I Internal Examination (2019-20)

B.Sc. Biotechnology II Year

Molecular Genetics

SET-A

Time: 1:30 Hours **Max. Marks-30**

Q1. Answer the following questions in very short: **1×7=7**

1). Who rediscovered Mendel's Law of Heredity?

Ans: De Vries, Carl Correns and Tshermark

2). How many contrasting traits mendal noted in Garden Pea?

Ans: Seven

3). Write down the Mendel's Dihybrid ratio for Phenotypes?

Ans: 9:3:3:1

4). Who is the father of genetics?

Ans: Gregor John Mendel

5). Write the genotype of man with blood group 'A'?

Ans: I^AI^A and I^Ai^A

6). Cite one example of complementary genes?

Ans: Flower colour of sweet Pea

7). What is the back cross?

Ans: A cross between a hybrid organism of an unknown genotype and a homozygous recessive organism.

Q2. Explain the following terms with examples: **4×2=8**

i). Co-Dominance

Ans: Codominance occurs when two versions, or “alleles,” of the same gene are present in a living thing, and both are expressed. Instead of one trait being dominant over the other, both traits appear.

Codominance is easy to spot in plants and animals that have more than one pigment color. Spotted cows and flowers with petals of two different colors are examples of codominance, for example.

Codominance also occurs in some less visible traits, such as blood type. The A and B alleles for blood type can both be expressed at the same time, resulting in type AB blood.

In genetics, "dominant" genes are those that are always expressed if they are found in an organism. Dominant genes may be expressed as co-dominant – where two different traits are both expressed alongside each other – or as dominant/recessive, where the presence of a dominant gene completely masks the presence of a recessive gene.

Examples of Codominance

Livestock

When a chicken with white feathers breeds with a chicken with black feathers, the result is an offspring chicken that grows up to have both black and white feathers.

Likewise, when a red cattle breeds with a red cattle, the resulting offspring may show both red and white hairs, resulting in a mixed coat pattern called "roan."

Rhododendron

Rhododendrons and other flowers may also exhibit codominance.

In the case of rhododendrons, the crossing of a red and white flower may yield a flower that has both red and white patches.

Many flowers show similar patterns of codominance, where both of the parental flower colors show up in different parts of the plant.

Blood Type

An example of codominance that occurs in humans is that of blood type.

There are three different versions of the gene for proteins that appear on the outside of our blood cells and help our body to identify the cells as their own. These alleles are A, B, and O.

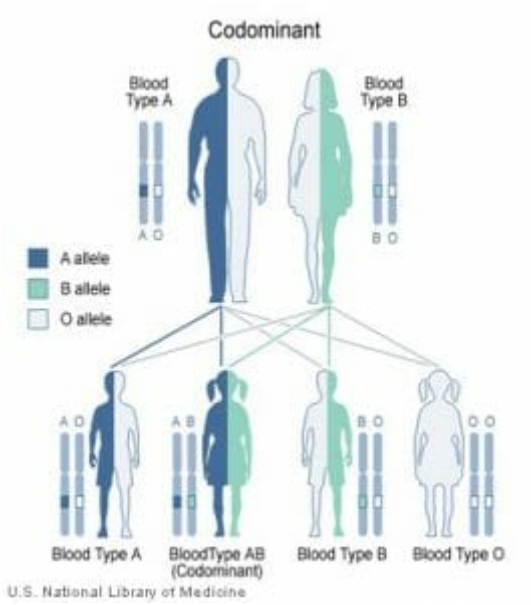
The "O" allele actually does not code for any protein at all, so people with the "O" trait lack both A and B proteins.

The A and B proteins, on the other hand, code for two different proteins. These proteins, like different colors in a flower, can appear together.

Someone who inherits an A allele from one parent and a B allele from the other will express both proteins in a codominant fashion, resulting in an AB blood type.

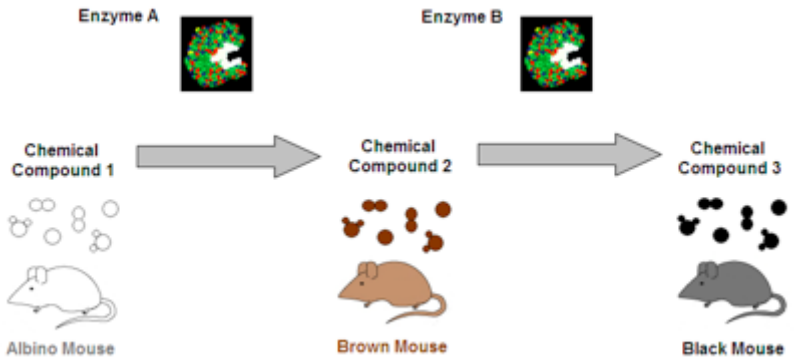
The "O" trait, on the other hand, is a good example of a dominant/recessive relationship: if either A or B is expressed, the "O" trait is not expressed.

This chart below illustrates how codominance can occur between A and B traits, while a dominant/recessive relationship exists between those traits and the O trait:

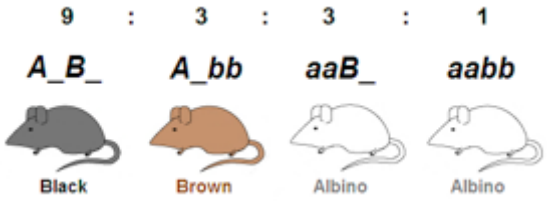


ii). Supplementary Genes

Ans: Supplementary genes are genes that both contribute to a single characteristic, where one gene can mask the effect of the other. You may also think of supplementary genes in terms of one gene producing a characteristic and the second as only being able to 'supplement' this characteristic.



