

University of Anbar

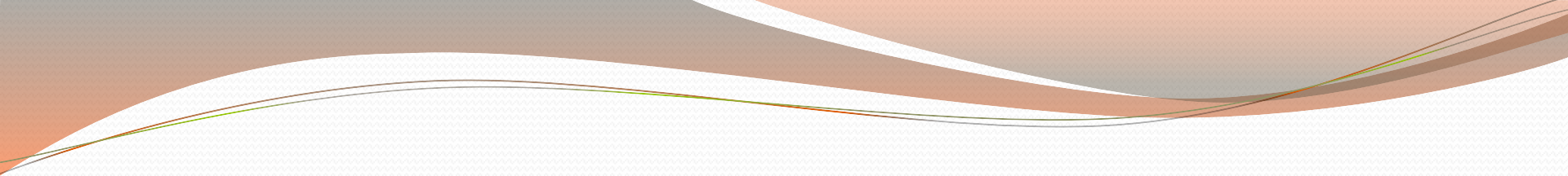
Department of Biotechnology

College of Science

“

Dr. Huda Musleh

Biotechnology 1



Lecture 3
Gene Transcription and Translation

In this lecture...

- Central Dogma
- RNA and proteins
- Codons
- Transcription
 - Initiation
 - Elongation
 - Termination
- Translation
- Post-translational modification
- Mutations

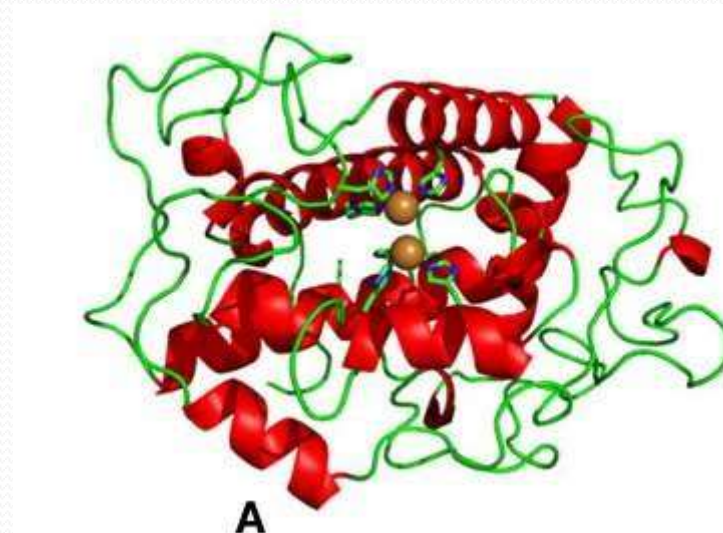
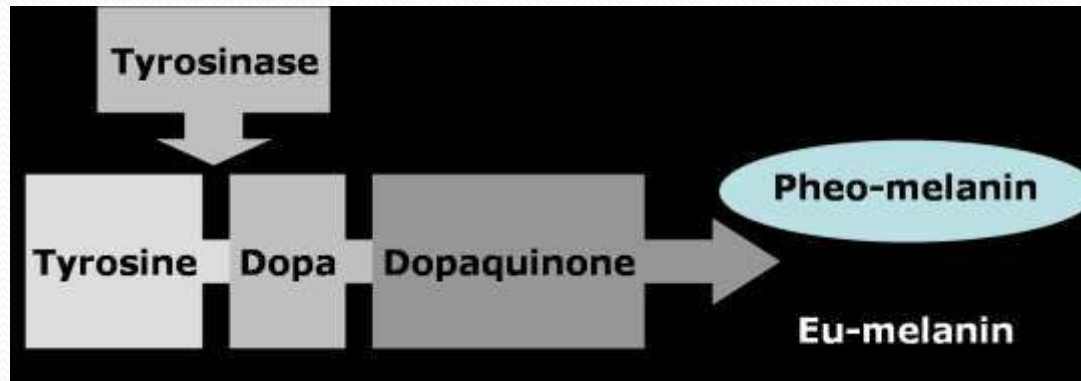
Proteins link genotype and phenotype



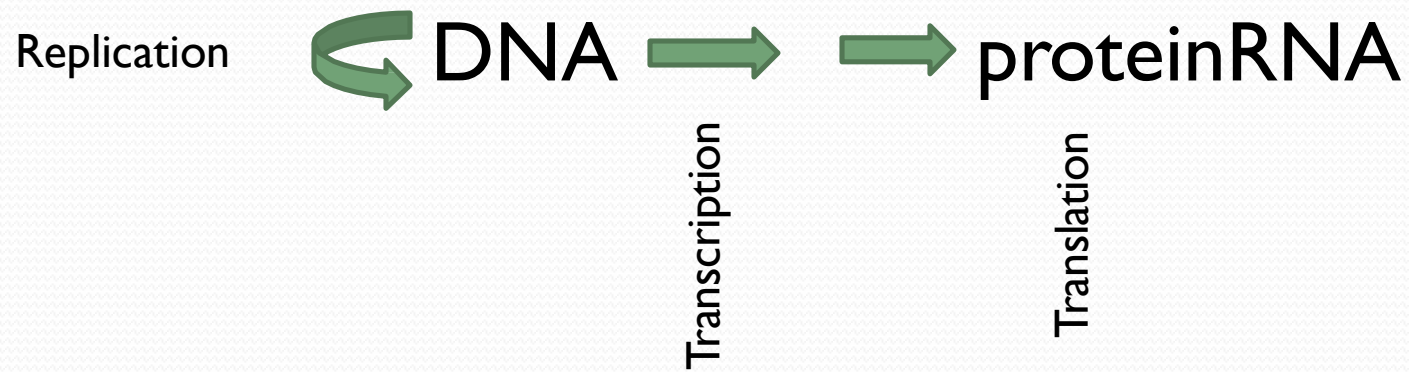
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- Albinism occurs when the protein tyrosinase is defective
- Tyrosinase directs the synthesis of melanin, a pigment found in skin and eyes
- Heterozygotes with one functional copy of tyrosinase produce enough melanin to not display the phenotype
- However, recessive homozygotes will lack all pigment

Melanin synthesis pathway and GPR413 structure



The Central Dogma



DNA sequence of tyrosinase gene

gi|209571475:5001-122888 Homo sapiens tyrosinase (oculocutaneous albinism IA) (TYR), RefSeqGene on chromosome 11

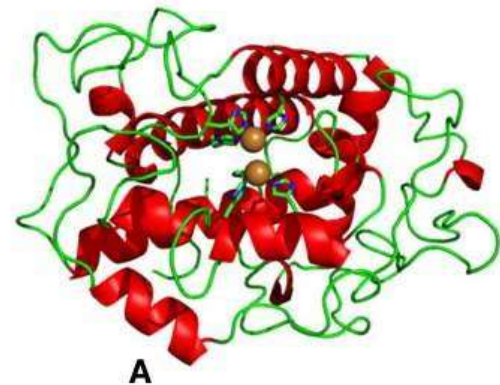
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CTGCTGTGGAGTTCCAGACCTCCGCTGGCCATT
TCCCTAGAGCCTGTGTCTCCTCTAAGAACCTGATGG
AGAAGGAATGCTGTCCACCGTGGAGCGGGGACAG
GAGTCCCTGTGGCCAGCTTTCAGGCAGAGGTTTCT
GTCAGAATATCCTTCTGTCCAATGCACCACTTGGG
CCTCAATTTCCCTTACAGGGGTGGATGACCGGGA
GTCGTGGCCTTCCGTCTTTTATAATAGGACCTGCC
AGTGCTCTGGCAACTTCATGGGATTCAACTGTGGAA
ACTGCAAGTTTGGCTTTTGGGGACCAAACTGCAC
AGAGAGACGACTCTTGGTGAGAAGAAACATCTTCG
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TATGTCATCCCCATAGGGACCTATGGCCAAATGA
AAAATGGATCAACACCCATGTTTAAACGACATCAATA
TTTATGACCTCTTTGTCTGGATGCATTATTATGT
GTCAATGGATGCACTGCTTGGGGGATCTGAAATCT
GGAGAGACATTGATTTTGCCCATGAAGCACCAGCT
TTTCTGCCTTGGCATAGACTCTTCTTGTTGCGGTGG
GAACAAGAAATCCAGAAGCTGACAGGAGATGAAA
ACTTCACTATTCCATATTGGGACTGGCGGGATGCAG
AAAAGTGTGACATTTGCACAGATGAGTACATGGG
AGGTCAGCACCCCACAAATCCTAACTTACTCAGCC
CAGCATCATTCTTCTCCTCTTGGCAGGTAAGATAT
GCTAGATATACGATGTCAGAGTAGGGAGGAACCTT
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AAATGGTGCCCTGTTAAGAACTCTCAATGTATCTT
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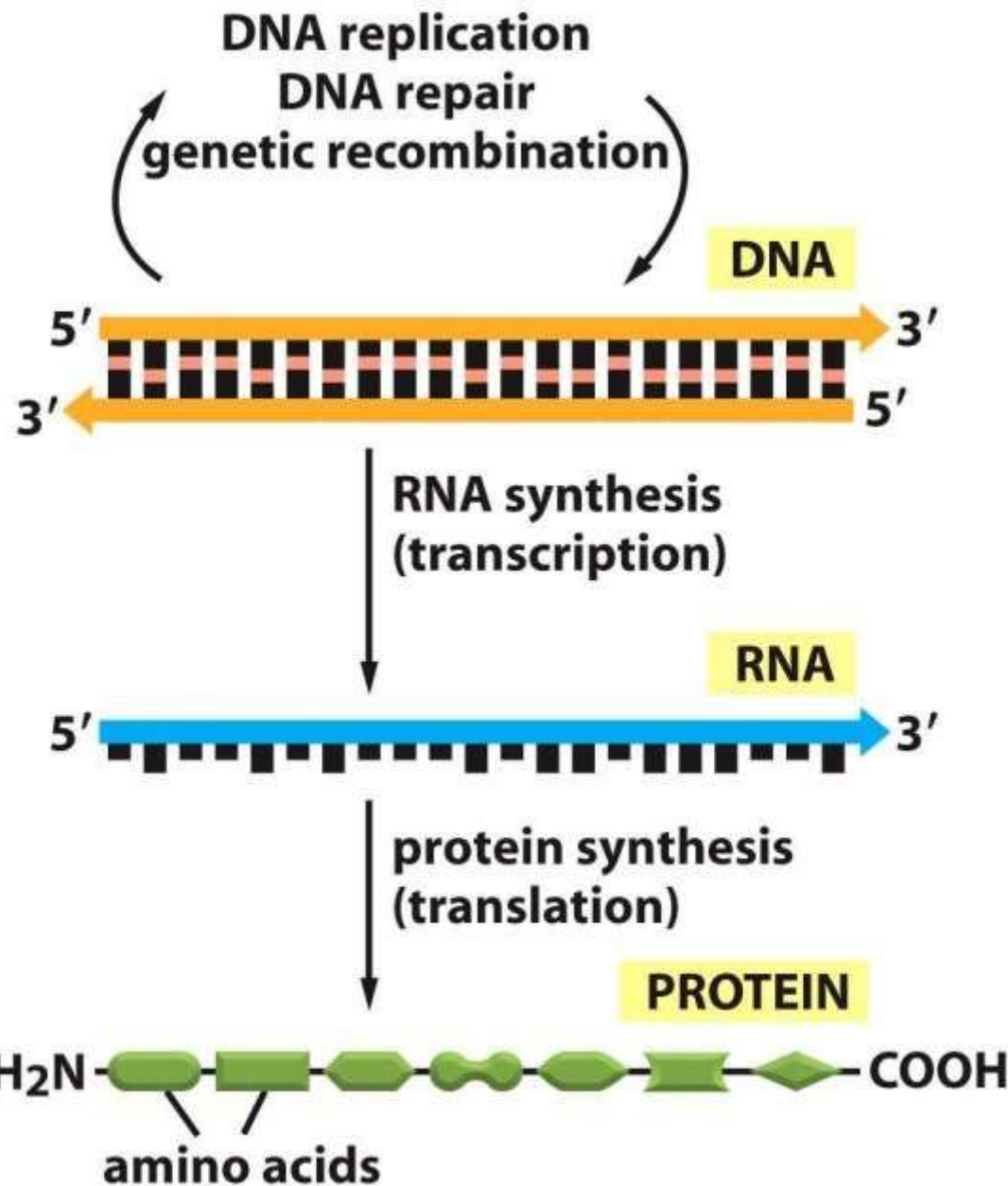
Primary structure of tyrosinase protein

>gi|403422|gb|AAB60319.1| tyrosinase [Homo sapiens]

```
MLLAVLYCLLWSFQTSAGHFPRACVSSKLNLMKECCPP
WSGDRSPCGQLSGRGSCQNILLSNAPLGPQFP
FTGVDDRESWPSVFYRNRTCQCSGNFMGFNCGNCKF
GFWGPNCTERRLLVRRNIFDLSAPEKDKFFAYLTL
AKHTISSDYVIPIGTYGQMKNGSTPMFNDINIYDLFWW
MHYYVSMDALLGGSEIWRDIDFAHEAPAFLPW
HRLFLLRWEQEIQKLTGDENFTIPYWDWRDAEKCDIC
TDEYMGQHPNPNLLSPASFFSSWQIVCSRLE
EYNHQSLCNGTPEGPLRRNPGNHDKSRTPLPSSADV
EFCLSLTQYESGSMDKAANFSFRNTLEGFASP
LTGIADASQSSMHNALHIYMNGTMSQVQGSANDPIFLL
HHAFVDSIFEQWLQRHRPLQEVYPEANAPIGH
NRESYMPFIPLYRNGDFFISSKDLGYDYSYLQSDPDSF
QDYIKSYLEQASRIWSWLLGAAMVGAVLTA
LLAGLVSLLCRHKRKQLPEEKQPLLMEKEDYHSLYQSHL
```

