CHAPTER 12 LECTURE SLIDES

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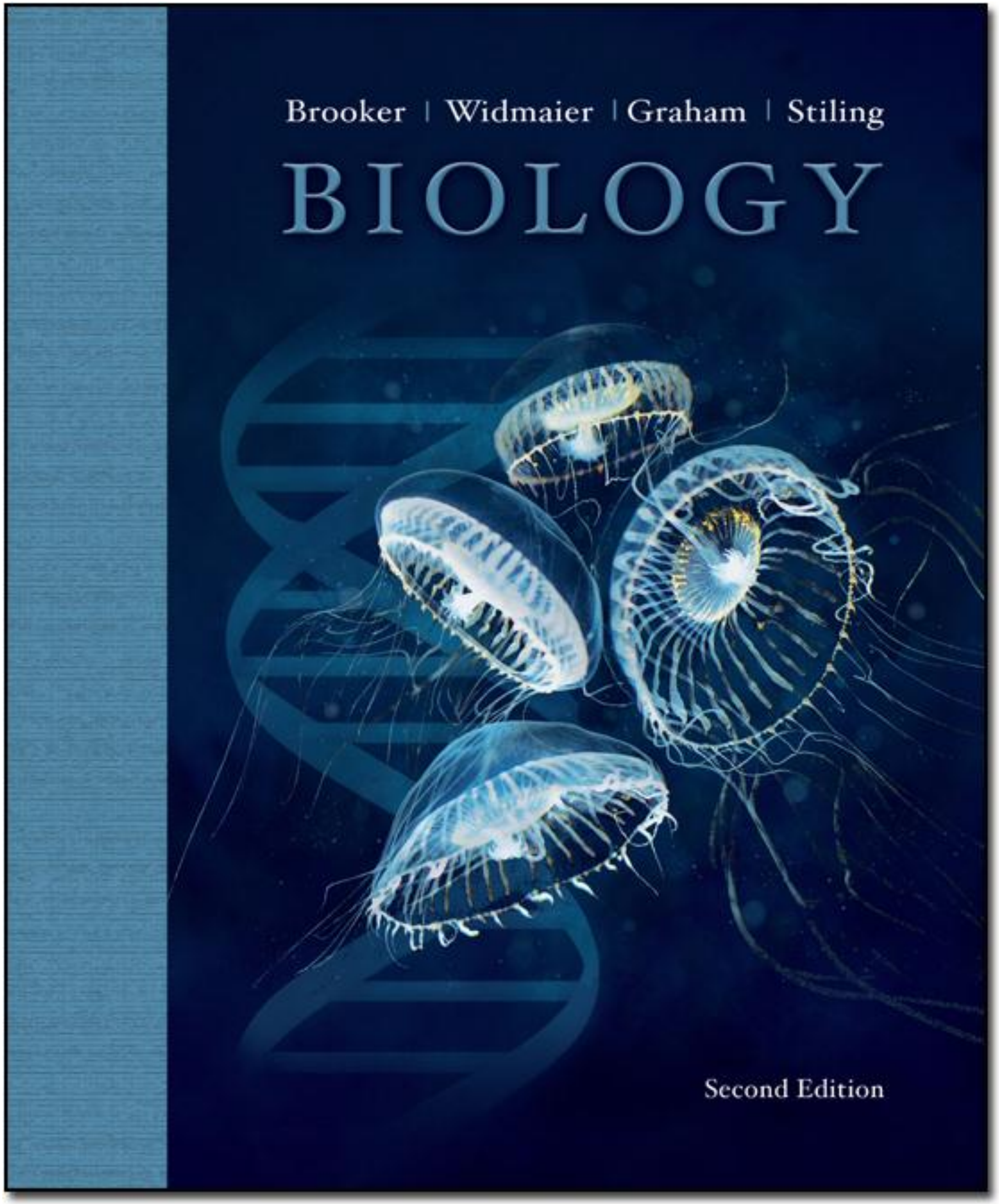
To run the animations you must be in **Slideshow View**. Use the buttons on the animation to play, pause, and turn audio/text on or off. Please note: once you have used any of the animation functions (such as Play or Pause), you must first click in the white background before you advance the next slide.



Gene expression

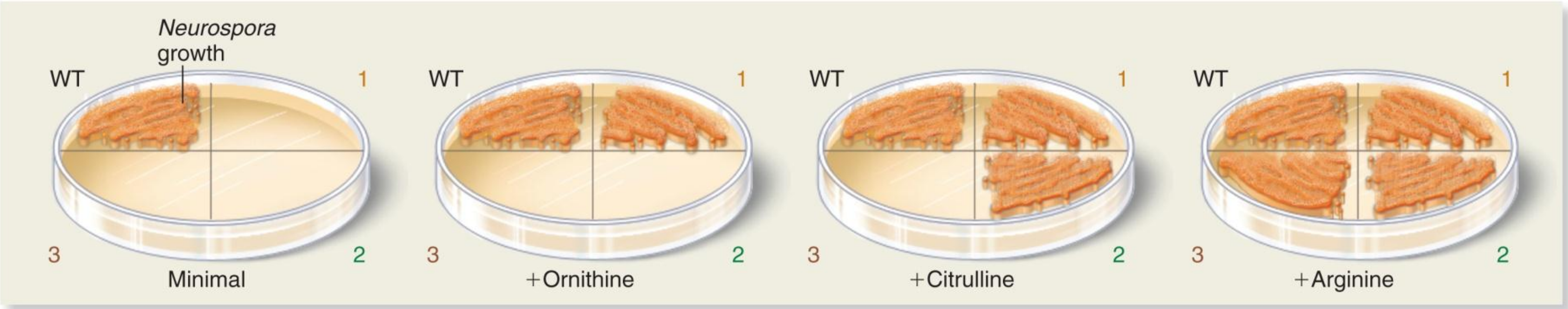
- Gene function at the level of traits
- Gene function at the molecular level
- Two levels tied together since the molecular level affects the structure and function of cells which determines what traits are expressed

- Beadle and Tatum became aware of Garrod’s work in the early 1940s
- Studied *Neurospora crassa*, common bread mold
- Minimum requirements for growth are carbon source (sugar), inorganic salts, and biotin
    - Has enzymes to synthesize other molecules it needs

- Mutant *Neurospora* strains would be unable to grow unless supplemented
  - Compare to wild-type or normal
- A single mutation resulted in the requirement for a single type of vitamin
- Stimulated research into other substances including arginine, an amino acid
  - Biochemical pathway already known

- Isolated several mutants requiring arginine for growth
- Examined for ability to grow in the presence of precursors
- 3 groups based on requirements
- Beadle and Tatum conclude that single gene controls the synthesis of a single enzyme
    - ☐ One gene – one enzyme hypothesis

(a) Simplified pathway for arginine synthesis

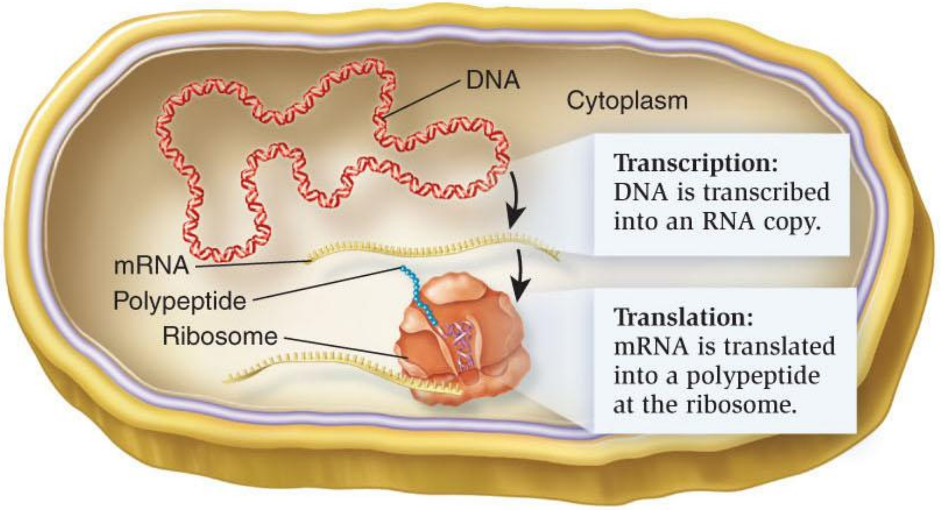


(b) Growth of strains on minimal and supplemented growth media

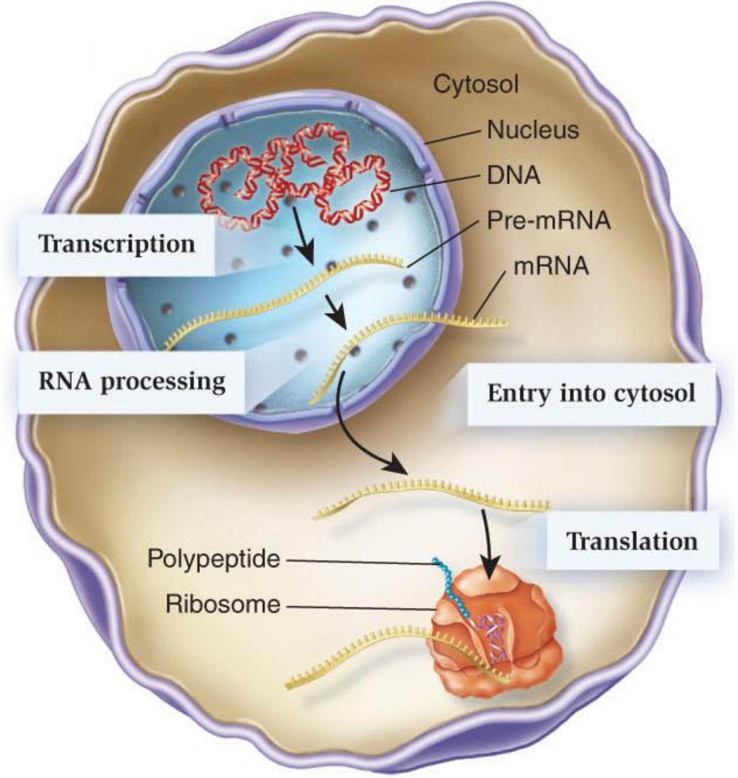
- One gene – one enzyme hypothesis has been modified
  - Enzymes are only one category of cellular proteins, genes also code for other proteins
  - Some proteins composed of one or more polypeptides
    - More accurate to say one gene encodes a polypeptide
    - Hemoglobin composed of 4 polypeptides 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



