group of glutamate is charged and hydrophilic** whereas that of **valine is non-polar and hydrophobic.**
- This substitution creates a **hydrophobic patch** on the outside of the protein structure that can stick to the hydrophobic region of an adjacent haemoglobin molecule's β -globin chain.
- As the red blood cell moves to a region of **low oxygen concentration** in actively respiring tissue, oxygen is released by the abnormal HbS and a different conformational change will occur.

Notes to self

- A hydrophobic patch will stick out from Hb S and cause Hb S to **polymerise** into **abnormal rigid rod-like fibres** that distort the biconcave shape of the red blood cell, resulting in the characteristic **sickle shape** of the cell.
- Normal circular biconcave shape of red blood cell → sickle shape under **low [O_2]**
- The polymers can be **broken up by binding oxygen to Hb S**. i.e. Hb S can still bind O_2. Sickling is reversible.

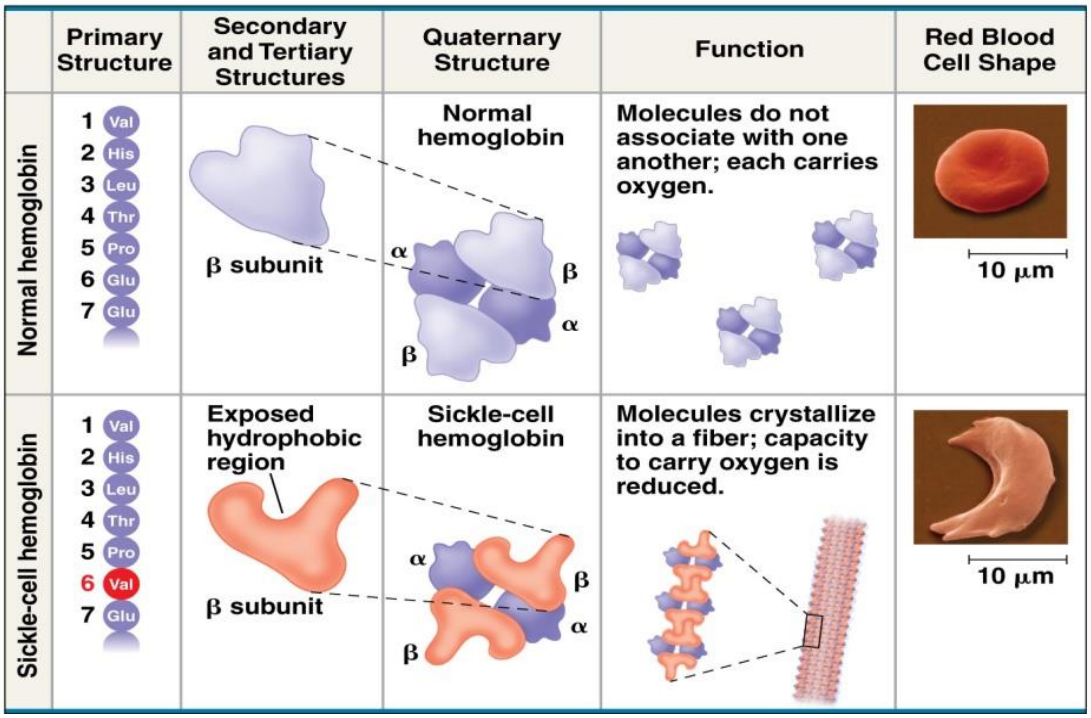


Fig.4: Differences in the protein structures of HbS (sickle-cell) and HbA (normal) molecules and their effects

In summary:

	Normal (Hb A)	**Sickle-cell anaemia (Hb S)**
Gene coding for β-globin chain	_____CTC_____	_____CAC_____
Resultant codon on mRNA	... … G**A**G…	… … G**U**G …
Resultant polypeptide chain	… **glutamate** …	… **valine** …
At low O_2 concentrations	Remain soluble	Polymerise into rigid rod-like fibres
Appearance of red blood cell	Disc-shaped	Sickle-shaped

Effects of the disease:

Notes to self

Sickle red blood cells are more **fragile** causing them to break up more easily. They are also actively destroyed in the spleen. This results in the **shortage of red blood cells** and **poor oxygen transport.** Possible consequences include:

- Patient suffers from anaemia, breathlessness and physical weakness
- Heart failure as the heart needs to work a lot harder
- Lack of energy in the form of ATP due to reduced cellular respiration

The sickle-shaped red blood cells, being pointed and elongated, may also get **lodged in small blood vessels** (capillaries) and therefore interfere with blood circulation → **Sickle-cell crisis**. Effects of this include:

- Depriving organs e.g. brain of oxygen
- Severe pain due to many localised blockages resulting in death of surrounding tissue
- Damage to organs especially those with numerous fine capillaries such as the spleen and lungs

Sickle cell anaemia is a **homozygous recessive disorder**. Sufferers would have **two copies** of the mutant form of the gene. They are said to have the **sickle cell disease**.

Heterozygous individuals have **one copy of the mutant and one copy of the normal form of the gene.** They are said to have the **sickle cell trait**. You will learn later in the lecture series for Genetics and Evolution that such individuals are resistant to the malaria parasite.

Example of disease: Cystic Fibrosis

Notes to self

Cystic fibrosis is more commonly seen in populations of Caucasians.

The **cystic fibrosis gene** normally codes for a membrane ion channel called the **cystic fibrosis transmembrane conductance regulator (CFTR).** CFTR controls the movement of **chloride ions, Cl^-** into or out of cells (& influences Na^+ transport indirectly).

More than 1000 different types cystic fibrosis of mutations have been reported.

- The most common mutation which occurs in 75% of cystic fibrosis patients, involves a **deletion of 3 nucleotides** on **exon 10 of chromosome 7**, resulting in the loss of an amino acid **phenylalanine** at position 508 of the CFTR polypeptide. Cystic fibrosis is an autosomal recessive disease.

- In affected patients, **cystic fibrosis transmembrane conductance regulators (CFTRs)** are either **missing or defective.**

Symptoms of cystic fibrosis

1. In the lungs, defective/missing CFTR → Cl^- not transported out of epithelial cells into lumen of air cavity→ Na^+ retained too (for charge balance) → Ψ more negative in the cell → water retained in cell as water moves into the epithelial cells from the lumen → mucus in lumen is undiluted → becomes thick → cannot flow easily → leads to congestion → mucus remains too long in respiratory tract → promotes bacteria growth → gives rise to lung infection → patient suffers from **severe breathing difficulty** (Fig. 5)

