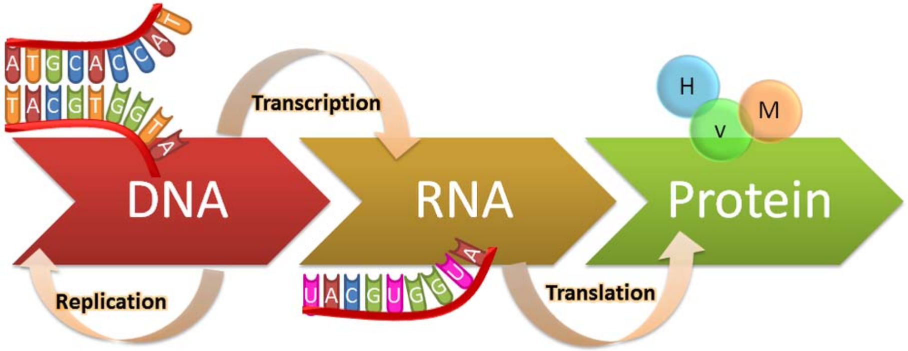
Transcription of Genes



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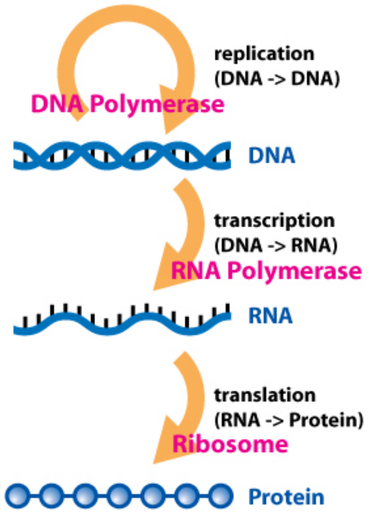
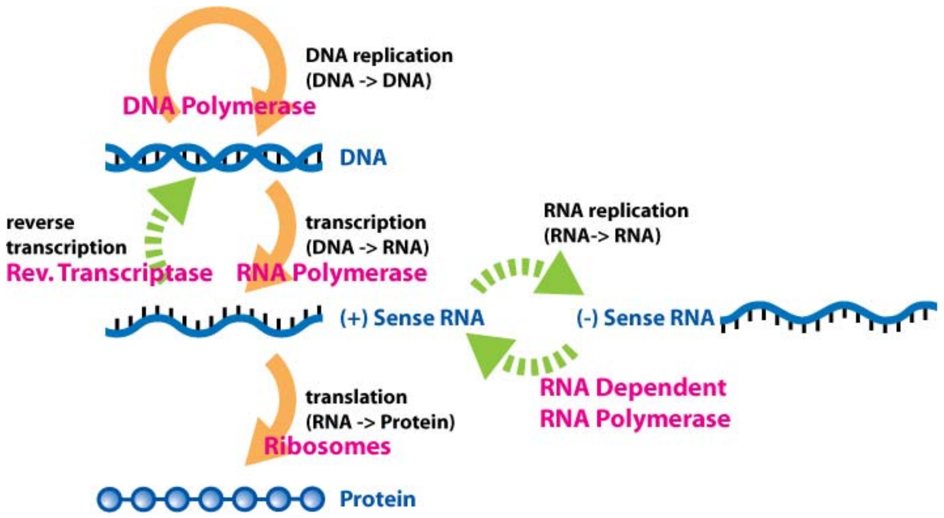
The Central Dogma of Molecular Biology



- Central dogma describes the flow of genetic information in living organisms
- Gene expression: is the process by which information from a gene is used in the synthesis of functional gene products (proteins or functional RNAs)
- Transcription is the first step in gene expression

The Central Dogma of Molecular Biology

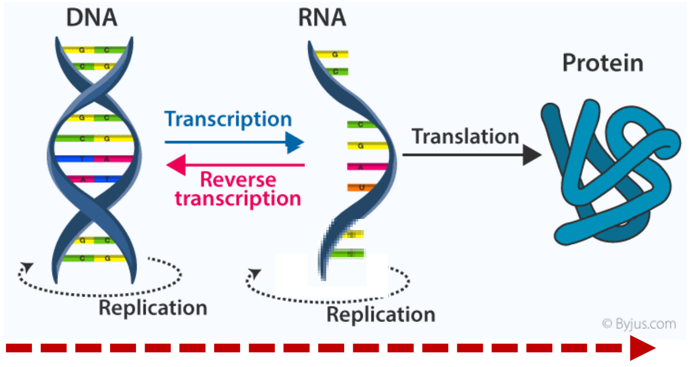


- The first one who used the term Central Dogma is the British molecular biologists **"Crick, 1957"** and he said that: DNA makes RNA which makes protein but once protein is synthesized, it can't flow back to nucleic acids

Classes of information transfer

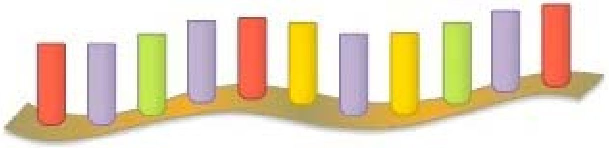
General	Special	Unknown
DNA → DNA	RNA → DNA	protein → DNA
DNA → RNA	RNA → RNA	protein → RNA
RNA → protein	DNA → protein	protein → protein

- General transfer
- Specific transfer
- Unknown transfer



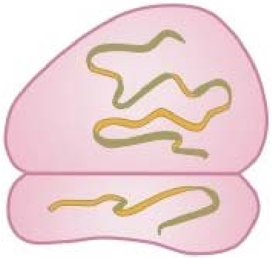
Function of different RNA Molecules

- There are different types of RNA molecules:
  1. Messenger RNA **(mRNA):** it is produced from the transcription of protein-coding genes. In eukaryotic cells, pre-mRNA (primary transcript) is modified to mature mRNA. In prokaryotes, single mRNA (bicistronic / polycistronic transcript) codes for different proteins



