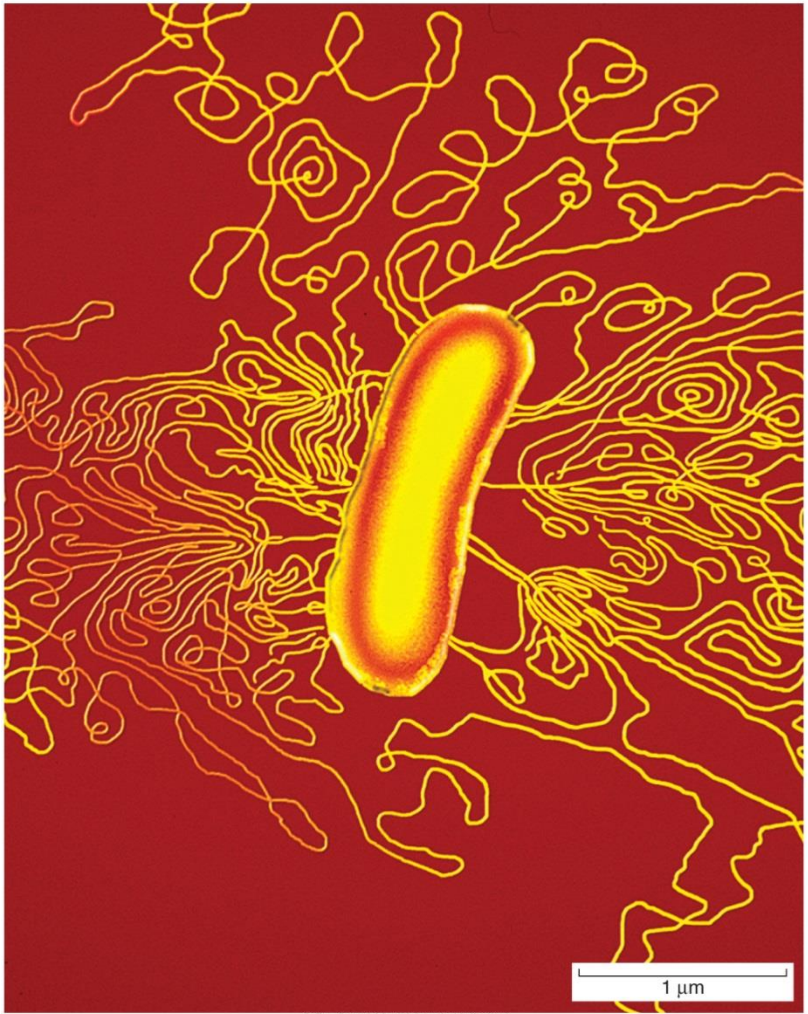
Genes and How They Work

Chapter 15



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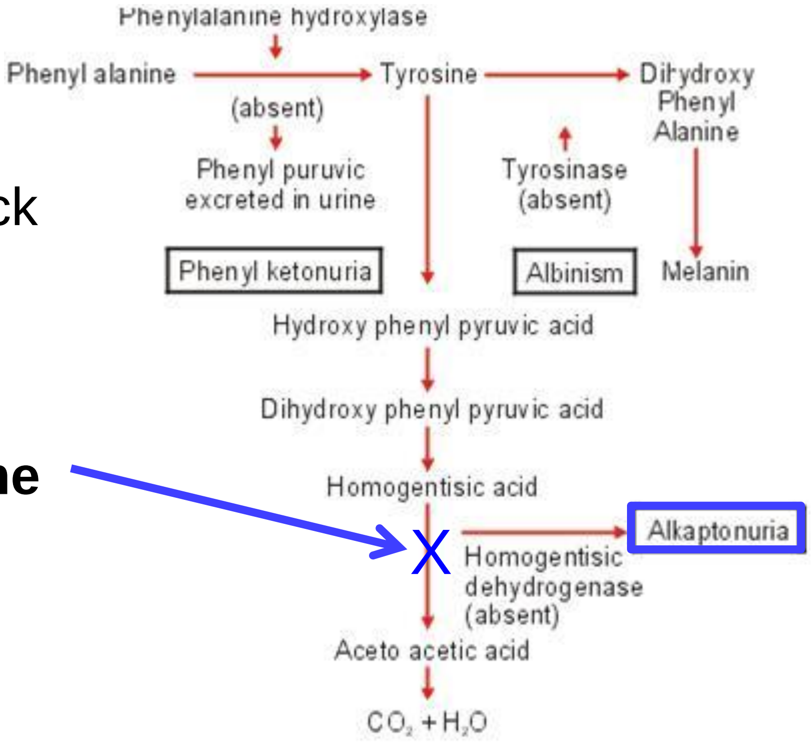
The Nature of Genes

- Early ideas to explain how **genes** work came from studying human diseases
- **Archibald Garrod** – 1902
  - Recognized that alkaptonuria (black urine disease) is inherited via a recessive allele
  - Proposed that patients with the disease lacked a functional **enzyme** for tyrosine degradation pathway



- These ideas <u>**connected**</u> **genes** to **enzymes**

The Nature of Genes

Beadle and Tatum (1941):

- Deliberately set out to create **mutations** in chromosomes and verify that they behaved in a Mendelian fashion in crosses

- Studied ***Neurospora crassa***
  - Used X-rays to damage DNA
  - Looked for nutritional mutations
    - Had to have minimal media supplemented to grow

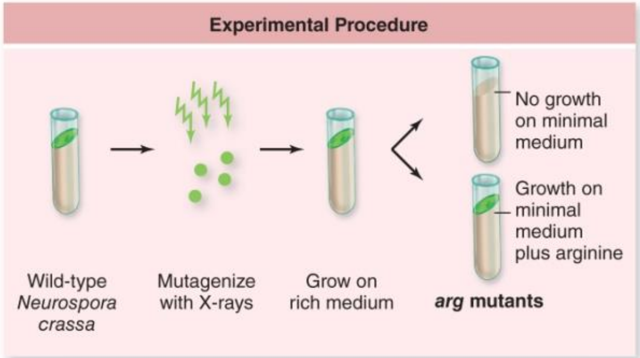
Neurospora crassa

Big Question: What information does DNA encode? -OR- What happens if you damage the DNA code?

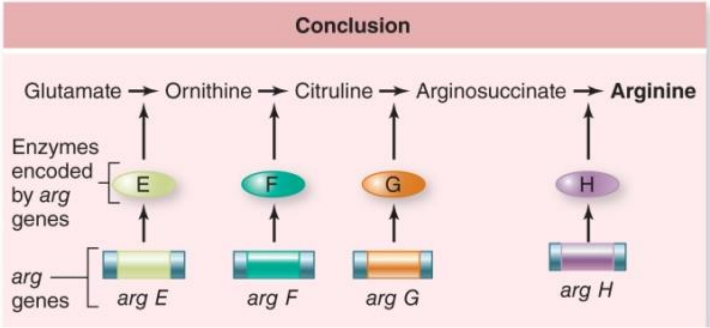
Image: http://biology.missouri.edu/news/shiu-lab-identifies-novel-players-in-gene-silencing-mechanism/

The Nature of Genes

- Beadle and Tatum looked for fungal cells **lacking** specific **enzymes**
  - The enzymes were required for the biochemical pathway producing the amino acid arginine
  - They identified mutants deficient in each enzyme of the pathway

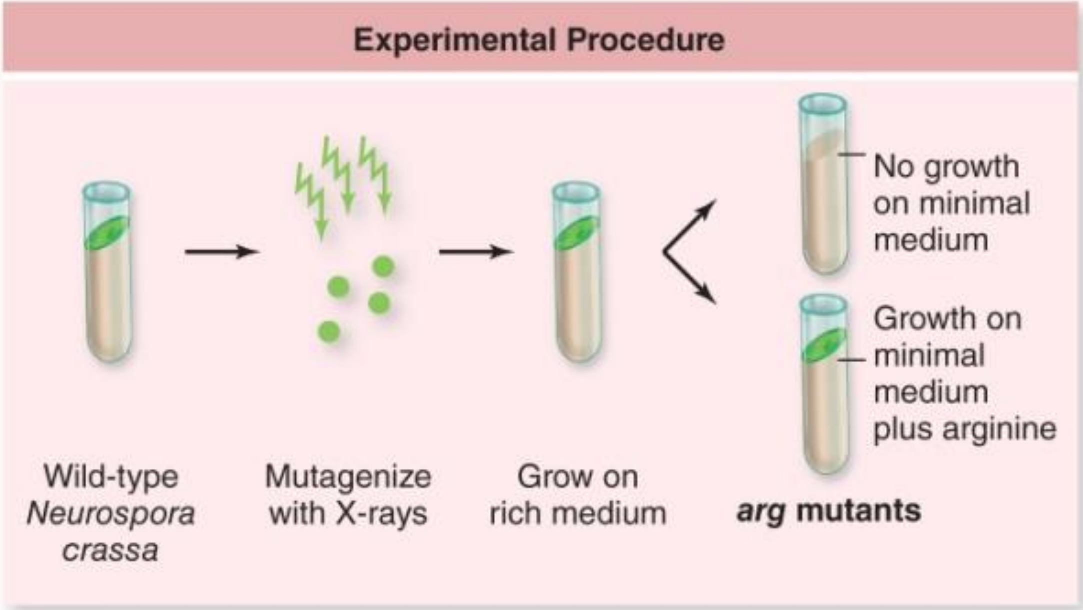


- **One-gene/one-enzyme** hypothesis has been modified to **one-gene/one-polypeptide** hypothesis



The Nature of Genes

Beadle and Tatum Experiment

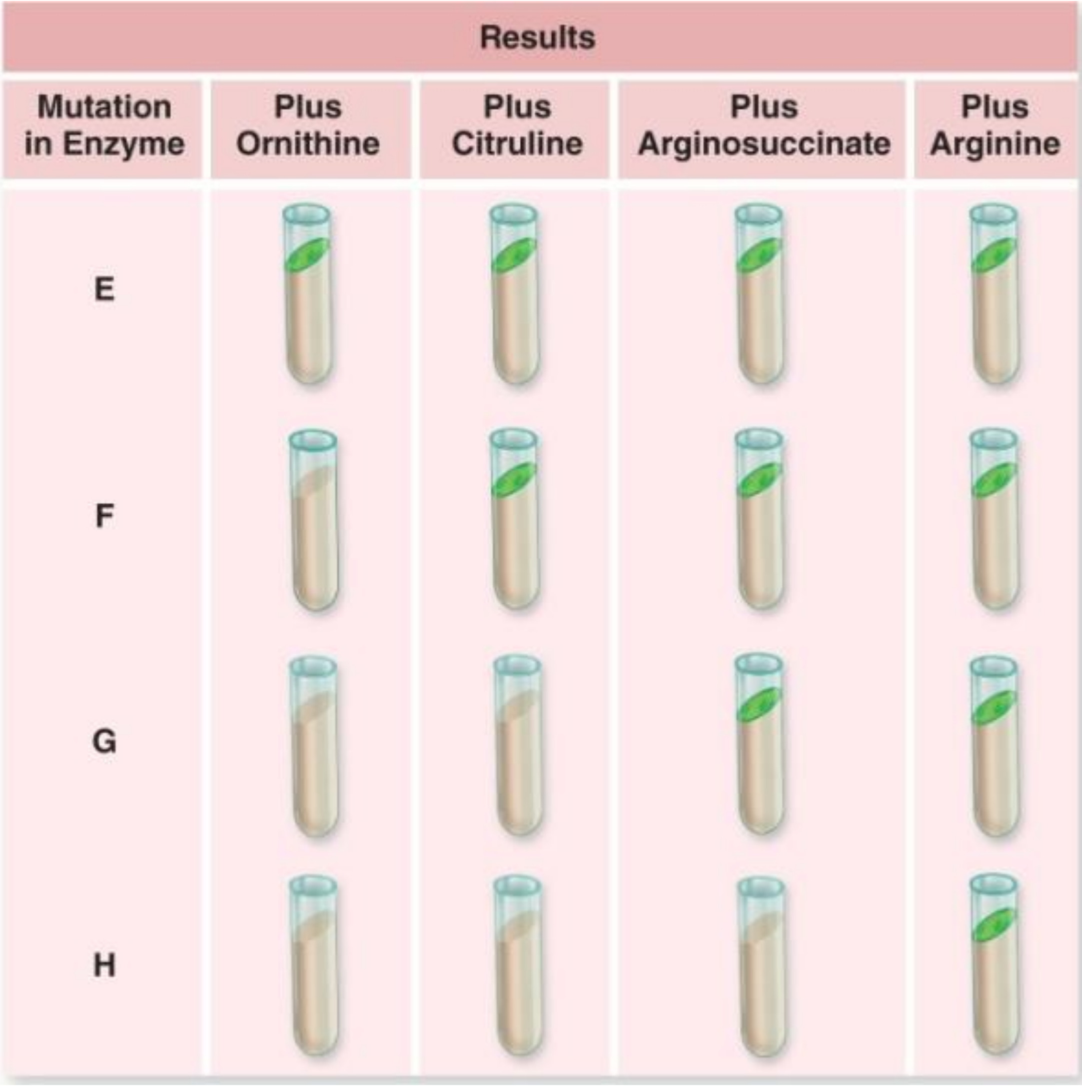


Step One:

- Create mutants of *Neurospora* using X-rays
- Look for mutants that cannot synthesize (make) arginine on their own, have to be given arginine in their media

The Nature of Genes

Beadle and Tatum Experiment

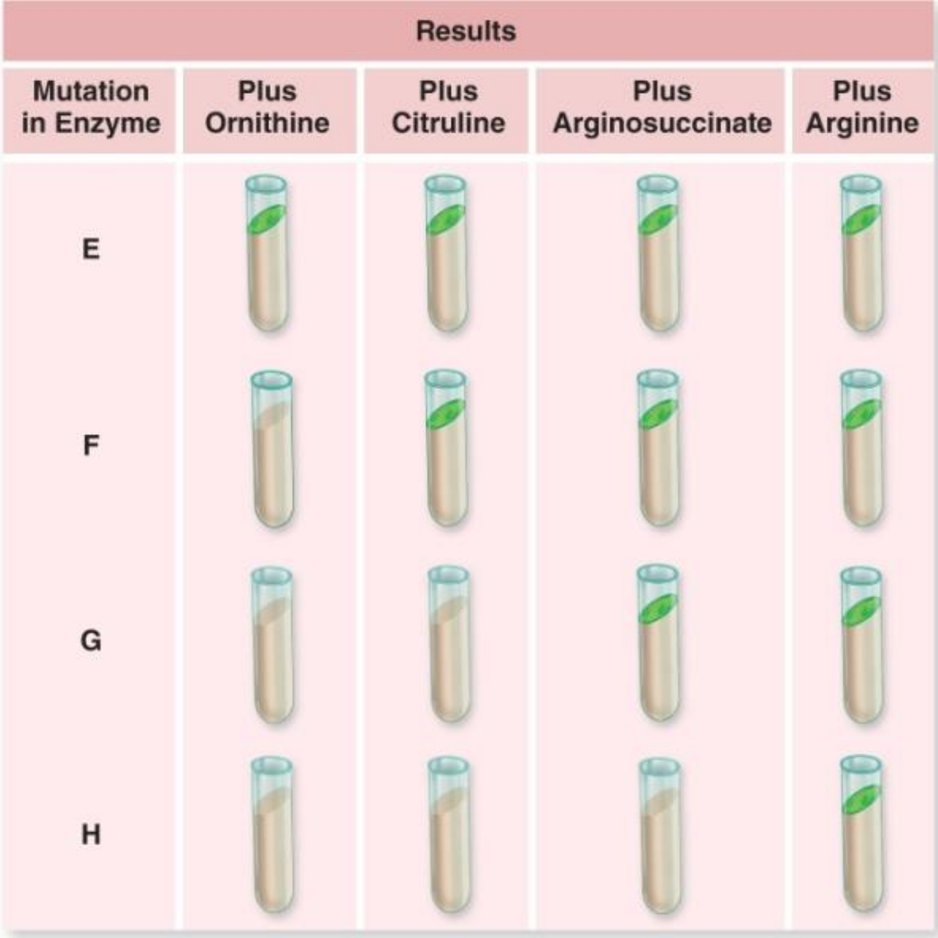


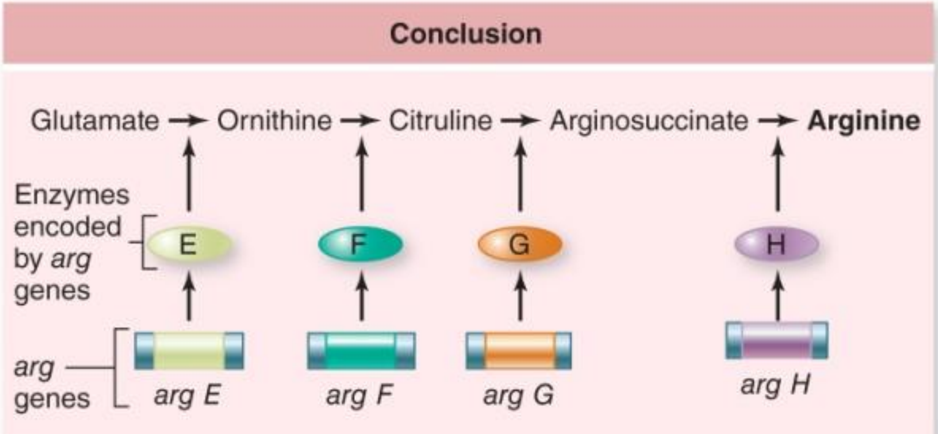
Step Two: **Analyze Results**

- Determine why mutation prevents the synthesis of Arginine
- Different enzyme mutations (1st column) lead to different growth
- ***Note:*** it takes several steps to make arginine (part of a metabolic pathway)

The Nature of Genes

Beadle and Tatum Experiment





Conclusions

- Each mutated enzyme disrupted one key enzyme in the metabolic pathway

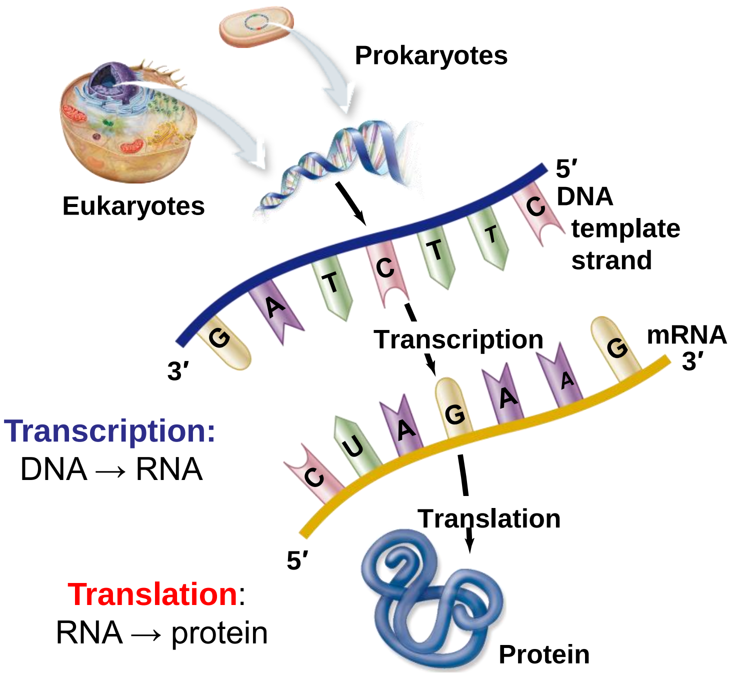
The Nature of Genes

Really Important!!

The Central Dogma:
DNA → RNA → protein

- First described by Francis Crick
- Information only flows from…

DNA → RNA → protein



Transcription:
DNA → RNA

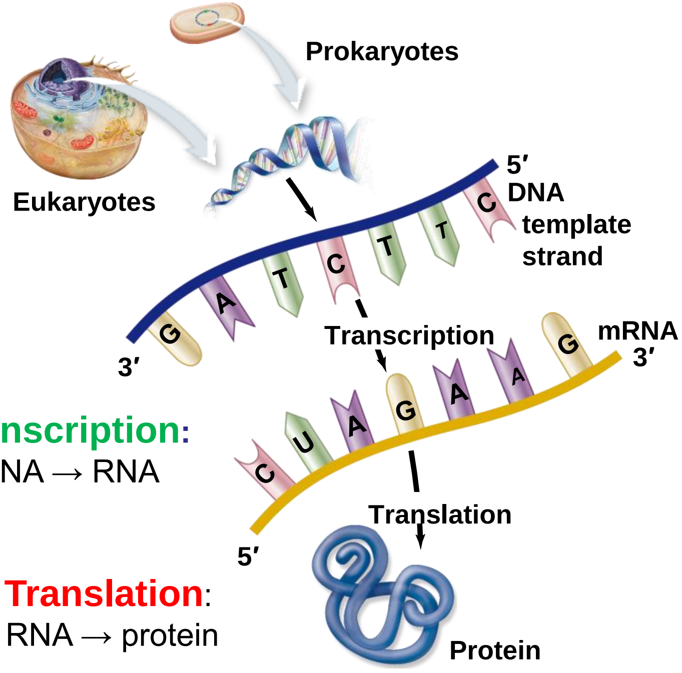
Translation:
RNA → protein

The Nature of Genes

Really Important!!

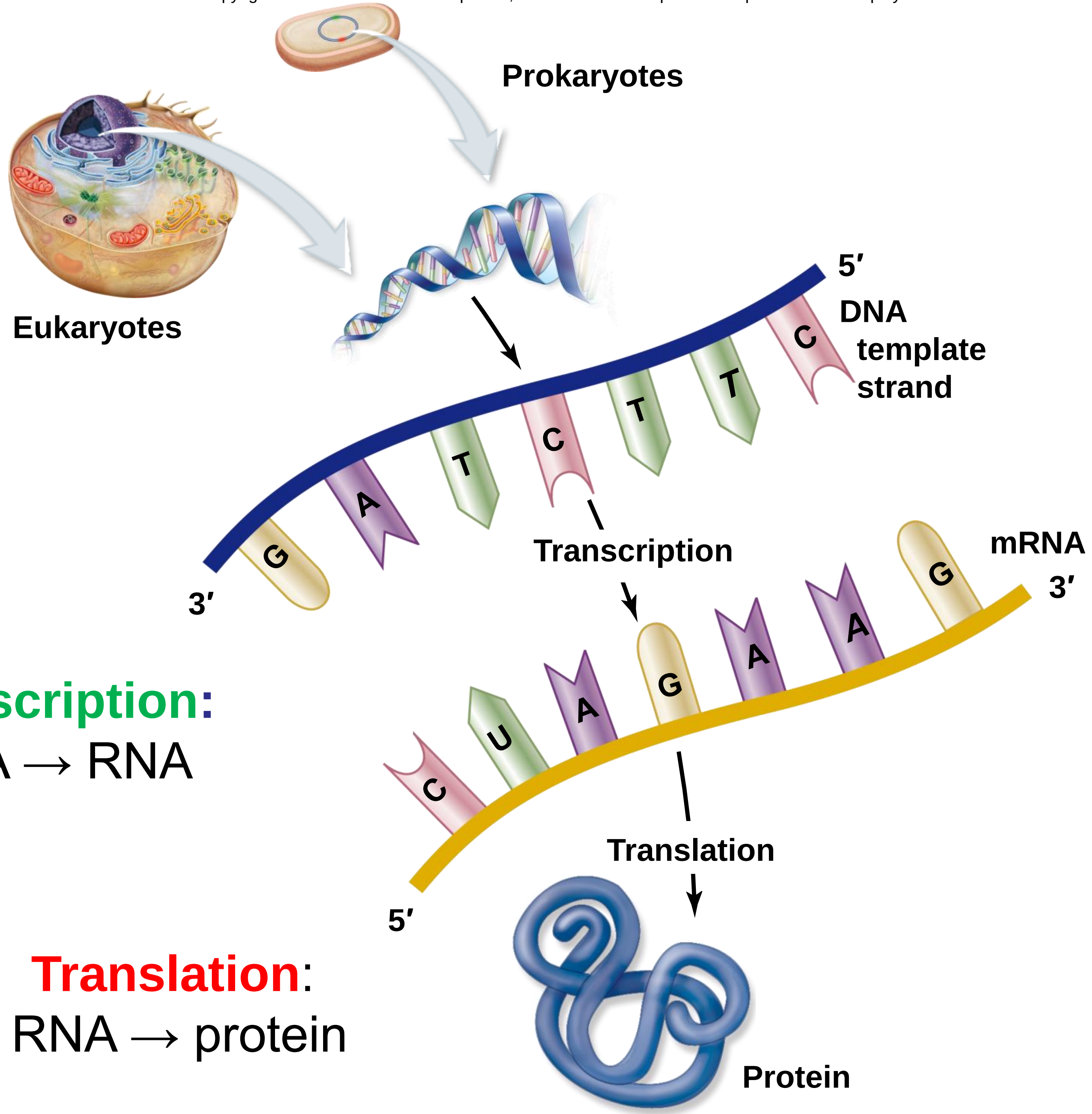
DNA → RNA → protein

- **Transcription** = DNA → RNA
- **Translation** = RNA → protein
- **Retroviruses** violate this order using reverse transcriptase to convert RNA genome into DNA



Transcription: DNA → RNA

Translation: RNA → protein



Transcription:
DNA → RNA

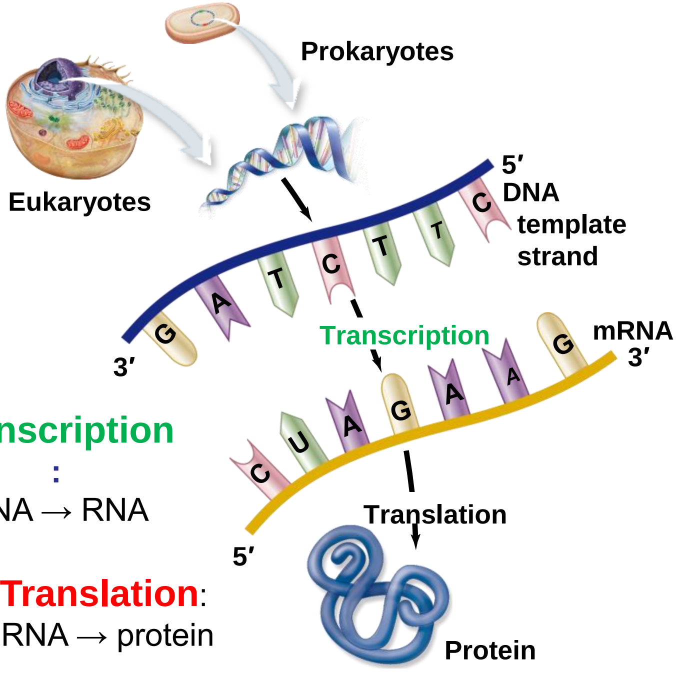
Translation:
RNA → protein

DNA → RNA → protein

- Transcription
  - DNA-directed synthesis of RNA
  - Only template strand of DNA used
  - U (uracil) in DNA replaced by T (thymine) in RNA
  - **mRNA** used to direct synthesis of polypeptides
- Translation
  - Synthesis of polypeptides
  - Takes place at ribosome
  - Requires several kinds of RNA

Transcription: DNA → RNA

Translation: RNA → protein



The Nature of Genes

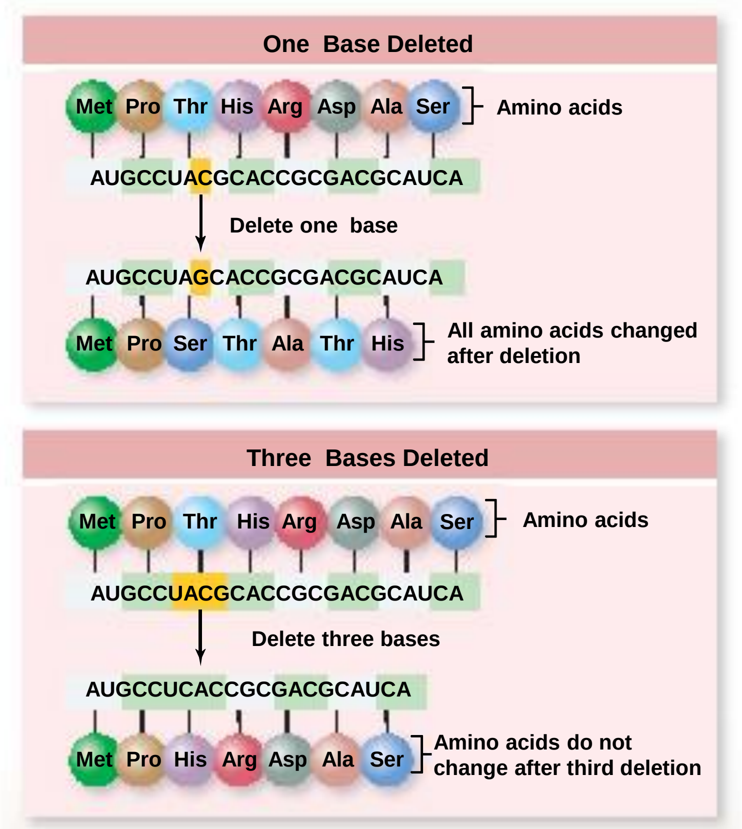
RNA

- All synthesized from DNA template by transcription
- Messenger RNA (**mRNA**)
- Ribosomal RNA (**rRNA**)
- Transfer RNA (**tRNA**)
- Small nuclear RNA (snRNA)
- Signal recognition particle RNA (SRP RNA)
- Micro-RNA (miRNA)

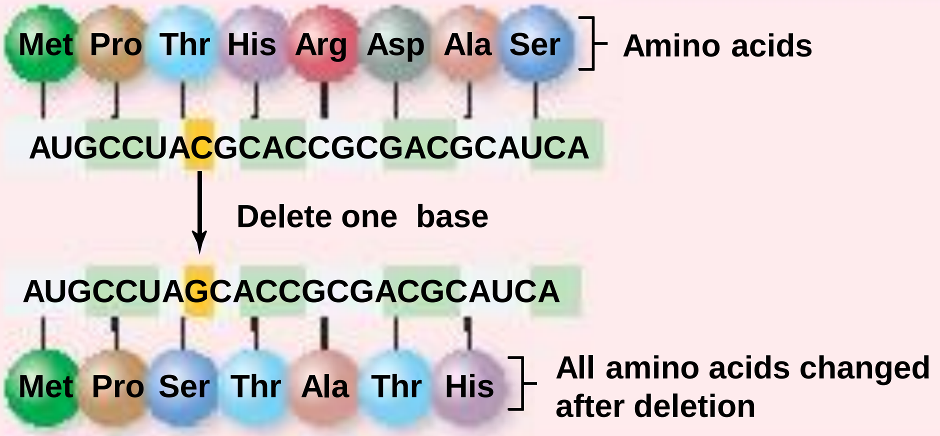
The Genetic Code

The Genetic Code:

- Francis Crick & Sydney Brenner determined how the *order of nucleotides* in DNA encoded amino acid order
- Introduced single nulcleotide insertions or deletions and looked for mutations
  - **Frameshift mutations**
- Indicates importance of **reading frame**



