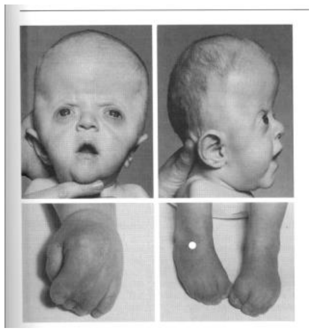
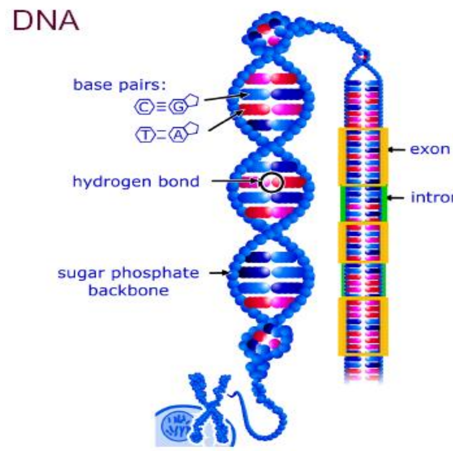
Gene & genome: transcription, translation, nucleus and control in gene expression



Defects in gene regulation can alter the development of an organism



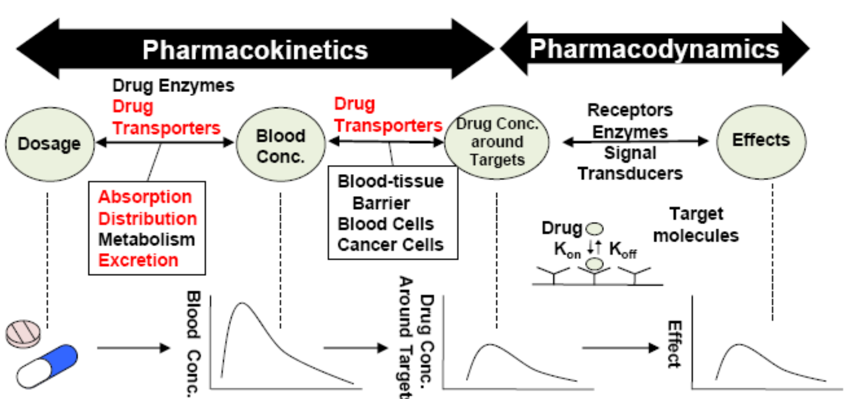
What can genome sequence tell us?

- Everything about the organism's life
- Its developmental program
- Disease resistance or susceptibility

Gene expression

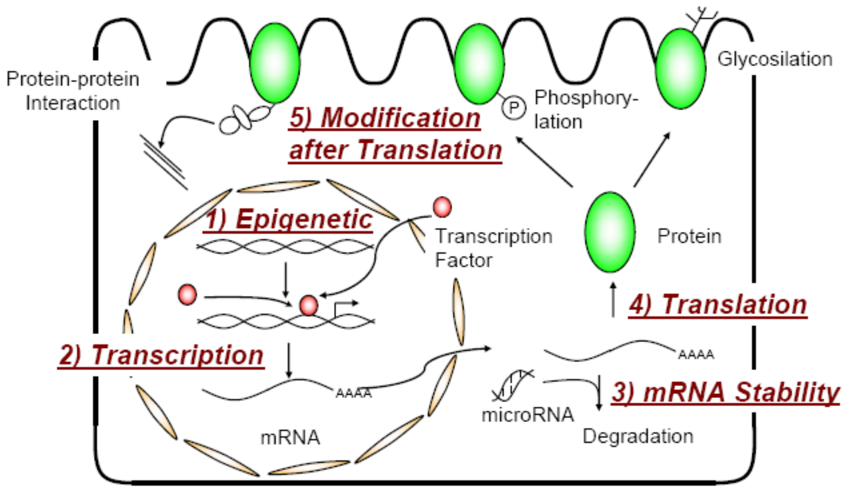
Some gene products are necessary only some of the time

- **Specialization**
  - **each cell of a multicellular eukaryote expresses only a small fraction of its genes** , e.g.Fending off predators or pathogens
- **Development,** In response to life events, e.g. Reproduction
  - **some gene expression must be controlled on a long-term basis for cellular differentiation & specialization during development**
- **Responding to organism's needs**
  - **cells of multicellular organisms must continually turn certain genes on & off in response to signals from their external & internal environment,** e.g.
  - Presence (or absence) of certain foods or toxins
  - Temperature change (heat shock, freezing)



Qualitative and quantative alteration of drug transporters can affect the PK/PD, and sometimes the drug effects. Quantitative alteration of drug transporters are directly related to the expression of drug transporters.