The dominant allele for gene A produces an enzyme responsible for the production of a pigment melanin (chemical compound 2). Thus as long the individual has one dominant allele A, they will be brown. However, the dominant allele for gene B produces an enzyme which increases the level of Melanin expression. Thus if an individual has at least one dominant allele for both gene A and gene B, they will be black. In a heterozygous cross (AaBb x AaBb) the following offspring are produced:



Note that although three of the albino individuals have the dominant allele B (which increases the expression of melanin), no melanin is produced to begin with and thus no colour can be produced. Therefore the final phenotypic ratio for Supplementary genes is always:

Ratio: 9 : 3 : 4

Q3. Write short notes on: **4×2=8**

i). Semiconservative model of DNA Replication

Ans: Replication is the process of formation of carbon copies. For this, DNA functions as its own template. Therefore, DNA replication is an autocatalytic function of DNA.

It usually occurs during S-phase of cell cycle when chromosomes are in highly extended form. As proposed by Watson and Crick, DNA replication is semiconservative (a type of replication in which one strand of the daughter duplex is derived from the parent while the other strand is formed anew).

This is carried out by the separation of two strands. The separated strands function as templates. The new strands built up over the templates of old strands will have complementary base pairs (A opposite T and G opposite C). The two daughters DNA molecules thus formed will be carbon copies of the parent molecule but shall have one new strand and one old strand.

Taylor et al (1957) fed dividing cells of root tips of Broad Bean (Vicia faba) with radioactive ^{3}H containing thymine instead of normal thymine. Thymine is incorporated into DNA which is the structural element of chromosomes. Taylor et al found that all the chromosomes became radioactive.

Labelled thymine was then replaced with normal one. Next generation came to have radioactivity in one of the two chromatids of each chromosome while in subsequent generation radioactivity was present in 50% of the chromosomes (Fig. 6.9). This is possible only if out of the two strands of a chromosome, one is formed afresh while the other is conserved at each replication this is semiconservative replication.

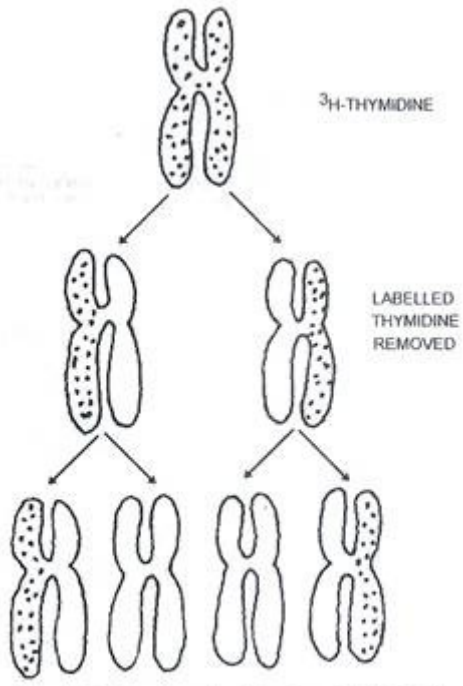


Fig. 6.9. Experiment of Taylor *et al* (1957) to show semi-conservative replication of chromosomes.

Semi-conservative replication of DNA was proved by the work of Mathew Meselson and Franklin Stahl (1958). They grew Escherichia coli for many generations in a medium having heavy isotope of nitrogen, in the form of $^{15}NH_4Cl$, till the bacterial DNA became completely labelled with heavy isotope.

The labelled bacteria were then shifted to fresh medium having normal or ^{14}N nitrogen. Samples were taken for each generation (one generation takes 20 minutes as E. coli divides in 20 minutes) and the DNA was tested for the heavy isotope of nitrogen through density gradient centrifugation using caesium chloride. Caesium chloride is highly water soluble heavy salt.

When spun in centrifuge at high speed (say 50,000 revolutions per minute) the salt forms a density gradient with heaviest most concentrated region at the bottom and successively less concentrated lighter one towards the surface. When DNA is mixed with caesium chloride it will settle down at a particular height in centrifugation, heavier towards the base and lighter one higher up (Fig. 6.10).

Fluoro- chrome called ethidium bromide is used to enhance contrast as the fluorochrome is specific for DNA. Meselson and Stahl found that DNA of the first generation was hybrid or intermediate (^{15}N and ^{14}N). It settled in caesium chloride solution at a level higher than the fully labelled DNA of parent bacteria ($^{15}N^{15}N$). The second generation of bacteria after 40 minutes contained two types of DNA, 50% light ($^{14}N^{I4}N$) and 50% intermediate ($^{15}N^{I4}N$).

The third generation of bacteria after 60 minutes contained two types of DNA, 25% intermediate ($^{15}N^{14}N$) and 75% light ($^{14}N^{14}N$) in 1 : 3 ratio. The fourth generation after 80 minutes contained 12.5% $^{15}N^{14}N$ and 87.5% $^{14}N^{14}N$ DNA in 1 : 7 ratio.

