

# General Biology

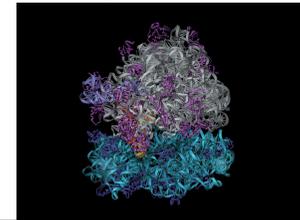
Course No: BNG2003  
Credits: 3.00

## 10. Genetics: From Genes to Proteins

Prof. Dr. Klaus Heese

- **Overview: The Flow of Genetic Information**

- The information content of DNA is in the form of specific sequences of nucleotides along the DNA strands
- The DNA inherited by an organism leads to specific traits by dictating the synthesis of proteins
- The process by which DNA directs protein synthesis, gene expression includes two stages, called transcription and translation
- The ribosome is part of the cellular machinery for translation, polypeptide synthesis



- Genes specify proteins via transcription and translation

### Evidence from the Study of Metabolic Defects

- In 1909, British physician Archibald Garrod was the first to suggest that genes dictate phenotypes through enzymes that catalyze specific chemical reactions in the cell

### Nutritional Mutants in Neurospora: Scientific Inquiry

- Beadle and Tatum causes bread mold to mutate with X-rays creating mutants that could not survive on minimal medium

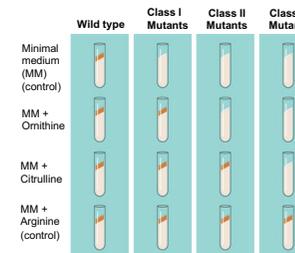
- Using genetic crosses, they determined that their mutants fell into three classes, each mutated in a different gene

#### EXPERIMENT

Working with the mold *Neurospora crassa*, George Beadle and Edward Tatum had isolated mutants requiring arginine in their growth medium and had shown genetically that these mutants fell into three classes, each defective in a different gene. From other considerations, they suspected that the metabolic pathway of arginine biosynthesis included the precursors ornithine and citrulline. Their most famous experiment, shown here, tested both their one gene-one enzyme hypothesis and their postulated arginine pathway. In this experiment, they grew their three classes of mutants under the four different conditions shown in the Results section below.

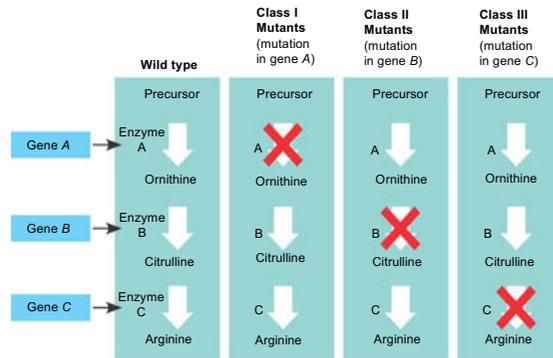
#### RESULTS

The wild-type strain required only the minimal medium for growth. The three classes of mutants had different growth requirements



**CONCLUSION**

From the growth patterns of the mutants, Beadle and Tatum deduced that each mutant was unable to carry out one step in the pathway for synthesizing arginine, presumably because it lacked the necessary enzyme. Because each of their mutants was mutated in a single gene, they concluded that each mutated gene must normally dictate the production of one enzyme. Their results supported the one gene–one enzyme hypothesis and also confirmed the arginine pathway. (Notice that a mutant can grow only if supplied with a compound made *after* the defective step.)



- Beadle and Tatum developed the “one gene–one enzyme hypothesis” - which states that the **function of a gene is to dictate the production of a specific enzyme**

*The Products of Gene Expression: A Developing Story*

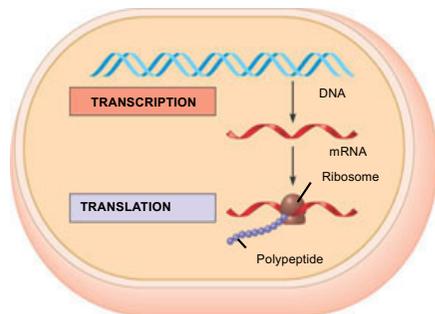
- As researchers learned more about proteins - they made minor revision to the one gene–one enzyme hypothesis
- genes code for polypeptide chains or for RNA molecules

*Basic Principles of Transcription and Translation*

- **Transcription** is the synthesis of RNA under the direction of DNA - producing messenger RNA (mRNA)
- **Translation** is the actual synthesis of a polypeptide, which occurs under the direction of mRNA – it occurs on ribosomes