Potential Regulatory Mechanisms of Drug Transporters' Expression



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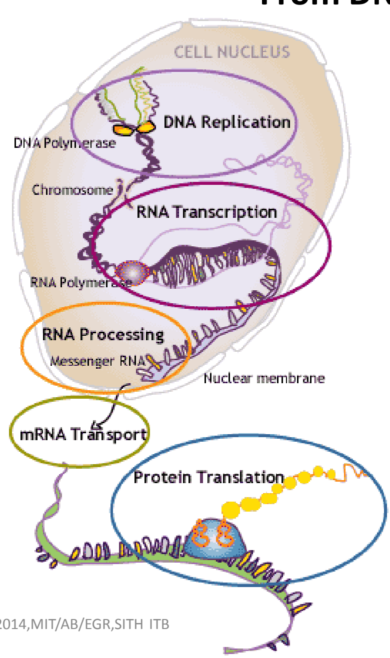
From DNA to Protein



- DNA carries the genetic information of a cell and consists of thousands of genes
- genes are transcribed into RNA (**transcription**)
- proteins are built based upon the code in the RNA (**translation**).

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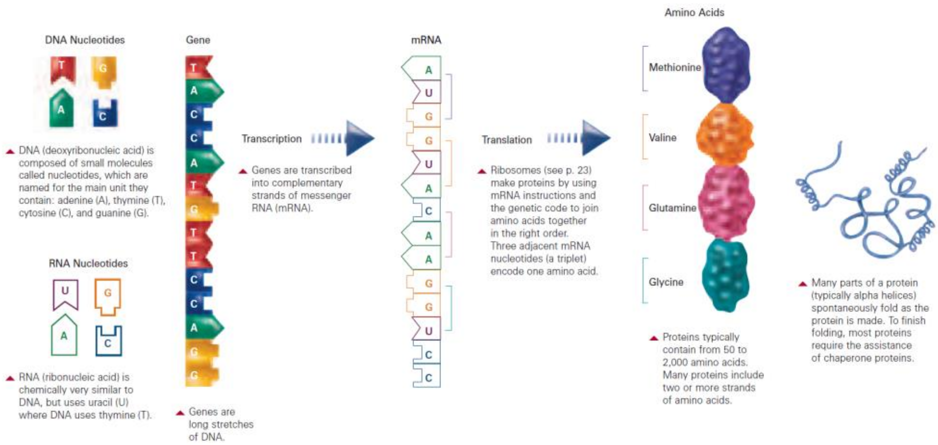
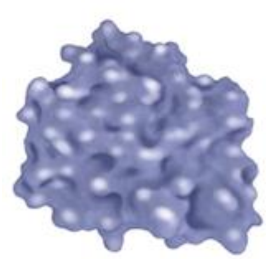
Genetic Codes



3D shape enables proteins to accomplish their function in your body

Proteins are made of amino acids like beads on a necklace.



To become active, proteins must fold into their final shape

Proteins are the worker molecules that make possible every activity in your body

Proteins have many different functions in our bodies. By studying the structures of proteins, we are better able to understand how they function normally and how some proteins with abnormal shapes can cause disease.

