Lecture Series 7

From DNA to Protein: Genotype to Phenotype

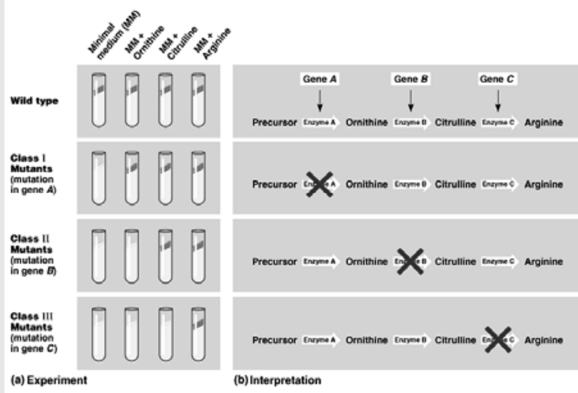
Reading Assignments

- Read Chapter 7
  From DNA to Protein

A. Genes and the Synthesis of Polypeptides

- Genes are made up of DNA and are expressed in the phenotype as polypeptides.
- Beadle and Tatum's experiments with the bread mold *Neurospora* resulted in mutant strains lacking a specific enzyme in a biochemical pathway. These results led to the one-gene, one-polypeptide hypothesis.

Beadle and Tatum's evidence for the one gene-one enzyme hypothesis

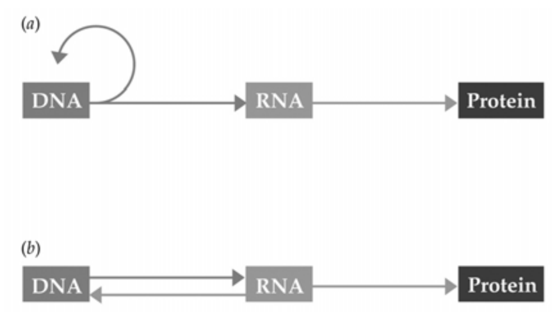


A. Genes and the Synthesis of Polypeptides

- Certain hereditary diseases in humans have been found to be caused by a defective enzyme.
- These observations supported the one-gene, one-polypeptide hypothesis.

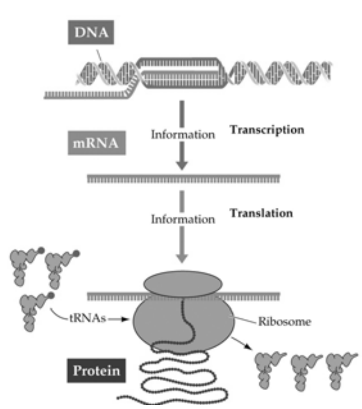
B. DNA, RNA, and the Flow of Information

- RNA differs from DNA in three ways: It is single-stranded, its sugar molecule is ribose rather than deoxyribose, and its fourth base is uracil rather than thymine.
- The **central dogma** of molecular biology is DNA → RNA → protein. Unidirectional when genes are expressed.

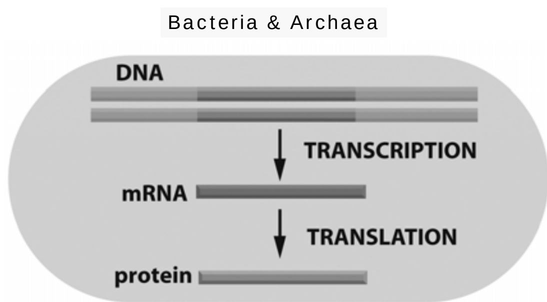


B. DNA, RNA, and the Flow of Information

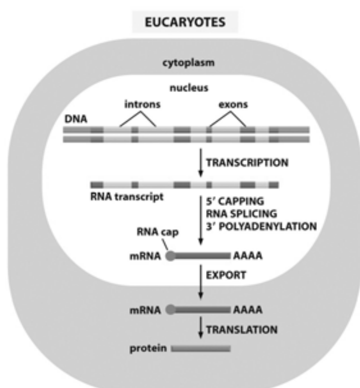
- A gene is expressed in two steps: First, DNA is transcribed to RNA; then RNA is translated into protein.
- In retroviruses, the rule for transcription is reversed: RNA → DNA. Other RNA viruses exclude DNA altogether, going directly from RNA to protein.