(a) Molecular gene expression in prokaryotes

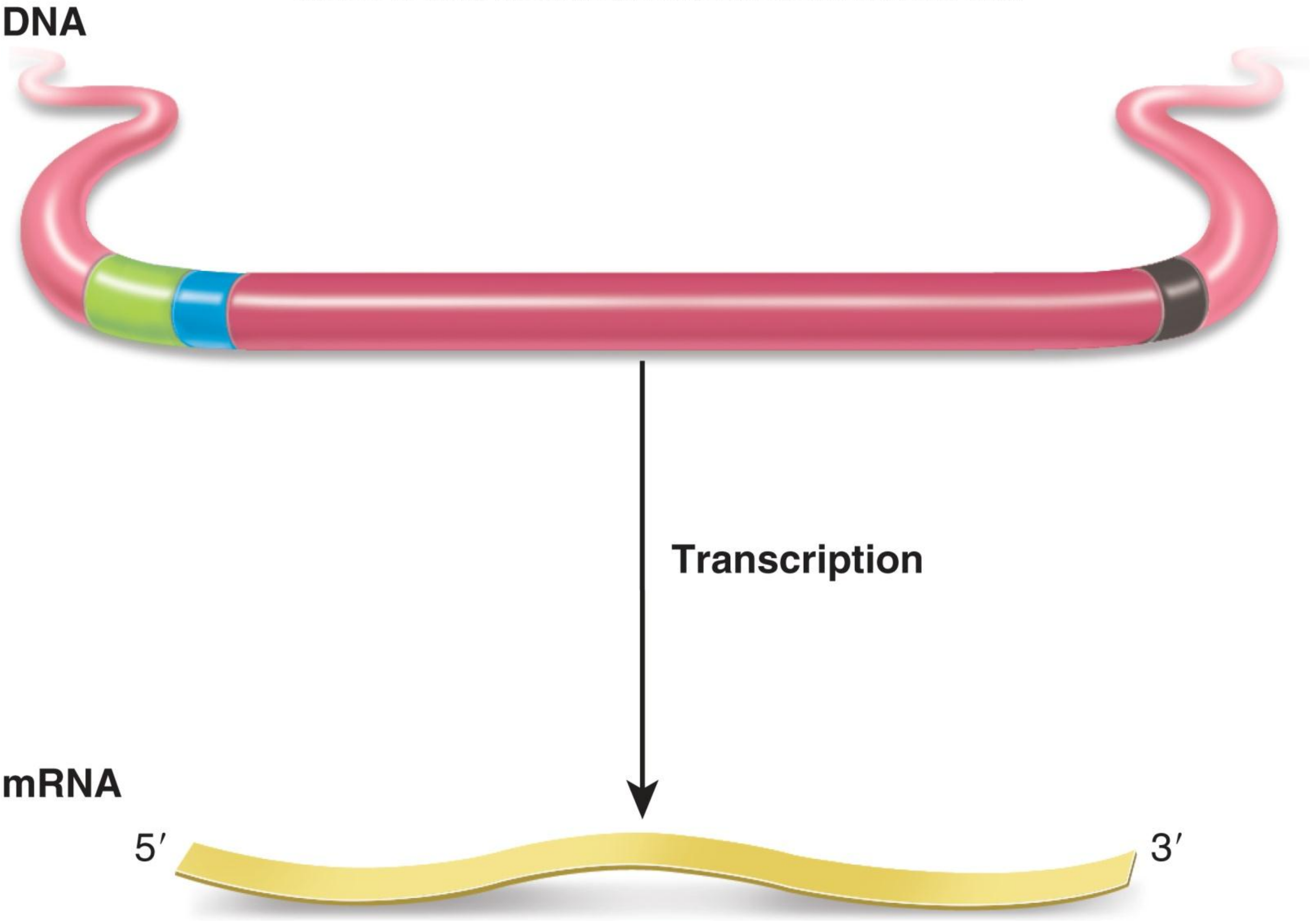


(b) Molecular gene expression in eukaryotes

- Genes constitute the genetic material
  - Blueprint for organisms’ characteristics
- 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
- Other genes code for the RNA itself
  - Transfer RNA (tRNA) - translates mRNA into amino acids
  - Ribosomal RNA (rRNA) - part of ribosomes


DNA
Transcription
mRNA
5′
3′

Three stages of transcription

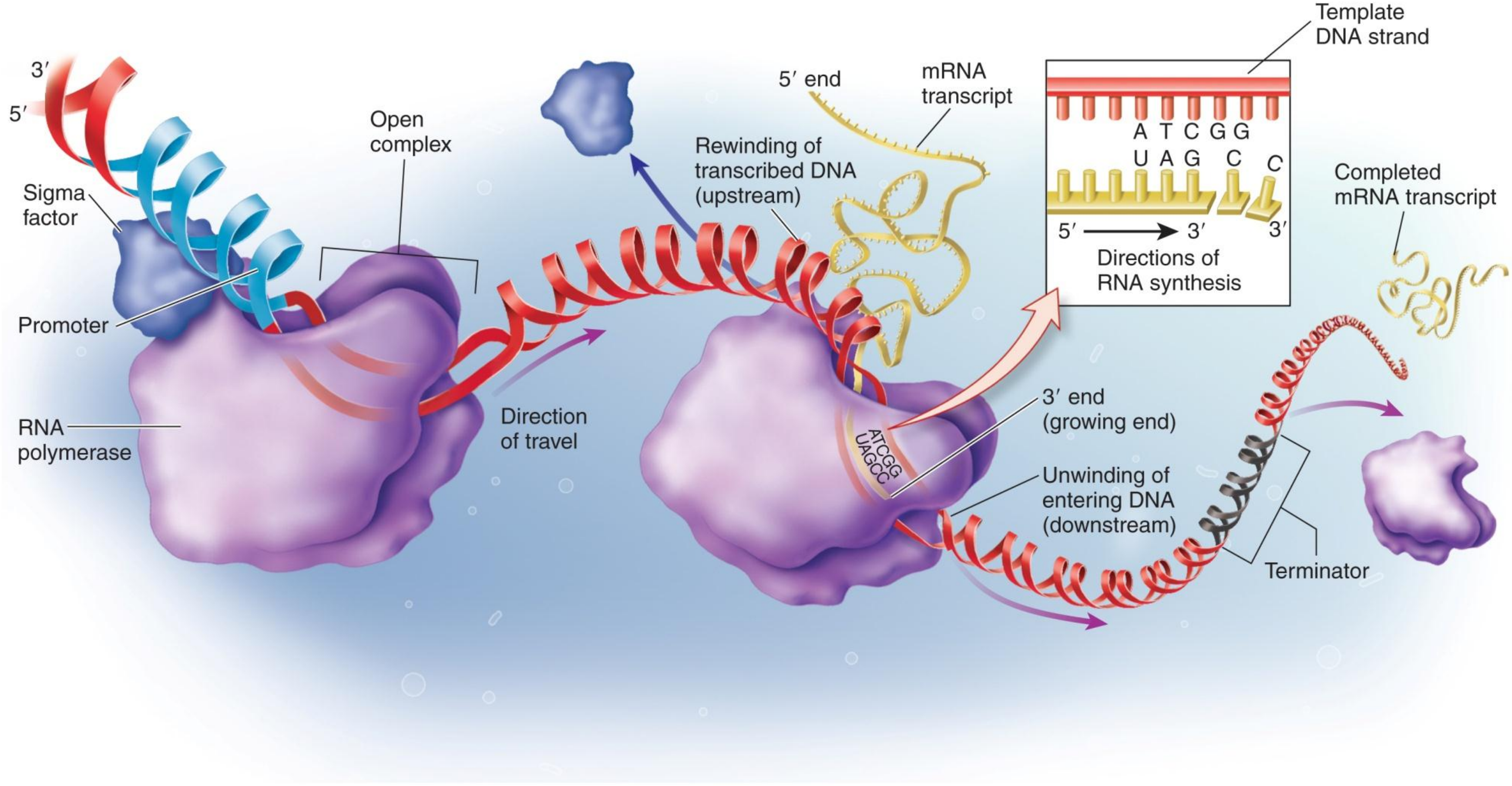
1. Initiation
2. Elongation
3. Termination

