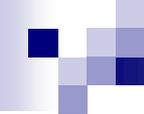


Gene expression

- Gene function at the level of traits
- Gene function at the molecular level

- Two levels tied together since the molecular level affects the structure and function of cells which determines what traits are expressed

- 
- One gene – one enzyme hypothesis has been modified
 - Enzymes are only one category of cellular proteins
 - More accurate to say one gene encodes a polypeptide
 - Hemoglobin composed of 4 polypeptides required for function
 - One gene – one polypeptide theory

Central dogma

■ Transcription

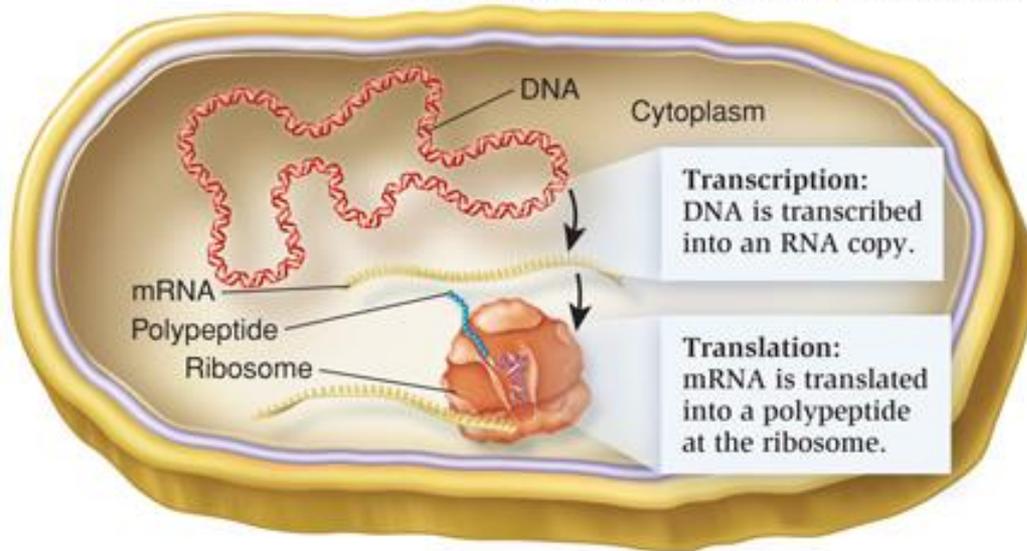
- Produces an RNA copy or transcript of a gene
- Structural genes produce messenger RNA (mRNA) that specifies the amino acid sequence of a polypeptide

■ Translation

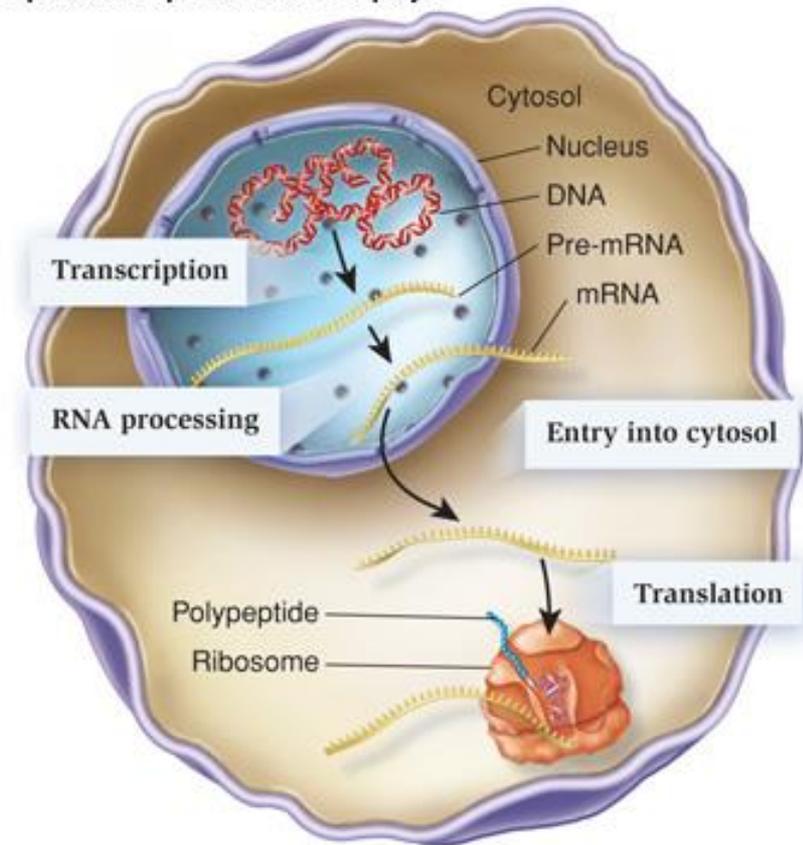
- Process of synthesizing specific polypeptide on a ribosome

■ Eukaryotes have additional intervening step called RNA processing where pre-mRNA is processed into functionally active mRNA

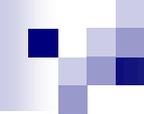
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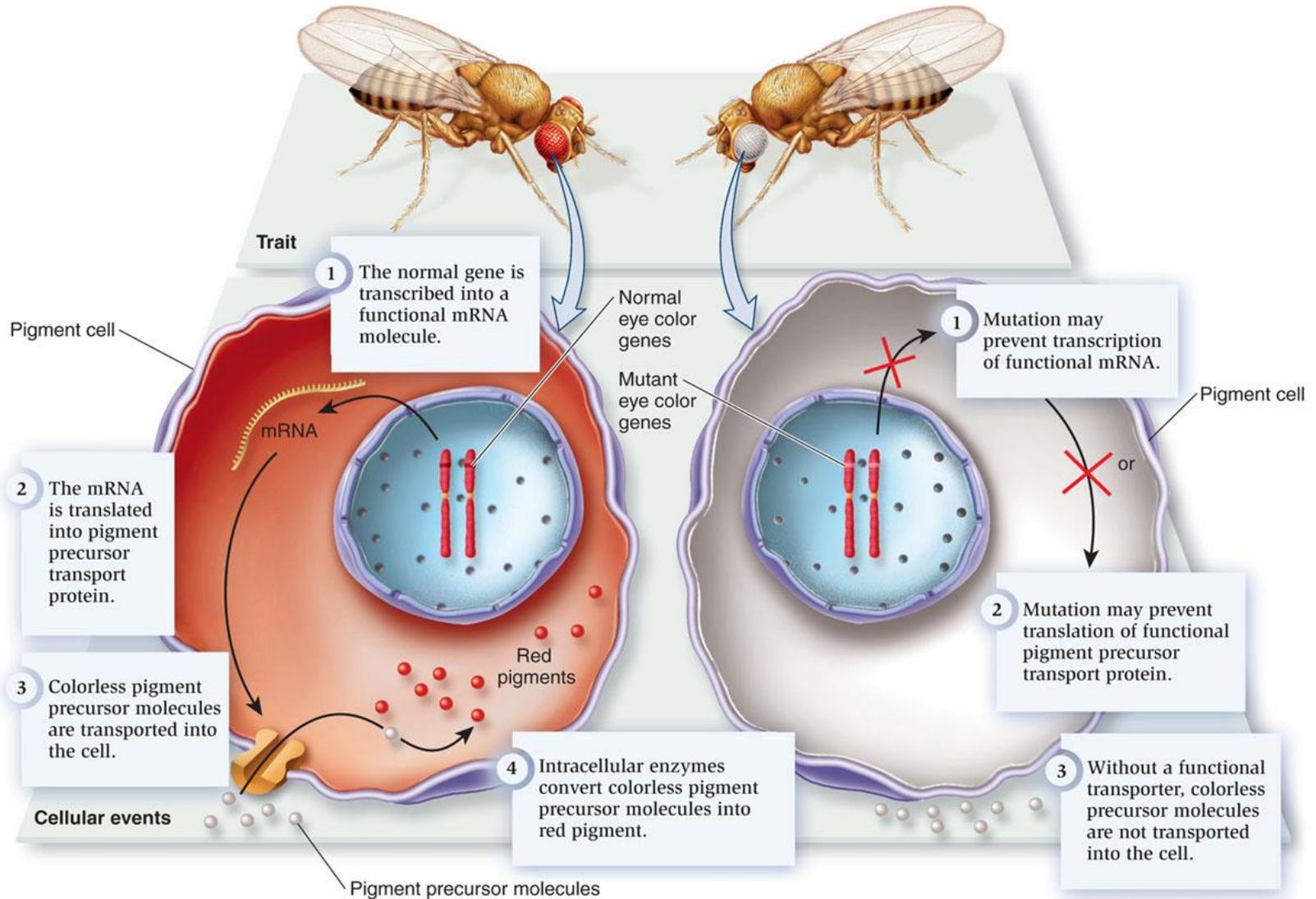