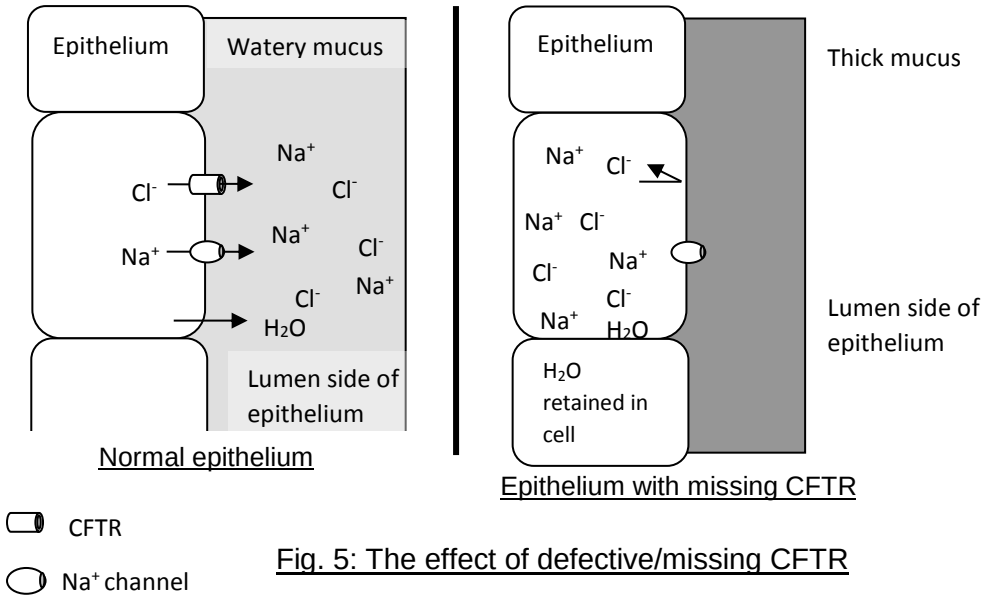


Fig. 5: The effect of defective/missing CFTR

2. In the pancreas, the pancreatic duct is choked by thick mucus preventing release of enzymes → **indigestion.**

3. In the intestines, thick mucus layer in intestines **reduces absorption** of digested food.

Notes to self

4. In the sweat, sweat gland produces sweat → as it rises up the duct towards the pore, upper duct reabsorbs Na^+ and Cl^- (as opposed to secreting NaCl into the lumen of lungs) due to opposite orientation of CFTR in the sweat ducts. Defective CFTR → no reabsorption of NaCl → **very salty and copious sweat** production. Basis of diagnosis → to measure [Cl^-] in sweat (Fig. 6).

5. Death usually by age 30

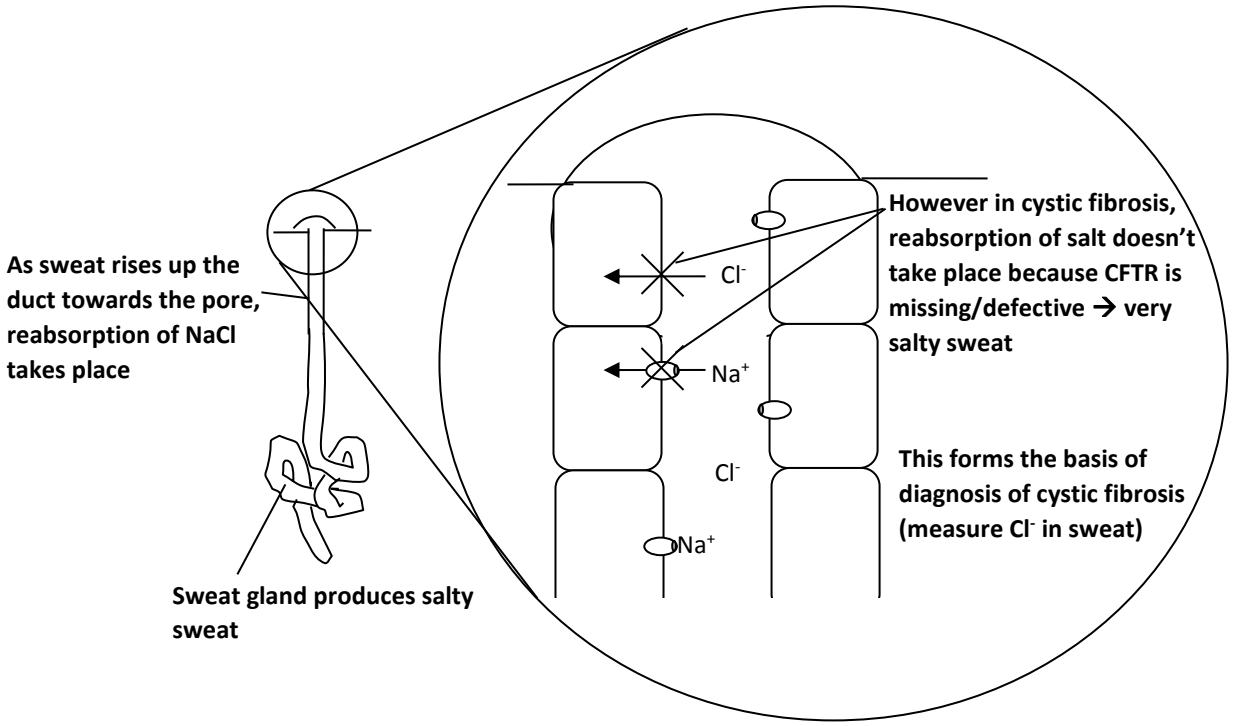


Fig. 6: Effect of cystic fibrosis on sweat production

7. Chromosomal mutations

Notes to self

Chromosomal aberrations

There are two major forms of chromosomal aberrations:

(I) Variation in chromosomal structure and
(II) Variation in chromosomal number

(I) Variation in the chromosomal structure

Alterations in chromosomal structure (usually involves **several gene loci**) can be brought about by the following mechanisms:

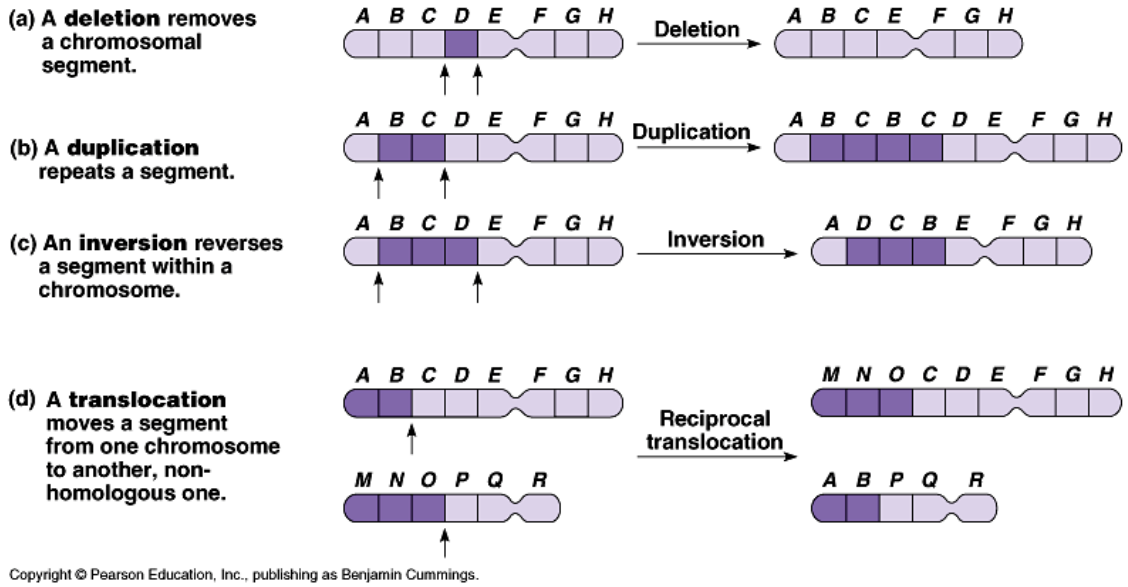


Fig. 7: Mechanisms for changes in chromosome structure

Deletions and **duplications** are especially likely to occur during crossing over. Non-sister chromatids of homologous chromosomes may break and re-join at incorrect places such that one chromatid may give up more genes than it receives. The products of such an unequal crossover are one chromosome with a deletion mutation and one with a duplication mutation. (Fig.8)

Variations in chromosome structures usually cause serious problems. Chromosome deletions frequently result in zygotic loss, stillbirths or infant deaths. Some survive a little longer.

The **phenotypic abnormalities** that result are usually due to the reduced or additional genetic material/dosage reflected in chromosomal deficiencies and duplications respectively.

You may then wonder how chromosomal **inversion and reciprocal translocations** may result in disease since the **amount of genetic material remains the same**.

Notes to self

However, these chromosomal aberrations may still alter the phenotype because the **gene's expression can be influenced by its new location among neighbouring genes**. e.g. Juxtaposition (placement of things side by side) of genes next to regulatory elements such as enhancers/silencers, which tend to increase/decrease rates of gene expression (you will learn more about these under the topic of control and organisation of eukaryotic gene expression).

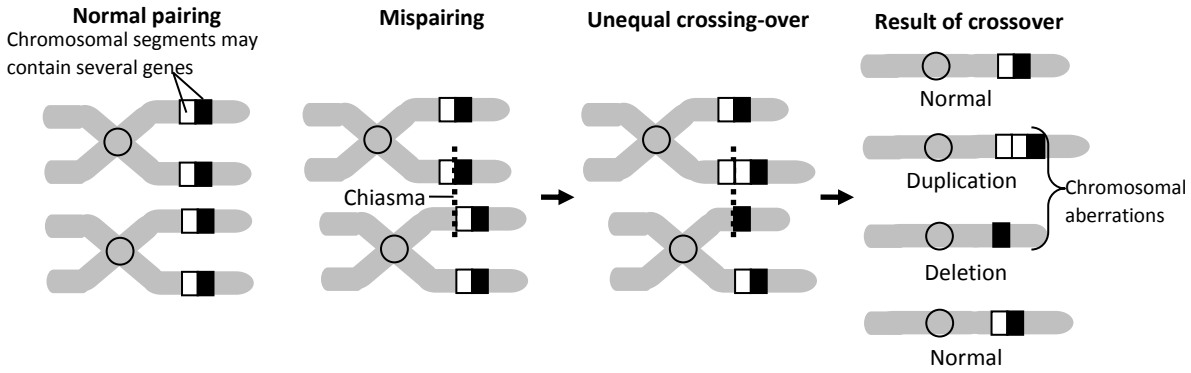


Fig.8: A proposed mechanism on how duplications and deletions of chromosomal segments may result in chromosomal aberrations