- In **prokaryotes**

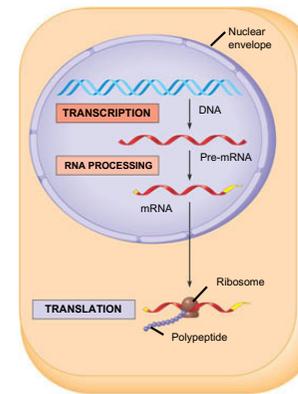
– transcription and translation occur together



**Prokaryotic cell.** In a cell lacking a nucleus, mRNA produced by transcription is immediately translated without additional processing.

- In **eukaryotes**

– RNA transcripts are modified before becoming true mRNA



**Eukaryotic cell.** The nucleus provides a separate compartment for transcription. The original RNA transcript, called pre-mRNA, is processed in various ways before leaving the nucleus as mRNA.

- Cells are governed by a cellular chain of commands

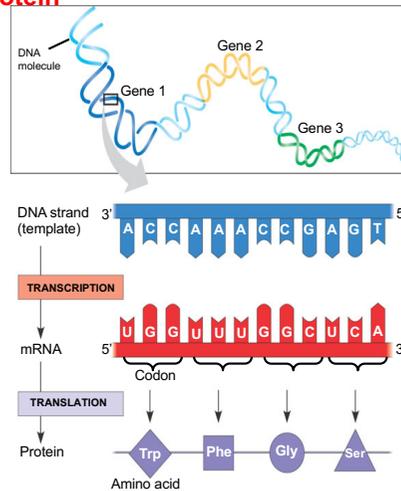
– DNA → RNA → protein

### The Genetic Code

- Genetic information is encoded as a sequence of **non-overlapping base triplets**, or codons

**Codons: Triplets of Bases**

- How many bases correspond to an amino acid?
- During transcription the gene determines the sequence of bases along the length of an mRNA molecule



### Cracking the Code

64 options

- A codon in messenger RNA is either translated into an amino acid or serves as a translational stop signal
- Codons must be read in the correct **reading frame** for the specified polypeptide to be produced
- The **genetic code is nearly universal** shared by organisms from the simplest bacteria to the most complex animals

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

- In laboratory experiments genes can be transcribed and translated after being transplanted from one species to another
- Transcription is the DNA-directed synthesis of RNA: *a closer look*

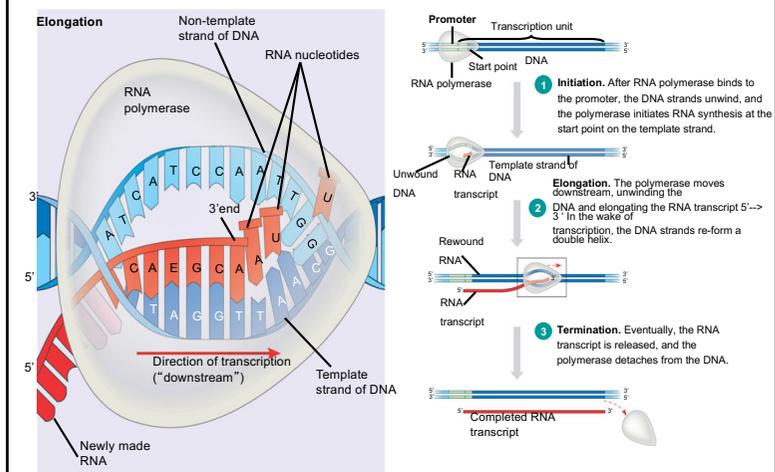
### Molecular Components of Transcription

- RNA synthesis
  - is catalyzed by RNA polymerase, which pries the DNA strands apart and hooks together the RNA nucleotides
  - follows the same base-pairing rules as DNA, except that **in RNA, uracil substitutes for thymine**



### Synthesis of an RNA Transcript

- The stages of transcription are: **Initiation, Elongation, Termination**



### RNA Polymerase Binding and Initiation of Transcription

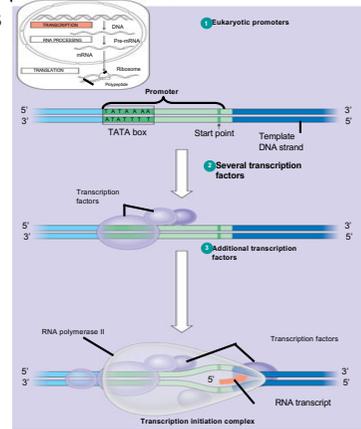
- **Promoters** signal the initiation of RNA synthesis
- **Transcription factors** help eukaryotic RNA polymerase recognize promoter sequences