This observation is possible only if the two strands of DNA duplex separate at the time of replication and act as a template for the synthesis of new complementary strands of DNA having normal or ^{14}N. This will produce two DNA duplexes with one old strand (^{15}N) and one new strand (^{14}N).

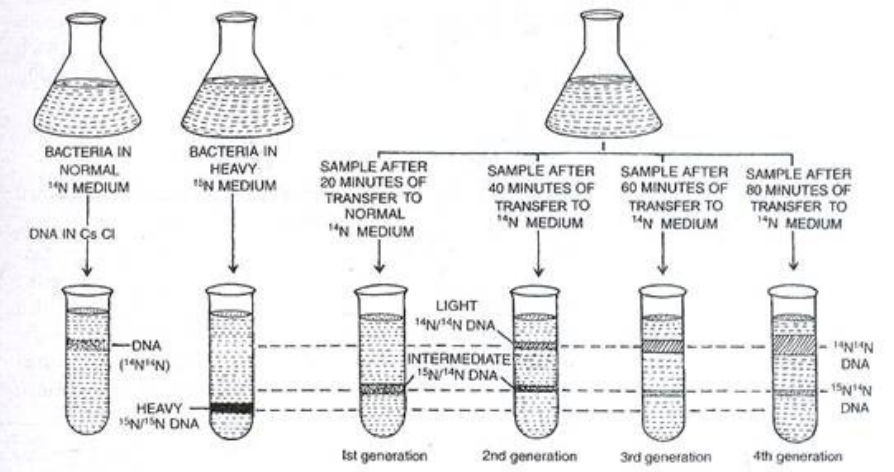


Fig. 6.10. Meselson and Stahl's Experiment.

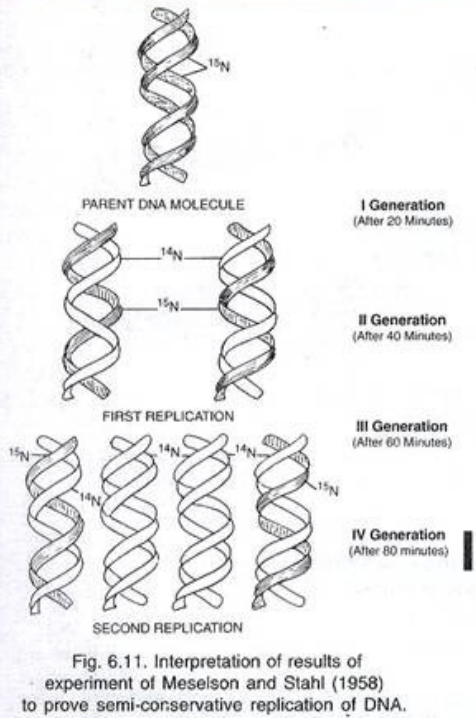


Fig. 6.11. Interpretation of results of experiment of Meselson and Stahl (1958) to prove semi-conservative replication of DNA.

Fig. 6.12. Showing result of semiconservative replication of DNA.

ii). Monohybrid Cross

Ans: A monohybrid cross is a genetic mix between two individuals who have homozygous *genotypes*, or genotypes that have completely dominant or completely recessive alleles, which result in opposite *phenotypes* for a certain genetic trait.

Monohybrid crosses are used by geneticists to observe how the offspring of *homozygous* individuals express the *heterozygous* genotypes they inherit from their parents. Typically, this mix determines the dominant genotype.

A monohybrid cross can also signify a genetic mix between two individuals who have heterozygous genotypes. These crosses confirm the dominance of an allele

Examples of Monohybrid Cross

Gregor Mendel's Peas

Although he did not know it at the time, Gregor Mendel used monohybrid crosses to identify dominant and recessive traits in his landmark experiments with peas.

Gregor Mendel focused on several different genetic traits, but we will focus on one: stem length. Imagine that two types of pea plants grow in a garden. One type of pea plant has long stems, while the other has short stems. For the sake of this example, assume that both types of pea plant have a homozygous genotype (LL and ll), and that long stems (LL) are dominant over short stems (ll).

A monohybrid cross, or breeding a long-stemmed pea plant with a short-stemmed pea plant, allows scientists, like Gregor Mendel, to determine the dominance of long stems or short stems. A monohybrid cross also permits scientists to evaluate how heterozygous offspring express the genes they inherit.

As mentioned before, breeding a long-stemmed pea plant with a short-stemmed pea plant creates offspring that all have a heterozygous genotype (Ll). As long stems are dominant, all offspring will have the long-stemmed phenotype. In different terms, and as modeled by Gregor Mendel's classic pea example observing the offspring of a monohybrid cross allows for determination of dominant genotypes and, by extension, dominant phenotypes.

Huntington's Disease

Huntington's Disease is a progressive degenerative condition that occurs in 4 to 15 of every 100,000 people in the United States. Having no cure, it is a certain death sentence for those diagnosed. While little is known about this condition, geneticists are sure that it is inherited via a dominant gene.

At the simplest level, a monohybrid cross was used to determine the genetic nature of Huntington's disease. Everyone carries the aptly-named *Huntingtin* gene, the gene

responsible for the complication. With this information, scientists paired the *Huntingtin* genes of an individual who is homozygous dominant for the condition (HH) with the *Huntingtin* genes of an individual who is homozygous recessive for the condition (hh).

