

17. DNA REPLICATION

The process by which a DNA molecule makes its identical copies is called DNA replication.

Modes of DNA Replication:

There are three possible modes of DNA replication (see Fig.)

- 1. Dispersive:** In dispersive mode of replication, the old DNA molecule would break into several pieces, each fragment would replicate and the old and new segments would recombine randomly to yield the progeny DNA molecule. Each progeny molecule would have both old and new segments along its length.
- 2. Conservative:** According to conservative scheme, the two newly synthesized strands following the replication of a DNA molecule would associate to form one double helix, while the other two old strands would remain together as one double helix.
- 3. Semi conservative:** In this model of DNA replication, each newly synthesized strand of DNA would remain associated with old strand against which it was synthesized. Thus, each progeny DNA molecule would consist of one old and one newly synthesized strand.

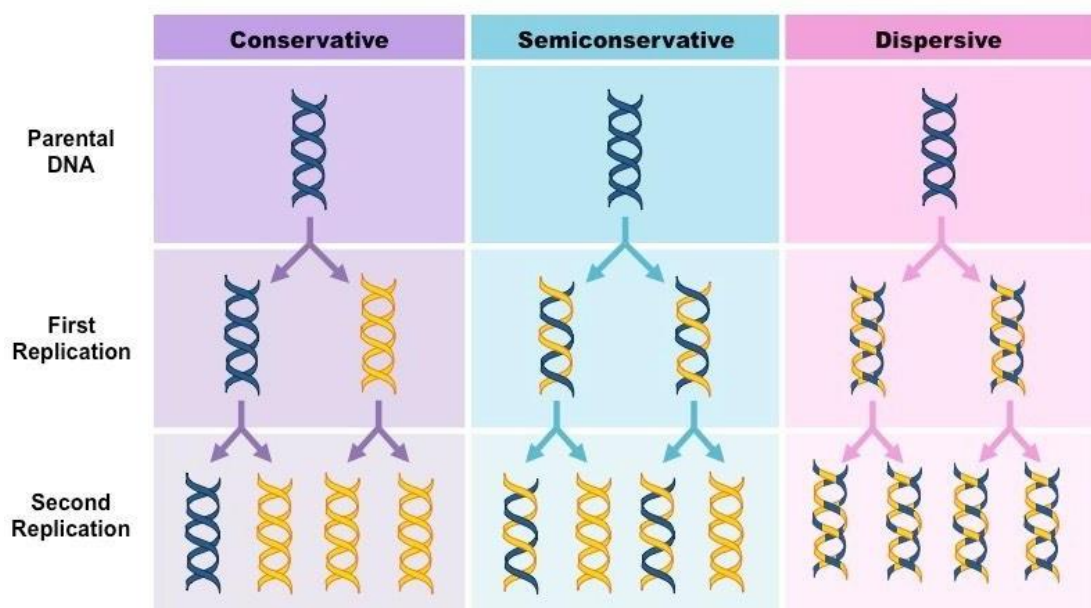


Fig.: Three different models of DNA replication

Evidence for Semi conservative replication:

The experiment conducted by Matthew Meselson and Franklin Stahl in 1958 on *E. coli* provided a conclusive proof that replication of DNA is by semi conservative model. Meselson and Stahl labelled DNA of *E. coli* bacteria with heavy nitrogen i.e. ^{15}N by growing them on a medium containing ^{15}N for many generations to replace the normal nitrogen (^{14}N). The density of normal and heavy nitrogen differs. The ^{14}N is lighter (1.710 g/cm³) than ^{15}N (1.724 g/cm³). It is possible to detect such minute differences in density through density gradient centrifugation. Distinct bands are formed in the centrifuge tube for different density DNA. DNA extracts of *E. coli* with ^{15}N gave a characteristic heavy band at one end of a tube that had been centrifuged at a high speed in an ultra-centrifuge. These labelled cells were then grown on a normal unlabelled media containing ^{14}N for one generation. DNA was again extracted and processed and it was found to consist of a hybrid DNA containing both ^{14}N and ^{15}N at the same time. This indicated that the DNA had not replicated in two separate labelled and unlabelled forms. The next generation of growth on unlabelled DNA was found to be in amounts equal to the partially labelled hybrid DNA. Additional generation of growth on unlabelled media gave a relative increase in the amount of unlabelled DNA. After two generations, half the DNA was with intermediate density and half with light bands which further confirm semi-conservative mode of DNA replication. After third generation, $\frac{3}{4}$ DNA was found with ^{14}N and $\frac{1}{4}$ with hybrid nitrogen ($^{14}\text{N}+^{15}\text{N}$). When the hybrid DNA was denatured by heating up to 100°C it was found to produce two separate single strands and in the ultracentrifuge density gradient, it was observed to form two separate bands; one band containing ^{15}N and the other ^{14}N . Thus, it was concluded that DNA replication was by semi-conservative mode. The major objection put forth for the semi-conservative replication was that the DNA molecule must unwind a number of times (1/10 of the total number of nucleotides) which cannot be accomplished without breaking within a short span of time say two minutes. Cairns (1968) provided evidence for this in his experiments with radio-active labelled *E. coli* chromosomes. The *E. coli* chromosome is a double stranded circular chromosome. It was shown that the two circular component strands separate during replication with each strand duplicating individually producing a θ shaped structures during replication. This indicates that unwinding and replication proceed simultaneously.

Method of semi-conservative replication:

DNA replication was found to begin at various initiation points, called origin of replication, and proceed bi-directionally. Two enzymes, DNA gyrase and DNA helicase induce unwinding of complementary strands of DNA. Single-strand DNA binding (SSB) proteins bind to the single-stranded DNA, stabilizing it and preventing it from reannealing. An enzyme, primase, initiates replication by synthesizing the primer. DNA polymerases synthesize the complementary strand by progressively adding deoxyribonucleotides. The DNA replication always proceeds in 5'→3' direction. During replication one strand of DNA can replicate continuously and the other strand discontinuously or in pieces. The continuously replicating strand is known as leading strand and the discontinuously replicating strand is known as lagging strand. The replication of lagging strand generates small polynucleotide fragments called 'Okazaki fragments' which are later joined together by the enzyme, DNA ligase (see Fig.)