### Elongation of the RNA Strand

- As RNA polymerase moves along the DNA, it continues to untwist the double helix, exposing about 10 to 20 DNA bases at a time for pairing with RNA nucleotides

### Termination of Transcription

- The mechanisms of termination are different in prokaryotes and eukaryotes

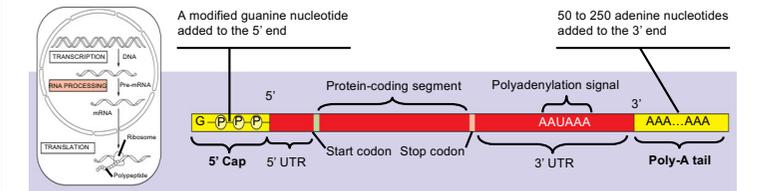


- **Eukaryotic cells modify RNA after transcription**

- **Enzymes in the eukaryotic nucleus modify pre-mRNA** in specific ways before the genetic messages are dispatched to the cytoplasm

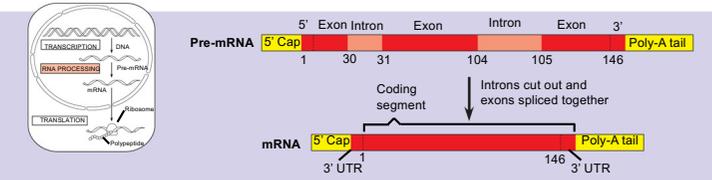
### Alteration of mRNA Ends

- Each end of a pre-mRNA molecule is modified in a particular way
  - the 5' end receives a modified nucleotide cap
  - the 3' end gets a poly-A tail



### Split Genes and RNA Splicing

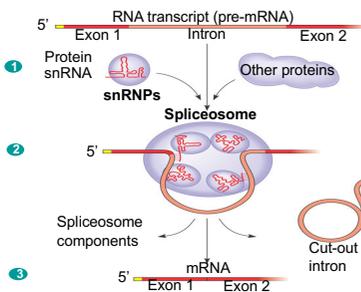
- RNA splicing removes introns and joins exons



- Is carried out by spliceosomes in some cases

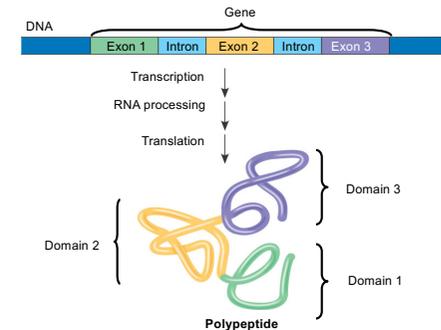
### Ribozymes

- Ribozymes are catalytic RNA molecules that function as enzymes and can splice RNA



### The Functional and Evolutionary Importance of Introns

- The presence of introns allows for **alternative RNA splicing**
- Proteins often have a modular architecture consisting of discrete structural and functional regions called domains
- In many cases different exons code for the different domains in a protein

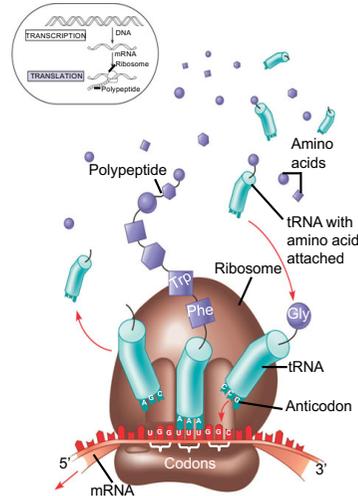


- **Translation: the basic concept**

- Translation is the RNA-directed synthesis of a polypeptide: *a closer look*

**Molecular Components of Translation**

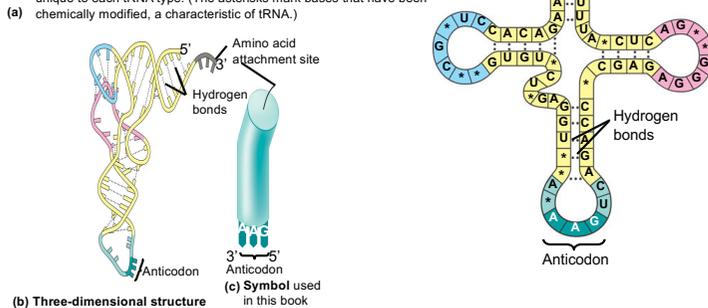
- A cell translates an mRNA message into protein with the help of **transfer RNA (tRNA)**
- Molecules of tRNA are not all identical
  - each carries a **specific amino acid** on one end
  - each has an **anticodon** on the other end