(a) Molecular gene expression in prokaryotes



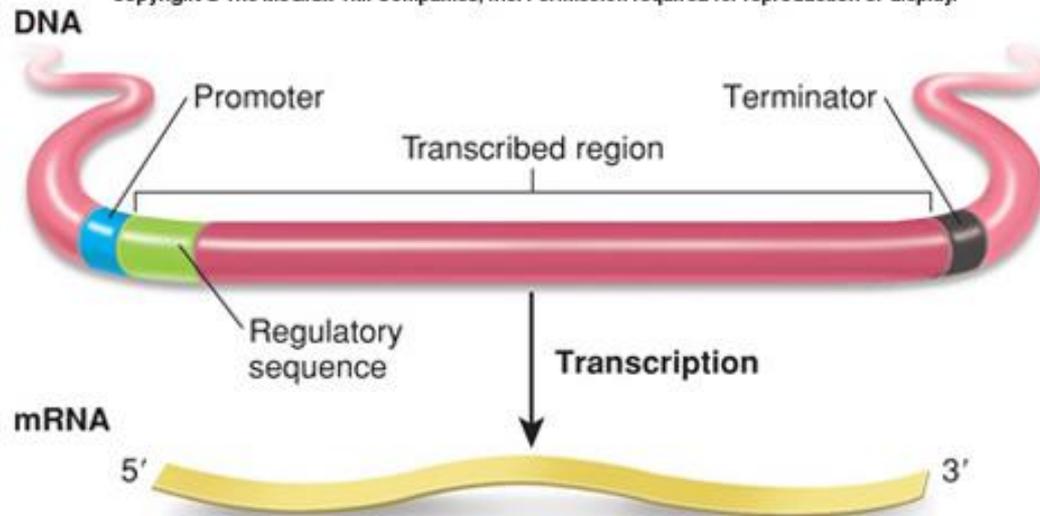
(b) Molecular gene expression in eukaryotes

- 
- Genes contain the information necessary to make the organism and allow it to favorably interact with its environment
 - Structural genes code for polypeptides
 - Polypeptide becomes a unit of function or protein
 - Activities of proteins determine structure and function of cells
 - Traits or characteristics of organism based on cellular activities



Transcription

- A gene is an organized unit of DNA sequences that enables a segment of DNA to be transcribed into RNA and ultimately results in the formation of a functional product
- Over 90% of all genes are structural
- Other genes code for the RNA itself
 - Transfer RNA (tRNA) – translates mRNA into amino acids
 - Ribosomal RNA (rRNA) - part of ribosomes

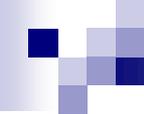


Promoter: Site for RNA polymerase binding; signals the beginning of transcription.

Regulatory sequence: Site for the binding of regulatory proteins. The role of regulatory proteins is to influence the rate of transcription. Regulatory sequences can be found in a variety of locations.

Transcribed region: Contains the information that specifies an amino acid sequence.

Terminator: Signals the end of transcription.



Three stages

1. Initiation
2. Elongation
3. Termination

Three stages

1. Initiation

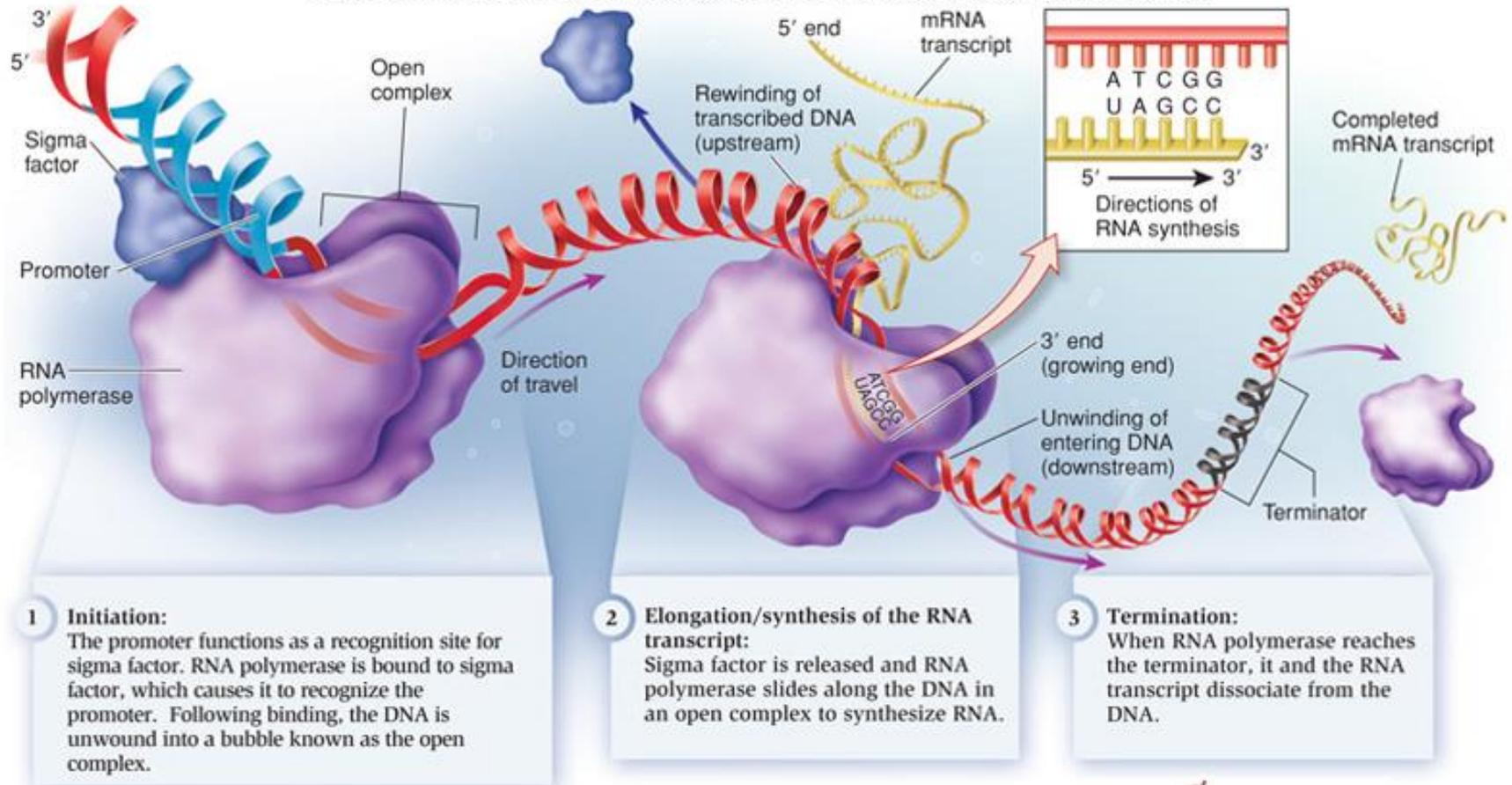
- Recognition step
- In bacteria, sigma factor causes RNA polymerase to recognize promoter region
- Catalytic portion of RNA polymerase has similar structure in all species

2. Elongation

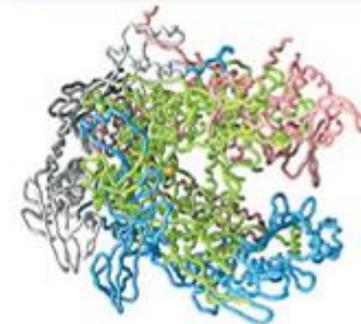
- RNA polymerase synthesizes RNA after release of sigma factor
- Open complex 10-15 base pairs long
- Template or coding strand used for RNA synthesis
 - Noncoding strand is not used
- Synthesized 5' to 3'
- Uracil substituted for thymine
- Behind open complex, DNA rewinds

3. Termination

- RNA polymerase reaches termination sequence
- Causes it and newly made RNA transcript to dissociate from DNA