Overview: the roles of transcription and translation in the flow of genetic information



Overview: the roles of transcription and translation in the flow of genetic information

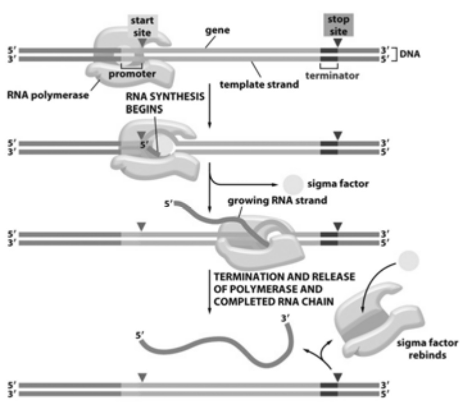


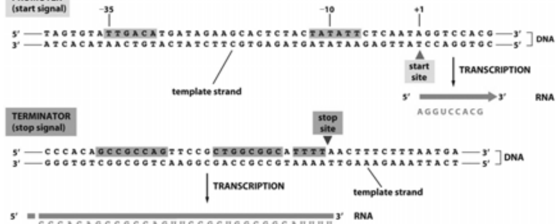
C. Transcription: DNA-Directed RNA Synthesis

- RNA is transcribed from a DNA template after the bases of DNA are exposed by unwinding of the double helix.
- In a given region of DNA, only one of the two strands can act as a template for transcription.
- RNA polymerase catalyzes transcription from the template strand of DNA.

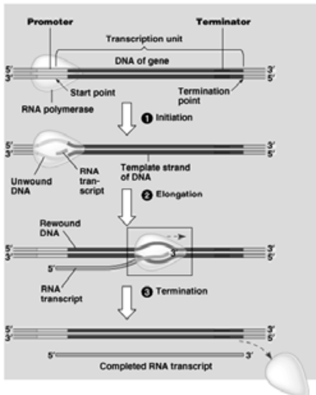
C. Transcription: DNA-Directed RNA Synthesis

- Three step process: Initiation, Elongation and Termination.
- The initiation of transcription requires that RNA polymerase recognize and bind tightly to a promoter sequence on DNA.
- RNA elongates in a 5'-to-3' direction, antiparallel to the template DNA.
- Special sequences and protein helpers terminate transcription.

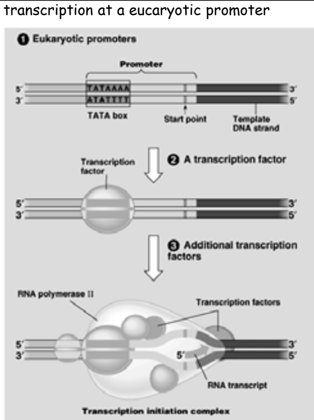




The stages of transcription



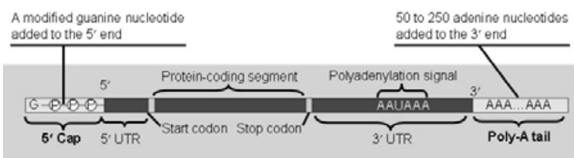
The initiation of transcription at a eucaryotic promoter



D. RNA Processing

- After transcription, the pre-mRNA is altered by the addition of a G cap at the 5' end and a poly A tail at the 3' end.
- UTR is untranslated region even though they are transcribed on the mRNA.