Examples of diseases:

Example 1: (Deletion)

Cri-du-chat ("Cat's cry" in French) syndrome

Disease comes about due to **deletion** in the short arm of **chromosome 5.**
A child born with this deletion is physically and mentally retarded, has a small head, broad face and saddle nose, widely spaced eyes, unusual facial features and a cry that sounds like the mewing of a distressed cat. Such individuals usually die in infancy or early childhood.

The signs and symptoms of *cri-du-chat* syndrome are probably related to the loss of multiple genes in this region. **Most cases** of *cri-du-chat* syndrome are not inherited. They result from a **chromosomal deletion** that occurs as a **random** event **during the formation of gametes** (eggs or sperm) or in early foetal development. Such people typically have no history of the disorder in their family.

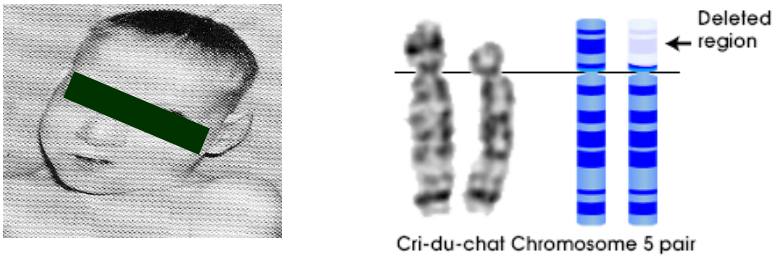


Fig.9: Chromosomal mutation in Cri-du-chat syndrome

Example 2: (translocation)

Notes to self

Chronic Myelogenous Leukemia (CML)

When a genetic aberration occurs in somatic cells, cancer may result.

In CML, most of the **chromosome 22** has been **translocated** onto the long arm of **chromosome 9**. In addition, the small distal portion of chromosome 9 is translocated to chromosome 22. The resultant chromosome 22 is called the "**Philadelphia chromosome**." This chromosome is **found only in tumour cells** of a person with CML.

The translocation brings **two genes next to each other and genes are transcribed and translated as one protein**. This protein causes increased cell proliferation and reduced apoptosis (programmed cell death) → **cancer**. It is unclear why this fusion product causes cancer.

CML **affects the stem cells** that develop into white blood cells. These tumor cells do not mature normally but proliferate rapidly.

CML usually occurs in people who are middle-aged or older, although it also can occur in children. When symptoms do appear, they may include a feeling of low energy, fever, lack of appetite, and night sweats. The spleen may also be enlarged.

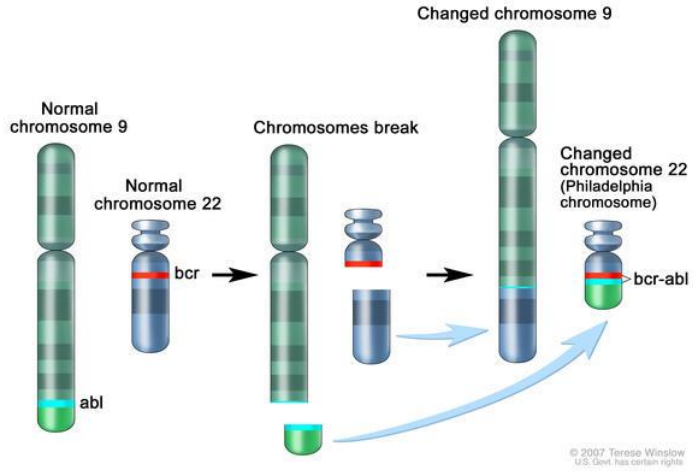


Fig.10: Chromosomal mutation in CML

Example 3: (duplication)

Charcot-Marie-Tooth syndrome (CMT)

In one form of CMT, a **chromosomal duplication on chromosome 17** results in high gene dosage (3 copies instead of a normal 2 copies) of a myelin sheath protein resulting in abnormal structure and function of the myelin sheath (an insulating sheath around nerve cells). This type of CMT is inherited in an autosomal dominant condition.

