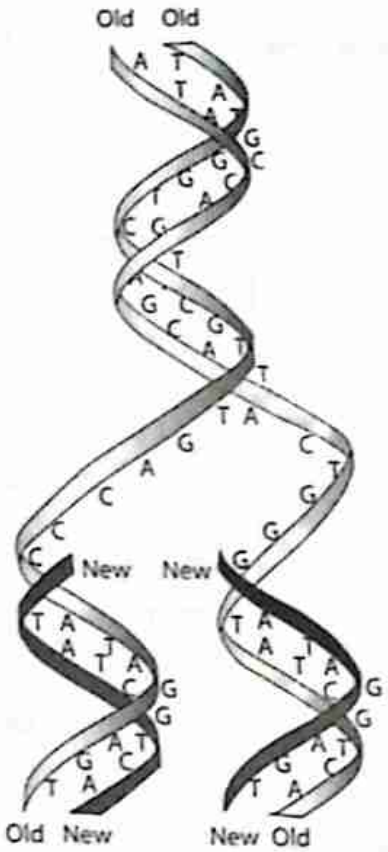

Figure 3 Watson–Crick model for semi-conservative DNA replication.

Mechanism of DNA Replication

The entire process of DNA replication involves following common steps (Figure 4):

1. **Recognition of the initiation point:** First, DNA helix unwinds by the enzyme helicase which use the energy of ATP and replication of DNA begin at a specific point, called initiation point or origin where replication fork begins.
2. **Unwinding of DNA:** The unwinding proteins bind to the nicked strand of the duplex and separate the two strands at DNA duplex. Topoisomerase (gyrase is a type of topoisomerase in *E. coli*) helps in unwinding of DNA.
3. **Single stranded binding protein (SSBP):** These proteins help in keeping DNA in single stranded position and also known as helix destabilizing protein (HDP).
4. **RNA priming:** The DNA directed RNA polymerase now synthesizes the primer strands of RNA (RNA primer). The priming RNA strands are complementary to the two strands of DNA and are formed of 50 to 100 nucleotides.
5. **Formation of DNA using RNA primers:** The new strands of DNA are formed in the 5′ → 3′ direction from the 3′→ 5′ template DNA by the addition of deoxyribonucleotides to the 3′ end of primer RNA.

   Addition of nucleotide is done by DNA polymerase III. The leading strand of DNA is synthesized continuously in 5′→3′ direction as one piece. The lagging strand of DNA is synthesized discontinuously in its opposite direction in short segments. These segments are called Okazaki fragments.
6. **Excision of RNA primers:** Once a small segment of an Okazaki fragment has been formed. The RNA primers are removed from the 5′ by the action of 5′→3′ exonuclease activity of DNA polymerase I.
7. **Joining of Okazaki fragments:** The gaps left between Okazaki fragments are filled with complimentary deoxyribonucleotide residues by DNA polymerase I. Finally, the adjacent 5′ and 3′ ends are joined by DNA ligase.