Three stages of transcription

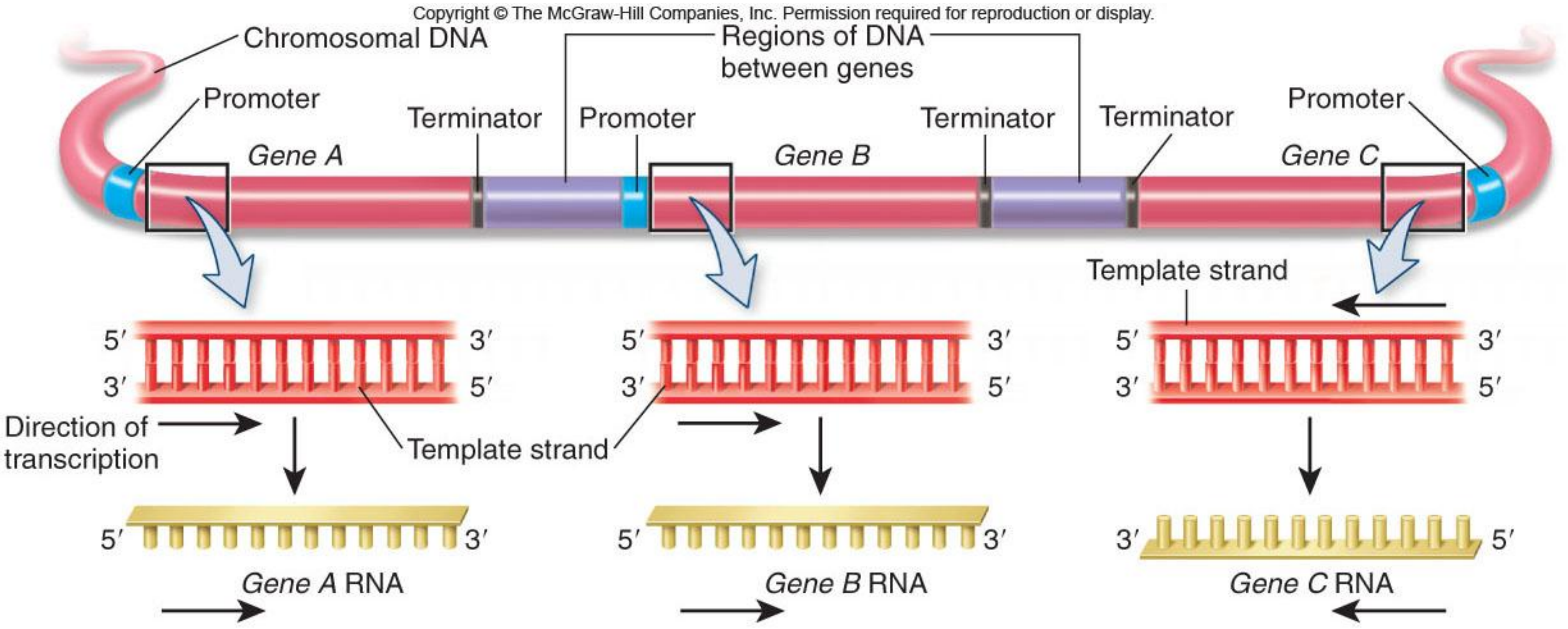
1. Initiation
   - Recognition step
   - In bacteria, sigma factor causes RNA polymerase to recognize promoter region
   - Stage completed when DNA strands separated near promoter to form open complex

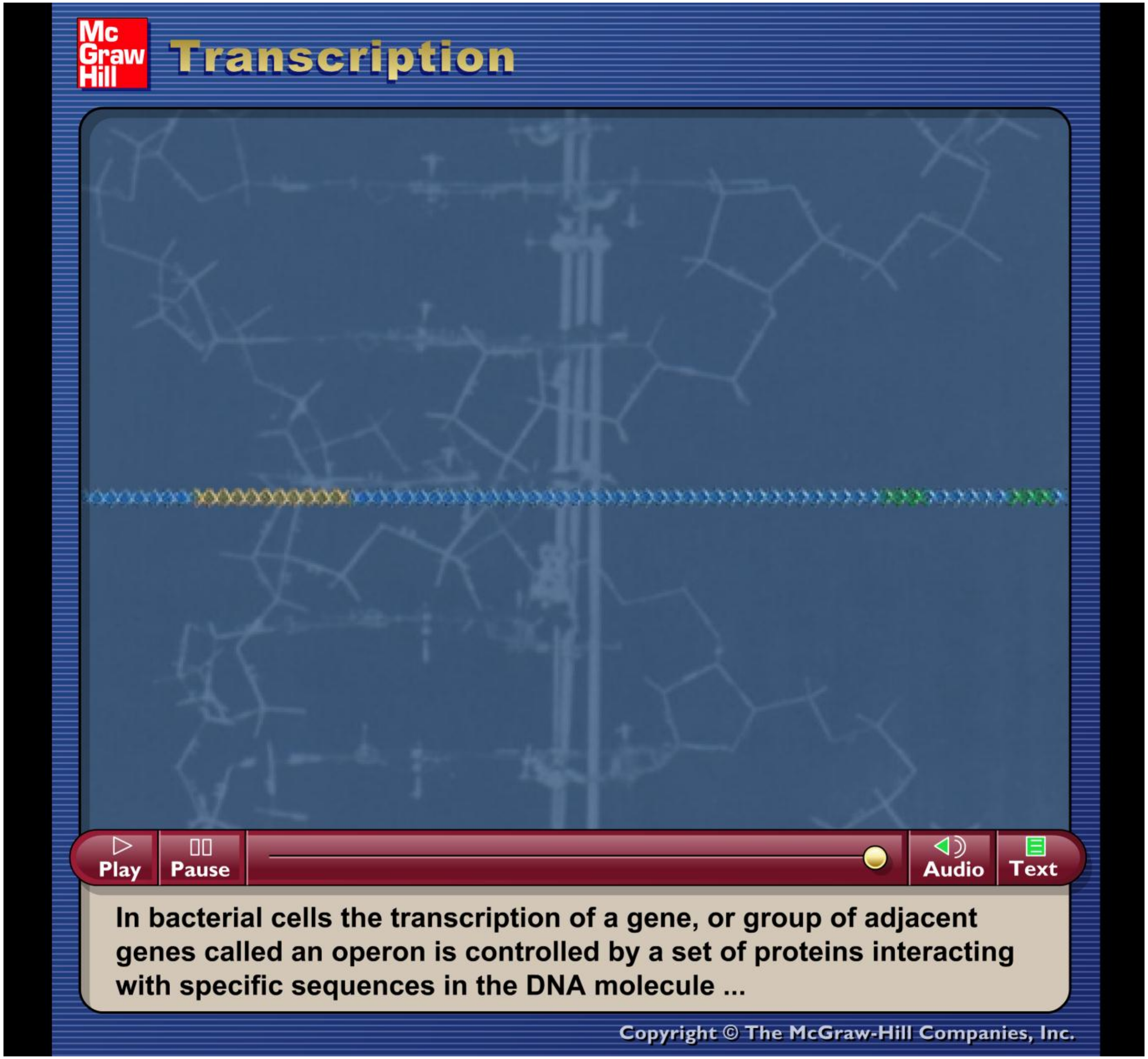
2. Elongation
   - RNA polymerase synthesizes RNA
   - Template or coding strand used for RNA synthesis
     - Noncoding strand is not used
   - Synthesized 5’ to 3’
   - Uracil substituted for thymine

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


Template DNA strand
3′
5′
5′ end
mRNA transcript
A T C G G
U A G C C
5′ → 3′
3′
Directions of RNA synthesis
Open complex
Rewinding of transcribed DNA (upstream)
Sigma factor
Completed mRNA transcript
Promoter
3′ end (growing end)
RNA polymerase
Direction of travel
ATCGG
UAGCC
Unwinding of entering DNA (downstream)
Terminator

- 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’





Transcription

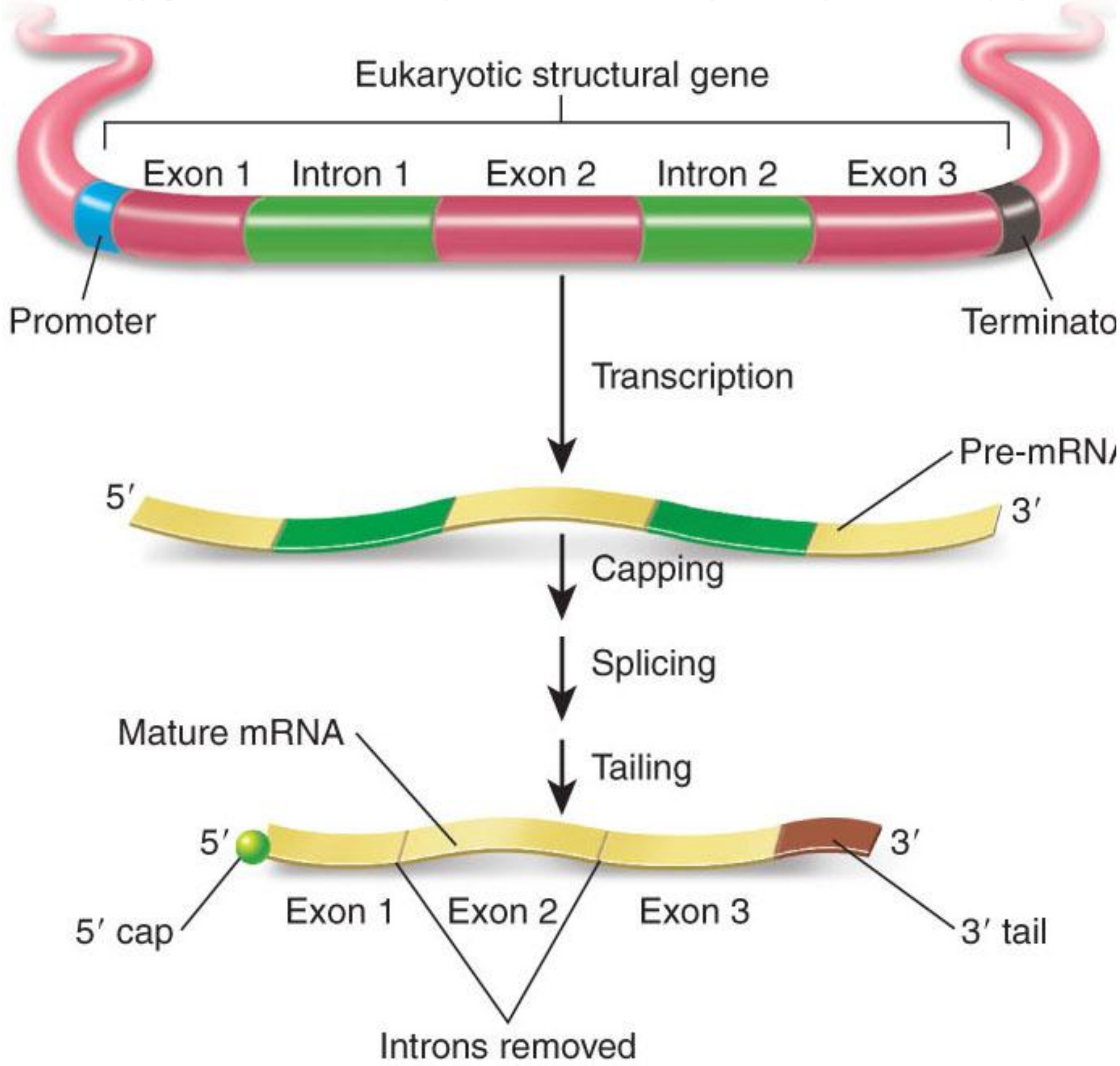
In bacterial cells the transcription of a gene, or group of adjacent genes called an operon is controlled by a set of proteins interacting with specific sequences in the DNA molecule ...