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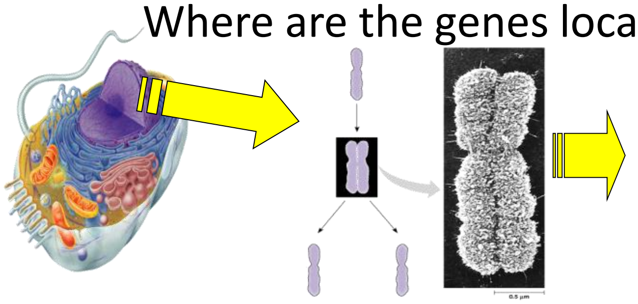
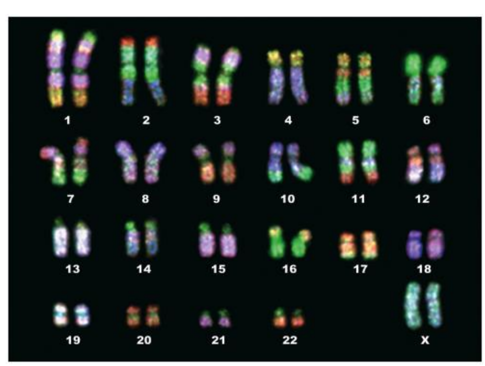
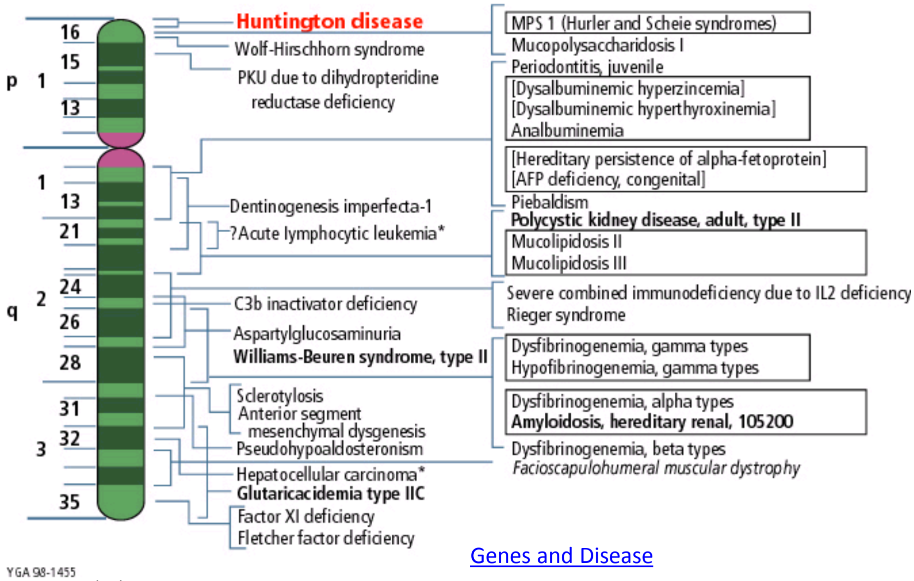
Where are the genes located?



- Genes are located on the chromosomes.
- Every species has a different number of chromosomes.
- There are two types of chromosomes: autosomes and sex chromosomes

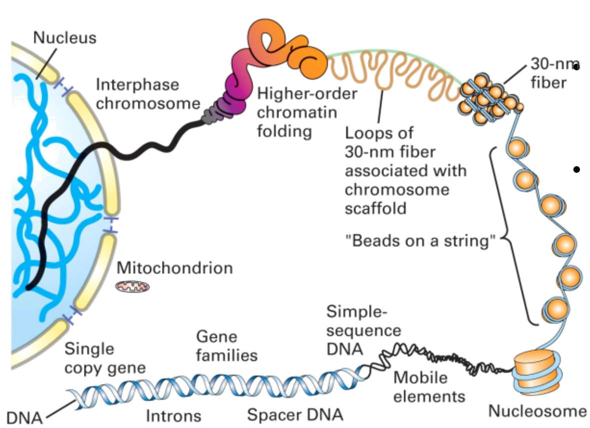


Chromosome 4



Genes and Disease

Eukaryotic Chromosome Structure



- Eukaryotic DNA is packaged into chromatin.
- Chromatin structure is directly related to the control of gene expression.
- Chromatin structure begins with the organization of the DNA into nucleosomes.
- Nucleosomes may block RNA polymerase II from gaining access to promoters.

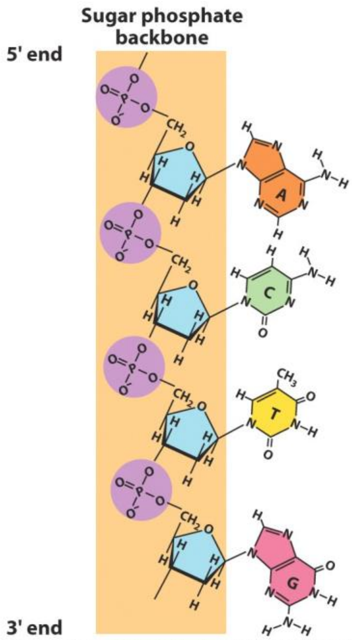


Figure 10-9c Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)

