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Lecture – 18

Transcription

by

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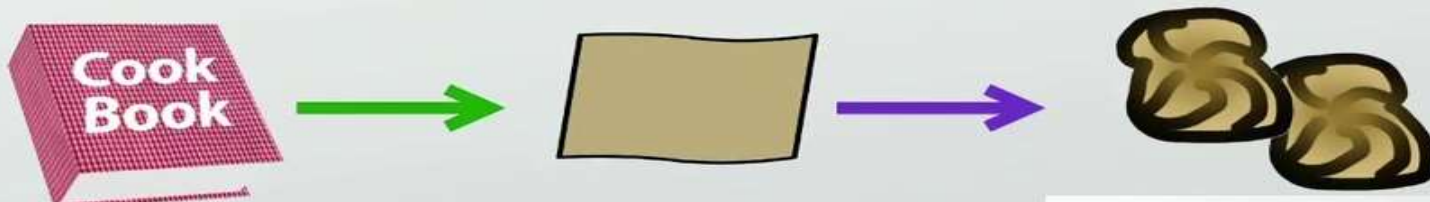
*Asst. Prof. **GPB***

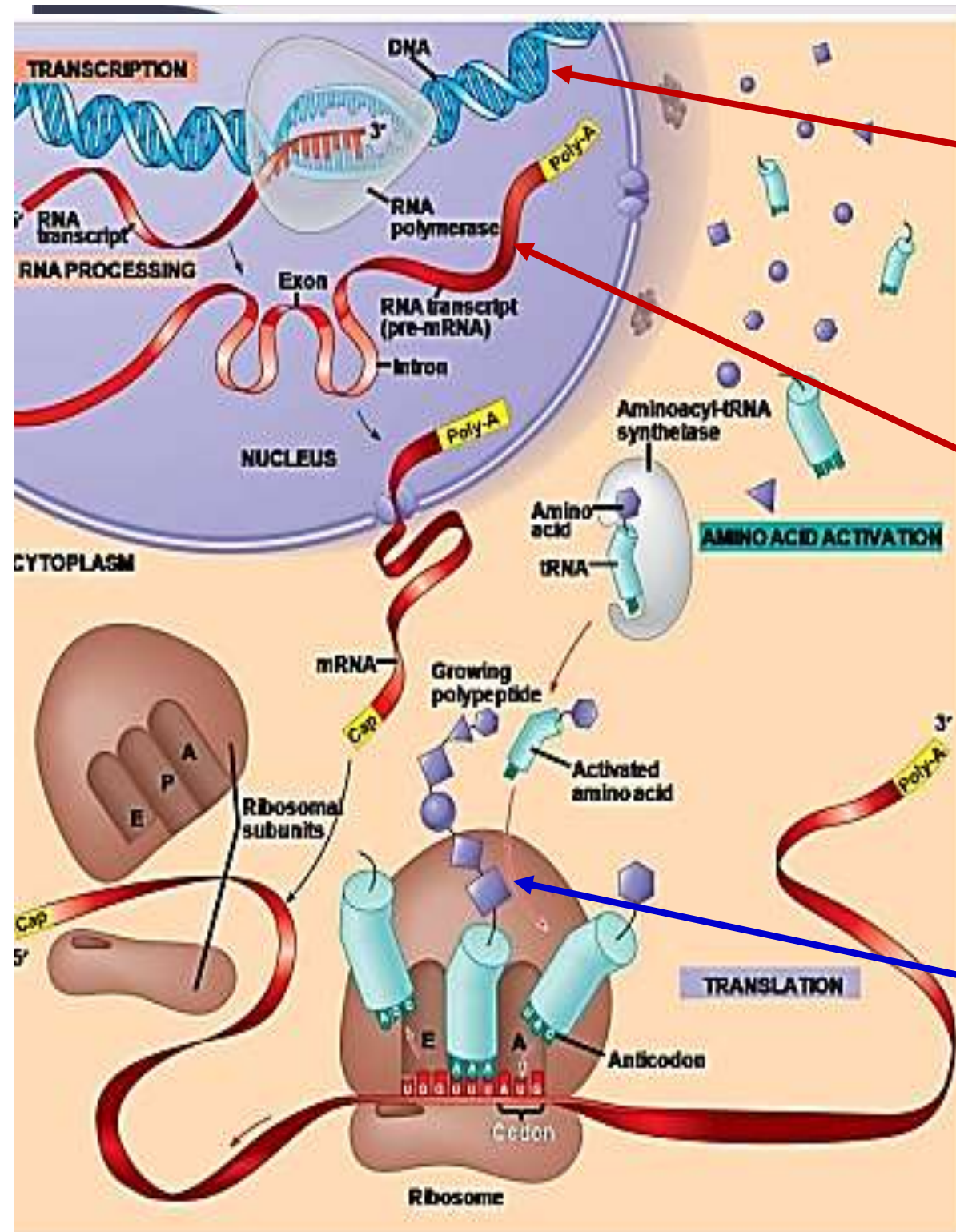
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Central dogma of molecular biology

- ❖ 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.
- ❖ However, **reverse transcription** was first reported by **Temin and Baltimore** in 1970.