One Base Deleted



Met Pro Thr His Arg Asp Ala Ser – Amino acids

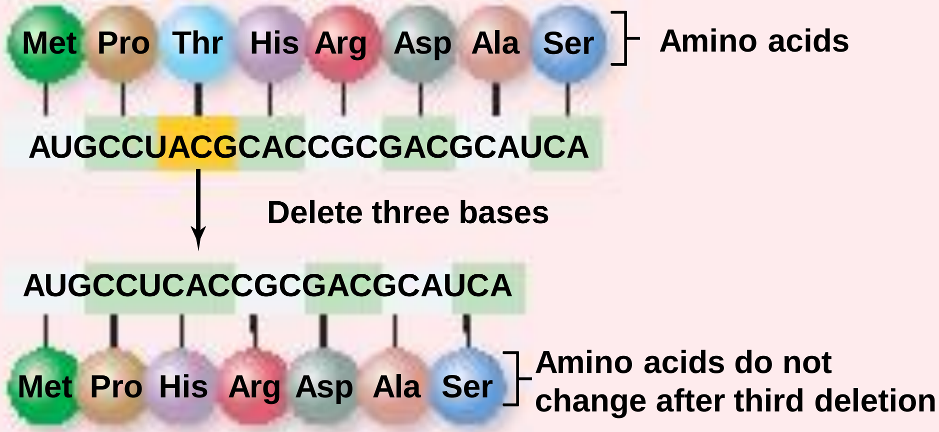
AUGCCUACGCACCGCGACGCAUCA

Delete one base

AUGCCUAGCACCGCGACGCAUCA

Met Pro Ser Thr Ala Thr His – All amino acids changed after deletion

Three Bases Deleted



Met Pro Thr His Arg Asp Ala Ser – Amino acids

AUGCCUACGCACCGCGACGCAUCA

Delete three bases

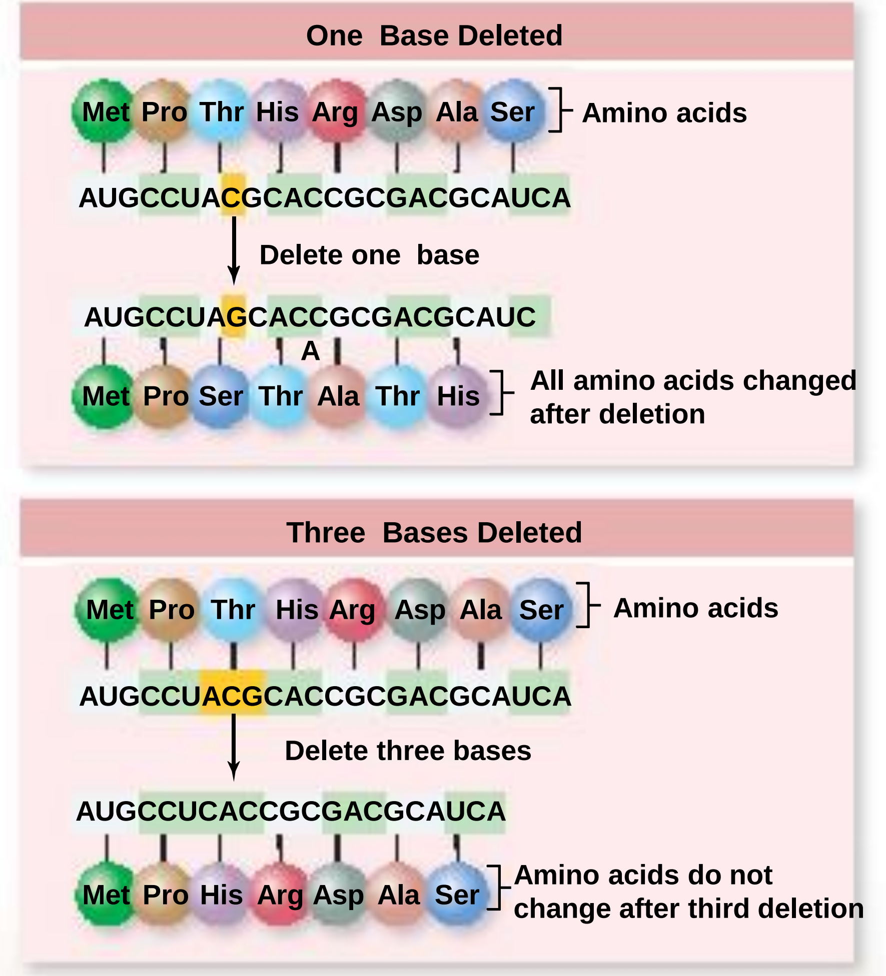
AUGCCUCACCGCGACGCAUCA

Met Pro His Arg Asp Ala Ser – Amino acids do not change after third deletion

The Genetic Code

The Genetic Code:

- **Codon** – blocks of three DNA nucleotides correspond to an amino acid (***triplet code***)



The Genetic Code

Alternative hypotheses for triplet arrangement

- **Spaced Codons**
  - Codon sequence in a gene punctuated
  - Only one word changes after deletion
- **Unspaced Codons**
  - Codons adjacent to each other
  - All words change

Sentence with Spaces

WHY DID THE RED BAT EAT THE FAT RAT

Delete one letter

WHY DID HE RED BAT EAT THE FAT RAT

Only one word changed

Sentence with No Spaces

WHYDIDTHEREDBATEATTHEFATRAT

Delete one letter

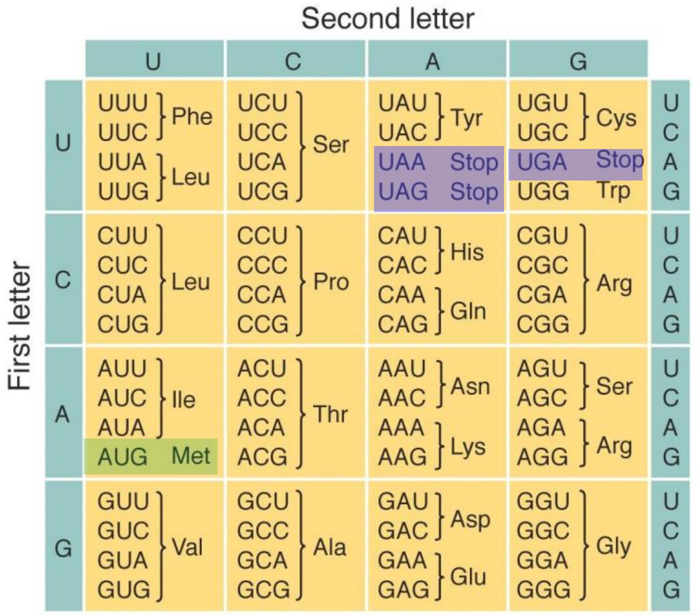
WHYDIDHEREDBATEATTHEFATRAT

All words after deletion changed

The Genetic Code

Marshall Nirenberg identified the codons that ***specify*** each amino acid

- **Stop codons**
  - 3 codons (UUA, UGA, UAG) used to terminate translation
- **Start codon** **Know this!**
  - Codon (**AUG**) used to signify the start of translation
  - Also codes for **methionine**



First letter	Second letter								Third letter
	U		C		A		G		
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	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

- Code is **degenerate**, meaning that some amino acids are specified by more than one codon

- Code is ***degenerate***, meaning that most amino acids are specified by *more than one codon, but unambigous*

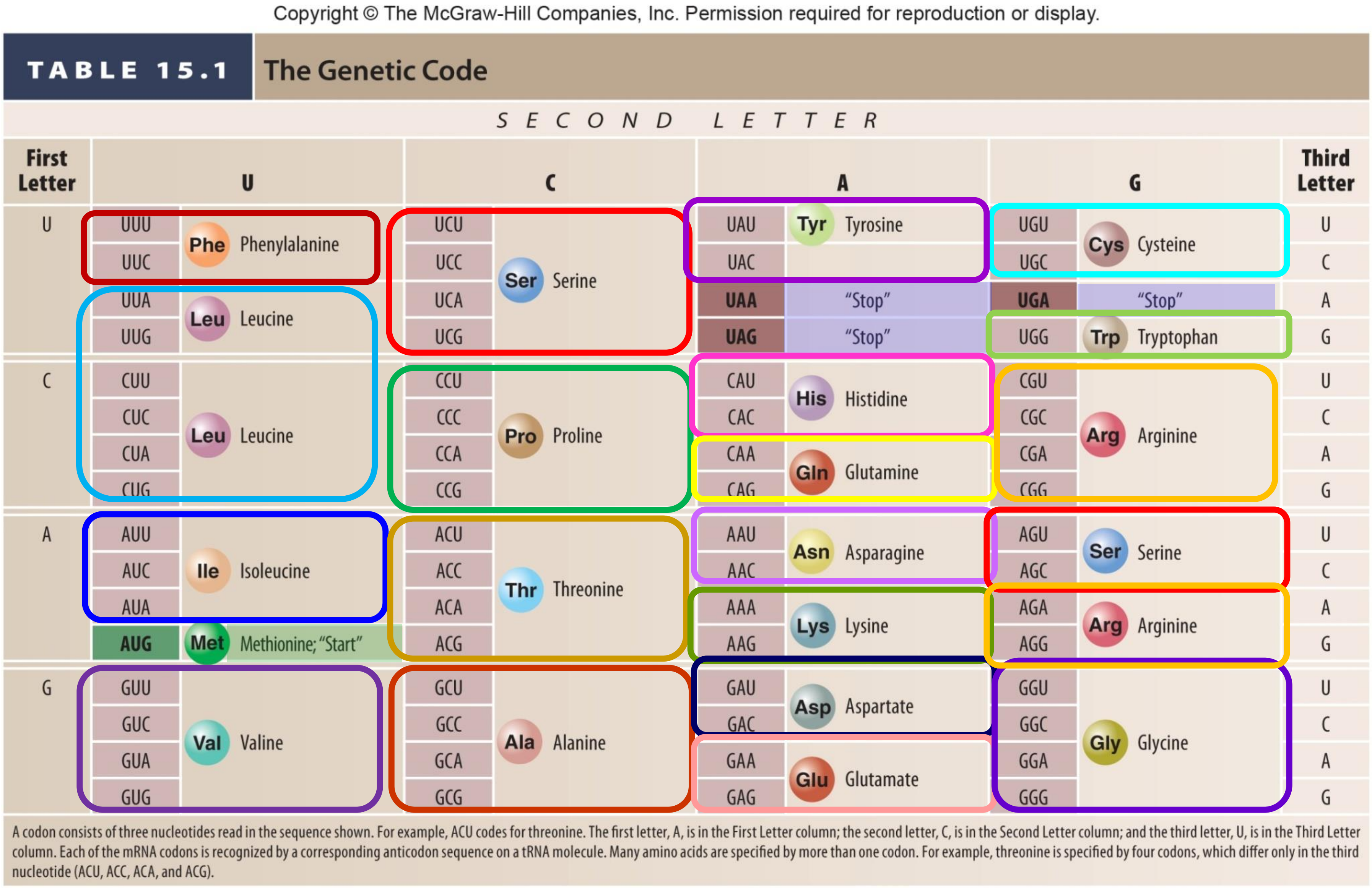


TABLE 15.1 The Genetic Code

SECOND LETTER

First Letter	U		C		A		G		Third Letter
U	UUU	**Phe** Phenylalanine	UCU	**Ser** Serine	UAU	**Tyr** Tyrosine	UGU	**Cys** Cysteine	U
	UUC		UCC		UAC		UGC		C
	UUA	**Leu** Leucine	UCA		**UAA**	"Stop"	**UGA**	"Stop"	A
	UUG		UCG		**UAG**	"Stop"	UGG	**Trp** Tryptophan	G
C	CUU	**Leu** Leucine	CCU	**Pro** Proline	CAU	**His** Histidine	CGU	**Arg** Arginine	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	**Gln** Glutamine	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	**Ile** Isoleucine	ACU	**Thr** Threonine	AAU	**Asn** Asparagine	AGU	**Ser** Serine	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	**Lys** Lysine	AGA	**Arg** Arginine	A
	AUG	**Met** Methionine; "Start"	ACG		AAG		AGG		G
G	GUU	**Val** Valine	GCU	**Ala** Alanine	GAU	**Asp** Aspartate	GGU	**Gly** Glycine	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	**Glu** Glutamate	GGA		A
	GUG		GCG		GAG		GGG		G

A codon consists of three nucleotides read in the sequence shown. For example, ACU codes for threonine. The first letter, A, is in the First Letter column; the second letter, C, is in the Second Letter column; and the third letter, U, is in the Third Letter column. Each of the mRNA codons is recognized by a corresponding anticodon sequence on a tRNA molecule. Many amino acids are specified by more than one codon. For example, threonine is specified by four codons, which differ only in the third nucleotide (ACU, ACC, ACA, and ACG).

The Genetic Code

Code practically universal

- Strongest evidence that all living things share **common ancestry**
- Advances in genetic engineering
- Mitochondria and chloroplasts have some differences in “stop” signals

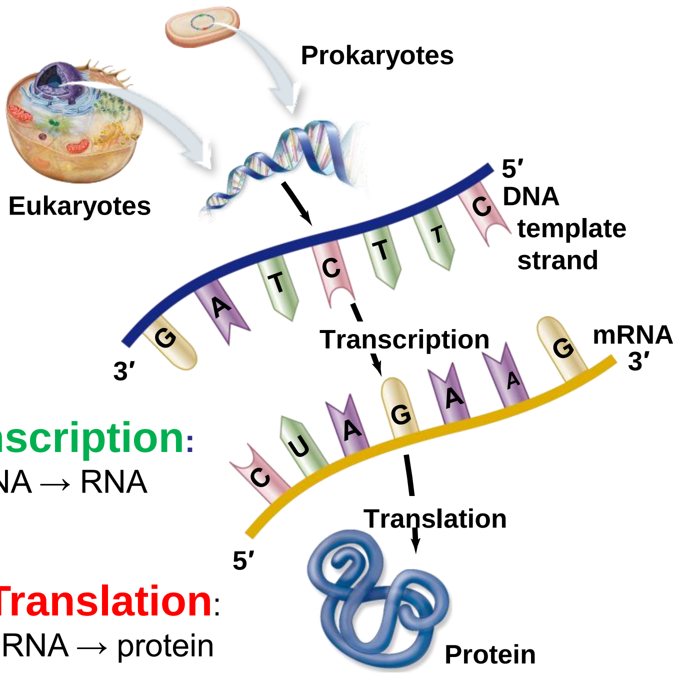
Image courtesy of the University of Missouri-Columbia, Agricultural Information

The Nature of Genes

DNA → RNA → protein

Really Important!!

- **Transcription** = DNA → RNA
- **Translation** = RNA → protein



Transcription: DNA → RNA

Translation: RNA → protein

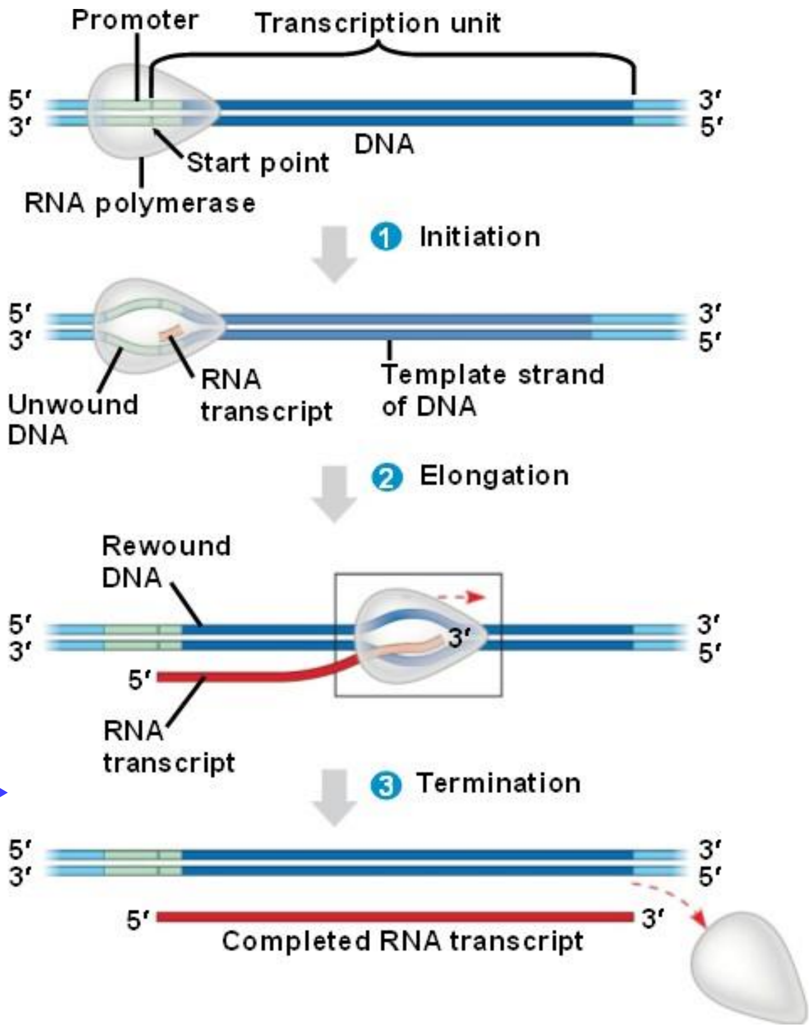
Prokaryotic Transcription

- Single RNA polymerase
- Initiation of mRNA synthesis does not require a primer
- Requires
  1. **Promoter**
  2. **Start site**
  3. **Termination site**

Transcription unit

Prokaryotic Transcription

- Transcription occurs in three major stages:
  1. **Initiation**
  2. **Elongation**
  3. **Termination**

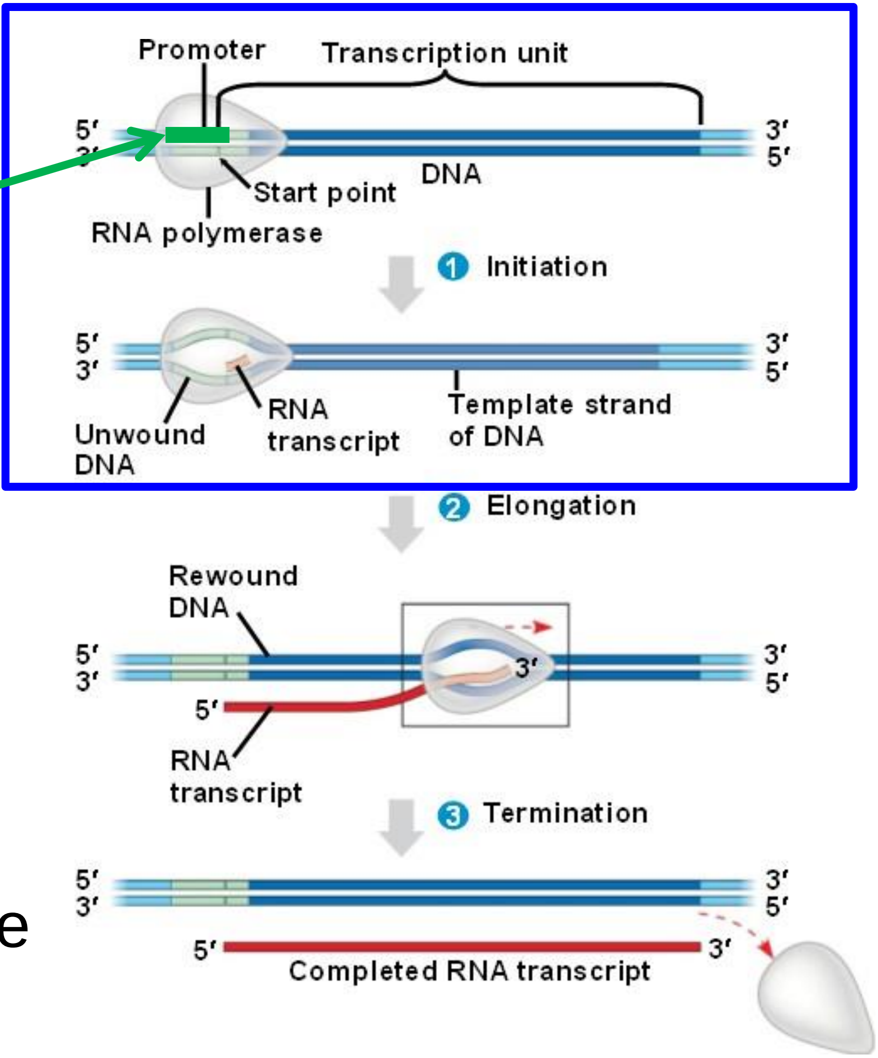


http://bio1151.nicerweb.com/Locked/media/ch17/transcription.html

Prokaryotic Transcription

Stage 1: Initiation

- RNA polymerase binds to the **promoter site**
- **Promoter**
  - Forms a recognition and binding site for the RNA polymerase
  - Found upstream of the start site
  - Promoter is not transcribed
  - *Asymmetrical* – indicates site of initiation and direction of transcription



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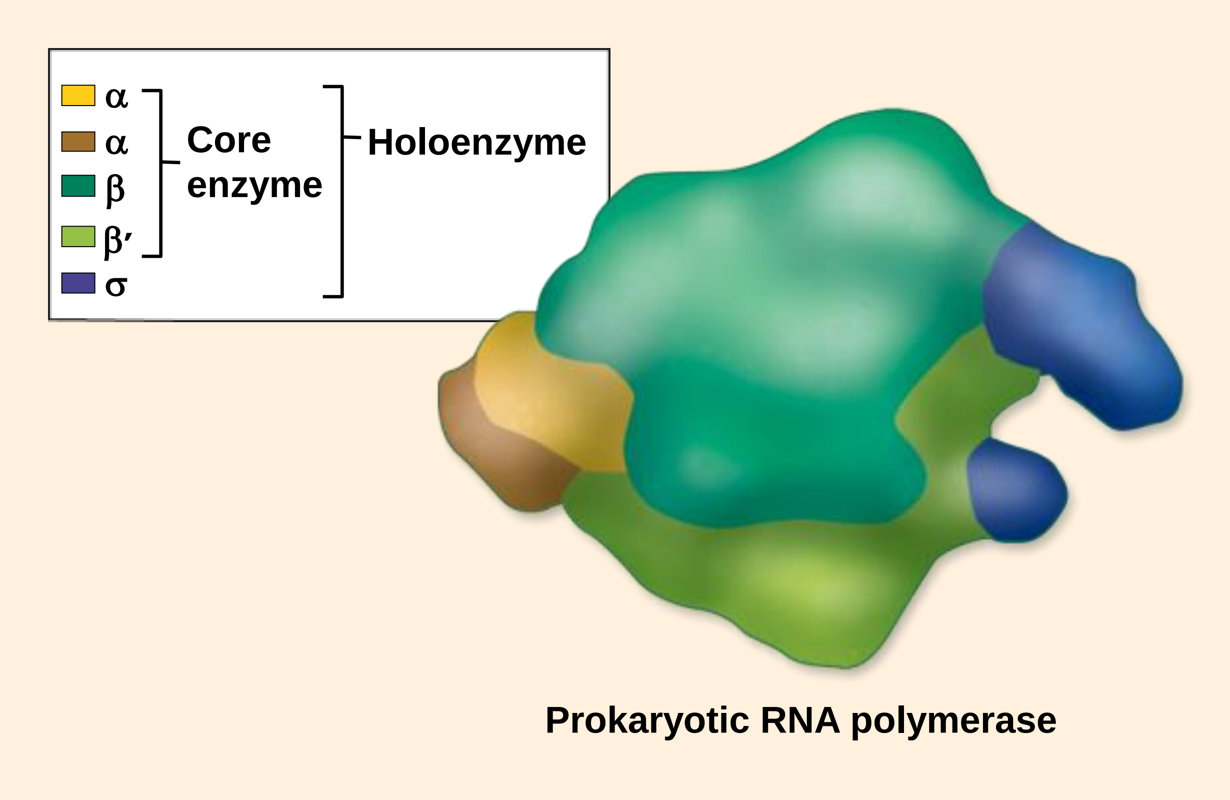
Prokaryotic Transcription

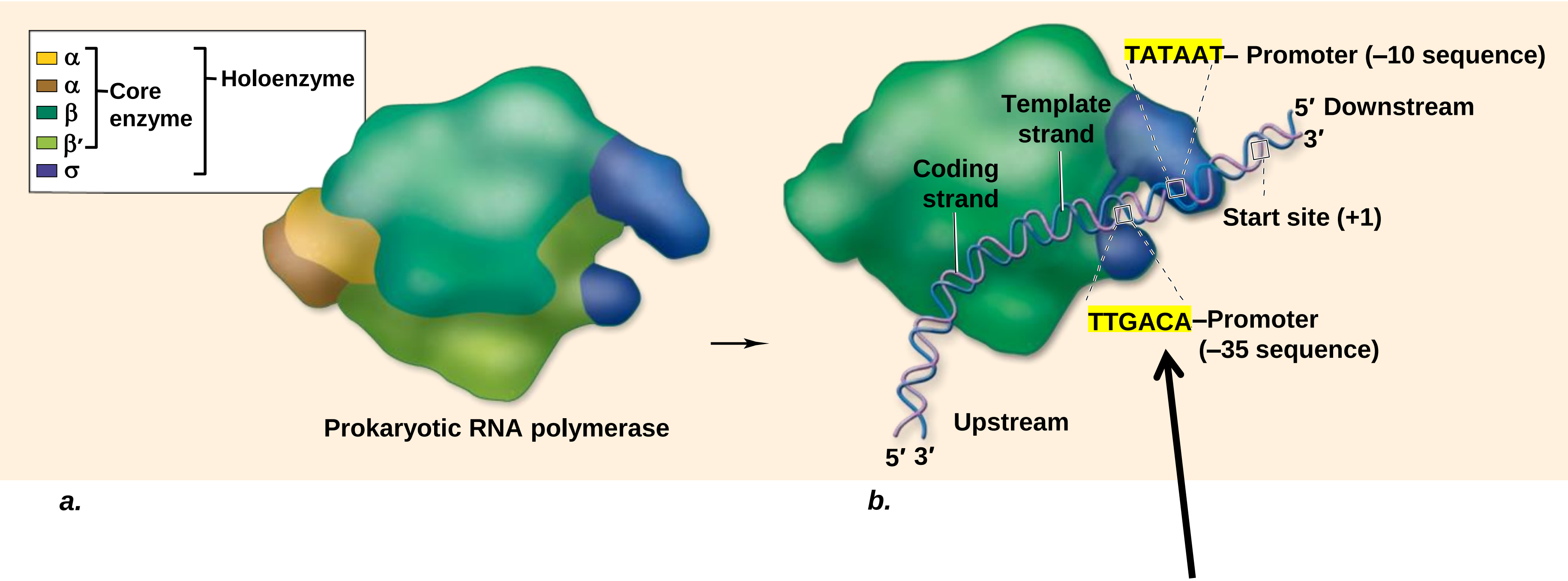


a.

Prokaryotic RNA Polymerase

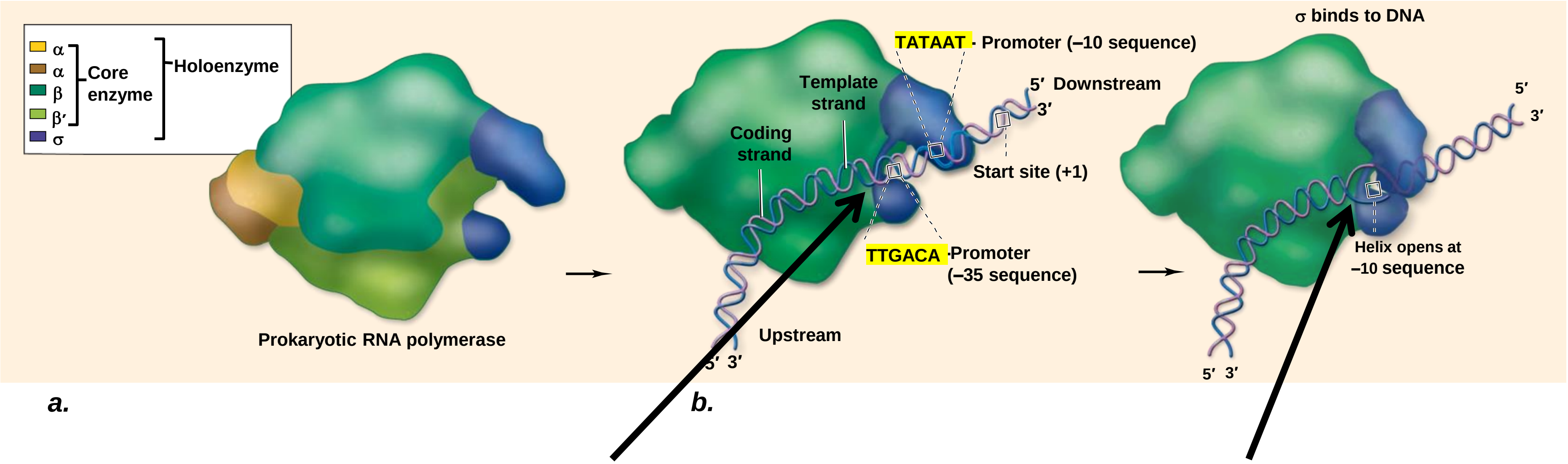
- Largeprotein that reads DNA and makes an RNA copy
- Made of several subunits

Prokaryotic Transcription



Prokaryotic **RNA Polymerase** binding to DNA at the **promoter** region of DNA

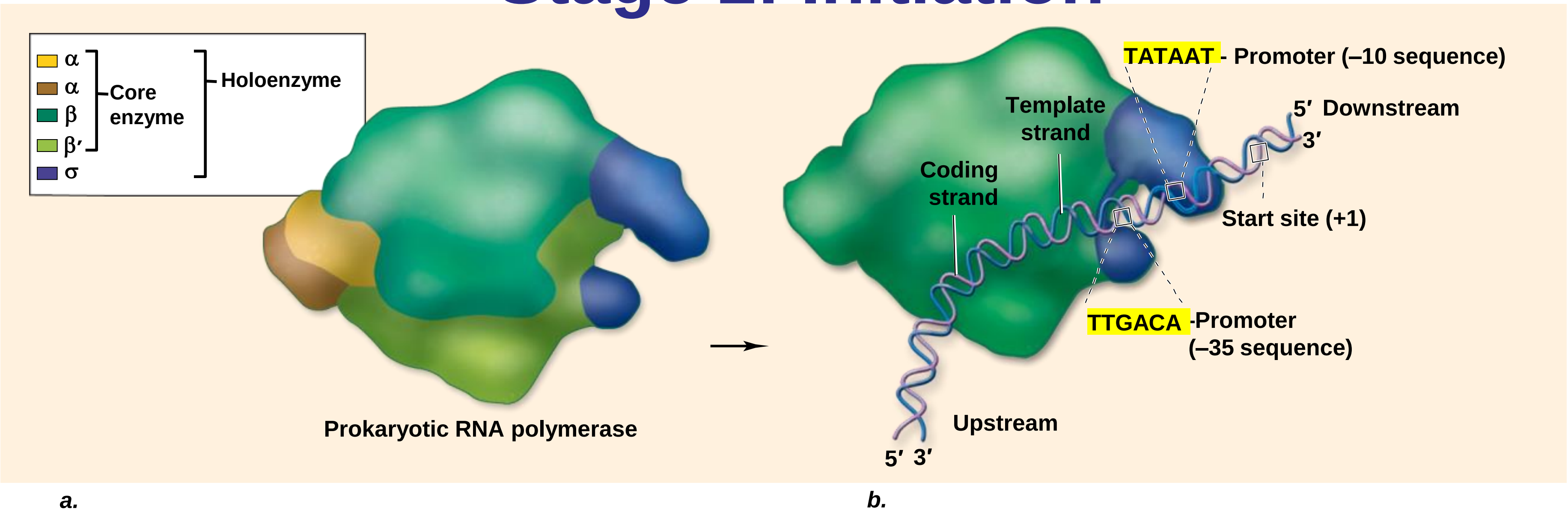
Stage 1: Initiation

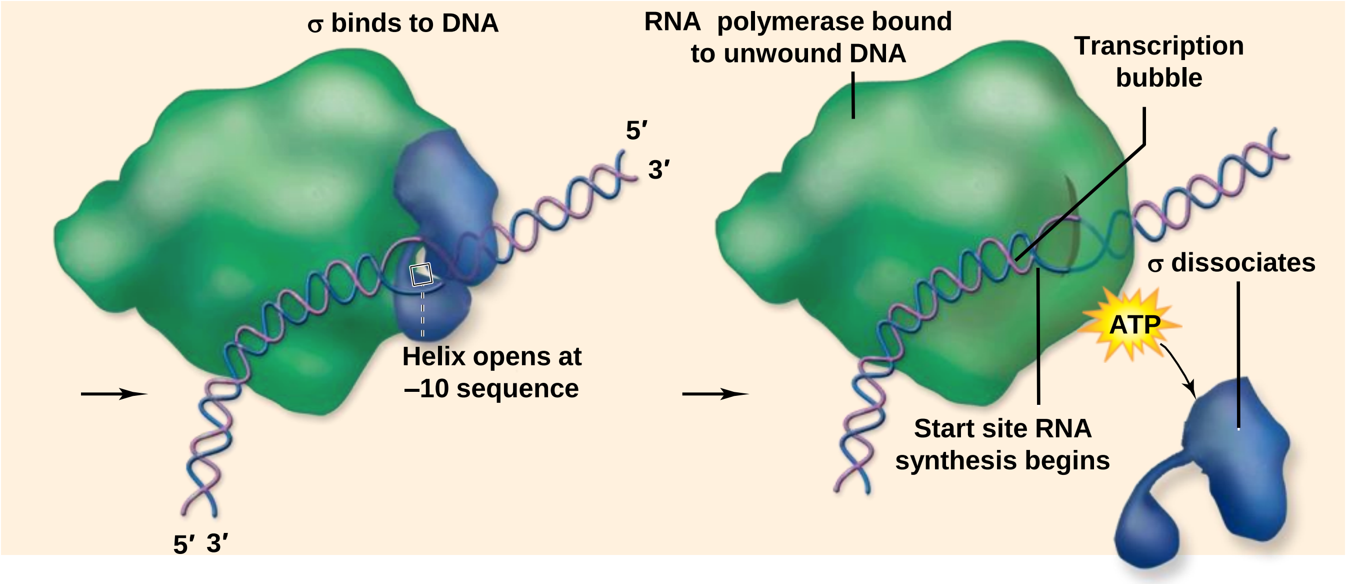


Prokaryotic RNA Polymerase binds **promoter** region of DNA

RNA Polymerase unwinds small region of DNA called **transcription bubble** (needs to read the DNA)