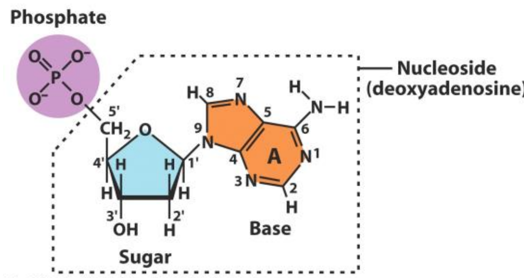
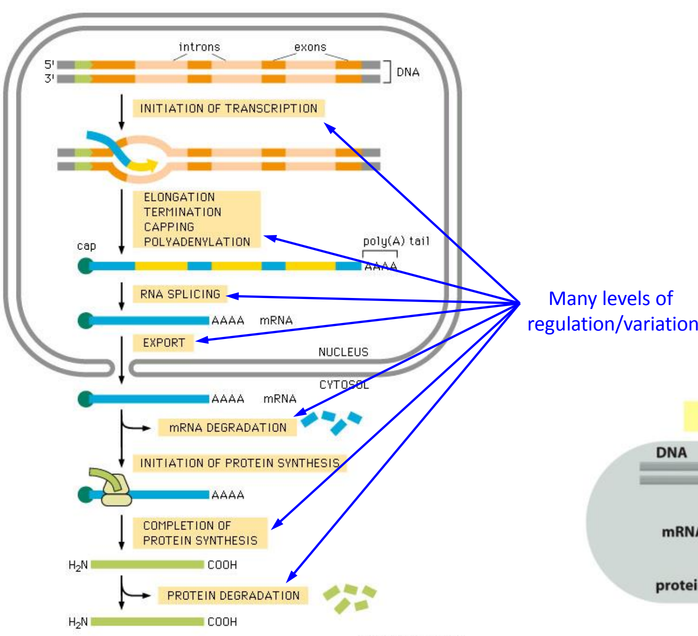
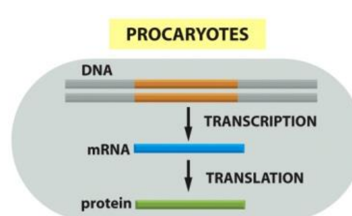


Figure 10-9b Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)

The production of a protein by a eukaryotic cell





- In prokayotes, transcription and translation occur in the cytoplasm.
- In eukaryotes, transcription occurs inside the nucleus in a two step sequence of events.
  - Pre-mRNA includes both introns and exons for the gene.
  - mRNA is only the coding portion (exons).
- Translation occurs in the cytoplasm at the ribosomes.
  - three (3) types of RNA
    - Messenger (mRNA)
    - Transfer (tRNA)
    - Ribosomal (rRNA)

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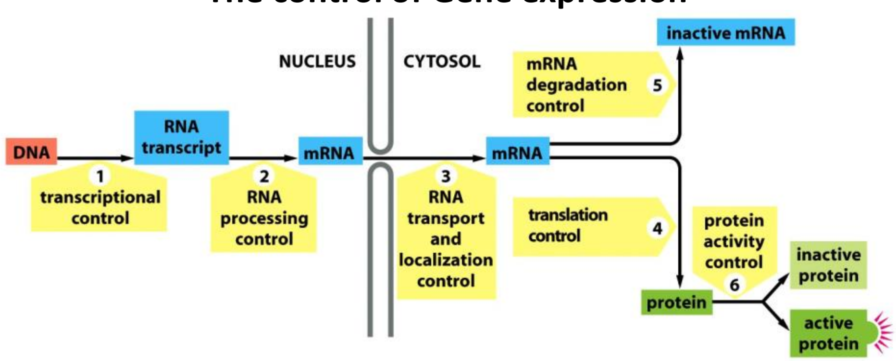
The control of Gene expression



- **The control of gene expression can occur at any step in the pathway from gene to functional protein :**
  - **unpacking DNA**
  - **transcription**
  - **mRNA processing**
  - **mRNA degradation**
  - **translation**
  - **protein processing**
  - **protein degradation**

Prokaryotic organisms regulate gene expression in response to their environment.