Although this example is highly abridged, the result remains that all offspring from the cross carried the dominant allele for Huntington's disease. While this experiment, if conducted on humans, would bring sad news to both parent and child, it would also highlight the dominant nature of the disease.

Confirming Dominant Traits

We have already discussed how scientists use monohybrid crosses to determine the dominant allele of a genotype. However, monohybrid crosses between homozygous individuals is often only the first step. Heterozygous crosses, in which both parents carry a dominant allele and a recessive allele, helps confirm whether a trait is dominant or recessive.

The model for this second step greatly resembles the process Gregor Mendel followed, with peas. Using stem length as an example, scientists breed two parents that both have long stems, with genotype Ll. In an ideal scenario, one in every four of their offspring will carry the genotype ll, and thus have a short stem. Because long stems occur more often than short stems in this second iteration, scientists can reasonably determine long stems are a dominant trait.

Q4. Write a detailed note on DNA replication in prokaryotes? 7

Ans: DNA replication has been extremely well studied in prokaryotes primarily because of the small size of the genome and the mutants that are available. *E. coli* has 4.6 million base pairs in a single circular chromosome and all of it gets replicated in approximately 42 minutes, starting from a single origin of replication and proceeding around the circle in both directions. This means that approximately 1000 nucleotides are added per second. The process is quite rapid and occurs without many mistakes.

DNA replication employs a large number of proteins and enzymes, each of which plays a critical role during the process. One of the key players is the enzyme DNA polymerase, also known as DNA pol, which adds nucleotides one by one to the growing DNA chain that are complementary to the template strand. The addition of nucleotides requires energy; this energy is obtained from the nucleotides that have three phosphates attached to them, similar to ATP which has three phosphate groups attached. When the bond between the phosphates is broken, the energy released is used to form the phosphodiester bond between the incoming nucleotide and the growing chain. In prokaryotes, three main types of polymerases are

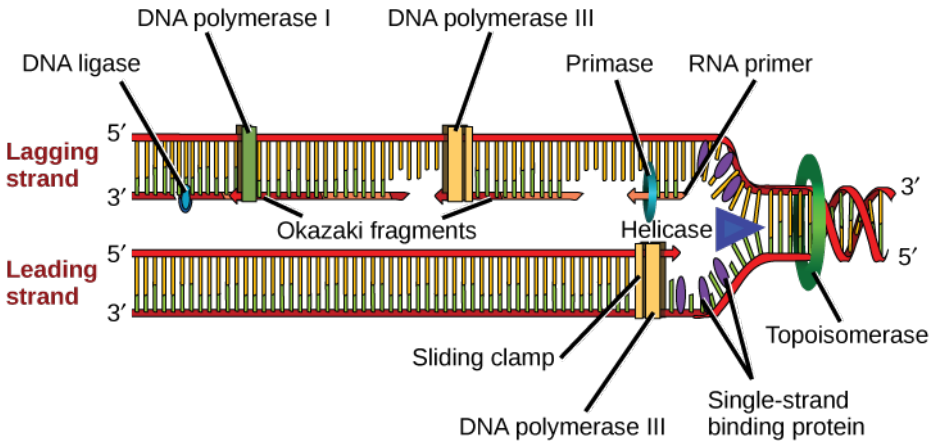
known: DNA pol I, DNA pol II, and DNA pol III. It is now known that DNA pol III is the enzyme required for DNA synthesis; DNA pol I and DNA pol II are primarily required for repair.

How does the replication machinery know where to begin? It turns out that there are specific nucleotide sequences called origins of replication where replication begins. In *E. coli,* which has a single origin of replication on its one chromosome (as do most prokaryotes), it is approximately 245 base pairs long and is rich in AT sequences. The origin of replication is recognized by certain proteins that bind to this site. An enzyme called helicase unwinds the DNA by breaking the hydrogen bonds between the nitrogenous base pairs. ATP hydrolysis is required for this process. As the DNA opens up, Y-shaped structures called replication forks are formed. Two replication forks are formed at the origin of replication and these get extended bi- directionally as replication proceeds. Single-strand binding proteins coat the single strands of DNA near the replication fork to prevent the single-stranded DNA from winding back into a double helix. DNA polymerase is able to add nucleotides only in the 5' to 3' direction (a new DNA strand can be only extended in this direction). It also requires a free 3'-OH group to which it can add nucleotides by forming a phosphodiester bond between the 3'-OH end and the 5' phosphate of the next nucleotide. This essentially means that it cannot add nucleotides if a free 3'-OH group is not available. Then how does it add the first nucleotide? The problem is solved with the help of a primer that provides the free 3'-OH end. Another enzyme, RNA primase, synthesizes an RNA primer that is about five to ten nucleotides long and complementary to the DNA. Because this sequence primes the DNA synthesis, it is appropriately called the primer. DNA polymerase can now extend this RNA primer, adding nucleotides one by one that are complementary to the template strand.

The replication fork moves at the rate of 1000 nucleotides per second. DNA polymerase can only extend in the 5' to 3' direction, which poses a slight problem at the replication fork. As we know, the DNA double helix is anti-parallel; that is, one strand is in the 5' to 3' direction and the other is oriented in the 3' to 5' direction. One strand, which is complementary to the 3' to 5' parental DNA strand, is synthesized continuously towards the replication fork because the polymerase can add nucleotides in this direction. This continuously synthesized strand is known as the leading strand. The other strand, complementary to the 5' to 3' parental DNA, is extended away from the replication fork, in small fragments known as Okazaki fragments, each requiring a primer to start the synthesis. Okazaki fragments are named after the Japanese

scientist who first discovered them. The strand with the Okazaki fragments is known as the lagging strand.