THE INFORMATION FOR PROTEIN SYNTHESIS IS IN THE DNA IN THE **NUCLEUS**.

THE INFO FROM THE DNA IS COPIED INTO mRNA, WHICH CAN LEAVE THE **NUCLEUS** AND GET TO THE RIBOSOMES IN THE **CYTOPLASM**.

THE PROTEINS ARE MADE IN THE **CYTOPLASM** IN THE **RIBOSOMES**

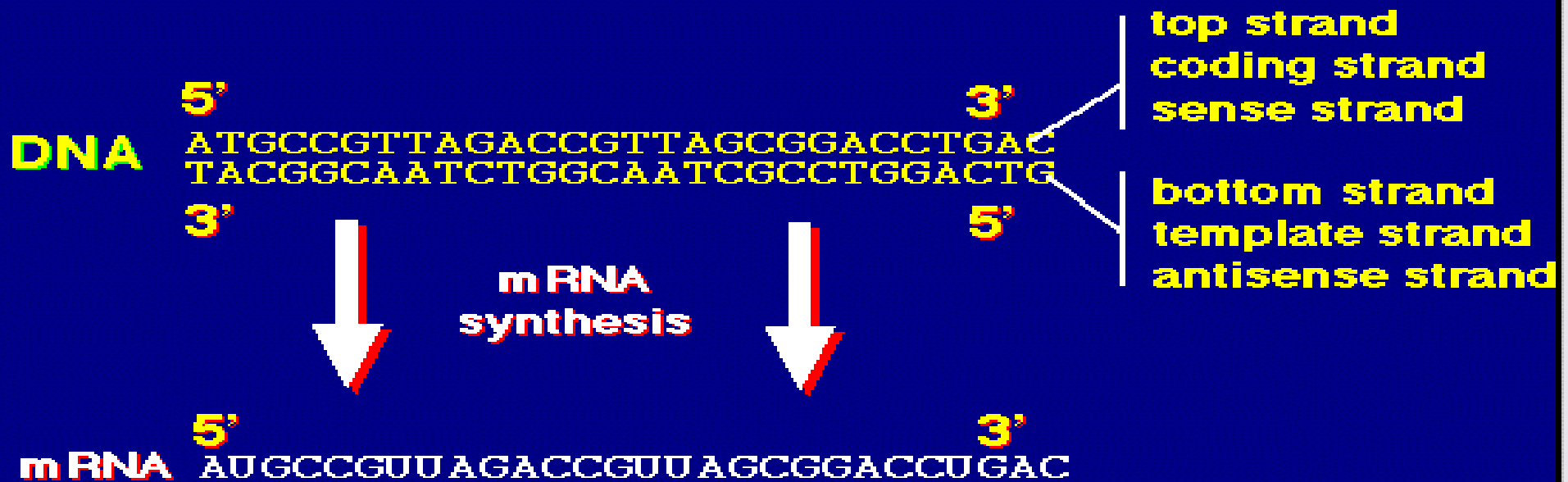
STEP 1: TRANSCRIPTION= making RNA


Location: in Eukaryotes-nucleus, in Prokaryotes-cytoplasm

RNA synthesis like DNA synthesis always occur in **5'-3' direction**.

As a result the orientation of antisense strand (template strand) of DNA duplex as a rule 3'-5' in relation to direction of transcription.

TRANSCRIPTION



- 
- Complementary nucleotides are added using the base pairing rules EXCEPT: A=U
 - The rest are the same C=G, T=A, G=C

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Question:

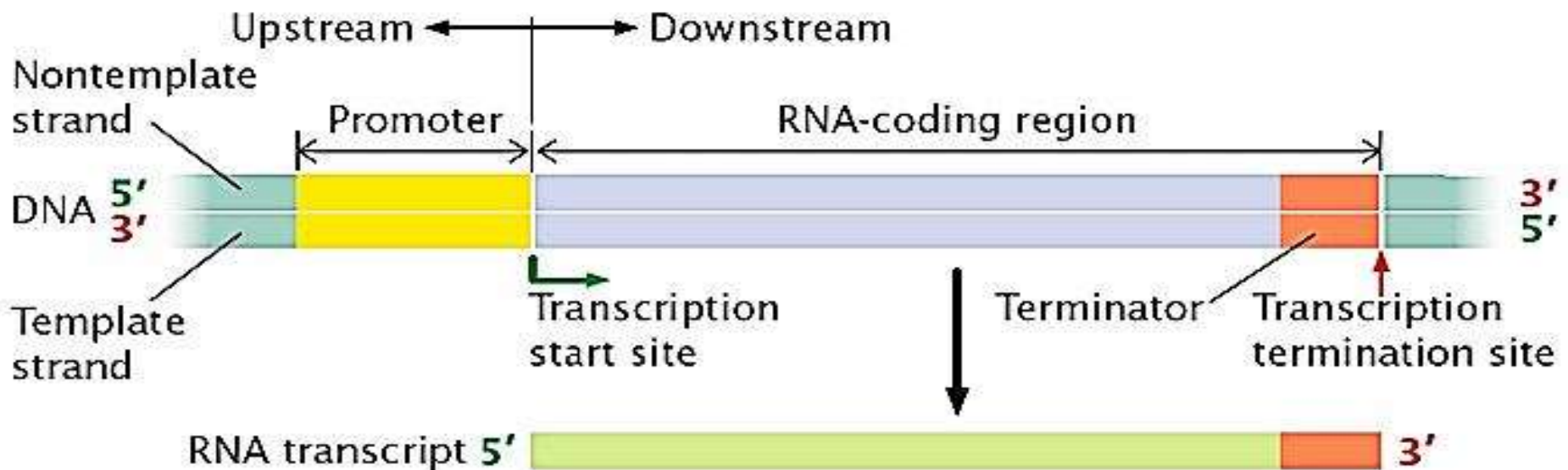
- What would be the complementary RNA strand for the following DNA sequence?

DNA 5'-GCGTATG-3'



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- A transcription unit define as the stretch of DNA, which transcribed as a single RNA molecule.
- A typical transcription unit has
 1. A promoter- at its beginning
 2. A start point
 3. A coding region- that transcribed
 4. A termination sequence- at its end