Messenger RNA (mRNA)

  2. Ribosomal RNA **(rRNA):** are specialized RNA molecules synthesized in the nucleolus. In the cytoplasm, they bind proteins to form ribosomes (the machinery to synthesize proteins)

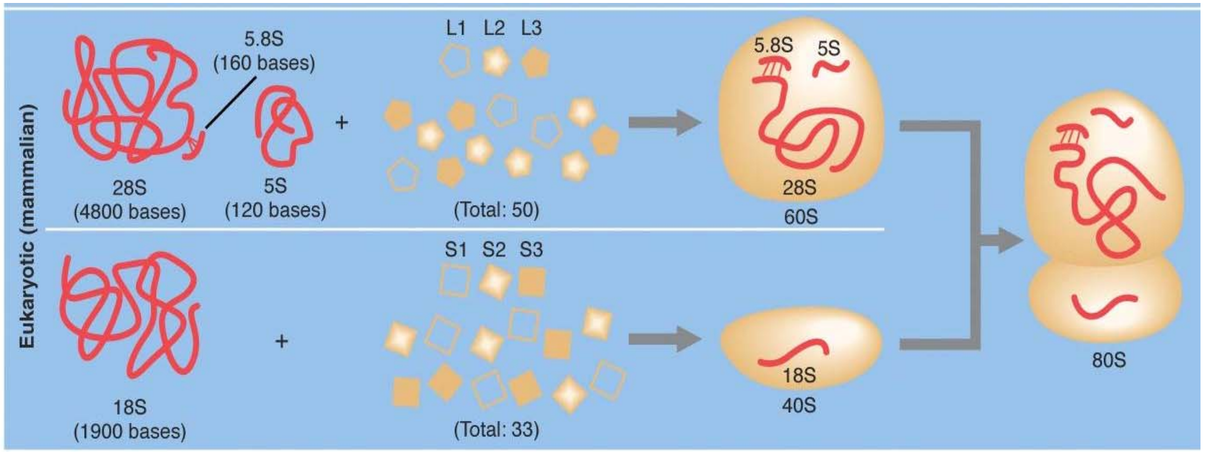


Ribosomal RNA (rRNA)

Function of different RNA Molecules

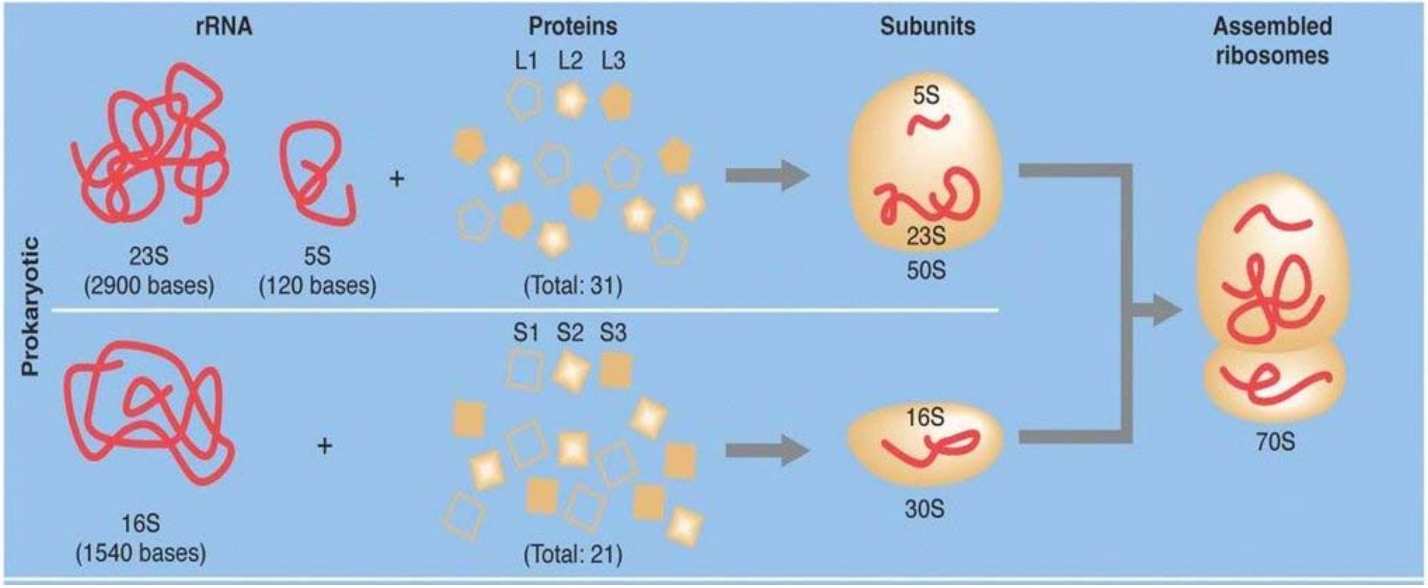
- Ribosome consists of small and large subunits. In eukaryotes (80S) the small subunit (40S) consists of 18S rRNA and 33 proteins while the large subunit (60S) contains: 5S rRNA, 5.8S rRNA, 28S rRNA and 50 proteins

S stands for Svedberg unit (a measure of sedimentation rate depending on particle size)