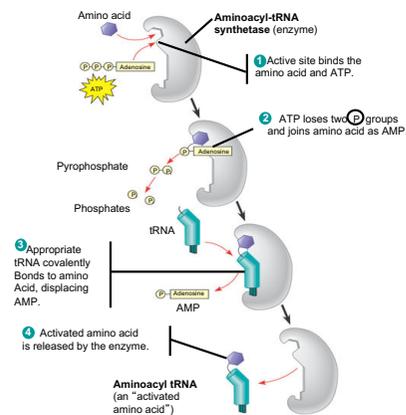
**The Structure and Function of Transfer RNA**

- A tRNA molecule
  - consists of a single RNA strand that is only about 80 nucleotides long
  - is roughly L-shaped

**Two-dimensional structure.** The four base-paired regions and three loops are characteristic of all tRNAs, as is the base sequence of the amino acid attachment site at the 3' end. The anticodon triplet is unique to each tRNA type. (The asterisks mark bases that have been chemically modified, a characteristic of tRNA.)

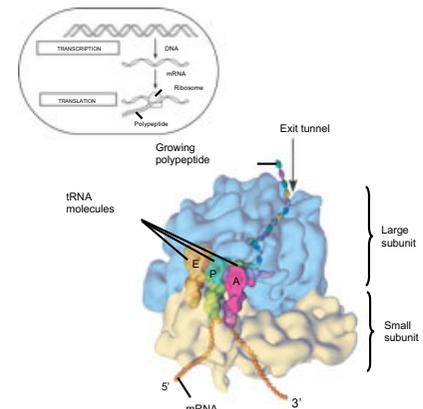


- A specific enzyme called an aminoacyl-tRNA synthetase
  - joins each amino acid to the correct tRNA



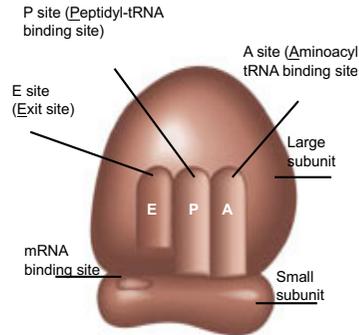
- **Ribosomes** facilitate the specific coupling of tRNA anticodons with mRNA codons during protein synthesis

- The ribosomal subunits are constructed of proteins and RNA molecules named ribosomal RNA or rRNA

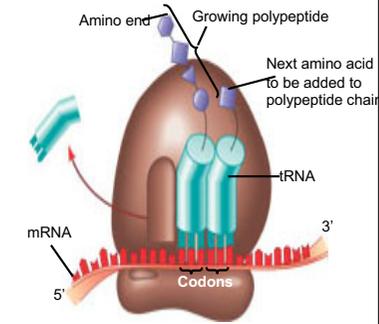


**Computer model of functioning ribosome.** This is a model of a bacterial ribosome, showing its overall shape. The eukaryotic ribosome is roughly similar. A ribosomal subunit is an aggregate of ribosomal RNA molecules and proteins.

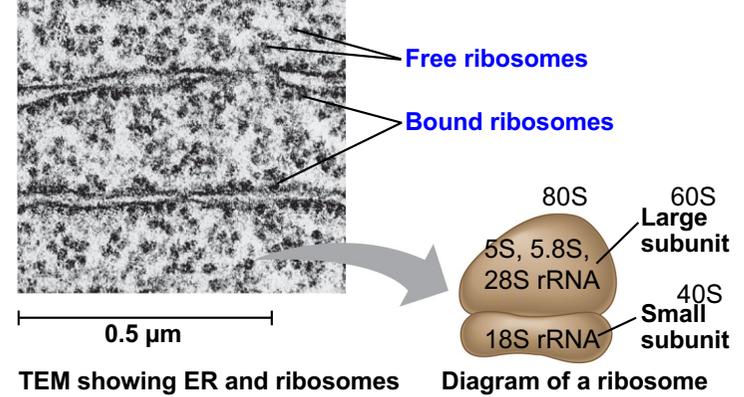
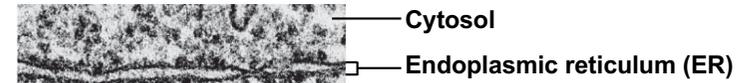
- The ribosome has three binding sites for tRNA: the P site, the A site and the E site