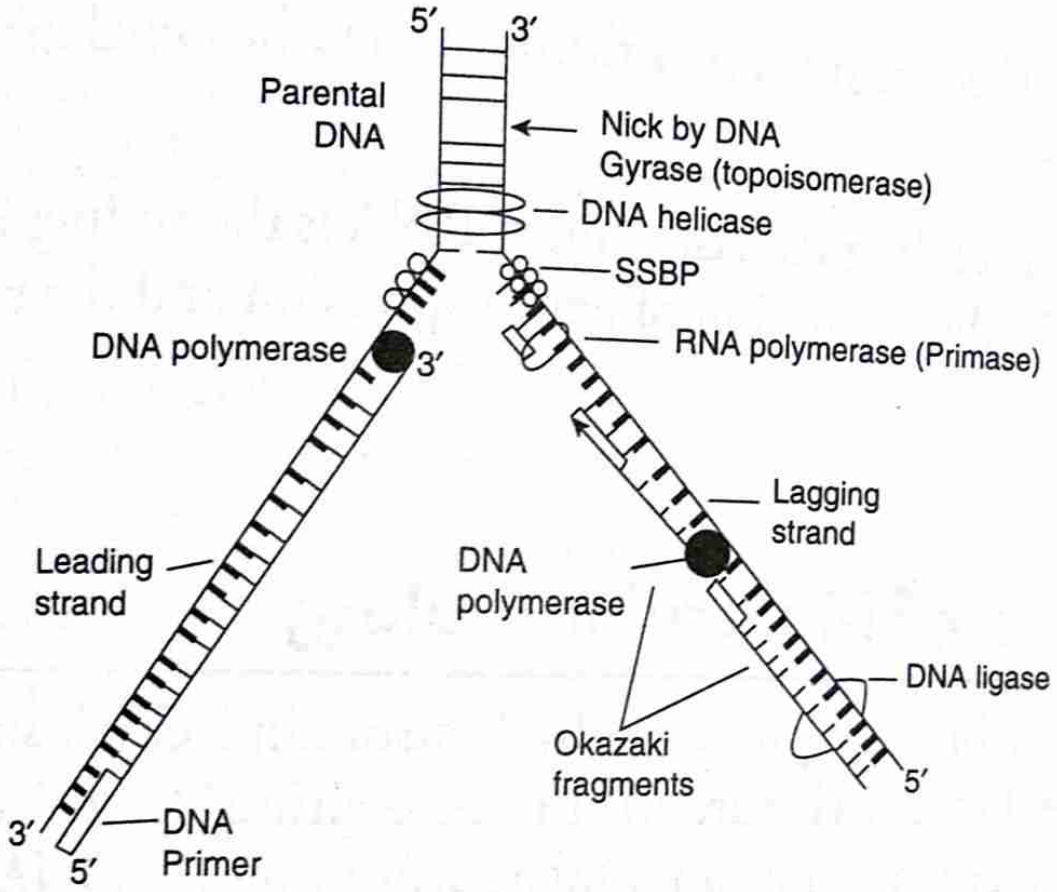

Figure 4 Replication of DNA.

7.4 Central Dogma of Molecular Biology

The central dogma in molecular biology proposed by Watson and Crick stated that the flow of information is from DNA → RNA → Protein (Figure 6). In some viruses (retroviruses) and related transposable elements (retrotransposons), RNA is used as a template to synthesize DNA. This process is called reverse transcription. It is catalyzed by the enzyme reverse transcriptase.

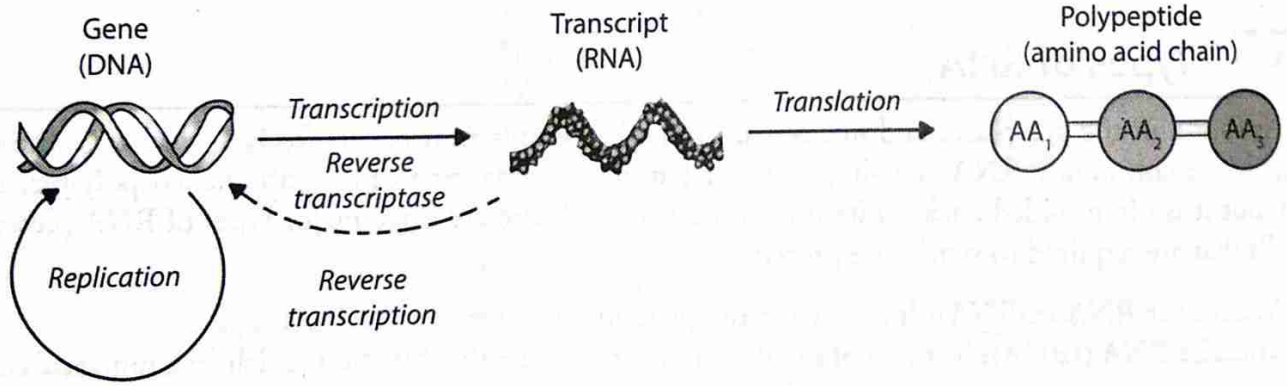

Figure 6 Central dogma in molecular biology.

7.5 Transcription

In 1961, François Jacob, Jacques Monod and Matthew Meselson discovered messenger RNA (mRNA). The mRNA is the complimentary copy of one of the two DNA strands that constitute the gene. However, thymine is replaced with uracil to pair with adenine. It serves as the intermediate between a gene and its polypeptide. The process by which RNA is synthesized from its DNA template is known as transcription. It is generally considered to be the first step in gene expression. The mechanism of transcription is basically similar to that of replication but it differs from it with respect to the following:

1. The precursors are ribonucleoside triphosphates rather than deoxyribonucleoside triphosphates.
2. In replication, once the process has initiated, the entire DNA of the organism is duplicated, however, in transcription, only a particular DNA segment is transcribed.
3. Only one strand of DNA is used as a template for the synthesis of a complementary RNA chain in any given region.
4. RNA chains can be initiated without any requirement for a pre-existing primer strand.

The reasons why only one DNA strand and not both are copied are as follows:

1. RNA molecule with two different sequences will be coded if both strands act as template. This would result in two different sequences of amino acids on transcription. This would disrupt the machinery of transfer of genetic information because one DNA segment will be coding for two proteins.

2. If both DNA strands code, two RNA strands will be produced at the same time which will be complementary to each other. As a result, they would form double stranded RNA which could not be translated into protein. This would defeat the purpose of transcription.

Process of Transcription

Although the basic features of transcription are the same in both prokaryotes and eukaryotes, but some features such as the promoter sequences are different.