Symptoms of CMT include weakness of lower foot, loss of balance, poor motor skills & muscle atrophy. Not a fatal disease and sufferers have a normal life expectancy.

(II) Variation in chromosomal number

Notes to self

ANEUPLOIDY

Aneuploidy is a condition where the cell **does not have** a chromosome number that is a **multiple of the haploid number. Chromosomes are present in extra or fewer copies** than in normal cells.

1. If chromosome is present in triplicate, the aneuploid cell is said to be **trisomic** e.g. 2n+1
2. If the cell is missing a chromosome, it is said to be **monosomic** e.g. 2n-1

Aneuploidy is a result of a **non-disjunction event** where:

1. Homologous chromosomes fail to separate properly during **meiosis I**

OR

2. When sister chromatids fail to separate properly during **meiosis II**

So one gamete receives two of the same type of chromosome and another gamete receives no copy (see Fig. 11).

If either of the aberrant gametes unites with a normal gamete at fertilisation, offspring will have abnormal number of a particular chromosome = **aneuploidy**

Mitosis will subsequently transmit the anomaly to all embryonic cells.

Non-disjunction can also occur during **mitosis**. If such an error occurs early in embryonic development, then the aneuploid condition is passed on to a large number of cells where the severity of the effect is more pronounced.
Aneuploidy causes genetic disorders.

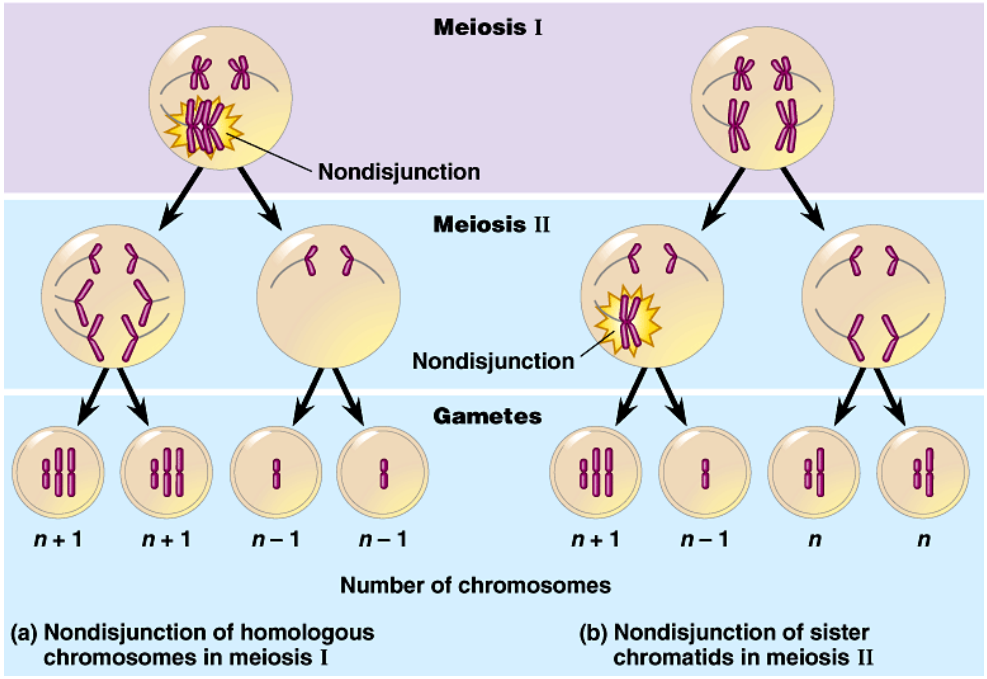


Fig. 11: Non-disjunction events during Meiosis

Examples of diseases:

Notes to self

Example 1: Down syndrome (Trisomy 21)

Down syndrome is result of an **extra chromosome 21**, so each body cell has a total of 47 chromosomes.

Most cases result from non-disjunction during meiosis I.