**Schematic model showing binding sites.** A ribosome has an mRNA binding site and three tRNA binding sites, known as the A, P, and E sites. This schematic ribosome will appear in later diagrams.



**Schematic model with mRNA and tRNA.** A tRNA fits into a binding site when its anticodon base-pairs with an mRNA codon. The P site holds the tRNA attached to the growing polypeptide. The A site holds the tRNA carrying the next amino acid to be added to the polypeptide chain. Discharged tRNA leaves via the E site.



TEM showing ER and ribosomes

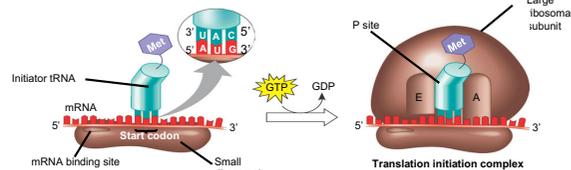
Diagram of a ribosome

## Building a Polypeptide

- We can divide translation into three stages: Initiation, Elongation and Termination

### Ribosome Association and Initiation of Translation

- The initiation stage of translation brings together mRNA, tRNA bearing the first amino acid of the polypeptide, and two subunits of a ribosome

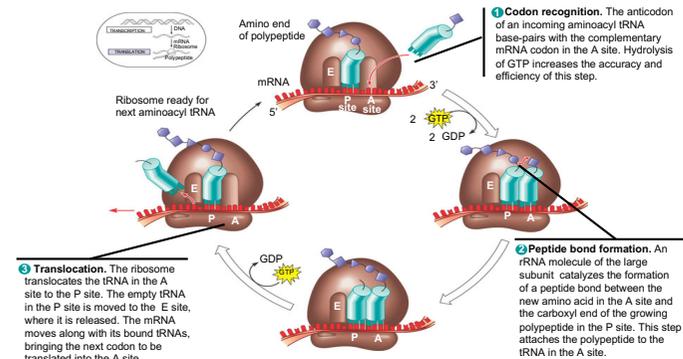


1 A small ribosomal subunit binds to a molecule of mRNA. In a prokaryotic cell, the mRNA binding site on this subunit recognizes a specific nucleotide sequence on the mRNA just upstream of the start codon. An initiator tRNA, with the anticodon UAC, base-pairs with the start codon, AUG. This tRNA carries the amino acid methionine (Met).

2 The arrival of a large ribosomal subunit completes the initiation complex. Proteins called initiation factors (not shown) are required to bring all the translation components together. GTP provides the energy for the assembly. The initiator tRNA is in the P site, the A site is available to the tRNA bearing the next amino acid.

## Elongation of the Polypeptide Chain

- In the elongation stage of translation amino acids are added one by one to the preceding amino acid



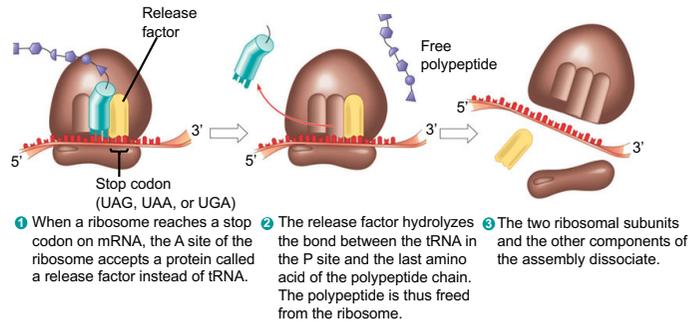
1 **Codon recognition.** The anticodon of an incoming aminoacyl tRNA base-pairs with the complementary mRNA codon in the A site. Hydrolysis of GTP increases the accuracy and efficiency of this step.