Function of different RNA Molecules

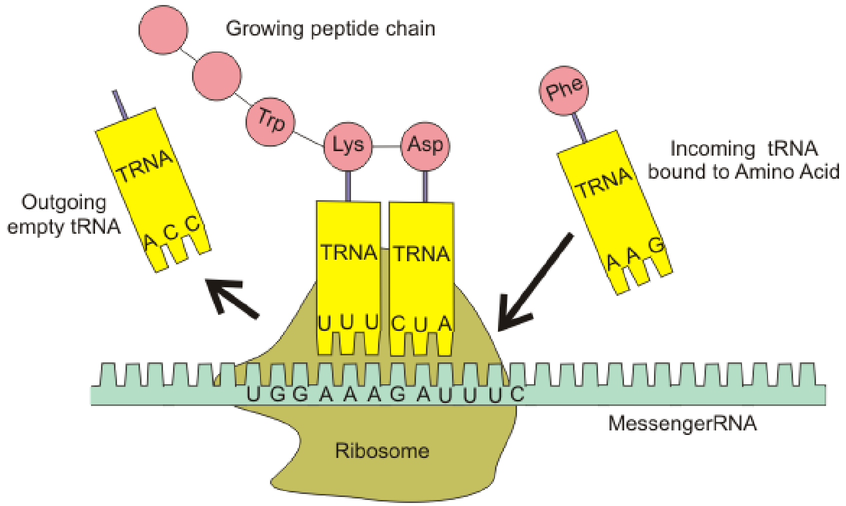
- In prokaryotes like bacteria, the ribosome (70S) consists of the small subunit (30S) and the large subunit (50S)



Function of different RNA Molecules

3. Transfer RNA **(tRNA):** are specialized molecules (adaptors) that collect the proper amino acid, bring it to the ribosome and attach it to the growing polypeptide chain

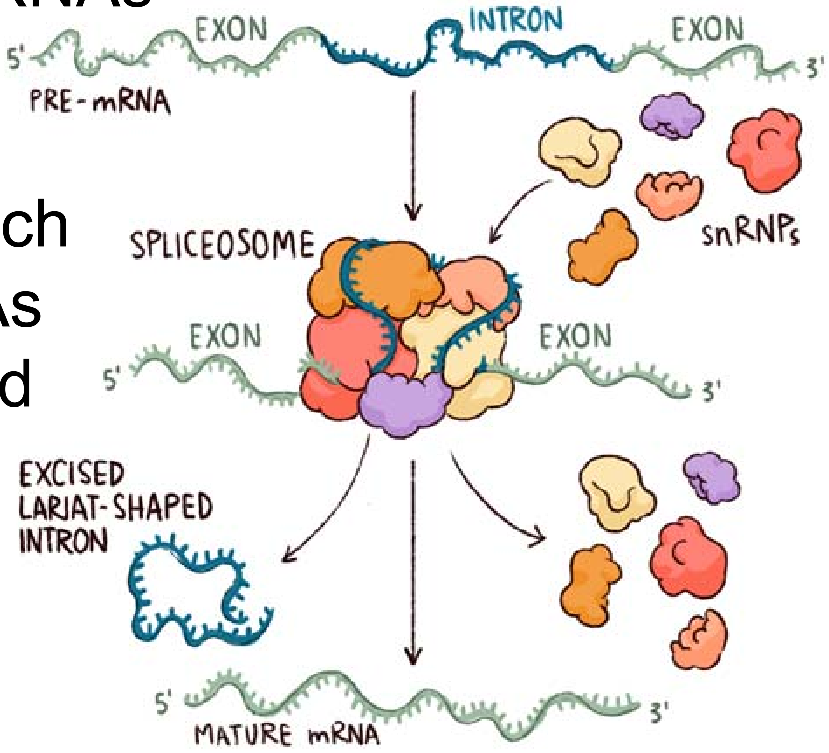
Transfer RNA (tRNA)