Stage 1: Initiation



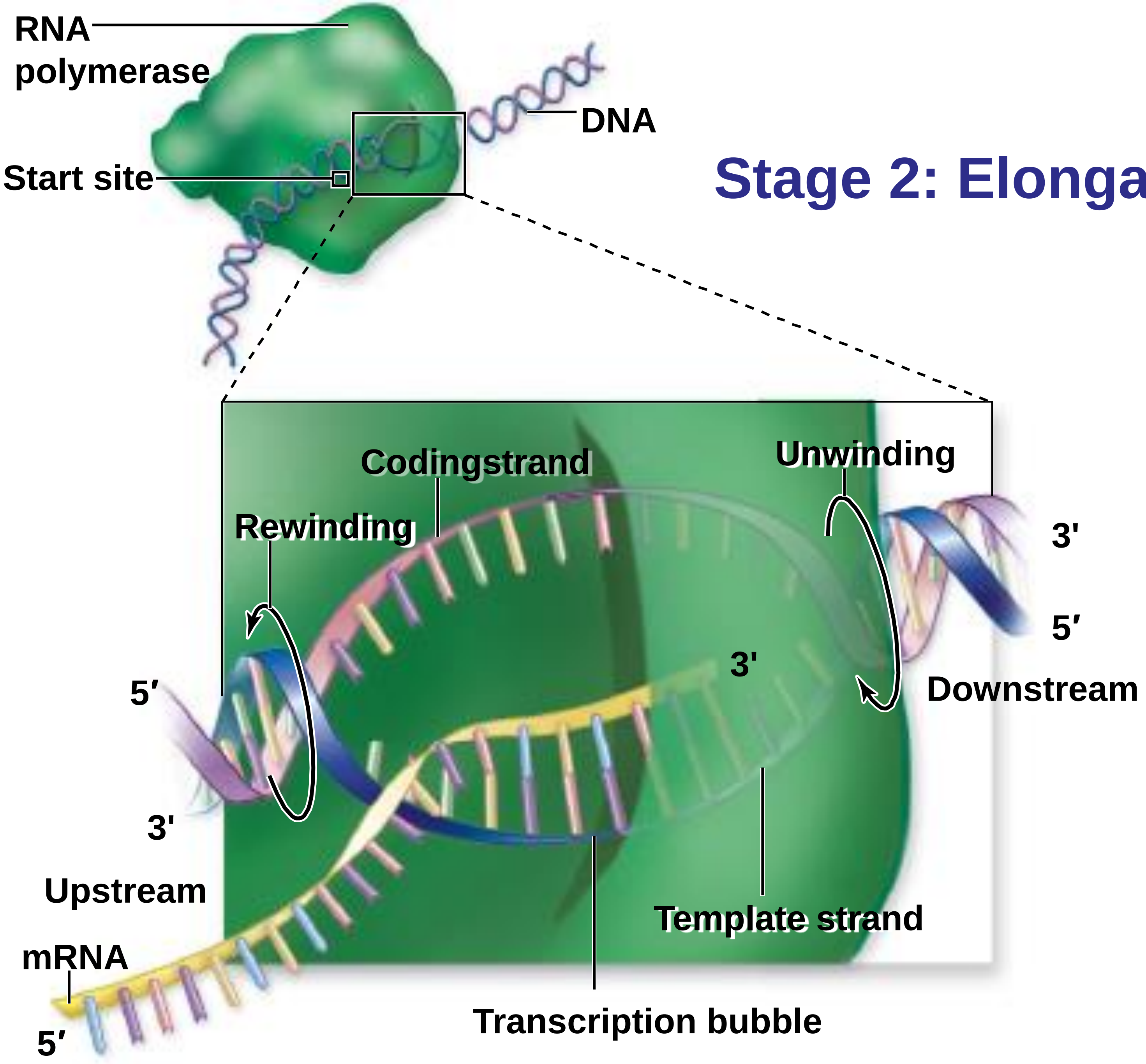


Prokaryotic Transcription

Stage 2: Elongation

- RNA transcript grows in the 5′-to-3′ direction as ribonucleotides are added
- **Transcription bubble** – contains RNA polymerase, DNA template, and growing RNA transcript
- After the transcription bubble passes, the now-transcribed DNA is rewound as it leaves the bubble

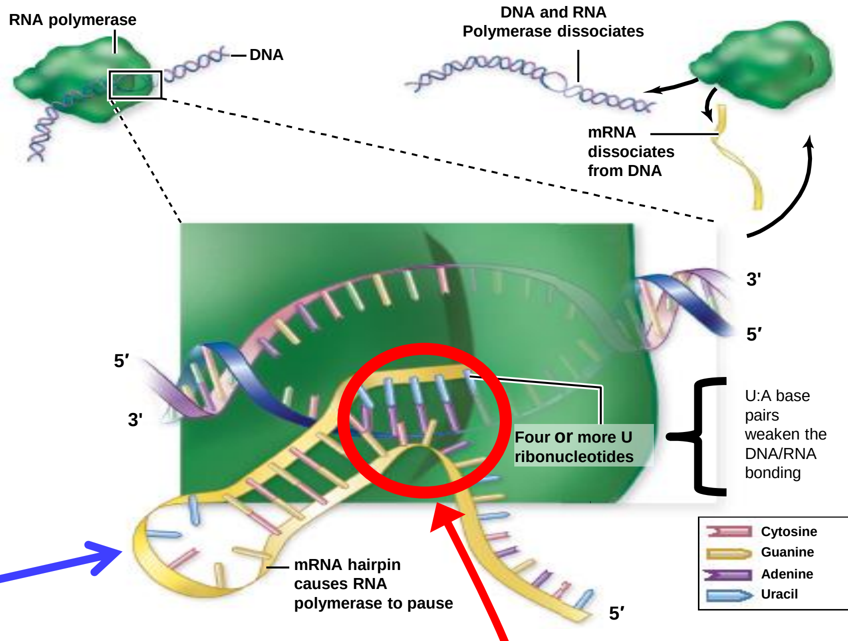
Stage 2: Elongation

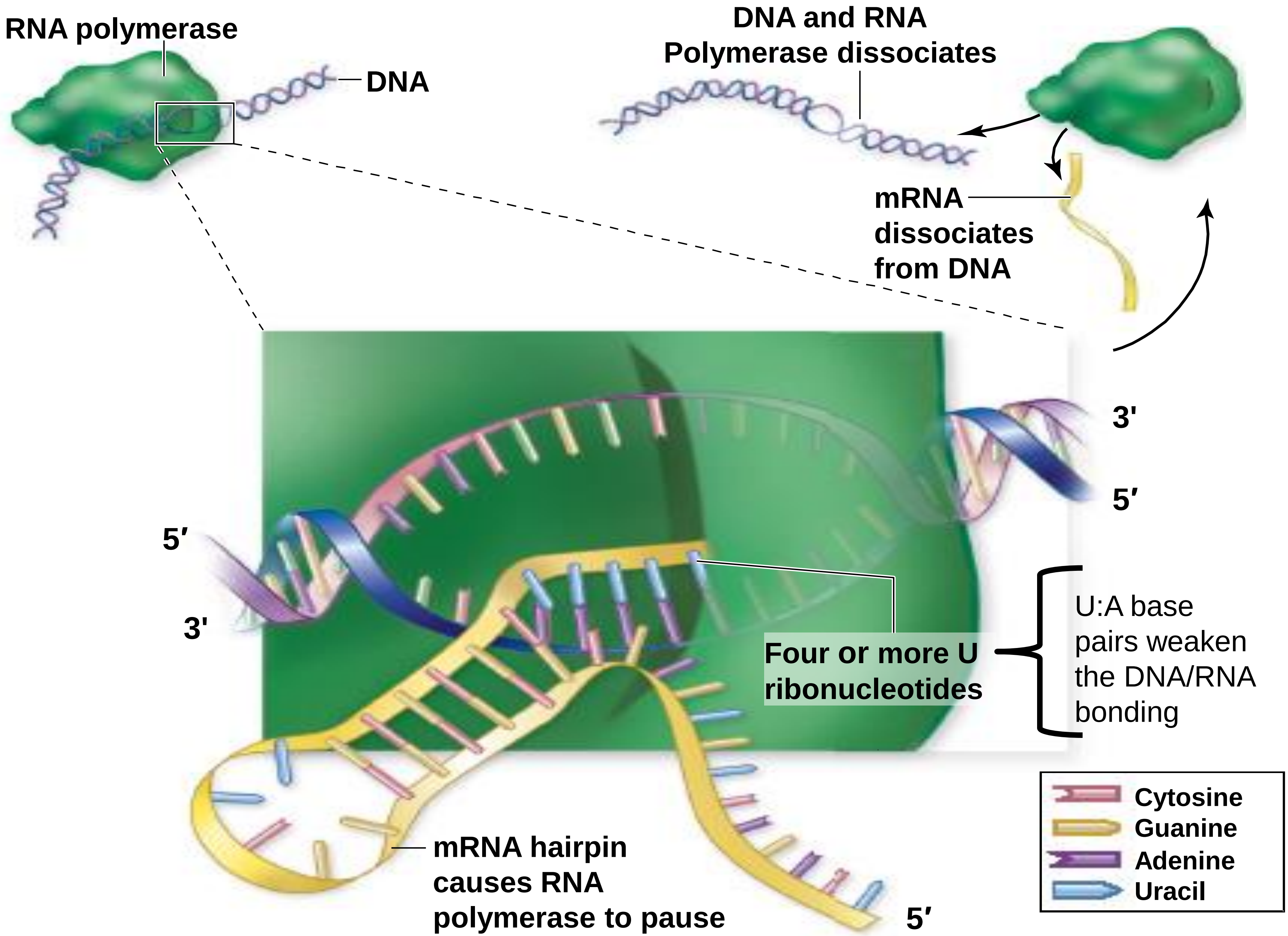


Prokaryotic Transcription

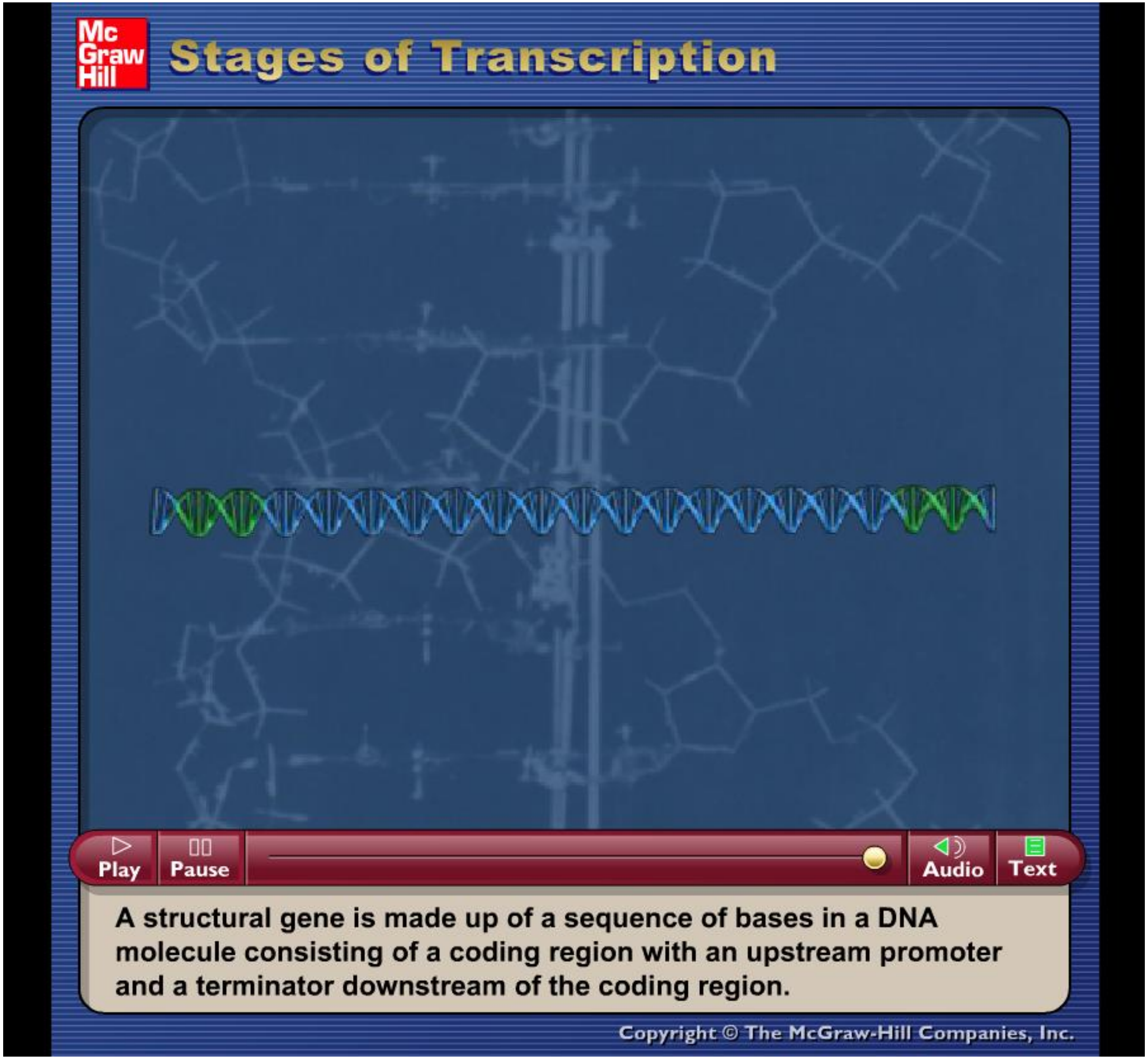
Stage 3: Termination

- Marked by sequence that signals “stop” to polymerase
  - Causes formation of phosphodiester bonds to cease
  - RNA–DNA hybrid within transcription bubble dissociates
  - RNA polymerase releases DNA
  - DNA rewinds
- ***Hairpin*** in RNA causes RNA polymerase to pause
- U:A base pairs weaken the DNA/RNA bonding





U:A base pairs weaken the DNA/RNA bonding

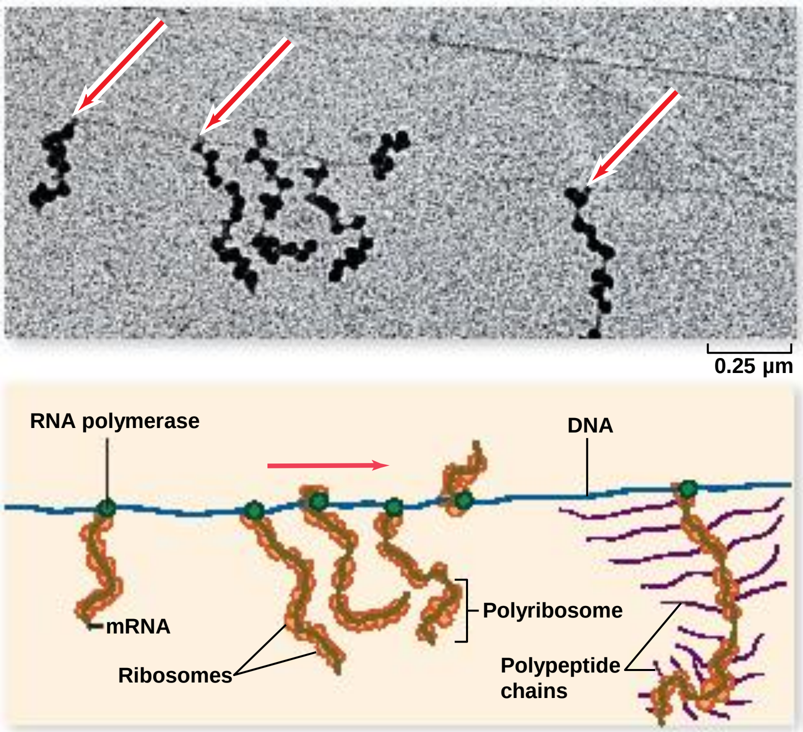


Stages of Transcription

A structural gene is made up of a sequence of bases in a DNA molecule consisting of a coding region with an upstream promoter and a terminator downstream of the coding region.

Prokaryotic Transcription

- Prokaryotic **transcription** is coupled to **translation**
  - mRNA begins to be translated before transcription is finished
  - **Polyribsomes** is a mRNA molecule with multiple ribosomes translating the mRNA



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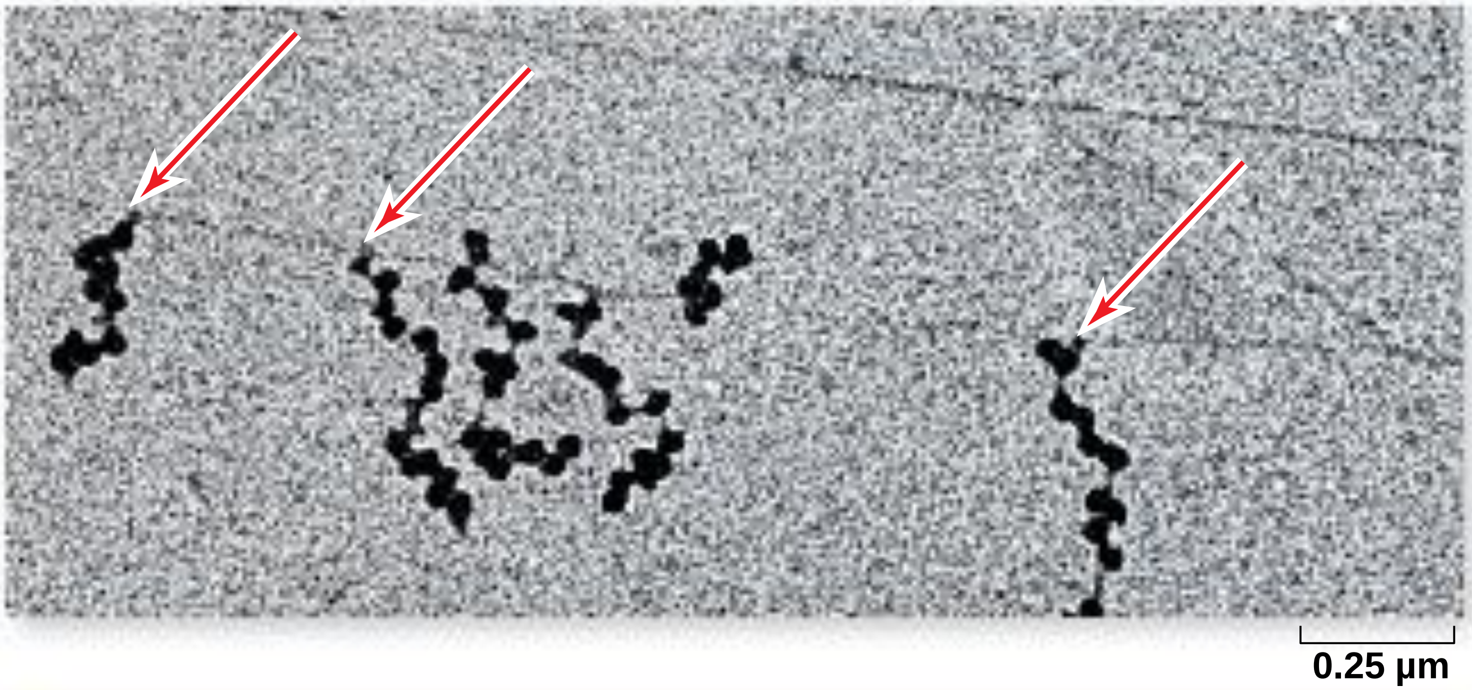

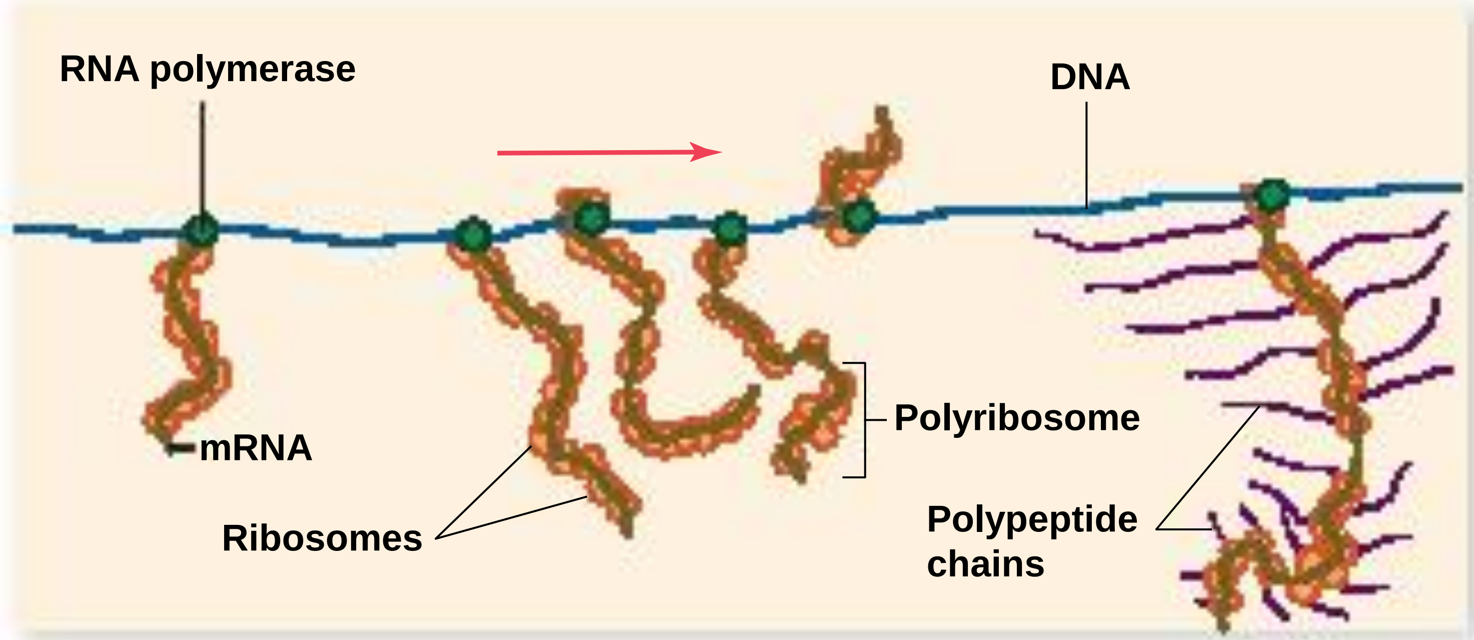

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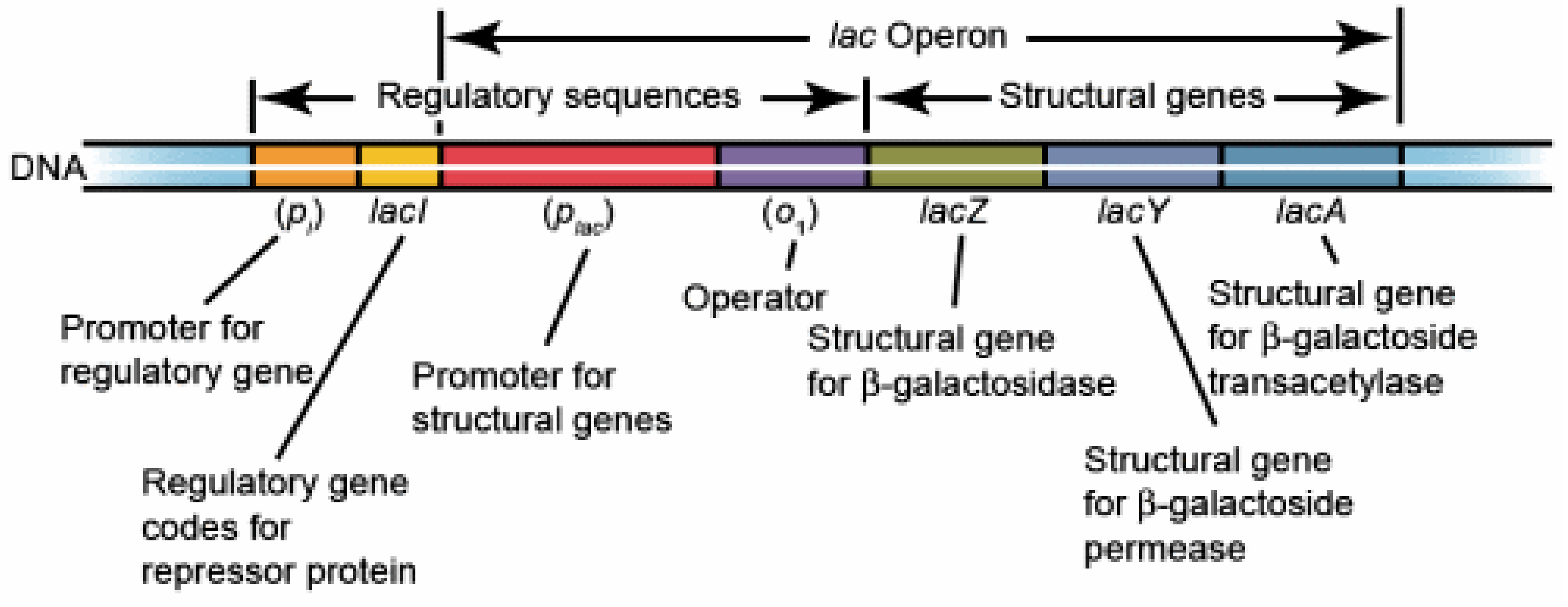
Prokaryotic Transcription

- **Operon**
  - Grouping of functionally related “structural” genes
  - Multiple enzymes for a pathway
  - Can be regulated together



http://www.answersingenesis.org/articles/aid/v4/n1/analysis-of-barry-halls-research

Eukaryotic Transcription

- 3 different RNA polymerases
  - RNA polymerase I transcribes rRNA
  - **RNA polymerase II** transcribes mRNA and some snRNA
  - RNA polymerase III transcribes tRNA and some other small RNAs

- Each RNA polymerase recognizes its own promoter

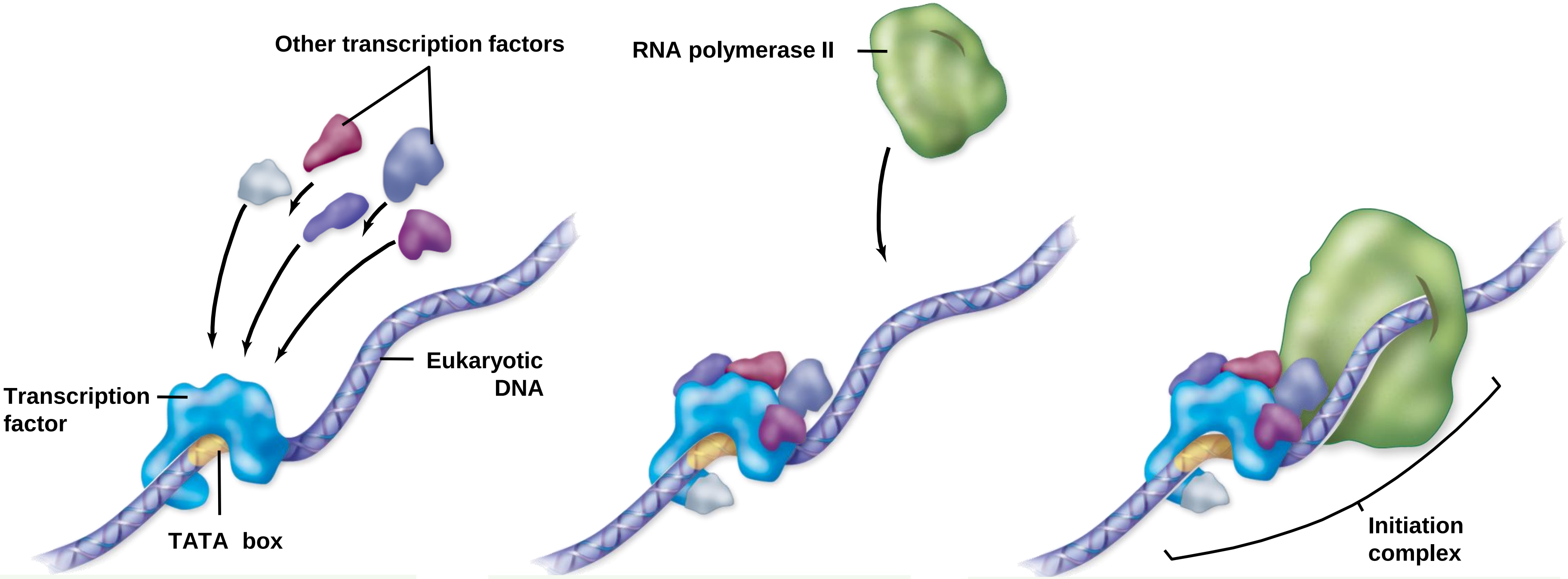
Eukaryotic Transcription

- **Initiation** of transcription
  - Requires a series of **transcription factors**
    - Necessary to get the RNA polymerase II enzyme to a **promoter** and to initiate gene expression
    - Interact with RNA polymerase to form initiation complex at promoter
- **Elongation**:
  - RNA transcribed from the DNA template
- **Termination**
  - Termination sites not as well defined

Eukaryotic Transcription

Initiation of Transcription

- Transcription factors bind to promoter region and recruit RNA polymerase
- Forms the initiation complex

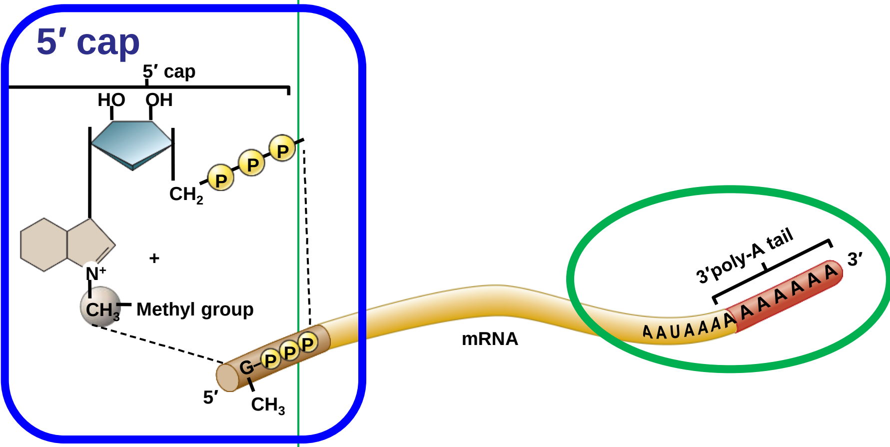


1. A transcription factor recognizes and binds to the TATA box sequence, which is part of the core promoter.
2. Other transcription factors are recruited, and the initiation complex begins to build.
3. Ultimately, RNA polymerase II associates with the transcription factors and the DNA, forming the initiation complex, and transcription begins.