2 **Peptide bond formation.** An rRNA molecule of the large subunit catalyzes the formation of a peptide bond between the new amino acid in the A site and the carboxyl end of the growing polypeptide in the P site. This step attaches the polypeptide to the tRNA in the A site.

3 **Translocation.** The ribosome translocates the tRNA in the A site to the P site. The empty tRNA in the P site is moved to the E site, where it is released. The mRNA moves along with its bound tRNAs, bringing the next codon to be translated into the A site.

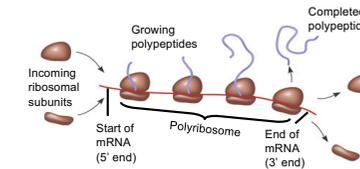
## Termination of Translation

- The final stage of translation is termination
  - when the ribosome reaches a stop codon in the mRNA

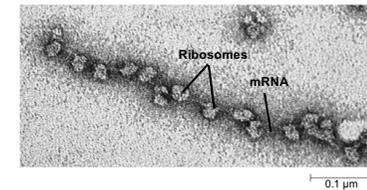


## Polyribosomes

- A number of ribosomes can translate a single mRNA molecule simultaneously - forming a polyribosome



(a) An mRNA molecule is generally translated simultaneously by several ribosomes in clusters called polyribosomes.



(b) This micrograph shows a large polyribosome in a prokaryotic cell (TEM).

## Completing and Targeting the Functional Protein

- Polypeptide chains undergo modifications after the translation process

### Protein Folding and Post-Translational Modifications

- After translation proteins may be modified (e.g. glycosylation) in ways that affect their three-dimensional shape (so called chaperone and co-chaperone proteins involved in folding (e.g. HSPs and FKBP)).

### Targeting Polypeptides to Specific Locations

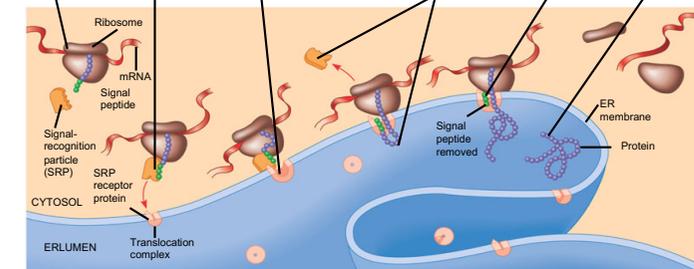
- Two populations of ribosomes are evident in cells - free and bound ribosomes (rough ER).
- Free ribosomes in the cytosol initiate the synthesis of all proteins

## Folding, Post-Translational modifications and Targeting

- Proteins destined for the endomembrane system or for secretion: a) must be transported into the ER; b) have signal peptides to which a signal-recognition particle (SRP) binds, enabling the translation ribosome to bind to the ER

- The signal mechanism for targeting proteins to the ER

- Polypeptide synthesis begins on a free ribosome in the cytosol.
- An SRP binds to the signal peptide, halting synthesis momentarily.
- The SRP binds to a receptor protein in the ER membrane. This receptor is part of a protein complex (a translocation complex) that has a membrane pore and a signal-cleaving enzyme.
- The SRP leaves, and the polypeptide resumes growing, meanwhile translocating across the membrane. (The signal peptide stays attached to the membrane.)
- The signal-cleaving enzyme cuts off the signal peptide.
- The rest of the completed polypeptide leaves the ribosome and folds into its final conformation.



## RNA plays multiple roles in the cell: a review

### RNA

- can hydrogen-bond to other nucleic acid molecules
- can assume a specific three-dimensional shape
- has functional groups that allow it to act as a catalyst

### Types of RNA in a Eukaryotic Cell

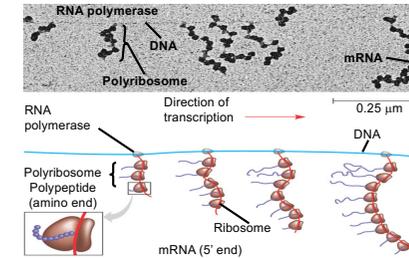
Type of RNA	Functions
Messenger RNA (mRNA)	Carries information specifying amino acid sequences of proteins from DNA to ribosomes.
Transfer RNA (tRNA)	Serves as adapter molecule in protein synthesis; translates mRNA codons into amino acids.
Ribosomal RNA (rRNA)	Plays catalytic (ribozyme) roles and structural roles in ribosomes.
Primary transcript	Serves as a precursor to mRNA, rRNA, or tRNA, before being processed by splicing or cleavage. 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.
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.
Small nucleolar RNA (snoRNA)	Aids in processing of pre-rRNA transcripts for ribosome subunit formation in the nucleolus.
Small interfering RNA (siRNA) and microRNA (miRNA)	Are involved in regulation of gene expression.