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

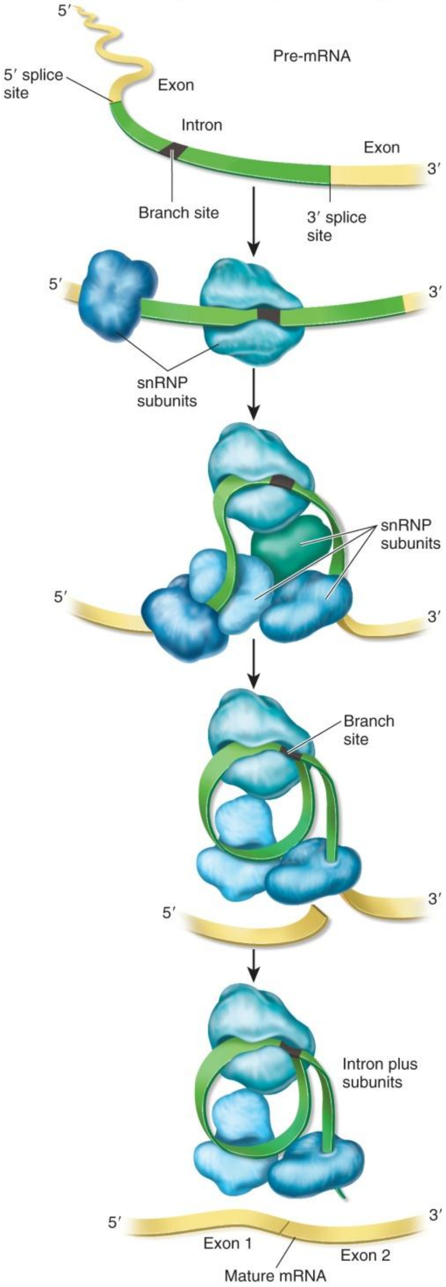
RNA processing

- 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


Eukaryotic structural gene
Exon 1
Intron 1
Exon 2
Intron 2
Exon 3
Promoter
Terminato
Transcription
Pre-mRN
5′
3′
Capping
Splicing
Tailing
Mature mRNA
5′
3′
5′ cap
Exon 1
Exon 2
Exon 3
3′ tail
Introns removed

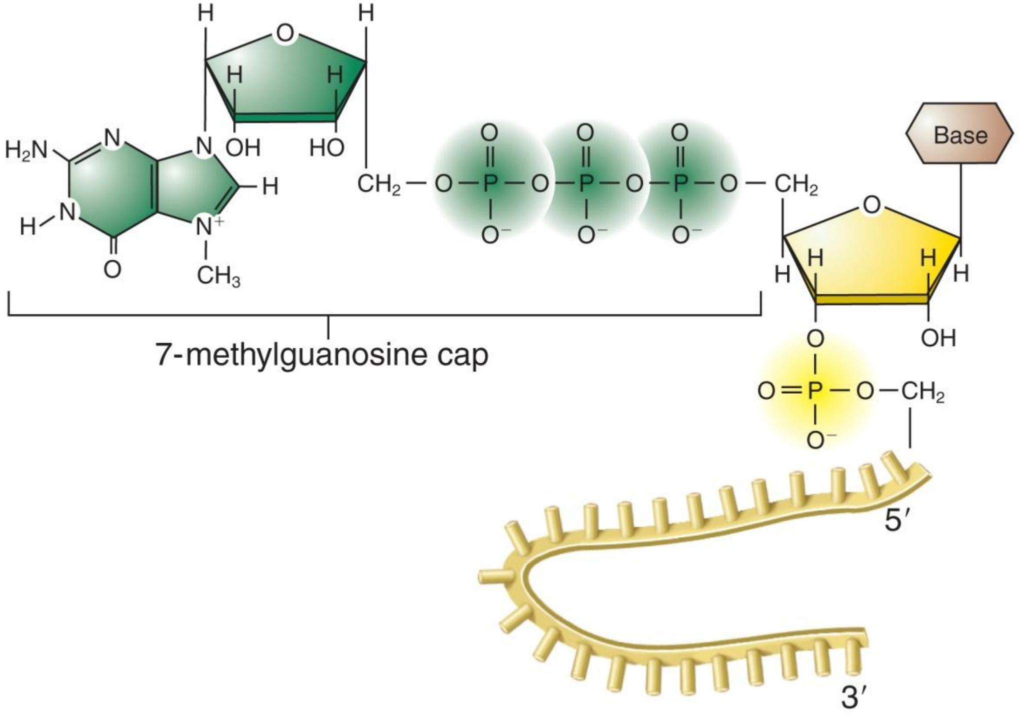
Splicing

- Introns found in many eukaryotic genes
  - Most structural genes have 1 or more introns
- Spliceosome – removes introns precisely
  - Composed of snRNPs – small nuclear RNA
- Alternative splicing – splicing can occur more than one way to produce different products
- rRNA and tRNA are self-splicing
  - Ribozyme

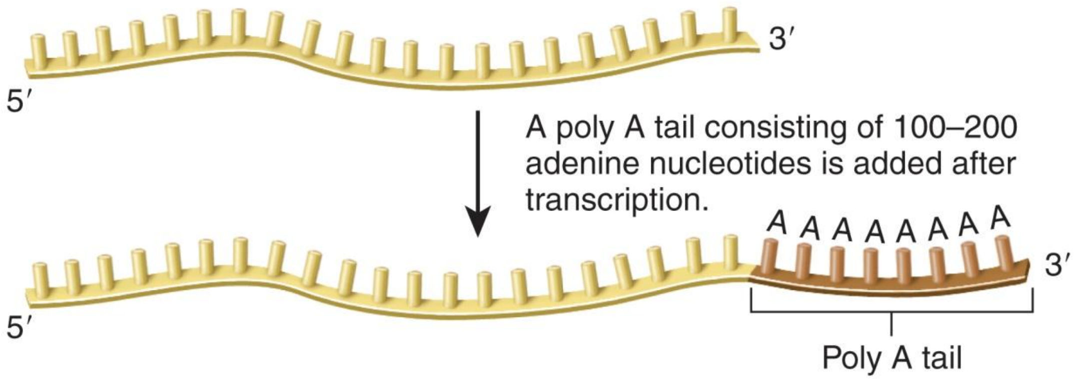

5′
Pre-mRNA
5′ splice site
Exon
Intron
Exon
3′
Branch site
3′ splice site
5′
3′
snRNP subunits
snRNP subunits
5′
3′
Branch site
3′
5′
Intron plus subunits
5′
3′
Exon 1
Exon 2
Mature mRNA

Additional RNA processing

- Capping
  - Modified guanosine attached to 5’ end
  - Needed for proper exit of mRNA from nucleus and binding to ribosome
- Poly A tail
  - 100-200 adenine nucleotides added to 3’ end
  - Increases stability and lifespan in cytosol
  - Not encoded in gene sequence



(a) Cap structure at the 5′ end of eukaryotic mRNA



A poly A tail consisting of 100–200 adenine nucleotides is added after transcription.

(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

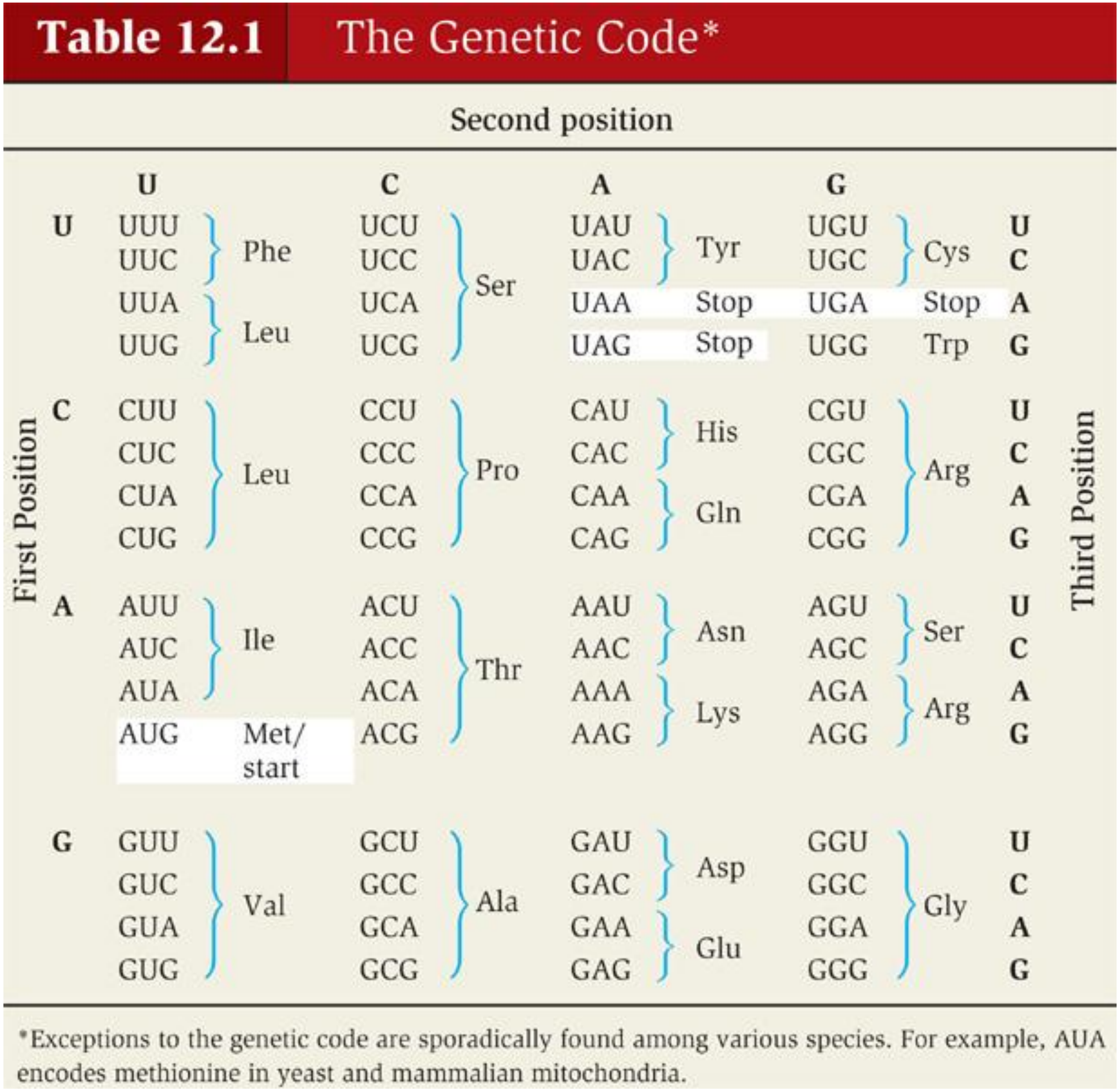
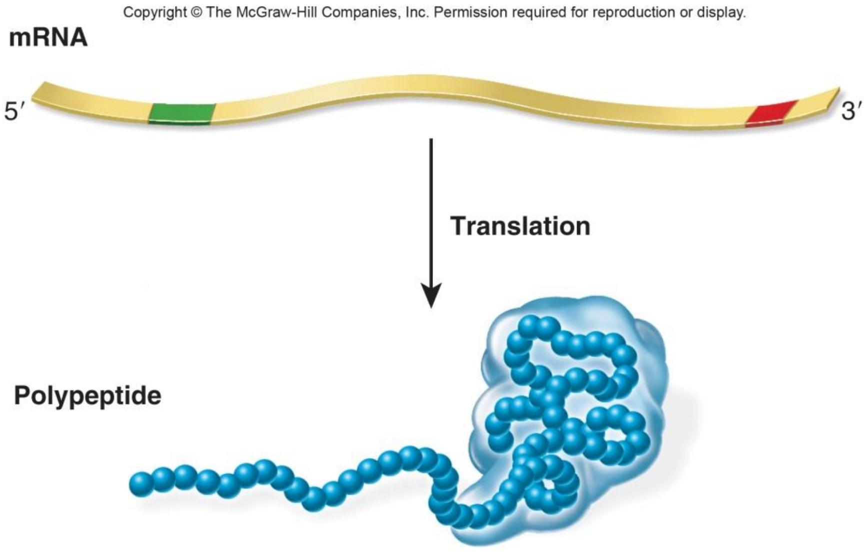


