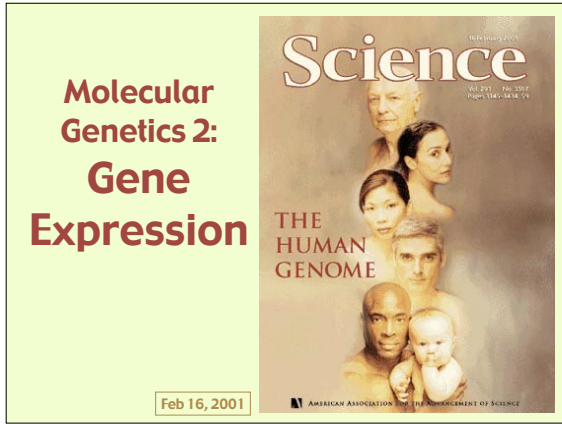


Gene Expression



Molecular Genetics

- Replication
 - **Precisely** copying all the genetic information (DNA)
 - S-stage of cell cycle
 - Exact replicas passed to daughter cells
- Gene Expression
 - Using a specific bit of the genetic information
 - Make a “working copy” of the needed bit (gene)
 - Take the working copy to the workshop (ribosome)
 - Use the copied instructions to build a specific protein

What is a gene?

- A unit of heredity (inherited information)
- Structure:
 - Short DNA sequence of nucleotides
 - One chromosome carries hundreds of genes
- Function:
 - Order of nucleotides in DNA determines order of amino acids in protein
 - **Each gene codes for a different polypeptide**

The Central Dogma of Biology

Gene Expression

- Copy the recipe from the master document (DNA gene) in the nucleus.
- Use the copy of the recipe (mRNA) to produce the protein on ribosomes in the cytoplasm.

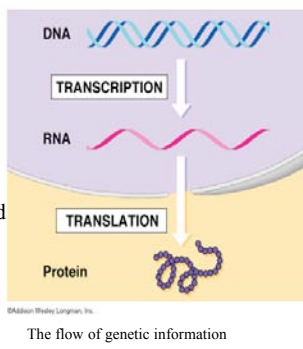
RNA nucleotides can base pair with DNA nucleotides

base pairing in transcription

Gene Expression

Genes are Expressed as Proteins

- 2 main stages
- 1) **Transcription**
 - DNA information copied to RNA
 - Occurs in the nucleus
- 2) **Translation**
 - RNA information used to construct a protein
 - Occurs in the cytoplasm



The flow of genetic information

I. Transcription: DNA to RNA

- A specific gene is “turned on”
- Making RNA strand from DNA gene
- Starts at **Promoter** - DNA region before a gene
- Ends at **Terminator** - DNA region at the end of a gene

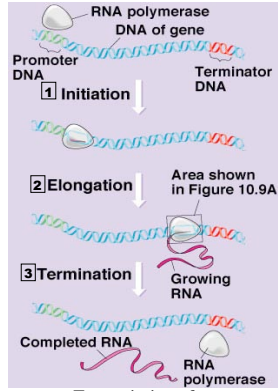
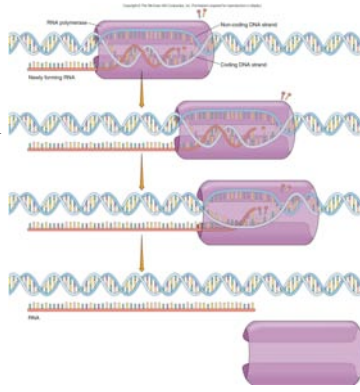


Figure 17.7 Transcription of a gene

- RNA polymerase moves along DNA strand and builds RNA strand



The stages of transcription: initiation, elongation, and termination

- Initiation.** After RNA polymerase binds to the promoter, the DNA strands unwind, and the polymerase initiates RNA synthesis at the start point on the template strand.
- Elongation.** The polymerase moves downstream, unwinding the DNA and elongating the RNA transcript 5' to 3'. In the wake of transcription, the DNA strands re-form a double helix.
- Termination.** Eventually, the RNA transcript is released, and the polymerase detaches from the DNA.