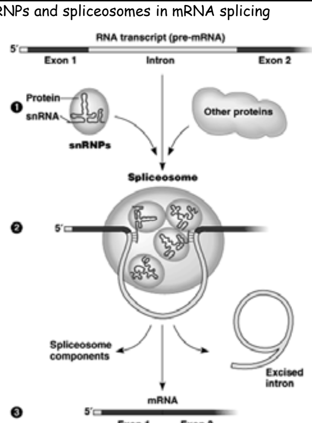
RNA processing; addition of the 5′ cap and poly(A) tail



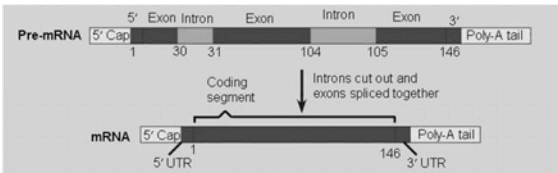
D. RNA Processing

- The introns are removed from the mRNA precursor by the spliceosome, a complex of RNA's and proteins (snRNPs).
- These RNA's are snRNAs which are ~300 bases long.
- Yet another ribozyme.

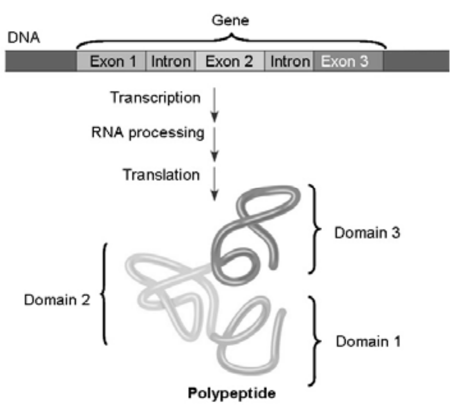
The roles of snRNPs and spliceosomes in mRNA splicing



RNA processing: RNA splicing



Correspondence between exons and protein domains



E. The Genetic Code

- The genetic code consists of triplets of nucleotides (codons). Since there are four bases, there are 64 possible codons.
- One mRNA codon indicates the starting point of translation and codes for methionine. Three stop codons indicate the end of translation. The other 60 codons code only for particular amino acids.

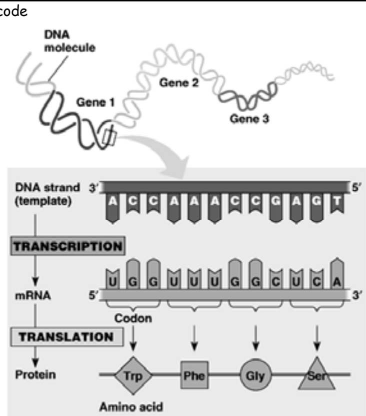
E. The Genetic Code

- Since there are only 20 different amino acids, the genetic code is redundant; that is, there is more than one codon for certain amino acids. However, a single codon does not specify more than one amino acid.
- The genetic code is degenerate but not ambiguous!

Second letter

First letter	U	C	A	G	Third letter
U	UUU UUC Phenylalanine; UUA UUG Leucine	UCU UCC UCA UCG Serine	UAU UAC Tyrosine; UAA Stop codon; UAG Stop codon	UGU UGC Cysteine; UGA Stop codon; UGG Tryptophan	U C A G
C	CUU CUC CUA CUG Leucine	CCU CCC CCA CCG Proline	CAU CAC Histidine; CAA CAG Glutamine	CGU CGC CGA CGG Arginine	U C A G
A	AUU AUC AUA Isoleucine; AUG Methionine; start codon	ACU ACC ACA ACG Threonine	AAU AAC Asparagine; AAA AAG Lysine	AGU AGC Serine; AGA AGG Arginine	U C A G
G	GUU GUC GUA GUG Valine	GCU GCC GCA GCG Alanine	GAU GAC Aspartic acid; GAA GAG Glutamic acid	GGU GGC GGA GGG Glycine	U C A G

The triplet code



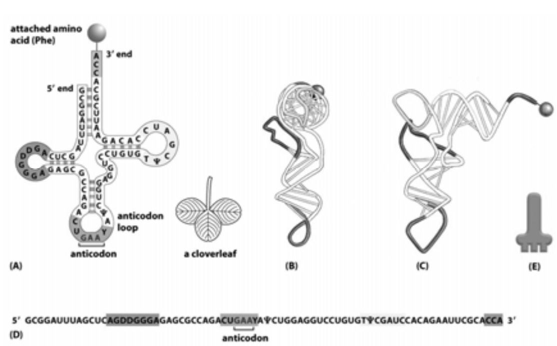
F. The Key Players in Translation

- In bacteria & archaea, translation begins before the mRNA is completed.
- In eucarya, transcription occurs in the nucleus and translation occurs in the cytoplasm.
- Translation requires three components: tRNA's, activating enzymes, and ribosomes.