Eukaryotic Transcription

mRNA modifications

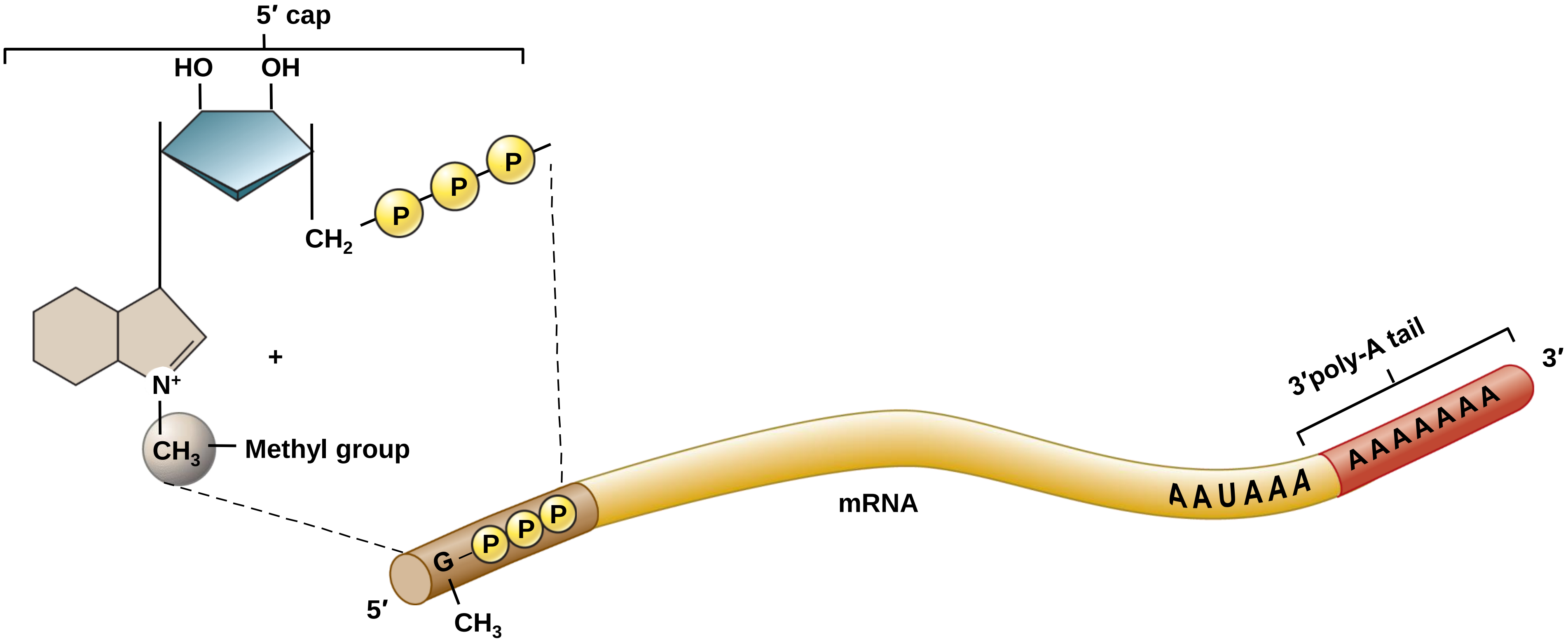
- In eukaryotes, the primary transcript must be modified to become **mature mRNA**
  - Addition of a **5′ cap**
    - Protects from degradation
    - Involved in translation initiation
  - Addition of a **3′ poly-A tail**
    - Created by poly-A polymerase
    - Protection from degradation
  - Removal of non-coding sequences (**introns**)
    - Pre-mRNA **splicing** done by spliceosome



Eukaryotic Transcription

mRNA modifications

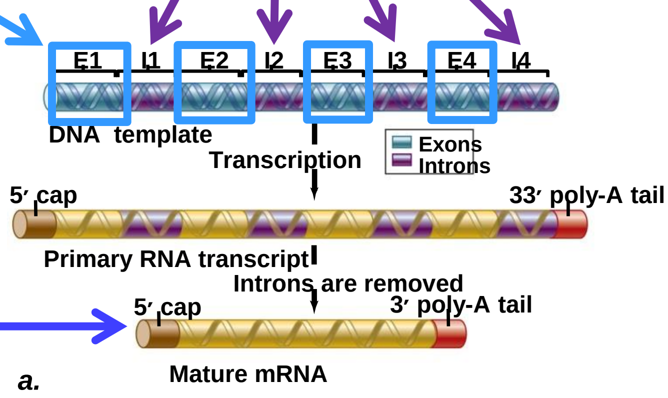
5′ cap



Eukaryotic Transcription

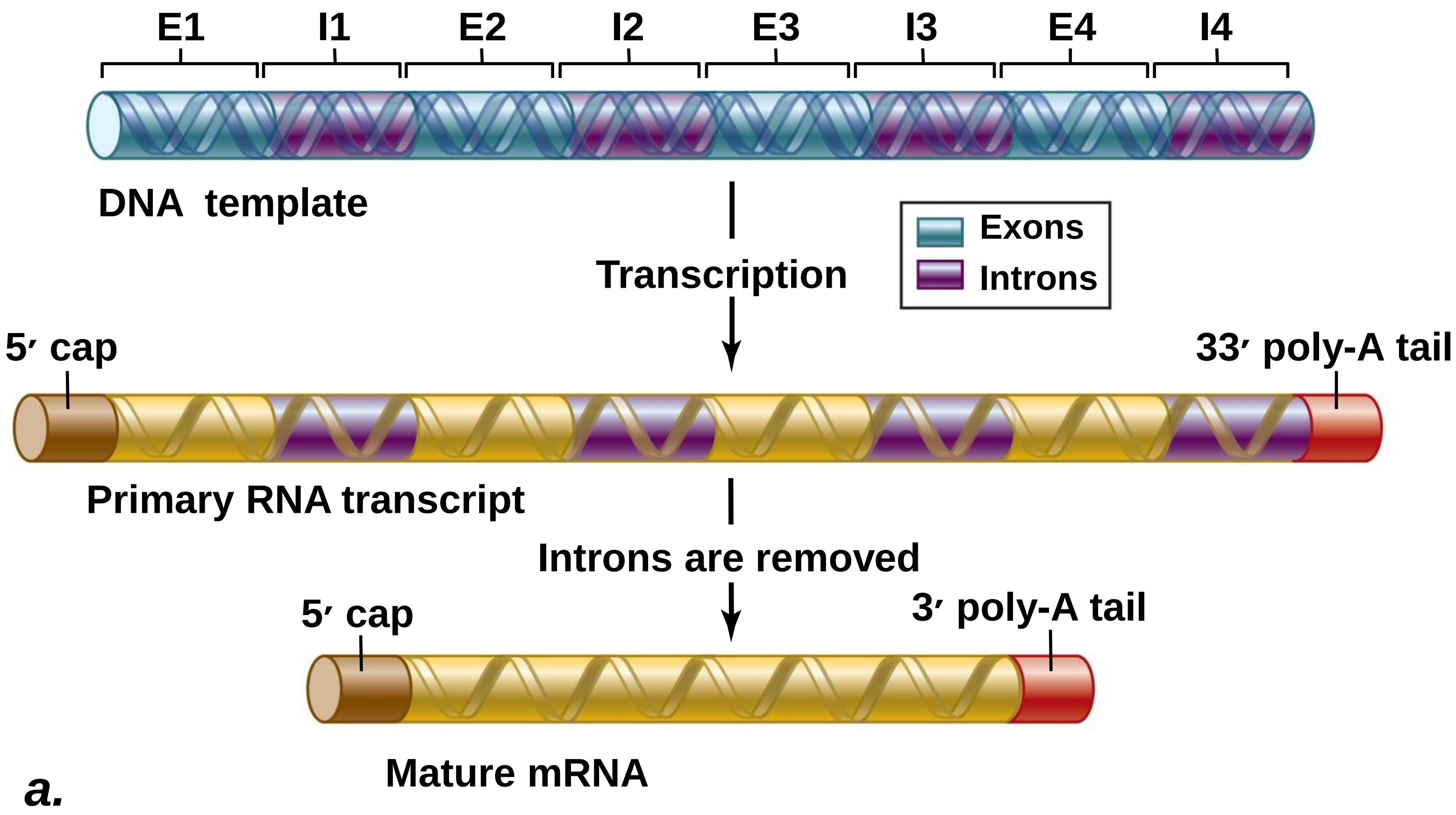
Eukaryotic pre-mRNA splicing

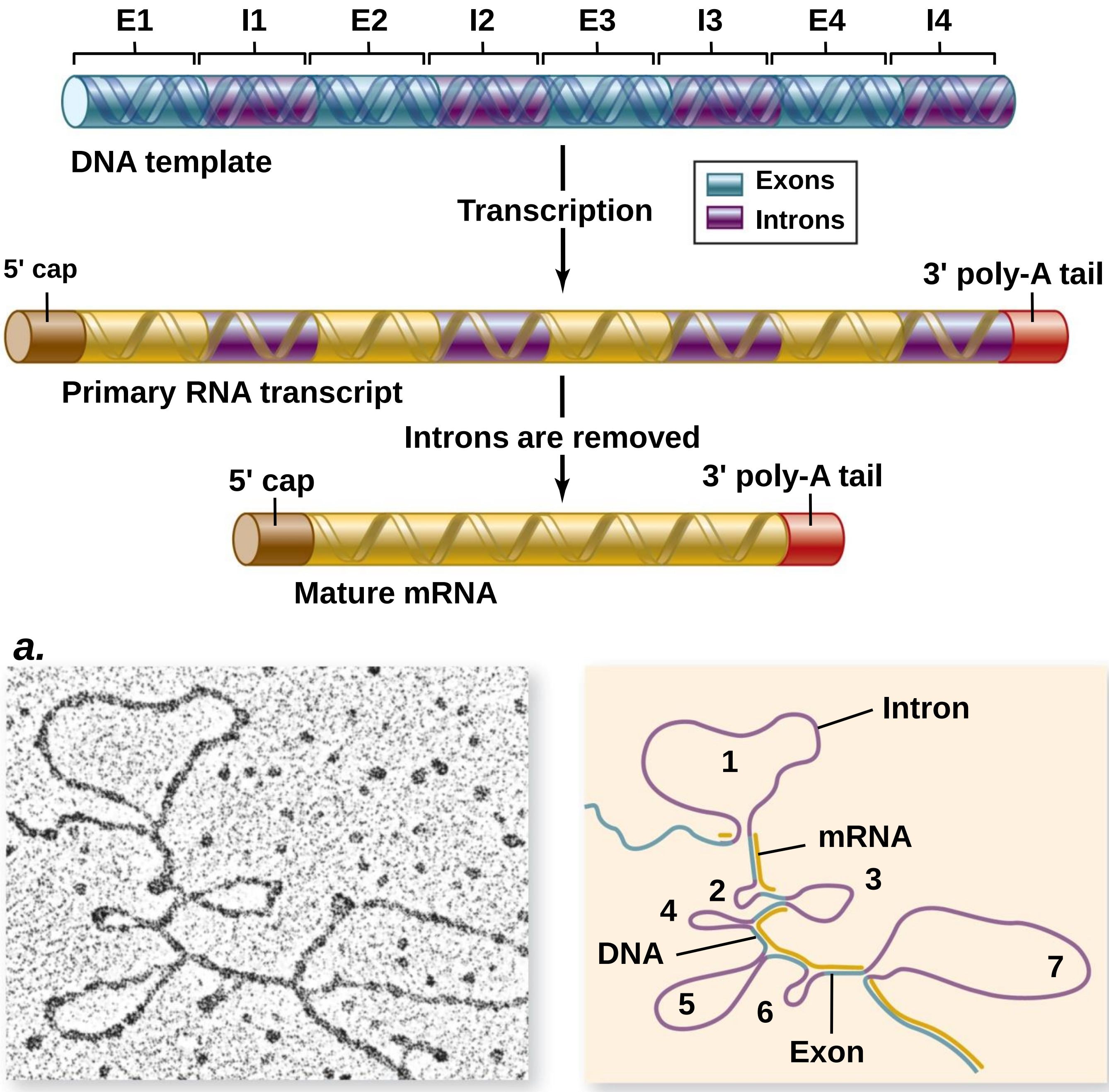
- **Introns** – non-coding sequences
- **Exons** – sequences that will be translated (*expressed*)
- Through post-transcriptional splicing, introns are removed before translation
  - Form “mature” mRNA



Eukaryotic Transcription

mRNA modifications: Splicing



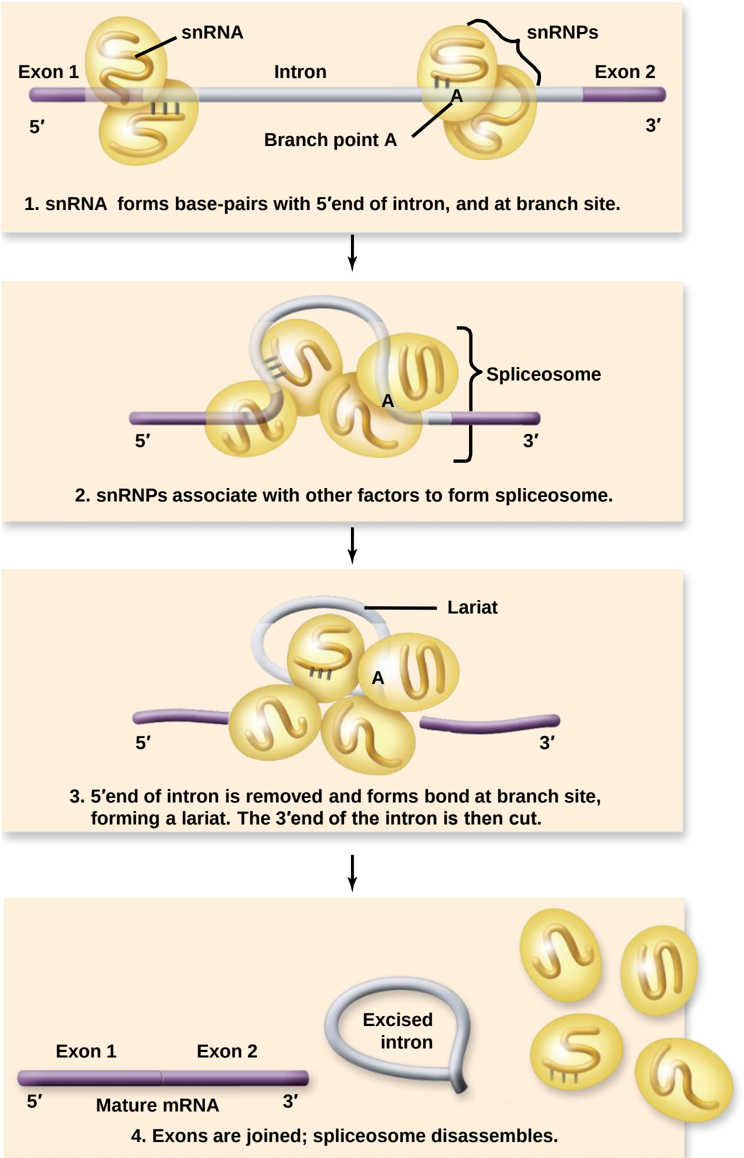


b: Courtesy of Dr. Bert O'Malley, Baylor College of Medicine

Eukaryotic Transcription

Eukaryotic pre-mRNA splicing

- Small ribonucleoprotein particles (**snRNPs “snurps”**) recognize the intron–exon boundaries
  - snRNPs cluster with other proteins to form **spliceosome**
    - Responsible for removing introns



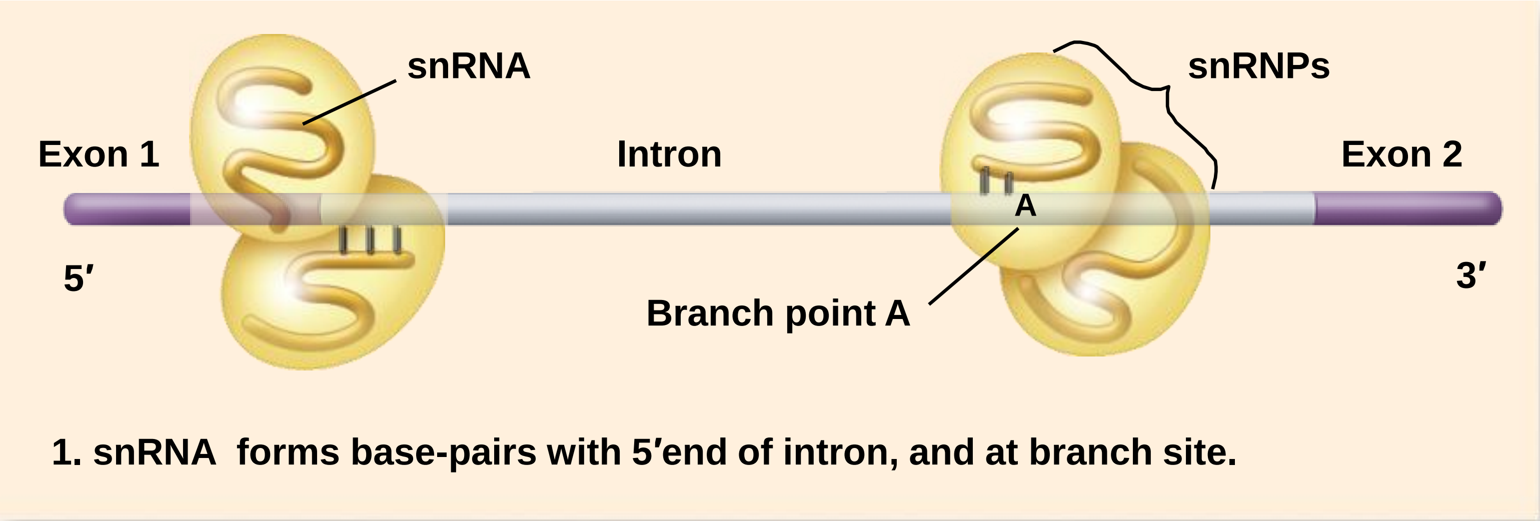
1. snRNA forms base-pairs with 5′end of intron, and at branch site.

2. snRNPs associate with other factors to form spliceosome.

3. 5′end of intron is removed and forms bond at branch site, forming a lariat. The 3′end of the intron is then cut.

4. Exons are joined; spliceosome disassembles.

mRNA modifications: Splicing



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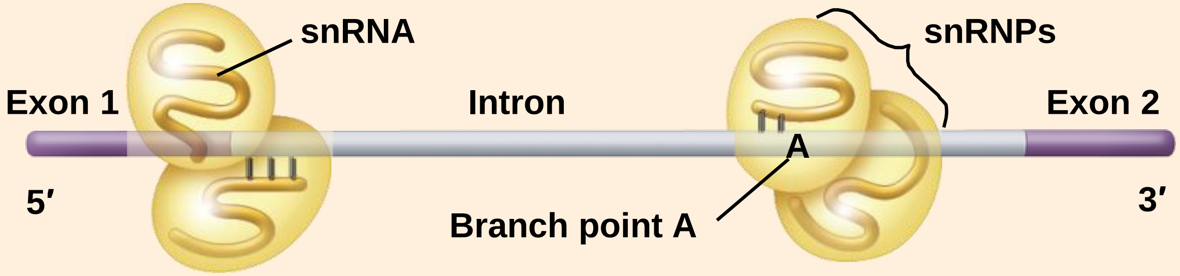

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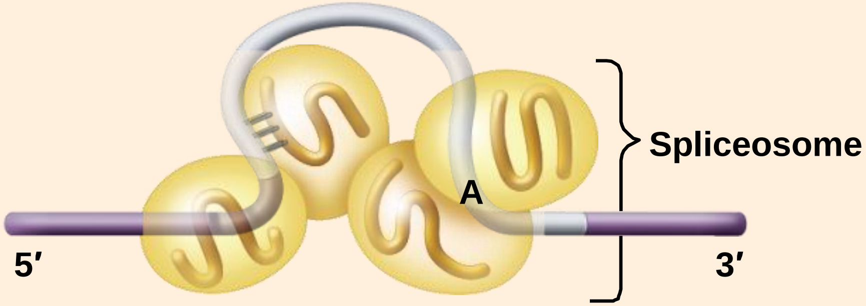

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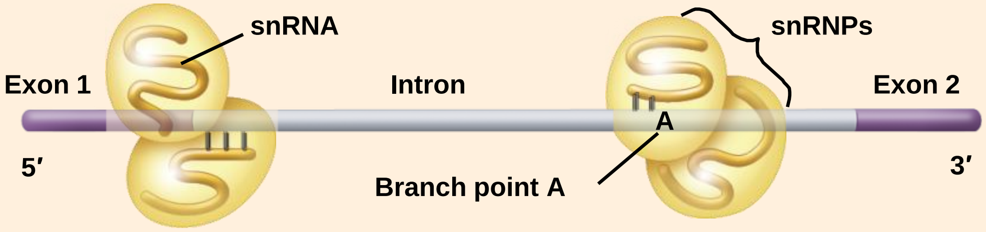

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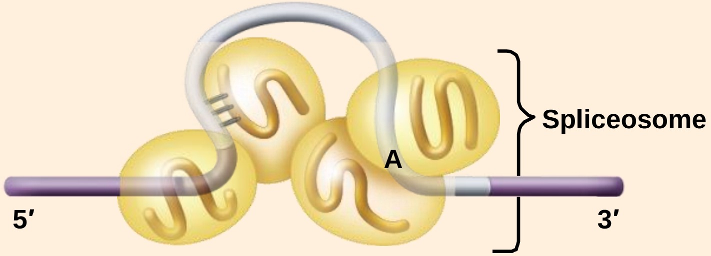

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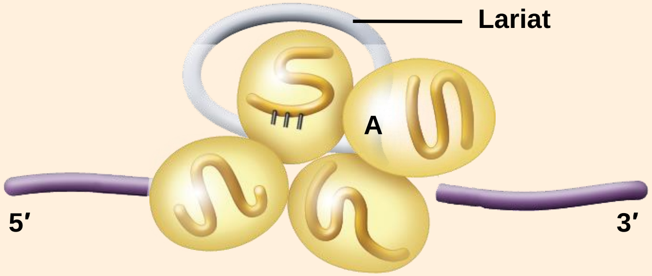

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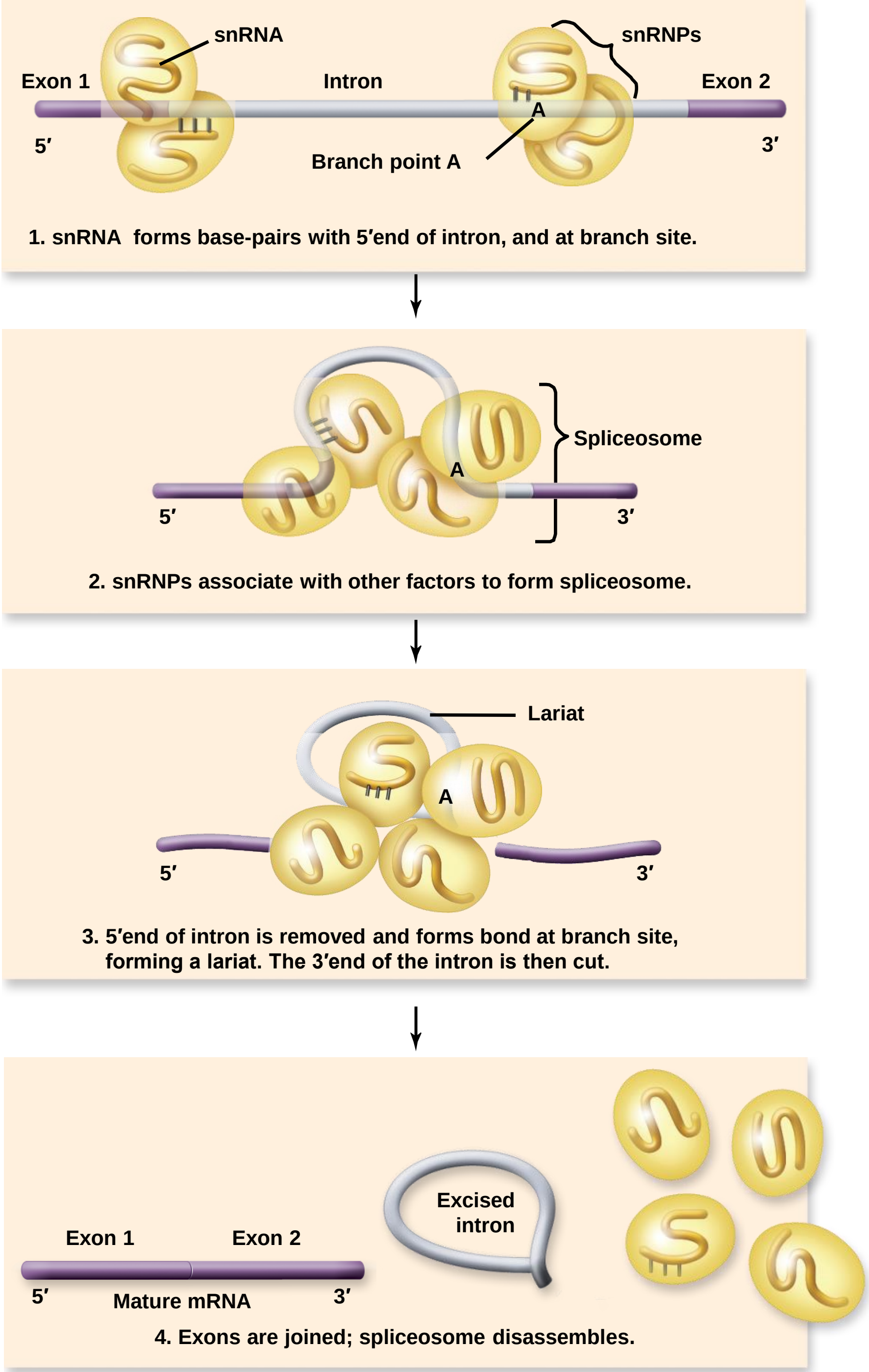

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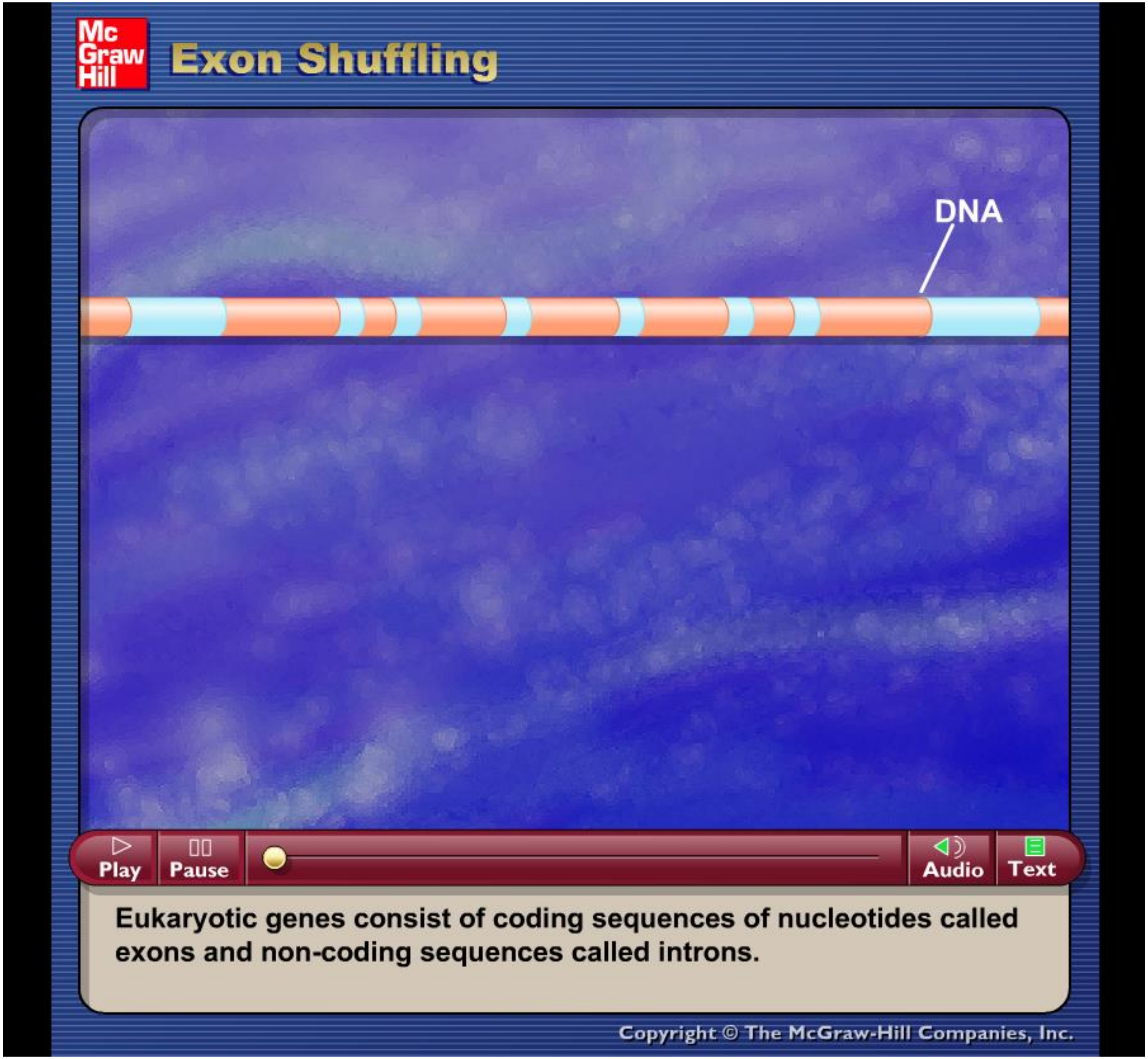
4. Exons are joined; spliceosome disassembles.

Eukaryotic Transcription

Alternative splicing

- Single primary transcript can be spliced into different mRNAs by the inclusion of different sets of exons
  - 15% of known human genetic disorders are due to altered splicing
  - 35 to 59% of human genes exhibit some form of alternative splicing
  - Explains how 25,000 genes of the human genome can encode the more than 80,000 different mRNAs



Exon Shuffling

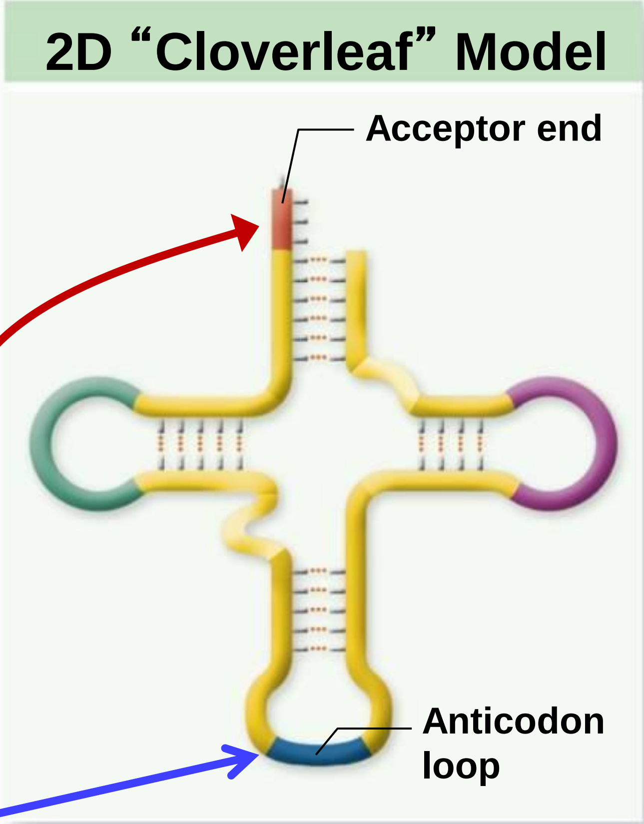
Eukaryotic genes consist of coding sequences of nucleotides called exons and non-coding sequences called introns.

tRNA and Ribosomes

tRNA (transfer RNA)

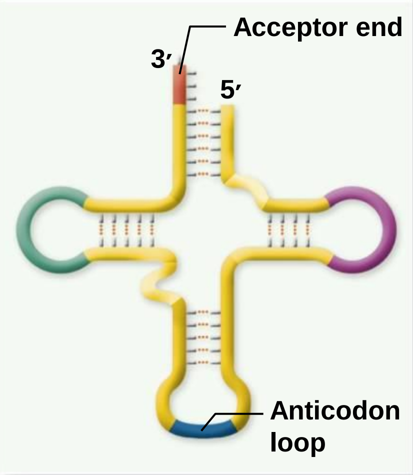
- molecules that carry amino acids to ribosome for incorporation into a polypeptide

- **Aminoacyl-tRNA synthetases** add amino acids to the **acceptor stem** of tRNA
- **Anticodon** loop contains three nucleotides complementary to mRNA codons

