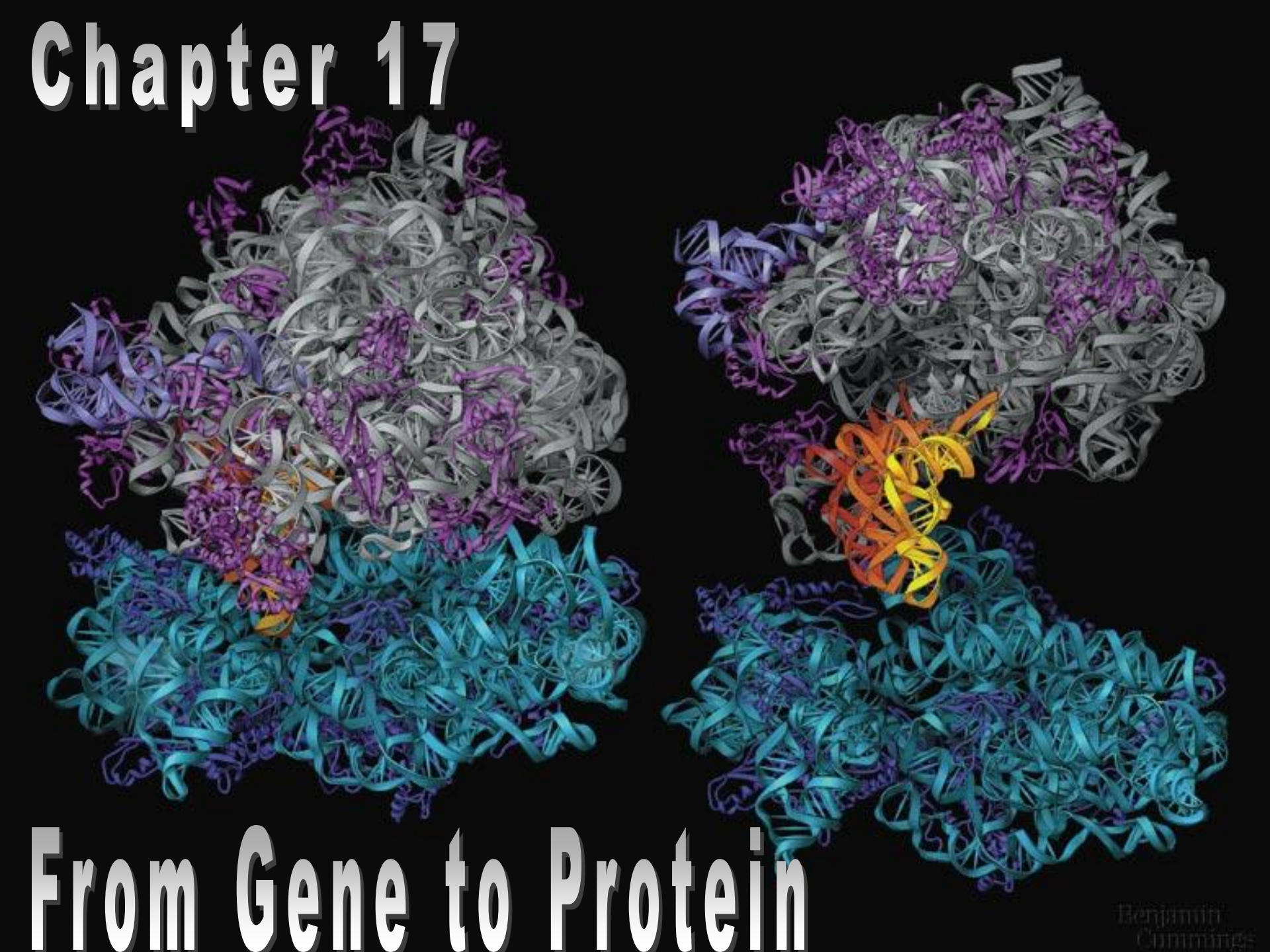
Chapter 17



From Gene to Protein

Gene Expression

- Process by which DNA directs the synthesis of proteins

- Scientists have developed the one gene – one polypeptide hypothesis
  - Each gene codes for a polypeptide

From Gene to Protein

- *Transcription* – the synthesis of RNA from the DNA template
  - *messenger RNA (mRNA)* – carries a genetic message from the DNA in the nucleus to the ribosome in the cytoplasm
- *Translation* – the actual synthesis of a protein at the ribosome which occurs under the direction of mRNA
  - *Ribosomal RNA (rRNA)* – the RNA that makes up the ribosome
  - *Transfer RNA (tRNA)* – RNA that is the interpreter of the mRNA strand
- *Codon* – mRNA base triplets that code for specific amino acids

Types of RNA in Eukaryotic Cells

Type of RNA	Functions
Messenger RNA (mRNA)	Carries information specifying amino acid sequences of proteins from DNA to ribosomes.
Transfer RNA (tRNA)	Plays catalytic (ribozyme) roles and structural roles in ribosomes.
Ribosomal RNA (rRNA)	Plays structural and catalytic (ribozyme) roles in ribosomes.

Primary transcript	Serves as a precursor to mRNA, rRNA, or tRNA and may be processed by splicing or cleavage . In eukaryotes, pre-mRNA commonly contains introns, noncoding segments that are spliced out as the primary transcript is processed. Some intron RNA acts as a ribozyme, catalyzing its own splicing.
Small nuclear RNA (snRNA)	Plays structural and catalytic roles in spliceosomes, the complexes of protein and RNA that splice pre-mRNA in the eukaryotic nucleus.
SRP RNA	Is a component of the signal-recognition particle (SRP), the protein-RNA complex that recognizes the signal peptides of polypeptides targeted to the ER.