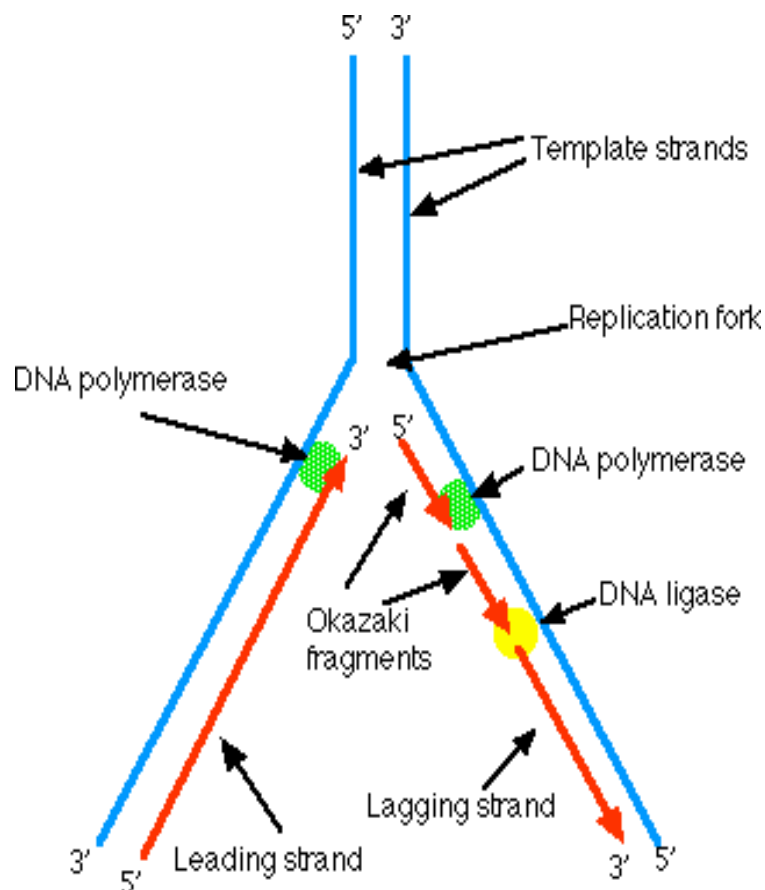
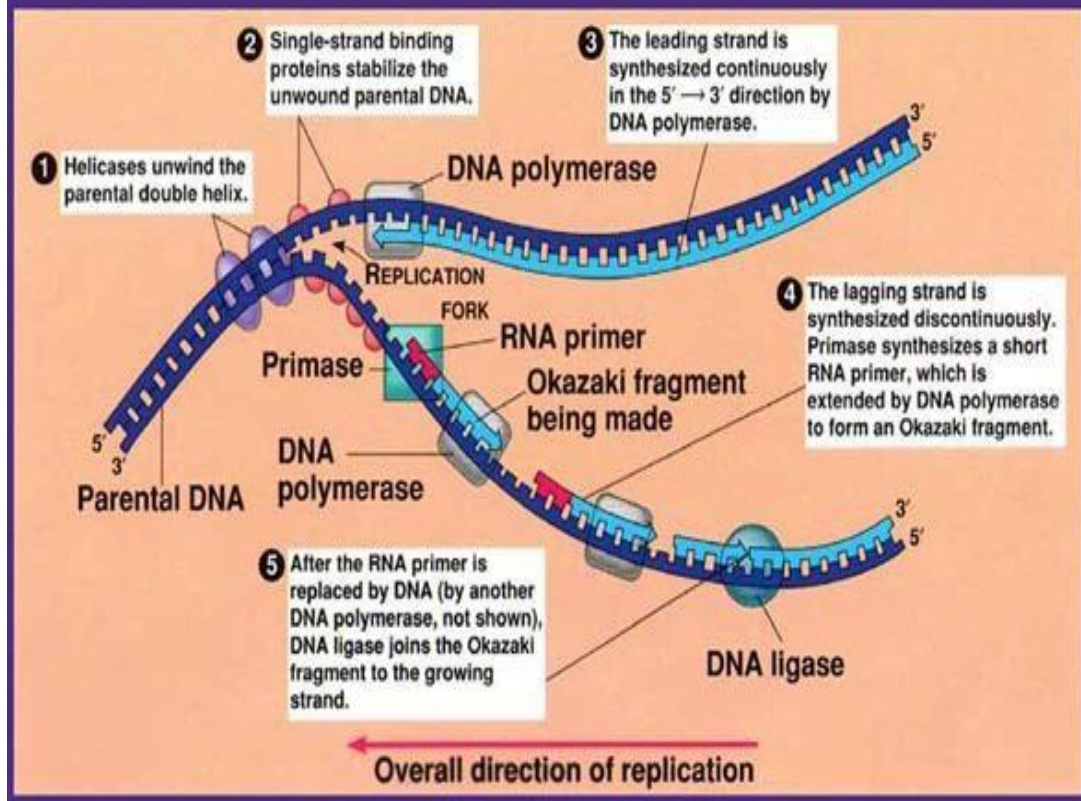


Fig: Generalised model of semi-conservative replication of DNA showing different enzymes involved during replication.

A SUMMARY OF DNA REPLICATION



RNA (Ribose nucleic acid)

Structure of RNA: RNA like DNA is a polynucleotide. RNA nucleotides have ribose sugar, which participate in the formation of sugar phosphate backbone of RNA. Thymine is absent and is replaced by Uracil. Usually RNA is a single stranded structure. Single stranded RNA is the genetic material in most plant viruses Eg. TMV. Double stranded RNA is also found to be the genetic material in some organisms. Eg.: Plant wound and tumour viruses. RNA performs non-genetic function. There are three main types or forms of RNA.