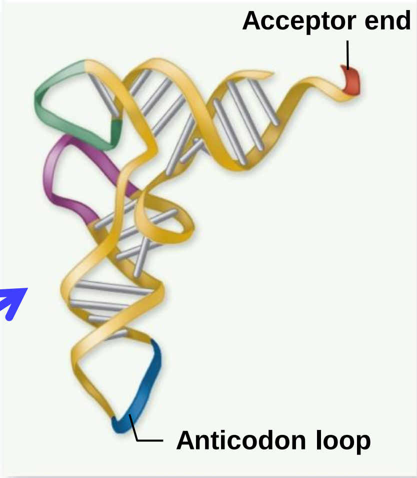


2D "Cloverleaf" Model

2D "Cloverleaf" Model

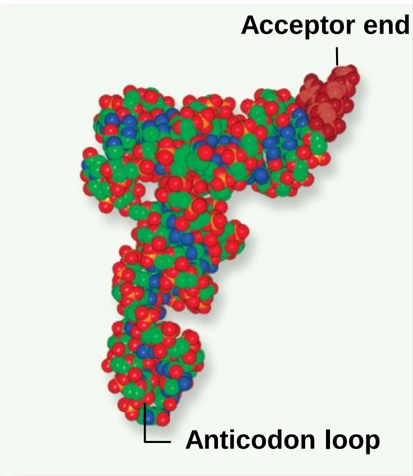


3D Ribbon-like Model

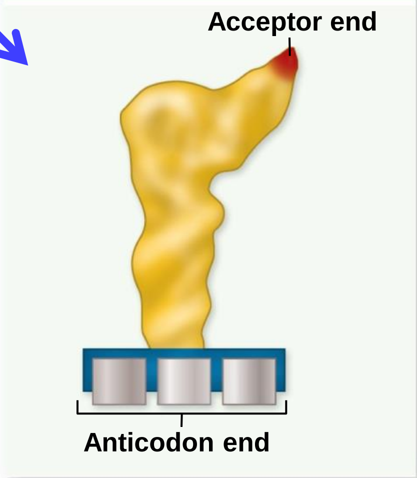


More realistic shapes

3D Space-filled Model



Icon

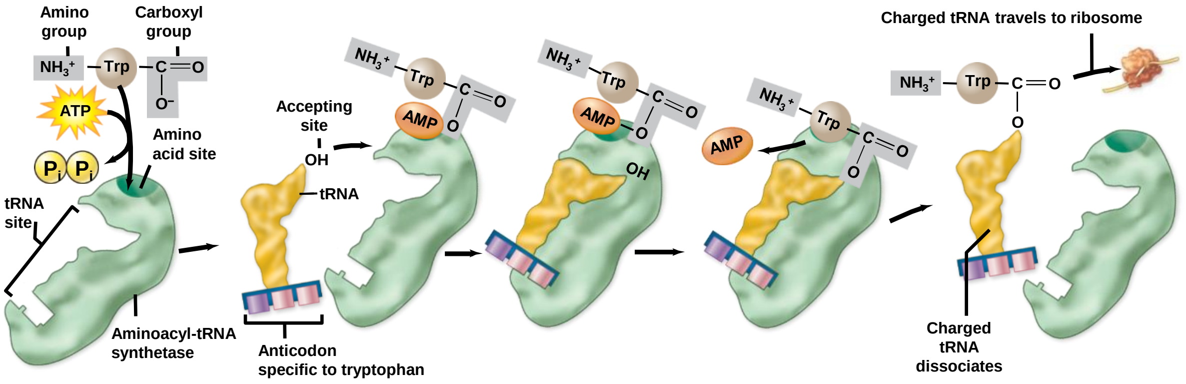


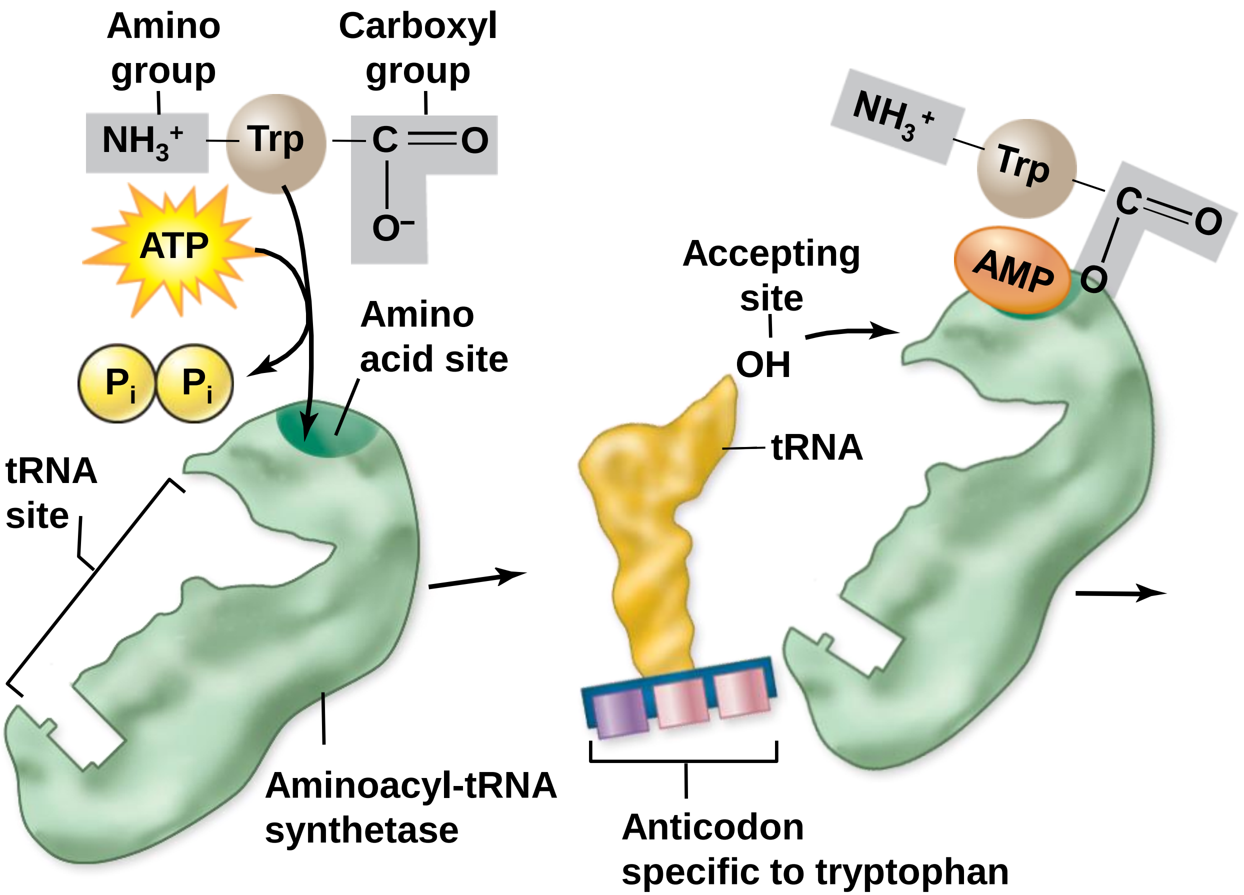
c: Created by John Beaver using ProteinWorkshop, a product of the RCSB PDB, and built using the Molecular Biology Toolkit developed by John Moreland and Apostol Gramada (mbt.sdsc.edu). The MBT is financed by grant GM63208

tRNA and Ribosomes

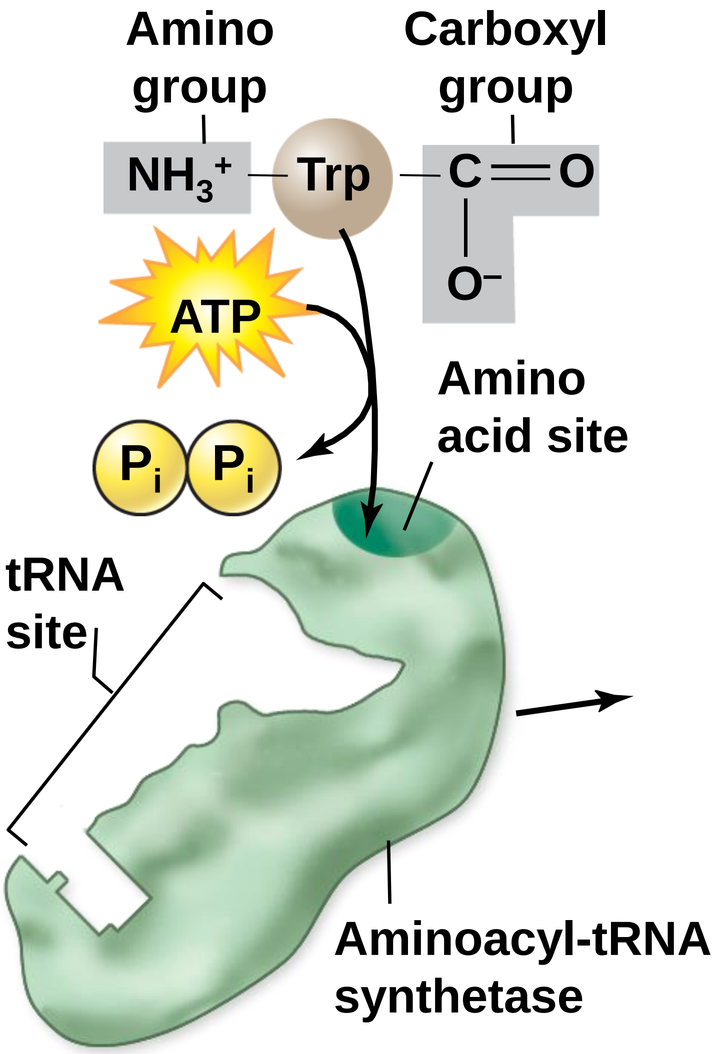
tRNA charging reaction:

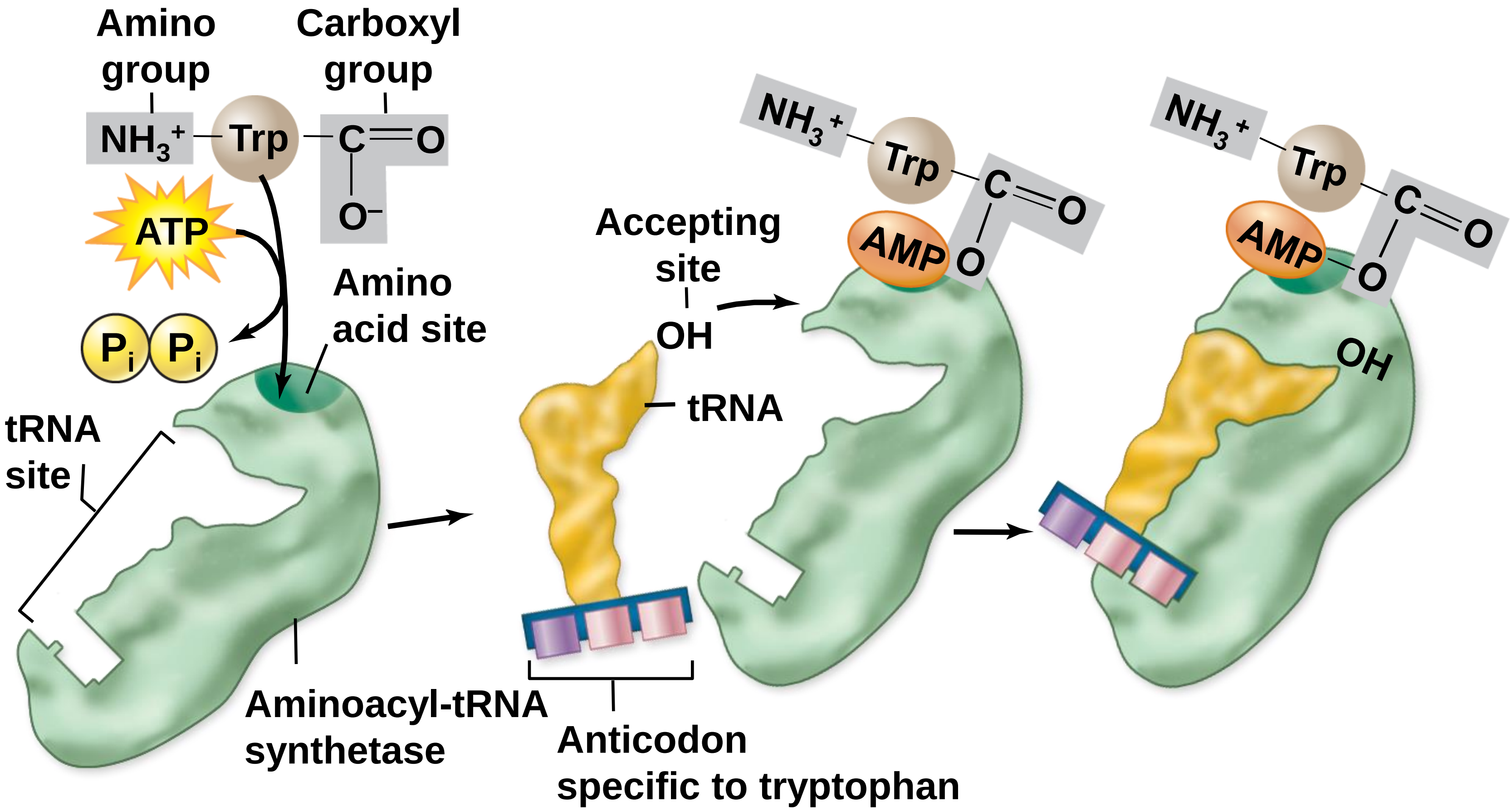
- Each **aminoacyl-tRNA synthetase** recognizes only 1 amino acid but several tRNAs
- Charged tRNA – has an amino acid added using ATP energy
  - Can undergo peptide bond formation without additional energy
- Ribosomes do not verify amino acid attached to tRNA





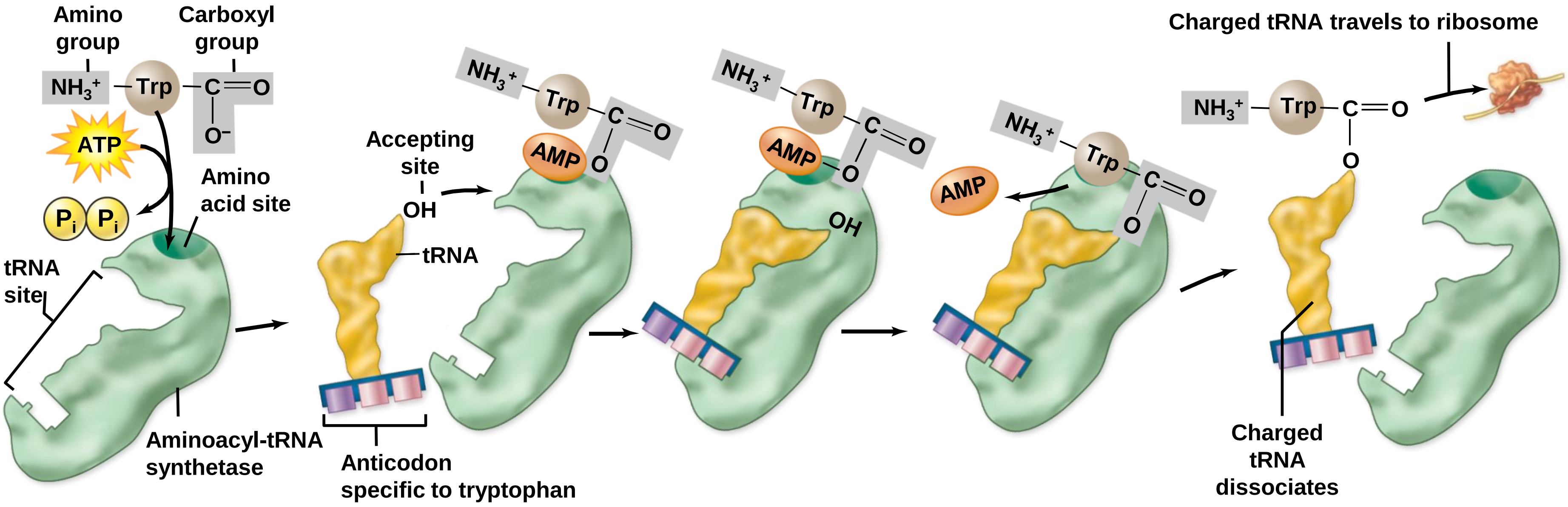
1. In the first step of the reaction, the amino acid is activated. The amino acid reacts with ATP to produce an intermediate with the carboxyl end of the amino acid attached to AMP. The two terminal phosphates (pyrophosphates) are cleaved from ATP in this reaction.


Amino group
Carboxyl group
NH_3^+
Trp
C=O
O^-
ATP
P_i P_i
Amino acid site
tRNA site
Aminoacyl-tRNA synthetase


Amino group
Carboxyl group
NH3+
Trp
C=O
O−
ATP
Pi Pi
Amino acid site
tRNA site
Aminoacyl-tRNA synthetase
Accepting site
OH
tRNA
Anticodon specific to tryptophan
NH3+
Trp
C=O
AMP
O
NH3+
Trp
C=O
AMP
O
OH

tRNA Charging Reaction:

- **Aminoacyl-tRNA synthetase** recognizes only 1 amino acid but several tRNAs
- Due to degenerate code



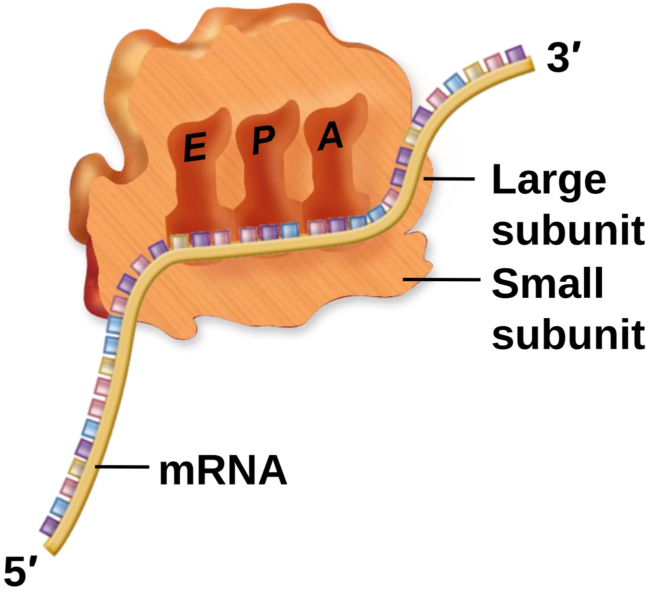
1. In the first step of the reaction, the amino acid is activated. The amino acid reacts with ATP to produce an intermediate with the carboxyl end of the amino acid attached to AMP. The two terminal phosphates (pyrophosphates) are cleaved from ATP in this reaction.

2. The amino acid-AMP complex remains bound to the enzyme. The tRNA next binds to the enzyme.

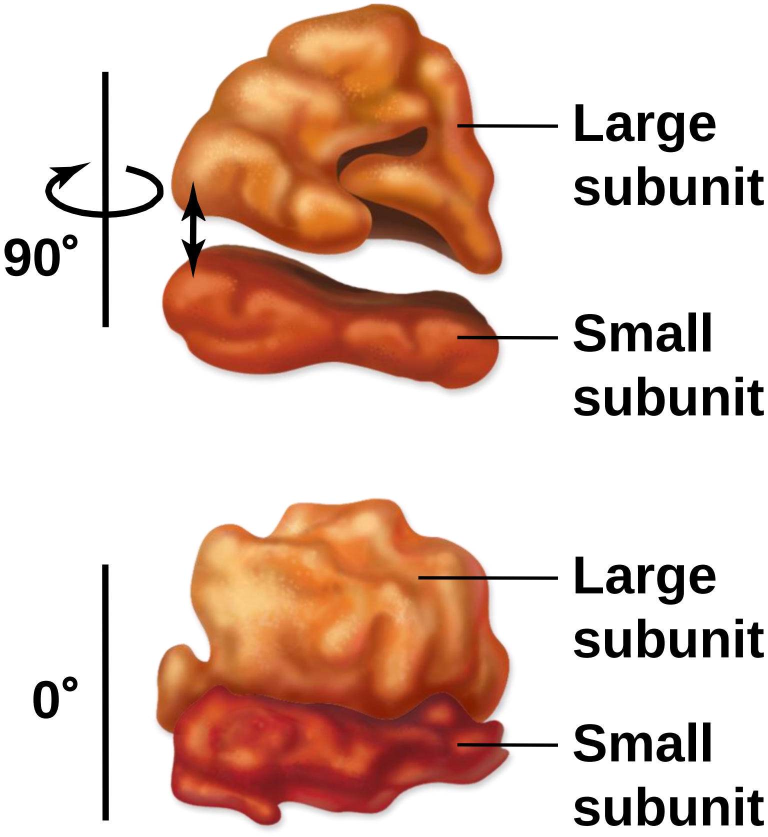
3. The second step of there action transfers the amino acid from AMP to the tRNA, Producing a charged tRNA and AMP. The charged tRNA consists of a specific amino acid attached to the 3′ accept or stem of it sRNA.

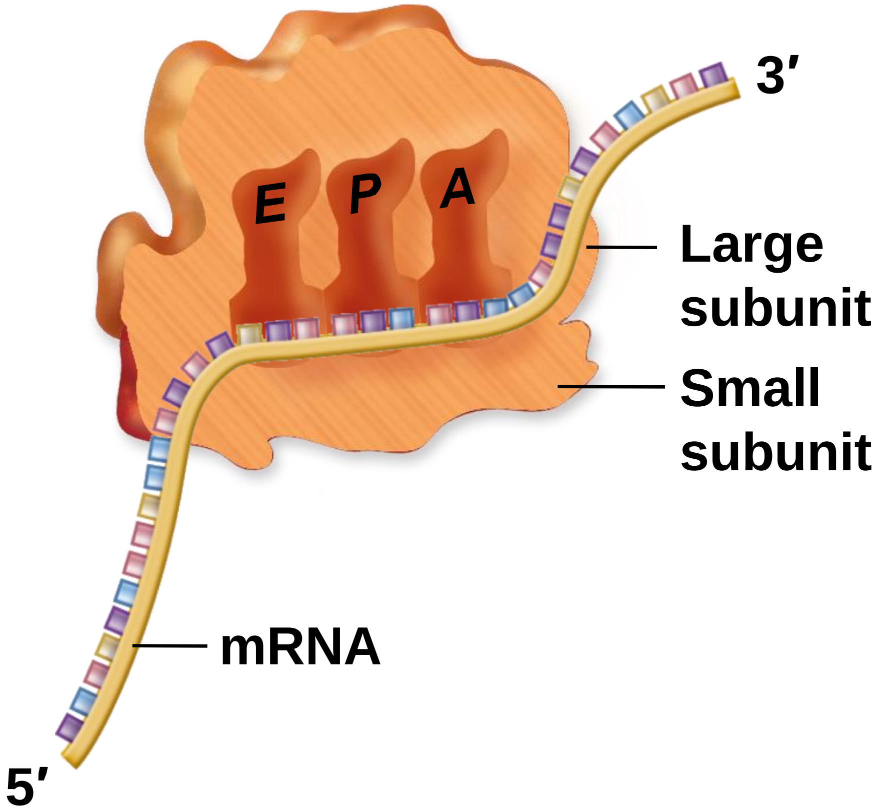
tRNA and Ribosomes

- **Ribosomes** have multiple tRNA binding sites
  - **A site**: binds the tRNA carrying the next **amino acid**
  - **P site**: binds the tRNA attached to the growing **peptide chain**
  - **E site**: binds the tRNA that carried the last amino acid, tRNA **exits** ribosome



Ribosomes


90°
Large subunit
Small subunit
0°
Large subunit
Small subunit


E
P
A
3′
Large subunit
Small subunit
mRNA
5′

tRNA and Ribosomes

- The **ribosome** has **two primary functions**
  1. Decode the mRNA
  2. Form peptide bonds

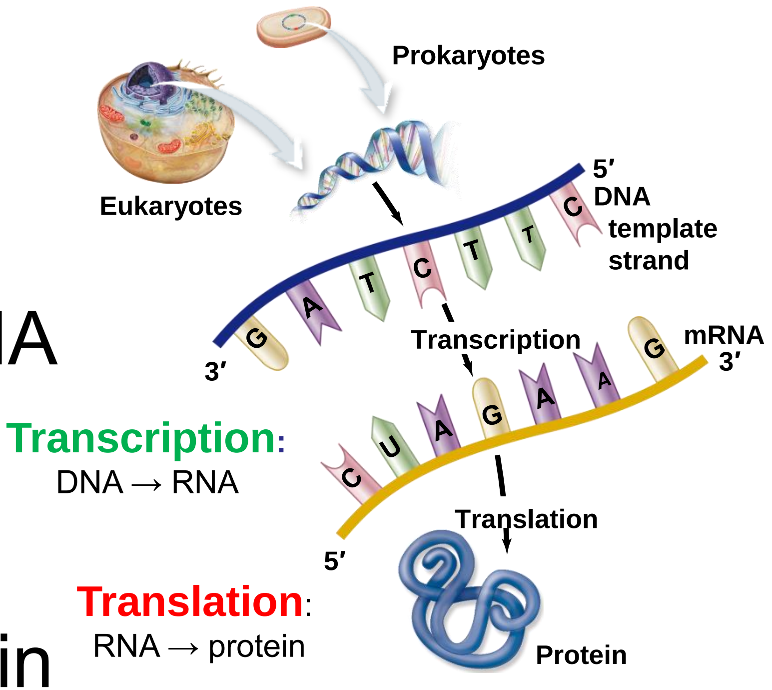
- **Peptidyl transferase**
  - Enzymatic component of the ribosome
  - Forms peptide bonds between amino acids

The Nature of Genes

DNA → RNA → protein

Really Important!!

- **Transcription** = DNA → RNA
- **Translation** = RNA → protein



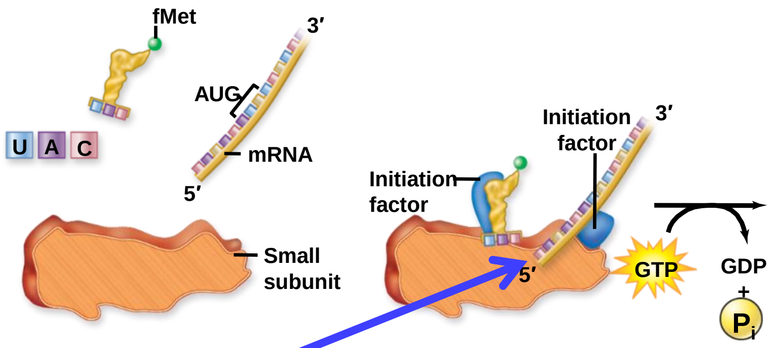
Translation

- Process by which the mRNA transcript is read by the ribosomes and used to make a polypeptide

- Occurs in 3 main stages
  1. **Initiation**
  2. **Elongation**
  3. **Termination**

- There are some important differences between translation in prokaryotes and eukaryotes

Translation

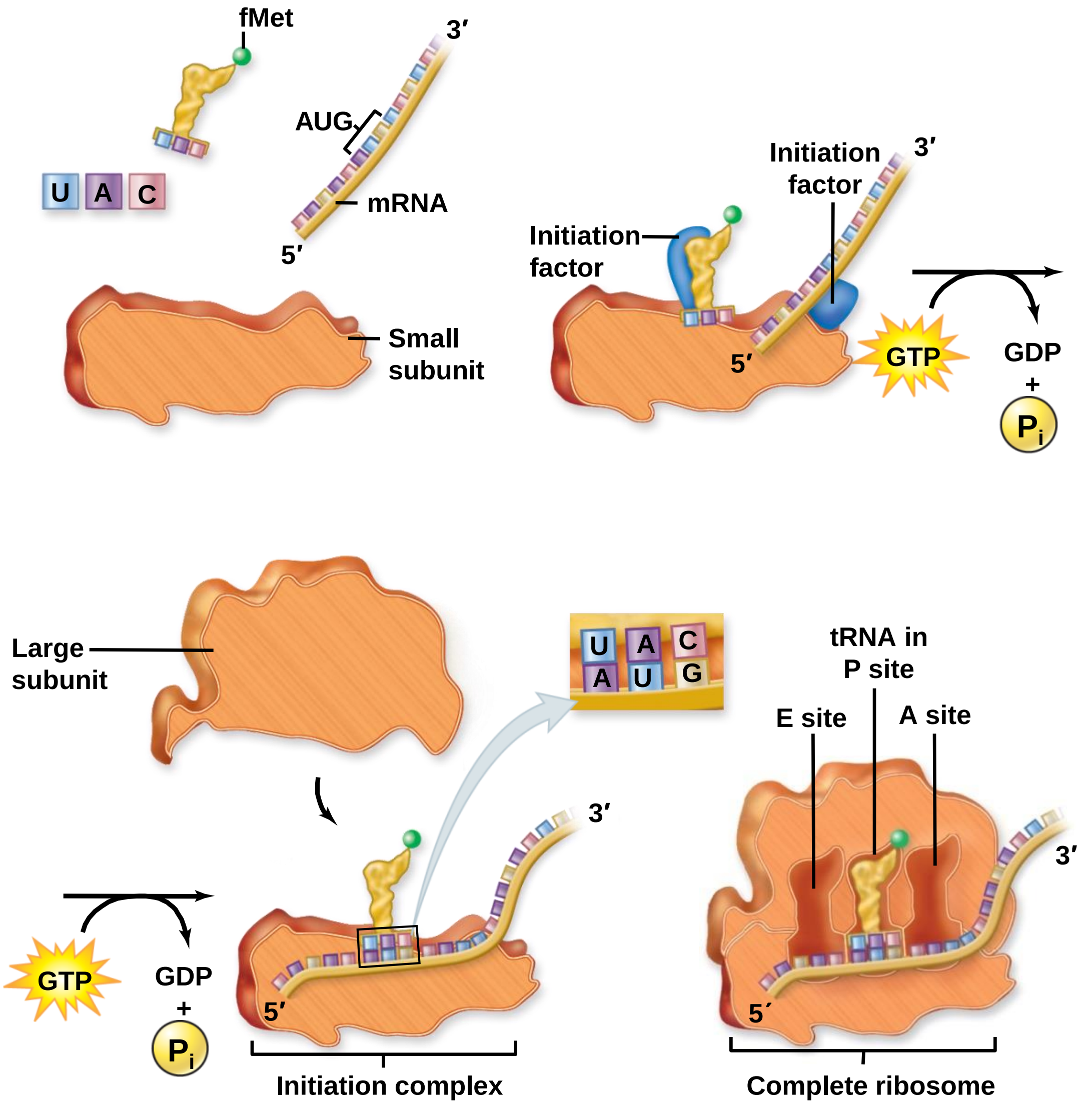


- In **prokaryotes**, **initiation complex** includes
  - **Initiator tRNA** charged with N-formylmethionine
  - Small ribosomal subunit
  - mRNA strand

- Ribosome binding sequence (RBS) of mRNA positions small subunit correctly

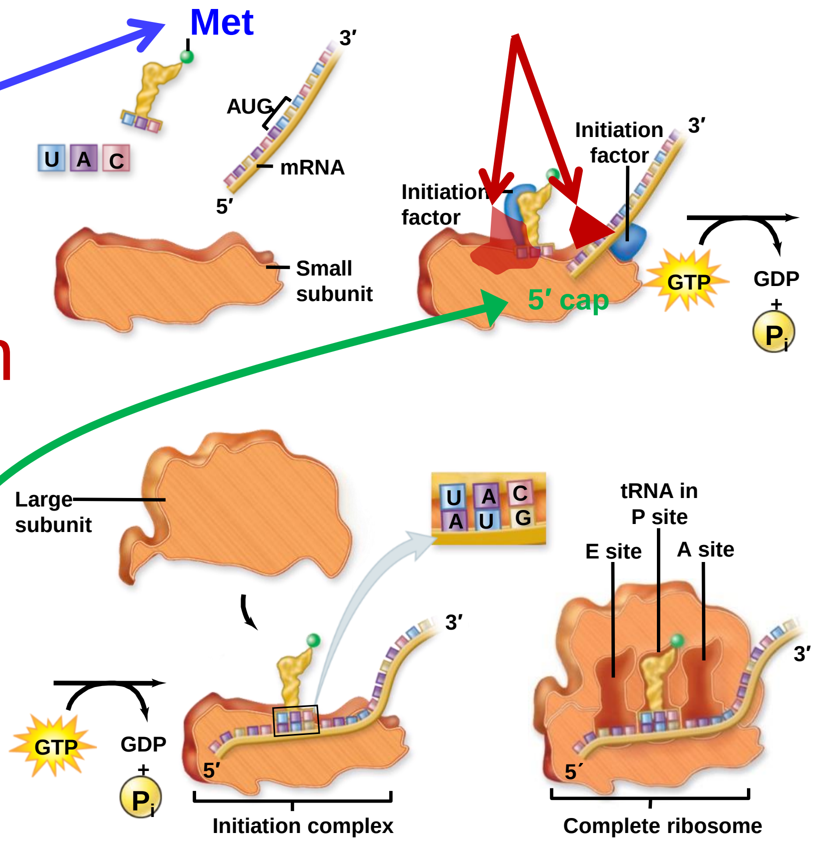
- Large subunit then added
  - Initiator tRNA aligned to P site with A and E sites empty

Prokaryotic Initiation Complex



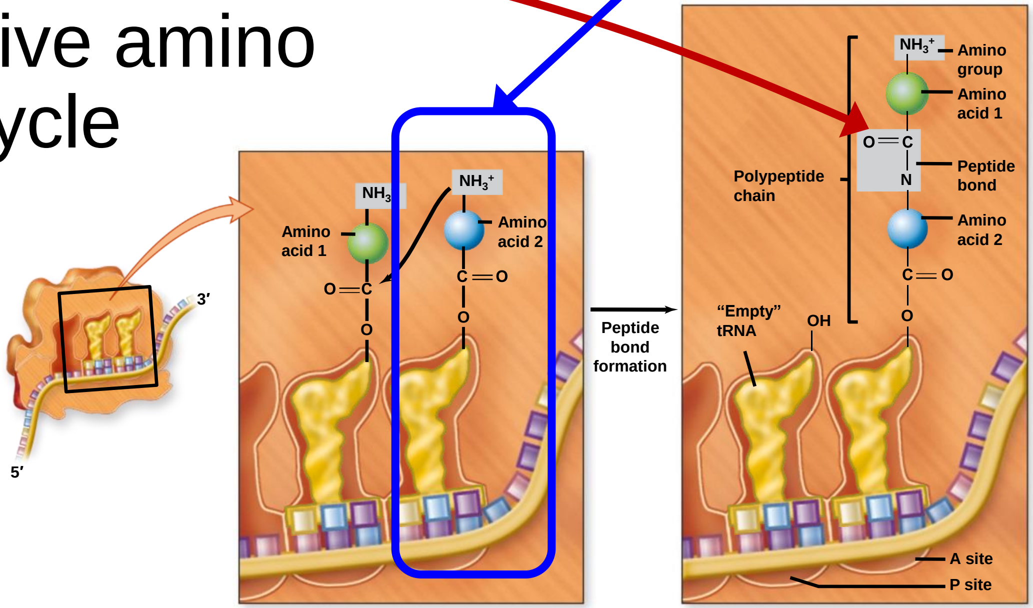
Translation

- **Initiations** in **eukaryotes** similar except…
  - Initiating amino acid is **methionine**
  - More complicated initiation complex
  - ***Lack*** of an RBS – small subunit binds to **5′ cap** of mRNA