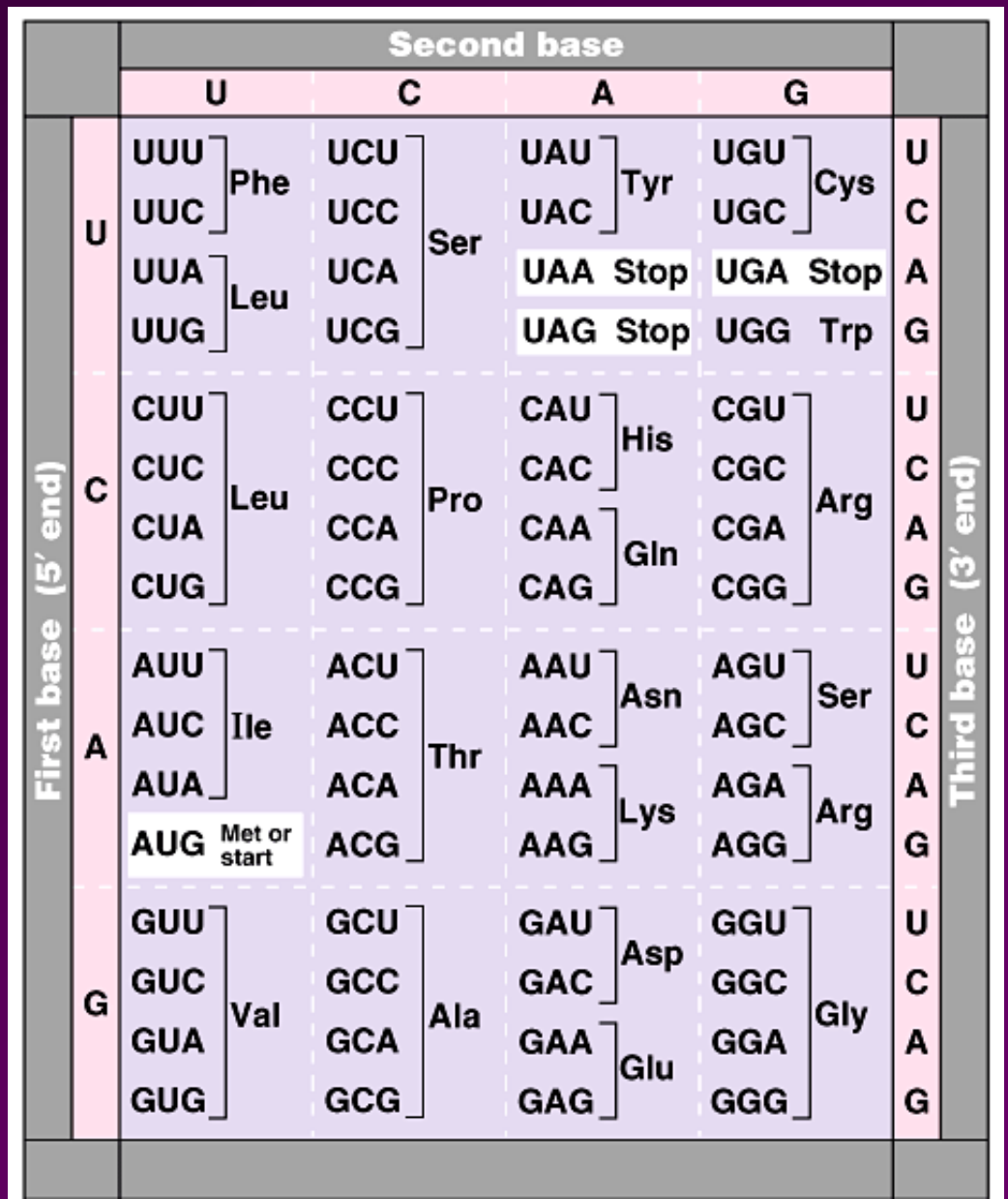
Types of RNA in Eukaryotic Cells

- RNAi = microRNA and small interfering RNA that inhibits gene expression by degrading mRNA

- Video

From Gene to Protein

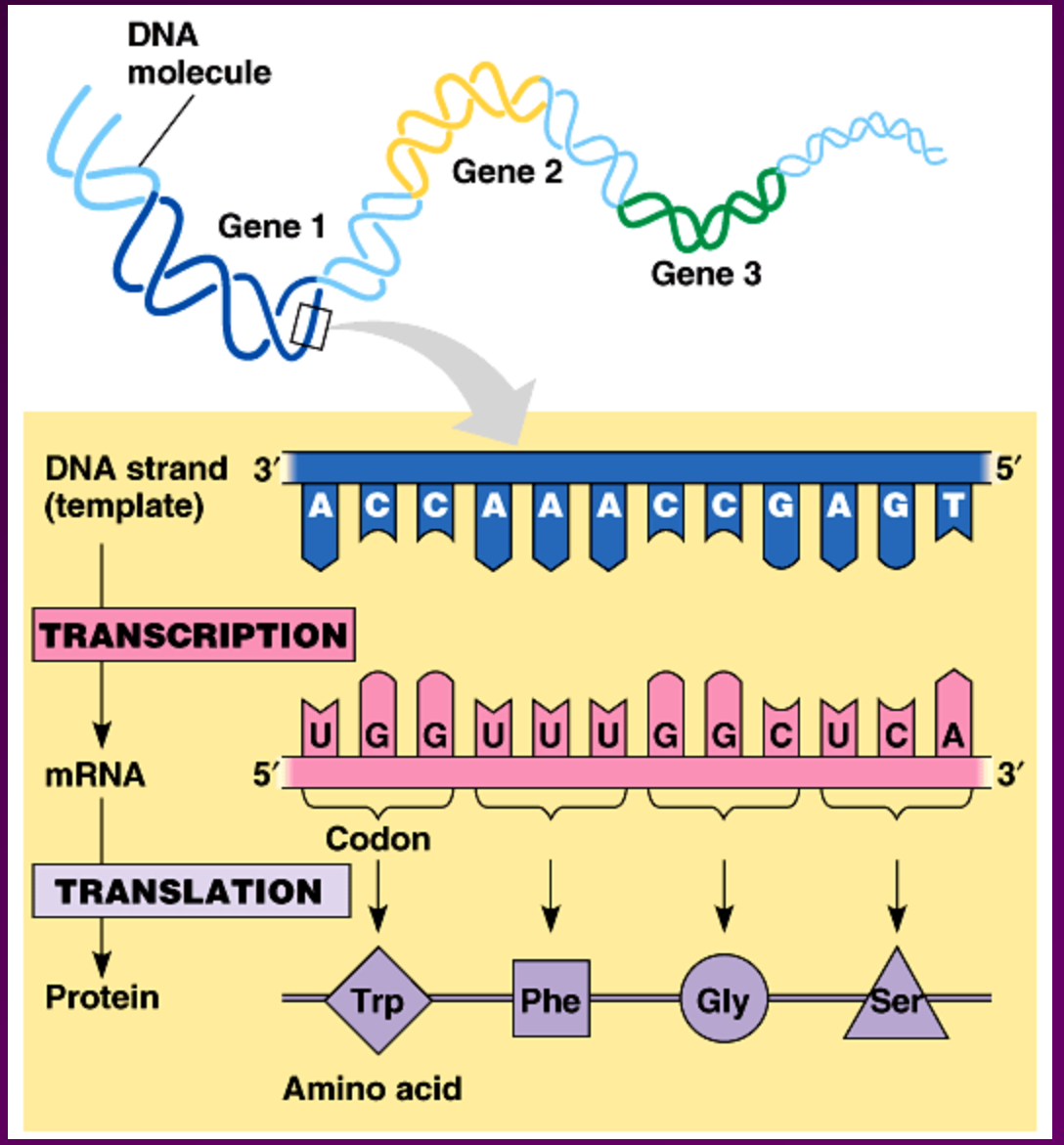
The Dictionary of the Genetic Code



First base (5′ end)	Second base: U	Second base: C	Second base: A	Second base: G	Third base (3′ end)
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 or 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