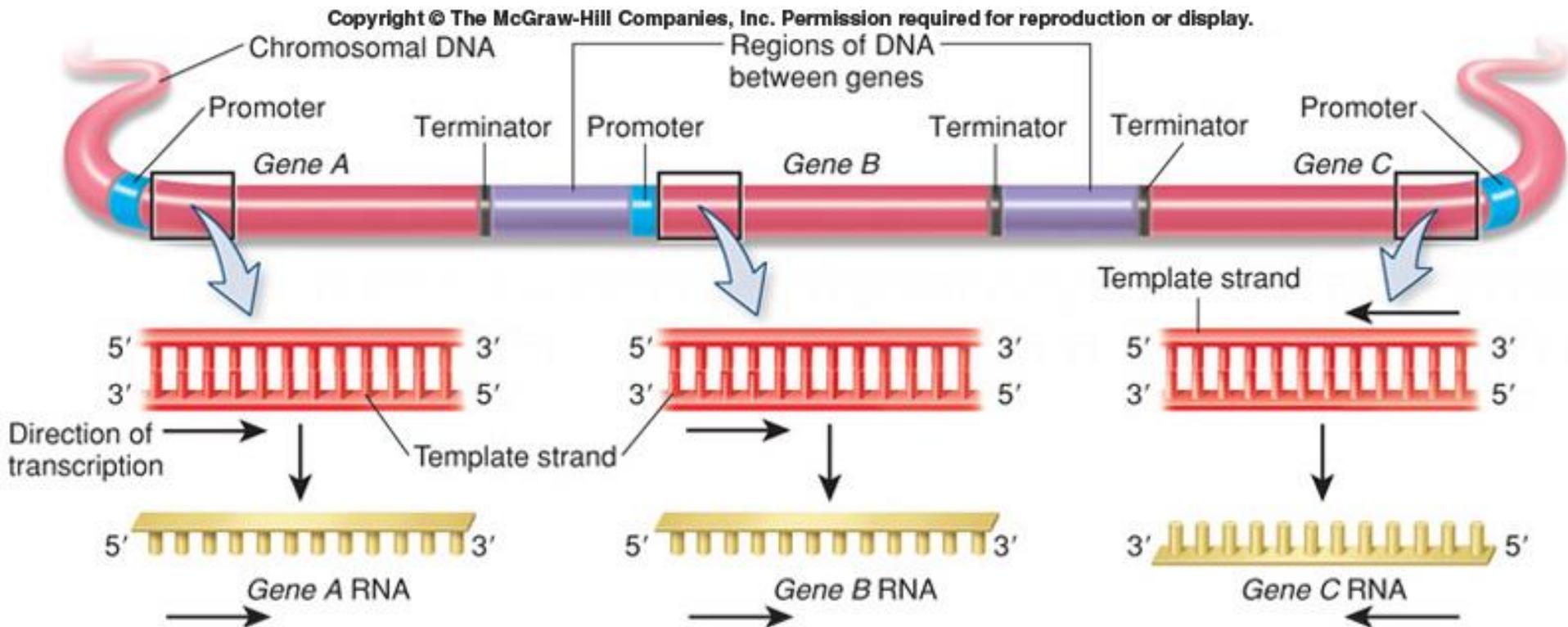


(a) Stages of transcription



(b) Structure of a bacterial RNA polymerase

- Direction of transcription and DNA strand used varies among genes
- In all cases, synthesis of RNA transcript is 5' to 3' and DNA template strand reads 3' to 5'

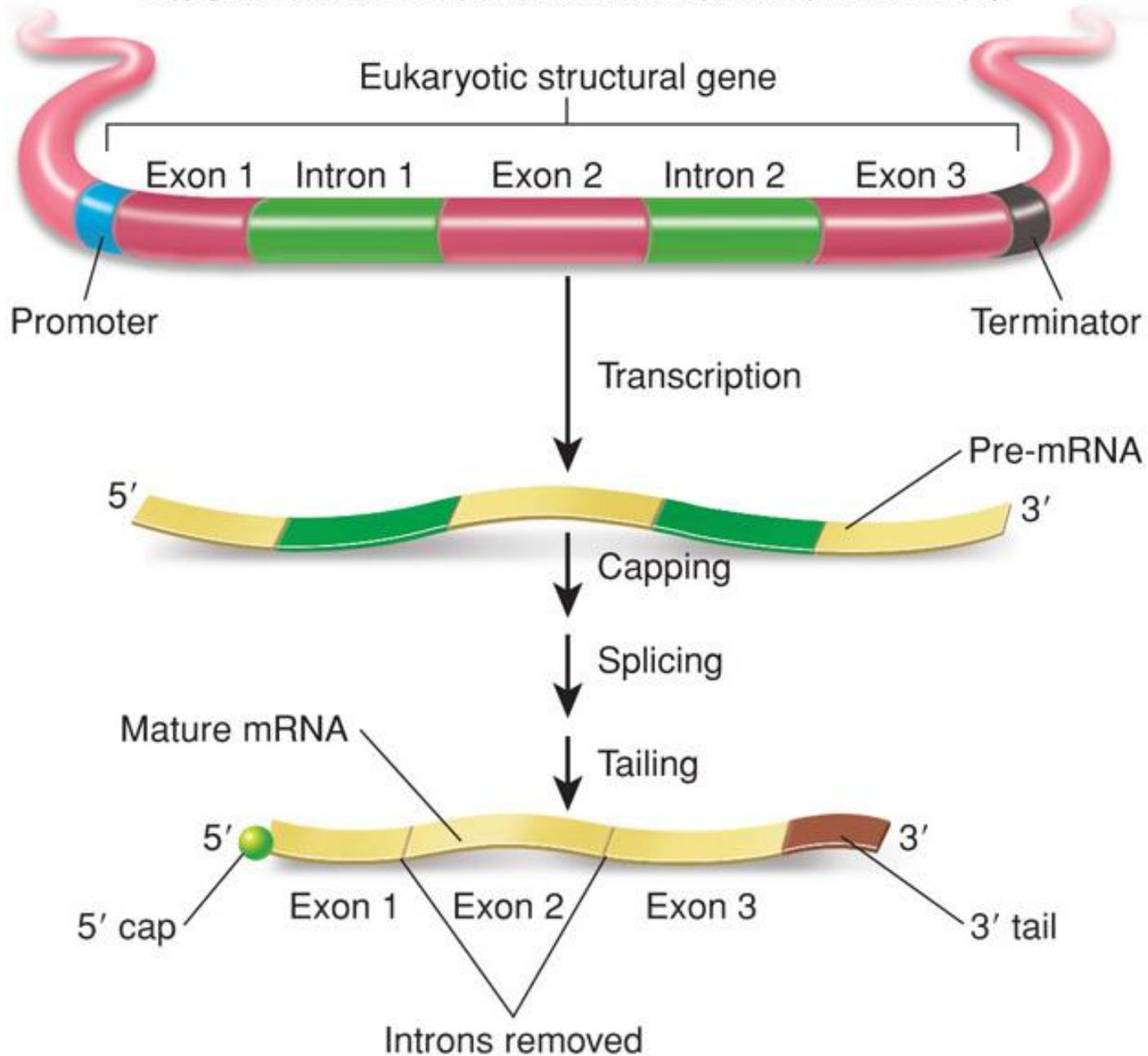


Eukaryotic transcription

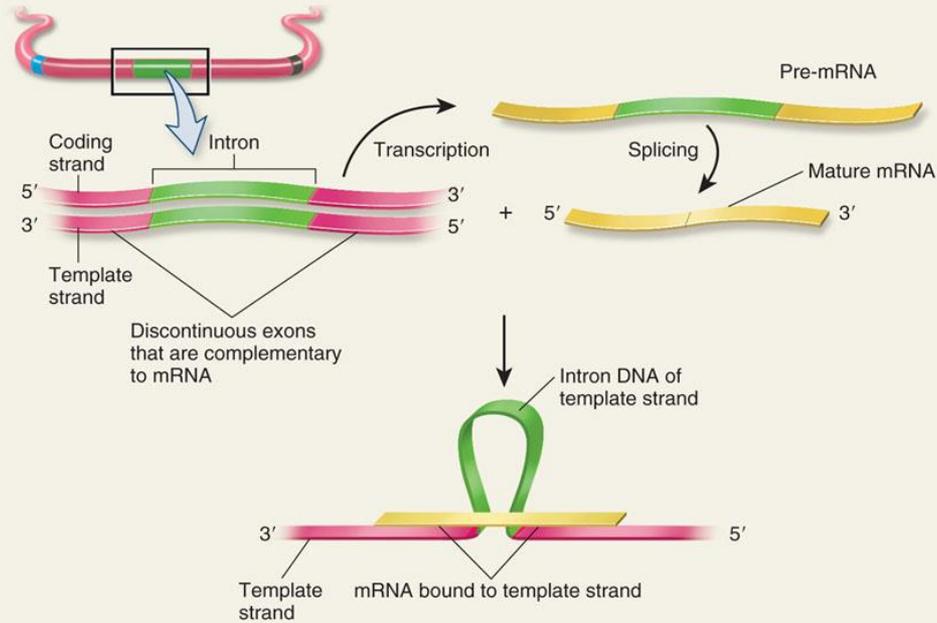
- Basic features identical to prokaryotes
- However, each step has more proteins
- 3 forms of RNA polymerase
 - RNA polymerase II – transcribes mRNA
 - RNA polymerase I and III – transcribes nonstructural genes for rRNA and tRNA
- RNA polymerase II requires 5 general transcription factors to initiate transcription