The leading strand can be extended by one primer alone, whereas the lagging strand needs a new primer for each of the short Okazaki fragments. The overall direction of the lagging strand will be 3' to 5', and that of the leading strand 5' to 3'. A protein called the sliding clamp holds the DNA polymerase in place as it continues to add nucleotides. The sliding clamp is a ring-shaped protein that binds to the DNA and holds the polymerase in place. Topoisomerase prevents the over-winding of the DNA double helix ahead of the replication fork as the DNA is opening up; it does so by causing temporary nicks in the DNA helix and then resealing it. As synthesis proceeds, the RNA primers are replaced by DNA. The primers are removed by the exonuclease activity of DNA pol I, and the gaps are filled in by deoxyribonucleotides. The nicks that remain between the newly synthesized DNA (that replaced the RNA primer) and the previously synthesized DNA are sealed by the enzyme DNA ligase that catalyzes the formation of phosphodiester linkage between the 3'-OH end of one nucleotide and the 5' phosphate end of the other fragment.



Once the chromosome has been completely replicated, the two DNA copies move into two different cells during cell division. The process of DNA replication can be summarized as follows.

DNA REPLICATION STEPS

1. DNA unwinds at the origin of replication.
2. Helicase opens up the DNA-forming replication forks; these are extended bidirectionally.
3. Single-strand binding proteins coat the DNA around the replication fork to prevent rewinding of the DNA.

4. Topoisomerase binds at the region ahead of the replication fork to prevent supercoiling.
5. Primase synthesizes RNA primers complementary to the DNA strand.
6. DNA polymerase starts adding nucleotides to the 3'-OH end of the primer.
7. Elongation of both the lagging and the leading strand continues.
8. RNA primers are removed by exonuclease activity.
9. Gaps are filled by DNA pol by adding dNTPs.
10. The gap between the two DNA fragments is sealed by DNA ligase, which helps in the formation of phosphodiester bonds.

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B.Sc. Biotechnology II Year

Molecular Genetics

SET-B

Time: 1:30 Hours **Max. Marks-30**

Q1. Answer the following questions in very short: **1×7=7**

1). On which plant did mendel work? Write its Botanical name?

Ans: Garden Pea (Pisum Sativum)

2). What are Mendel's factor called in modern Terminology?

Ans: Genes

3). What do the letters P, F1 and F2 represent in Heredity?

Ans: P- Parental generation, F1- First flial generation, F2- Second flial Generation

4). Which term have been used for the heredity units and by whom?

Ans: Term Factor: Correns, Elements: Mendel, Genes: Johenson

5). Write the genotype of man with blood group 'B'?

Ans: I^BI^B and I^Bi^B

6). Why mendel selected pea plant for his experiment?

Ans: Self pollinated as well as cross pollinated and have seven contrasting region

7). What is the test cross?

Ans: A cross between a hybrid organism and recessive parent.

Q2. Explain the following: **4×2=8**

i). Recessive Epistasis

Ans: **Epistasis** (which means "standing upon") occurs when the phenotype of one locus masks, or prevents, the phenotype of another locus. Thus, following a dihybrid cross fewer than the typical four phenotypic classes will be observed with epistasis. As we have already discussed, in the absence of epistasis, there are four phenotypic classes among the progeny of a dihybrid cross. The four phenotypic classes correspond to the genotypes: *A_B_, A_bb, aaB_,* and *aabb*. If either of the singly homozygous recessive genotypes (i.e. *A_bb* or *aaB_*) has the same phenotype as the double homozygous recessive (*aabb*), then a **9:3:4** phenotypic ratio will be obtained. The B locus encodes a gene for an important step in the production of

melanin. The dominant allele, *B* is more efficient at pigment production than the recessive *b* allele, thus *B_* hair appears black, and *bb* hair appears brown. A second locus, which we will call *E*, controls the deposition of melanin in the hairs. At least one functional *E* allele is required to deposit any pigment, whether it is black or brown. Thus, all retrievers that are *ee* fail to deposit any melanin (and so appear pale yellow), regardless of the genotype at the *B* locus.

ii). Garden Pea Plant as experimental Model

Ans: Mendel chose to experiment with peas because they possessed four important qualities:

1. Peas had been shown to be true-breeding (all offspring will have the same characteristic generation after generation).
2. Peas exhibit a variety of contrasting traits (purple vs. white flowers; round vs. wrinkled seeds).
3. The shape of the pea flower protected it from foreign pollen. Peas usually reproduce by self-pollination, in which pollen produced by a flower fertilizes eggs in the same flower.
4. Pea plants grow quickly and do not require much space.