Quaternary structure of tyrosinase protein

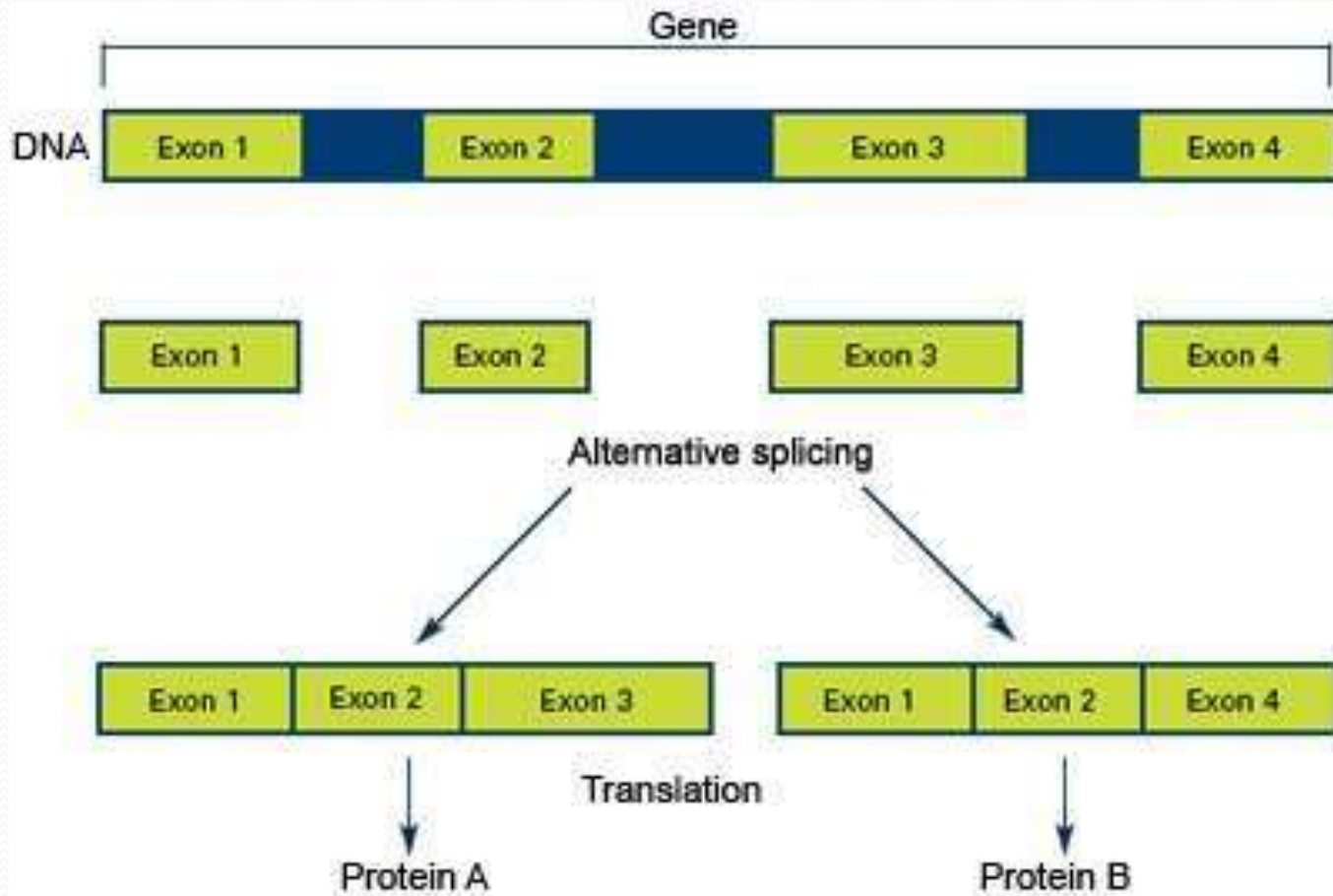




Proteins have an N terminus and a C terminus

Figure 6-2 Molecular Biology of the Cell 5/e (© Garland Science 2008)

One gene, one protein hypothesis

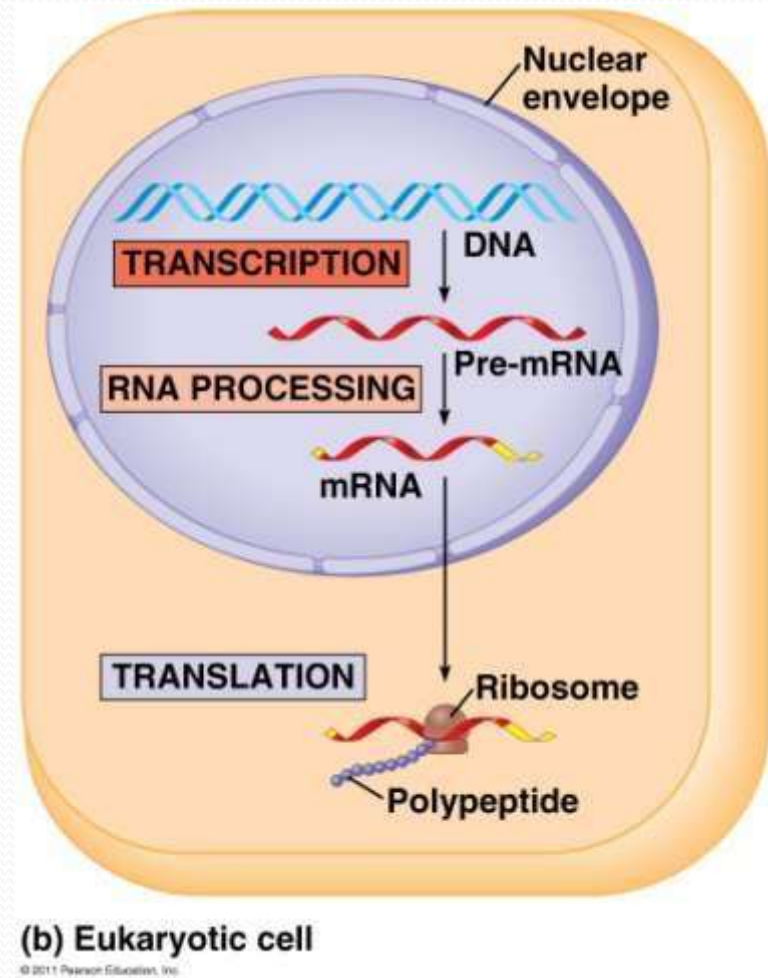


How do genes produce proteins?

Gene expression: the process by which genes produce proteins

Two stages:

- Transcription
- Translation



Genes can be expressed at different efficiencies

- Gene A is transcribed much more efficiently than gene B
- This allows the amount of protein A in the cell to be greater than protein B
- The lower expression of gene B is a reason behind incomplete dominance

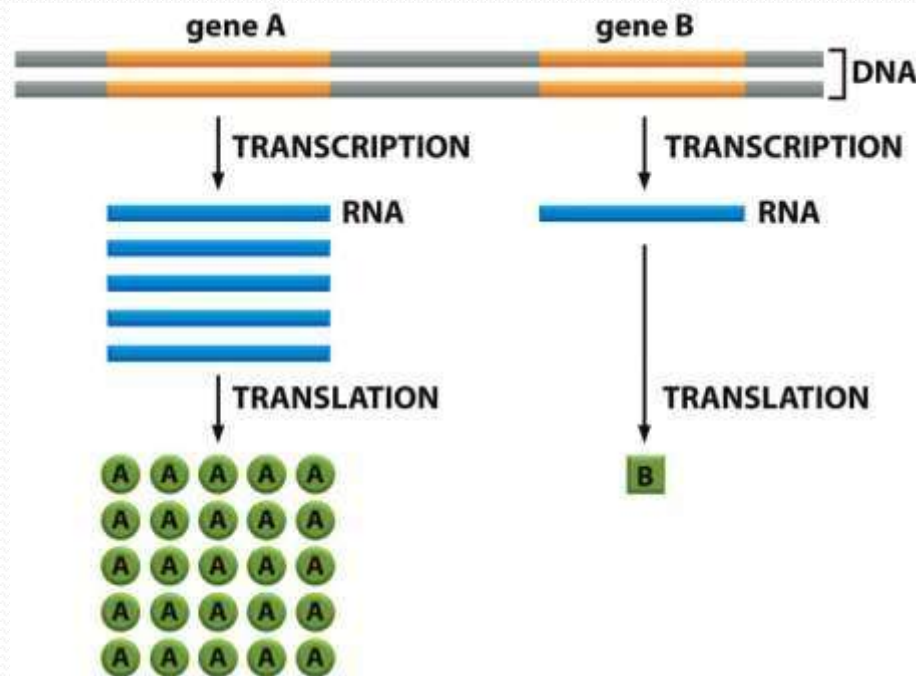


Figure 5-3 Molecular Biology of the Cell 5/e (© Garland Science 2008)

A reminder: what is RNA?

- RNA is the bridge between genes and the proteins for which they code

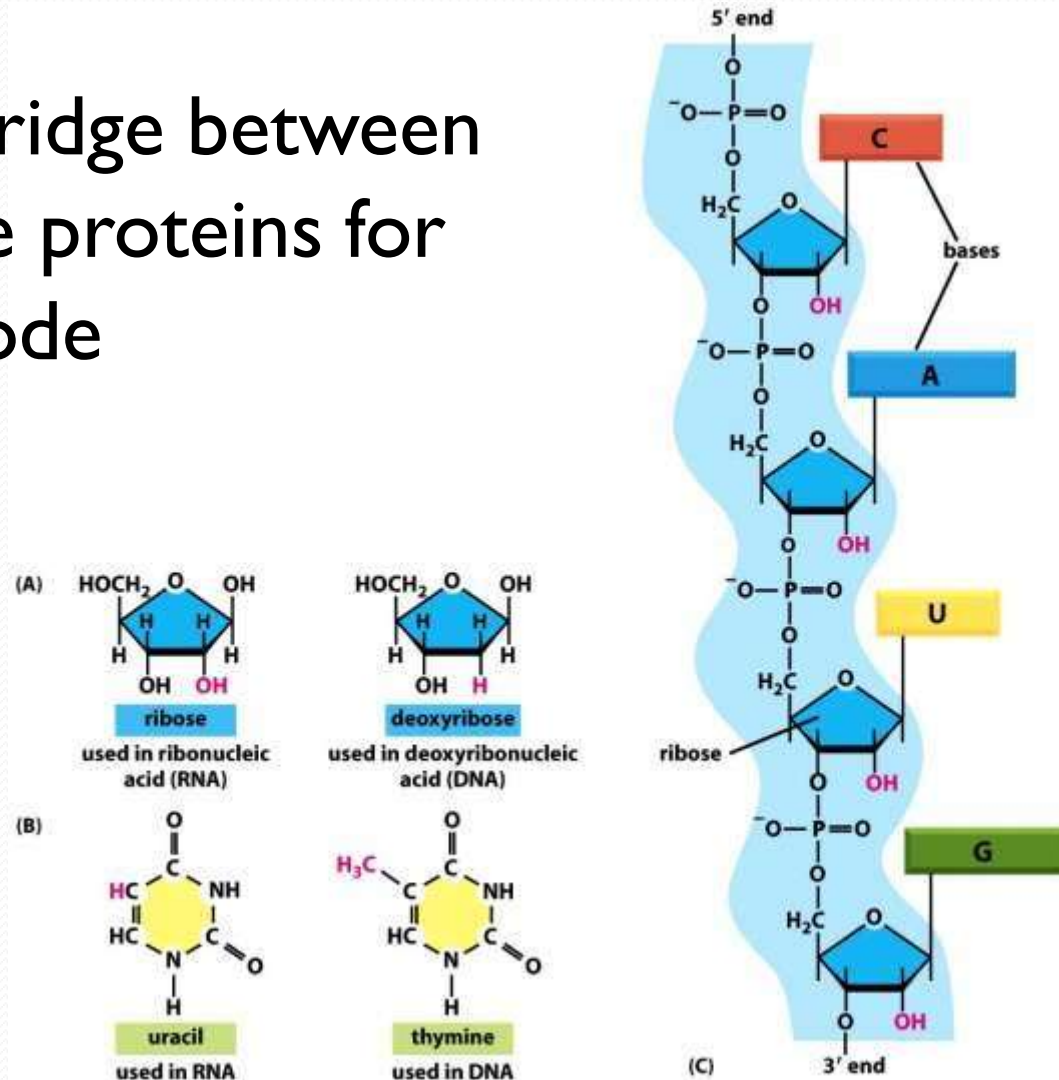


Figure 6-4 Molecular Biology of the Cell 5/e (© Garland Science 2008)

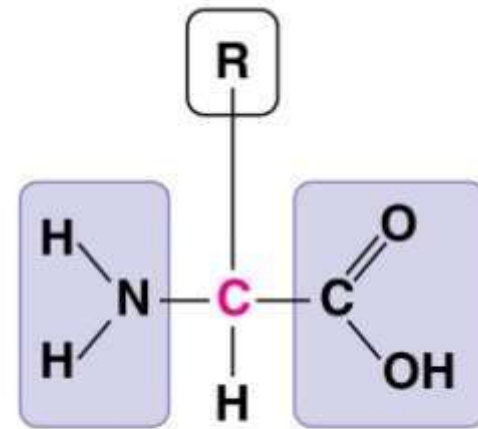
Type of RNA	Functions
Messenger RNA (mRNA)	Carries information specifying amino acid sequences of proteins from DNA to ribosomes
Transfer RNA (tRNA)	Serves as translator molecule in protein synthesis; translates mRNA codons into amino acids
Ribosomal RNA (rRNA)	Plays catalytic (ribozyme) roles and structural roles in ribosomes
Primary transcript	Is a precursor to mRNA, rRNA, or tRNA, before being processed; some intron RNA acts as a ribozyme, catalyzing its own splicing
Small nuclear RNA (snRNA)	Plays structural and catalytic roles in spliceosomes, the complexes of protein and RNA that splice pre-mRNA

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A reminder: what are proteins made of?