RNA processing

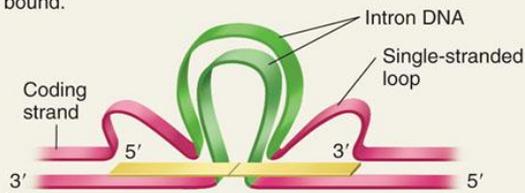
- Bacterial mRNAs can be translated into polypeptides as soon as they are made
- Eukaryotic mRNAs are made in a longer pre-mRNA form that requires processing into mature mRNA
- Introns- transcribed but not translated
- Exons- coding sequence found in mature mRNA
- Splicing- removal of introns and connection of exons
- Other modifications also occur – addition of tails and caps



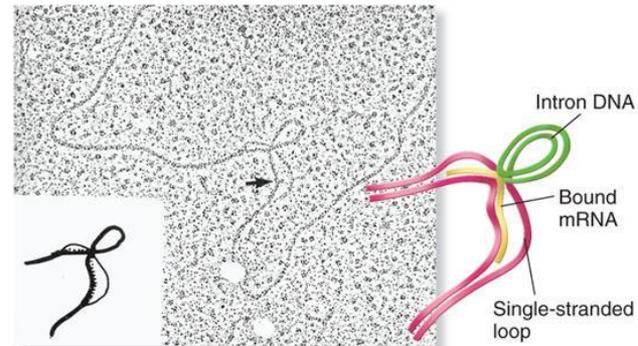
1 Mature mRNA binds to template strand, which causes the intron DNA to loop out.



2 The coding strand then binds to the template strand, but it loops out where the RNA is already bound.



(a) Schematic drawing of mature mRNA binding to DNA containing an intron

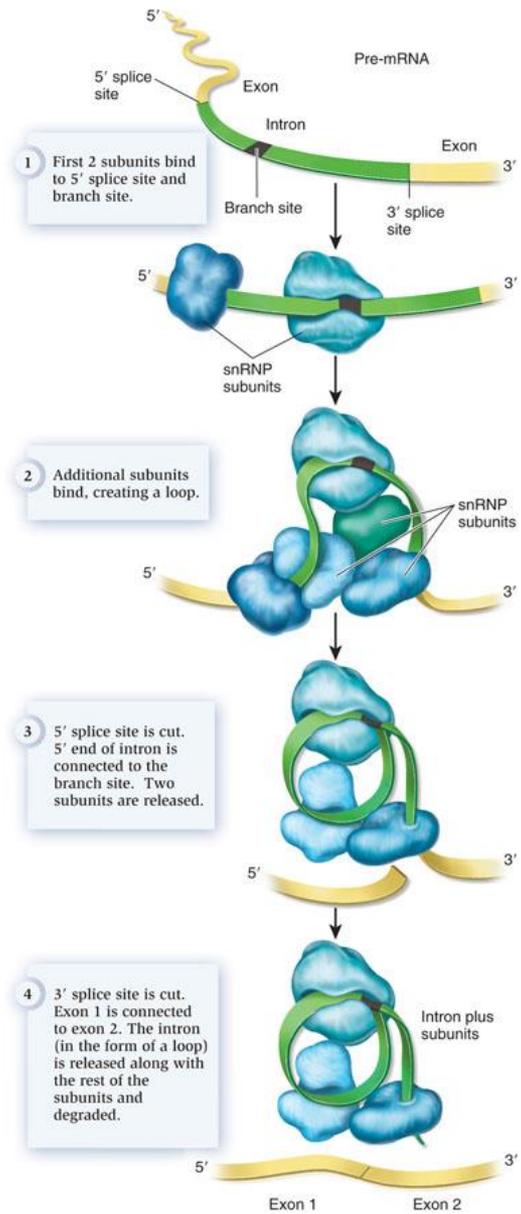


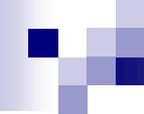
(b) Results after electron microscopy

Tilghman et al. Intervening sequence of DNA identified in the structural portion of a mouse beta-globin gene. PNAS 1978, Vol 75. f. 2, p. 727

- Introns found in many eukaryotic genes
- Widespread in complex eukaryotes
 - Human dystrophin gene has 79 exons and 78 introns
- Introns are rare in all prokaryotes
- Introns removed from eukaryotic pre-mRNA using a spliceosome composed of snRNPs (snurps)
 - Nuclear *RNA* and a set of *proteins*

- Intron RNA is defined by particular sequences within the intron and at the intron-exon boundaries
 - 5' splice site, branch site, 3' splice site
- Spliceosome subunits recognize intron sequences
- Binding causes intron to loop outward
- Two exons brought closer together
- Alternative splicing- function of spliceosome regulated so that single gene can encode 2 or more polypeptides
- Introns in rRNA and tRNA are self-splicing





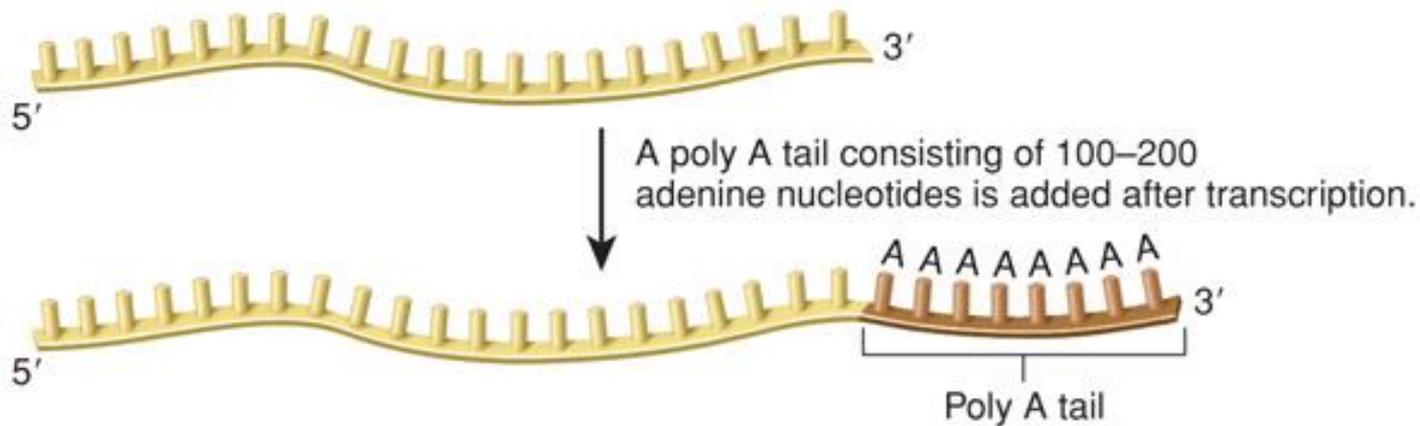
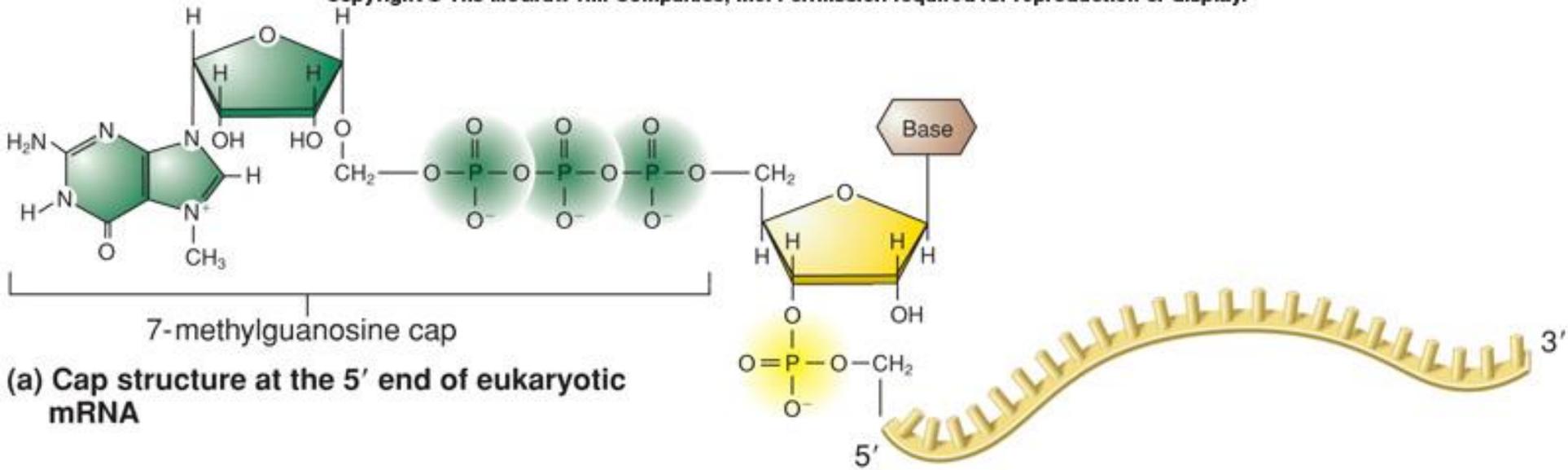
■ 5' cap and capping