From Gene to Protein

Overview of Protein Synthesis

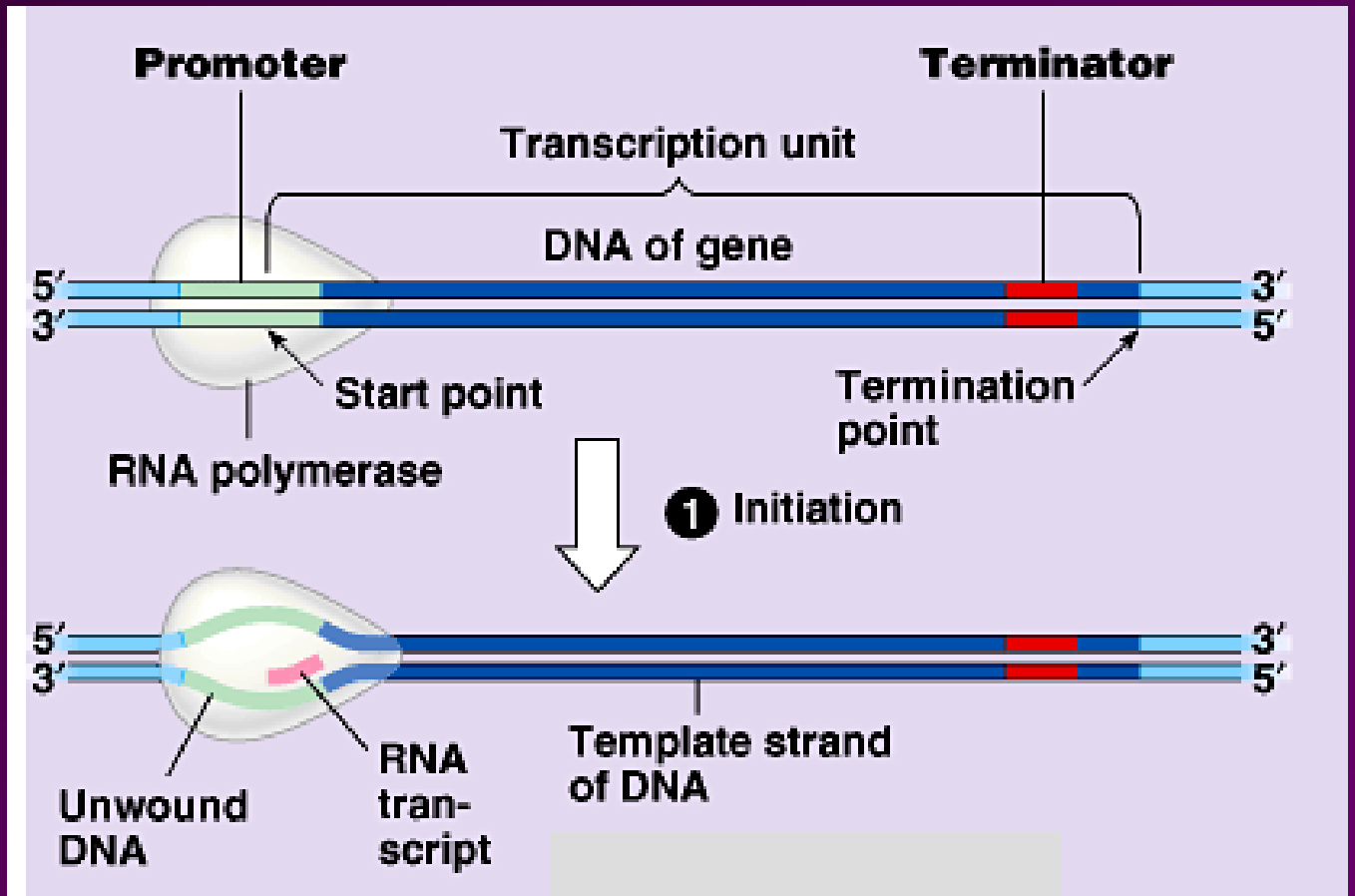


Transcription

- In transcription, mRNA carries the information from DNA to the cell's protein-synthesizing machinery
  - Transcribed from template strand of a gene
- RNA polymerase
  - Enzyme binds to "Promoter Region" – marked by TATA box and start sequence (about 25 base pairs of TATATATAT)
  - Pries DNA strand apart
  - Hooks together RNA nucleotides
  - Add to 3' end only
- Promoter – DNA sequence that initiates transcription
- Terminator – signals end of transcription

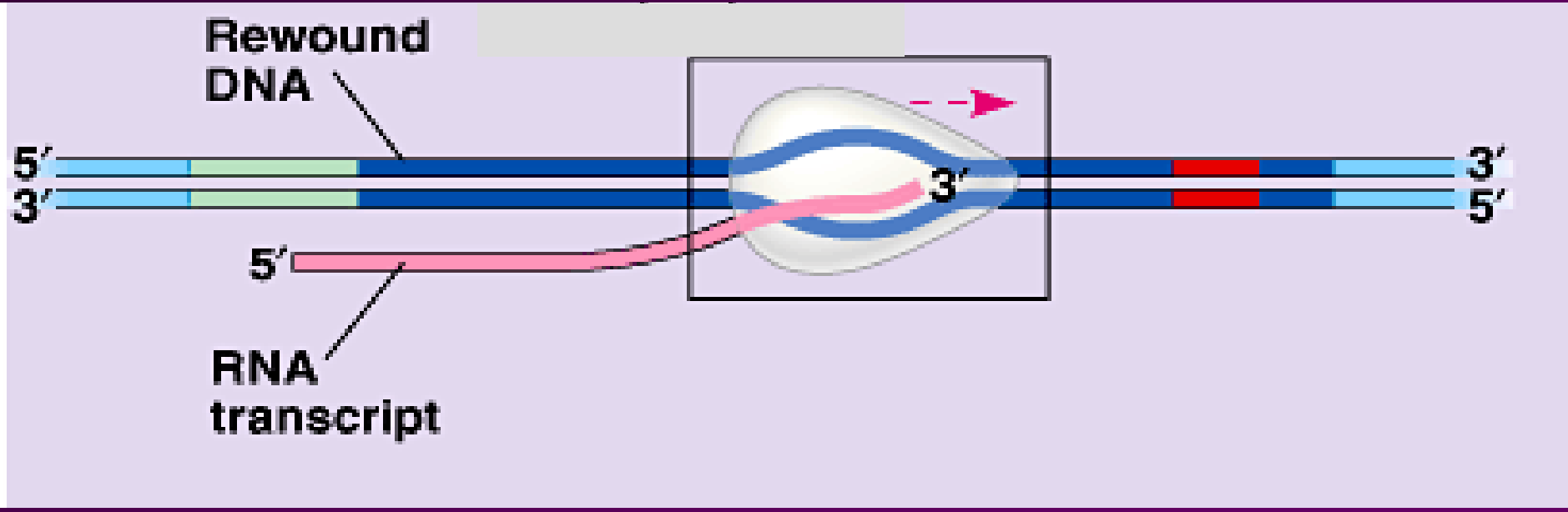
Transcription

1. Initiation – transcription factors (a collection of proteins) mediate binding of RNA polymerase and the initiation of transcription



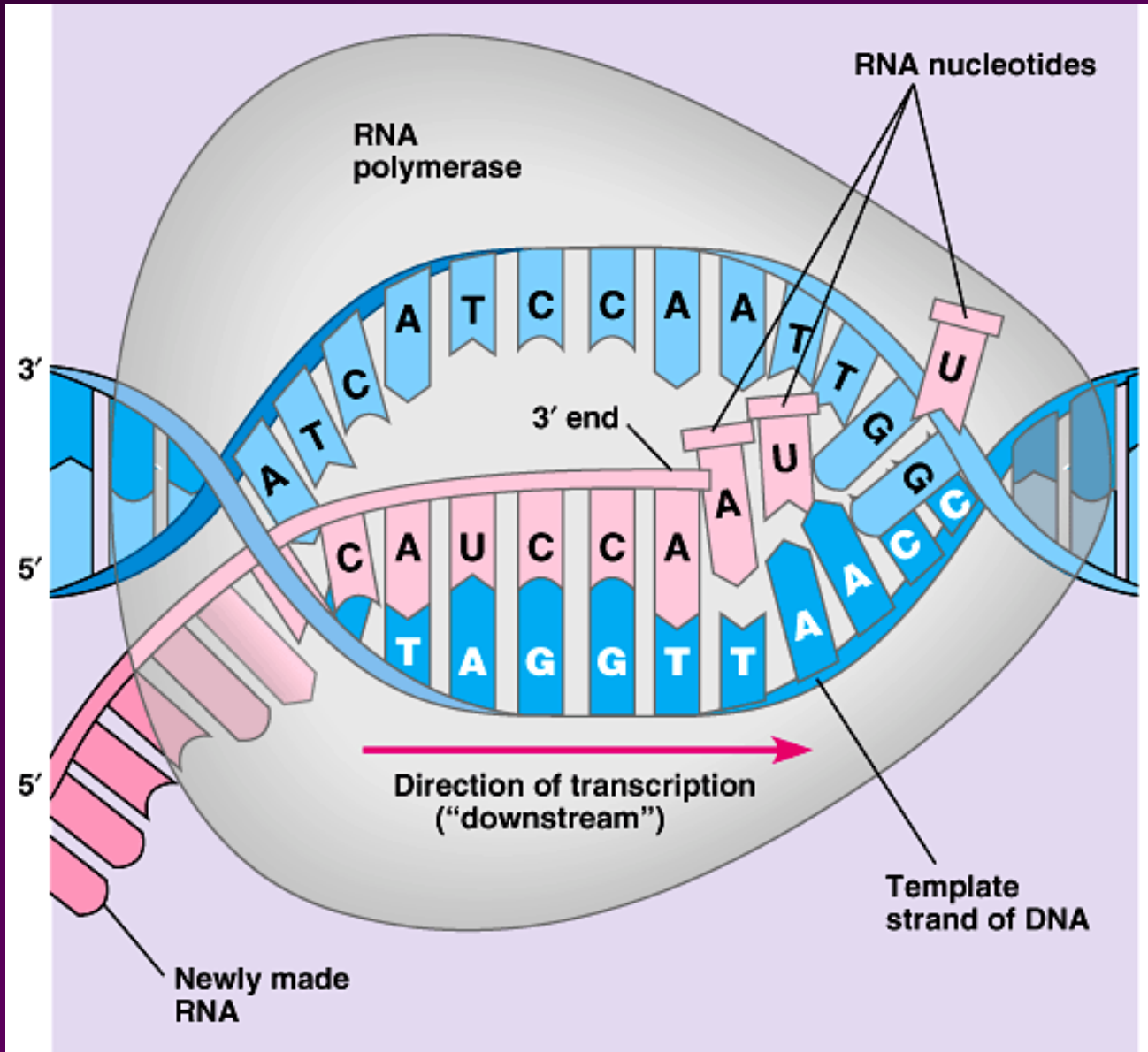
Transcription

2. Elongation – as nucleotides are added to the 3’ end, the RNA molecule pulls away from DNA strand and double helix re-forms