- Monomers of proteins are **amino acids**

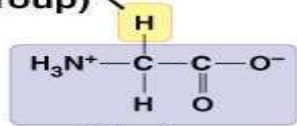
There are 20 amino acids
Each has a different property
depending on its R group/side
chain



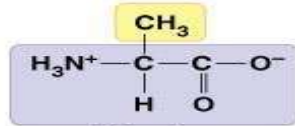
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Nonpolar side chains; hydrophobic

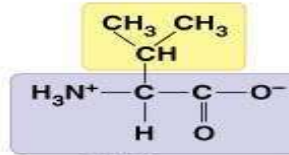
Side chain
(R group)



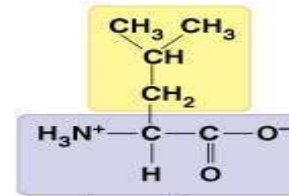
Glycine
(Gly or G)



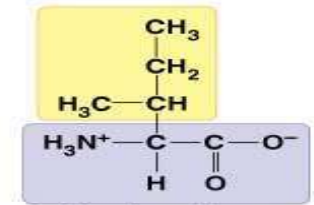
Alanine
(Ala or A)



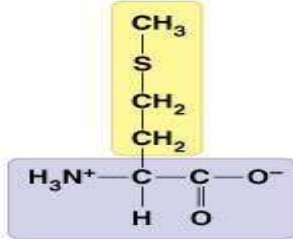
Valine
(Val or V)



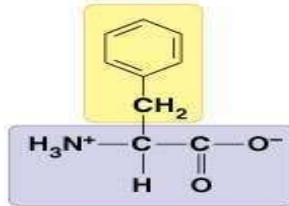
Leucine
(Leu or L)



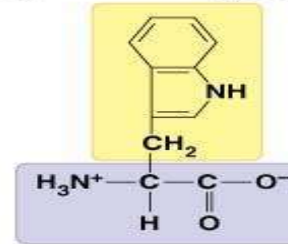
Isoleucine
(Ile or I)



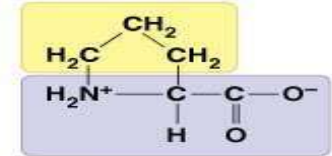
Methionine
(Met or M)



Phenylalanine
(Phe or F)

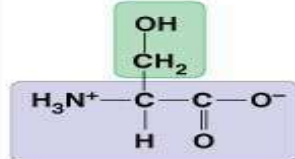


Tryptophan
(Trp or W)

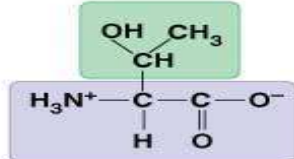


Proline
(Pro or P)

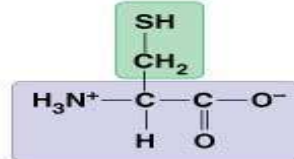
Polar side chains; hydrophilic



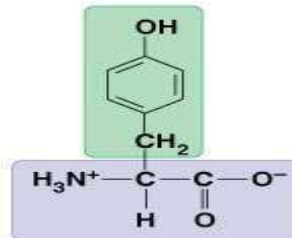
Serine
(Ser or S)



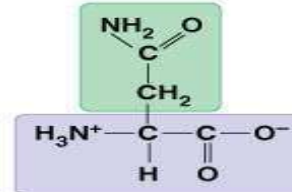
Threonine
(Thr or T)



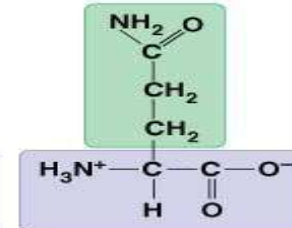
Cysteine
(Cys or C)



Tyrosine
(Tyr or Y)



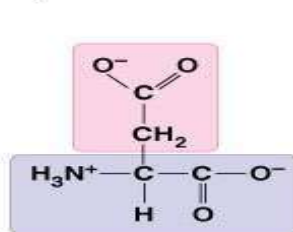
Asparagine
(Asn or N)



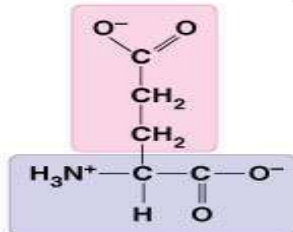
Glutamine
(Gln or Q)

Electrically charged side chains; hydrophilic

Acidic (negatively charged)

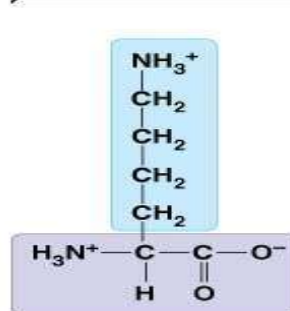


Aspartic acid
(Asp or D)

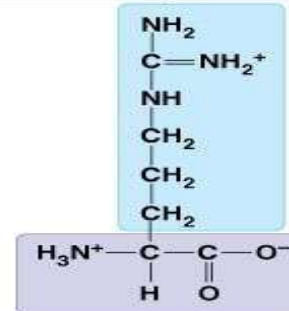


Glutamic acid
(Glu or E)

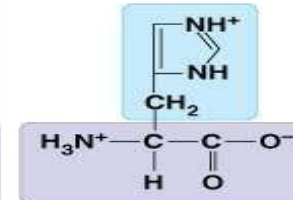
Basic (positively charged)



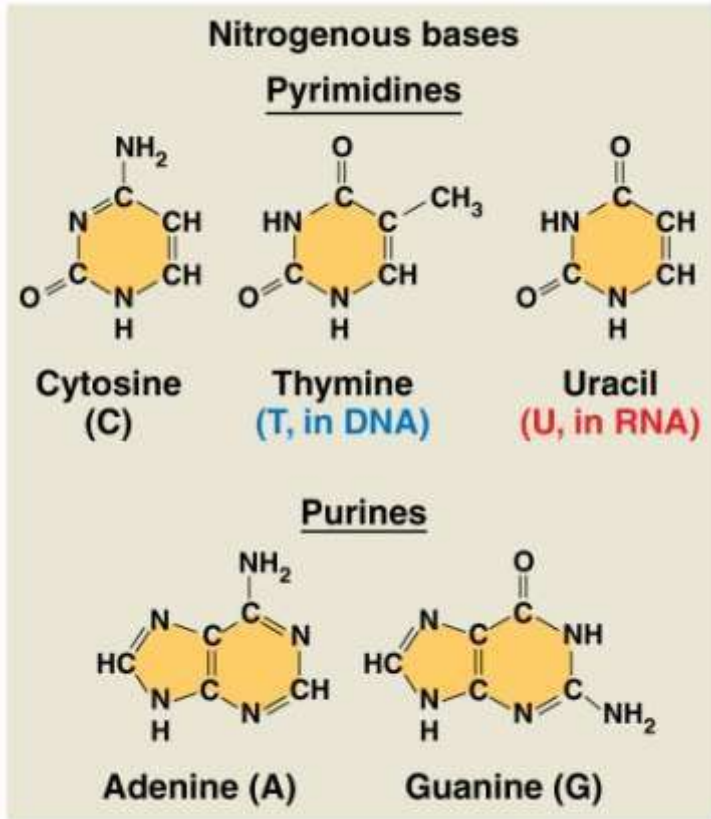
Lysine
(Lys or K)



Arginine
(Arg or R)

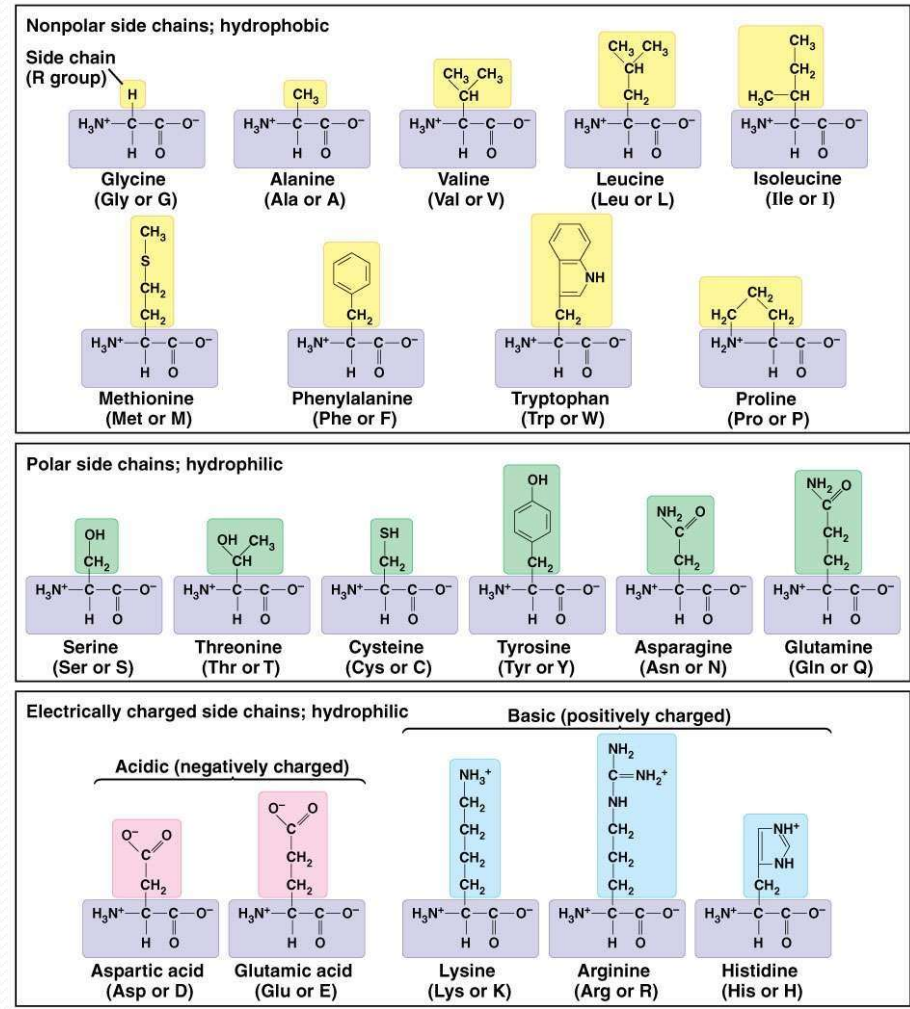


Histidine
(His or H)



?

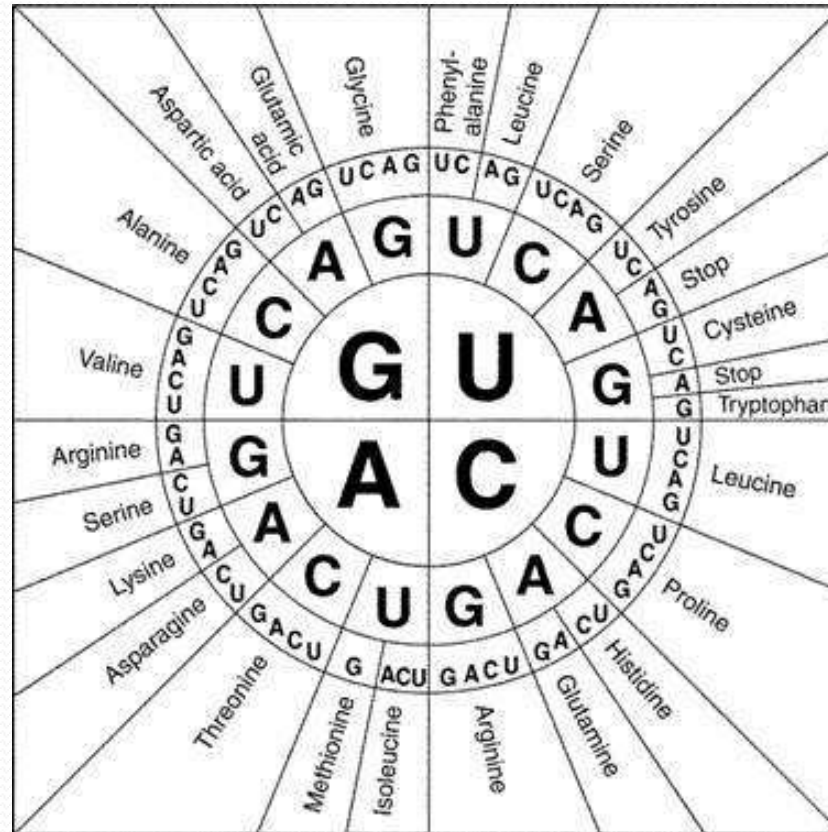
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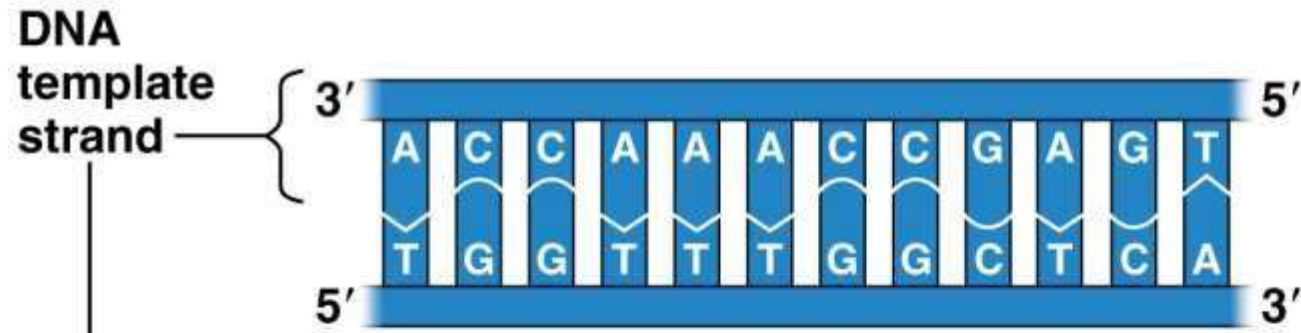


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Codons

- A **codon** is three nucleotides in a row on an RNA molecule that codes for a single amino acid
- A specific three-nucleotide sequence encodes for each amino acid