Down syndrome includes characteristic facial features, short stature, heart defects, susceptibility to respiratory infection and mental retardation. Most are sexually underdeveloped and sterile.

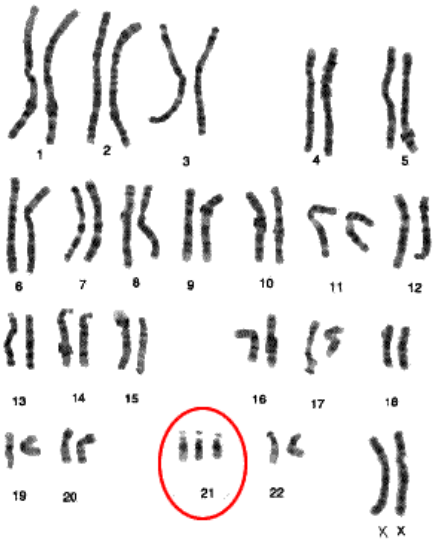


Fig.12: Karyotype of an individual with trisomy 21

Example 2 (Non-disjunction of sex chromosomes): Klinefelter syndrome (XXY)

Males with an extra X chromosome suffer from Klinefelter syndrome. These individuals have male sex organs, but the testes are abnormally small and the man is sterile. Though extra X chromosome is inactivated, some breast enlargement and other female body characteristics are common. Affected individual is usually of normal intelligence

Example 3 (Non-disjunction of sex chromosomes): Turner syndrome (monosomy X)

Monosomy X, is the only known viable monosomy in humans. These XO individuals are phenotypically female, but are sterile and their sex organs do not mature. When provided with estrogen replacement therapy, girls with Turner syndrome do develop secondary sex characteristics.

Q. What is the consequence of a non-disjunction event occurring in a somatic cell of an adult?

It depends. Could be harmless as very few cells are affected since only the daughter cells carry the aneuploidy or it could be harmful especially if the affected chromosomes lead to imbalance in the expression of critical genes.

Interesting Science Fact – Did You Know?

Although females have 2 X chromosomes, one X chromosome in each cell becomes almost completely inactivated during embryonic development. As a result, the cells of females and males have the same effective dose (one copy) of genes with loci on the X chromosome. This is called dosage compensation. Either one of the X chromosomes in a cell gets inactivated and this is random.

Non-disjunction of sex chromosomes produces a variety of aneuploid conditions. Most of these aneuploid conditions upset genetic balance less than those involving autosomes. This may be because Y chromosome carries fewer genes and extra X chromosomes become inactivated in somatic cells.

POLYPLOIDY

Polyploidy is a condition where there are **three or more times the haploid number of chromosomes** in the nucleus, e.g. 3n (triploidy), 4n (tetraploidy), 5n etc.

This condition can result from:

1. Non-disjunction of entire chromosome sets in mitosis. → producing 4n somatic cells
2. Non-disjunction of entire chromosome sets in meiosis → producing 2n gametes formed. E.g.:
   - 2n gamete fuse with 2n gamete → 4n zygote
   - 2n gamete fuse with n gamete(normal) → 3n zygote

Polyploidy is fairly common in the plant kingdom, but in the animal kingdom, polyploidy species are less common, although there are some amphibian and fish examples.

An example of a **tetraploid mammal** is the burrowing rodent, *Tympanoctomys barrerae*. This is a naturally occurring tetraploid species.

Fig.13: *Tympanoctomys barrerae*

In the genus *Chrysanthemum*, the basic number of chromosome is 9, and species are known to have 18, 36, 54, 72 and 90 chromosomes (2n,4n, 6n etc.).

Q. Naturally-occurring species of the genus Chrysanthemum have ploidy numbers which are even e.g. 2n, 4n, 6n etc. Can you suggest a reason why these species tend not to have odd sets of the basic 9 chromosomes i.e. 3x9 = 27, 5x9 = 45, 7x9 = 64 etc?

Chromosomes cannot pair up as homologs during prophase I of meiosis and form viable gametes. Organisms may survive but are infertile unless they propagate vegetatively.