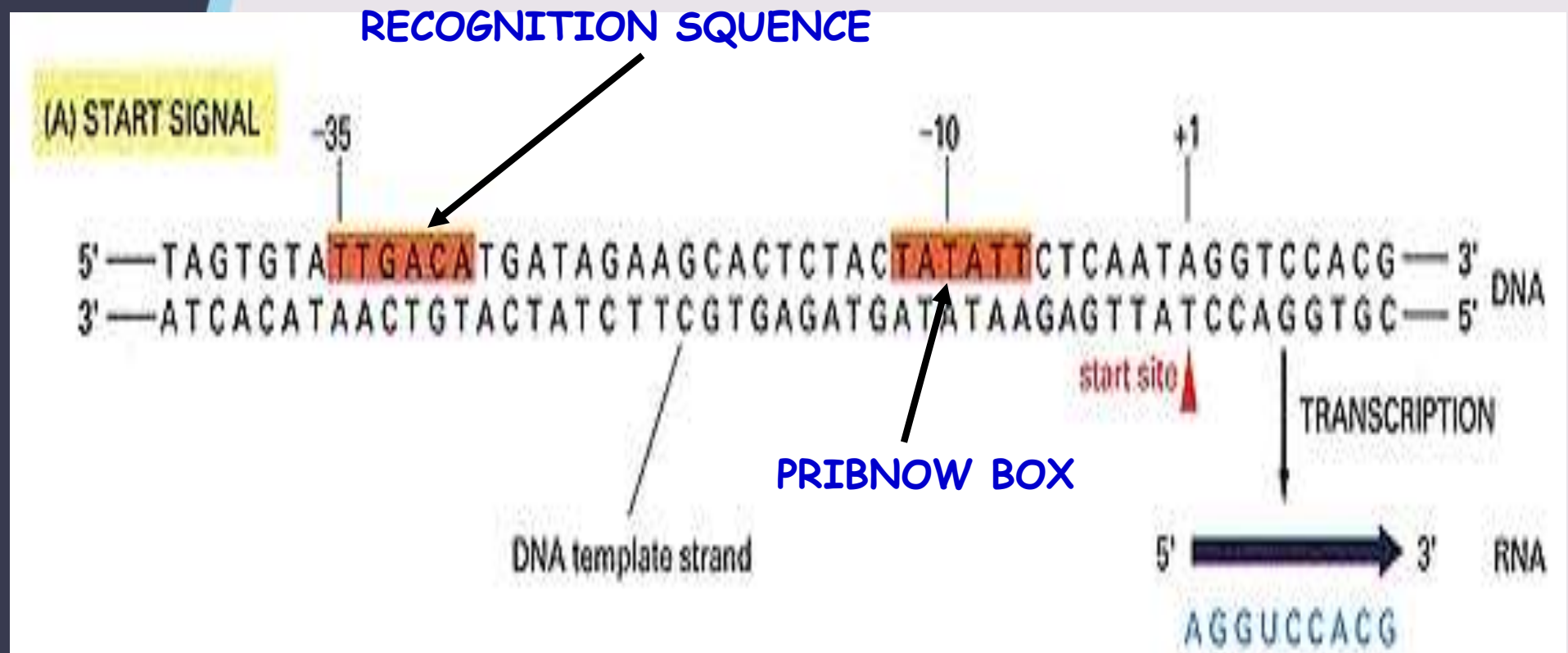


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Transcription: Initiation

- ❑ RNA polymerase binds to a segment of DNA and opens up the double helix
- ❑ RNA polymerase recognizes the **promoter region** which is a sequence of DNA rich in A and T bases (**TATA box**) found only on one strand of the DNA.
- ❑ An RNA polymerase cannot recognize the TATA box and other landmarks of the promoter region on its own. Another protein, a **transcription factor** that recognizes the TATA box, binds to the DNA before the RNA polymerase can do so.

- For transcription to be initiated, both promoter sequences must be present in their correct locations. The nucleotide sequences in the promoters are slightly different from one another, which means the RNA polymerase will bind in only 1 orientation, thus RNA polymerase can only face 1 way during transcription. This ensures transcription will proceed in only 1 direction.





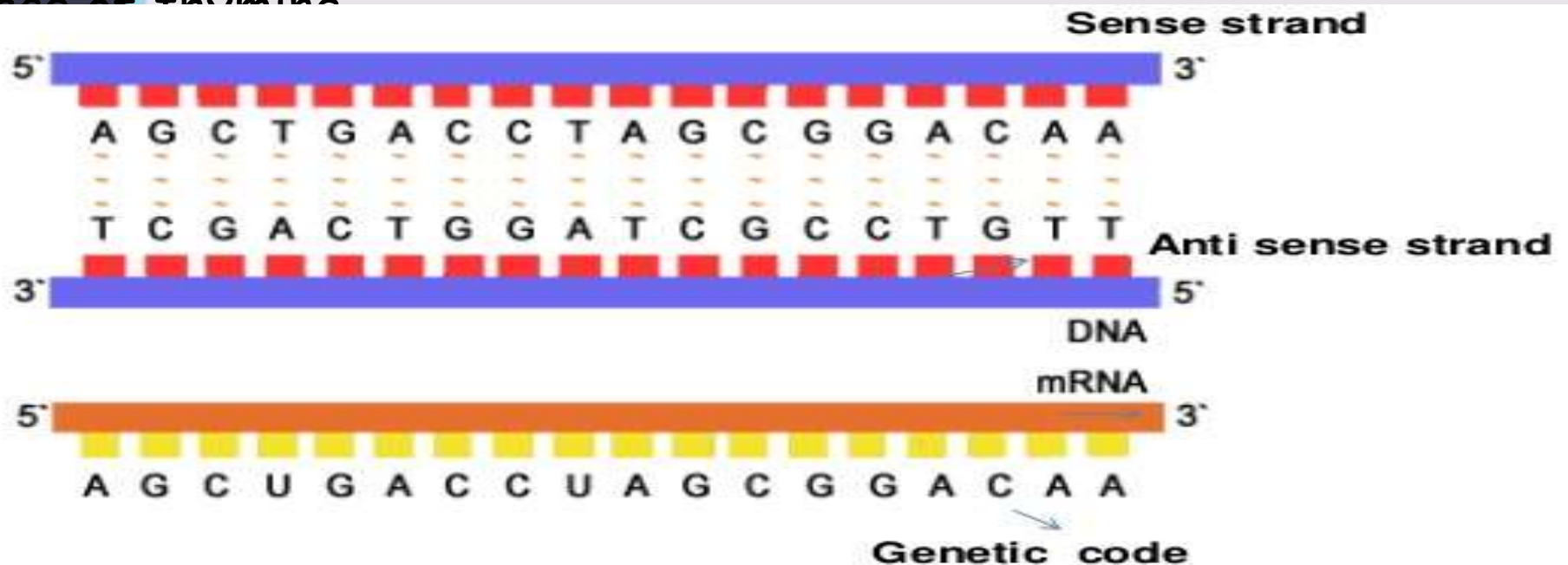
RNA Polymerase

RNA Polymerase I	RNA Polymerase II	RNA Polymerase III
Located in nucleolus	Present in nucleoplasm (outside nucleolus)	Present in nucleoplasm (outside nucleolus)
Catalyzes the synthesis of r-RNA	Catalyzes the synthesis of m-RNA	Transcribe the gene for small nuclear RNA and t-RNA