Table 12.1 The Genetic Code*

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

*Exceptions to the genetic code are sporadically found among various species. For example, AUA encodes methionine in yeast and mammalian mitochondria.

- 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



Reading frame

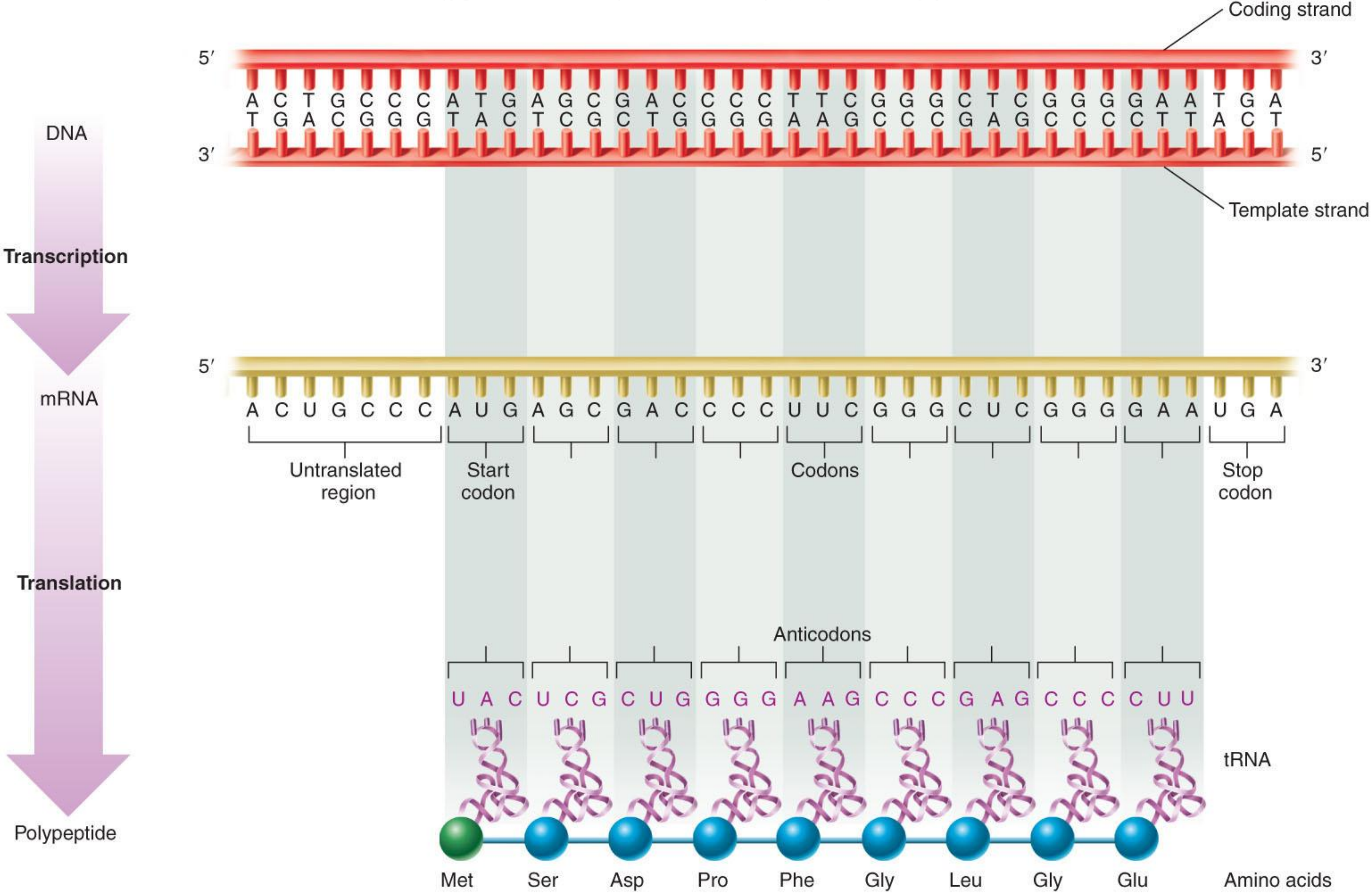
- Start codon defines reading frame

5’ –AUAAGGAGGUUACG(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’ –AUAAGGAGGUUACG(AUG)(UCA)(GCA)(GGG)(CUU)(UAC)C – 3’
Met –Ser -Ala -Gly -Leu -Tyr

- DNA sequence of gene transcribed into mRNA
- mRNA
  - Codon – set of 3 RNA nucleotides
  - T of DNA substituted for U of RNA
- tRNA
  - Anticodon – 3 RNA nucleotide part of tRNA molecule
  - Allows binding of tRNA to mRNA codon


Coding strand
5′
A C T G C C C A T G A G C G A C C C T T C G G G C T C G G G G A A T G A
3′
T G A C G G G T A C T C G C T G G G A A G C C C G A G C C C C T T A C T
3′
5′
Template strand
DNA
Transcription
mRNA
5′
A C U G C C C A U G A G C G A C C C U U C G G G C U C G G G G A A U G A
3′
Untranslated region
Start codon
Codons
Stop codon
Translation
Anticodons
U A C U C G C U G G G G A A G C C C G A G C C C C U U
tRNA
Polypeptide
Met
Ser
Asp
Pro
Phe
Gly
Leu
Gly
Glu
Amino acids

Nirenberg and Leder found the RNA triplets can promote the binding of tRNA to ribosomes

- 1964 found that an RNA triplet can act like a codon within an mRNA molecule
- Experiment establishes relationship between triplet sequence and specific amino acids
- Used radiolabeled amino acids bound to tRNA
- Complex of tRNA, RNA triplet and ribosome could be filtered by size

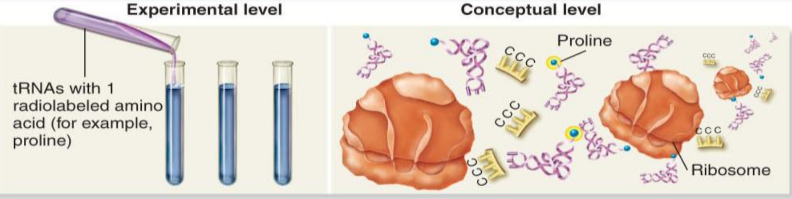
FEATURE INVESTIGATION

HYPOTHESIS A triplet RNA can bind to a ribosome and promote the binding of the tRNA that carries the amino acid that the triplet RNA specifies.

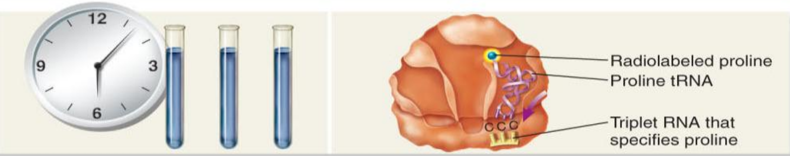
STARTING MATERIALS Components of an *in vitro* translation system, including ribosomes and tRNAs. Preparations containing all of the different tRNA molecules were given 1 radiolabeled amino acid; the other 19 amino acids were nonlabeled. For example, in 1 sample, radiolabeled glycine was added and the other 19 amino acids were nonlabeled. In a different sample, radiolabeled proline was added and the other 19 amino acids were nonlabeled. The tRNA preparation also contained the enzymes that attach amino acids to tRNAs.