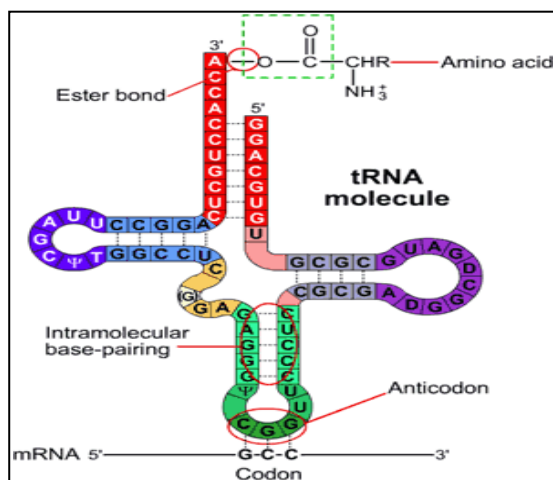
1. **messenger RNA (m-RNA):** It constitutes about 5-10% of the total cellular RNA. It is a single stranded base for base complimentary copy of one of the DNA strands of a gene. It provides the information for the amino acid sequence of the polypeptide specified by that gene. Generally, a single prokaryotic mRNA molecule codes for more than one polypeptide; such a mRNA is known as polycistronic mRNA. All eukaryotic mRNAs are monocistronic i.e. coding for a single polypeptide specified by a single cistron.
2. **ribosomal RNA (r - RNA):** Occur in association with proteins and is

organized into special bodies of about 200^0A diameter called ribosomes. The size of ribosomes is expressed in terms of 'S' units, based on the rate of sedimentation in an ultracentrifuge. It constitutes about 80% of the total cellular RNA. The function of rRNA is binding of mRNA and tRNA to ribosomes.

- 3. transfer RNA (t- RNA):** It is also known as soluble RNA(sRNA). It constitutes about 10-15% of total RNA of the cell. It is a class of RNA which is of small size of 3S type and generally have 70 – 80 nucleotides. The longest t – RNA has 87 nucleotides. Its main function is to carry various types of amino acids and attach them to mRNA template for synthesis of protein. Each t– RNA species has a specific anticodon which base pairs with the appropriate m – RNA codon (see Fig.).

The nucleotide sequence of the first tRNA (yeast alanyl tRNA) was determined by Robert Holley (1965), who proposed the clover leaf model of secondary structure of tRNA. Each tRNA is specific for each amino acid. tRNA molecule contains the sequence of CCA at the 3'end, which is called

amino acid attachment site. At the CCA end, it is joined to the single amino acid molecule for which that tRNA is specific. For example, a tRNA molecule specific for lysine cannot bind to the arginine. tRNA consists of three loops; (a) DHU-loop or D-loop (aminoacyl recognition region), (b) anticodon loop and (c) thymine loop (ribosome attachment region). Anticodon loop contains a short sequence of bases, which permits temporary complementary pairing with the codons of mRNA.



IMPORTANT DIFFERENCES BETWEEN DNA AND RNA

SL. No.	Particulars	DNA	RNA
1	Strands.	Usually two, rarely one.	Usually one, rarely two.
2	Sugar.	Deoxyribose.	Ribose.
3	Bases.	Adenine, guanine, cytosine and thymine.	Adenine, guanine, cytosine and uracil.
4	Pairing.	AT and GC.	AU and GC.
5	Location.	Mostly in chromosomes, some in mitochondria and chloroplasts.	In chromosomes and ribosomes.
6	Replication.	Self-replicating.	Formed from DNA. Self replication only in some viruses.
7	Size.	Contains up to 4.3 million nucleotides.	Contains up to 12,000 nucleotides.
8	Function,	Genetic role.	Protein synthesis, genetic in some viruses.
9	Types.	There are several forms of DNA.	Three types, viz., mRNA, tRNA and rRNA.

CENTRAL DOGMA

(Dogma = Principle)

In 1958 Crick proposed that the information present in DNA (in the form of base sequences) is transferred to RNA and then from RNA it is transferred to proteins in the form of amino acid sequence and that this information does not flow in the reverse direction i.e. from protein to RNA to DNA. This relationship between DNA, RNA and protein is known as central dogma. DNA molecules however provide the information for their own replication.