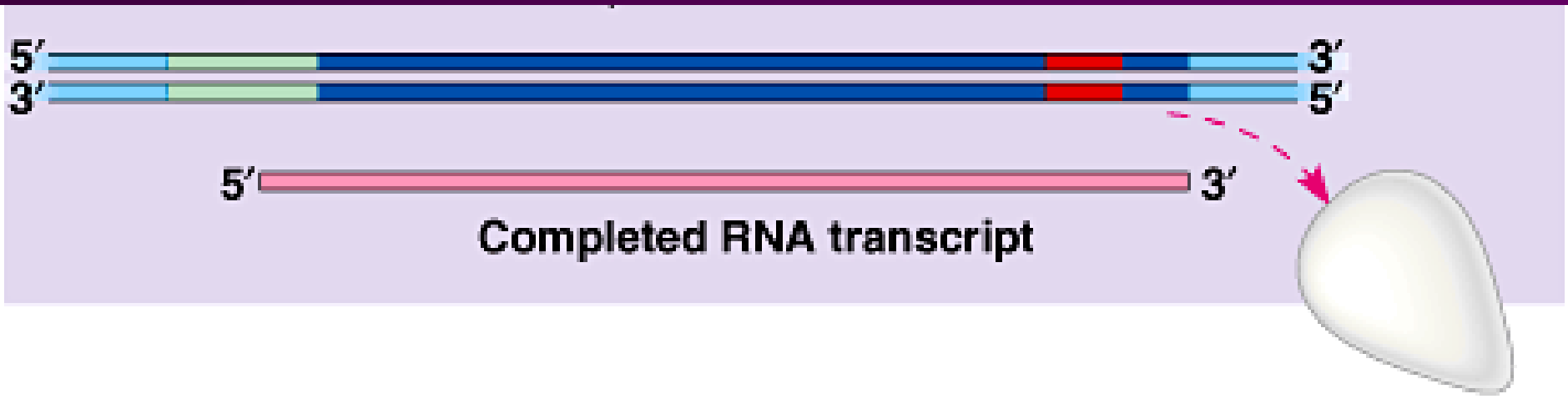
Transcription

2. Elongation



Transcription

3. Termination – termination sequence (AAUAAA) is reached and the RNA transcript is released
    - RNA polymerase detaches from the DNA



Average Transcription

- Average transcription unit = 8000 nucleotides
- Average protein = 400 amino acids

- Most eukaryotic genes contain non-coding sequences (introns) and coding sequences (exons).
- Enzymes remove the introns and join the exons when forming mRNA in a process called “Gene Splicing”

Modifying pre mRNA in Eukaryotes

RNA Processing:

- Attach 5’ GTP cap and a poly-A tail
  - Helps with exporting RNA from nucleus, protects RNA from degradation, helps attach to ribosome

RNA Splicing:

- Introns and exons – introns are removed from pre mRNA
  - snRNPs make up a spliceosome that cuts the introns and removes them

Modifying pre mRNA in Eukaryotes

RNA Splicing:

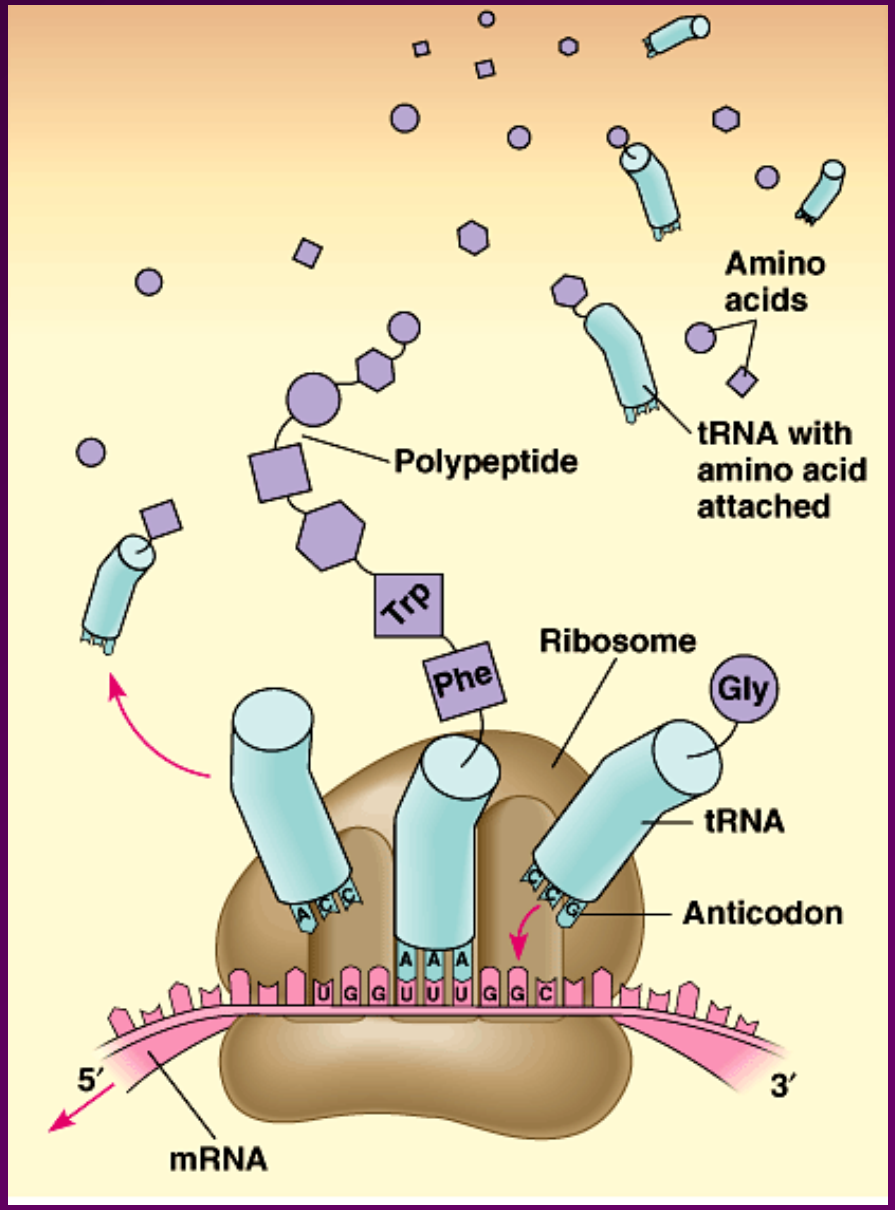
- snRNPs = small nuclear ribonucleoproteins
  - Part of a group of RNA molecules called ribozymes (RNA molecules that act as enzymes)

Alternative RNA Splicing:

- Removal of an exon with an intron to produce different combinations of exons
  - This results in more than one polypeptide per gene