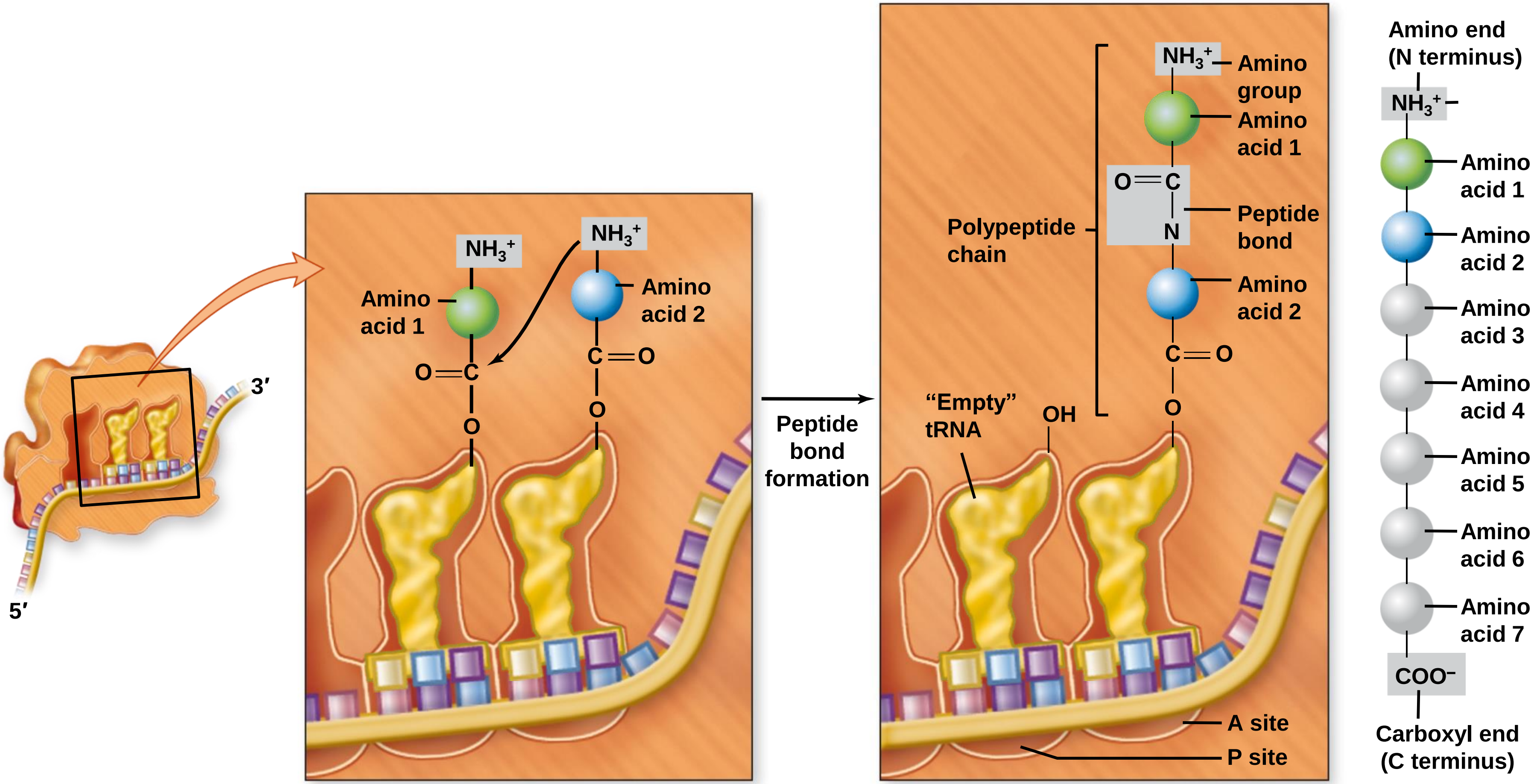


Translation

- **Elongation** adds amino acids
  - 2nd charged tRNA can bind to empty A site
  - Requires elongation factor called EF-Tu to bind to tRNA and GTP (not shown)
  - Peptide bond can then form
  - Addition of successive amino acids occurs as a cycle

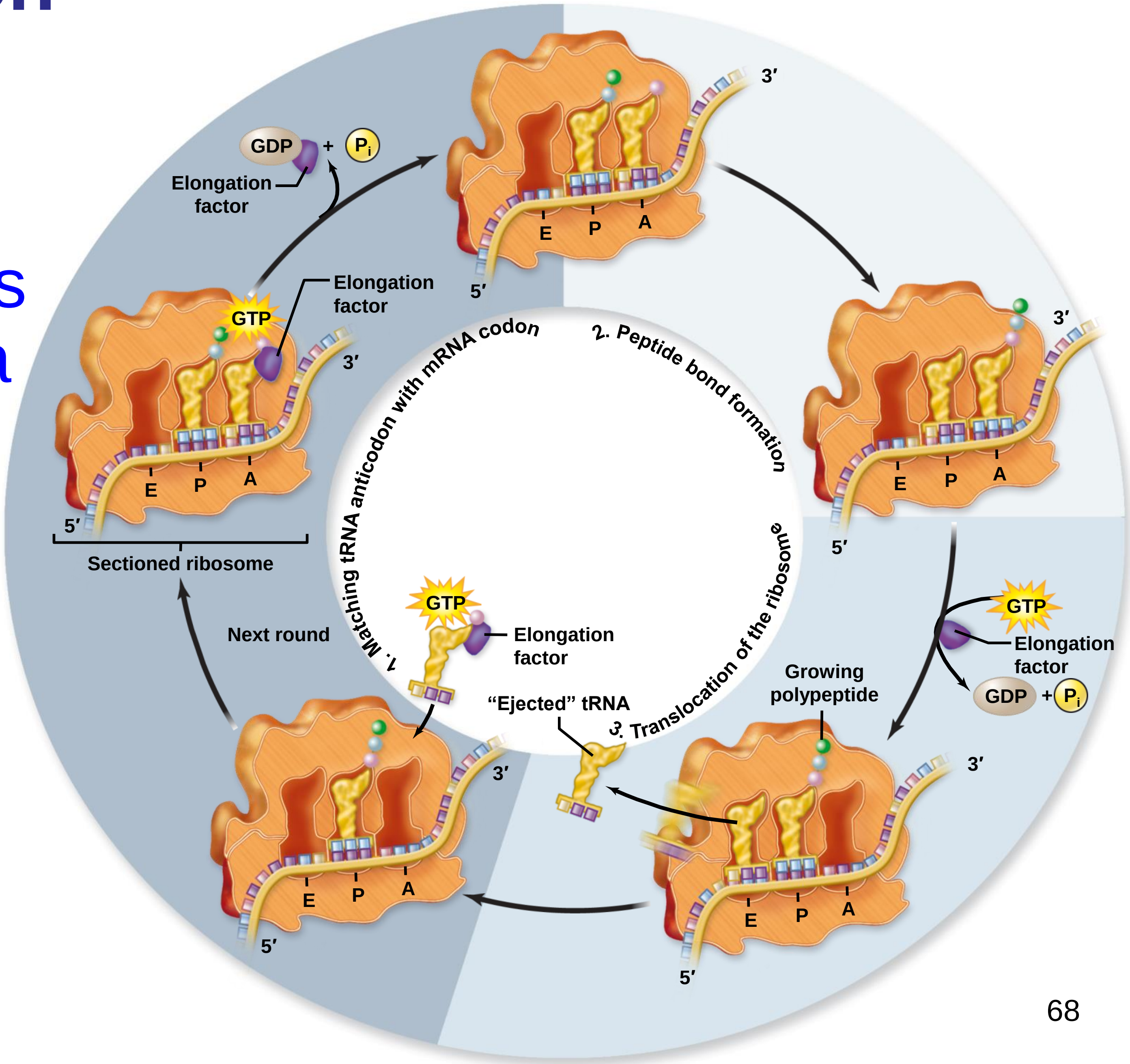


Elongation


3′
5′
NH_3^+
Amino acid 1
O═C
O
NH_3^+
Amino acid 2
C═O
O
Peptide bond formation
NH_3^+
Amino group
Amino acid 1
O═C
N
Peptide bond
Polypeptide chain
Amino acid 2
C═O
O
"Empty" tRNA
OH
A site
P site
Amino end (N terminus)
NH_3^+
Amino acid 1
Amino acid 2
Amino acid 3
Amino acid 4
Amino acid 5
Amino acid 6
Amino acid 7
COO^-
Carboxyl end (C terminus)

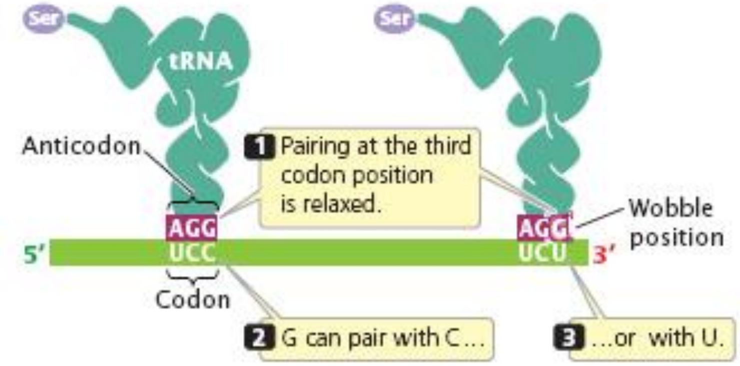
Elongation

Addition of successive amino acids occurs as a cycle



Translation

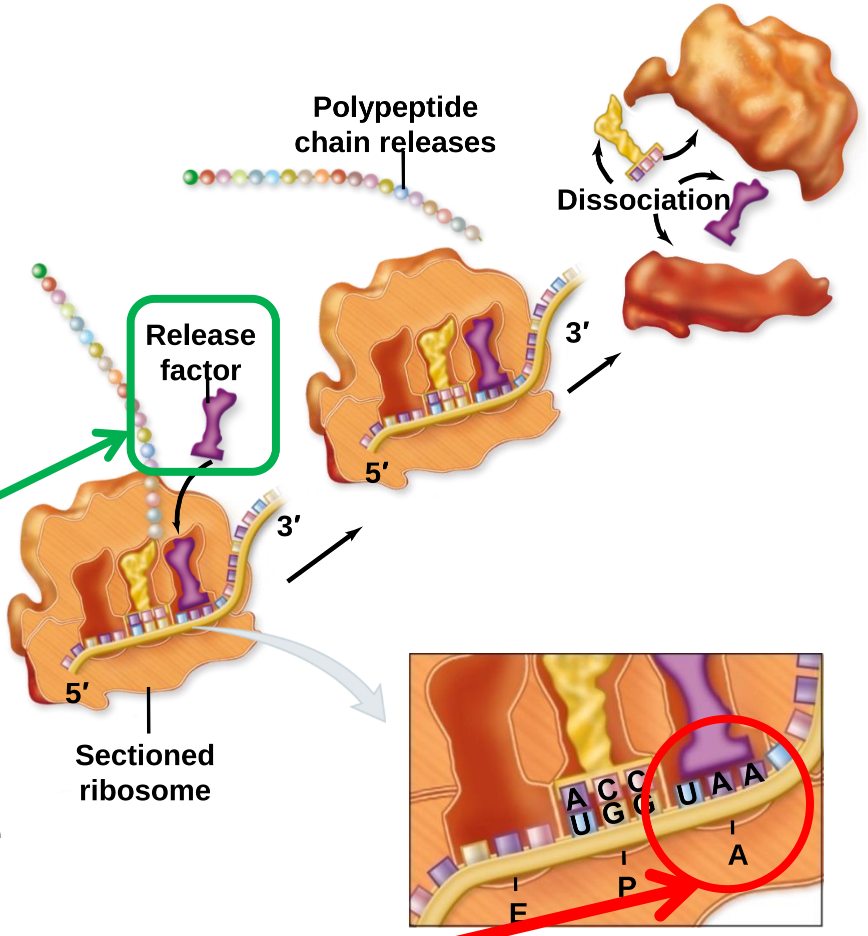
- There are fewer tRNAs than codons
- **Wobble pairing** allows less stringent pairing between the 3′ base of the codon and the 5′ base of the anticodon
  - This allows fewer tRNAs to accommodate all codons
  - Allowed by degenerate code



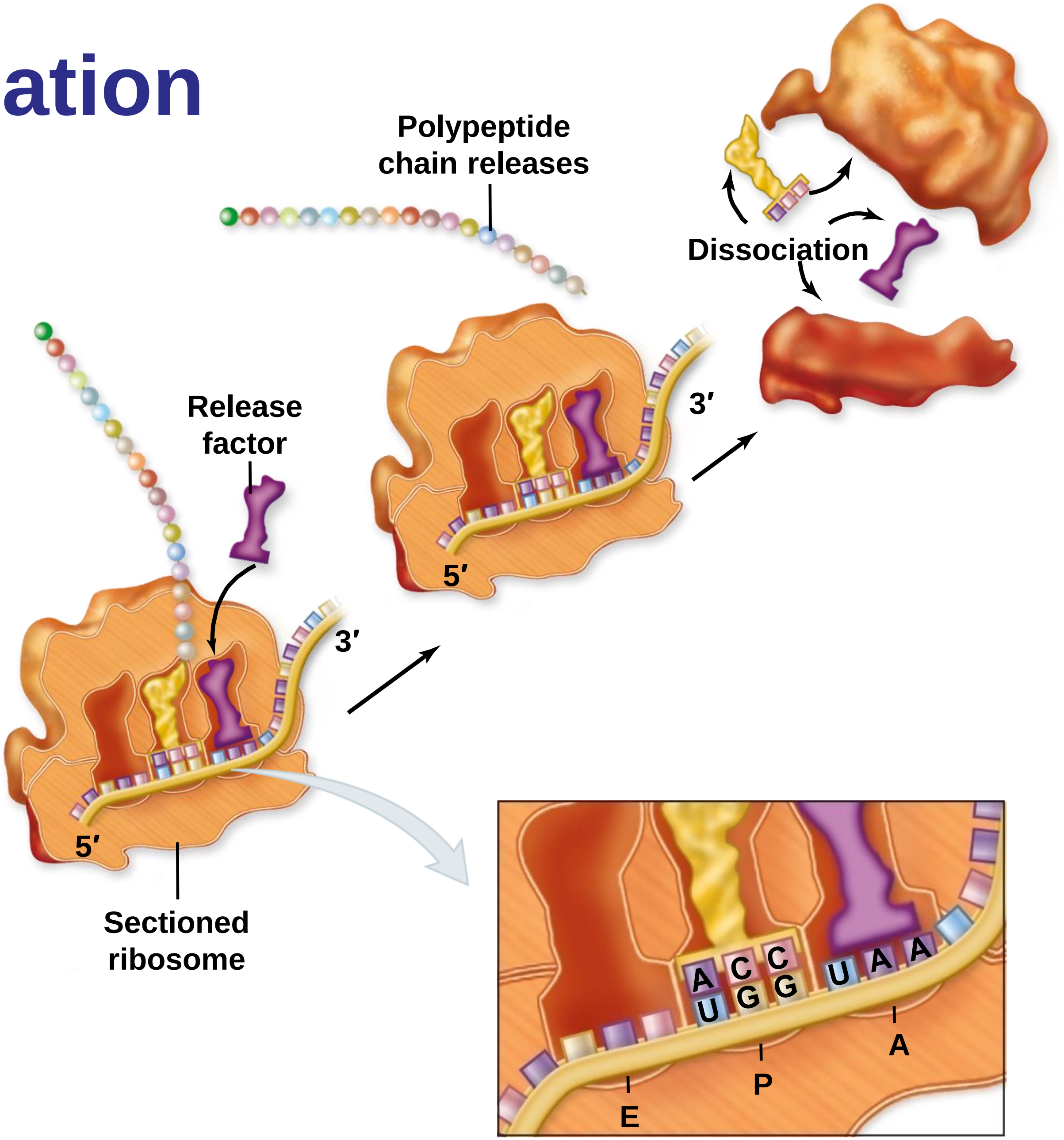
http://www.nature.com/scitable/content/wobble-may-exist-in-the-pairing-of-4489

Translation

- **Termination**
  - Elongation continues until the ribosome encounters a **stop codon**
  - Stop codons are recognized by **release factors** which release the polypeptide from the ribosome



Termination



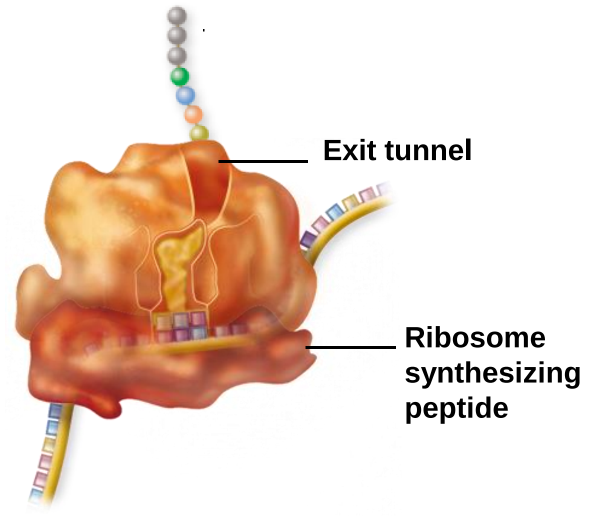
How Translation Works

The genetic information stored in DNA is transcribed into messenger RNA and then translated into protein.

Protein Targeting

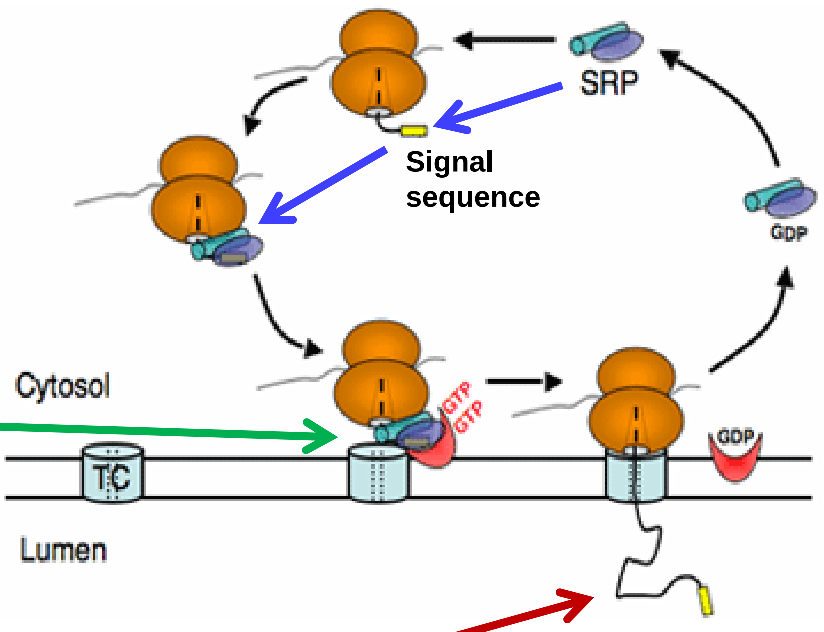
- In eukaryotes, translation may occur in the cytoplasm or the rough endoplasmic reticulum (RER)
  - – Free ribosomes → cytoplasm
  - – Bound ribosomes → RER

Free ribosome



Protein Targeting

- Bound ribsomes → RER
  - **Signal sequences** at the beginning of polypeptide sequence bind to the **signal recognition particle (SRP)**
  - The signal sequence & SRP are recognized by RER receptor proteins
  - Docking holds ribosome to RER ("bound" ribosome)
  - Beginning of the protein-trafficking pathway

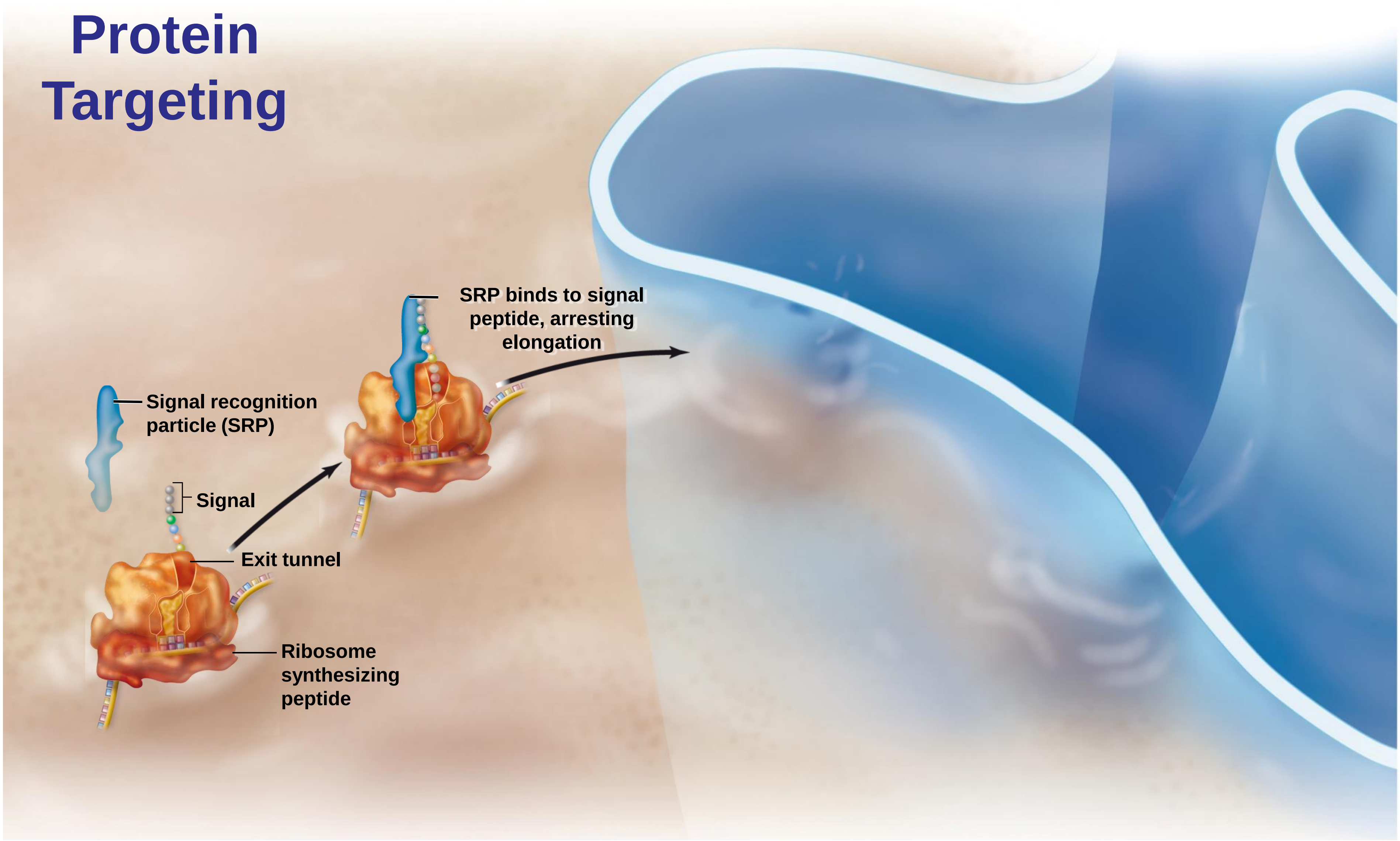


http://mbg.cornell.edu/faculty-staff/faculty/ke.cfm

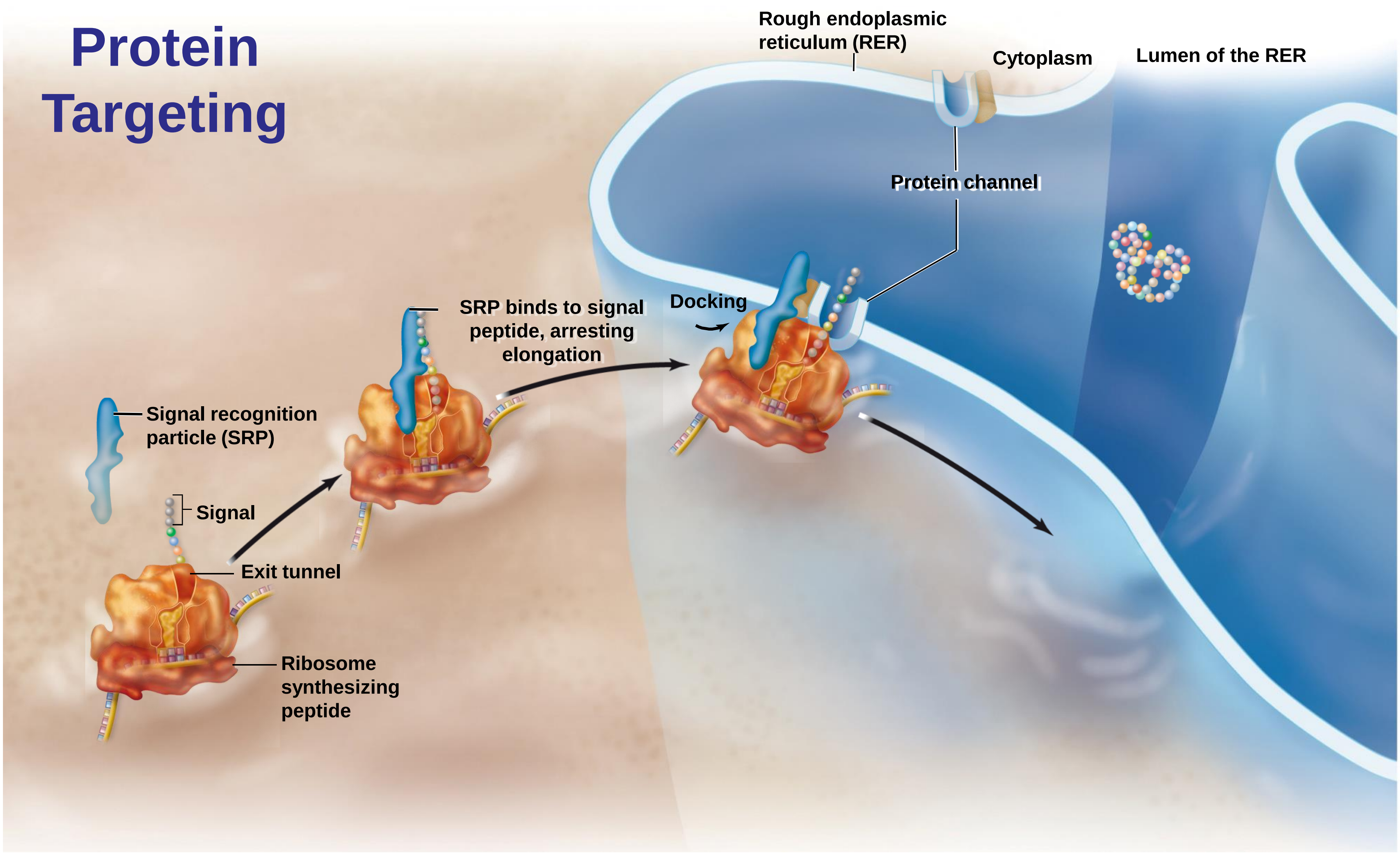
Protein Targeting

Fig. 15.22-1

Protein Targeting



Protein Targeting



Protein Targeting

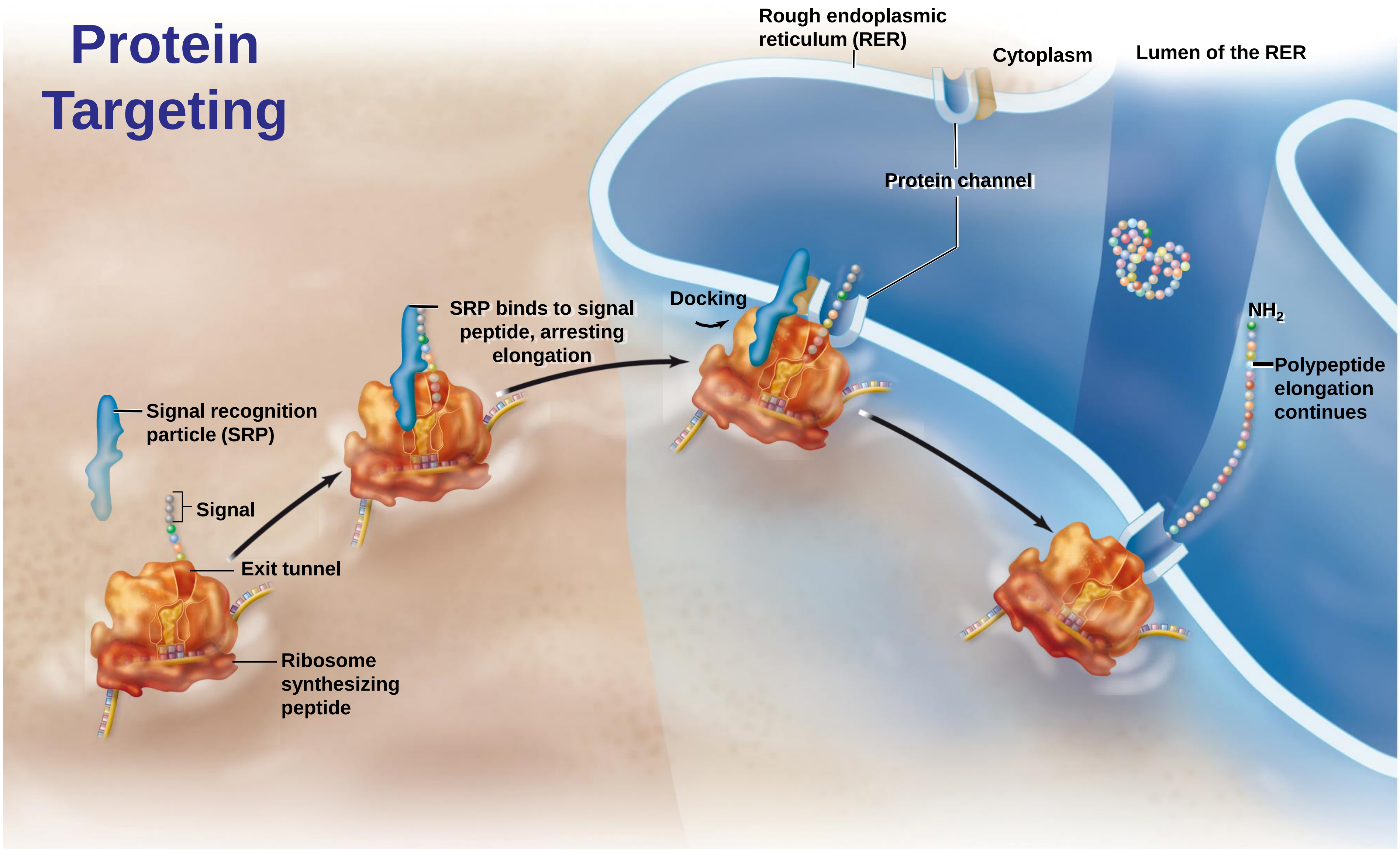
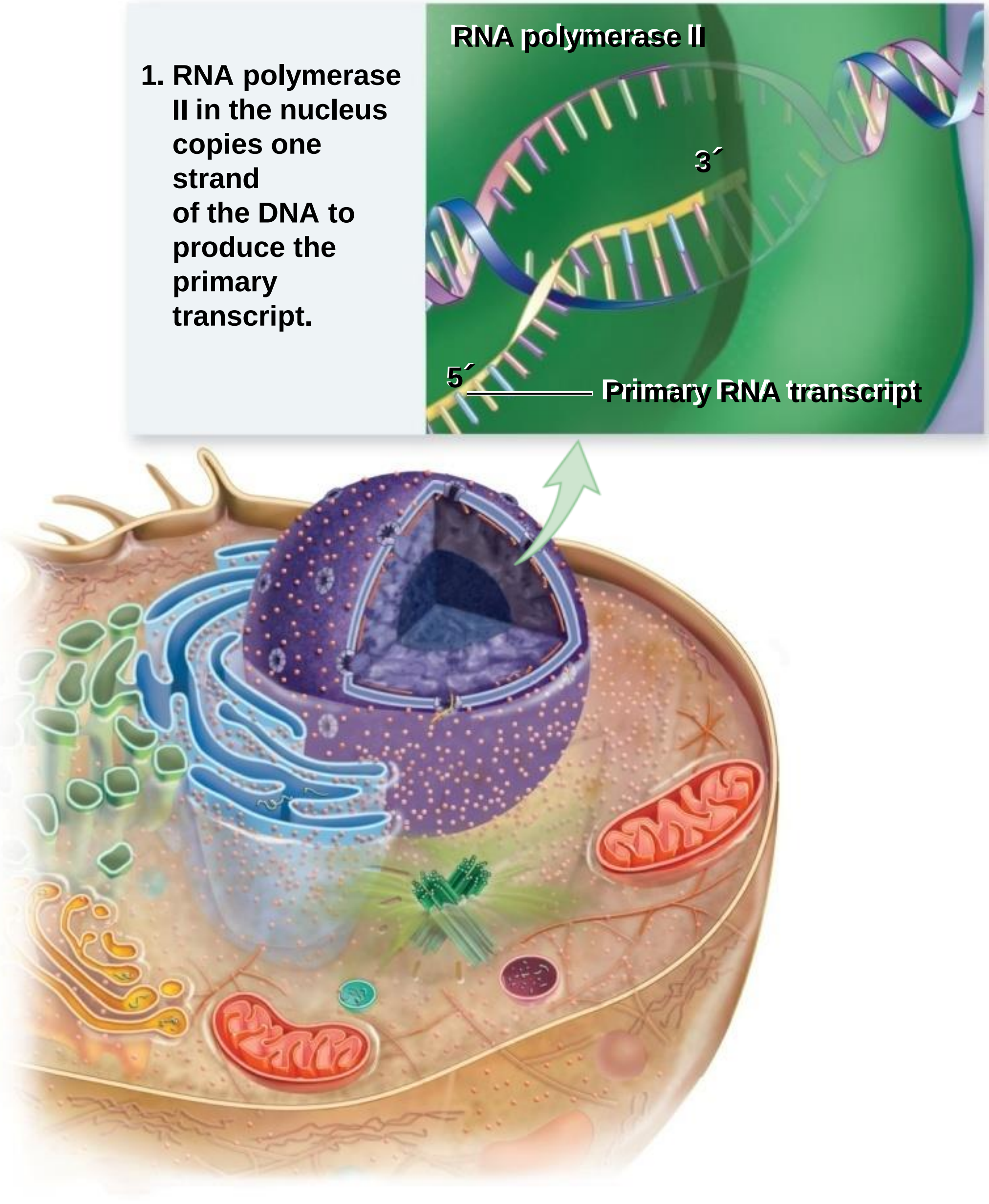
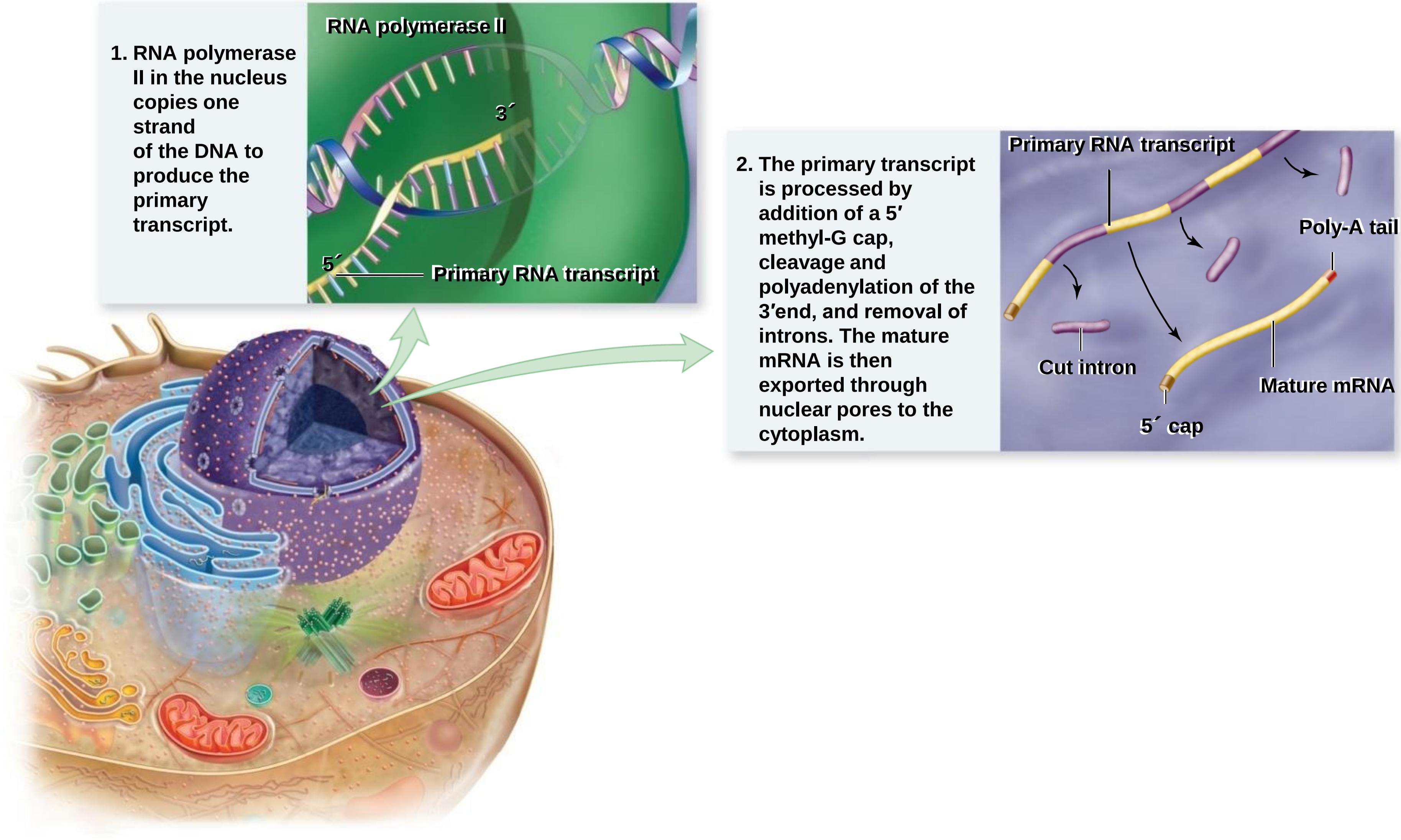