Experimental level **Conceptual level**

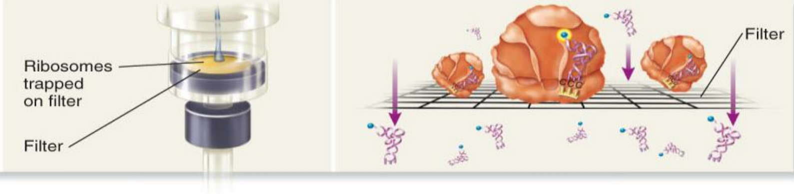
1 Mix together triplet RNAs of a specific sequence and ribosomes. In the example shown here, the triplet is 5′–CCC–3′. Add a tRNA sample to this mixture that contains 1 radiolabeled amino acid. (Note: Only 3 tubes are shown here. Because there are 20 different amino acids, this would be done in 20 different tubes.)



2 Allow time for triplet RNA to bind to the ribosome, and for the appropriate tRNA to bind to the triplet RNA.



3 Pour mixture through a filter that allows the passage of unbound tRNA but does not allow the passage of ribosomes.



4 Count radioactivity on the filter.

5 **THE DATA**

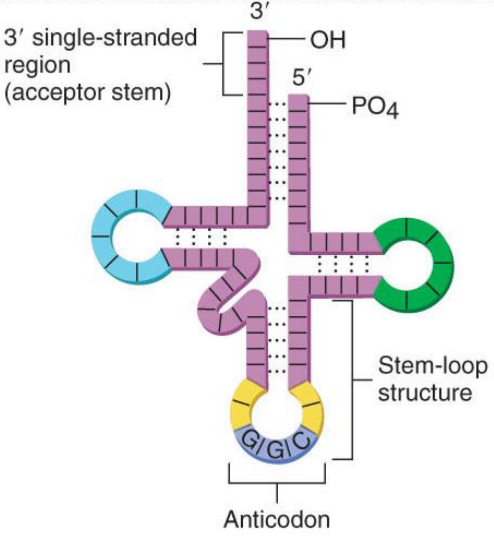
Triplet	Radiolabeled amino acid trapped on the filter	Triplet	Radiolabeled amino acid trapped on the filter
5′ – AAA – 3′	Lysine	5′ – GAC – 3′	Aspartic acid
5′ – ACA – 3′, 5′ – ACC – 3′	Threonine	5′ – GCC – 3′	Alanine
5′ – AGA – 3′	Arginine	5′ – GGU – 3′, 5′ – GGC – 3′	Glycine
5′ – AUA – 3′, 5′ – AUU – 3′	Isoleucine	5′ – GUU – 3′	Valine
5′ – CCC – 3′	Proline	5′ – UAU – 3′	Tyrosine
5′ – CGC – 3′	Arginine	5′ – UGU – 3′	Cysteine
5′ – GAA – 3′	Glutamic acid	5′ – UUG – 3′	Leucine

Translation

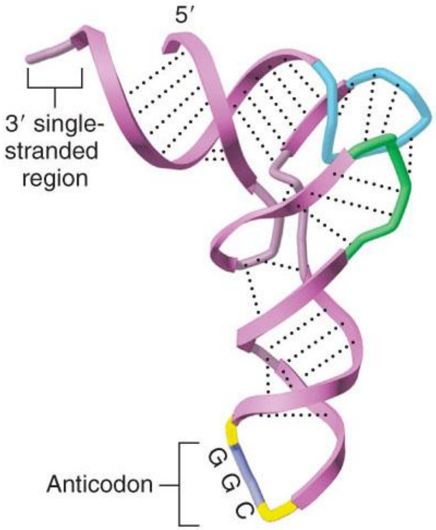
- Requires more components
- mRNA, tRNA, ribosomes, translation factors
- Most cells use a substantial amount of energy on translation

tRNA

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



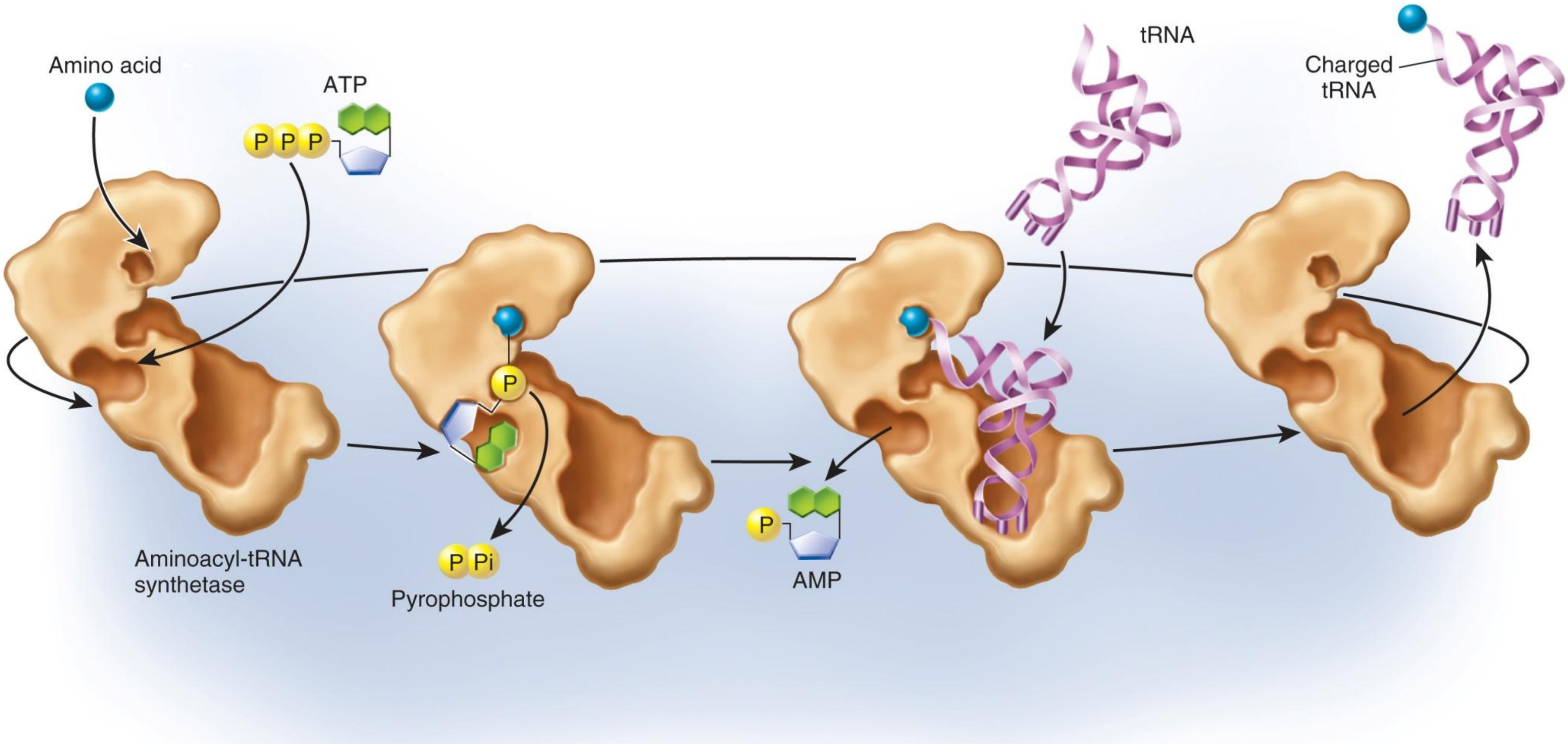
(a) Secondary structure of tRNA



(b) Three-dimensional structure of tRNA

Aminoacyl-tRNA synthetase

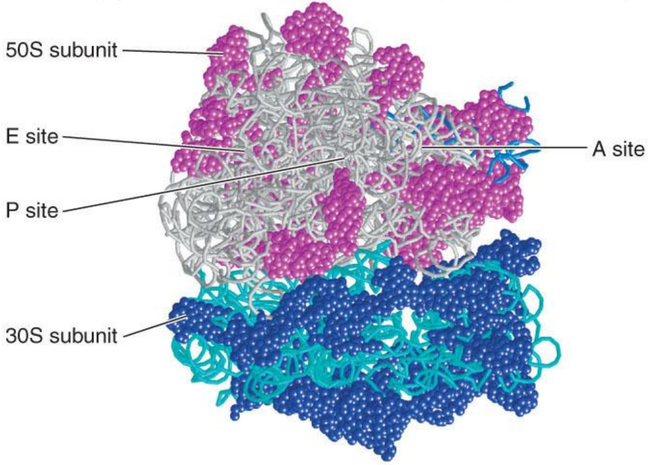
- Catalyzes the attachment of amino acids to tRNA
  - One for each of 20 different amino acids
- Reactions result in tRNA with amino acid attached or charged tRNA or aminoacyl tRNA
- Ability of aminoacyl-tRNA synthetase to recognize appropriate tRNA has been called the second genetic code


Amino acid
ATP
P P P
tRNA
Charged tRNA
P
P
AMP
P Pi
Pyrophosphate
Aminoacyl-tRNA synthetase