Eukaryotic cells regulate gene expression to maintain **homeostasis** in the organism.

e.g. Heart genes are not expressed in brain cells

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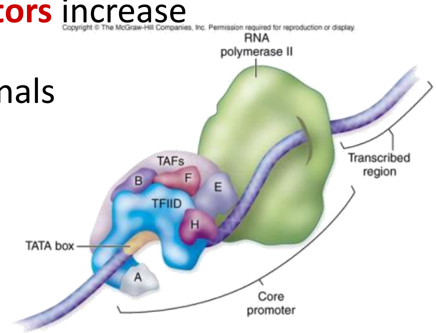
Figure 7-5 *Molecular Biology of the Cell* (© Garland Science 2008)

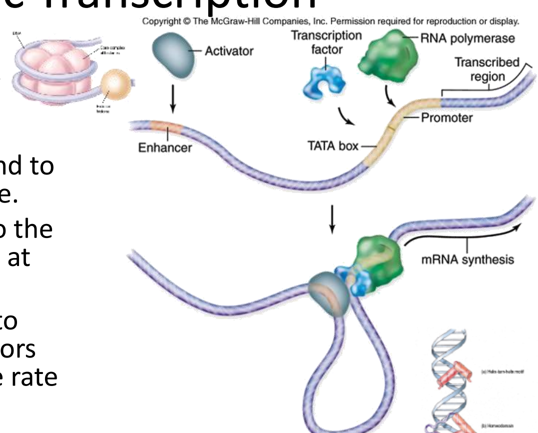
Eukaryotic Regulation

- Controlling the expression of eukaryotic genes requires **transcription factors**.
  - **general transcription factors** are required for transcription initiation
    - required for proper binding of RNA polymerase to the DNA
  - **specific transcription factors** increase transcription in certain cells or in response to signals

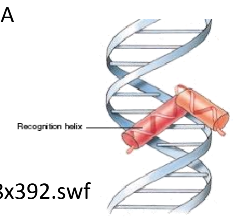


Eukaryotic Transcription

- Transcriptional control of gene expression occurs in eukaryotes despite the tight packaging of DNA into nucleosomes.
- General transcription factors bind to the **promoter** region of the gene.
- RNA polymerase II then binds to the promoter to begin transcription at the **start site (+1)**.
- **Enhancers** are DNA sequences to which specific transcription factors (**activators**) bind to increase the rate of transcription.



Regulatory proteins identify specific sequences on the DNA double helix, without unwinding it, by inserting DNA-binding motifs into the major groove of the double helix where the edges of the bases protrude.



http://www.as.wvu.edu/~dray/219files/Transcription_588x392.swf

Translation

- The genetic code
- Amino acids in a protein are determined by a degenerate, triplet code
- The code was determined using synthetic RNAs
- The first, poly(U) → polyphenylalanine
- The genetic code is nearly universal

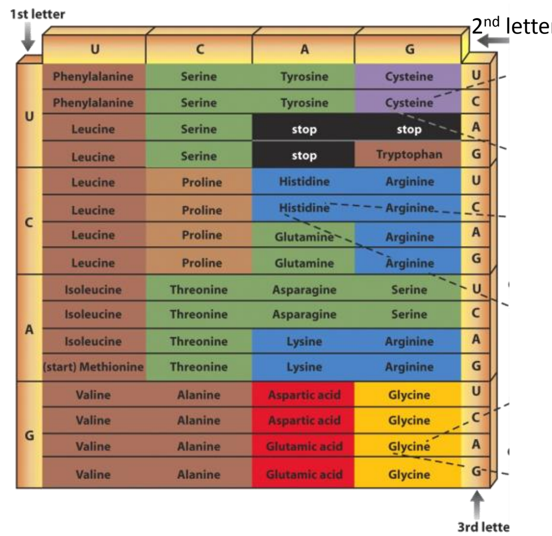


Figure 11-41 Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)

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Translation

- The genetic code
- Codon assignments are nonrandom;
- Codons for same amino acid tend to be similar
- Benefits:
  - Less likely for a mutation to alter the amino acid sequence
    - Synonymous vs nonsynonymous mutations
  - Amino acids with similar chemical properties are encoded by similar codons

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Translation

- Translation - converting the nucleic acid information to amino acid information
  A. Each tRNA is linked to a specific amino acid
  B. Each tRNA is also able to recognize a particular codon in the mRNA
  C. Interaction between successive codons in mRNA & specific aa-tRNAs leads to synthesis of polypeptide with an ordered amino acid sequence