TRANSCRIPTION

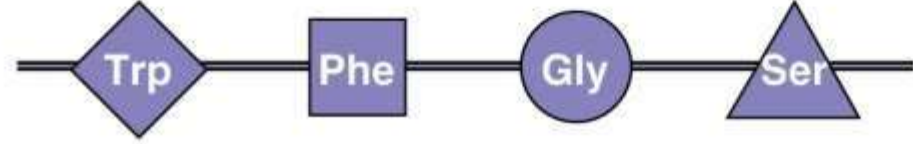
mRNA



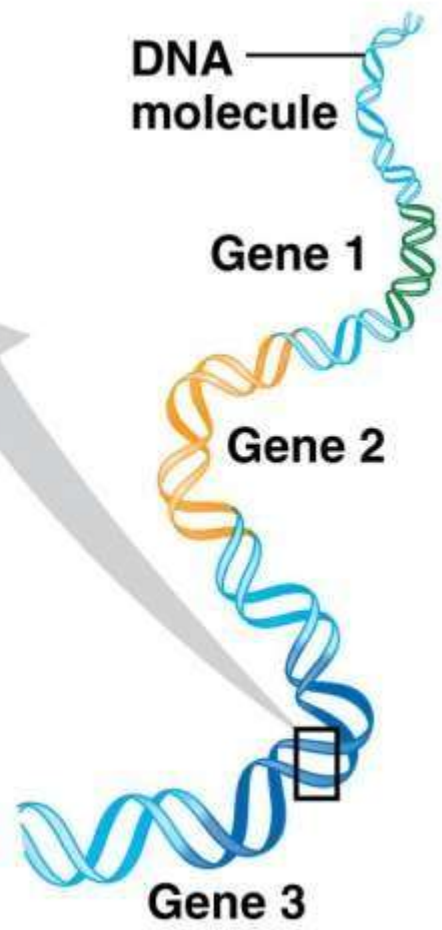
Codon

TRANSLATION

Protein



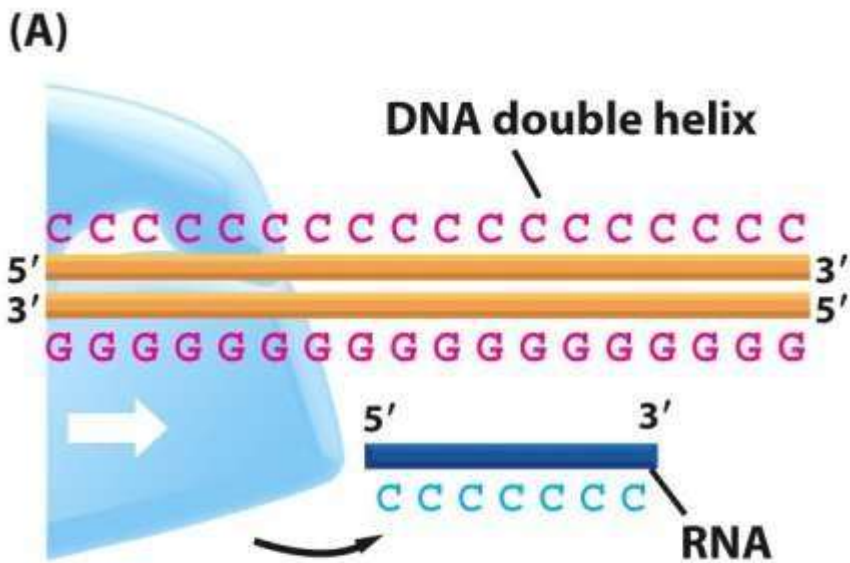
Amino acid



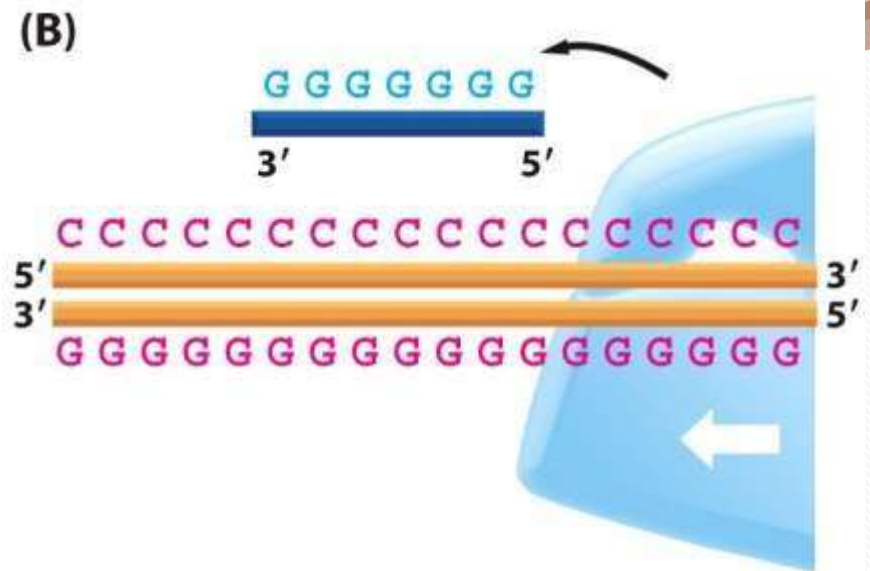
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Template Strand

- During transcription, one of the two DNA strands, called the **template strand**, provides a template for ordering the sequence of complementary nucleotides in an RNA transcript
 - The template strand is always the same strand for a given gene
 - However, different genes may be on opposite strands



an RNA polymerase that moves from left to right makes RNA by using the bottom strand as a template



an RNA polymerase that moves from right to left makes RNA by using the top strand as a template

Figure 6-13 Molecular Biology of the Cell 5/e (© Garland Science 2008)

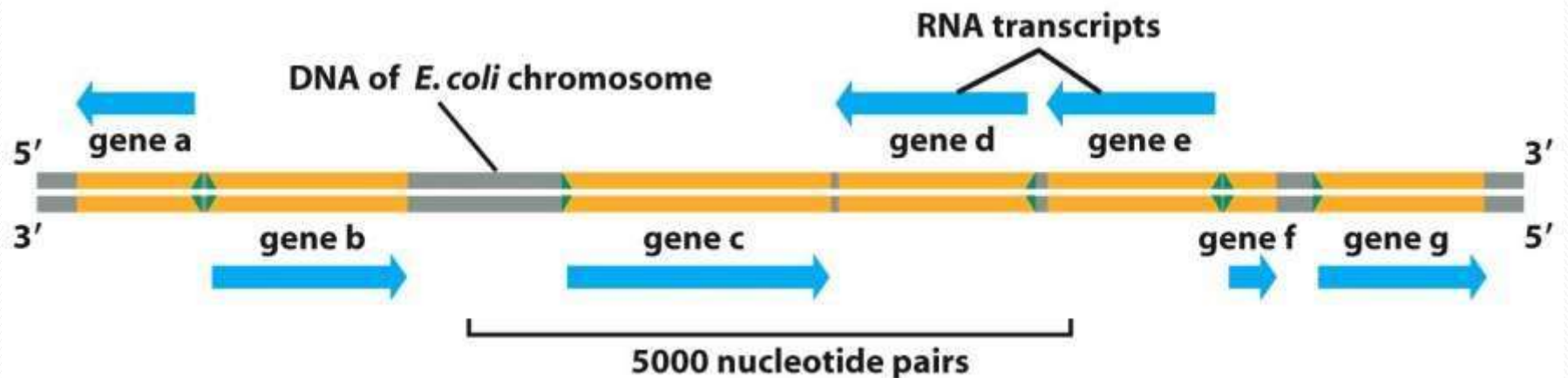


Figure 6-14 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Evolution of the code

- The genetic code is nearly universal, shared by the simplest bacteria to the most complex animals
 - Some species prefer certain codons (**codon bias**)
- Genes can be transcribed and translated after being transplanted from one species to another



(a) Tobacco plant expressing a firefly gene

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(b) Pig expressing a jellyfish gene



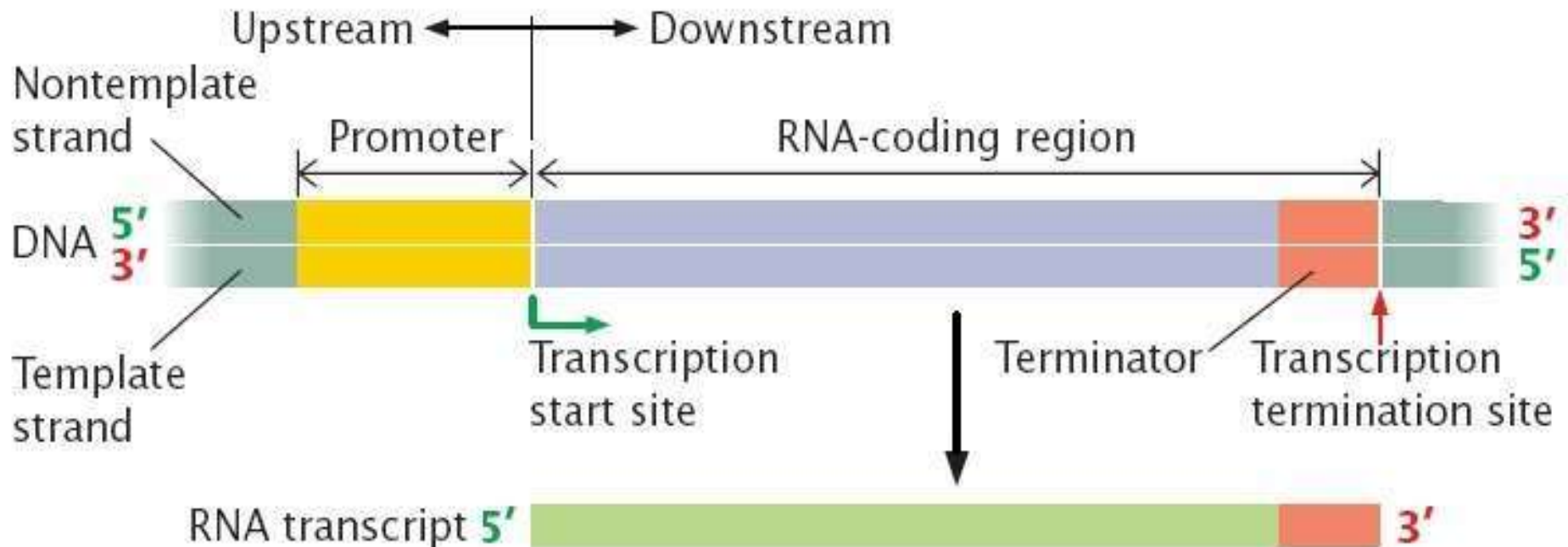
Transcription

DNA → RNA

- DNA is transcribed into a special type of RNA called **messenger RNA**, or **mRNA**
- mRNA synthesis is catalyzed by **RNA polymerase**, which pries the DNA strands apart and hooks together the RNA nucleotides
- The RNA is complementary to the DNA template strand
 - RNA synthesis follows the same base-pairing rules as DNA, except that uracil substitutes for thymine

The Transcription Unit

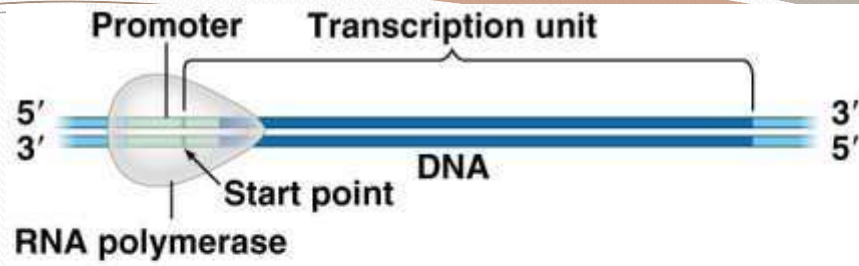
- The stretch of DNA that is used in transcription