Fig. 15.23 (page 298)

Overview of Gene Expression in Eukaryotes

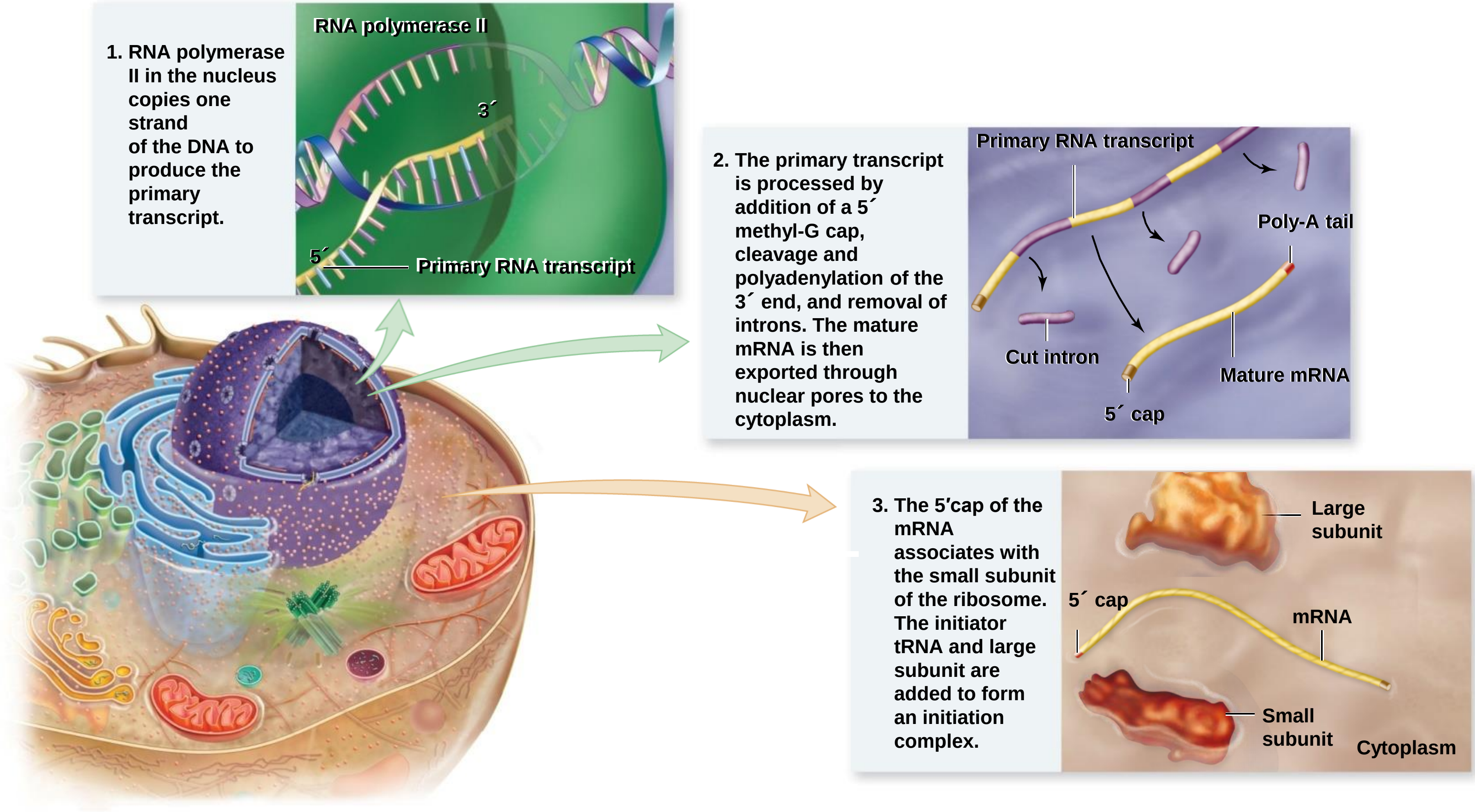
1. **RNA polymerase II in the nucleus copies one strand of the DNA to produce the primary transcript.**





1. RNA polymerase II in the nucleus copies one strand of the DNA to produce the primary transcript.

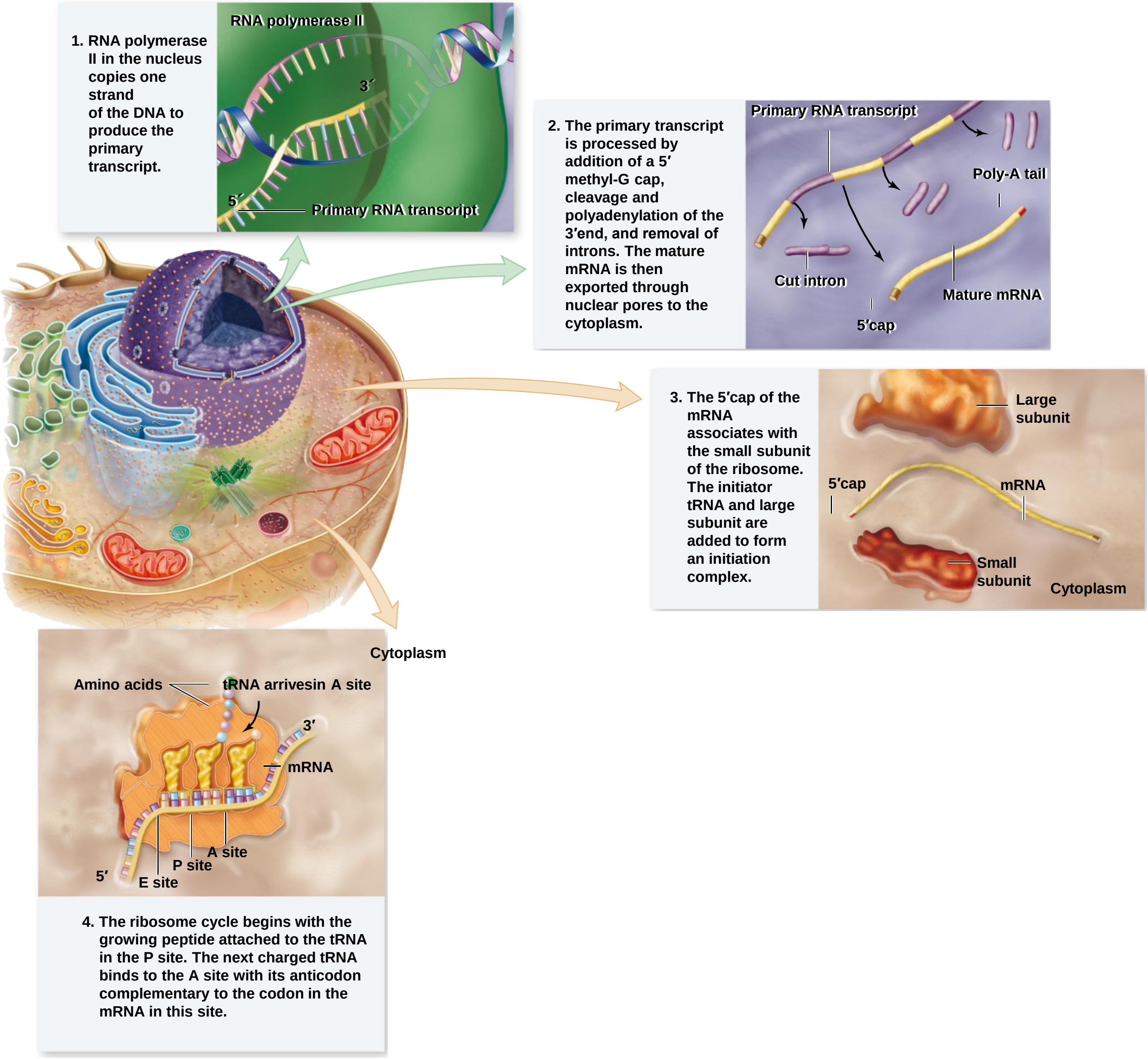
2. The primary transcript is processed by addition of a 5′ methyl-G cap, cleavage and polyadenylation of the 3′end, and removal of introns. The mature mRNA is then exported through nuclear pores to the cytoplasm.



1. RNA polymerase II in the nucleus copies one strand of the DNA to produce the primary transcript.

2. The primary transcript is processed by addition of a 5´ methyl-G cap, cleavage and polyadenylation of the 3´ end, and removal of introns. The mature mRNA is then exported through nuclear pores to the cytoplasm.

3. The 5′cap of the mRNA associates with the small subunit of the ribosome. The initiator tRNA and large subunit are added to form an initiation complex.



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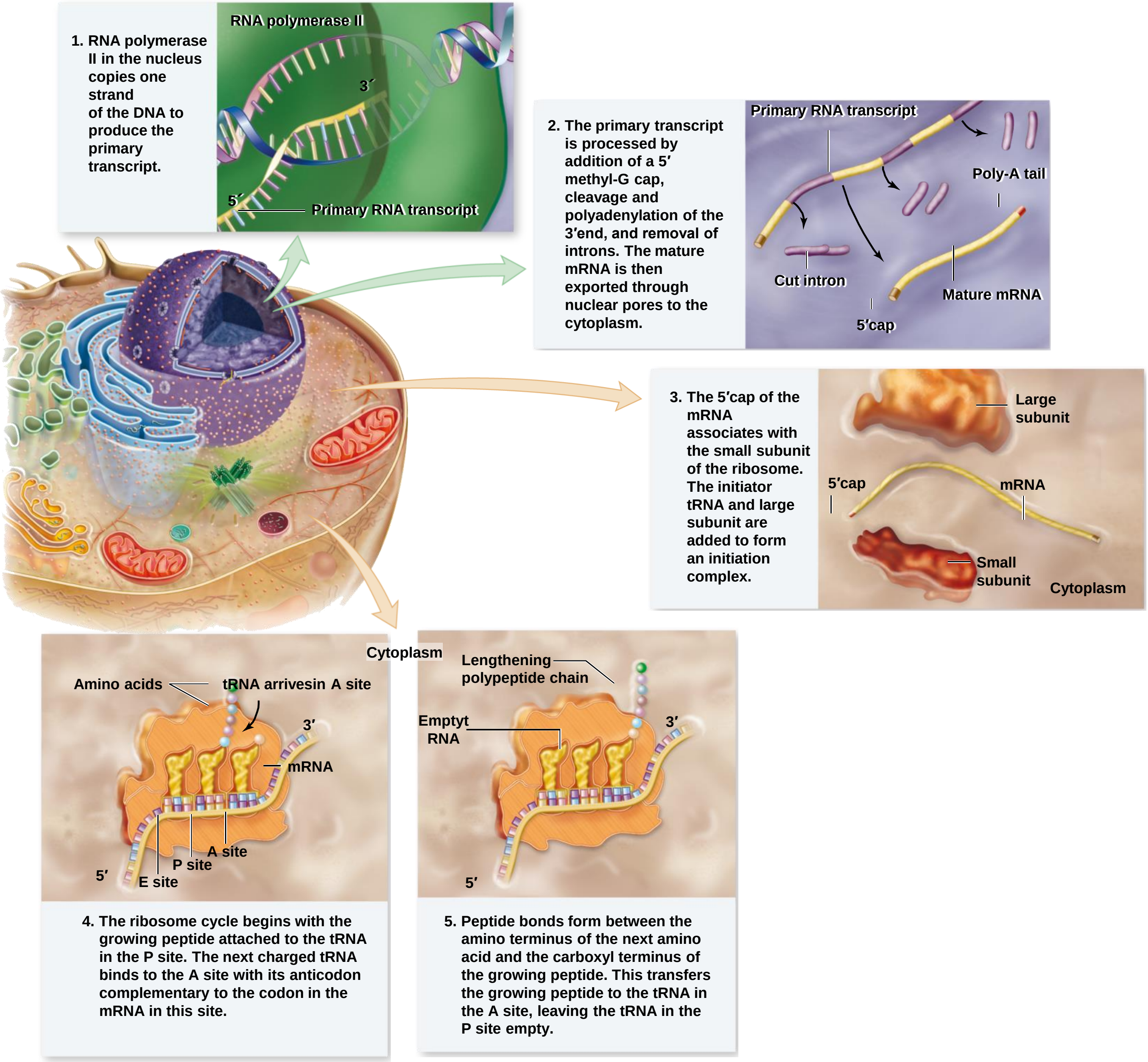

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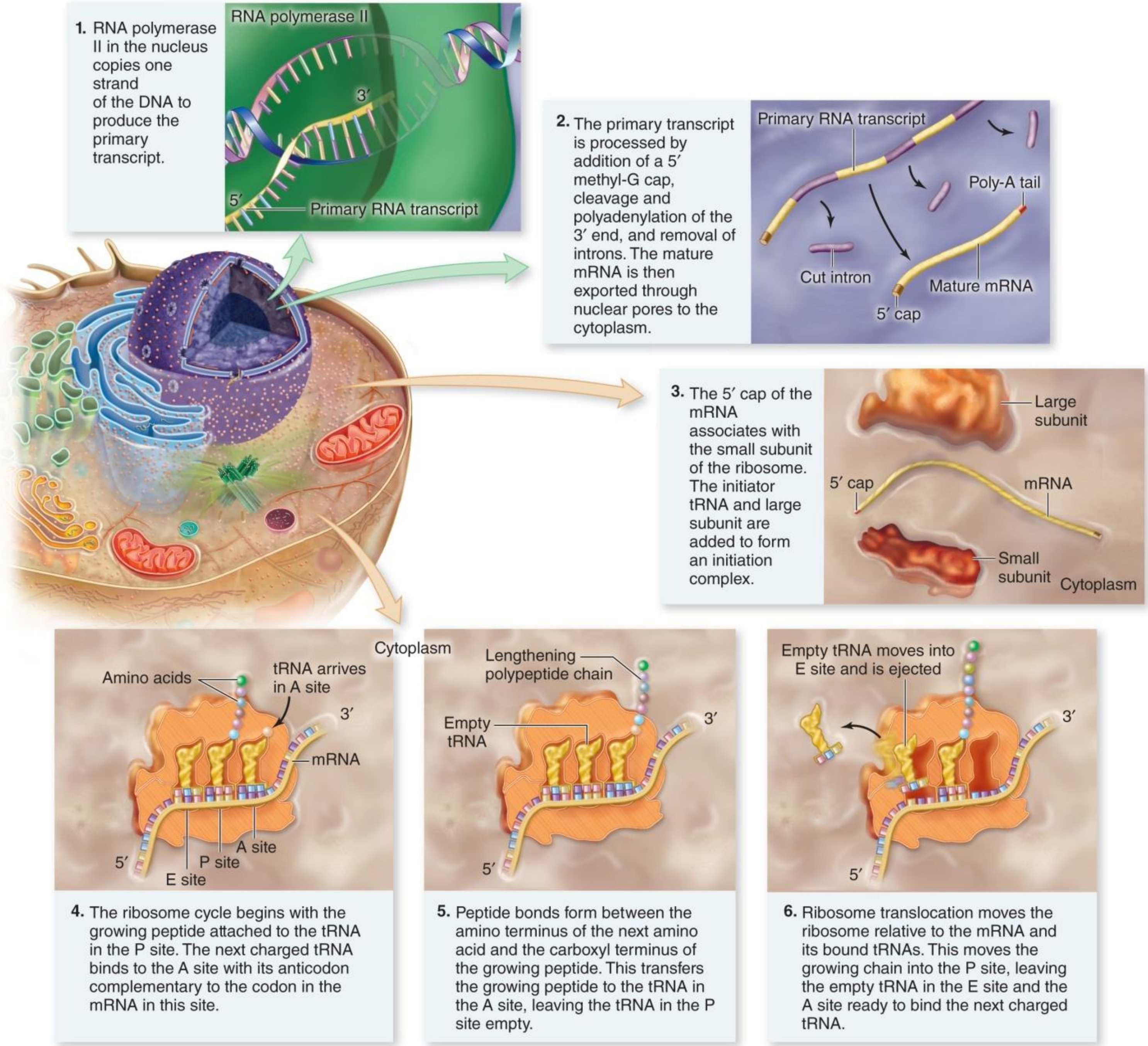

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6. Ribosome translocation moves the ribosome relative to the mRNA and its bound tRNAs. This moves the growing chain into the P site, leaving the empty tRNA in the E site and the A site ready to bind the next charged tRNA.

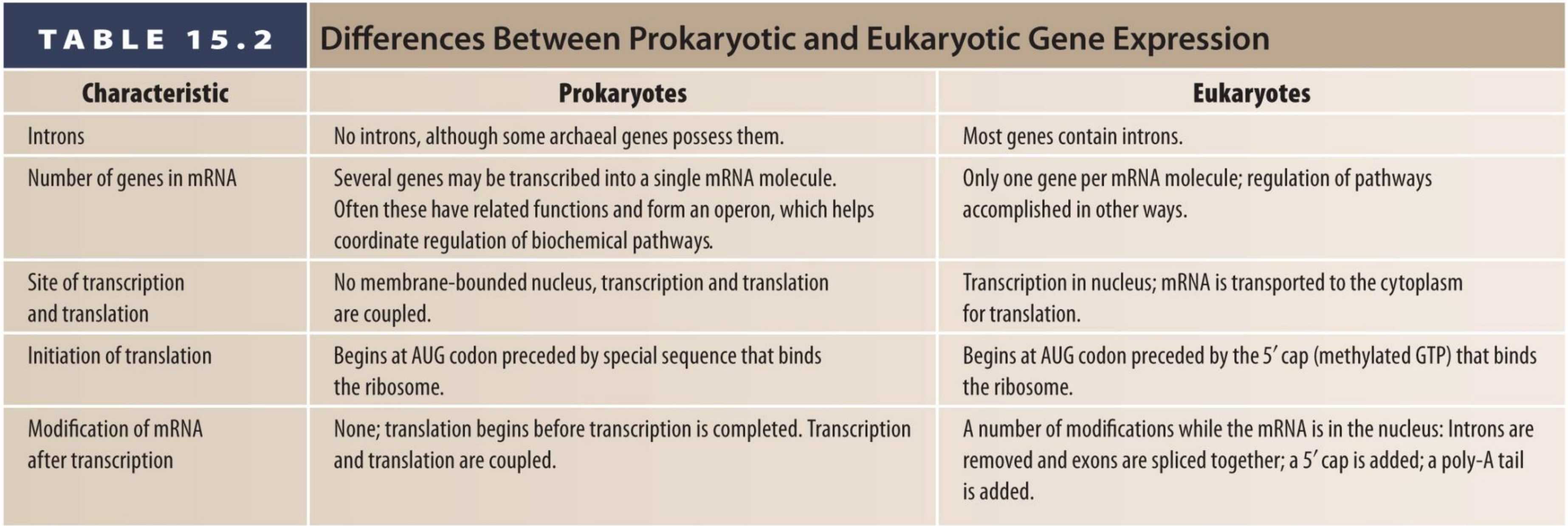


TABLE 15.2 Differences Between Prokaryotic and Eukaryotic Gene Expression

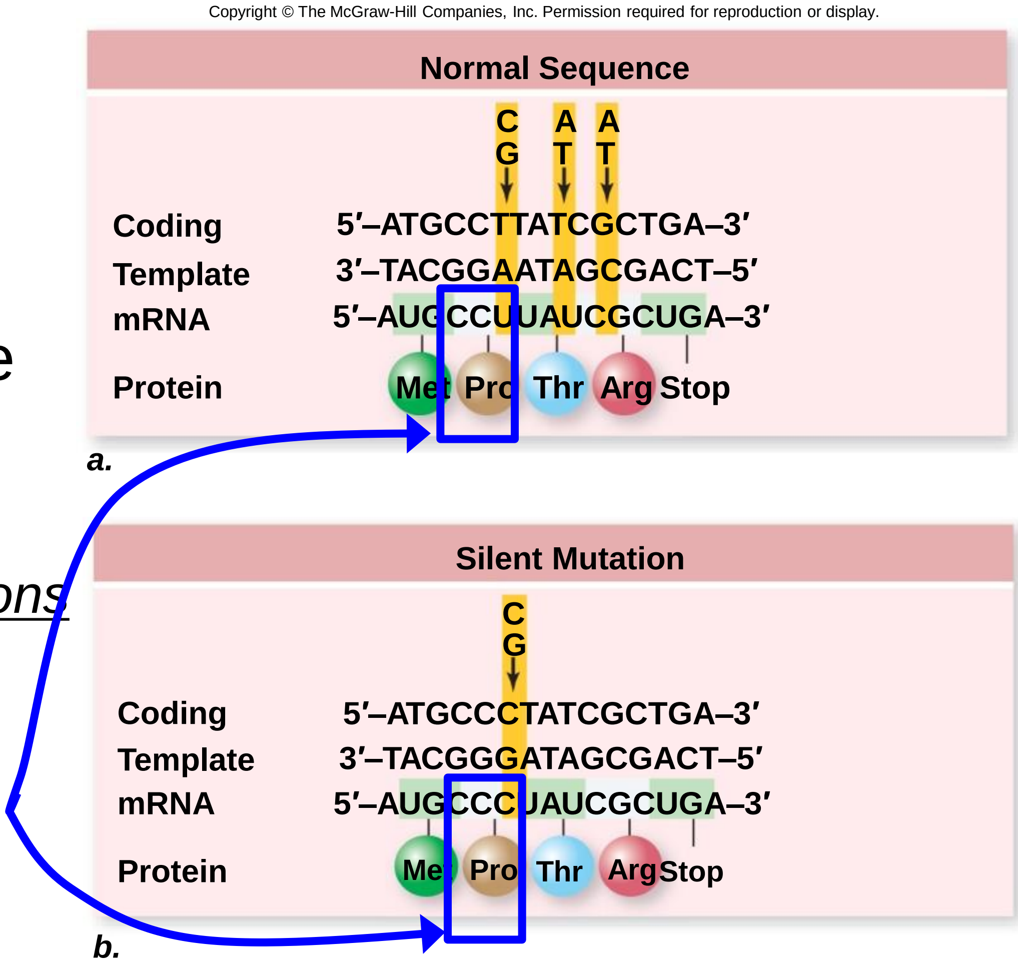
Characteristic	Prokaryotes	Eukaryotes
Introns	No introns, although some archaeal genes possess them.	Most genes contain introns.
Number of genes in mRNA	Several genes may be transcribed into a single mRNA molecule. Often these have related functions and form an operon, which helps coordinate regulation of biochemical pathways.	Only one gene per mRNA molecule; regulation of pathways accomplished in other ways.
Site of transcription and translation	No membrane-bounded nucleus, transcription and translation are coupled.	Transcription in nucleus; mRNA is transported to the cytoplasm for translation.
Initiation of translation	Begins at AUG codon preceded by special sequence that binds the ribosome.	Begins at AUG codon preceded by the 5′ cap (methylated GTP) that binds the ribosome.
Modification of mRNA after transcription	None; translation begins before transcription is completed. Transcription and translation are coupled.	A number of modifications while the mRNA is in the nucleus: Introns are removed and exons are spliced together; a 5′ cap is added; a poly-A tail is added.

Mutation: Altered Genes

Point mutations alter a single base

- **Base substitution** – substitute one base for another
  - **Silent mutation** – same amino acid inserted
    - Due to degenerate code
    - *Reduces affect of mutations*

CCU and CCC both code for proline



- Code is ***degenerate***, meaning that most amino acids are specified by *more than one codon, but unambigous*

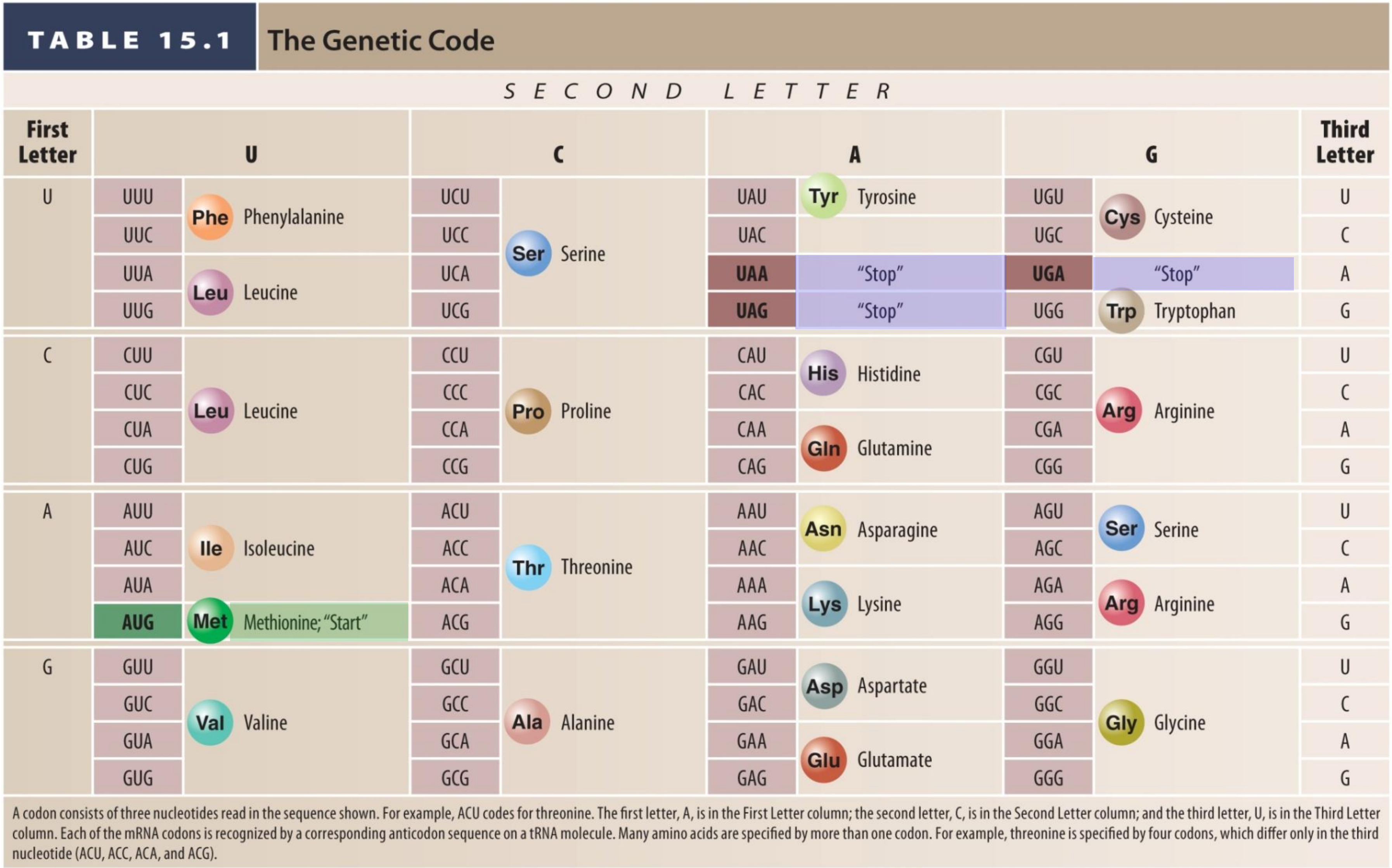


TABLE 15.1 The Genetic Code

SECOND LETTER

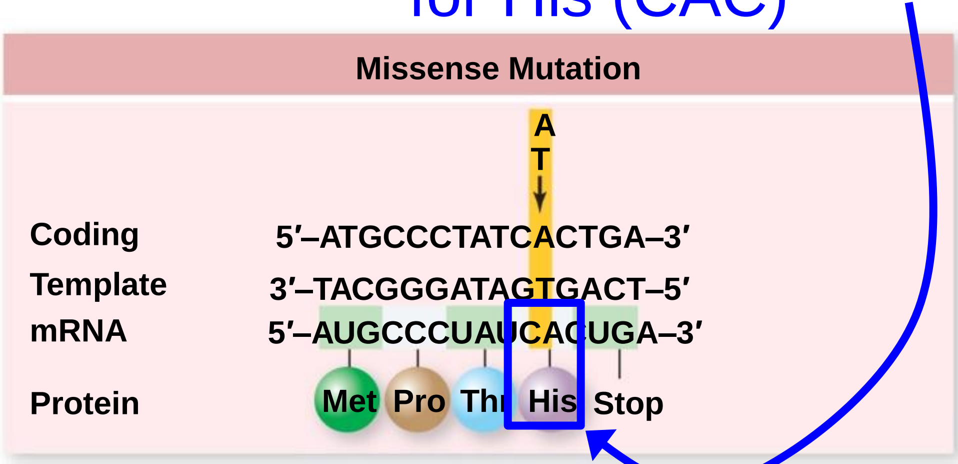
First Letter	U		C		A		G		Third Letter
U	UUU	**Phe** Phenylalanine	UCU	**Ser** Serine	UAU	**Tyr** Tyrosine	UGU	**Cys** Cysteine	U
	UUC		UCC		UAC		UGC		C
	UUA	**Leu** Leucine	UCA		**UAA**	"Stop"	**UGA**	"Stop"	A
	UUG		UCG		**UAG**	"Stop"	UGG	**Trp** Tryptophan	G
C	CUU	**Leu** Leucine	CCU	**Pro** Proline	CAU	**His** Histidine	CGU	**Arg** Arginine	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	**Gln** Glutamine	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	**Ile** Isoleucine	ACU	**Thr** Threonine	AAU	**Asn** Asparagine	AGU	**Ser** Serine	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	**Lys** Lysine	AGA	**Arg** Arginine	A
	AUG	**Met** Methionine; "Start"	ACG		AAG		AGG		G
G	GUU	**Val** Valine	GCU	**Ala** Alanine	GAU	**Asp** Aspartate	GGU	**Gly** Glycine	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	**Glu** Glutamate	GGA		A
	GUG		GCG		GAG		GGG		G

A codon consists of three nucleotides read in the sequence shown. For example, ACU codes for threonine. The first letter, A, is in the First Letter column; the second letter, C, is in the Second Letter column; and the third letter, U, is in the Third Letter column. Each of the mRNA codons is recognized by a corresponding anticodon sequence on a tRNA molecule. Many amino acids are specified by more than one codon. For example, threonine is specified by four codons, which differ only in the third nucleotide (ACU, ACC, ACA, and ACG).