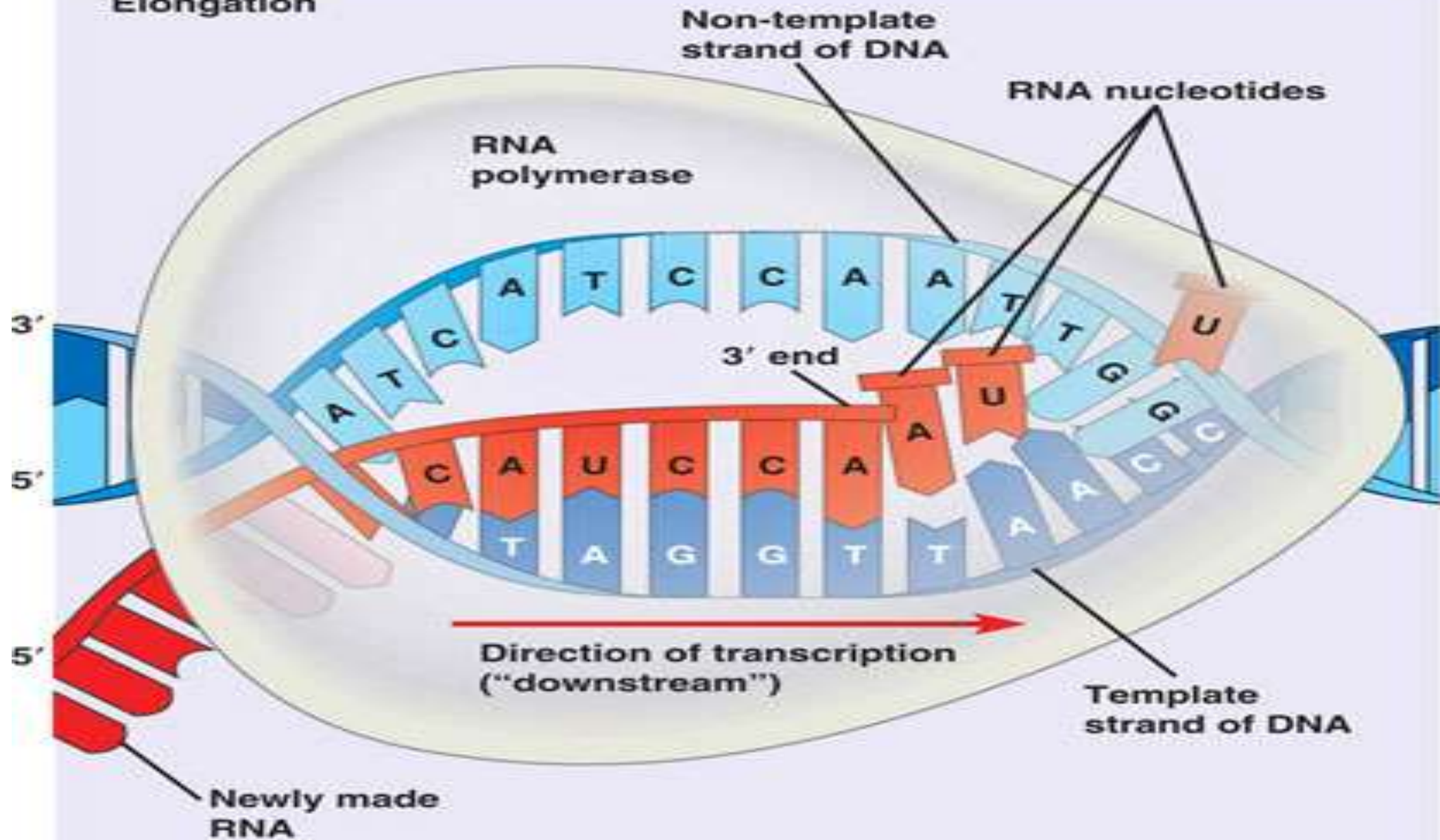
Transcription : Elongation

- ❖ The RNA polymerase uses only one of the strands of DNA as a template for mRNA synthesis. This is called the **template strand or sense strand**. The **coding strand or anti-sense strand** contains the complementary nucleotide sequence to the sense strand.
- ❖ RNA polymerases can add nucleotides only to the 3' end of a DNA sequence. Thus, an RNA molecule elongates in the 5' to 3' direction.
- ❖ The coding strand is exactly identical to the mRNA, but mRNA has uracil in place of thymine.



mRNA SYNTHESIS BY TRANSCRIPTION

Elongation



- RNA polymerase uses the template strand to transcribe. Thus the RNA is complimentary to the template.