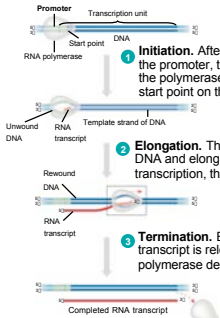


Figure 17.7

1. The initiation of transcription

Transcription initiation complex in eukaryotic cells

- Promoter**
 - A region of ~25 bases “upstream” from the gene
 - Contains the transcription start point
 - Often includes a “TATA box”
- Transcription factors**
 - Proteins that bind to the promoter
 - Facilitate binding of the polymerase here
- RNA polymerase**
 - Catalyses the synthesis of RNA 5' to 3'
 - Starts moving “downstream” from the start point

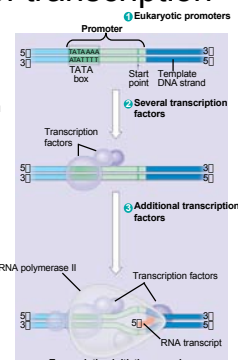
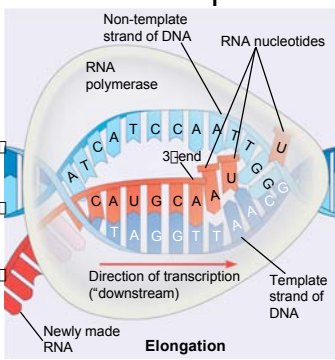


Figure 17.8 Transcription initiation complex

2. Transcriptional Elongation

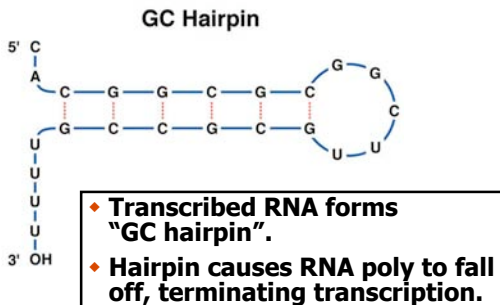


RNA-polymerase II

- Moves 3' to 5' down template DNA
- Un-zips 10–20 DNA bases at a time
- Ribo-nucleotides align by base pairing with template DNA of gene at 3'-end of elongating RNA strand
- RNA synthesized 5' to 3' (~60 nucleotides per sec)
- RNA strand separates from DNA as polymerase passes
- DNA re-zips

Figure 17.7

3. Termination (prokaryotes)



3. Termination (eukaryotes)

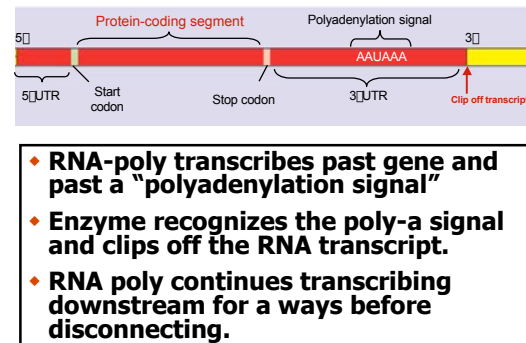
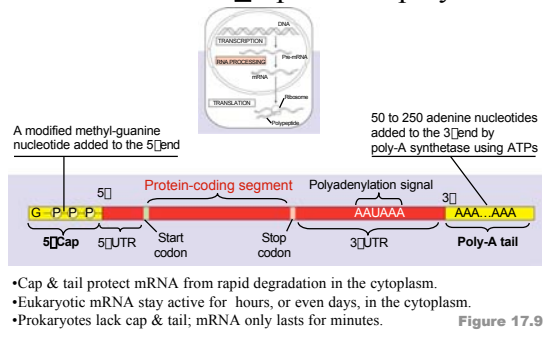


Table 17.1 Types of RNA in a Eukaryotic Cell * Each type produced by transcription from DNA.