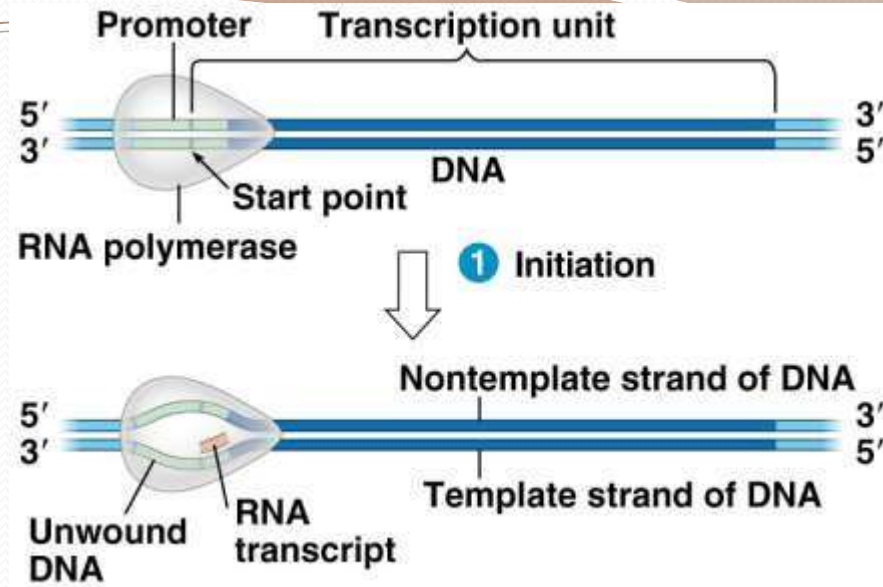


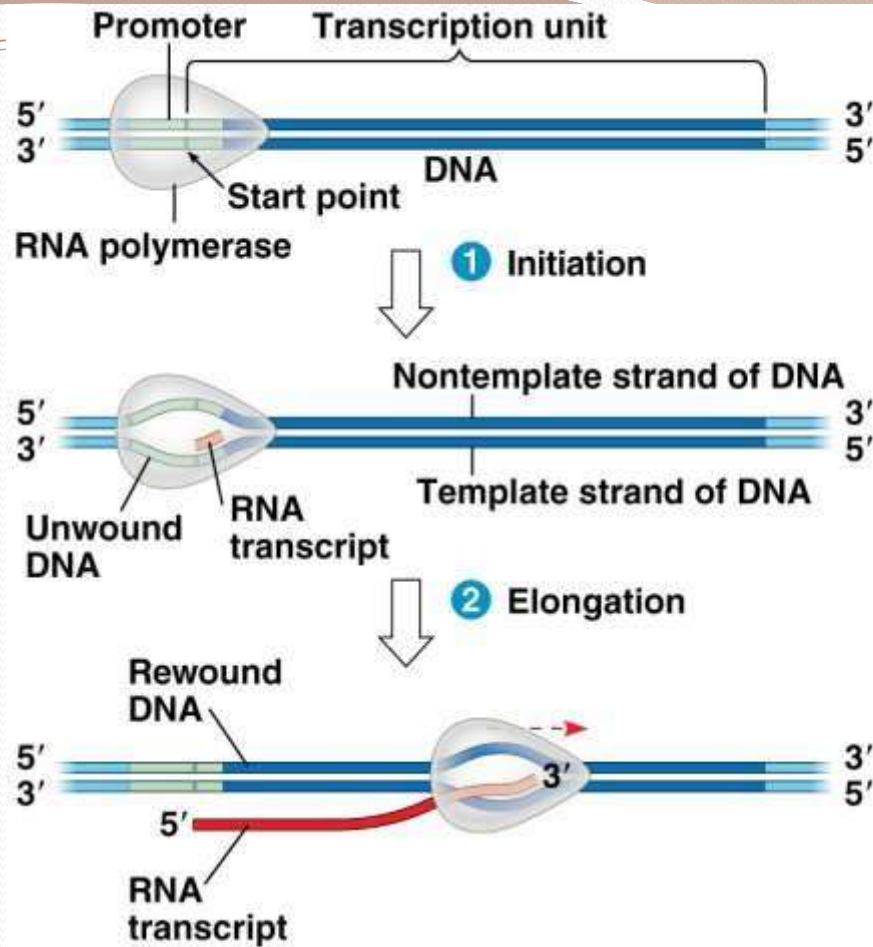
Transcription

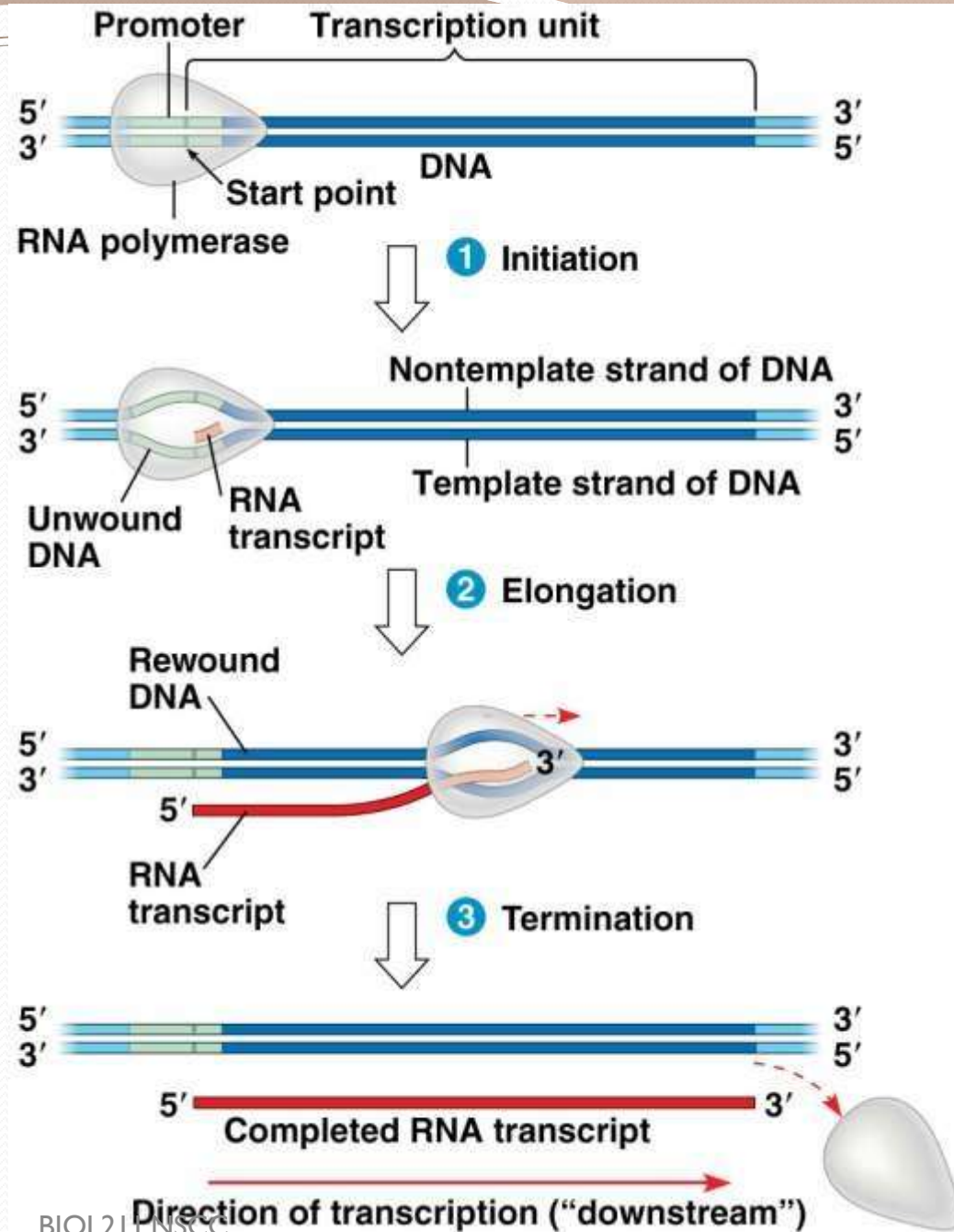
Generic process for both prokaryotes and eukaryotes

- **Initiation**
 - RNA polymerase binds to the promoter of a gene and begins to unwind the DNA
- **Elongation**
 - RNA pol 'reads' the template strand in 3' to 5' direction and adds complementary ribonucleotides
- **Termination**
 - RNA pol hits a stop signal (prokaryotes) or falls off (eukaryotes)









Transcription: Initiation

- **Promoters** control when, how, and at what level a gene is transcribed
 - Composed of a distinct sequence of nucleotides
- **Transcription factors** help RNA polymerase bind to the promoter
 - Transcription factors bind to areas in and around the promoter and provide a “landing pad” for RNA pol

Regulators of gene transcription

- Promoters
- Enhancers
 - Upstream sequences in eukaryotes that help to control the expression of genes
 - Can be thousands of nucleotides away from the protein-coding region
- Silencers
 - When transcription factors bind, they prevent a gene from being transcribed

Promoters in prokaryotes

- Prokaryotes
 - Highly conserved nucleotide sequences at -10 and -35 base pairs upstream of the start of the gene
 - -10 sequence is TATA, called “TATA box”

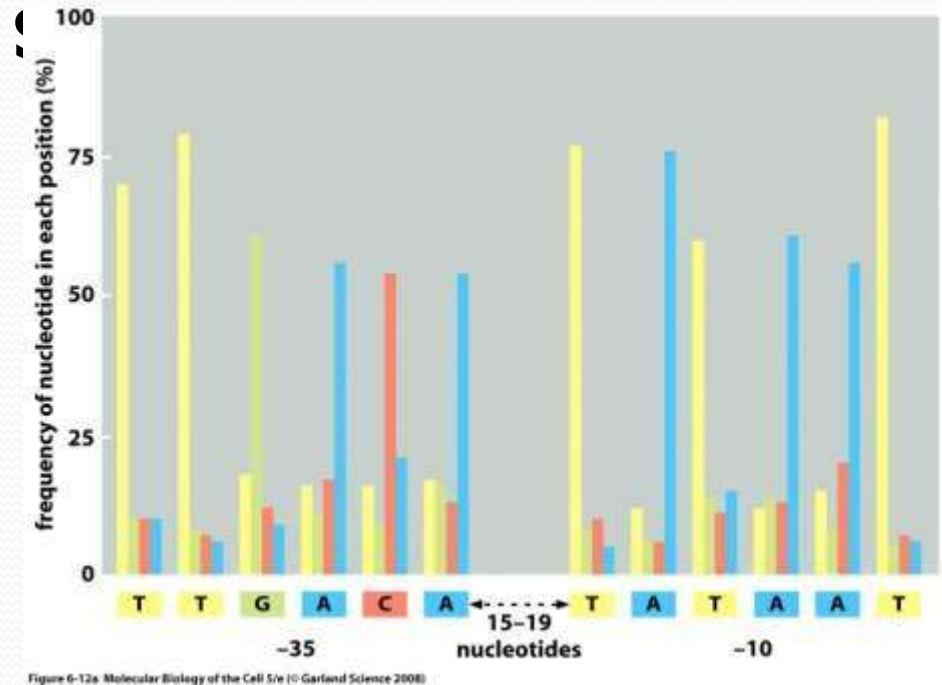


Figure 6-12a Molecular Biology of the Cell 5/e (© Garland Science 2008)

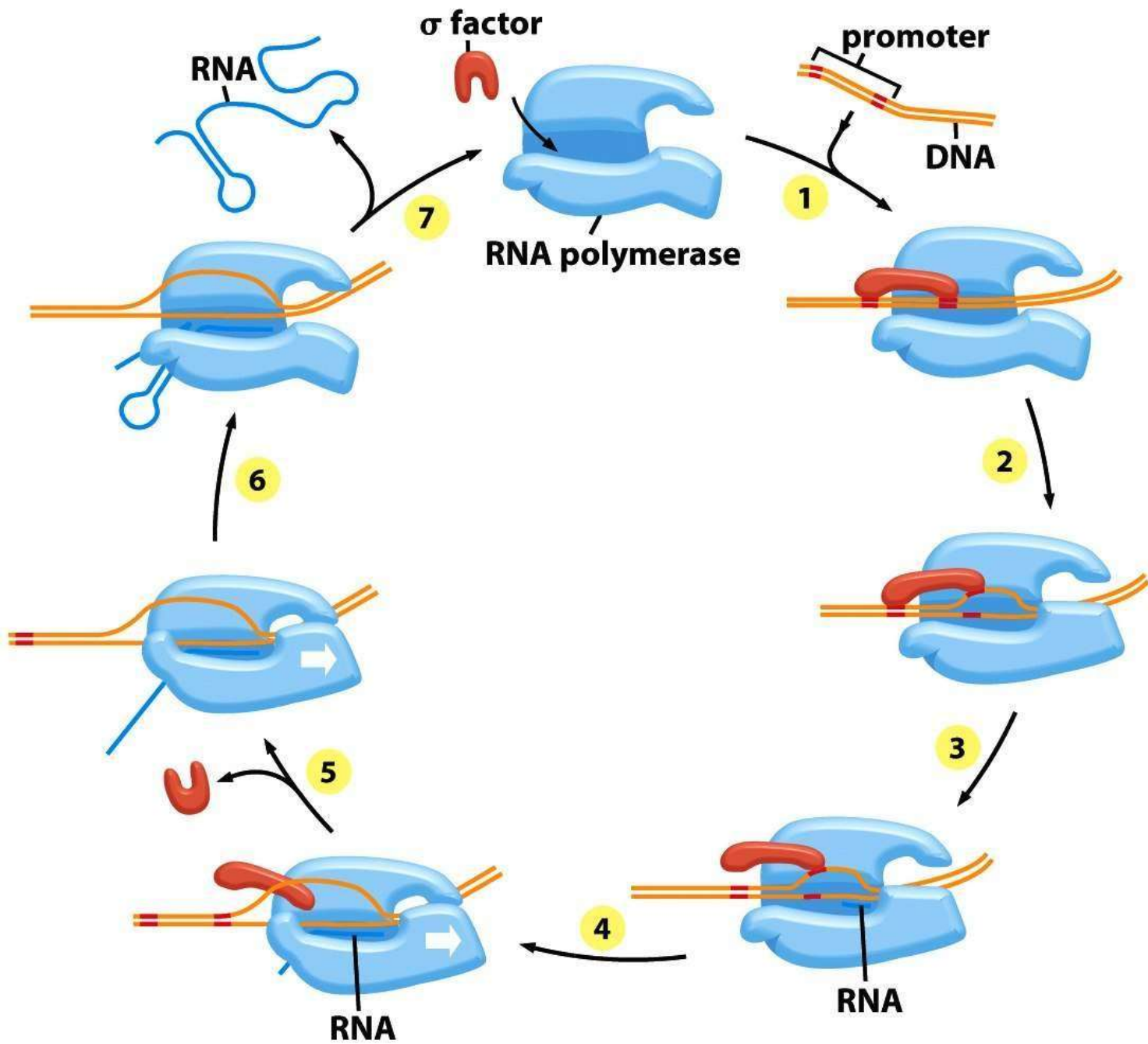


Figure 6-11 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Promoters in eukaryotes

- Eukaryotes
 - A “basal promoter” made of a TATA box and other elements ~25bp upstream
 - A conserved region around the start of the gene called “Inr” for initiation
 - Many different transcription factors

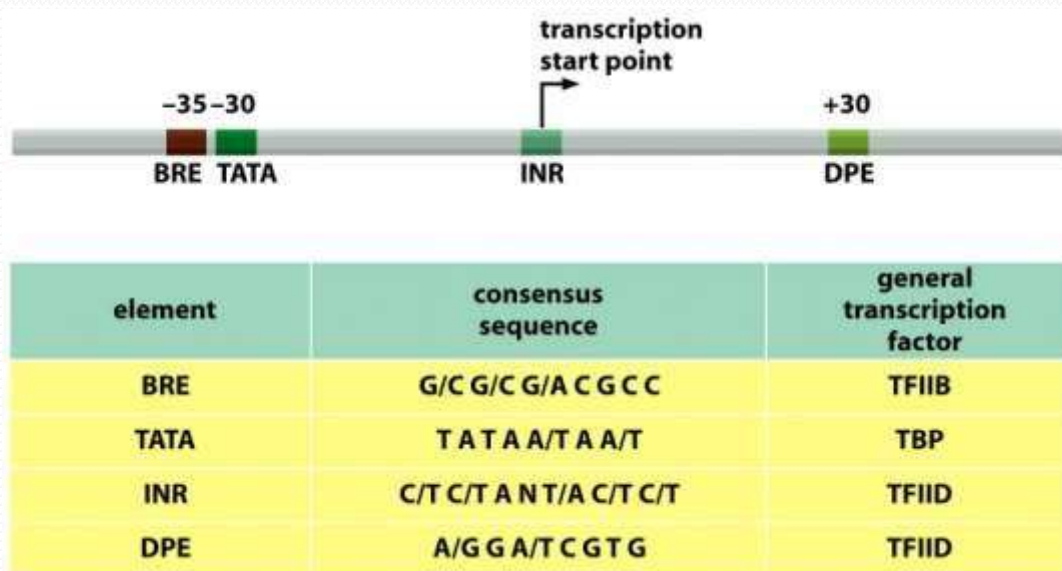
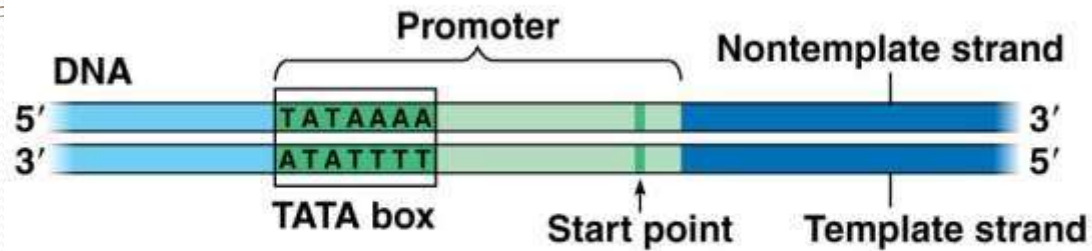
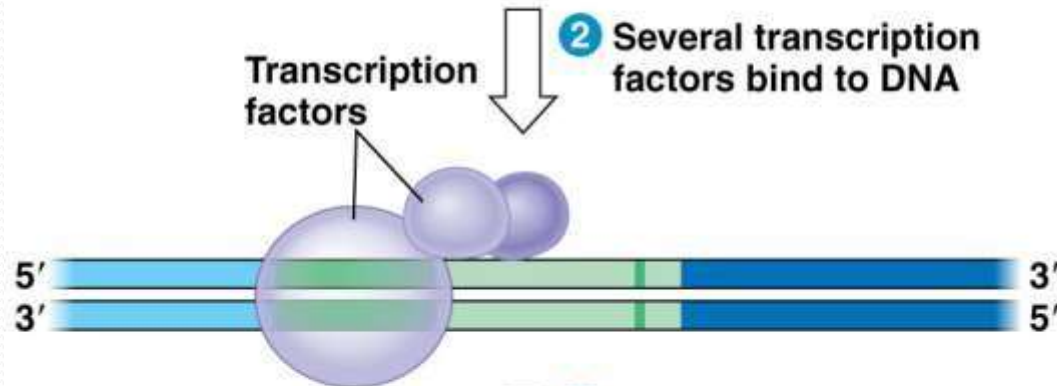