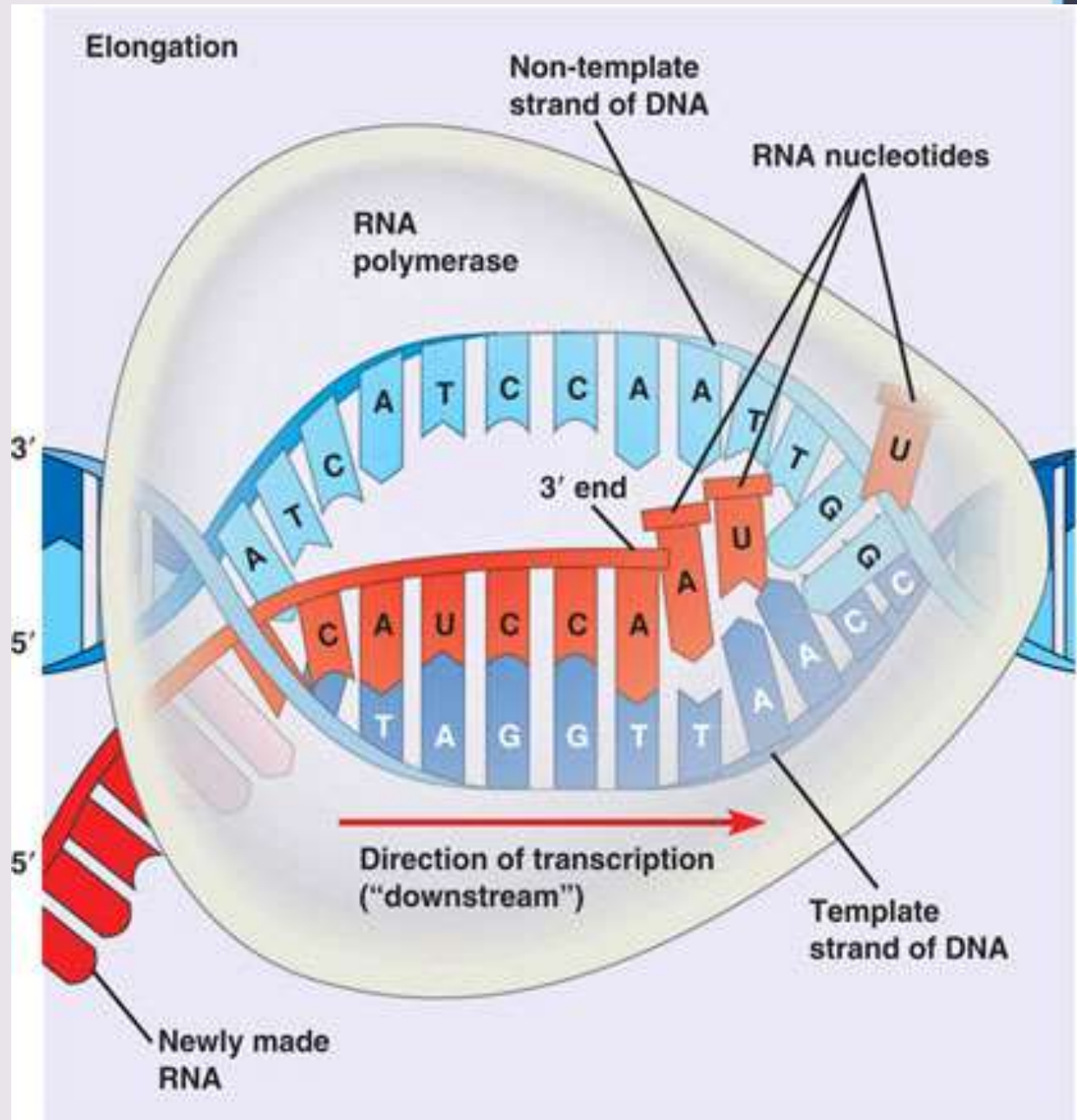


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Transcription: Termination

- As the RNA polymerase molecule passes, the DNA helix re-forms. Synthesis continues until the end of a gene is reached where RNA polymerase recognizes a **terminator sequence**.