Translation

- Codon – Anticodon pairing
- Similar to typical basepairing but allows for *third position wobble*
- The first two positions must pair exactly but the third is more relaxed
- Anticodon U can pair with A or G on mRNA
- Anticodon I (derived from G) can pair with U, C, or A
- Allows for fewer required tRNAs
  – Leucine (6 codons) requires only 3 different tRNAs

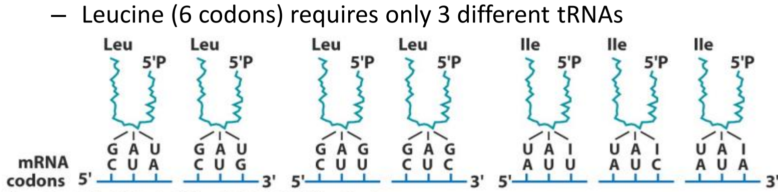
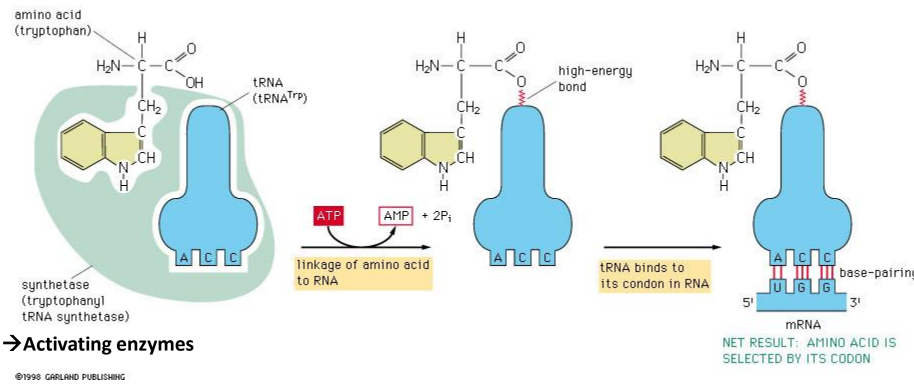


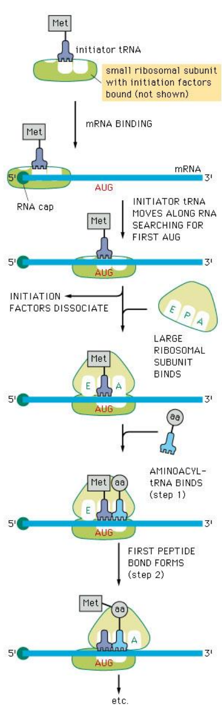
Figure 11-44 Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)

Translation of the genetic code: two adaptors that act one after another



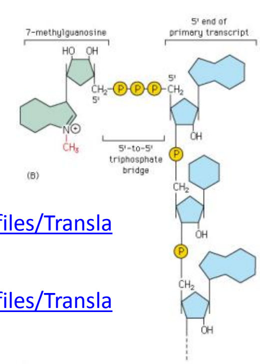
→**Activating enzymes**

TRANSLATION : Activating enzymes "read" the genetic code.
Each kind of activating enzyme recognizes and binds to a specific amino acid, such as tryptophan; it also recognizes and binds to the tRNA molecules with anticodons specifying that amino acid, such as ACC for tryptophan. In this way, activating enzymes link the tRNA molecules to specific amino acids.



The initiation phase of protein synthesis in eukaryotes

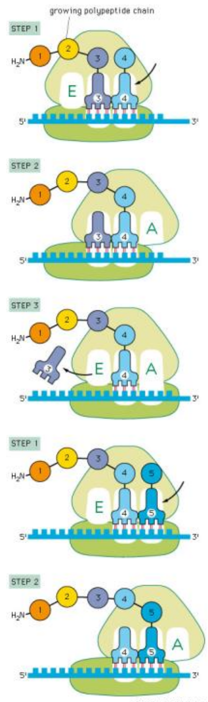
1. Initiation complex (small ribosomal subunit + initiation factors) binds mRNA and searches for start codon
2. Large ribosomal subunit adds to the complex
3. Translation starts
4. . . .