Peptide Synthesis

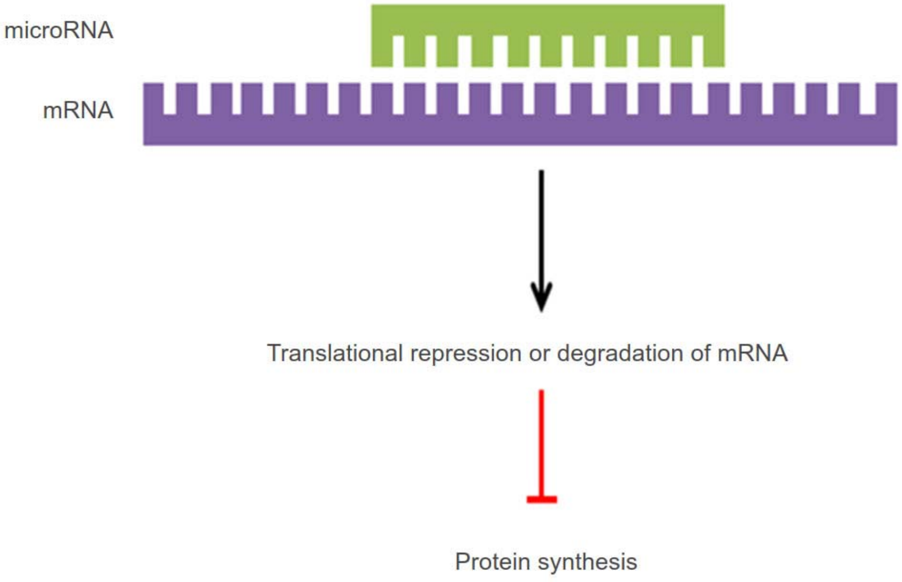
Function of different RNA Molecules

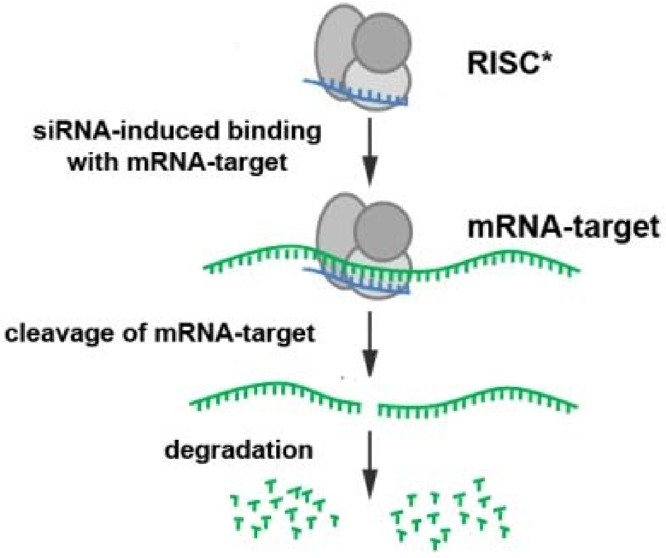
4. Small nuclear RNA **(snRNA):** is a class of small RNA molecules (150 nucleotides) which play an important role in pre-mRNA processing by removing introns (RNA splicing). snRNAs are main components of spliceosome (a large protein-RNA complex) which consists of 5 types snRNAs (U1,U2,U4,U5 and U6) and over 150 proteins. The RNA-protein complex is known as snRNPs or "snurps".



Function of different RNA Molecules

5. Small regulatory RNAs: short RNAs with average size of 21-27 including small interfering RNA **(siRNA)** and microRNA **(miRNA)** have a role in gene silencing and regulation of gene expression by base-pairing with complementary sequence of target mRNA molecules.





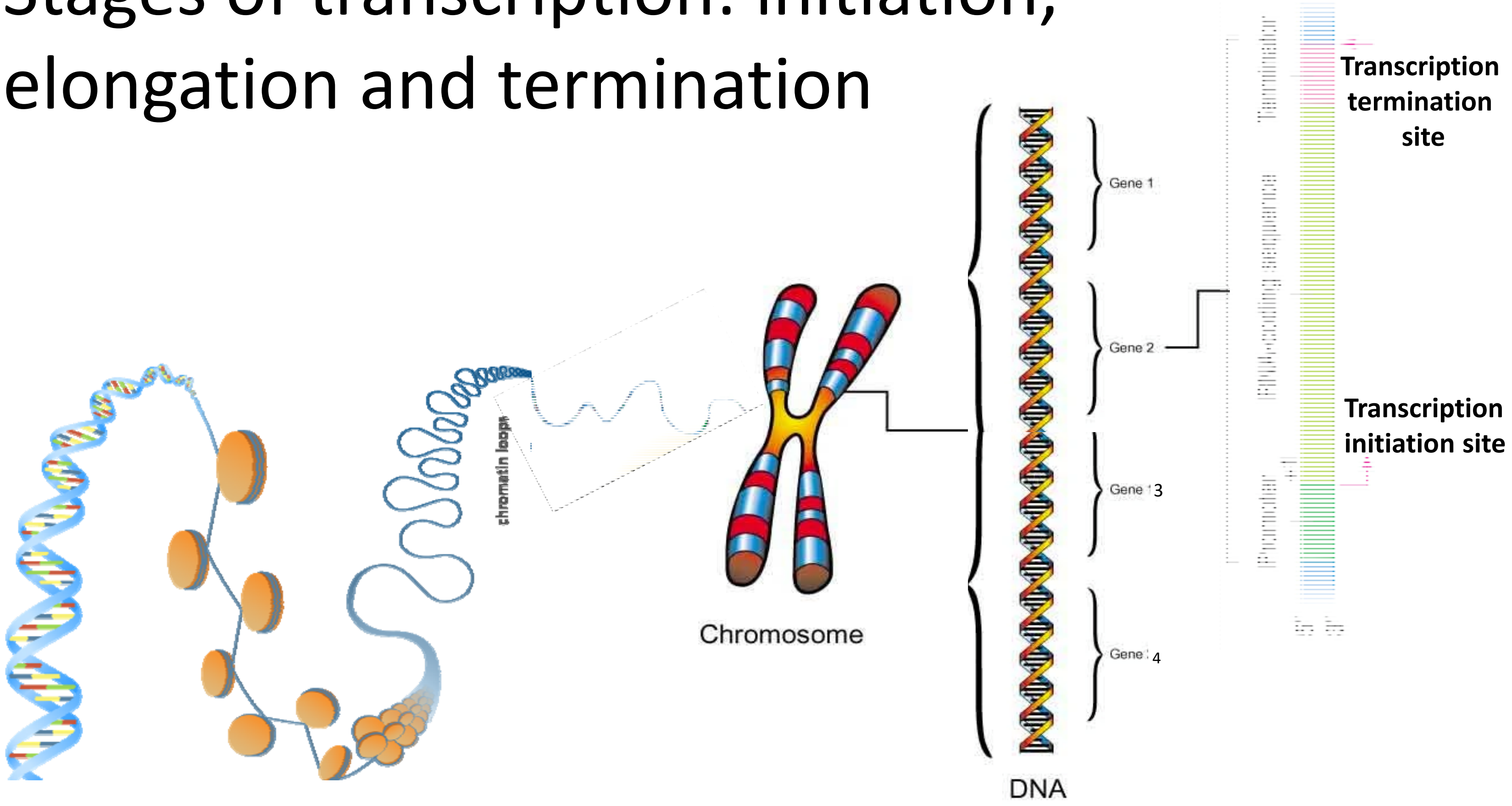
RNA Polymerase Enzyme

- **RNA polymerase:** is an enzyme which synthesizes RNA from DNA template
- Three classes of RNA polymerases in Eukaryotic cells:

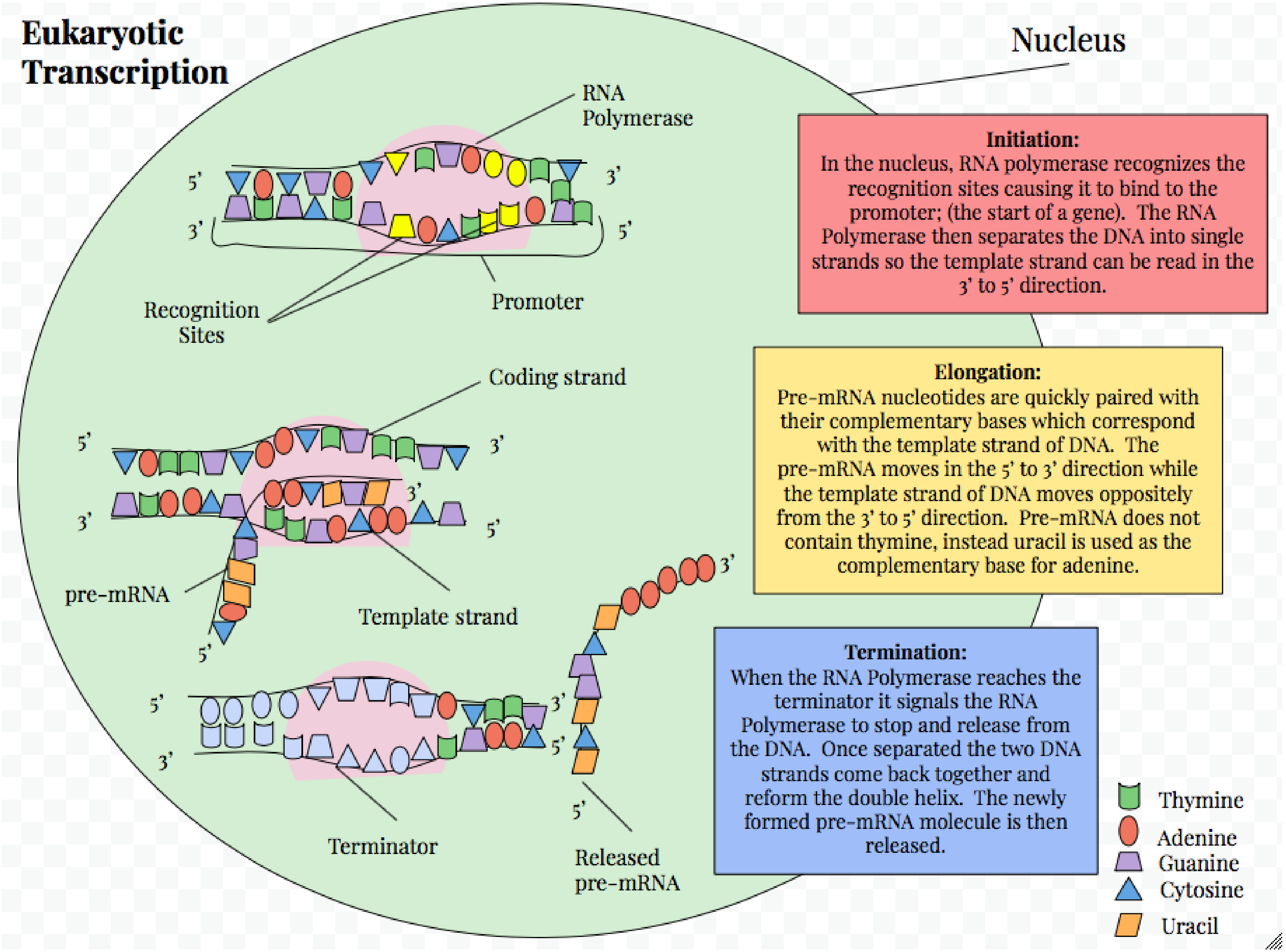
Form	Product
RNA Polymerase I	All rRNAs except 5S rRNA
RNA Polymerase II	All mRNAs, miRNAs, some snRNAs
RNA Polymerase III	All tRNAs, 5S rRNA, other small RNAs

Stages of Transcription

- Gene transcription in eukaryotes is more complex compared to prokaryotes
- Stages of transcription: initiation, elongation and termination



Stages of Transcription



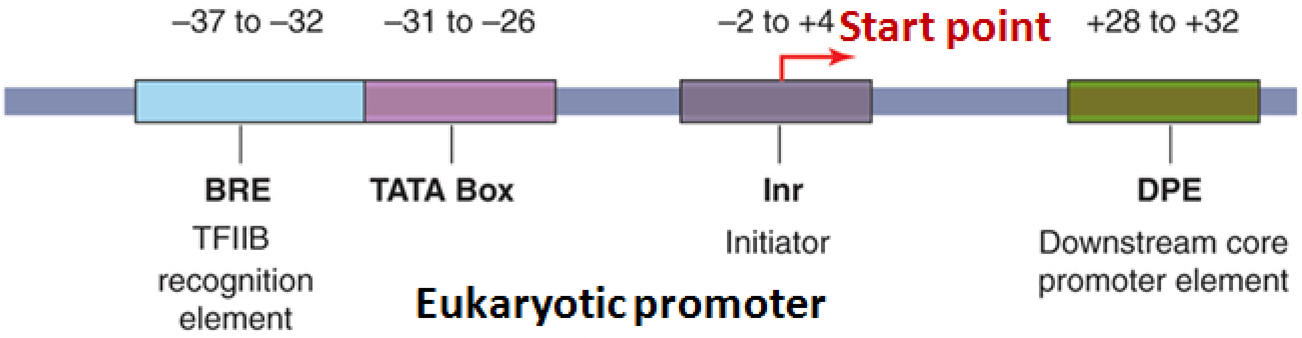
Initiation:
In the nucleus, RNA polymerase recognizes the recognition sites causing it to bind to the promoter; (the start of a gene). The RNA Polymerase then separates the DNA into single strands so the template strand can be read in the 3' to 5' direction.