F. The Key Players in Translation

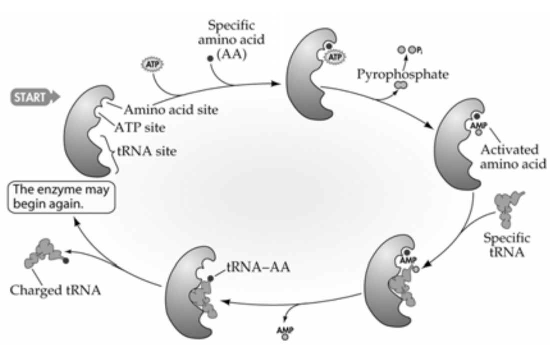
- In translation, amino acids are linked in codon-specified order in mRNA.
- This is achieved by an adapter, transfer RNA (tRNA), which binds the correct amino acid and has an anticodon complementary to the mRNA codon.

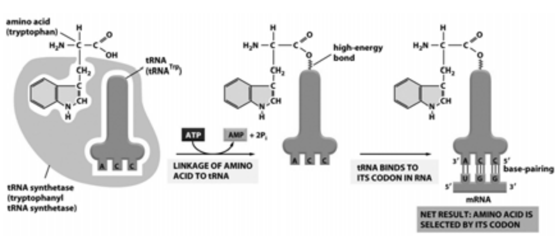
The structure of transfer RNA (tRNA)



F. The Key Players in Translation

- The aminoacyl-tRNA synthetases, a family of activating enzymes, attach specific amino acids to their appropriate tRNA's, forming charged tRNA's.
- These are the **ultimate translators** in the cell.

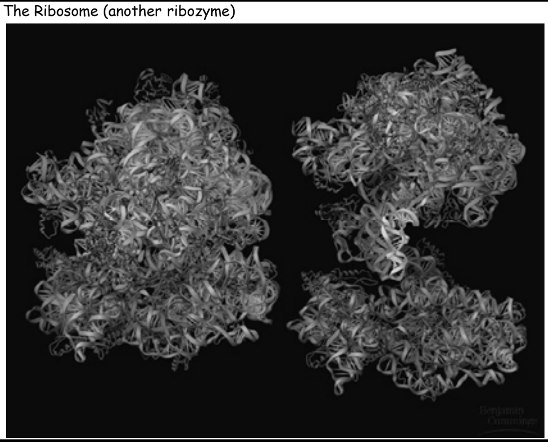




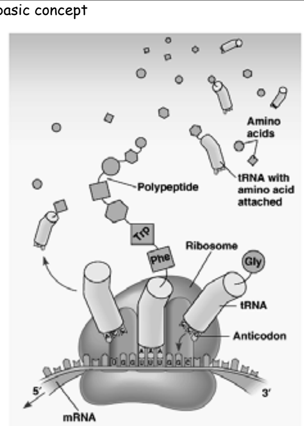
F. The Key Players in Translation

- The mRNA meets the charged tRNA's at a ribosome.
- The ribosome is the staging area for protein synthesis or translation.
- Ribosomes are roughly 60% RNA and 40% proteins.