5´ end capping of eukaryotic mRNA molecules

http://www.as.wvu.edu/~dray/219files/Translation_588x392.swf

http://www.as.wvu.edu/~dray/219files/TranslationAdvanced.wmv



mRNA translation mechanism

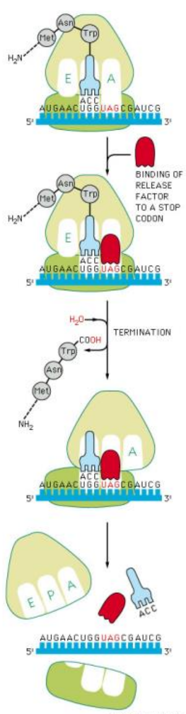
Step1: An aminoacyl-tRNA molecule binds to the A-site on the ribosome

Step2: A new peptide bond is formed

Step3: The small subunit moves a distance of three nucleotides along the mRNA chain ejecting the spent tRNA molecule

Step4: The next aminoacyl-tRNA molecule binds to the A-site on the ribosome

Step5: . . .



The final phase of protein synthesis

→ binding of release factor to a stop codon terminates translation

→ the completed polypeptide is released

→ the ribosome dissociates into its two separate subunits

Translation

- Polyribosomes

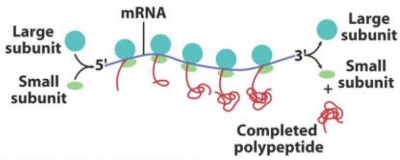


Figure 11-51a Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)

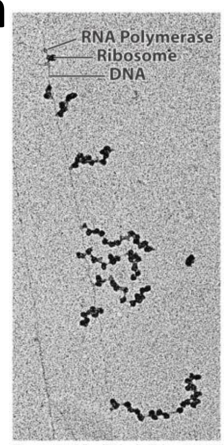


Figure 11-52a Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)

Prokaryote

Eukaryote

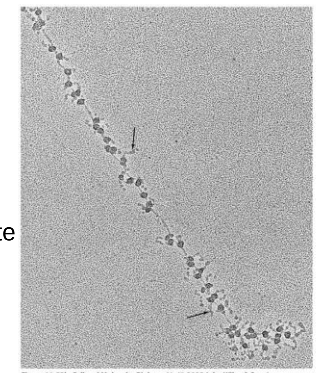


Figure 11-52b Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)

Note the difference –
Due to presence/absence of nuclear membrane

Translation Inhibitors are important antibiotics

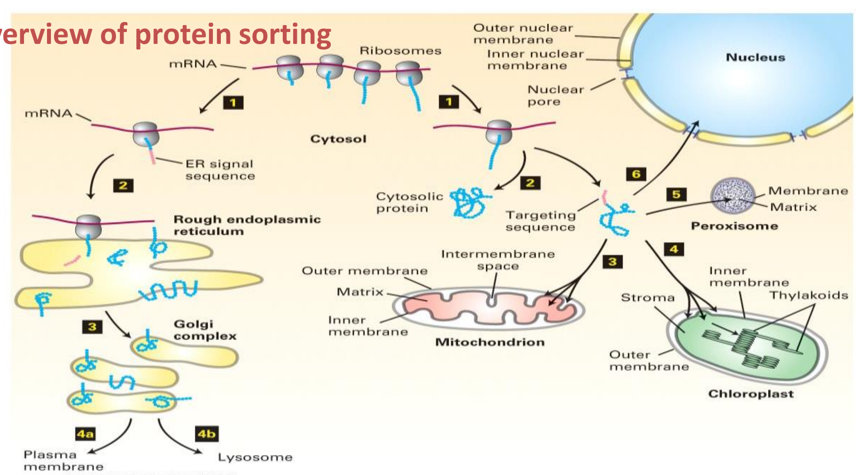
TABLE 29.4 Antibiotic inhibitors of protein synthesis

Antibiotic	Action
Streptomycin and other aminoglycosides	Inhibit initiation and cause misreading of mRNA (prokaryotes)
Tetracycline	Binds to the 30S subunit and inhibits binding of aminoacyl-tRNAs (prokaryotes)
Chloramphenicol	Inhibits the peptidyl transferase activity of the 50S ribosomal subunit (prokaryotes)
Cycloheximide	Inhibits the peptidyl transferase activity of the 60S ribosomal subunit (eukaryotes)
Erythromycin	Binds to the 50S subunit and inhibits translocation (prokaryotes)
Puromycin	Causes premature chain termination by acting as an analog of aminoacyl-tRNA (prokaryotes and eukaryotes)