Transcription in Prokaryotes

The DNA-dependent RNA polymerase of prokaryotes catalyzes synthesis of all types of RNA. The process of transcription can be divided into three stages (Figure 7):

1. **Initiation of a new RNA chain:** The three steps in RNA chain initiation are:
   (a) Recognition and binding of the RNA polymerase holoenzyme to a promoter region in DNA by the sigma subunit (initiation factor) of RNA polymerase. Localized unwinding of the two strands of DNA by RNA polymerase, which is an essential prerequisite to the synthesis of a new RNA chain. This provides a template strand that can base-pair with incoming ribonucleotides.
   (b) Formation of phosphodiester bonds between the first few ribonucleotides in the nascent RNA chain.
2. **Elongation of the chain:** The elongation phase of RNA synthesis is then carried out by the core enzyme. The covalent extension of RNA chains takes place within this transcription bubble that is formed by a locally unwound segment of DNA.
3. **Termination of transcription and release of the nascent RNA molecule:** Termination of RNA chains occurs when the RNA polymerase comes across the termination signal, which is a nucleotide sequence that specifies RNA chain termination. At this point, the transcription complex dissociates, releasing the nascent RNA molecule. It can rho dependent or independent.

Transcription in Eukaryotes

In eukaryotes, multiple RNA polymerases are required instead of only one as in prokaryotes. Besides, the control sequences are much more complicated in eukaryotes than in prokaryotes (Figure 7). The additional complexities in case of transcription in eukaryotes are discussed as follows:

1. **Initiation:** The conserved sequence that is closest to the start site of transcription is called TATA box. It is centered at position -30. An initiation complex is formed by the transcription factors (TBP, TFII) at this site before RNA polymerase binds and initiates transcription.
2. **Elongation:** When the growing RNA chains are only about 30 nucleotides long, the 5′ ends of eukaryotic pre-mRNAs are modified by the addition of 7-methyl guanosine (7-MG) triphosphate caps (a process known as capping. These caps have two main functions:
   (a) They are recognized by protein factors involved in the initiation of translation.
   (b) They protect the growing RNA chains from degradation by nucleases.
3. **Termination:** Transcription proceeds 1000 to 2000 nucleotides beyond the site that will become the 3′ terminus. Then the distal segment is removed by endonucleolytic cleavage. Then, the enzyme poly (A) polymerase adds poly(A) tails made up of adenylate residues to the 3′ ends of the transcripts. It is also known as tailing or polyadenylation. In eukaryotes, the genes contain non-coding sequences (introns) intervening between coding sequences (exons). Eukaryotic mRNA transcripts can therefore, undergo extensive post-transcriptional processing while still in the nucleus. These introns are removed from the RNA transcripts by a mechanism called RNA splicing. Now, the spliced mRNA can be exported to the cytoplasm for translation.