Synthesis of Proteins

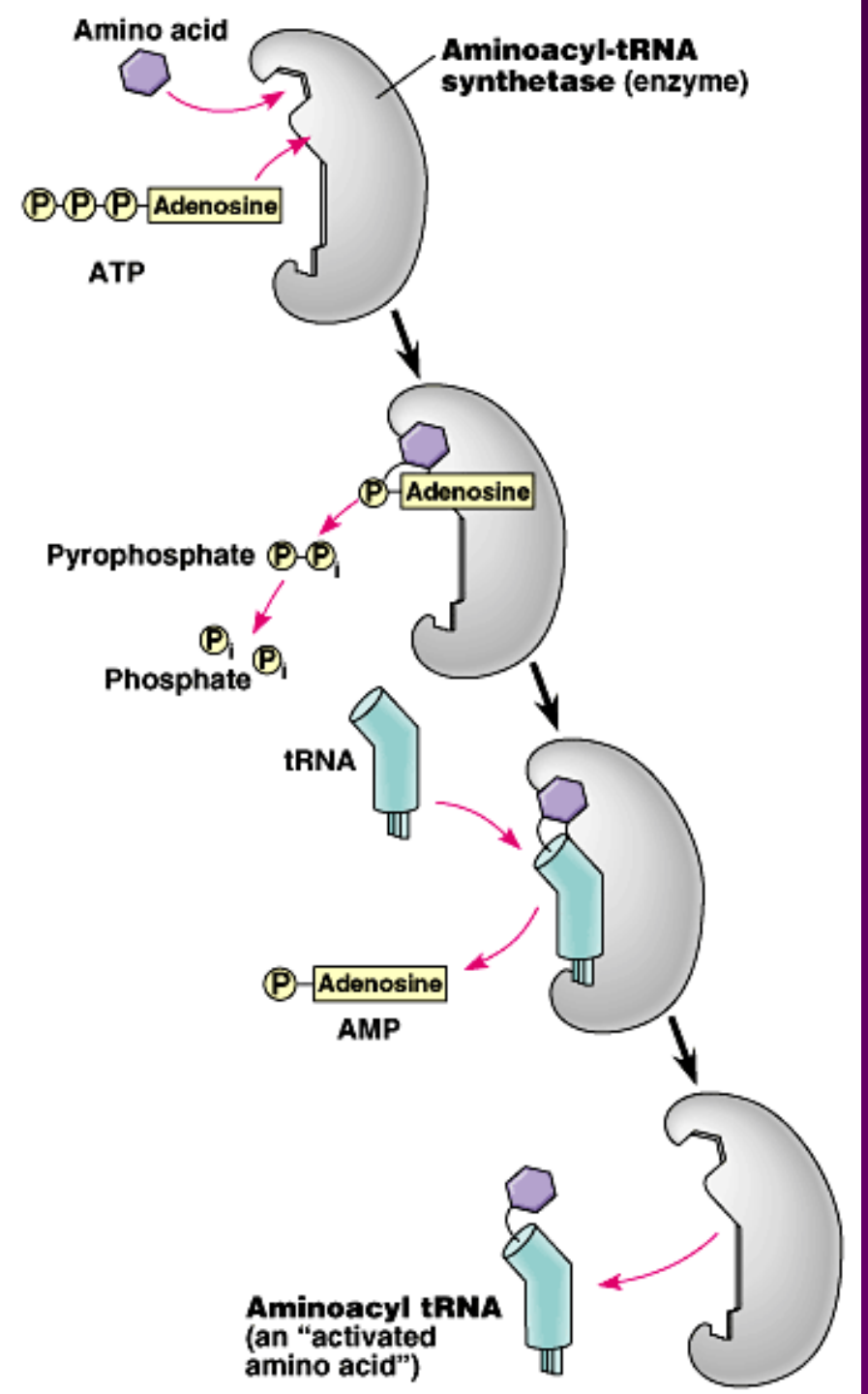
- Translation – a cell interprets a genetic message and builds a protein accordingly
- tRNA (transfer RNA) – transfers amino acids from the cytoplasm's amino acid pool to a ribosome



Translation

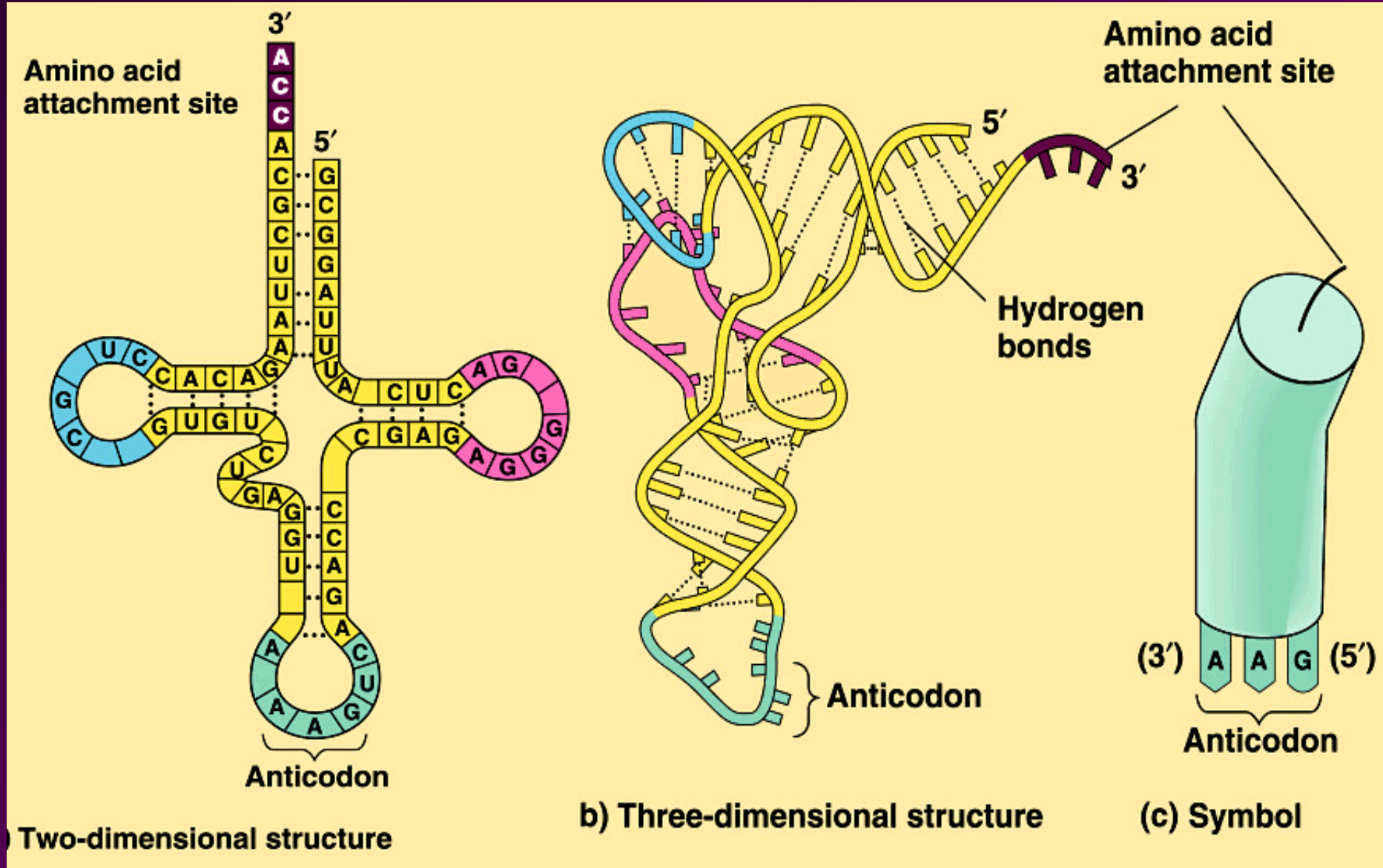
- tRNA consists of a single RNA strand that is about 80 nucleotides long
- 3D structure
- 45 tRNAs – some anticodons can recognize 2 or more different codons
- Aminoacyl-tRNA Synthetase – enzyme the correctly joins tRNA and amino acids
  - Driven by ATP hydrolysis

Translation



Translation

Structure of tRNA



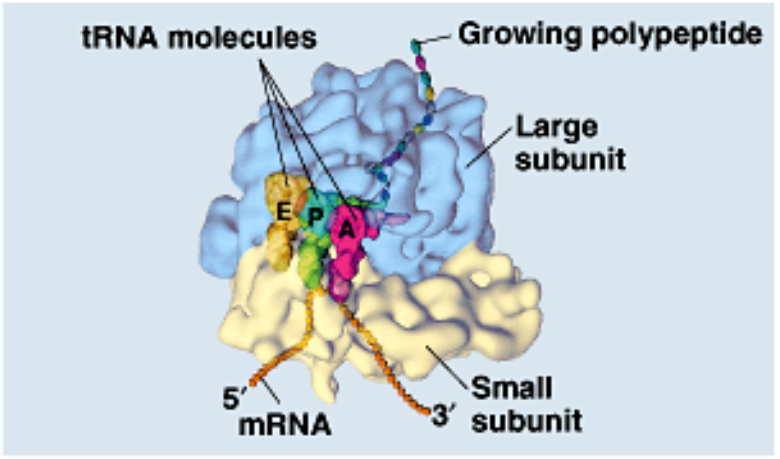
Translation

Ribosomes

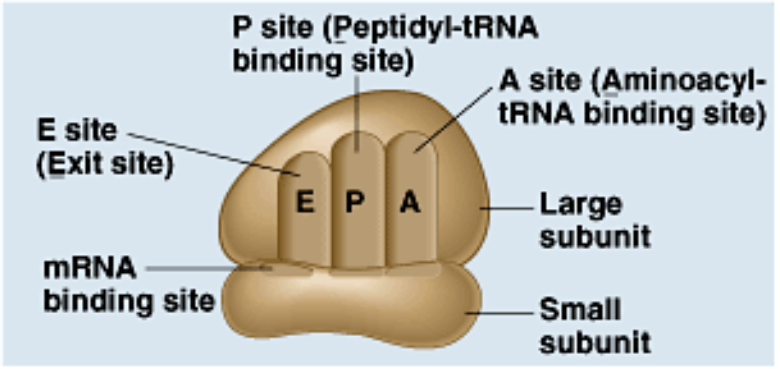
- Large and small subunits made up of proteins and RNA molecules (rRNA)
- 3 binding sites for tRNA
  - P site (peptidyl – tRNA) holds tRNA carrying the growing polypeptide chain
  - A site (aminoacyl – tRNA) holds the tRNA carrying the next amino acid to be added to the chain
  - E site – Exit site