Post-Translational Modification

- New polypeptides usually fold themselves spontaneously into their active conformation. However, some proteins are helped and guided in the folding process by chaperone proteins
- Many proteins have sugars, phosphate groups, fatty acids, and other molecules covalently attached to certain amino acids. Most of this is done in the endoplasmic reticulum.
- Many proteins are targeted to specific organelles within the cell. Targeting is accomplished through “signal sequences” on the polypeptide. In the case of proteins that go into the endoplasmic reticulum, the signal seqeunce is a group of amino acids at the N terminal of the polypeptide, which are removed from the final protein after translation.

Overview of protein sorting



Proteins have a variety of roles that they must fulfil:
1. they are the enzymes that rearrange chemical bonds.
2. they carry signals to and from the outside of the cell,and within the cell.
3. they transport small molecules.
4. they form many of the cellular structures.
5. they regulate cell processes, turning them on and off and controlling their rates.

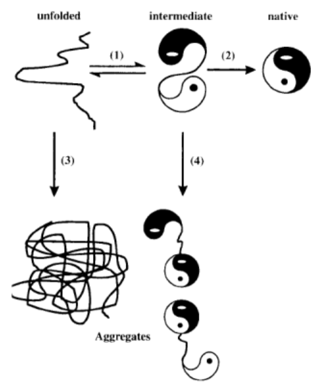
Mystery of Protein Folding

Alzheimer's disease. Cystic fibrosis. Mad Cow disease. An inherited form of emphysema. Even many cancers

In the hereditary disease familial amyloidotic polyneuropathy (FAP), peripheral nerves and other organs are damaged by deposits of amyloid-type protein. Extensive genetic studies have shown that the disease results from mutations in the protein transthyretin

The key is to find a small molecule, a drug that can either stabilize the normally folded structure or disrupt the pathway that leads to a misfolded protein. Although many molecular biologists and protein chemists believe this will be quite difficult, others are more optimistic.

Folding and aggregation during protein renaturation. Correct folding reactions, leading to the native state(1,2). Irreversible aggregation reactions, starting from different conformations during the renaturation process(3,4)



Folding and aggregation during protein renaturation. Correct folding reactions, leading to the native state [(1), (2)]. Irreversible aggregation reactions, starting from different conformations during the renaturation process [(3), (4)]. [From *FASEB J.* **10**, 52 (1996)]

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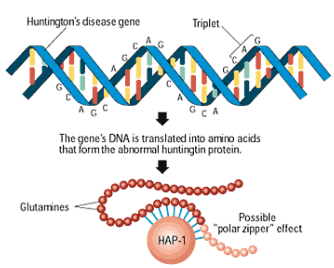
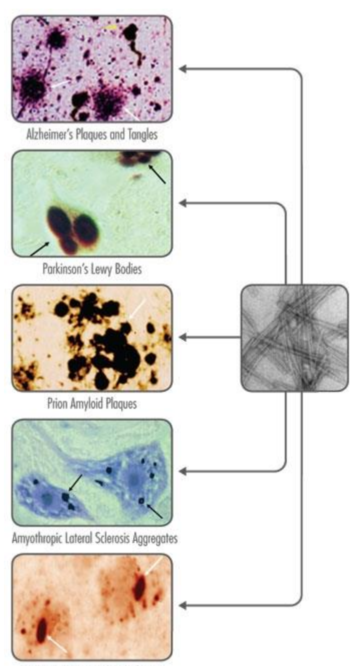


Huntington's Disease



Huntington's disease

- Rare autosomal dominant degenerative neurological disease
  - 1st described in 1872 by Dr. Huntington
  - most common in white Europeans
  - 1st symptoms at age 30-50
  - death comes ~12 years after onset
- Mutation on chromosome 4
  - CAG repeats, CAG codes for glutamine amino acid
  - 40-100+ copies, normal = 11-30 CAG repeats
- Abnormal (huntingtin) protein produced
  - chain of charged glutamines in protein
  - bonds tightly to brain protein, HAP-1

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Genetic modification of humans

- Once we know the genes responsible for particular diseases, should we "cure" the diseases?
- Should we also modify genes responsible for traits such as height or beauty?
- Should we allow the cloning of human beings?