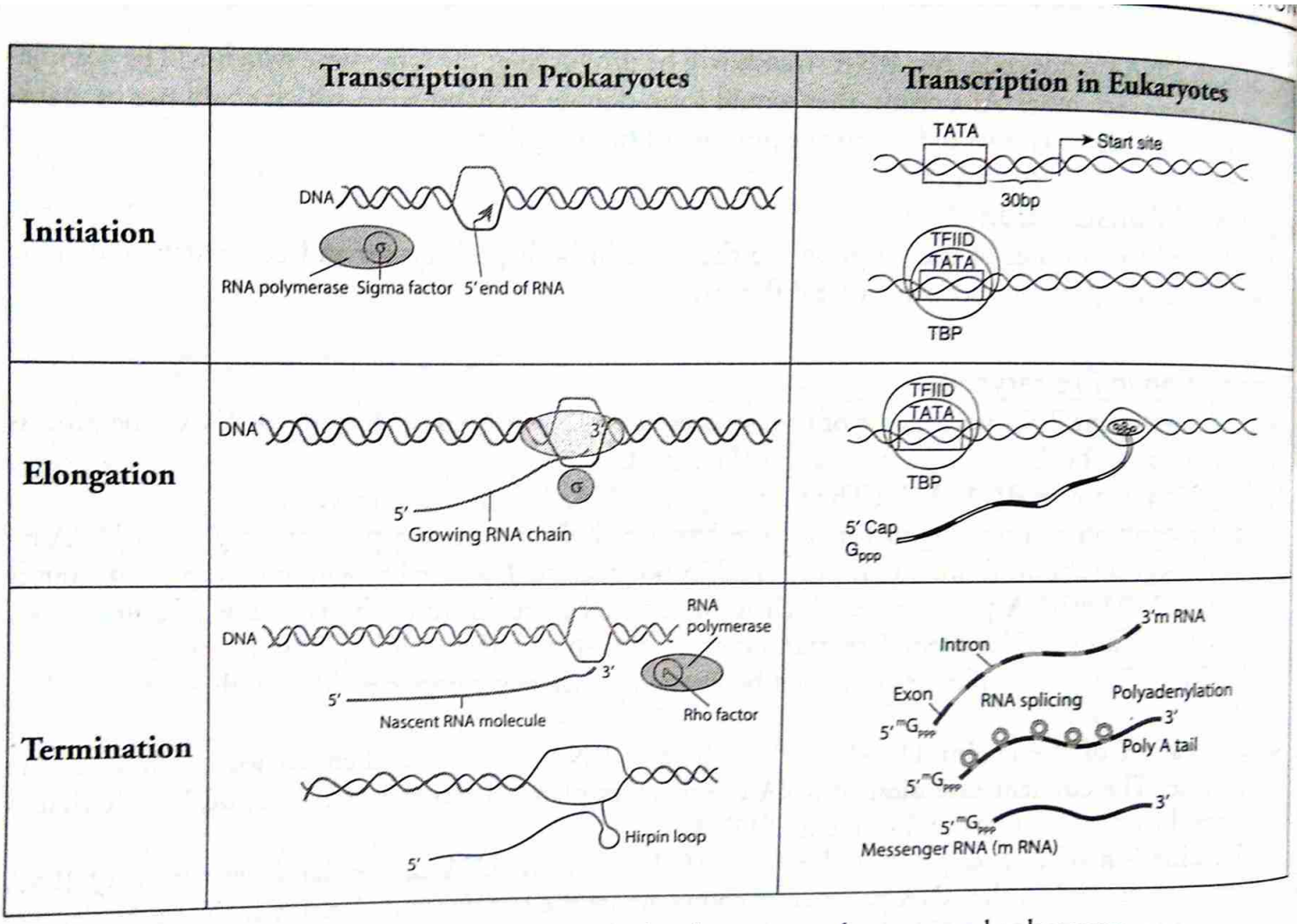

Figure 7 Comparison of transcription between prokaryotes and eukaryotes.

7.6 Genetic Code

From the determination of structure of DNA, it became clear that the sequence of amino acids in the polypeptide was determined by the nucleotides present in the DNA or gene. Genetic code thus refers to the manner in which the nucleotide sequences of DNA encode the information for making proteins.

Salient Features of the Genetic Code

1. **Triplet:** A single amino acid is specified by a sequence of three nucleotides in mRNA, that is, called codon. Due to triplet nature, it consists of 64 codons (Figure 8).
2. **Universal:** A codon specifies the same amino acid in all organisms from viruses to human beings.
3. **Commaless:** There is no pause, so it reads continuously.
4. **Non-overlapping:** No overlapping between adjacent nucleotide.
5. **Initiation codon:** The synthesis of polypeptide chain initiated by initiation codon that is AUG that codes for methionine.
6. **Termination codon:** Termination is done by terminator codons UAA, UGA or UAG which do not code for any amino acid. These are also called nonsense codon.
7. **Degenerate:** A single amino acid may be specified by many codons, that is, called degeneracy. Degeneracy is due to the last base in codon, which is known as Wobble base. Thus, first two codons are more important in determining the amino acid and third one is different without affecting the coding this is known as Wobble hypothesis, which establishes an economy of tRNA molecule and put forward by Crick.

Steps in Translation

The sequence of RNA which has a start codon on side and the stop codon on the other and codes for polypeptide is called the translational unit of the mRNA. Some sequences are not translated in the mRNA. They are present before the start codon at the 5′-end and after the stop codon at the 3′-end. They are known as untranslated regions (UTRs). However, they aid efficient translation. The process of translation is divided into initiation, elongation and termination of polypeptide chains.

The steps involved in translation are:

1. An mRNA molecule binds to a small ribosomal subunit. The anticodon UAC on a tRNA molecule binds with the mRNA "start" codon, AUG, at the P site.
2. A large ribosomal subunit binds to the small subunit, forming a functional ribosome.
3. A tRNA molecule binds to the next mRNA codon, at the A site.
4. A bond is formed between the amino acids of the two tRNA molecules.
5. A bond between the P site amino acid and its tRNA molecule breaks, and the tRNA molecule at the P site falls away. The ribsome moves along the mRNA molecule. So the bound tRNA molecule at the A site moves to the P site. Another tRNA molecule binds to the next mRNA codeon, at the A site.
6. Elongation steps repeat 2–3 times until the next mRNA codon is a stop codon. The process then moves to 7.
7. When the next mRNA codon is a stop codon (in this example, UAA), a release factor binds to the mRNA. This triggers the release of the polypeptide molecule. The final tRNA molecule and the release factor are released.
8. The ribosome disassociates into its large and small subunits. The process can then start again.