Translation

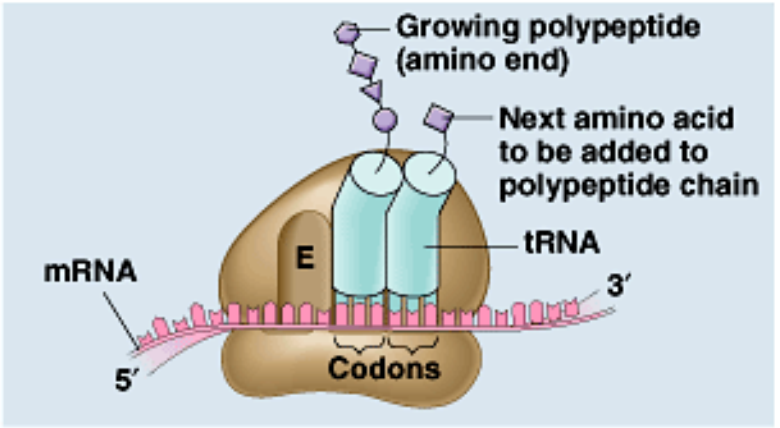
Anatomy of a Functioning Ribosome



(a) Computer model of functioning ribosome



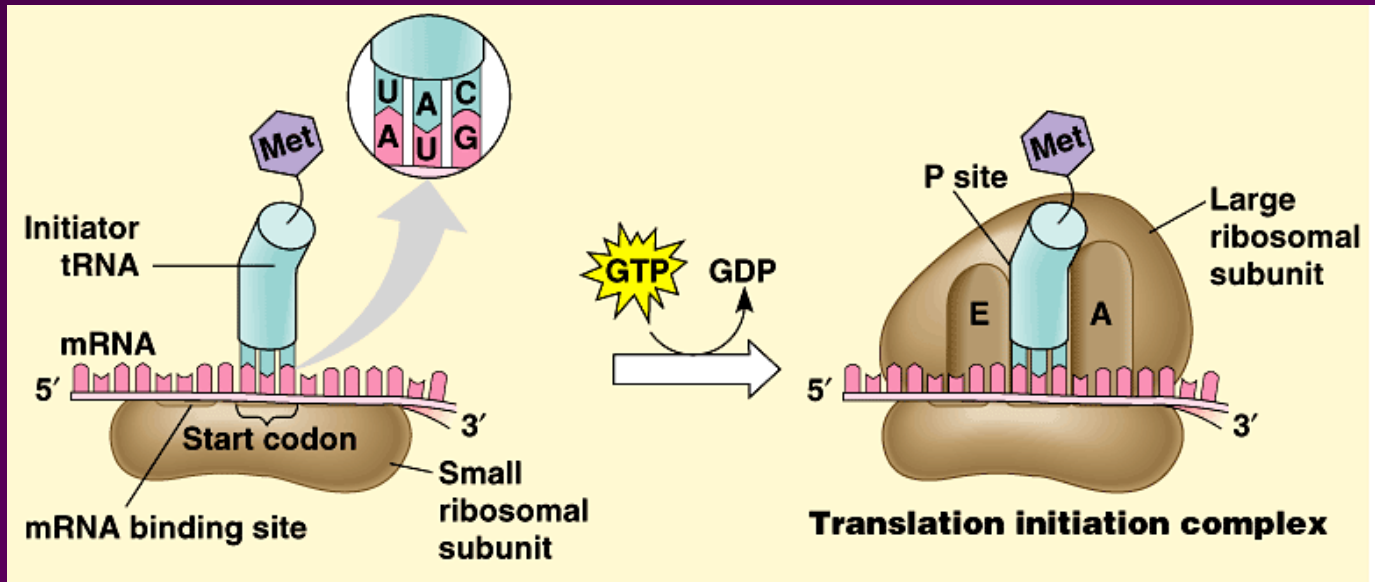
(b) Schematic model showing binding sites



Translation

Building a Polypeptide

1. Initiation
   - Small ribosomal subunits binds to mRNA and tRNA (special initiator) at the 5' end
   - AUG = methionine – this the start codon
   - GTP (guanosine triphosphate) provides the energy



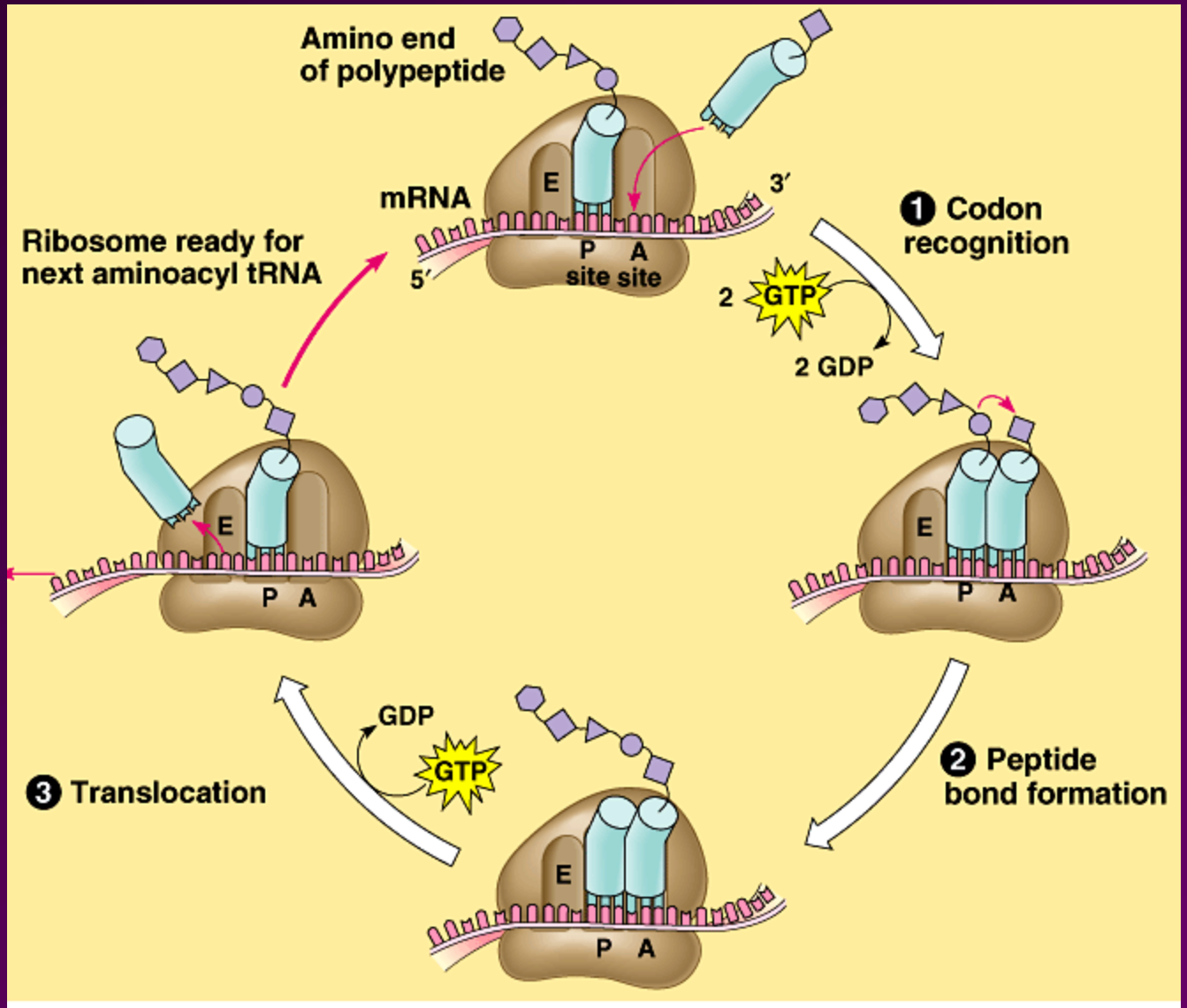
Translation

Building a Polypeptide

2. Elongation
    - Codon recognition (uses 2 GTP) – mRNA codon in A site forms hydrogen bond with anticodon of incoming tRNA
    - Peptide bond formation – rRNA of large subunit catalyses formation of a peptide bond that joins P-A sites amino acids
    - Translocation – (uses 1 GTP) ribosome moves the tRNA in the A site with its polypeptide to the P site