Type of RNA	Functions
Messenger RNA (mRNA)	Carries information specifying amino acid sequences of proteins from DNA to ribosomes.
Transfer RNA (tRNA)	Serves as adaptor 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 nuclear RNA (snRNA)	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.

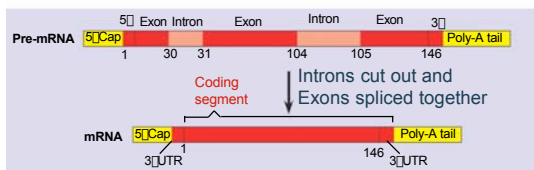
Although each RNA molecule has only a single polynucleotide chain, it is not a smooth linear structure. **Within strand complementary base pairing:** Regions of complementary AU or GC pairs allow the molecule to fold on itself forming helical structures called hairpin loops.

Processing of "primary transcript" RNA: addition of the 5' cap and the poly-A tail



Processing of "primary transcript" RNA: RNA splicing

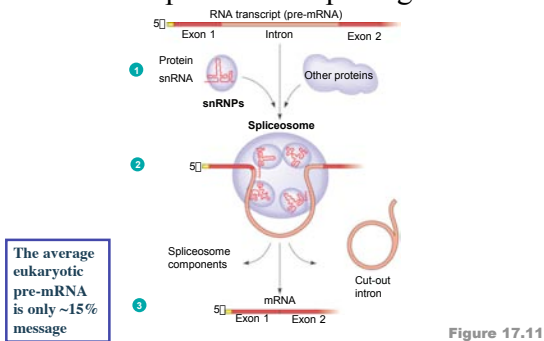
- Introns: "intervening sequences" — non-coding regions of DNA within a gene
- Must be excised from pre-mRNA before translation.



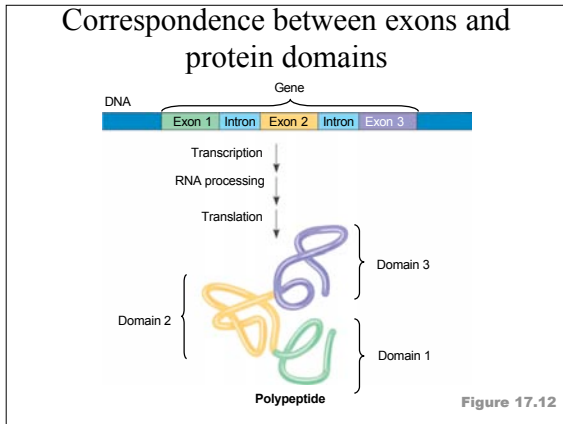
UTR: Un-Translated Region outside of coding segment.

Figure 17.10

The roles of snRNPs and spliceosomes in pre-mRNA splicing

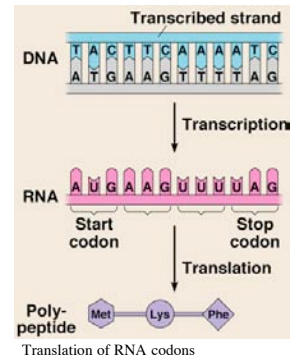


Gene Expression



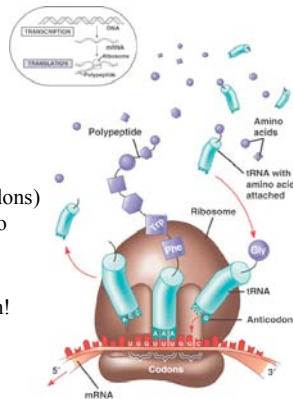
II. Translation: the sequence of mRNA **codons** determines the sequence of amino acids in the polypeptide

- **Codons** (“words”) are RNA nucleotide triplets
- Each codon represents a specific amino acid



II. Translation

- On the ribosome
- tRNAs translate the sequence of 3-base nucleotide “words” (codons) into a sequence of amino acids in a polypeptide
- NOTE: the mRNA is not “turned into” protein!