- 7-methylguanosine covalently attached to 5' end
- Needed for proper exit of mRNA from nucleus and binding to ribosome

■ 3' poly A tail

- 100-200 adenine nucleotides added to 3' end
- Increases stability and lifespan in cytosol
- Not encoded in gene sequence

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(b) Addition of a poly A tail at the 3' end of eukaryotic mRNA

Translation

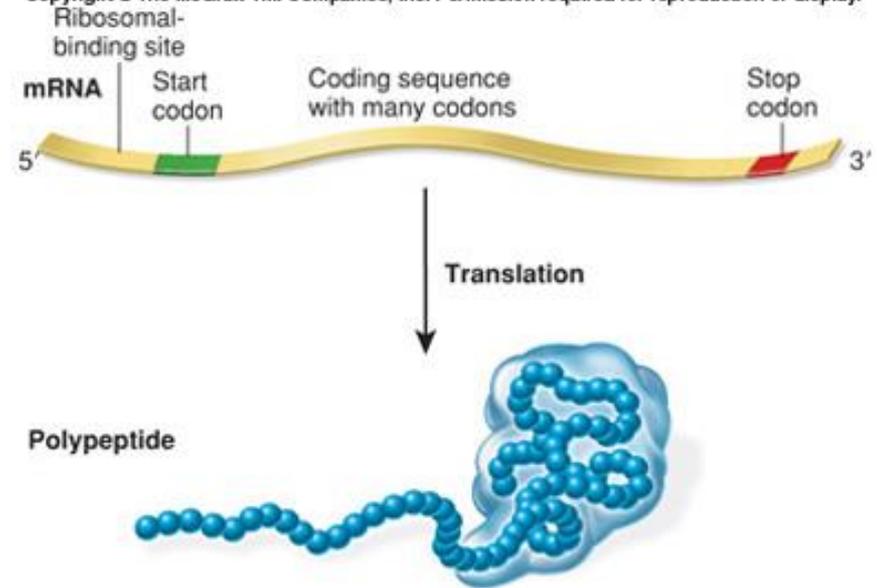
- Genetic code – sequence of bases in an mRNA molecule
- Read in groups of three nucleotide bases or codons
- Most codons specify a particular amino acid
 - Also start and stop codons
- Degenerate- more than one codon can specify the same amino acid

Table 12.1 The Genetic Code*

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

*Exceptions to the genetic code are sporadically found among various species. A few examples are as follows: AUA encodes methionine in yeast and mammalian mitochondria; UGA encodes tryptophan in mammalian mitochondria; CUU, CUA, CUC, and CUG encode threonine in yeast mitochondria; AGA and AGG act as stop codons in ciliated protozoa and in yeast and mammalian mitochondria; and UAA and UAG encode glutamine in ciliated protozoa.

- Bacterial mRNA
- 5' ribosomal-binding site
- Start codon usually AUG
- Typical polypeptide is a few hundred amino acids in length
- 1 of 3 stop codons
 - Termination or nonsense codons
 - UAA, UAG or UGA



Ribosomal-binding site: The site for ribosome binding.

Start codon: Specifies the first amino acid in a polypeptide sequence.

Coding sequence: A 3-nucleotide sequence within each codon specifies a particular amino acid. The order of codons within mRNA determines the sequence of amino acids of a polypeptide.

Stop codon: Specifies the end of translation.

Reading frame

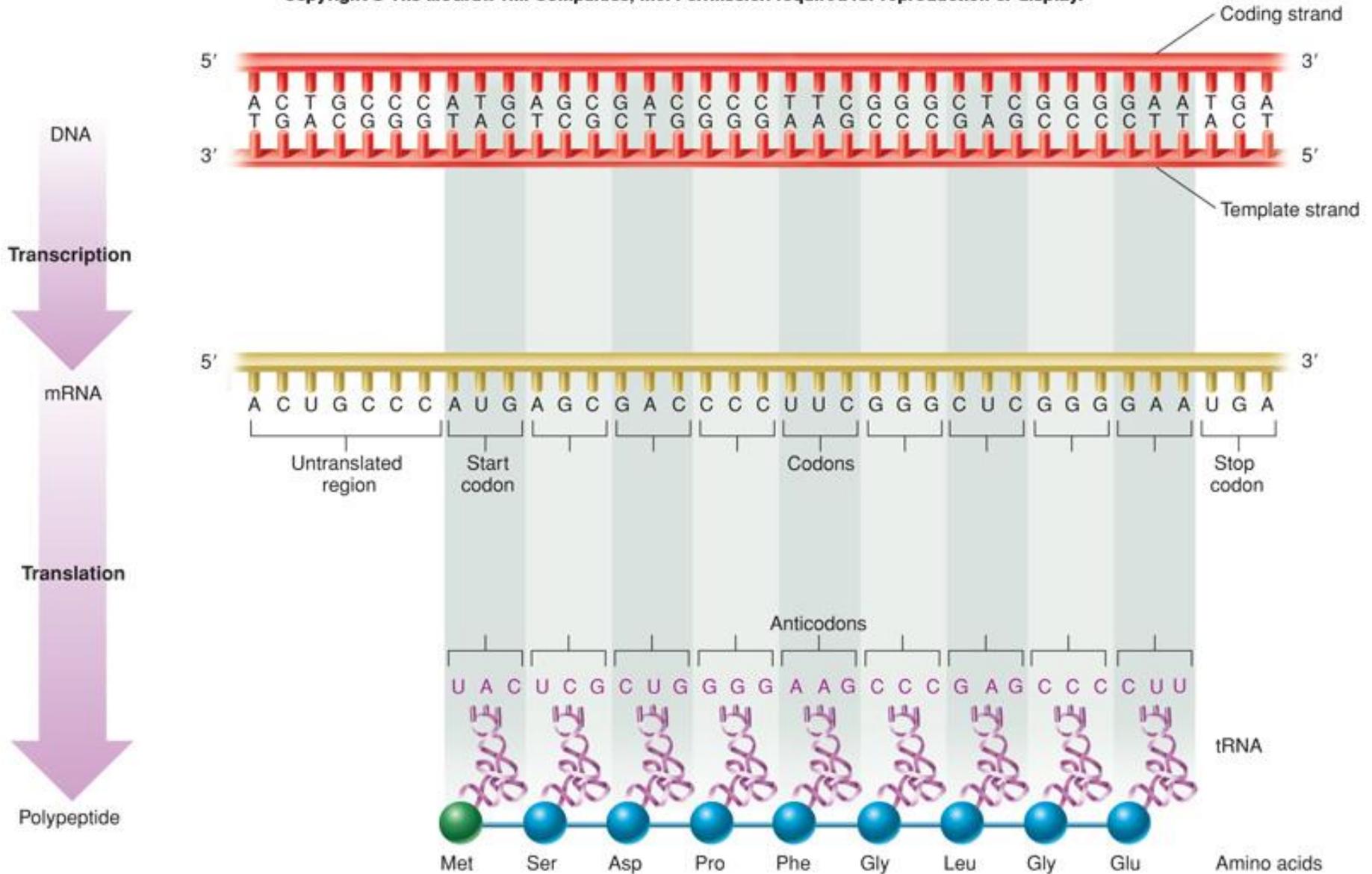
- Start codon defines reading frame

5' -AUAAGGAGGUACG(AUG)(CAG)(CAG)(GGC)(UUU)(ACC) - 3'
Met -Gln -Gln -Gly -Phe -Thr