Elongation:
Pre-mRNA nucleotides are quickly paired with their complementary bases which correspond with the template strand of DNA. The pre-mRNA moves in the 5' to 3' direction while the template strand of DNA moves oppositely from the 3' to 5' direction. Pre-mRNA does not contain thymine, instead uracil is used as the complementary base for adenine.

Termination:
When the RNA Polymerase reaches the terminator it signals the RNA Polymerase to stop and release from the DNA. Once separated the two DNA strands come back together and reform the double helix. The newly formed pre-mRNA molecule is then released.

Transcription Initiation

- **Promoter:** is a stretch of DNA sequence (non-coding DNA) where RNA polymerase can bind to start the transcription. Promoters are located upstream the genes that they regulate
- Promotors are found in Eukaryotes and Prokaryotes
- Eukaryotic promoter is a short DNA sequence (~100 bp) which consists of consensus sequences such as TATA box, BRE, INR and DPE



Eukaryotic promoter

Transcription Initiation

- General transcription factors **(GTFs):** are DNA-binding proteins which recognise specific regions in promoter and correctly position the RNA polymerase II at the transcription start site (TSS)
- There are five types of GTFs: TFIIB, TFIID (TBP and TAFs subunits), TFIIE, TFIIF and TFIIH

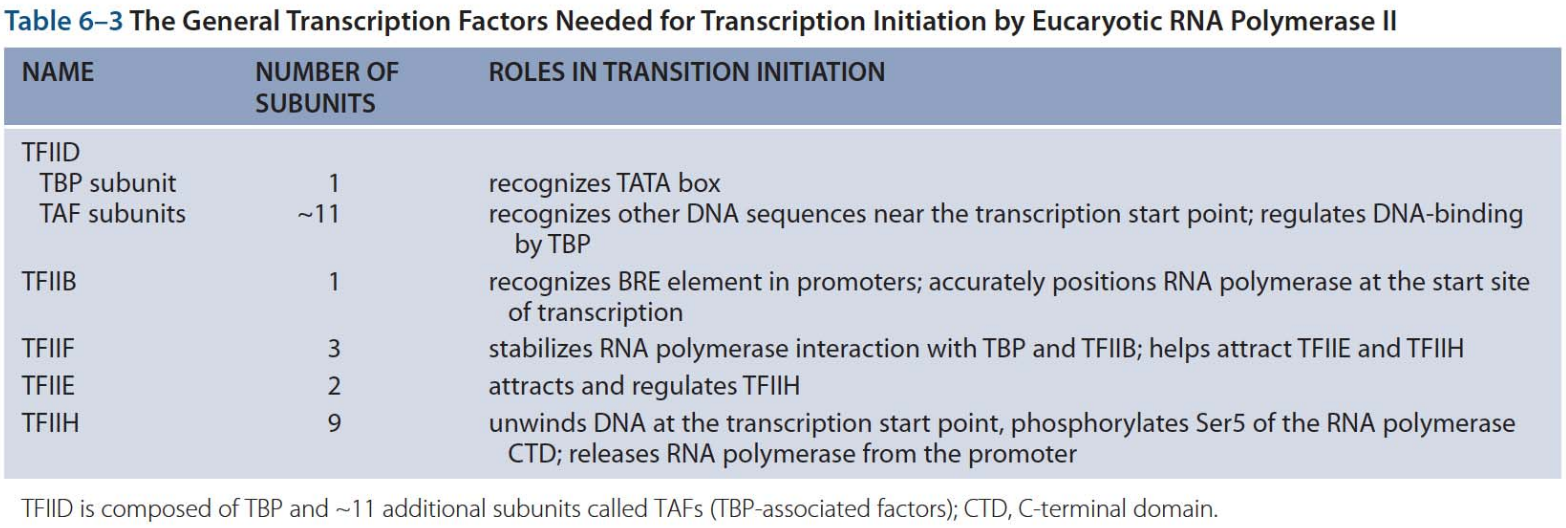


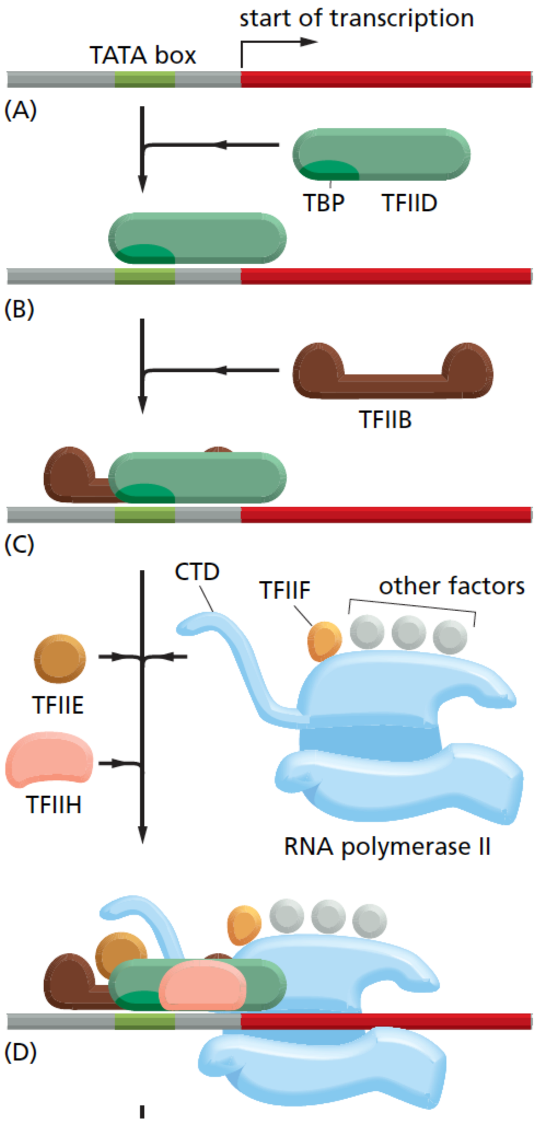
Table 6–3 The General Transcription Factors Needed for Transcription Initiation by Eucaryotic RNA Polymerase II