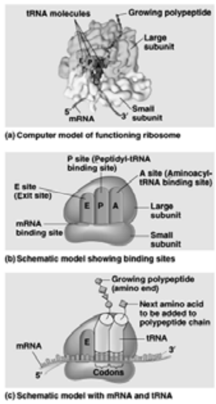
The Ribosome (another ribozyme)



Translation: the basic concept



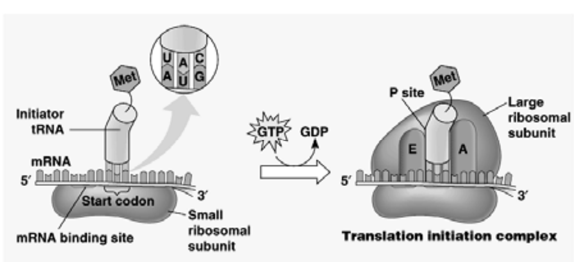
The anatomy of a functioning ribosome



G. Translation: RNA-Directed Polypeptide Synthesis

- Three step process: Initiation, Elongation and Termination.
- An initiation complex consisting of an amino acid-charged tRNA and a small ribosomal subunit bound to mRNA triggers the beginning of translation.
- Initiation complex includes the use of various initiations factors and of 1 GTP.

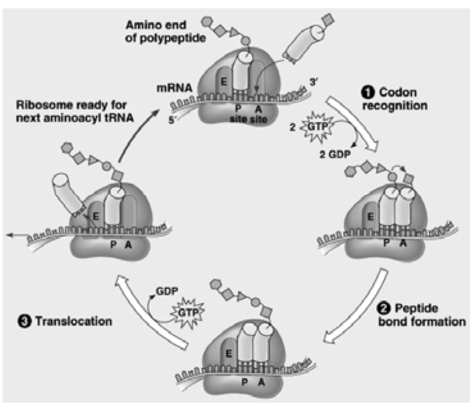
The initiation of translation



G. Translation: RNA-Directed Polypeptide Synthesis

- Polypeptides grow from the N terminus toward the C terminus. The ribosome moves along the mRNA one codon at a time.
- Elongation has three steps: Codon Recognition, Peptide Bond Formation and Translocation.
- Elongation also requires elongation factors and 3 GTPs per amino acid added.

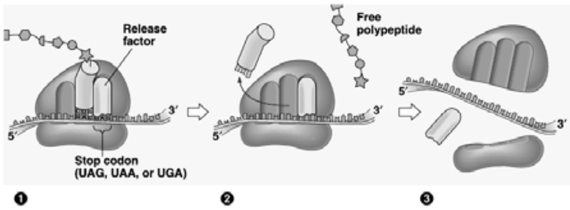
The elongation cycle of translation



G. Translation: RNA-Directed Polypeptide Synthesis

- The presence of a stop codon in the A site of the ribosome causes translation to terminate.

The termination of translation



RNA World: Peptide Bond Formation

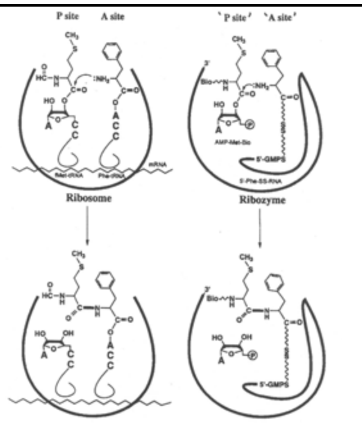
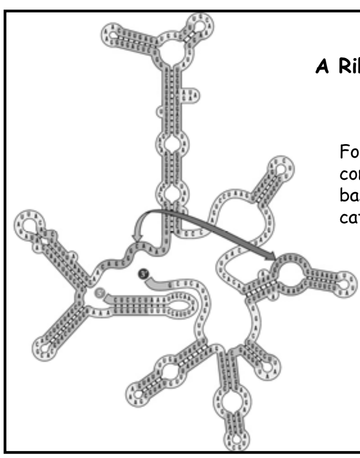
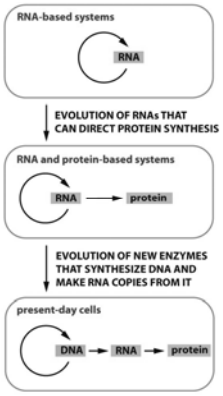


Figure 1 Peptide bond formation by a ribosome (left) and by a ribozyme (right).