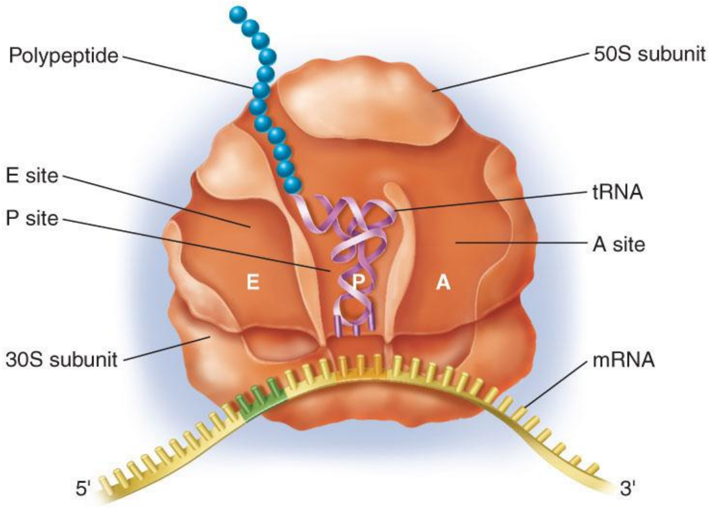
Ribosomes

- 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

- 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

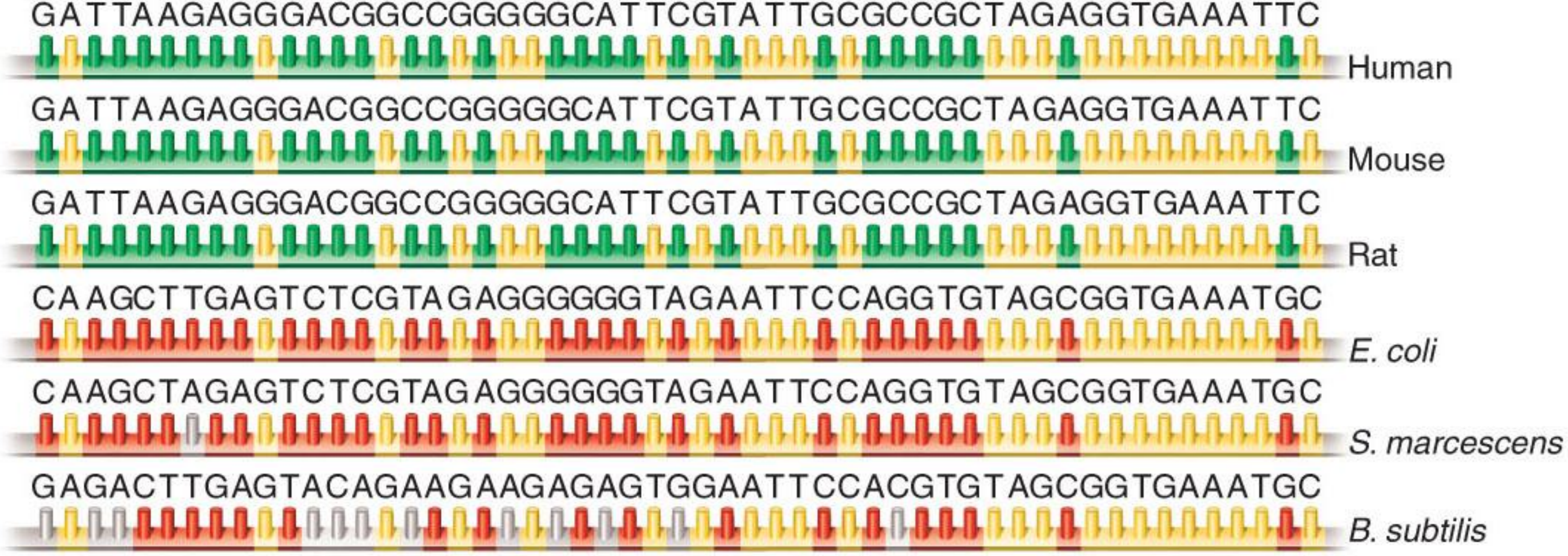


(b) Schematic model for ribosome structure

a: © Tom Pantages

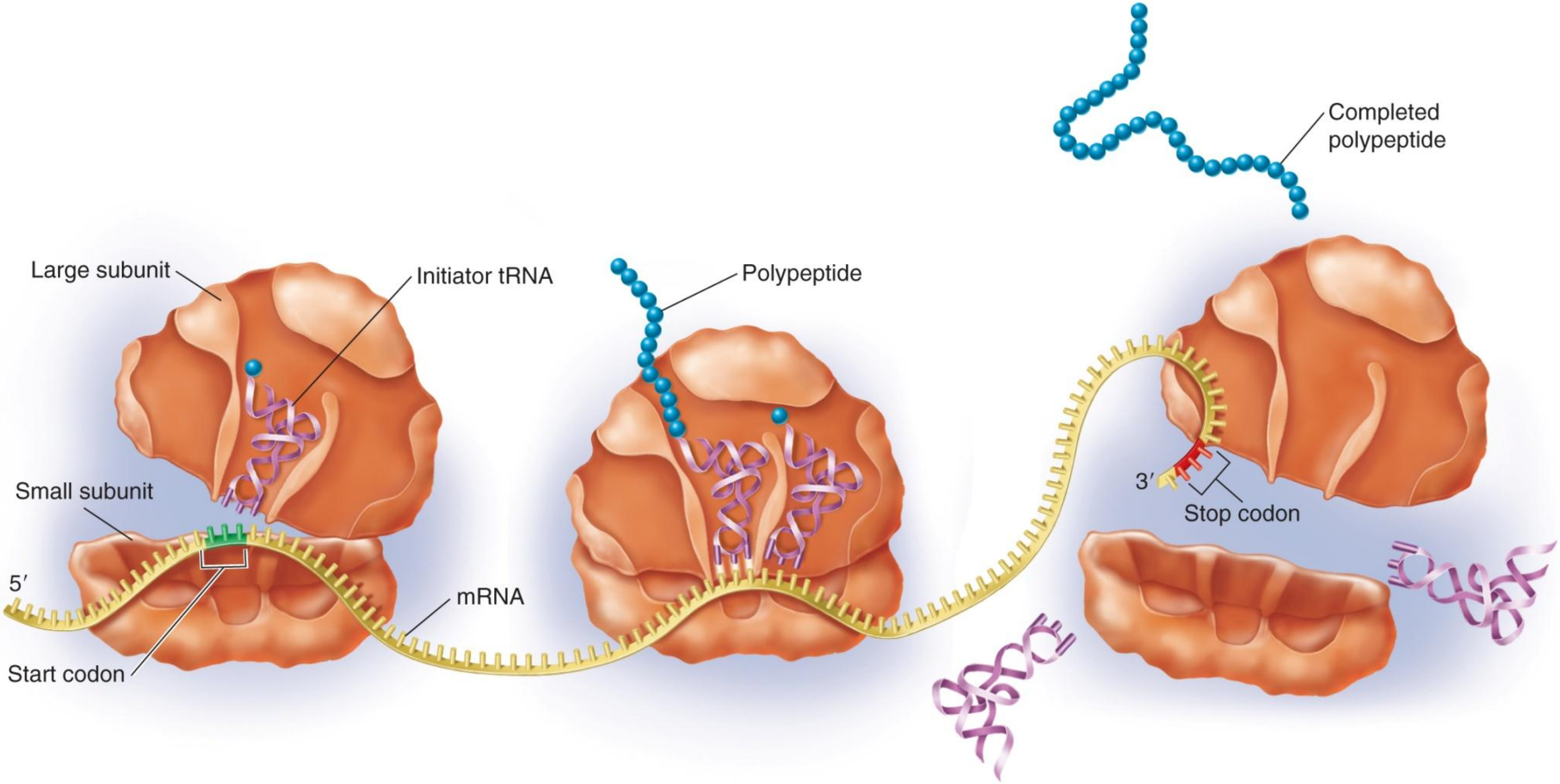
Comparisons of small subunit rRNAs among different species provide basis for establishing evolutionary relationships

- Components for translation arose in ancestor that gave rise to all living species
- All organisms have evolutionarily related translational components
- Gene for small subunit rRNA (SSU rRNA) found in all genomes
- Gene evolution involves changes in DNA sequences
- Identical sequences are evolutionarily conserved
  - Critical function not subject to change
- Gene sequences more similar in more closely related species


GATTAAGAGGGACGGCCGGGGGCATTCGTATTGCGCCGCTAGAGGTGAAATTC
Human
GATTAAGAGGGACGGCCGGGGGCATTCGTATTGCGCCGCTAGAGGTGAAATTC
Mouse
GATTAAGAGGGACGGCCGGGGGCATTCGTATTGCGCCGCTAGAGGTGAAATTC
Rat
CAAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGC
E. coli
CAAGCTAGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGC
S. marcescens
GAGACTTGAGTACAGAAGAAGAGAGTGGAATTCCACGTGTAGCGGTGAAATGC
B. subtilis

3 Stages of Translation

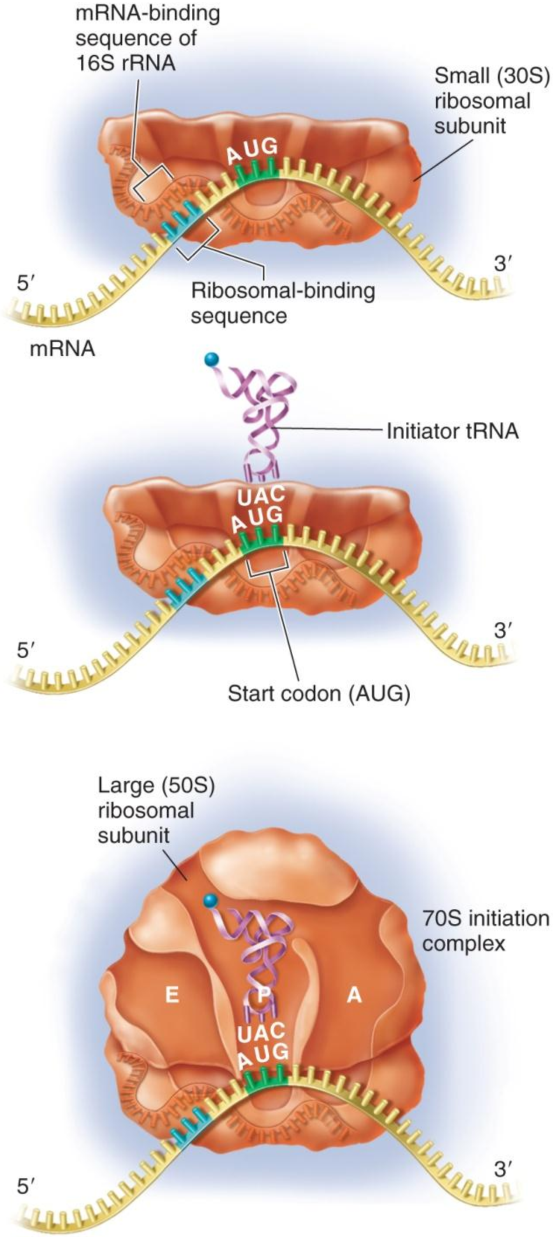
1. Initiation
    - mRNA, first tRNA and ribosomal subunits assemble
2. Elongation
    - Synthesis from start codon to stop codon
3. Termination
    - Complex disassembles at stop codon releasing completed polypeptide