Translation

Building a Polypeptide



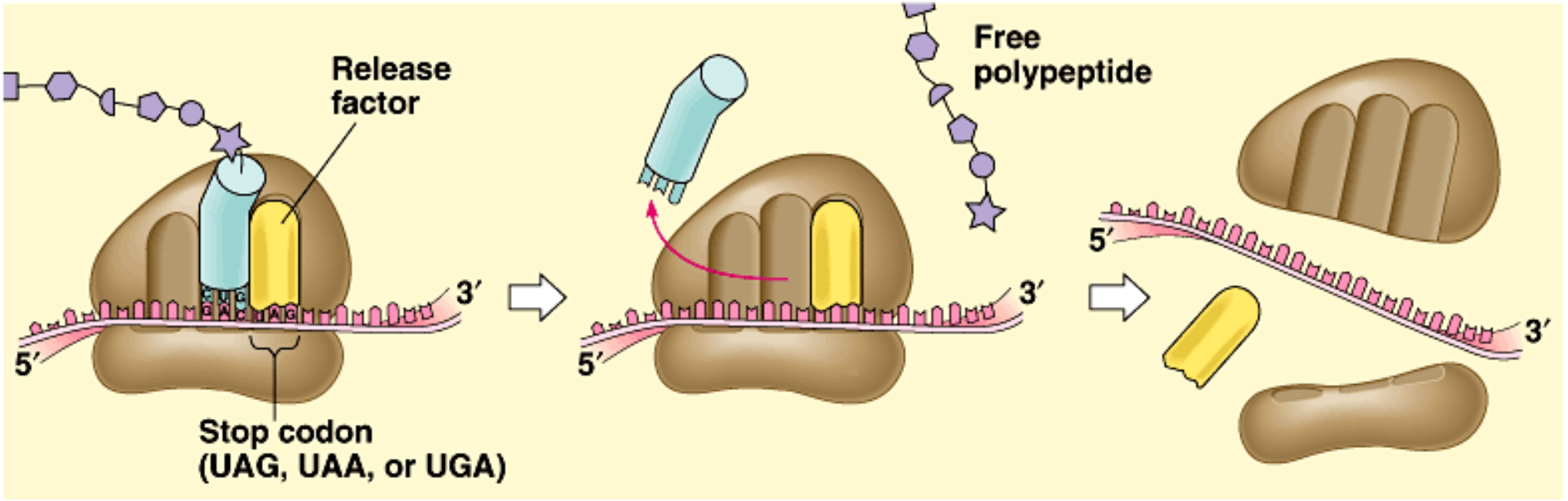
Translation

Building a Polypeptide

3. Termination
    - Stop codons (UAA, UGA, and UAG)
    - Protein called *release factor* binds to the stop codon in the A site
    - Causes addition of water to the polypeptide chain
    - Hydrolyses the completed polypeptide from the tRNA in the P site

Translation

Building a Polypeptide



Translation

From Polypeptide to Functional Protein

- Genes determine a protein's primary structure and primary structure determines conformation
- Amino acids may be chemically modified by the addition of sugars, lipids, or phosphate groups

Protein Synthesis

- http://www.wisc-online.com/objects/index_tj.asp?objid=AP1302

- https://www.youtube.com/watch?v=yqESR7E4b_8
- DNA replication and protein synthesis
- https://www.youtube.com/watch?v=5oyQXR9gJrs

Animations

- Transcription
- http://www.ncc.gmu.edu/dna/mRNAanim.htm

- Translation
- http://www.ncc.gmu.edu/dna/ANIMPROT.htm

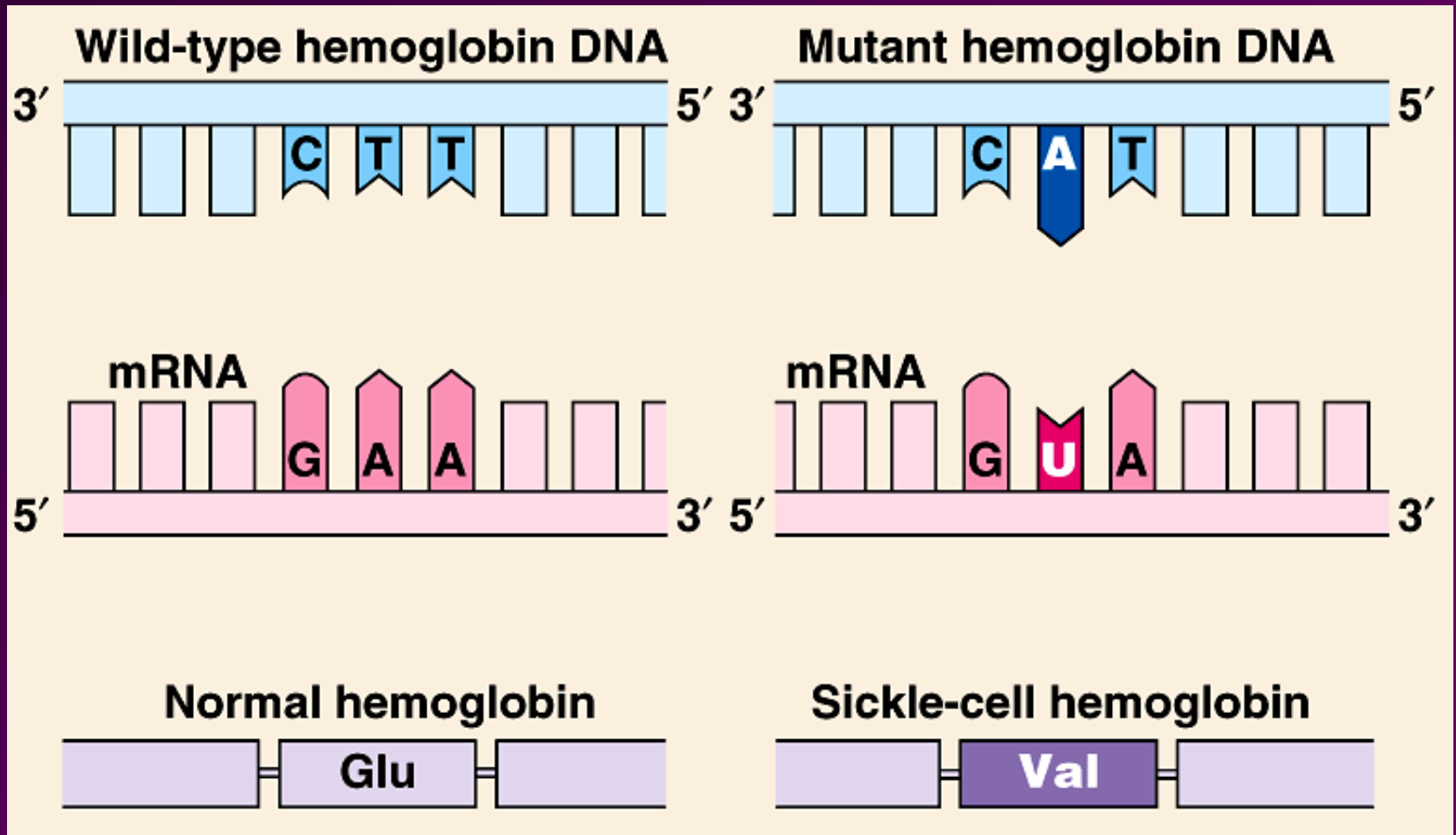
Point Mutations

- Change in one base pair of a gene
- *Mutations* – changes in the genetic material of a cell