Mutation: Altered Genes

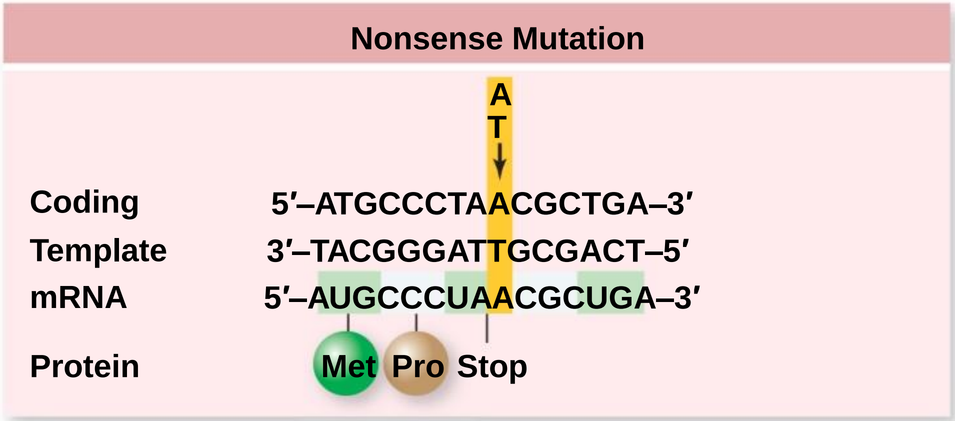
- **Missense mutation**
  - changes amino acid with substitution
  - **Transitions**
  - **Transversions**

Substitute Arg (CGC) for His (CAC)

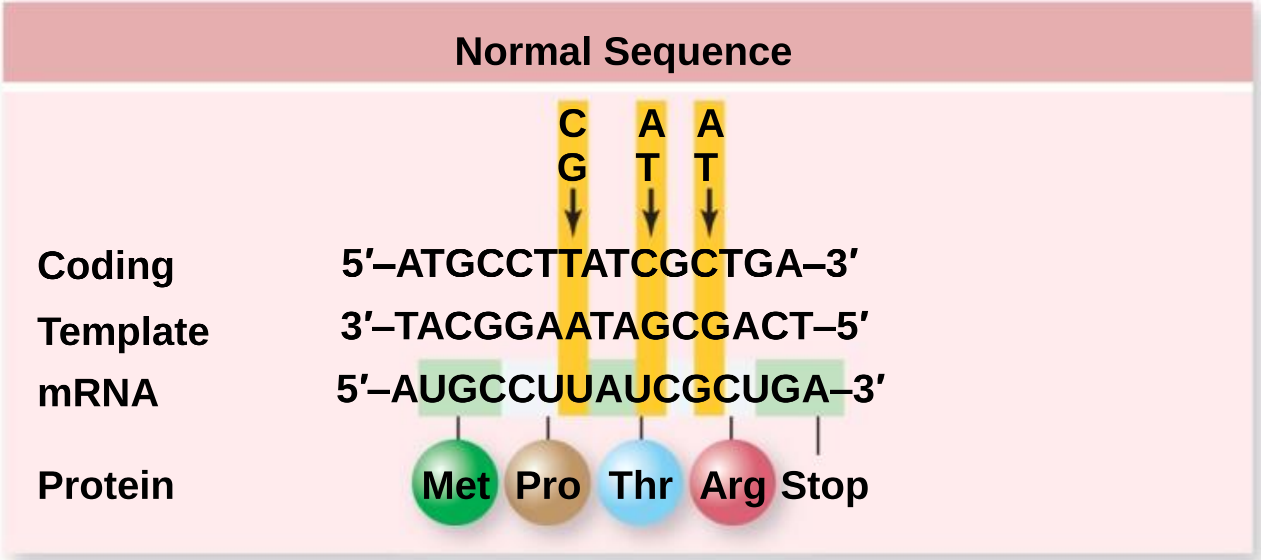


c.

- **Nonsense mutations**
  - changed to stop codon



d.



Normal Sequence

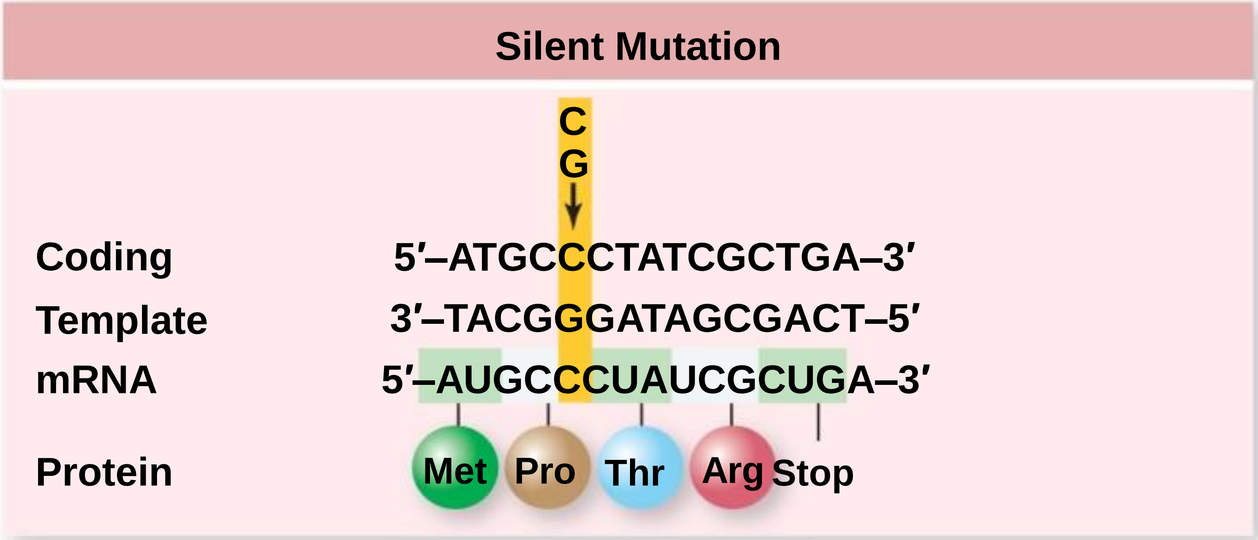
Coding 5′–ATGCCTTATCGCTGA–3′

Template 3′–TACGGAATAGCGACT–5′

mRNA 5′–AUGCCUUAUCGCUGA–3′

Protein Met Pro Thr Arg Stop

a.



Silent Mutation

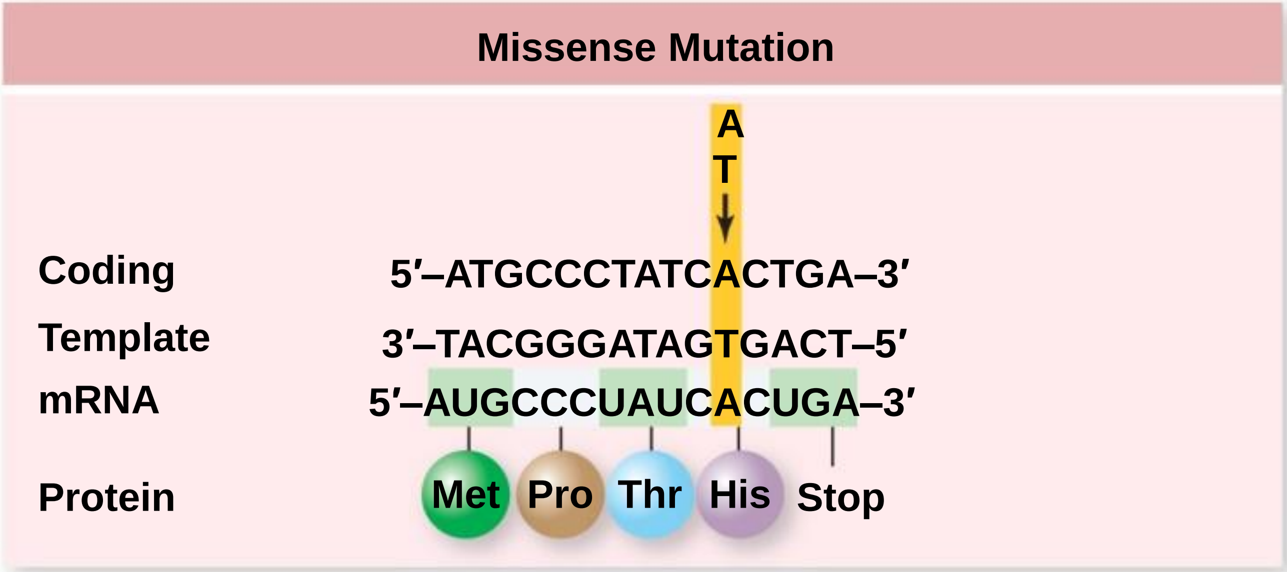
Coding 5′–ATGCCCTATCGCTGA–3′

Template 3′–TACGGGATAGCGACT–5′

mRNA 5′–AUGCCCUAUCGCUGA–3′

Protein Met Pro Thr Arg Stop

b.



Missense Mutation

A
T

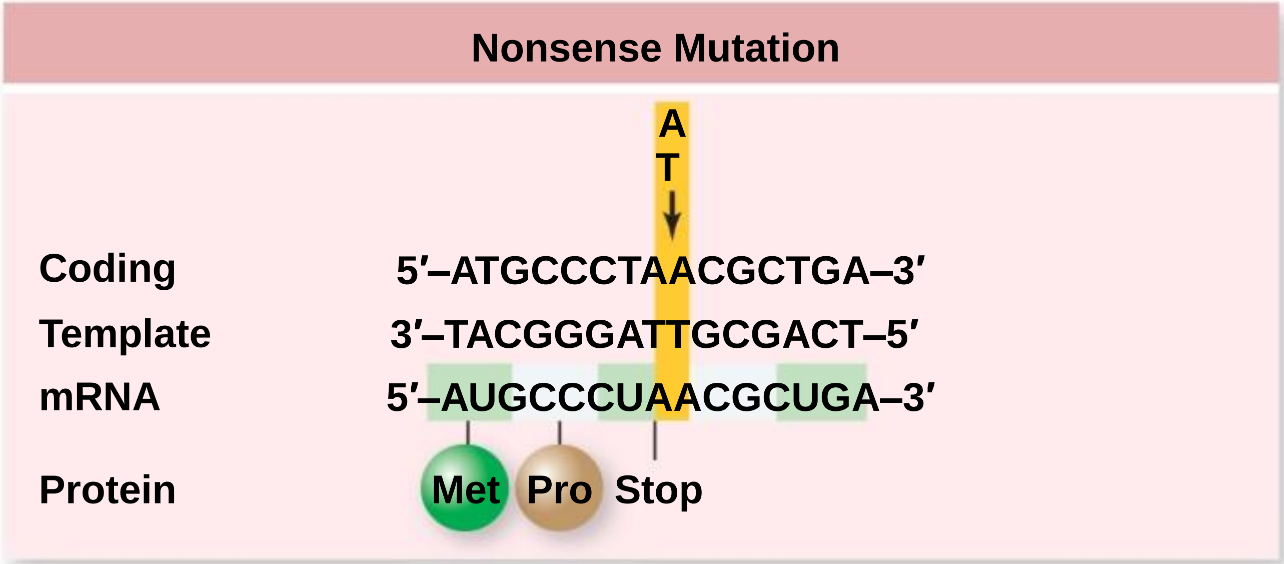
Coding 5′–ATGCCCTATCACTGA–3′

Template 3′–TACGGGATAGTGACT–5′

mRNA 5′–AUGCCCUAUCACUGA–3′

Protein Met Pro Thr His Stop

c.



Nonsense Mutation

A
T

Coding 5′–ATGCCCTAACGCTGA–3′

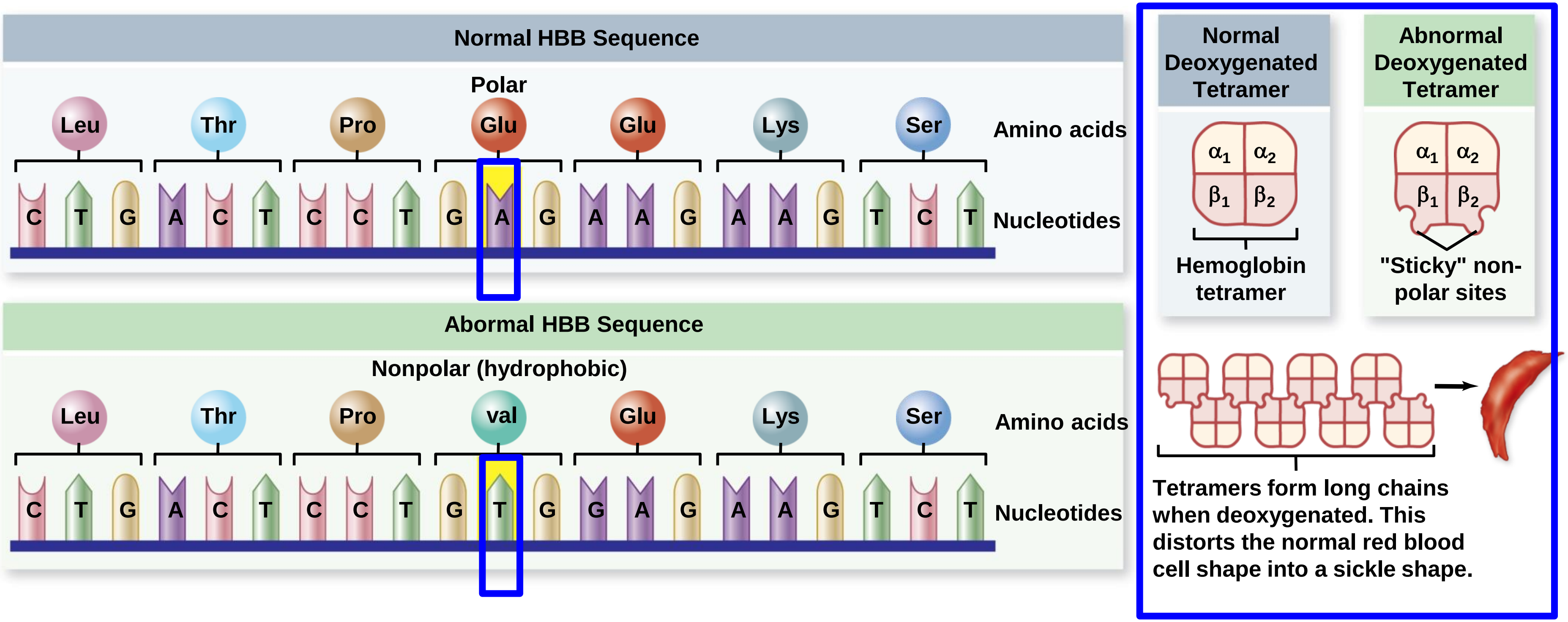
Template 3′–TACGGGATTGCGACT–5′

mRNA 5′–AUGCCCUAACGCUGA–3′

Protein Met Pro Stop

d.

Mutation: Altered Genes

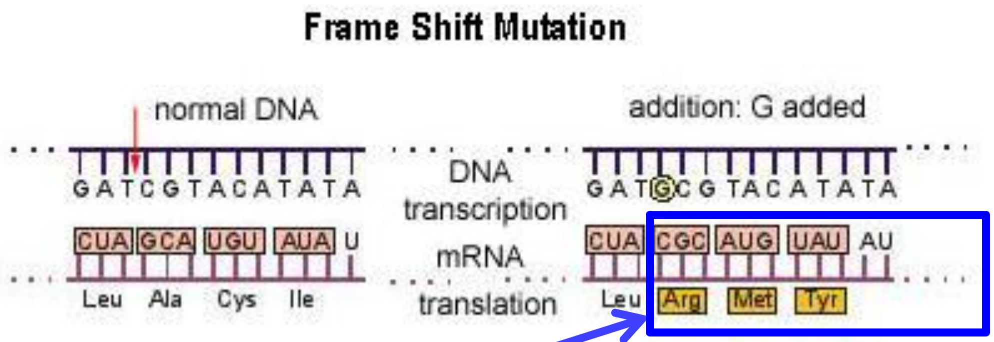


Tetramers form long chains when deoxygenated. This distorts the normal red blood cell shape into a sickle shape.

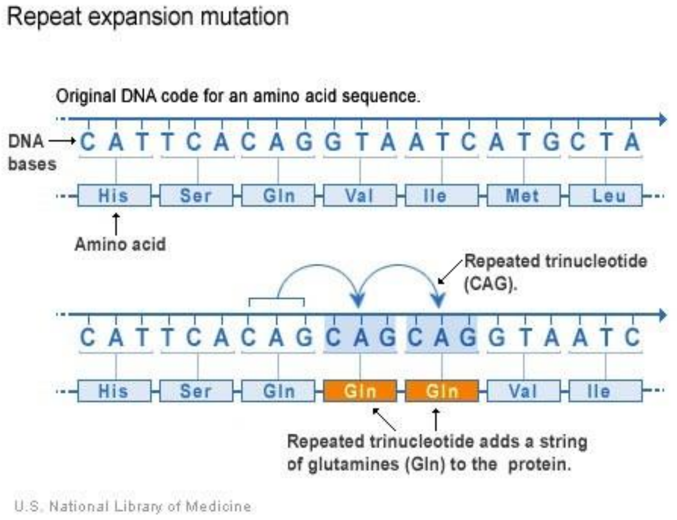
In hemoglobin gene, a base substitution (A replaced with T) causes Valine to be substituted for Glutamate. This causes the hemoglobin molecules to stick together, causing sickle cell anemia http://staff.tuhsd.k12.az.us/gfoster/standard/bmut.htm

Mutation: Altered Genes

- **Frameshift mutations**
  - Addition or deletion of a single base
  - Much more profound consequences
  - Alter reading frame *downstream of mutation*



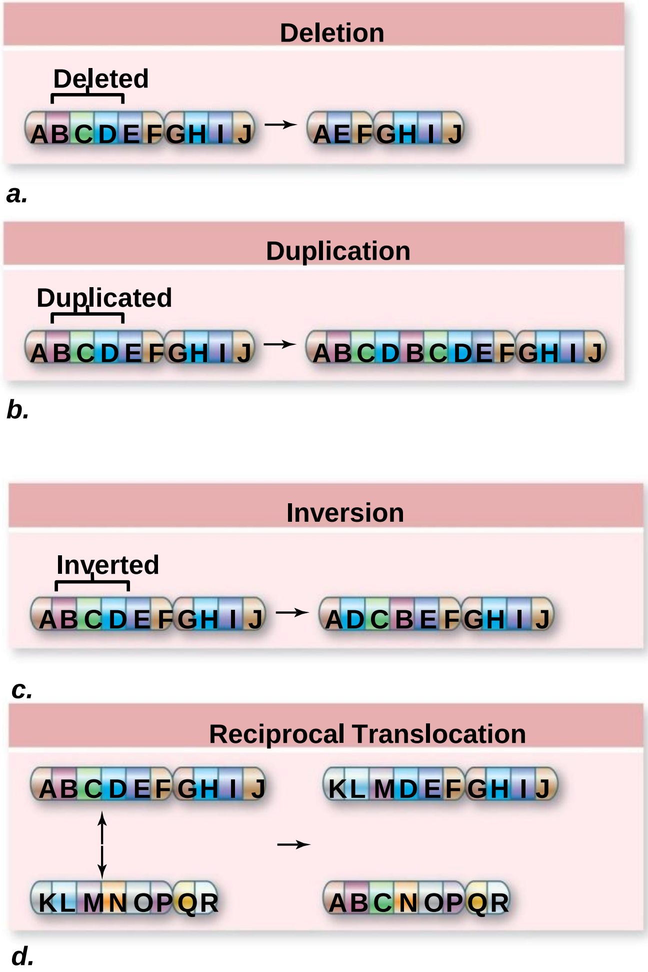
  - Triplet repeat expansion mutation
    - Huntington disease
    - Repeat units expand in disease allele relative to normal protein → degenerates neurons



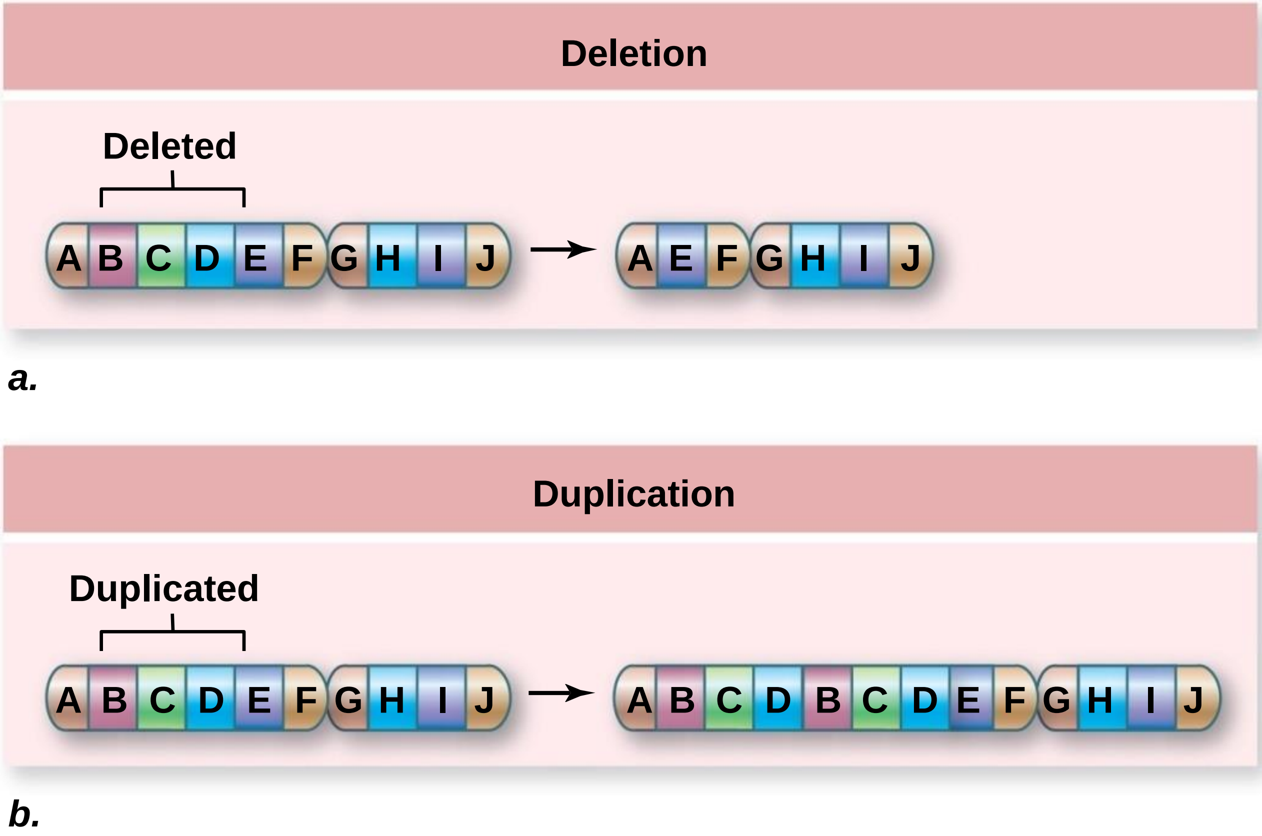
http://ghr.nlm.nih.gov/handbook/illustrations/repeatexpansion

Chromosomal mutations

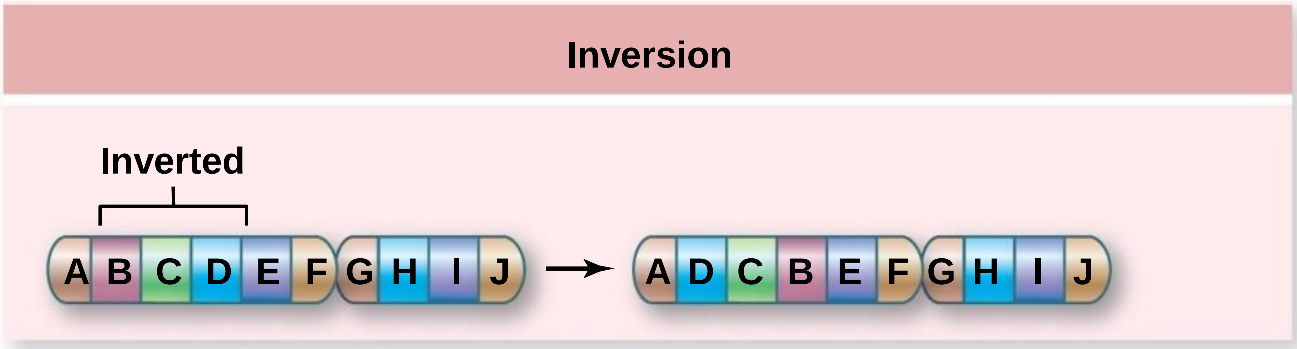
- Change the structure of a chromosome
  - **Deletions** – part of chromosome is lost
  - **Duplication** – part of chromosome is copied
  - **Inversion** – part of chromosome in reverse order
  - **Translocation** – part of chromosome is moved to a new location



Chromosomal mutations



Chromosomal mutations



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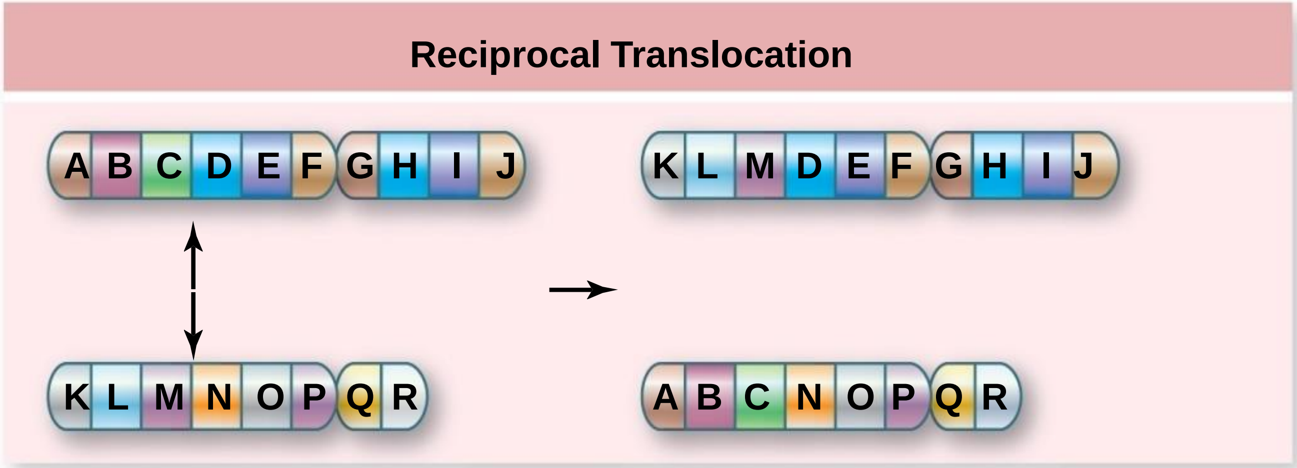

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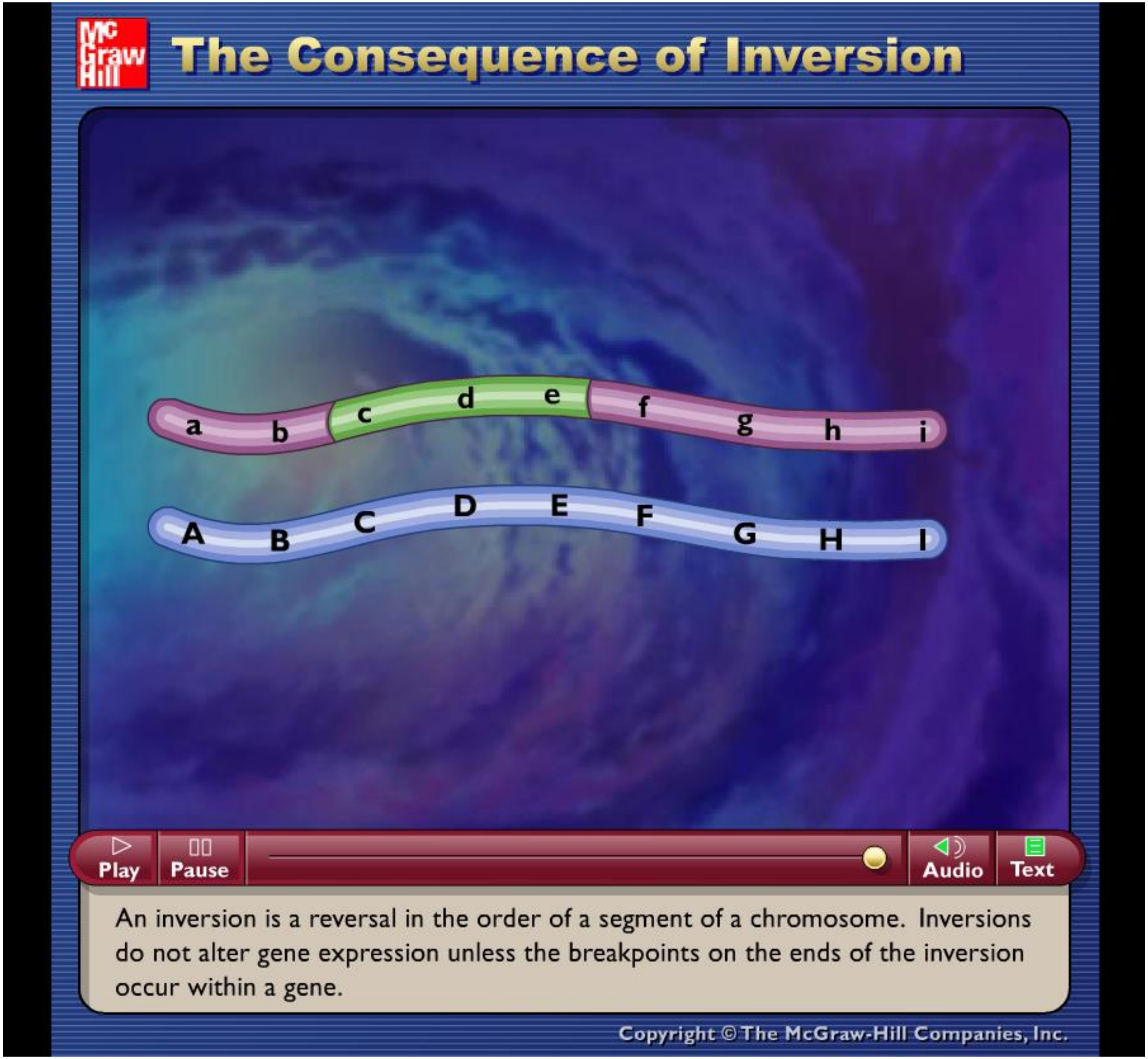


The Consequence of Inversion

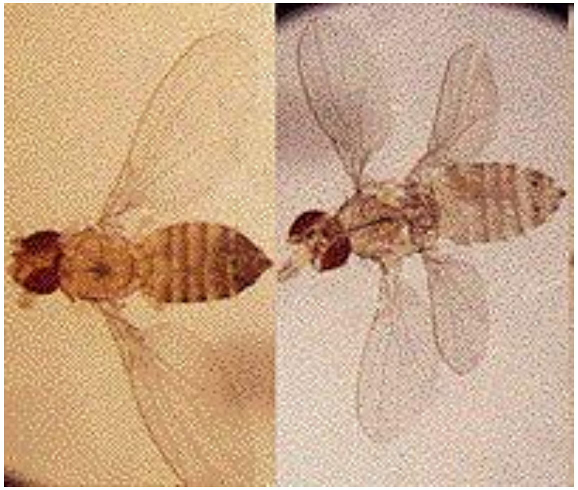
An inversion is a reversal in the order of a segment of a chromosome. Inversions do not alter gene expression unless the breakpoints on the ends of the inversion occur within a gene.

http://www.philly.com/philly/blogs/evolution/White-Horses-and-other-Beautiful-Mutants.html

Mutation: Altered Genes

What processes cause additional genetic variation??

- **Mutations** are the starting point for **evolution**
  - – Source of “new” alleles
  - – Too much change, however, is harmful to the individual with a greatly altered genome
  - – Balance must exist between amount of new variation and health of species



http://www.blackwellpublishing.com/ridley/a-z/Mutation.asp