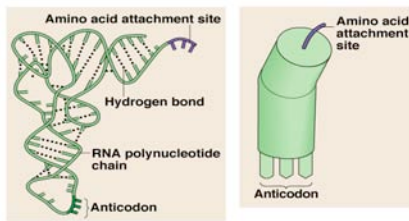
The Genetic Code

Table 7.2
AMINO-ACID-mRNA NUCLEIC ACID DICTIONARY

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

- specific mRNA codons are associated with specific amino acids
- 64 codons, but only 20 aa's
 - Many codons are redundant
 - Some are start/stop signals

Transfer RNAs Carry Amino Acids



Structure and symbol of transfer RNA

- tRNA molecules match amino acids to the appropriate codon
- tRNA **anticodon** - a triplet sequence on tRNA that base pairs with a mRNA codon

An aminoacyl-tRNA synthetase joins a specific amino acid to a specific tRNA

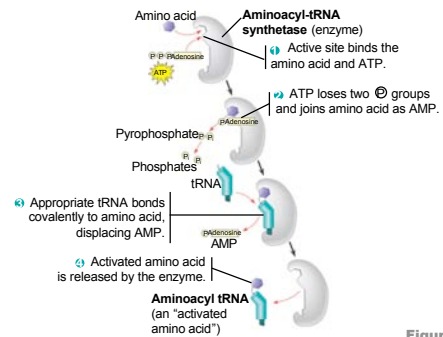


Figure 17.15

Gene Expression

Ribosomes: the site of translation

Ribosomes are bound to ER and free in cytoplasm

Ribosome: Made of rRNA and protein

The Ribosome

(b) 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.

Initiation of Translation

The initiation of translation

- mRNA binds to ribosome at **start codon [AUG]**
- First tRNA binds to mRNA (anticodon to codon)

Elongation

- Next tRNA binds to A site
- Adjacent amino acids linked
- tRNA in P site leaves
- Ribosome moves to next codon