- Addition of a U shifts the reading frame and changes the codons and amino acids specified

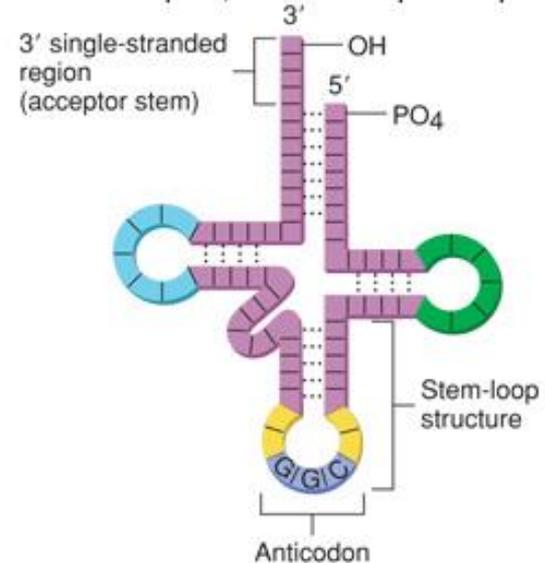
5' -AUAAGGAGGUACG(AUG)(UCA)(GCA)(GGG)(CUU)(UAC)C - 3'
Met -Ser -Ala -Gly -Leu -Tyr

- 
- DNA sequence of gene transcribed into mRNA
 - mRNA
 - Codons
 - T of DNA substituted for U of RNA
 - tRNA
 - Anticodon allows binding of tRNA to mRNA codon

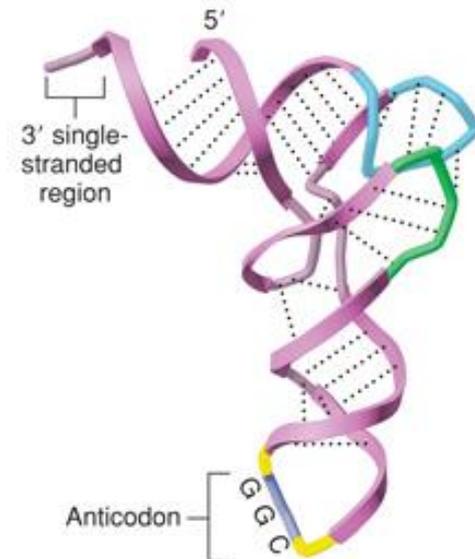


tRNA

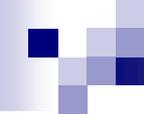
- Different tRNA molecules encoded by different genes
- tRNA^{ser} carries serine
- Common features
 - Cloverleaf structure
 - Anticodon
 - Acceptor stem for amino acid binding

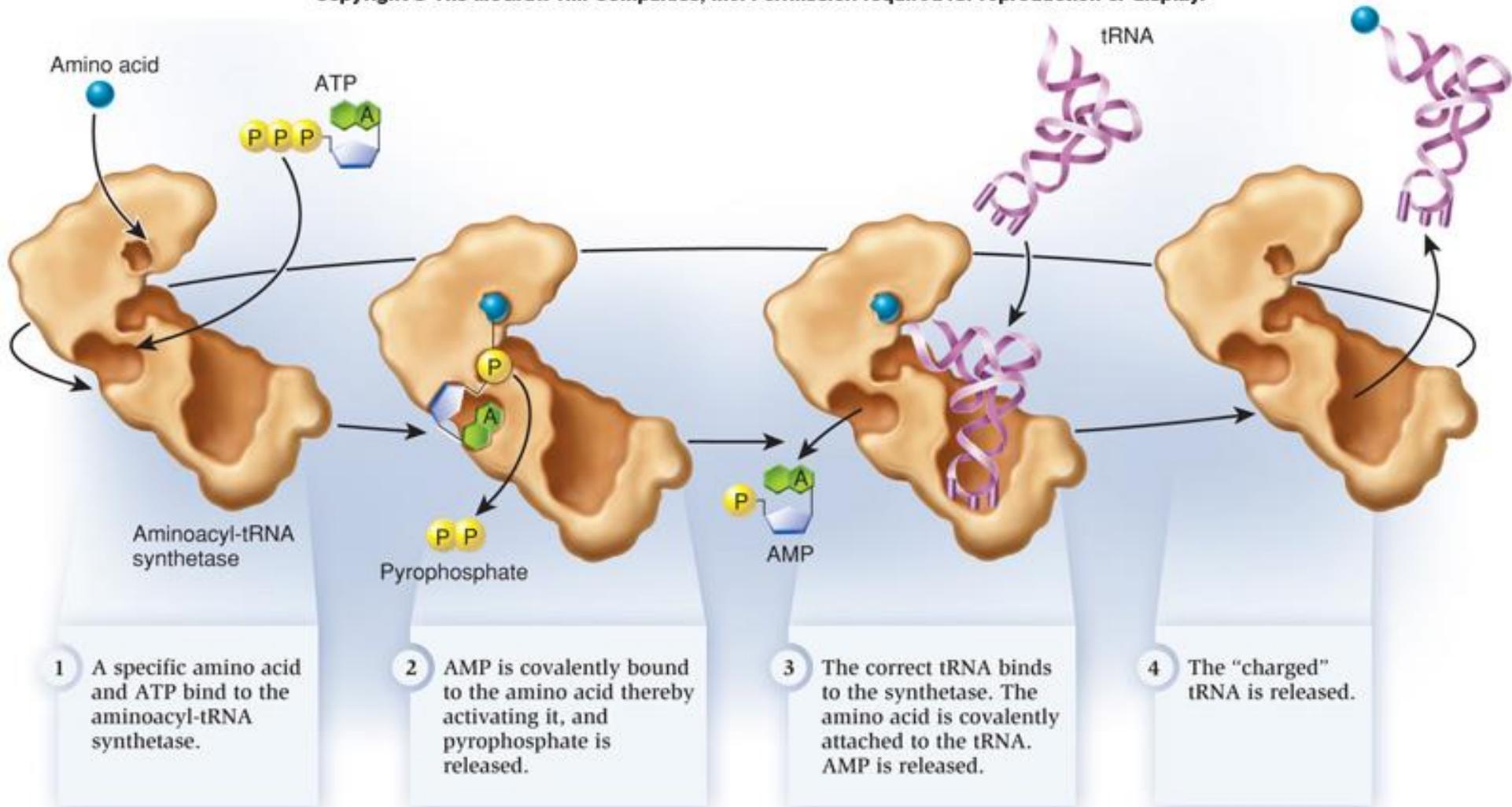


(a) Secondary structure of tRNA



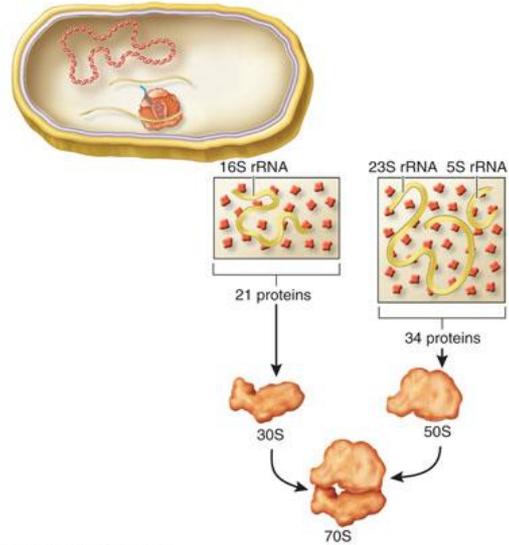
(b) Three-dimensional structure of tRNA

- 
- Aminoacyl-tRNA synthetase catalyzes the attachment of amino acids to tRNA
 - One for each of 20 different amino acids
 - 2 reactions result in tRNA with amino acid attached or charged tRNA or aminoacyl tRNA
 - Ability of aminoacyl-tRNA synthetase to recognize appropriate tRNA has been called the second genetic code