NAME	NUMBER OF SUBUNITS	ROLES IN TRANSITION INITIATION
TFIID		
TBP subunit	1	recognizes TATA box
TAF subunits	~11	recognizes other DNA sequences near the transcription start point; regulates DNA-binding by TBP
TFIIB	1	recognizes BRE element in promoters; accurately positions RNA polymerase at the start site of transcription
TFIIF	3	stabilizes RNA polymerase interaction with TBP and TFIIB; helps attract TFIIE and TFIIH
TFIIE	2	attracts and regulates TFIIH
TFIIH	9	unwinds DNA at the transcription start point, phosphorylates Ser5 of the RNA polymerase CTD; releases RNA polymerase from the promoter

TFIID is composed of TBP and ~11 additional subunits called TAFs (TBP-associated factors); CTD, C-terminal domain.

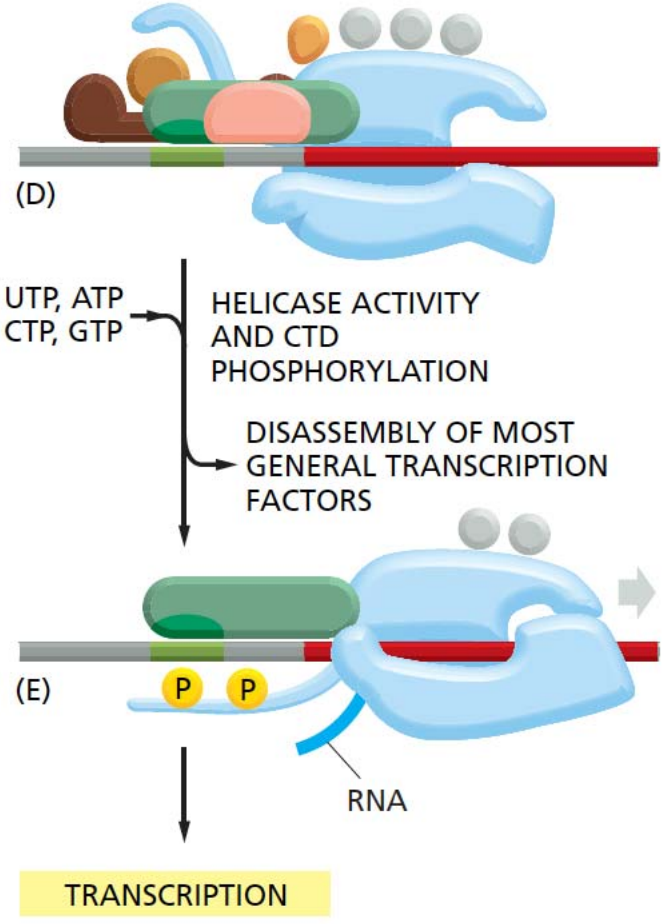
Transcription Initiation

- Pre-initiation complex **(PIC)** formation: is the assembly of GTFs along with RNA polymerase II at the promoter region. It is an important step in the transcription initiation process
- TFIID initially binds TATA box region via its TBP subunit. TFIIB subsequently binds BRE region of the promoter



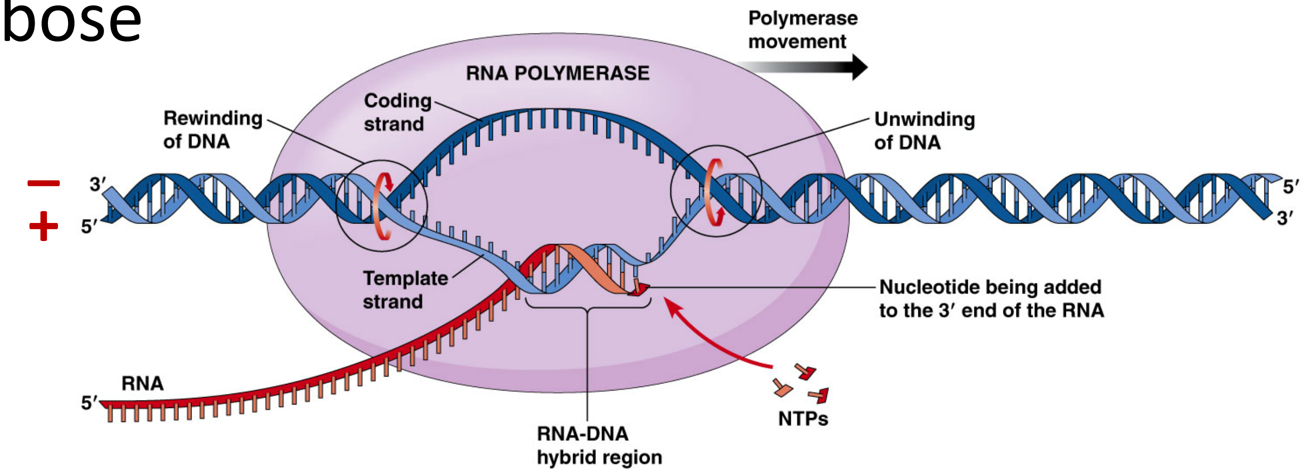
Transcription Initiation

- TFIIH can perform several enzymatic steps required for the transcription elongation.
  1. It unwinds (unzips) the double helix at the transcription start site (TSS) via its helicase activity
  2. It phosphorylates Ser5 located at the tail of RNA polymerase II called CTD (C-terminal domain) via its kinase activity. The CTD tail consists of 52 tandem repeats of 7-amino acids sequence (Ser2, Ser5). This event is important for RNA polymerase II to escape the promoter and start elongation