Figure 6-17 Molecular Biology of the Cell 5/e (© Garland Science 2008)

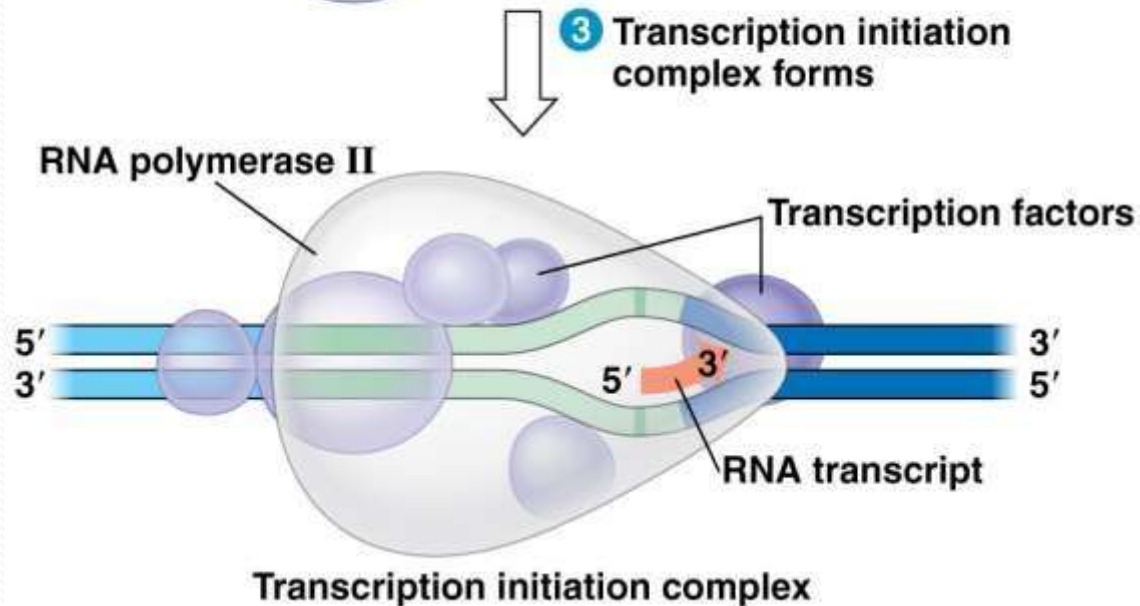
1 A eukaryotic promoter



2 Several transcription factors bind to DNA

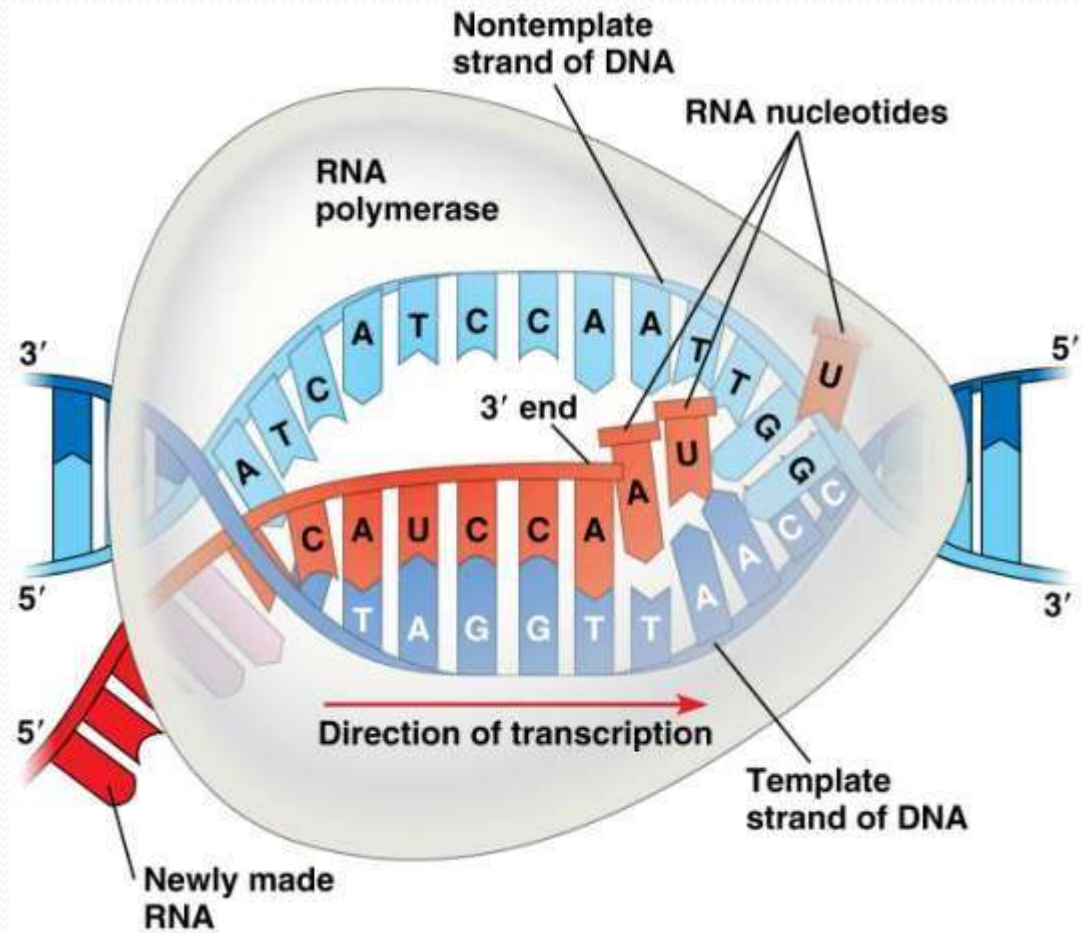


3 Transcription initiation complex forms

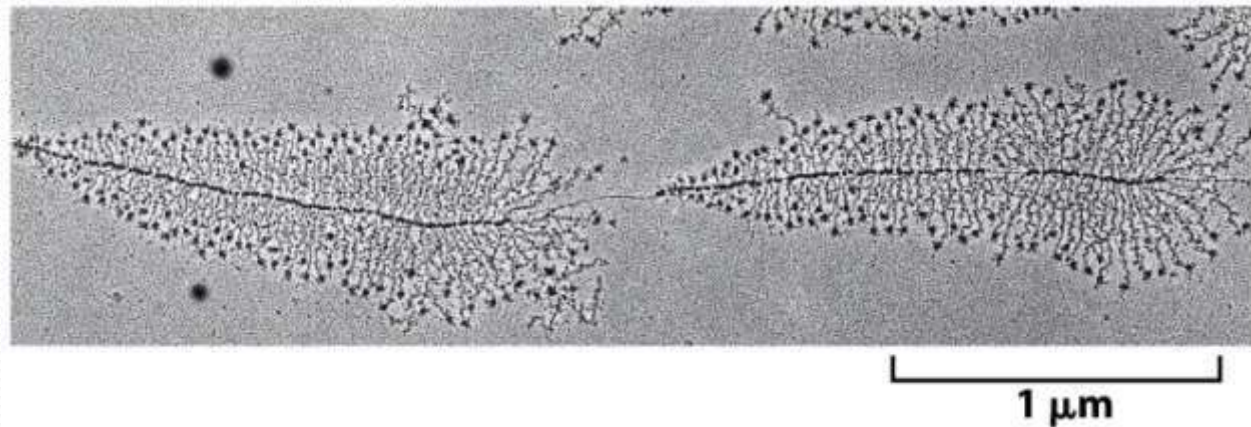


Transcription: Elongation

- As RNA polymerase moves along the DNA, it untwists the double helix, 10 to 20 bases at a time
- Transcription progresses at a rate of 40 nucleotides per second in eukaryotes
 - Actually 24/s in eukaryotes and 60/s in prokaryotes
- A gene can be transcribed simultaneously by several RNA polymerases
- Nucleotides are added to the 3' end of the growing RNA molecule



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Transcription:Termination

- The mechanisms of termination are different in bacteria and eukaryotes
 - In bacteria, the polymerase stops transcription at the terminator signal and the mRNA can then be translated without further modification

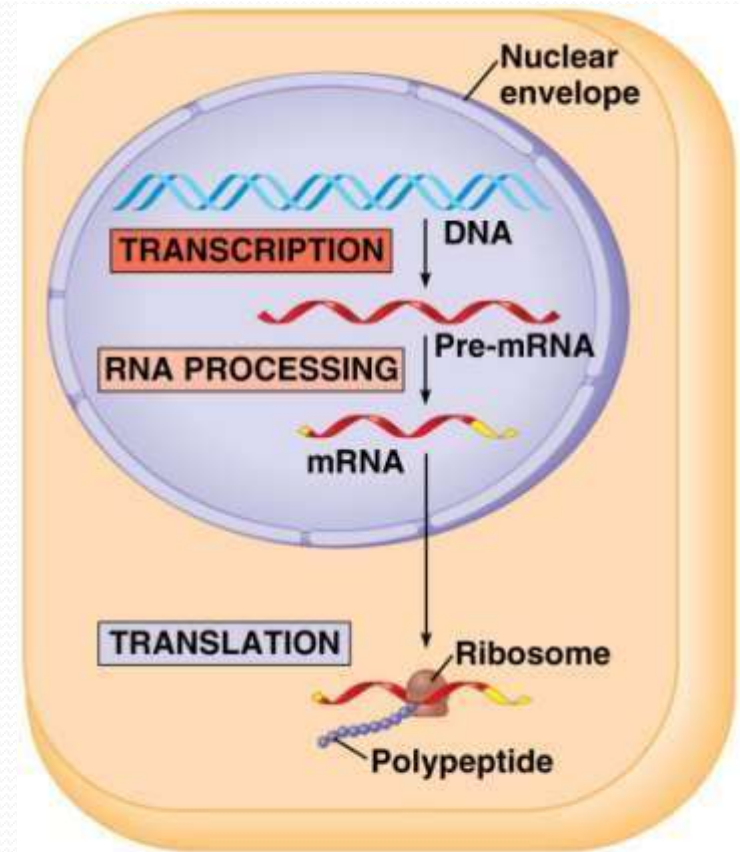
Transcription: Termination

- In eukaryotes, RNA polymerase II transcribes the **polyadenylation signal sequence**
- The RNA transcript is released 10–35 nucleotides past this polyadenylation sequence
 - What exactly boots RNA pol off after the poly-A tail is unknown

RNA Processing

- In eukaryotes, before mRNA can be translated it has to first be processed
 - Prokaryotes don't need processing
- Pre-mRNA is renamed to mRNA after being processed

DNA → pre-mRNA → mRNA → protein



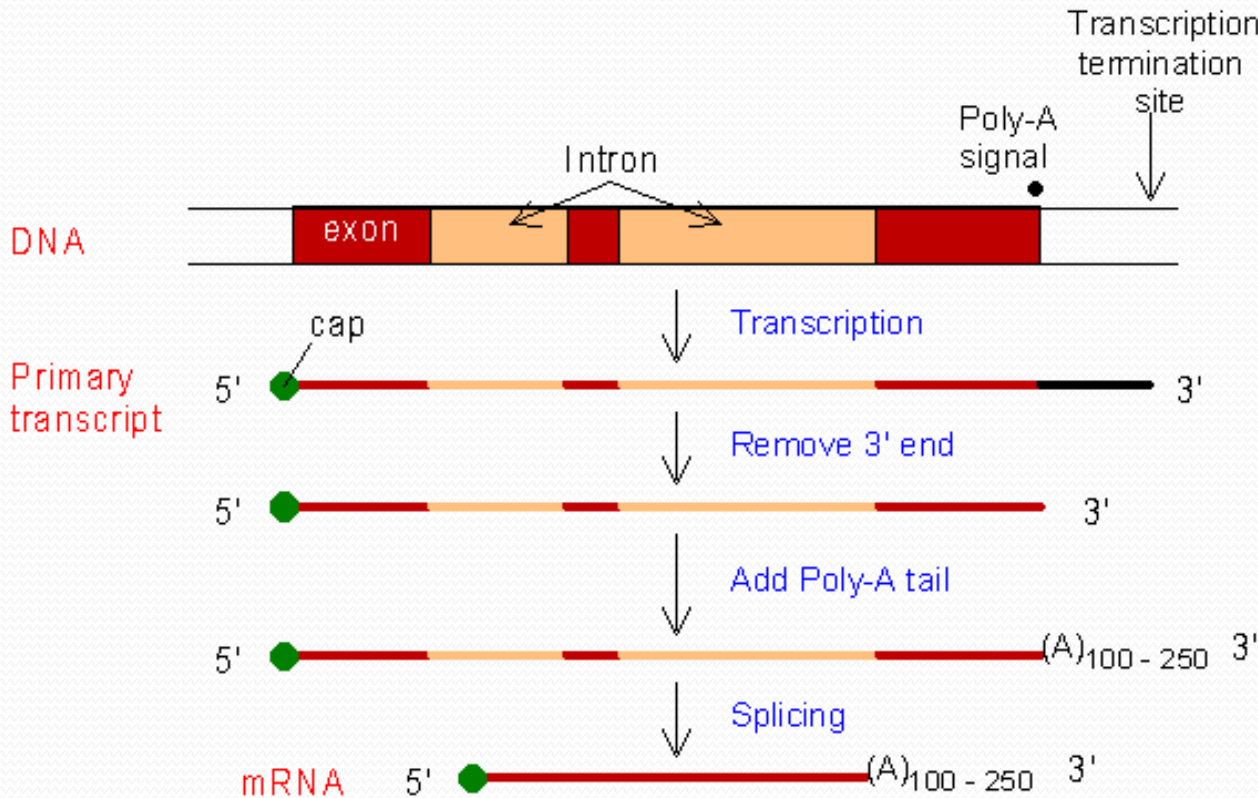
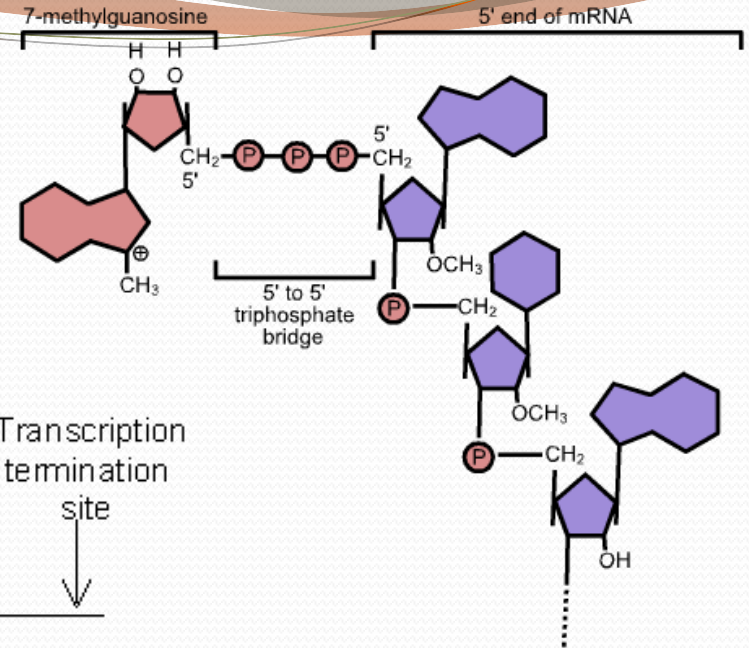
(b) Eukaryotic cell