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- Once the RNA polymerase leaves the promoter region, a new RNA polymerase can bind there to begin a new mRNA transcript.
- Do we need new mRNA every time?
 - NO, the same mRNA may be used to make many copies of the same protein.
- Since prokaryotes lack a membrane bound nucleus translation can begin even before the mRNA dissociates. However the pre-mRNA from eukaryotic cells needs some modification before it leave the nucleus.
- The genetic information's copied in m-RNA are stored in the form of **CODON (Genetic code)**



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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.

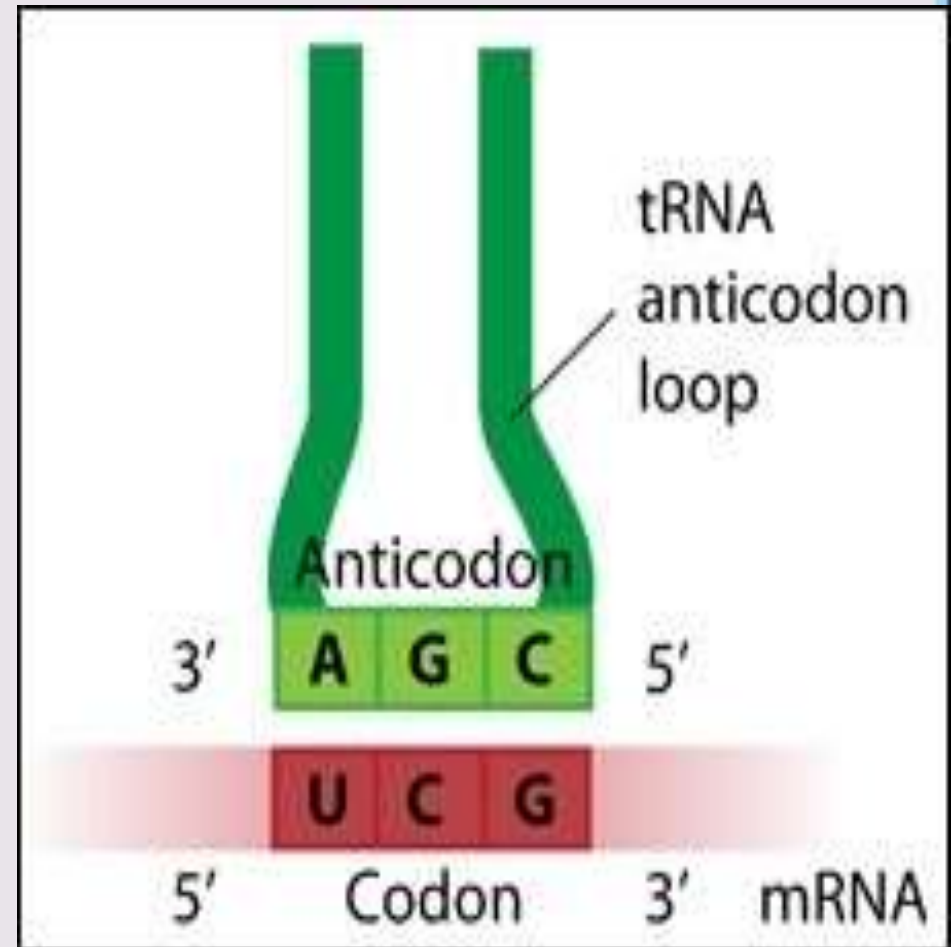


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❖ The number and the sequence of bases in mRNA specifying an amino acid is known as a **codon**. While the set of bases in t-RNA which base pair with a codon of mRNA is known as **anti-codon**.

❖ 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**.

❑ First evidence of genetic code as triplet revealed by **F.H.C. Crick (1961)** from genetic analysis of Proflavin- induced mutation in **rII locus of phase T4**.

❑ **Three codons, UAA (Ochre), UAG (Amber) and UGA (Opal)** do not code for any amino acid and hence they are called **nonsense codons or 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 but in prokaryotes it is N-formyl methionine**.



Codon Dictionary

Second Letter

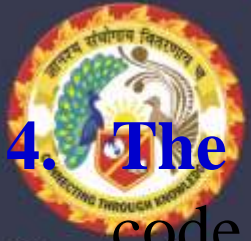
		Second Letter					
		U	C	A	G		
1st letter	U	UUU Phe UUC UUA Leu UUG	UCU UCC Ser UCA UCG	UAU Tyr UAC UAA Stop UAG Stop	UGU Cys UGC UGA Stop UGG Trp	U C A G	3rd letter
	C	CUU CUC Leu CUA CUG	CCU CCC Pro CCA CCG	CAU His CAC CAA Gln CAG	CGU CGC Arg CGA CGG	U C A G	
	A	AUU AUC Ile AUA AUG Met	ACU ACC Thr ACA ACG	AAU Asn AAC AAA Lys AAG	AGU Ser AGC AGA Arg AGG	U C A G	
	G	GUU GUC Val GUA GUG	GCU GCC Ala GCA GCG	GAU Asp GAC GAA Glu GAG	GGU GGC Gly GGA GGG	U C A G	



Nature / Properties of Genetic code

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- 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.