The traits that Mendel studied are listed below:

- Form of ripe seed (R) – smooth or wrinkled
- Color of seed albumen (Y) – yellow or green
- Color of flower (P) – purple or white
- Form of ripe pods (I) – inflated or constricted
- Color of unripe pods (G) – green or yellow
- Position of flowers (A) – axial or terminal
- Length of stem (T) – tall or dwarf

Q3. Write short notes on: **4×2=8**

i). Types of RNA

Ans: **Types of RNA**

In both prokaryotes and eukaryotes, there are three main types of RNA – messenger RNA (mRNA), ribosomal RNA (rRNA), and transfer RNA (tRNA). These 3 types of RNA are discussed below:

Messenger RNA (mRNA)

mRNA accounts for just 5% of the total RNA in the cell. mRNA is the most heterogeneous of the 3 types of RNA in terms of both base sequence and size. It carries complimentary genetic code copied, from DNA during transcription, in the form of triplets of nucleotides called codons.

Each codon specifies a particular amino acid, though one amino acid may be coded for by many different codons. Although there are 64 possible codons or triplet bases in the genetic code, only 20 of them represent amino acids. There are also 3 stop codons, which indicate that ribosomes should cease protein generation by translation.

As part of post-transcriptional processing in eukaryotes, the 5' end of mRNA is capped with a guanosine triphosphate nucleotide, which helps in mRNA recognition during translation or protein synthesis. Similarly, the 3' end of an mRNA has a poly-A tail or multiple adenylate residues added to it, which prevents enzymatic degradation of mRNA. Both the 5' and 3' end of an mRNA imparts stability to the mRNA.

Ribosomal RNA (rRNA)

rRNAs are found in the ribosomes and account for 80% of the total RNA present in the cell. Ribosomes are composed of a large subunit called the 50S and a small subunit called the 30S, each of which is made up of its own specific rRNA molecules. Different rRNAs present in the ribosomes include small rRNAs and large rRNAs, which belong to the small and large subunits of the ribosome, respectively.

rRNAs combine with proteins and enzymes in the cytoplasm to form ribosomes, which act as the site of protein synthesis. These complex structures travel along the mRNA molecule during translation and facilitate the assembly of amino acids to form a polypeptide chain. They interact with tRNAs and other molecules that are crucial to protein synthesis.

In bacteria, the small and large rRNAs contain about 1500 and 3000 nucleotides, respectively, whereas in humans, they have about 1800 and 5000 nucleotides, respectively. However, the structure and function of ribosomes is largely similar across all species.

Transfer RNA (tRNA)

tRNA is the smallest of the 3 types of RNA, possessing around 75-95 nucleotides. tRNAs are an essential component of translation, where their main function is the transfer of amino acids during protein synthesis. Therefore, they are called transfer RNAs.

Each of the 20 amino acids has a specific tRNA that binds with it and transfers it to the growing polypeptide chain. tRNAs also act as adapters in the translation of the genetic sequence of mRNA into proteins. Thus, they are also called adapter molecules.

tRNAs have a clover leaf structure which is stabilized by strong hydrogen bonds between the nucleotides. They normally contain some unusual bases in addition to the usual 4, which are formed by methylation of the usual bases. Methyl guanine and methylcytosine are two examples of methylated bases.

Other types of RNA

Beyond the primary role of RNA in protein synthesis, several varieties of RNA exist that are involved in post-transcriptional modification, DNA replication, and gene regulation. Some forms of RNA are only found in particular forms of life, such as in eukaryotes or bacteria.

Small Nuclear RNA (snRNA)

snRNA is involved in the processing of pre-messenger RNA (pre-mRNA) into mature mRNA. They are very short, with an average length of only 150 nucleotides.

Regulatory RNAs

A number of types of RNA are involved in regulation of gene expression, including micro RNA (miRNA), small interfering RNA (siRNA) and antisense RNA (aRNA).

miRNA (21-22 nt) is found in eukaryotes, and acts through RNA interference (RNAi). miRNA can break down mRNA that it is complementary to, with the aid of enzymes. This can block the mRNA from being translated, or accelerate its degradation.

siRNA (20-25 nt) are often produced by breakdown of viral RNA, though there are also endogenous sources of siRNAs. They act similarly to miRNA. An mRNA may contain regulatory elements itself, such as riboswitches, in the 5' untranslated region or 3' untranslated region; these cis-regulatory elements regulate the activity of that mRNA.

Transfer-messenger RNA (tmRNA)

Found in many bacteria and plastids. tmRNA tag the proteins encoded by mRNAs that lack stop codons for degradation, and prevents the ribosome from stalling due to the missing stop codon.

Ribozymes (RNA enzymes)

RNAs are now known to adopt complex tertiary structures and act as biological catalysts. Such RNA enzymes are known as ribozymes, and they exhibit many of the features of a classical enzyme, such as an active site, a binding site for a substrate and a binding site for a cofactor, such as a metal ion.

One of the first ribozymes to be discovered was RNase P, a ribonuclease that is involved in generating tRNA molecules from larger, precursor RNAs. RNase P is composed of both RNA and protein; however, the RNA moiety alone is the catalyst.

Double-stranded RNA (dsRNA)

This type of RNA has two strands bound together, as with double stranded DNA. dsRNA forms the genetic material of some viruses.

ii). Incomplete Dominance

Ans: Incomplete Dominance Definition

Incomplete dominance is when a dominant allele, or form of a gene, does not completely mask the effects of a recessive allele, and the organism's resulting physical appearance shows a blending of both alleles. It is also called semi-dominance or partial dominance. One example is shown in roses. The allele for red color is dominant over the allele for white color, but heterozygous roses, which have both alleles, are pink. Note that this is different from codominance, which is when both alleles are expressed at the same time.