Completed polypeptide
Large subunit
Initiator tRNA
Polypeptide
Small subunit
5′
mRNA
Start codon
3′
Stop codon

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


mRNA-binding sequence of 16S rRNA
Small (30S) ribosomal subunit
AUG
5′
3′
Ribosomal-binding sequence
mRNA
Initiator tRNA
UAC
AUG
5′
3′
Start codon (AUG)
Large (50S) ribosomal subunit
70S initiation complex
E
P
A
UAC
AUG
5′
3′

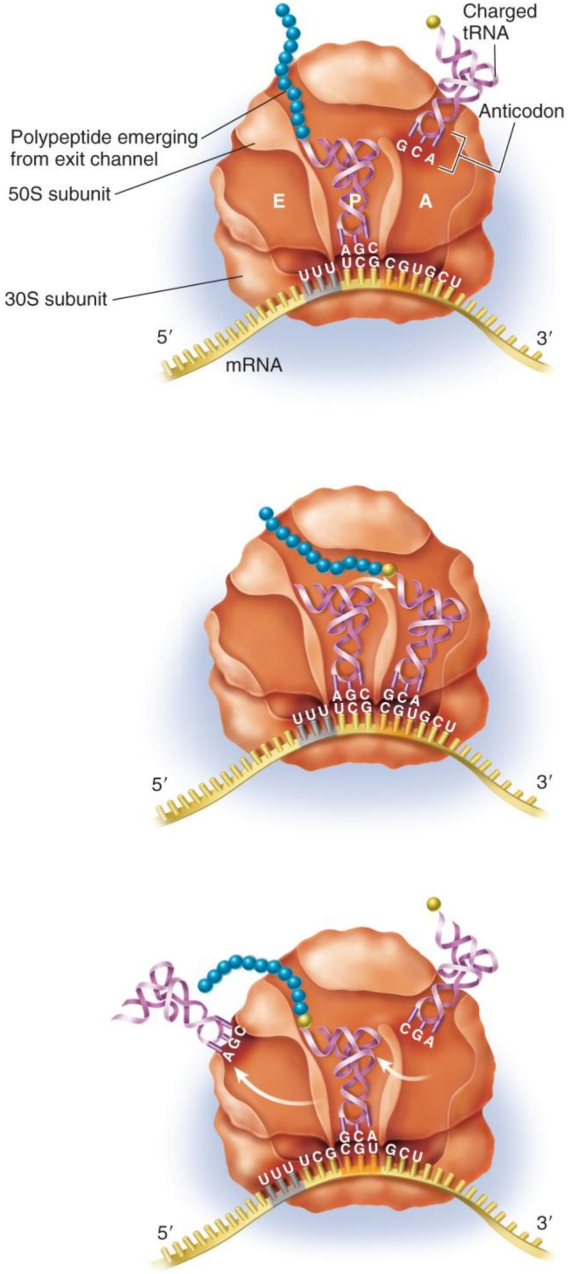
- 2 eukaryotic differences in initiation
  - Instead of a ribosomal-binding sequence, mRNAs have guanosine cap at 5’ end
    - Recognized by cap-binding proteins
  - Position of start codon more variable
    - In many cases, first AUG codon used as start codon

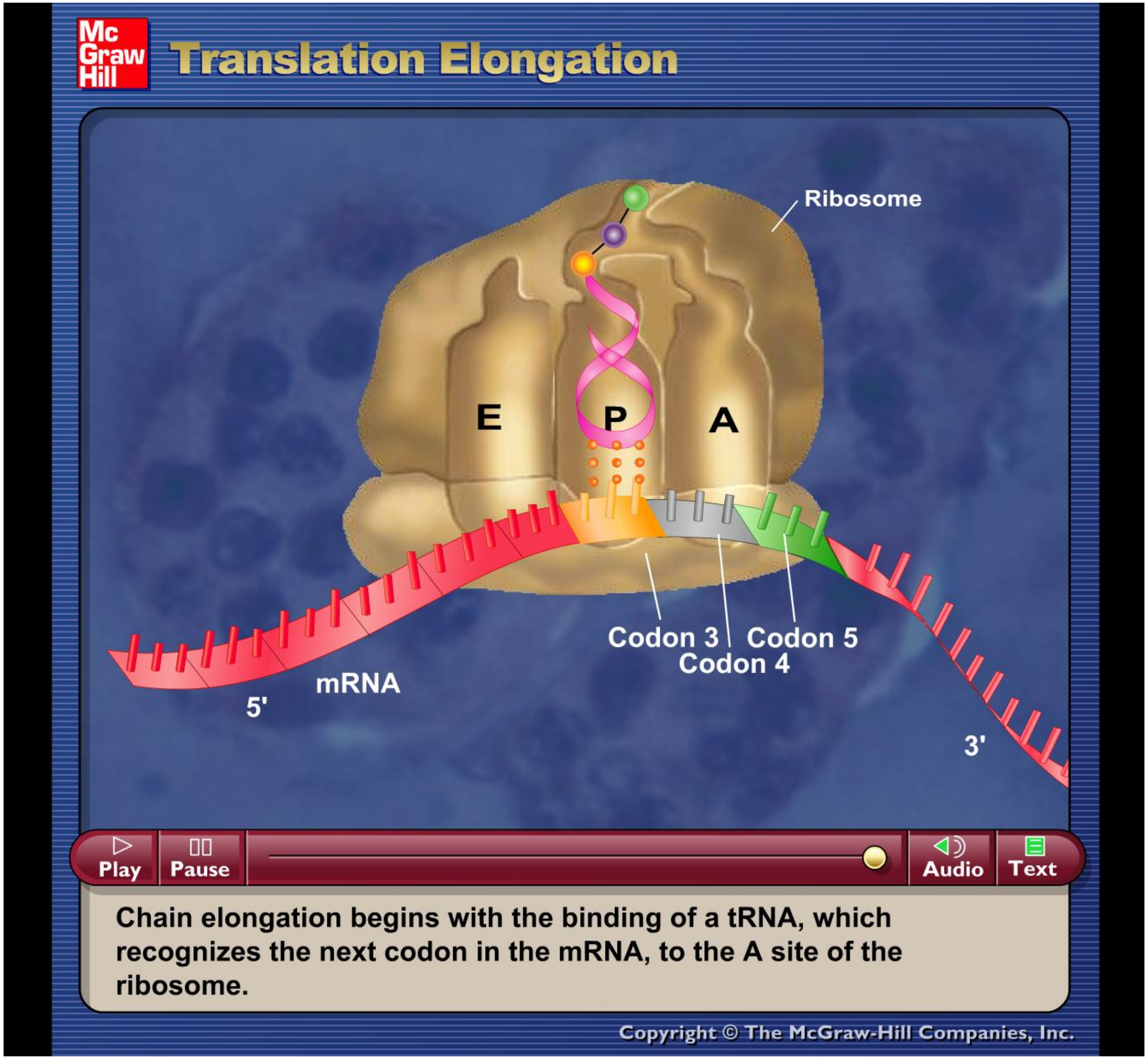
Elongation

1. Aminoacyl tRNA brings a new amino acid to the A site
   - Binding occurs due to codon/ anticodon recognition
   - Elongation factors hydrolzye 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
    - rRNA catalyzes peptide bond formation – ribosome is a ribozyme

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


Charged tRNA
Anticodon
Polypeptide emerging from exit channel
50S subunit
30S subunit
E
P
A
GCA
AGC
UUU UCG CGUGCU
5′
3′
mRNA
AGC GCA
UUU UCG CGUGCU
5′
3′
AGC
CGA
GCA
UUU UCG CGU GCU
5′
3′



Translation Elongation

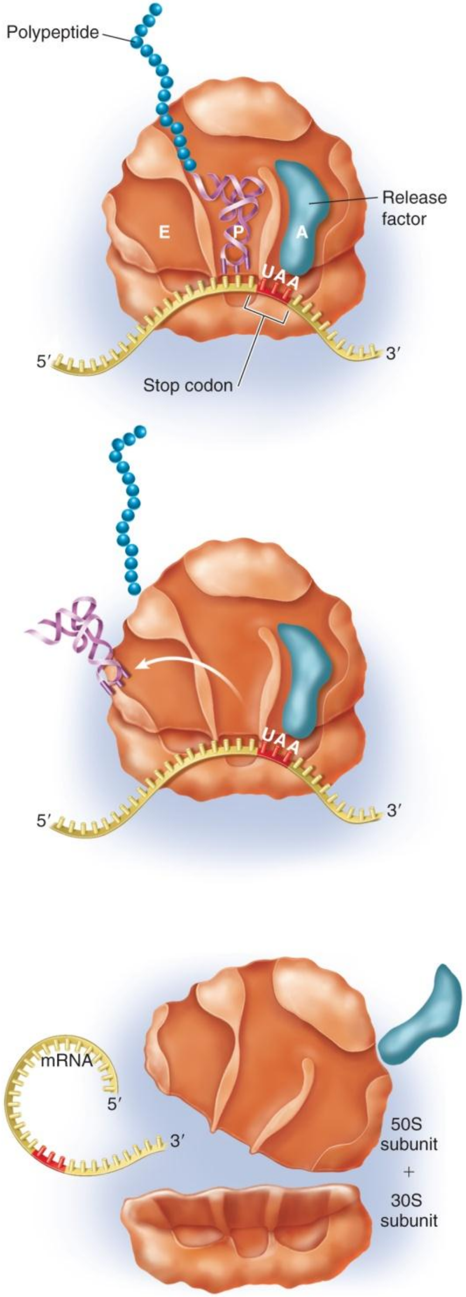
Chain elongation begins with the binding of a tRNA, which recognizes the next codon in the mRNA, to the A site of the ribosome.

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
Release factor
E
P
A
UAA
5′
3′
Stop codon
UAA
5′
3′
mRNA
5′
3′
50S subunit
+
30S subunit