A Ribozyme from a Protist



Folding brings together complementary but distant base sequences allowing catalytic activity to occur



H. Regulation of Translation

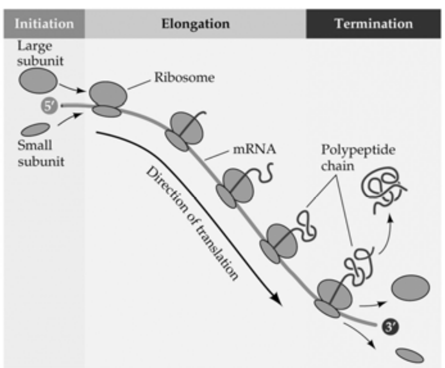
- Many antibiotics work by blocking events in translation.

12.2 *Antibiotics that Inhibit Bacterial Protein Synthesis*

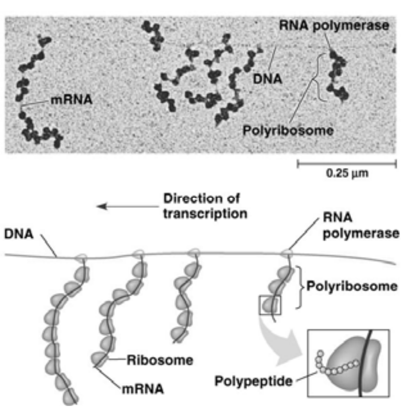
ANTIBIOTIC	STEP INHIBITED
Chloromycetin	Formation of peptide bonds
Erythromycin	Translocation of mRNA along ribosome
Neomycin	Interactions between tRNA and mRNA
Streptomycin	Initiation of translation
Tetracycline	Binding of tRNA to ribosome

H. Regulation of Translation

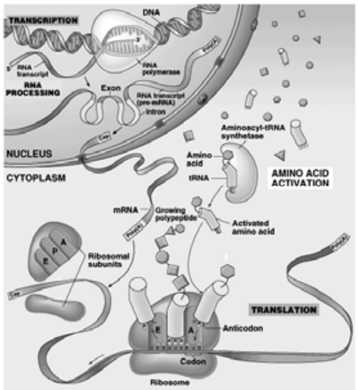
- In a polysome, more than one ribosome moves along the mRNA at one time.



Coupled transcription and translation in bacteria

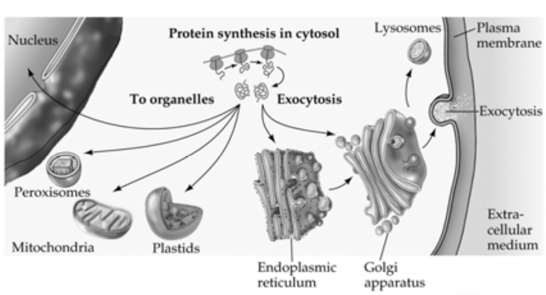


A summary of transcription and translation in a eucarya cell



I. Posttranslational Events

- Signals contained in the amino acid sequences of proteins direct them to cellular destinations.



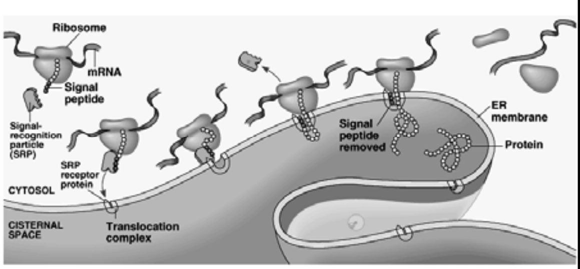
I. Posttranslational Events

- Protein synthesis begins on free ribosomes in the cytoplasm. Many proteins destined for the nucleus, mitochondria, and plastids are completed there and have signals that allow them to bind to and enter destined organelles.

I. Posttranslational Events

- Proteins destined for the ER, Golgi apparatus, lysosomes, and outside the cell complete their synthesis on the ER surface. They enter the ER by the interaction of a hydrophobic signal sequence with a channel in the membrane.

Rem: The signal mechanism for targeting proteins to the ER



I. Posttranslational Events

- Covalent modifications of proteins after translation include proteolysis, glycosylation, and phosphorylation.