ncRNA = non-coding RNA

- Comparing gene expression in prokaryotes and eukaryotes reveals key differences

- Prokaryotic cells lack a nuclear envelope

- allowing translation to begin while transcription is still in progress



- In a eukaryotic cell the nuclear envelope separates transcription from translation; extensive RNA processing occurs in the nucleus

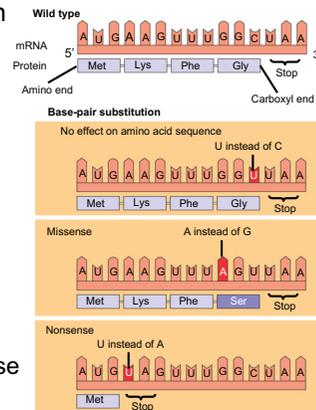
## Types of Point Mutations

- Point mutations within a gene can be divided into two general categories

- base-pair substitutions
- base-pair insertions or deletions

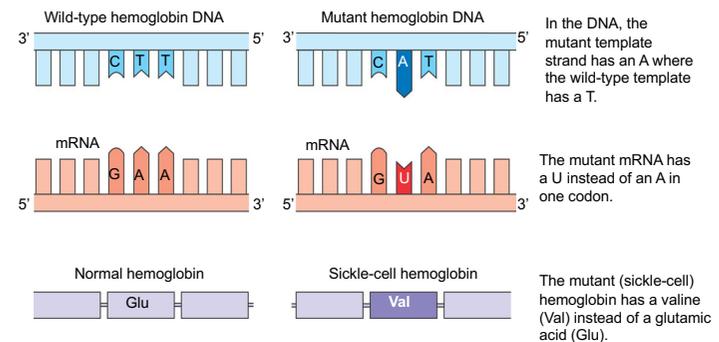
- A base-pair **substitution**

- is the replacement of one nucleotide and its partner with another pair of nucleotides
- can cause missense or nonsense



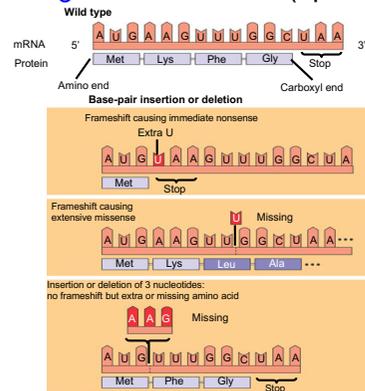
- Point mutations can affect protein structure and function

- Mutations are changes in the genetic material of a cell
- Point mutations are changes in just one base pair of a gene
- The change of a single nucleotide in the DNA's template strand leads to the production of an abnormal protein



## Insertions and Deletions

- Insertions and deletions are additions or losses of nucleotide pairs in a gene and may produce frameshift mutations (**change of natural ORF** (open reading frame))

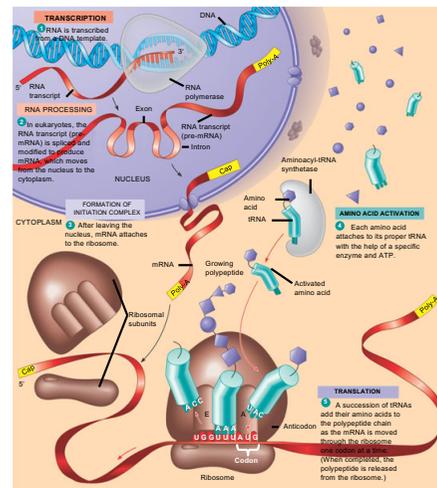


## Mutagens

- Spontaneous mutations
  - can occur during DNA replication, recombination, or repair
- Mutagens are physical or chemical agents that can cause mutations

## What is a gene? revisiting the question

- A gene is a region of DNA whose final product is either a polypeptide or an RNA molecule
- A summary of transcription and translation in a eukaryotic cell



## Overview of four basic molecular genetic processes