Mechanisms of Incomplete Dominance

Many genes show complete dominance. This means that if an individual is heterozygous for a particular gene, the dominant allele will completely mask the recessive allele. Many of the properties that the Austrian monk Gregor Mendel studied in his famous pea plants were controlled by genes that showed complete dominance. For example, the dominant flower color was purple, and the recessive color was white. Plants that were heterozygous were also purple, since purple was the dominant allele, even though they also had the white allele. A plant only had white flowers if it was homozygous for the recessive allele, which means that it had two copies of that allele. (This is also why two purple plants sometimes produced white ones; a proportion of the offspring received two recessive alleles.

The *ee* genotype is therefore said to be **epistatic** to both the B and b alleles, since the homozygous *ee* phenotype masks the phenotype of the B locus. The B/b locus is said to be **hypostatic** to the *ee* genotype. Because the masking allele is in this case is recessive, this is called **recessive epistasis**.

	EB	*Eb*	*eB*	*eb*
EB	*EEBB*	*EEBb*	*EeBB*	*EeBb*
Eb	*EEBb*	*EEbb*	*EeBb*	*Eebb*
eB	*EeBB*	*EeBb*	*eeBB*	*eeBb*
eb	*EeBb*	*Eebb*	*eeBb*	*eebb*

Q4. Write a detailed note on DNA replication in Eukaryotes? 7

Ans: DNA replication

- DNA replication is fundamental process occurring in all living organism to copy their DNA. The process is called replication in sense that each strand of ds DNA serve as template for reproduction of complementary strand.

General feature of DNA replication

- DNA replication is semi conservative
- It is bidirectional process
- It proceed from a specific point called origin
- It proceed in 5'-3' direction
- It occur with high degree of fidelity
- It is a multi-enzymatic process

DNA replication occurs by three steps

1. Initiation:

- Initiation complex formation
- Closed complex formation
- Open complex formation

2. Elongation:

- Leading strand synthesis
- Lagging strand synthesis

3. Termination

DNA replication in Eukaryotes

DNA replication in eukaryotes occur only in S-phase of cell cycle. However pre-initiation occur in G1 pahse. Due to sheer size of chromosome in eukaryotes, chromosome chromosome contains multiple origin of replication. ARS (autonomously replicating sequence) in case of yeast is origin for replication.

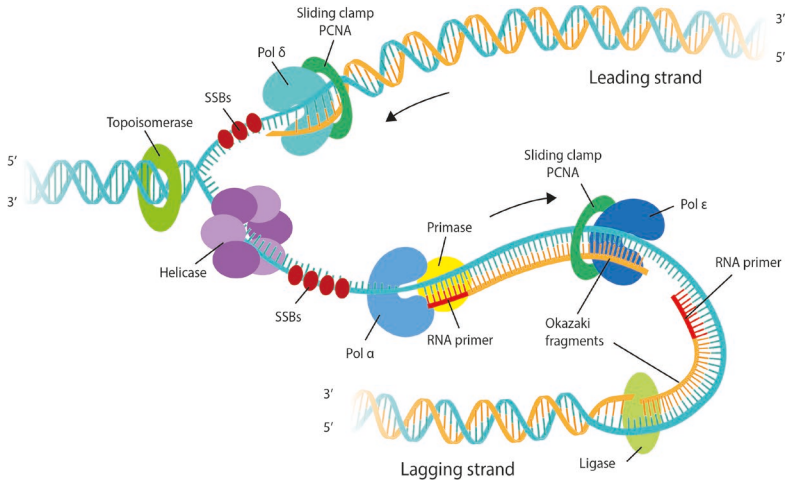
Steps in DNA replication

1. **Initiation**

- The first steps is the formation of pre-initiation replication complex (pre-RC). It occurs in two stage. 1st stage requires, there is no CDK activities. It occurs in early G1 phase.

It is known as licensing but licensed pre-RC cannot initiate replication at G1 phase. 2nd stage is binding of ORC (origin recognition complex).

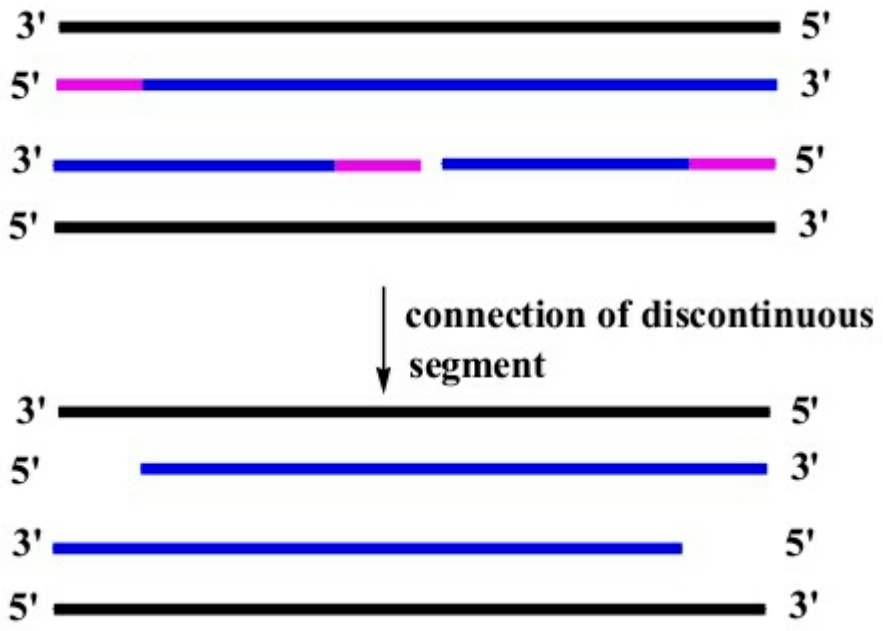
- The replication begins with binding of ORC to the origin. ORC is a hexamer of related protein and remains bounded even after DNA replication occurs. Furthermore ORC is analogue of prokaryotic dnaA protein.
- After binding of ORC to origin, cdc6/cdc18 and cdtl coordinate the loading of MEM (mini chromosome maintainance) to origin.
- MEM complex is thought to be major eukaryotic helicase.
- After binding of MEM complex to pre-RC, cdtl get displaced. Then DdK phosphorylates MEM, which activates its helicase activity. Again DdK and CdK recruit another protein called cdc45 which then recruit all the DNA replicating protein such that the origin get fired and replication begins.