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RNA Processing

- A pre-mRNA is processed in three particular ways:
 - The 5' end receives a modified nucleotide **5' cap**
 - The 3' end gets a **poly-A tail**
 - Exons are spliced out
- These modifications share several functions
 - They seem to facilitate the export of mRNA
 - They protect mRNA from hydrolytic enzymes
 - They help ribosomes attach to the 5' end

RNA Processing



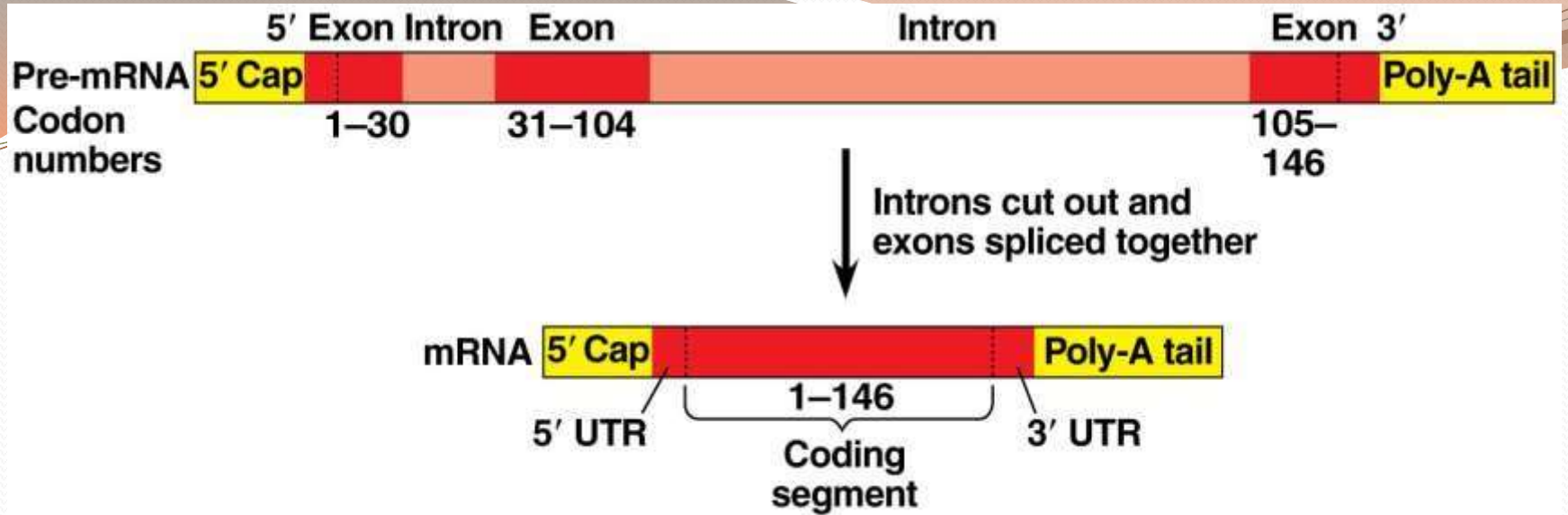
mRNA splicing

Most eukaryotic genes and their RNA transcripts •
have long noncoding stretches of nucleotides that
lie between coding regions

These noncoding regions are called intervening •
sequences, or **introns**

The other regions are called **exons** because they •
are eventually expressed, usually translated into
amino acid sequences

RNA splicing removes introns and joins exons, •
creating an mRNA molecule with a continuous
coding sequence



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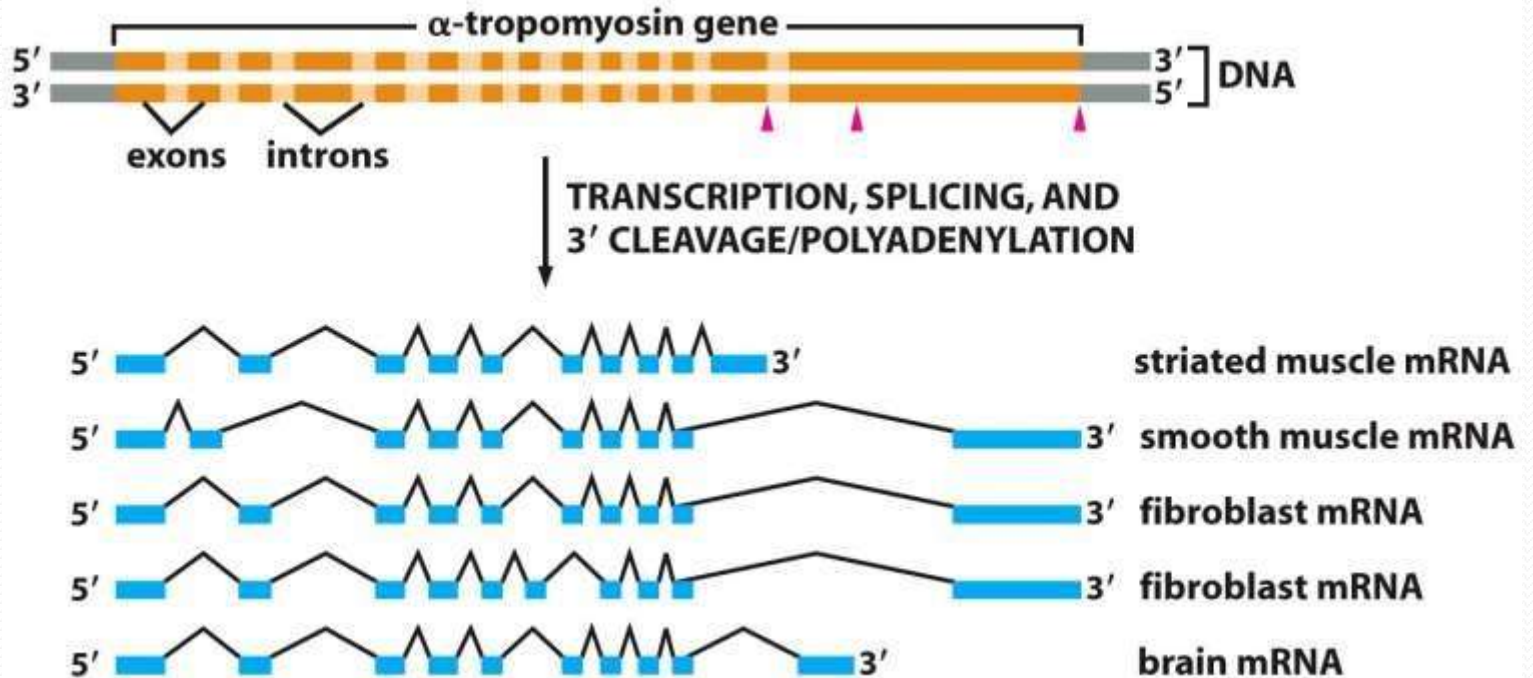
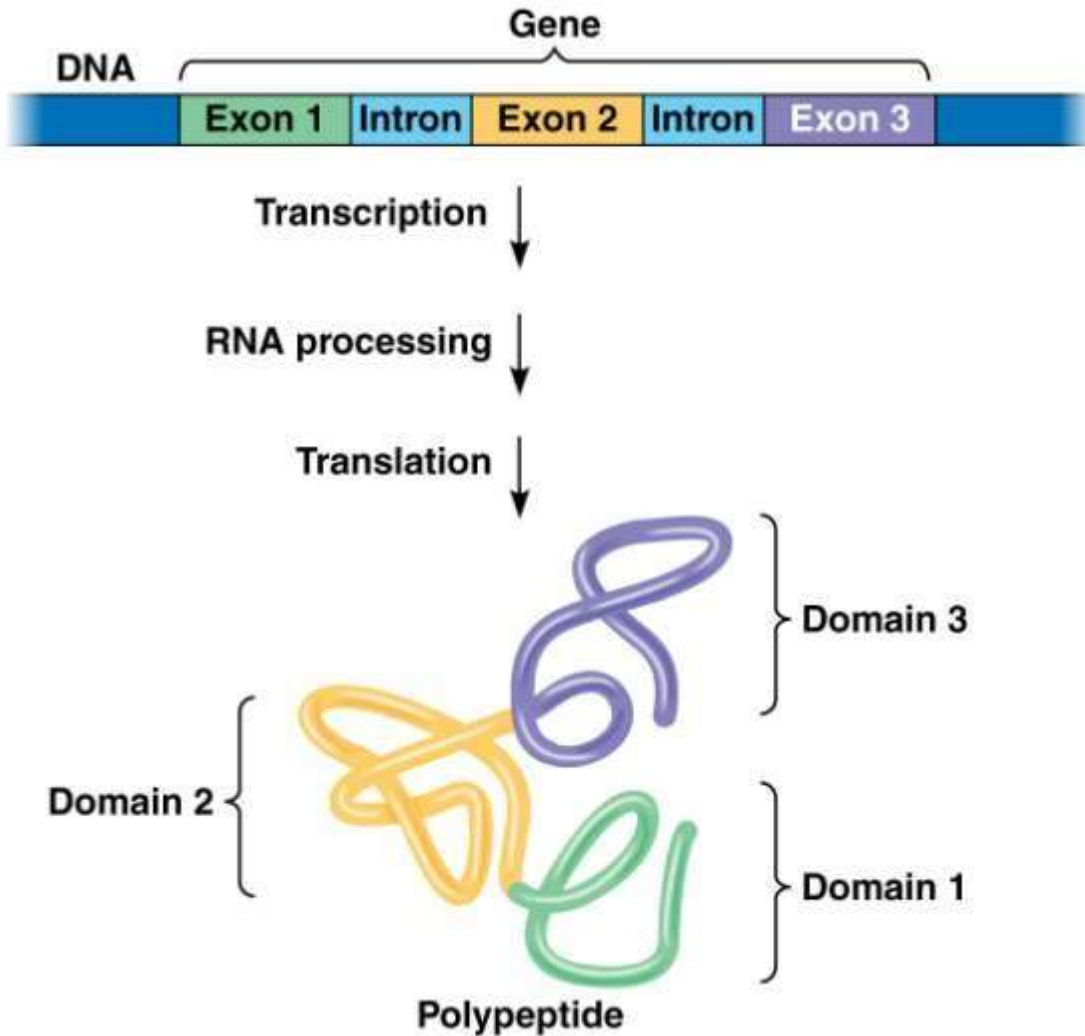


Figure 6-27 Molecular Biology of the Cell 5/e (© Garland Science 2008)