Process continues

Polypeptide elongation

Translation: Termination

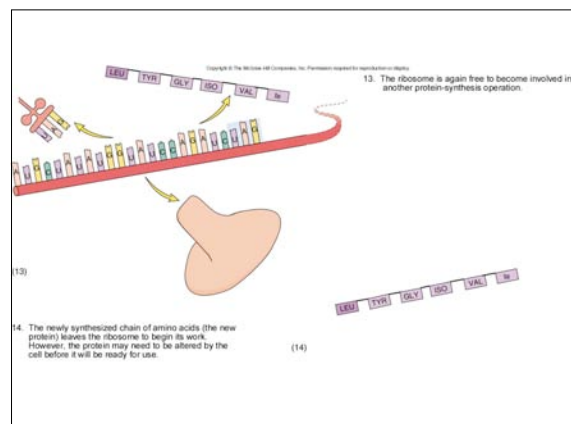
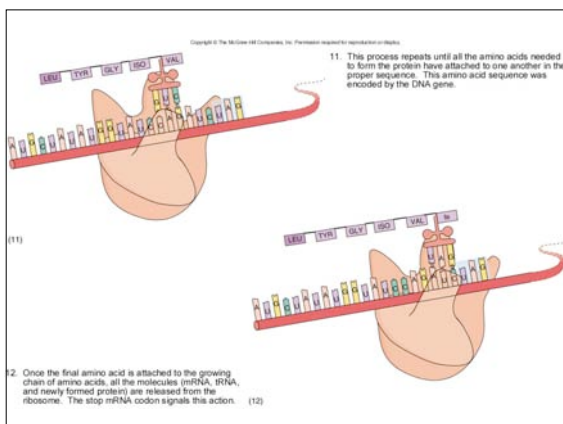
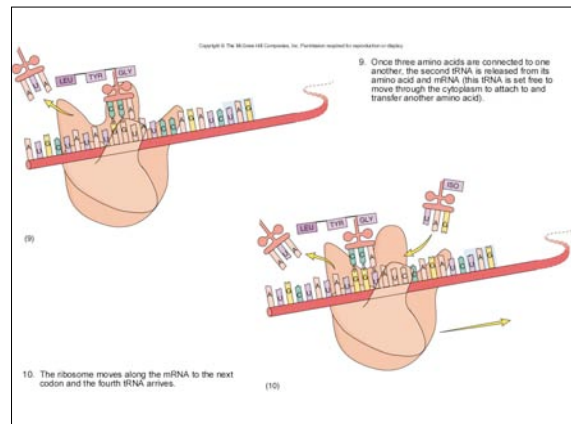
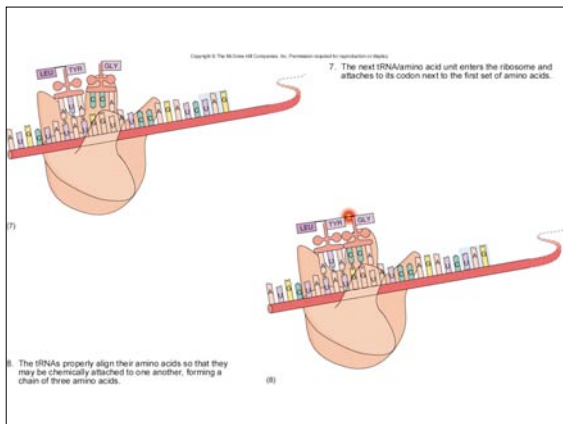
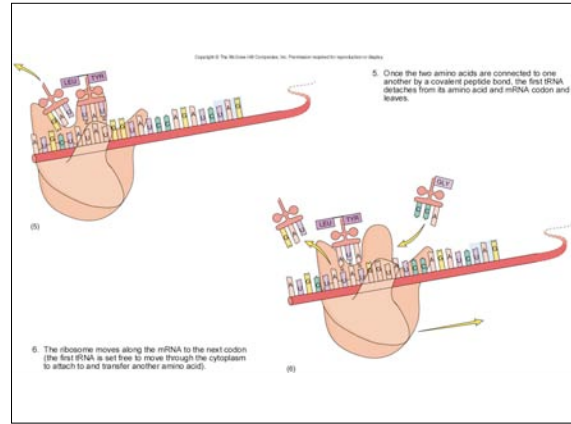
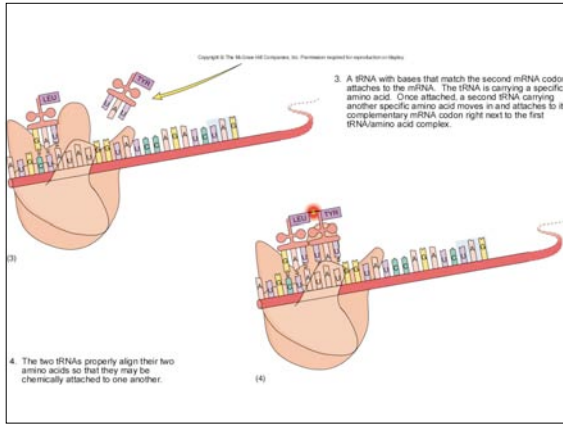
Step 5 Termination
The ribosome recognizes a stop codon. The polypeptide is terminated and released.

- When ribosome reaches **stop codon**, a releasing factor (protein) binds to the codon instead of a tRNA.
- Polypeptide is released from tRNA.
- Ribosome then released from mRNA.