1. Base-pair substitution – replacement of one nucleotide and its partner in the complementary DNA strand with another
    - Could be *silent* – no effect
    - Could have little effect – *missense mutation*
    - Could have a significant effect – *nonsense mutation*

Point Mutations

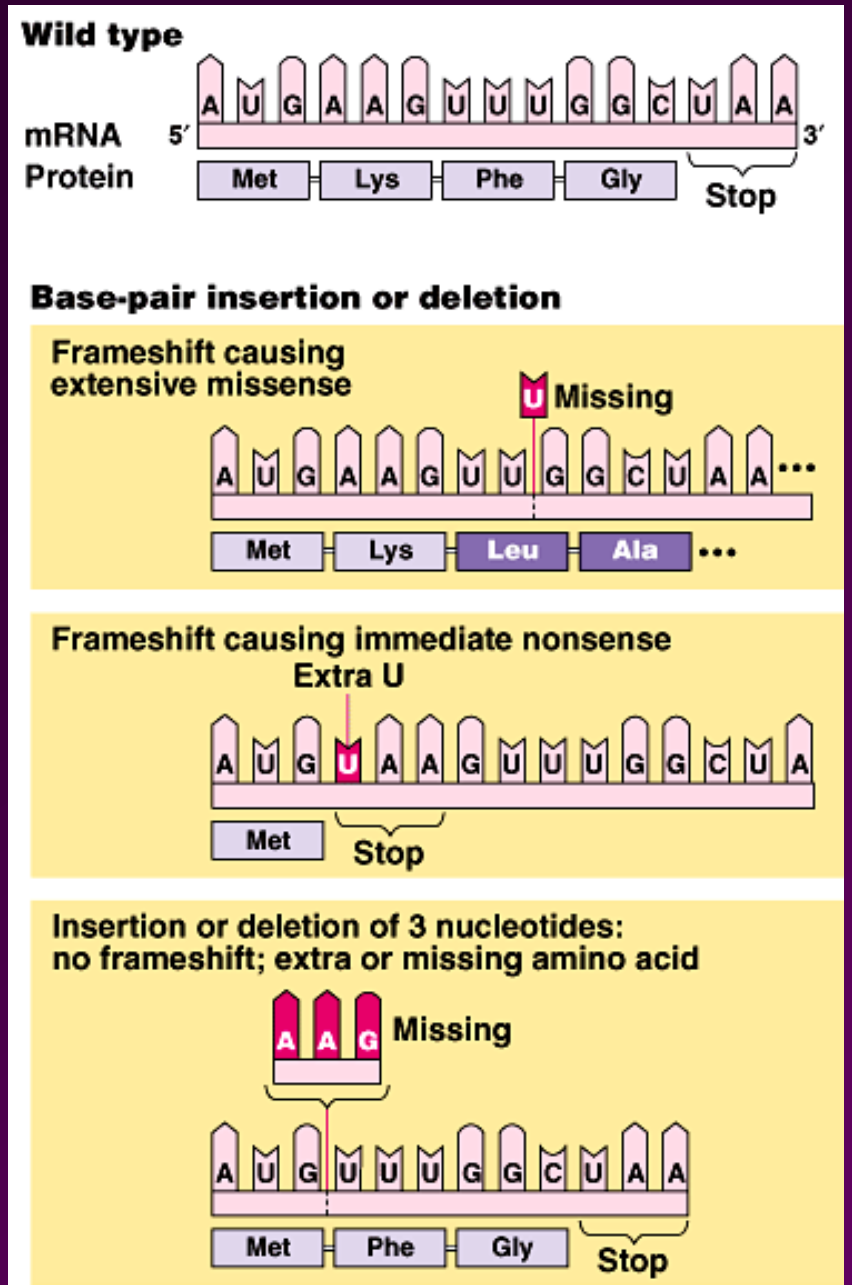
- *Sickle-Cell Disease* – single base pair mutation produces a defective protein that forms hemoglobin

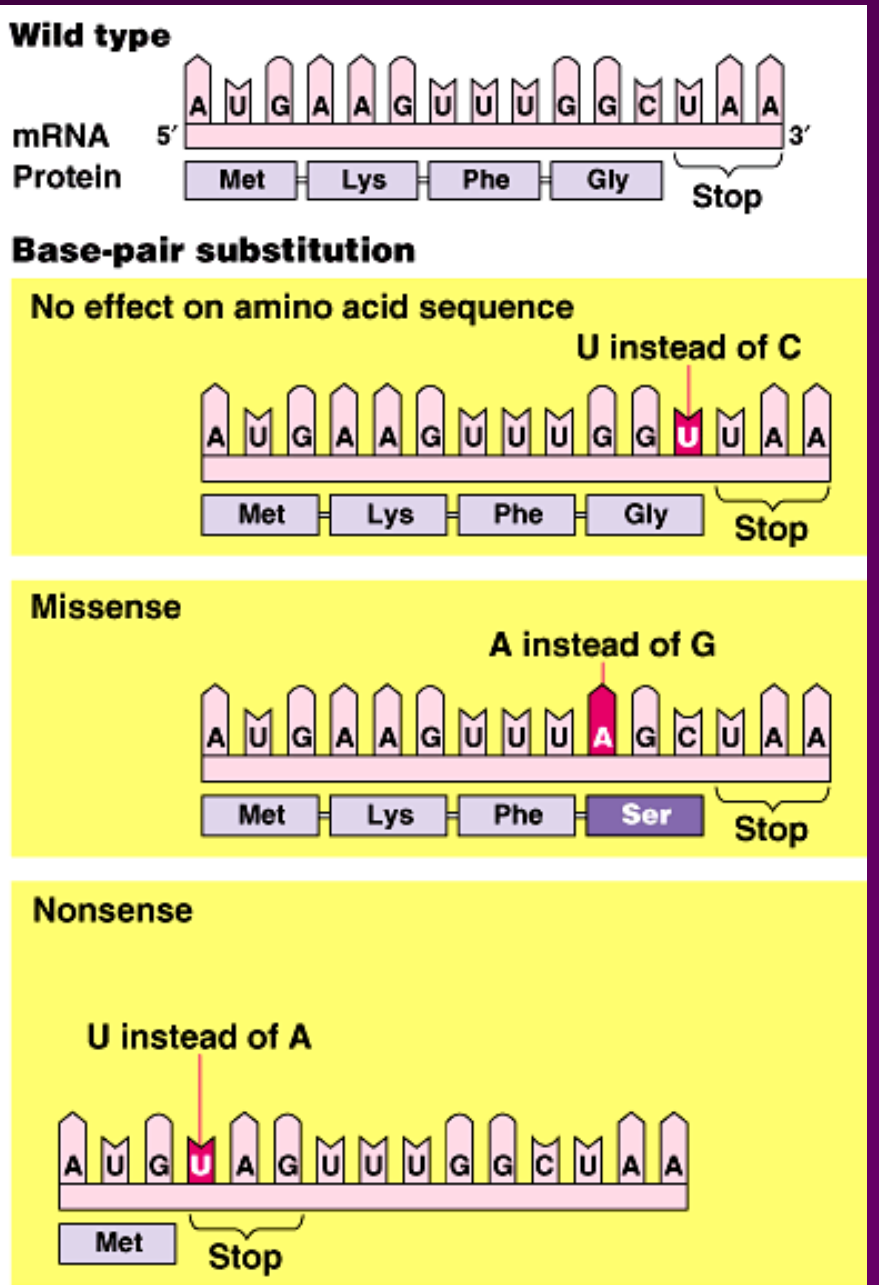


Point Mutations

2. Insertion and Deletion – addition or loss of nucleotide pairs in a gene.
   - May alter the reading frame – *frameshift mutation*

Point Mutations





Mutagens

- Environmental factors that can cause spontaneous errors during DNA replication, repair, or recombination
- Examples include X-rays, UV light, and carcinogens

What are Genes?

- A region of DNA whose final product is either a polypeptide or an RNA molecule

Summary of Transcription and Translation