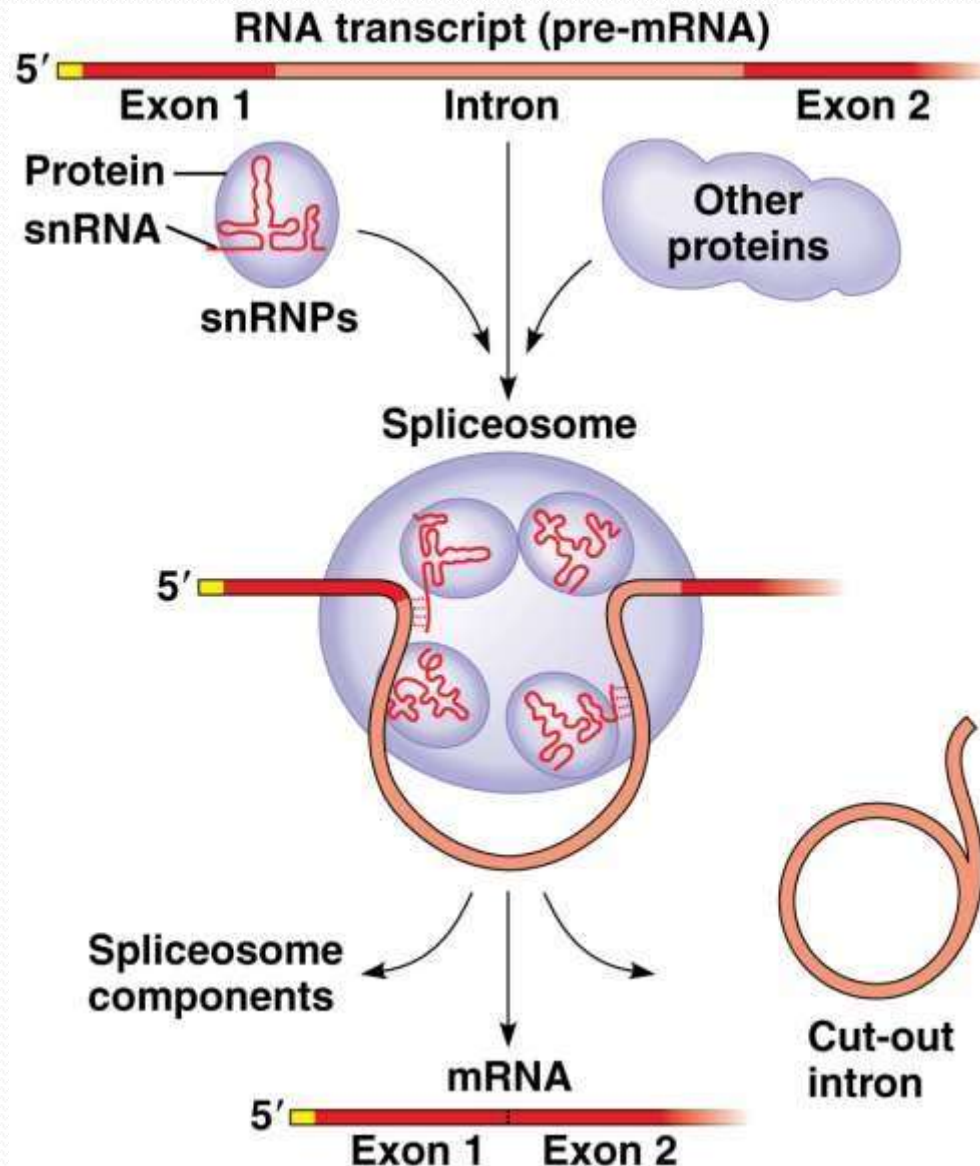
Alternative Splicing

The rearrangement of exons during splicing •



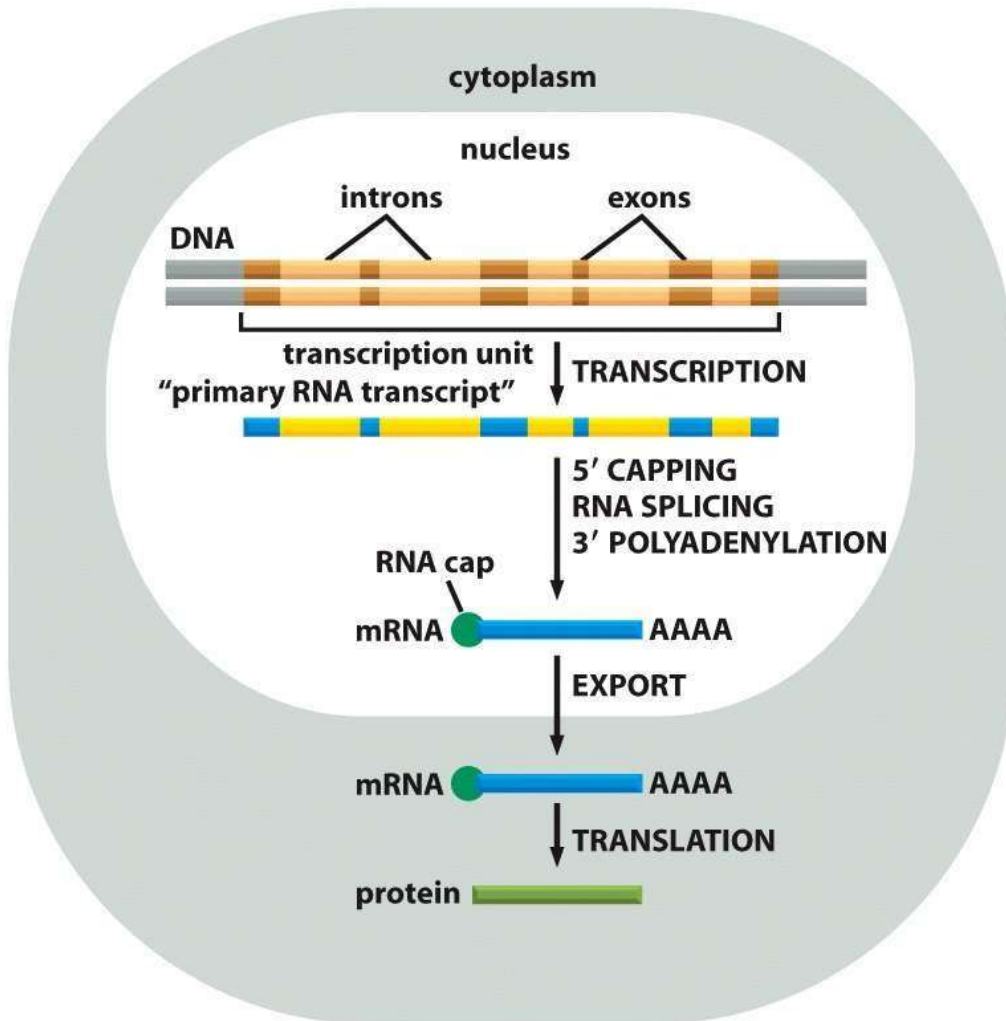
How do we splice?

- Protein complexes called **spliceosomes** recognize and splice out introns
- **Spliceosomes** consist of a variety of proteins and several small nuclear ribonucleoproteins (snRNPs) that recognize the splice sites



(A)

EUCARYOTES



(B)

PROCARYOTES

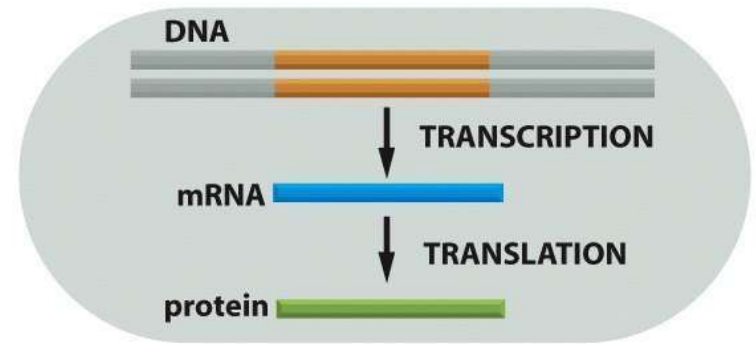
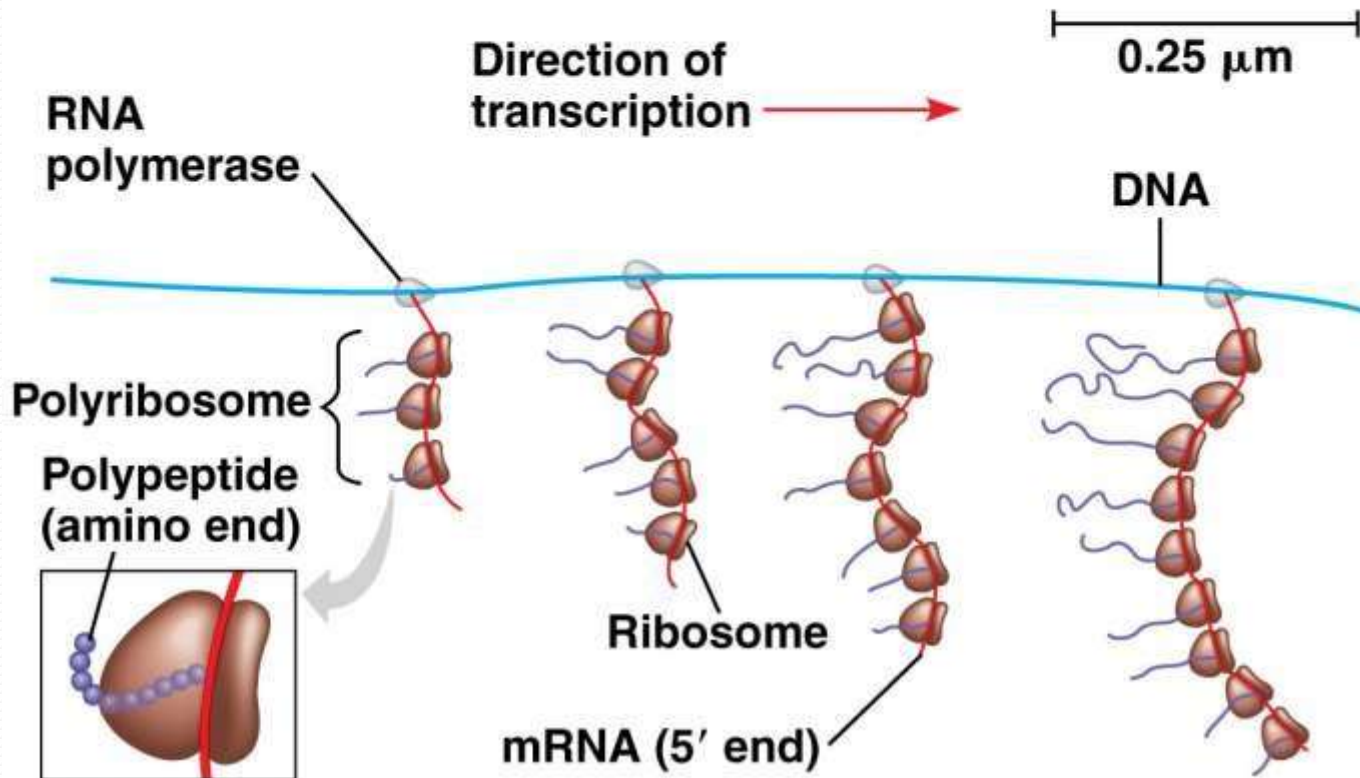
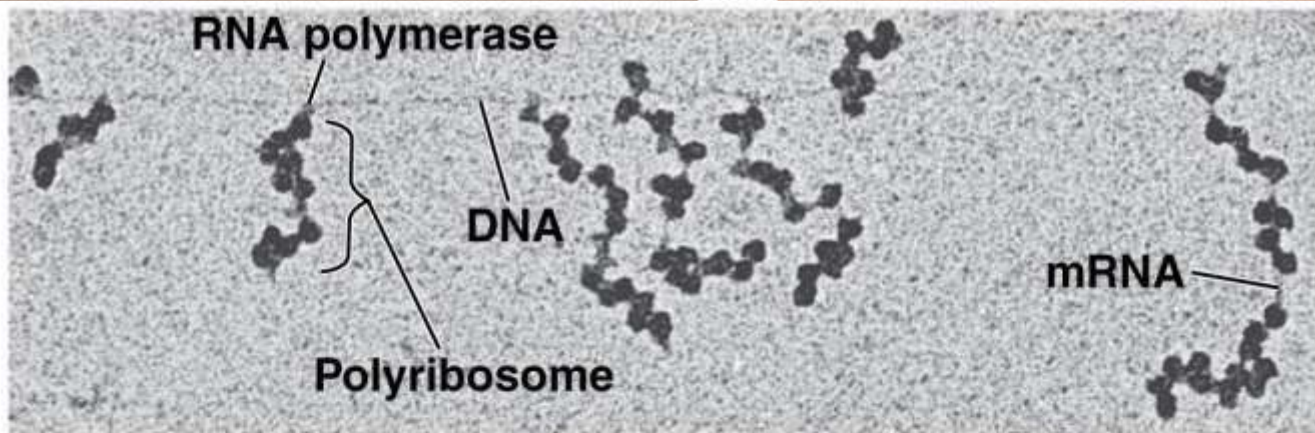


Figure 6-21 Molecular Biology of the Cell 5/e (© Garland Science 2008)

- Bacteria and eukarya differ in their RNA polymerases, termination of transcription, and ribosomes; archaea tend to resemble eukarya in these respects
 - Bacteria can simultaneously transcribe and translate the same gene
 - In eukarya, transcription and translation are separated by the nuclear envelope
 - In archaea, transcription and translation are likely coupled



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- **After processing, mRNA moves through nuclear**

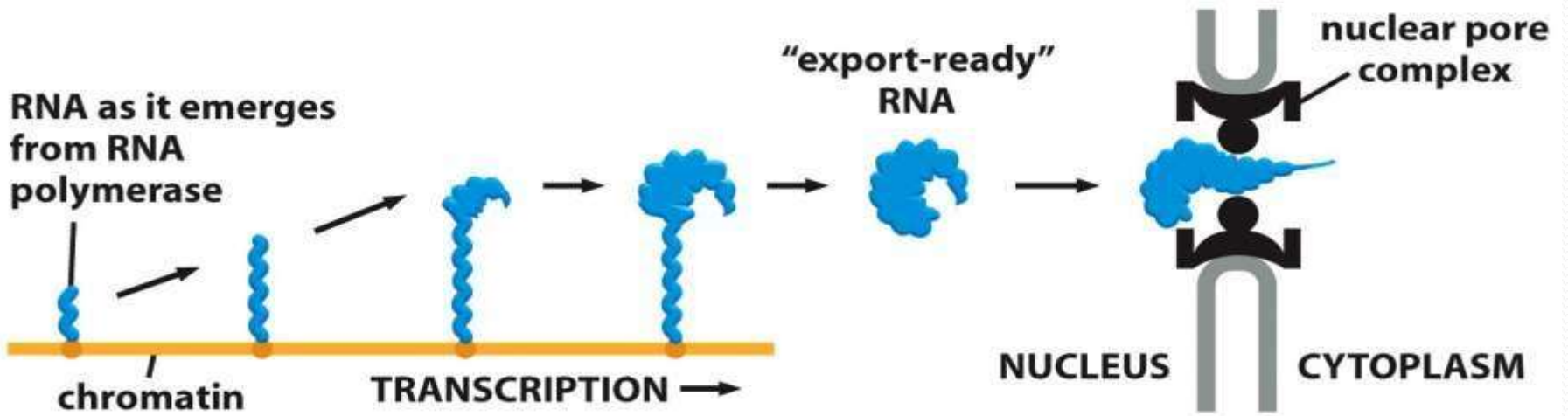


Figure 6-39a Molecular Biology of the Cell 5/e (© Garland Science 2008)

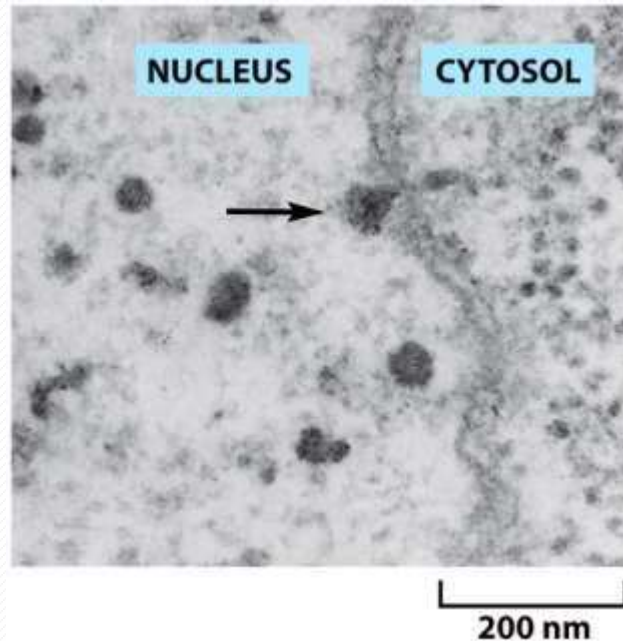


Figure 6-39b Molecular Biology of the Cell 5/e (© Garland Science 2008)



- Reference

- - Molecular biology of the cell, Garland Science , 2008.