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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 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**



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7. The code is degenerate: In most cases, several codons code for the same amino acid. **Means one amino acid is coded by more than one codon.** 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

Amino acid	Number of codon
tryptophan and methionine (Two)	1
Nine codones	2
Isoleucine (One)	3
Valine, Proline, Threonine, Alanine and Glycine (Five)	4
Lucine, Arginine and Serine (Three)	6

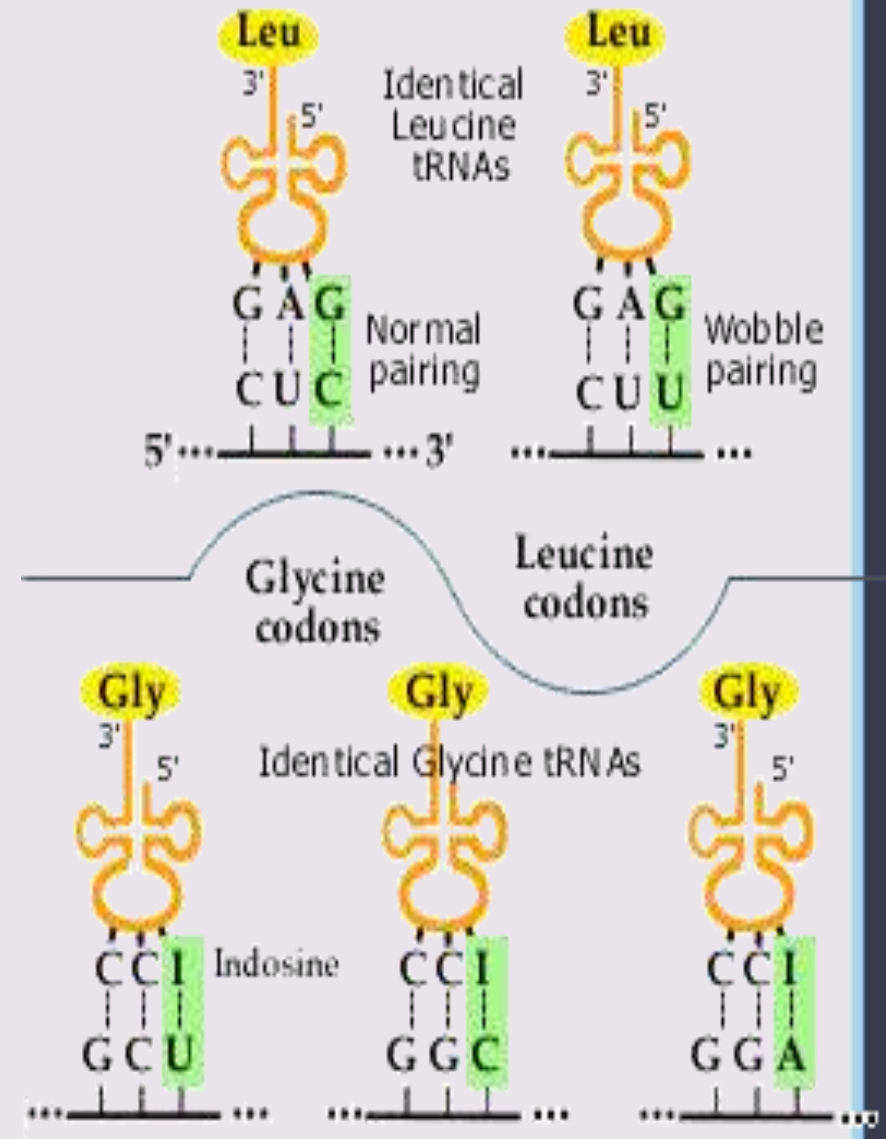


Wobble hypothesis:

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

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❖ 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.





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In other word the hydrogen bonding between bases in the anticodon of t-RNA and codon of m-RNA appear to follow strict (tight) base pairing only for first two bases of codon third base of codon show less strength

Base pairing predicted by wobble hypothesis

Codon	Anti-Codon
A	U or I
G	C or U or I
C	G or I
U	A or G or I



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.



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