2. Elongation:

- DNA polymerase δ synthesizes and adds dNTPs at 3' end of RNA primer.
- The leading and lagging strands are synthesized in the similar fashion as in prokaryotic DNA replication.

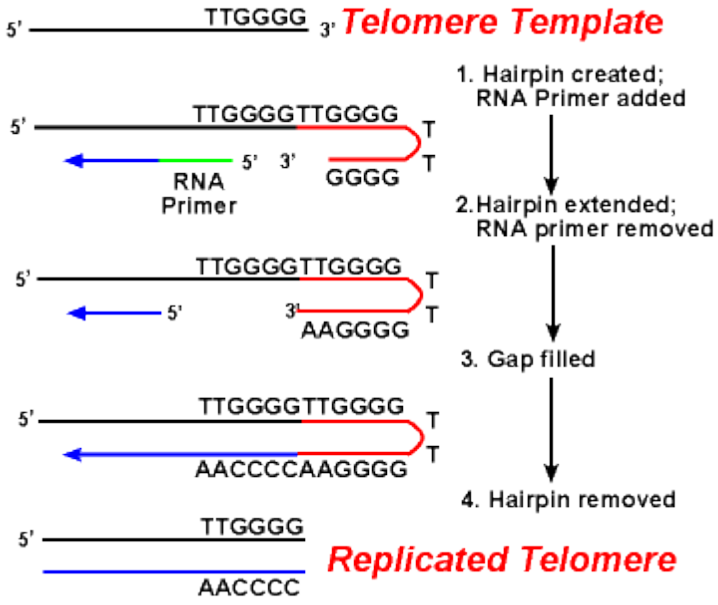
3. **Termination:**



- At the end of DNA replication the RNA primer are replaced by DNA by 5'-3'exonuclease and polymerase activity of DNA polymerase ε.
- Exonuclease activity of DNA polymerase removes the RNA primer and polymerase activity adds dNTPs at 3'-OH end preceding the primer.
- In case of bacteria, with circular genome, the replacement of RNA primer with DNA is not a problem because there is always a preceding 3'-OH in a circular DNA.
- But in eukaryotic organism with linear DNA, there is a problem. When RNA primer at 5' end of daughter strand is removed, there is not a preceding 3'-OH such that the DNA polymerase can use it to replace by DNA. So, at 5' end of each daughter strand there is a gap (missing DNA). This missing DNA cause loss of information contain in that region. This gap must be filled before next round of replication.
- For solving this end replication problem; studies have found that linear end of DNA called telomere has G:C rich repeats. This sequence is known as telomere sequence. This repeat of telomere sequence is different among different organisms. Telomere in human cell consists of repeats of TTAGGG/AATCCC. Each species has its own species specific telomere repeats. These telomere sequence donot codes anything but it is essential to fill the gap in daughter strand and maintain the integrity of DNA.

Telomere replication: end replication problem in Eukaryotic DNA

- There is an enzyme found in eukaryotic cell called telomerase.
- Telomerase is a DNA polymerase (RNA dependent DNA polymerase) which adds many copies of telomere sequence at 3'-OH end of template strand. Like other DNA polymerase, terlomerase also adds deoxyribonucleotide at 3'-OH end. Unlike other DNA polymerase, telomerase adds DNA at 3'-OH end of parent strand not at the daughter strand and also it synthesizes the same sequences over and over in absence of template strand.



- First telomerase binds to 3'-OH end of parent strand by hybridization between its AACCCCAAC RNA sequences and TTGGGG DNA sequences (telomere sequences of T*etrahymena*).
- The telomerase adds TTG at 3' end of parent strand. After adding TTG sequences, telomerase translocates along 5'-3' end of parent strand. Now the telomerase adds GGGTTG to 3' end by using its CCCAAC sequence. Again telomerase translocates and adds GGGTTA sequence. This process is continued for many time. The parent strand become more longer than daughter strand. Now RNA polymerase (PRIMASE) synthesize RNA primer by copying the parent strand in 5'-3' direction using telomere sequence as template.
- The DNA polymerase can now extend the primer in 5'-3' direction by adding deoxyribonucleotide to 3' end.

- The primer is now removed and it won’t be replaced because it is an extra sequence added by copying telomere sequence.
- Finally the integrity of daughter strand is maintained.