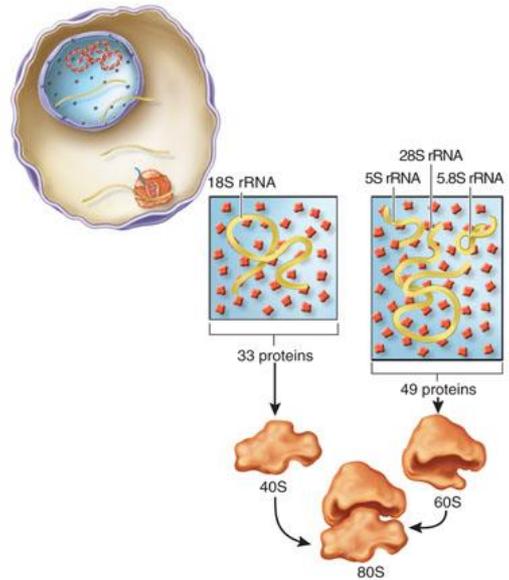


Ribosomes

- Prokaryotes have one kind
- Eukaryotes have distinct ribosomes in different cellular compartments
 - Focus on cytosolic ribosomes
- Composed of large and small subunits
- Structural differences between prokaryotes and eukaryotes exploited by antibiotics to inhibit bacterial ribosomes only

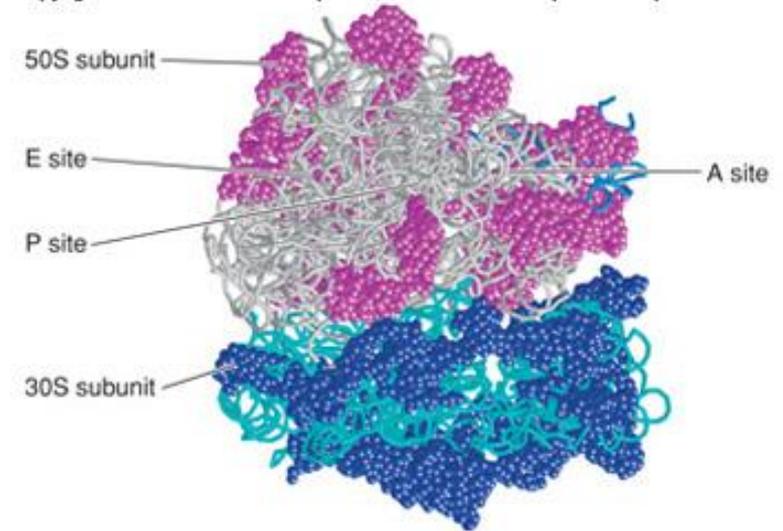


(a) Bacterial ribosomes

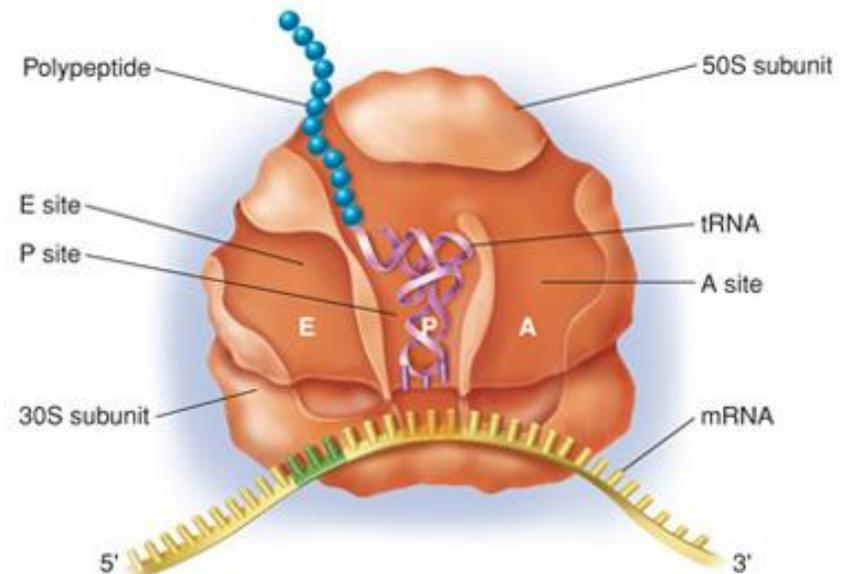


(b) Eukaryotic ribosomes

- Overall ribosome shape determined by rRNA
- Discrete sites for tRNA binding and polypeptide synthesis
- P site- peptidyl site
- A site- aminoacyl site
- E site- exit site



(a) Bacterial ribosome model based on X-ray diffraction studies



(b) Schematic model for ribosome structure
a: © Tom Pantages

Stages of Translation

1. Initiation

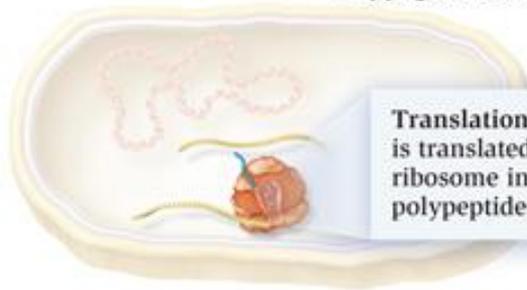
- mRNA, first tRNA and ribosomal subunits assemble

2. Elongation

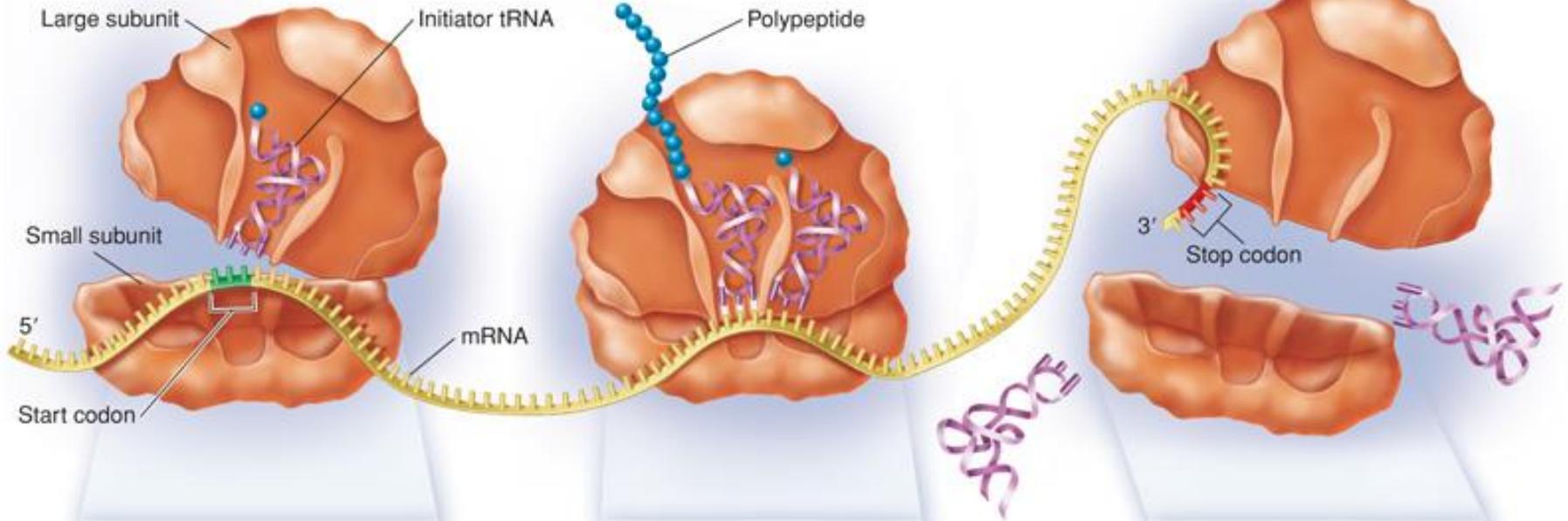
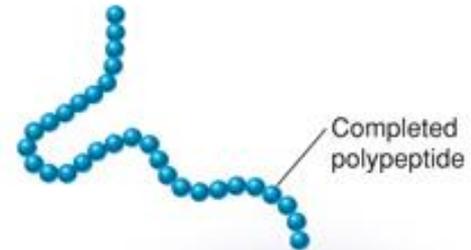
- Synthesis from start codon to stop codon

3. Termination

- Complex disassembles at stop codon releasing completed polypeptide



Translation: mRNA is translated at the ribosome into a polypeptide.



1 Initiation: mRNA, tRNA, and the ribosomal subunits form a complex.

2 Elongation: The ribosome travels in the 5' to 3' direction and synthesizes a polypeptide.

3 Termination: The ribosome reaches a stop codon and all of the components disassemble, releasing a completed polypeptide.