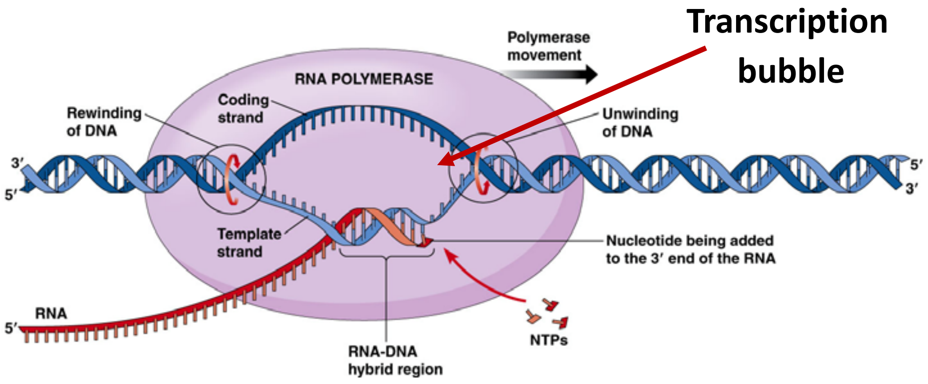
Transcription Elongation

- Elongation: the extension of the newly synthesized transcript
- The template strand (antisense strand,–): 3’-5’ strand which is used to synthesize the RNA molecule. It is complementary to RNA or transcript strand
- The coding strand (sense strand, +):5’-3’ strand which resembles the RNA strand except T is replaced with U and deoxyribose is changed to ribose



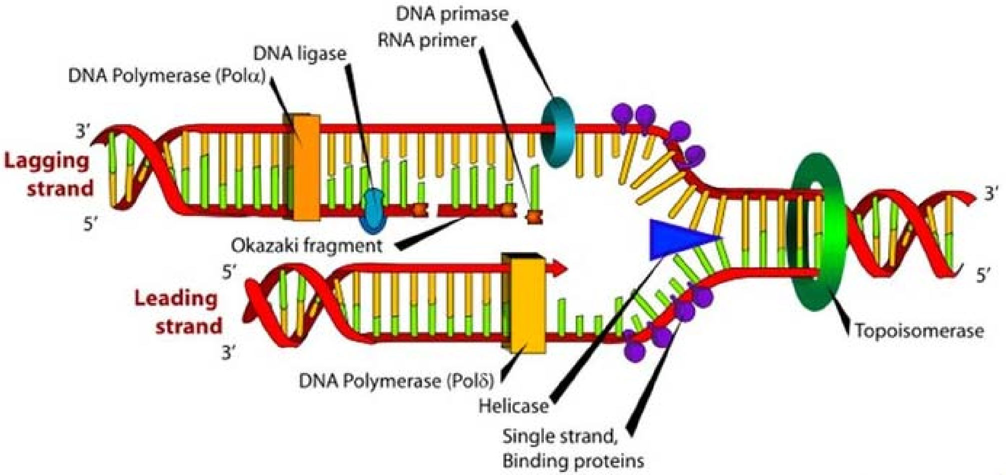
Transcription Elongation

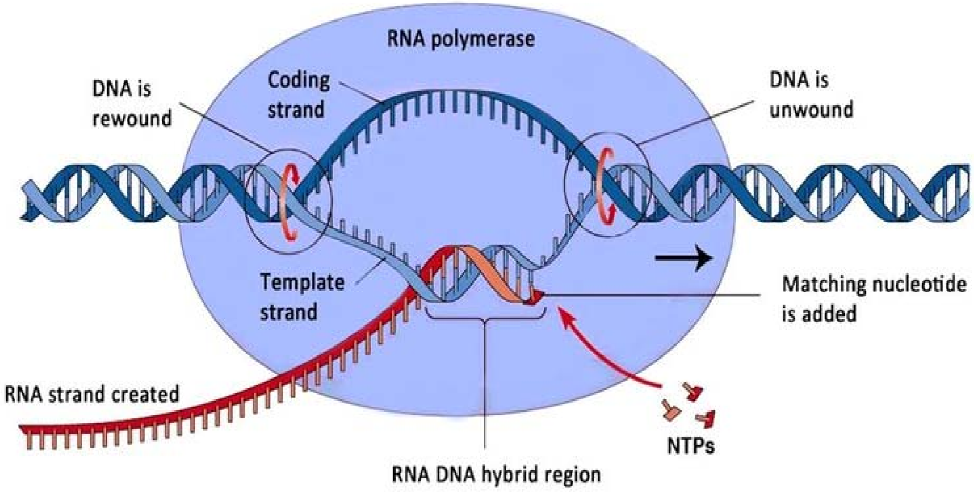
- RNA polymerase II can only read 3’-5’ DNA strand and elongate the growing RNA strand in the 5’-3’ direction (add only to free 3’end)
- A polymerase II unwinds/ unzips the helical DNA and creates a bubble that exposes the single strands of DNA during the elongation process
- Transcription bubble: is a region (10-20 nucleotides) where the two single strands of DNA are separated and exposed



DNA & RNA polymerases

- What are the differences between DNA polymerase and RNA polymerase enzymes?





Transcription Termination

- Transcription termination: in eukaryotes is not well understood. RNA polymerase II meets a termination signal and knows that it is the end of transcription so will stop at that point.
- RNA polymerase II detaches from the template strand to initiate another round of transcription.
- To reinitiate transcription, soluble phosphatases remove the phosphates on CTD tail of RNA polymerase II
- The mature mRNA will leave to cytoplasm to start the second step in gene expression: **Translation**