However, in cells infected by certain RNA viruses Eg: TMV, the viral RNA produces new copies of itself with the help of RNA replicase. In some viruses, the DNA is synthesized from RNA in the presence of an enzyme, reverse transcriptase. This process is known as reverse transcription. This was first reported by Temin and Baltimore in 1970.

GENETIC CODE

There are 20 amino acids involved in protein synthesis and there are only 4 bases in the DNA coding for all the amino acids. Nirenberg who has found that three bases code for one amino acid and thus it is possible to code for all the amino acids.

The number and the sequence of bases in mRNA specifying an amino acid is known as a codon. While the set of bases in tRNA which base pair with a codon of mRNA is known as anti-codon. The sequence of bases in an anti-codon is exactly the opposite of that present in the codon. Since the codon and anticodon segments run anti-parallel to each other when they base pair, Codons are written in 5'→3' direction, whereas anticodons are usually written in 3'→5' direction. The set of all the codons that specify the 20 amino acids is termed as the genetic code.

It is clear from various experiments that a sequence of three nucleotides in mRNA code for an amino acid and hence the genetic code is called the triplet code. Three codons, UAA, UAG and UGA do not code for any amino acid and hence they are called nonsense codons. These codons are also known as stop codons or termination codons as they provide stop signal for termination. The codon i.e. AUG is known as start codon or initiation codon as it starts the synthesis of polypeptide chain.

This codon also codes for amino acid methionine. In eukaryotes, the starting amino acid is methionine, while in prokaryotes it is N-formyl methionine.

Nature / Properties of Genetic code; There are several important features or properties of a genetic code.

1. **The code is triplet:** The triplet code was first suggested by Gammow in 1954. In a triplet code, three RNA bases code for one amino acid.
2. **The code is universal:** The same genetic code is applicable to all forms of organisms from microbes to human beings.
3. **The code is comma less:** The genetic code is without a comma or break. The codons are continuous and there are no breaks between the codons. A change or deletion of a single base in the code will alter the entire sequence of amino acid to be synthesized.
4. **The code is non-overlapping:** Three nucleotides or bases code for one amino acid and six bases will code for two amino acids. In a non-overlapping code, one base or letter is read only once.
5. **The code is non-ambiguous:** Out of the 64 codons, 61 code for 20 different amino acid, while 3 are nonsense codons. None of the codons code for more than one amino acid.
6. **The code is degenerate:** In most cases, several codons code for the same amino acid. Only two amino acids, viz., tryptophan and methionine are coded by one codon each. Nine amino acids are coded by two codons each, one amino acid (isoleucine) by three codons each, five amino acids by four codons each and three amino acids by six codons each. This type of redundancy of genetic code is called degeneracy of genetic code. Such a system provides protection to the organisms against many harmful mutations. If one base of codon is mutated, there are other codons, which will code for the same amino acid and thus there will be no alteration in polypeptide chain.
7. **The code has polarity:** The code has a definite direction for reading of message, which is referred to as polarity. Reading of code in opposite direction will naturally specify for another amino acid example: GUC codes for valine, if reversed, CUG codes for Leucine.

Crick postulated the wobble hypothesis in 1966 to account for the degeneracy of genetic code.

Wobble hypothesis: The first two bases on the codon are usually the important ones, the third base may be in some cases one of the four bases and the triplet would still code for the same amino acid as long as the first two bases are the same and have the same sequence. This concept is known as the Wobble hypothesis.

Exceptions to the Universality of the genetic code: Some exceptions to the universality of the genetic code are known in ciliates (unicellular protozoa) and mitochondria. These exceptions generally involve, initiation or termination codons (and result from either production or the absence of tRNAs representing certain codons). For example, ciliates read UAA and UAG as glutamine instead of termination signals. Similarly, in mitochondria, UGA does not specify termination, it means the same as UGG and codes for tryptophan. This change is found in yeast, invertebrates and vertebrates but not in plants