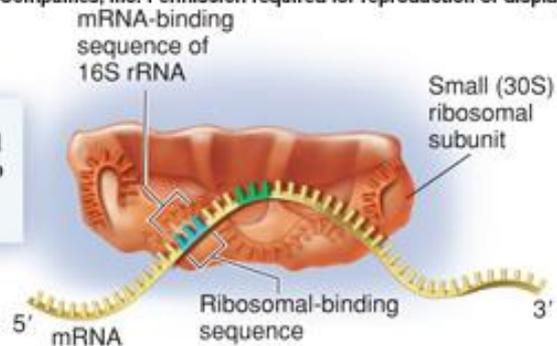
Initiation

- mRNA, first tRNA and ribosomal subunits assemble
- Requires help of ribosomal initiation factors
- Also requires input of energy (GTP hydrolysis)

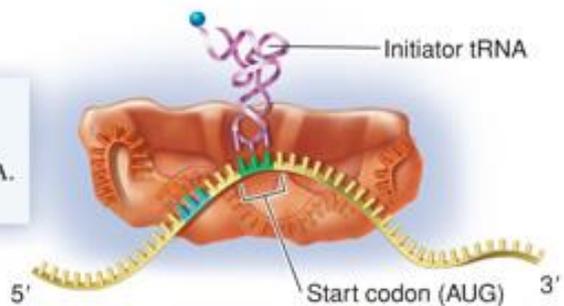
■ Bacteria

- mRNA binds to small ribosomal subunit facilitated by ribosomal-binding sequence
- Start codon a few nucleotides downstream
- Initiator tRNA recognizes start codon in mRNA
- Large ribosomal subunit associates
- At the end, the initiator tRNA is in the P site

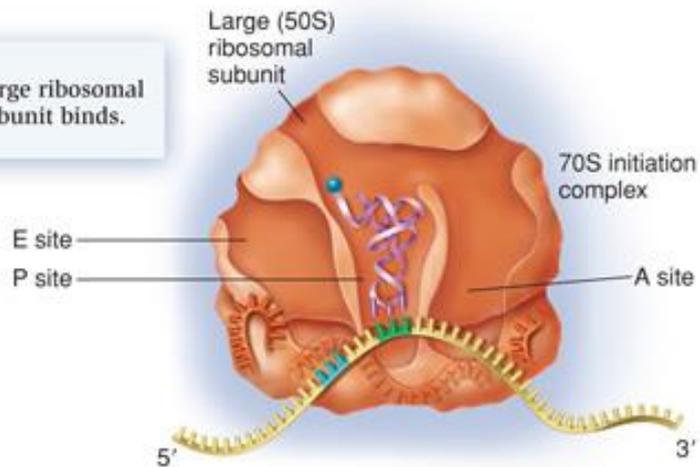
1 Small ribosomal subunit binds to mRNA.

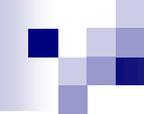


2 Initiator tRNA binds to start codon in mRNA.



3 Large ribosomal subunit binds.



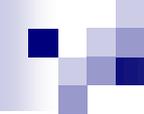


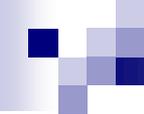
■ Eukaryotic differences in initiation

- Instead of a ribosomal-binding sequence, mRNAs have 7-methylguanosine cap at 5' end
 - Recognized by cap-binding proteins
- Position of start codon more variable
 - In many cases, first AUG codon used as start codon

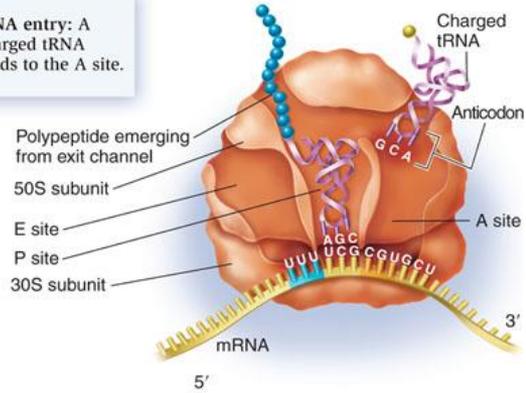
Elongation

1. Aminoacyl tRNA brings a new amino acid to the A site
 - Binding occurs due to codon/ anticodon recognition
 - Elongation factors hydrolyze GTP to provide energy to bind tRNA to A site
 - Peptidyl tRNA is in the P site
 - Aminoacyl tRNA is in the A site

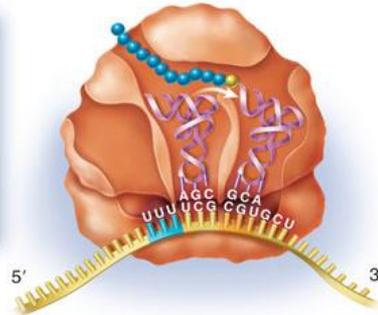
- 
- 
2. A peptide bond is formed between the amino acid at the A site and the growing polypeptide chain
 - The polypeptide is removed from the tRNA in the P site and transferred to the amino acid at the A site – peptidyl transfer reaction

- 
3. Movement or translocation of the ribosome toward the 3' end of the mRNA by one codon
- Shifts tRNAs at the P and A sites to the E and P sites
 - The next codon is now at the A spot
 - Uncharged tRNA exits from E spot

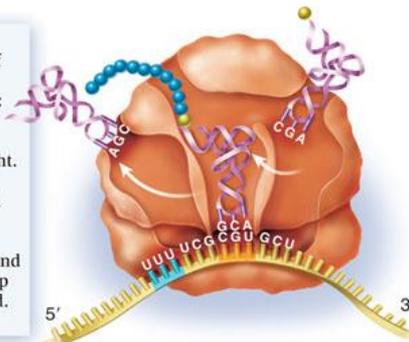
1 tRNA entry: A charged tRNA binds to the A site.



2 Peptidyl transfer reaction: A bond forms between the polypeptide chain and the amino acid in the A site. The polypeptide is transferred to the A site.

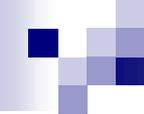


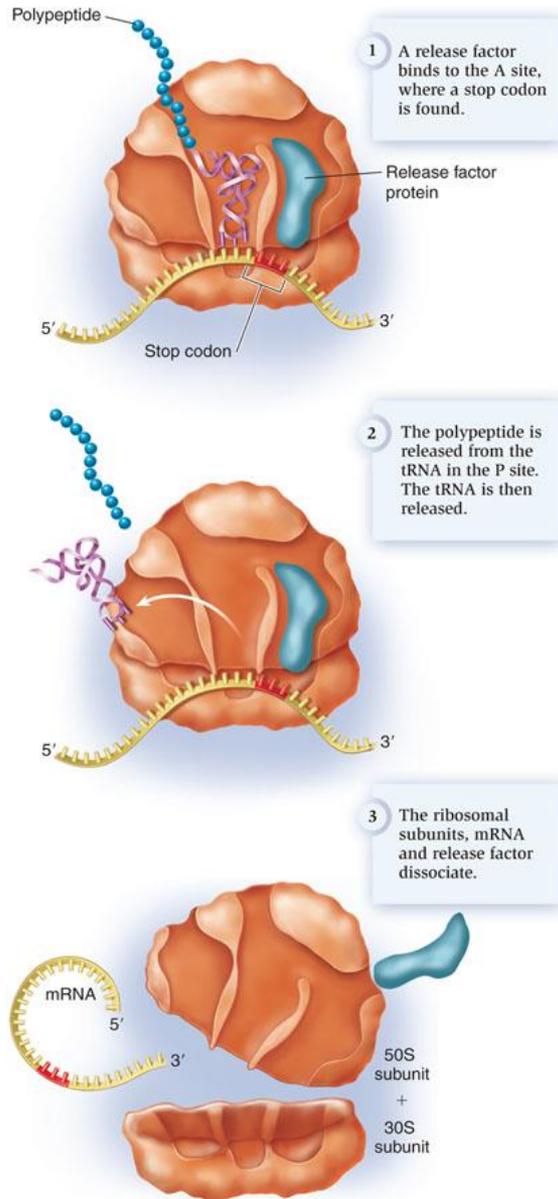
3 Translocation of ribosome and release of tRNA: The ribosome translocates 1 codon to the right. The uncharged tRNA is released from the E site. This process is repeated again and again until a stop codon is reached.



Termination

- When a stop codon is found in the A site, translation ends
- 3 stop codons- UAA, UAG, UGA
- Recognized by release factors

- 
- Completed polypeptide attached to a tRNA in the P site and stop codon in the A site
 1. Release factor binds to stop codon at the A site
 2. Bond between polypeptide and tRNA hydrolyzed to release polypeptide
 3. Ribosomal subunits and release factors disassociate



- Polypeptide chain has directionality
- Parallels 5' to 3' orientation of mRNA
- N-terminus or amino terminus is first amino acid
- C-terminus or carboxyl terminus is last amino acid