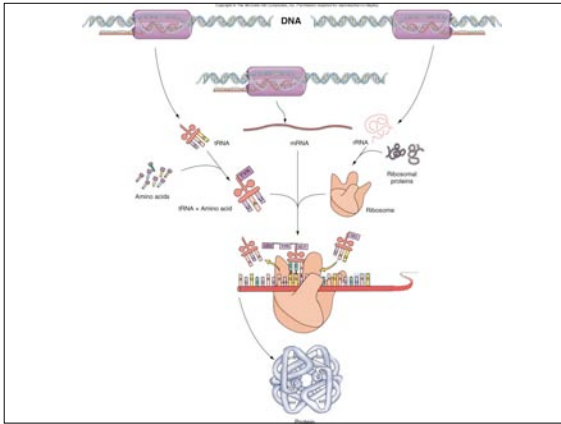
Basic Steps of Translation

- An mRNA molecule is placed in the small portion of a ribosome so that six nucleotides (two codons) are locked into position.
- The larger ribosomal unit is added to the ribosome-mRNA combination.

Gene Expression



Gene Expression



Making lots of protein

- Many copies of mRNA can be made from one gene
- Many ribosomes can make protein from the same mRNA
- Amplification of information allows rapid production of proteins

Not done yet!

Protein Shape Determines Function

- Post-translation modification
- Specific 3-D shape
- **Shape is critical to function**

Ribbon model of lysozyme protein

- **Denaturation** = loss of shape ➔ loss of function

Regulation of Gene Expression

Critical to have the right protein at the right place and right time.

- **Initiation of transcription***
 - “Transcription factors”
- Post-transcriptional modification
 - Alternative splicing of mRNA — “RNA processing”
- Translation
- Post-translational modification*

* Major regulative processes
(Topics for future lectures)

Review

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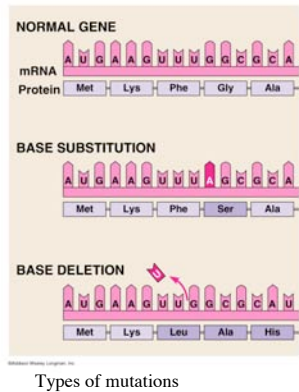
Prokaryotes & Eukaryotes

The diagram compares the two processes. In prokaryotes, transcription of the bacterial chromosome produces mRNA, which is then translated into protein in the cytoplasm. In eukaryotes, transcription of DNA in the nucleus produces a primary RNA transcript containing introns. This transcript undergoes processing (removal of introns, addition of a 5' cap and poly-A tail) to become mature mRNA, which is then translated into protein in the cytoplasm.

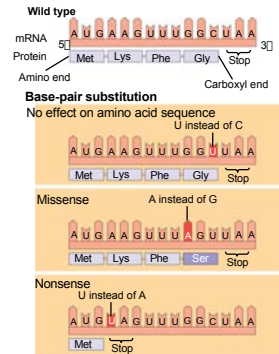
Gene Expression

Mutations

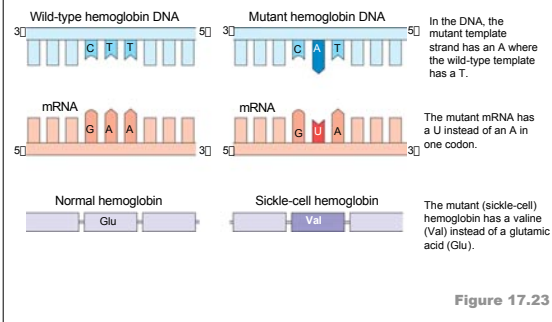
- Single base changes:
 - Substitution** changes one aa in protein chain
 - Deletion or Insertion** leads to **Frame Shifts**; changes entire aa sequence downstream



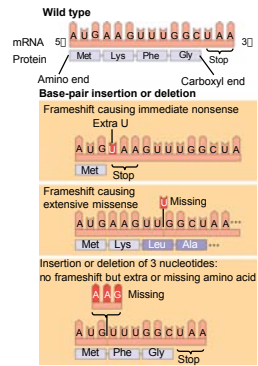
Base-pair substitution



A missense point mutation: The molecular basis of sickle-cell disease



Base-pair insertion or deletion



A problem of origins: which came first?