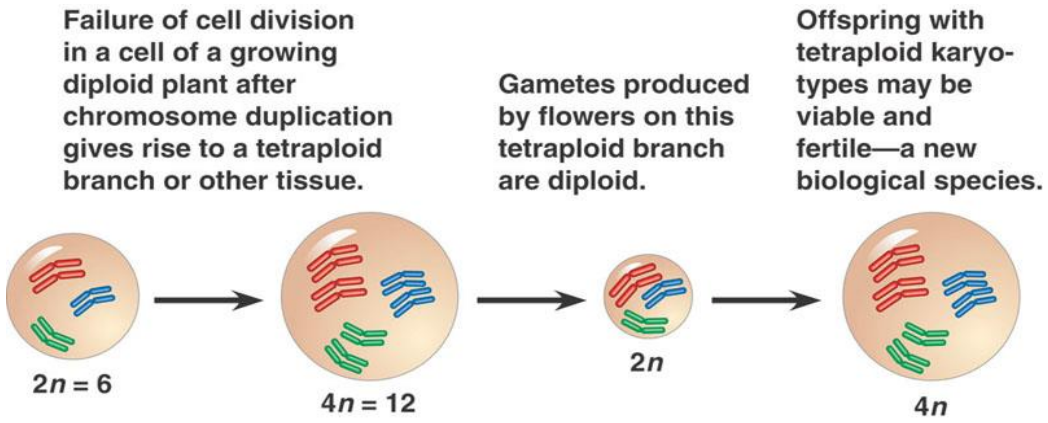
FOR YOUR INFORMATION ONLY

Autopolyploidy

The genus *Chrysanthemum* represents a type of polyploidy, known as **autopolyploidy**, in which all chromosomes in the polyploid species derive from a **single diploid ancestral** species.

This can occur when a failure of cell division can double a cell's chromosome number from diploid (2n) to tetraploid (4n). This condition prevents tetraploids from successfully interbreeding with diploid plants of the original population – triploid offspring of such unions are infertile. However, the tetraploid plants can still produce fertile tetraploid offspring by self-pollinating or mating with other tetraploids.

Note: When the newly-occurring tetraploids are unable to interbreed with the original diploid plants, this means that the tetraploids are now considered a new species.



FOR YOUR INFORMATION ONLY

Allopolyploidy

Where the polyploid species have complete sets of chromosomes from **two or more different ancestral species,** such polyploids are known as allopolyploids.

Two different species interbreed and produce a hybrid that is infertile. They are infertile because the haploid set of chromosomes from one species cannot pair during meiosis with the haploid set from another species. Though infertile, the hybrid can still propagate itself asexually as most plants can. In subsequent generations, various mechanisms (see Fig. 14) can change the sterile hybrid into a fertile one known as an allopolyploid. The allopolyploids are fertile, but cannot interbreed with either parental species, thus the hybrid is now a new biological species.

Many important agricultural crops such as oats, cotton, potatoes, tobacco and wheat are allopolyploids. The wheat used for bread, *Triticum aestivum*, is an allohexaploid (6 sets of chromosomes, 2 sets from 3 different species). Plant geneticists create new polyploids by using chemicals that induce meiotic and mitotic errors.

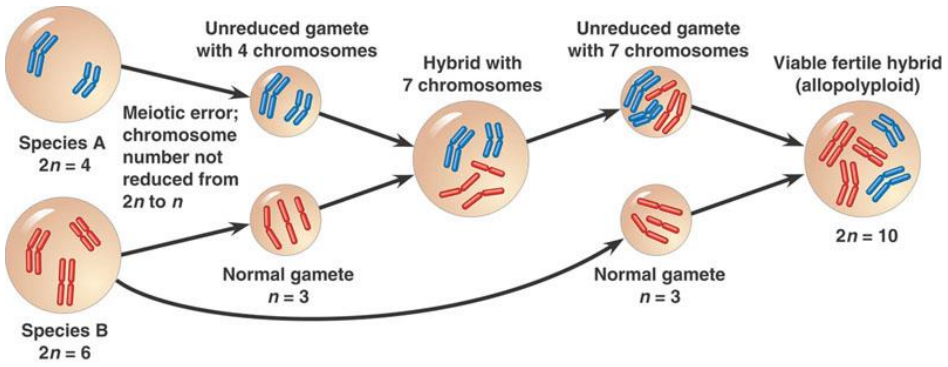


Figure 14: Allopolyploid speciation in plants