

Chapter 4

Expression of Genetic Information



Genetic Code

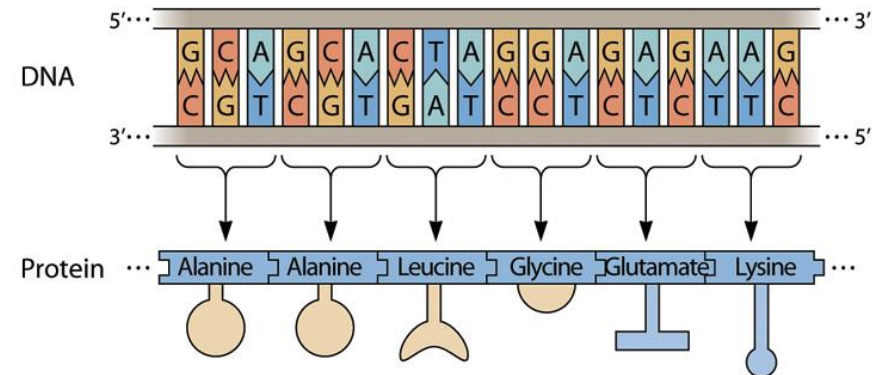
- Information in DNA → amino acid sequence in protein

mRNA

5' GCA GCA CUA GGA GAG AAG 3'

- Codon: triplet of RNA bases

20 amino acids in nature



Codon (nt)

Amino acid

1

4

2

16

3

64

Genetic Code

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

Genome

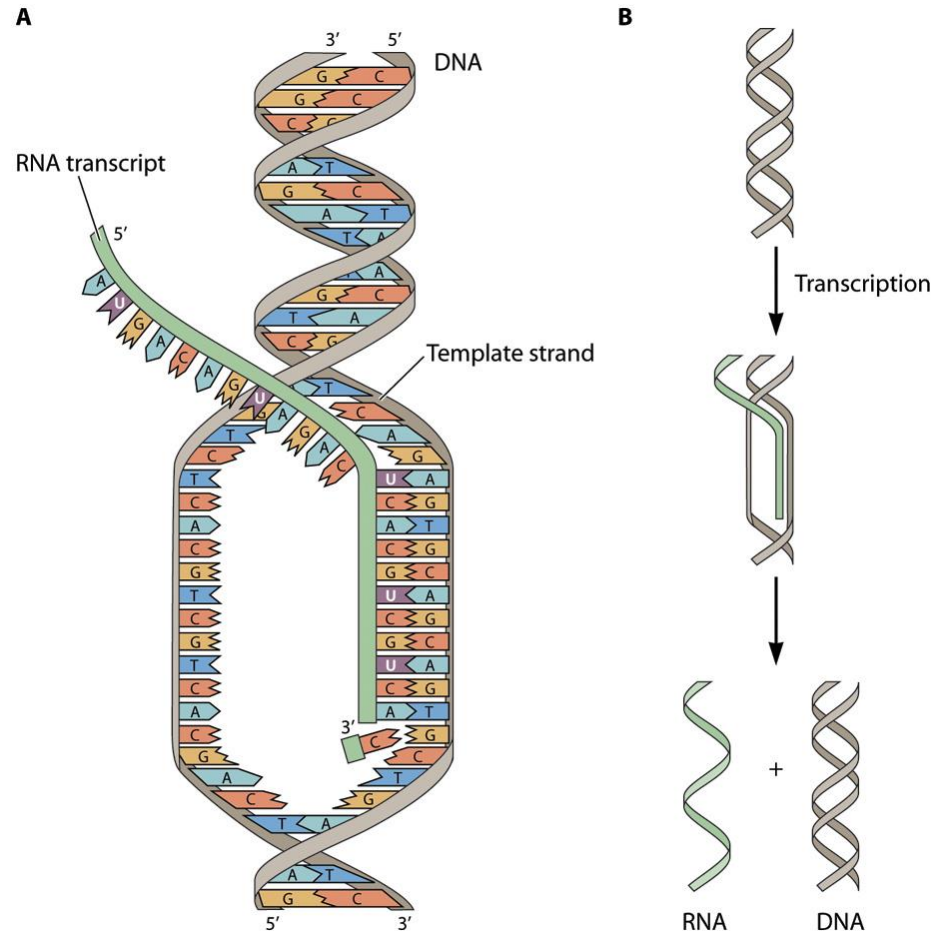
- Gene
 - The complete stretch of DNA needed to determine the amino acid sequence of a protein
- Genome
 - The complete set of genetic material in an organism
 - Human genome project
 - 1990-2003
 - U.S. department of energy and the National Institute of Health
 - 3.2×10^9 bp, 19,000~20,000 genes
 - 98% is noncoding DNA

Protein Synthesis

- Transcription
 - From DNA to mRNA (messenger RNA)
- Translation
 - From mRNA to protein
 - tRNA (transfer RNA) matches the codon to amino acid
 - Ribosome (made of Proteins and RNAs)
 - Protein synthesis

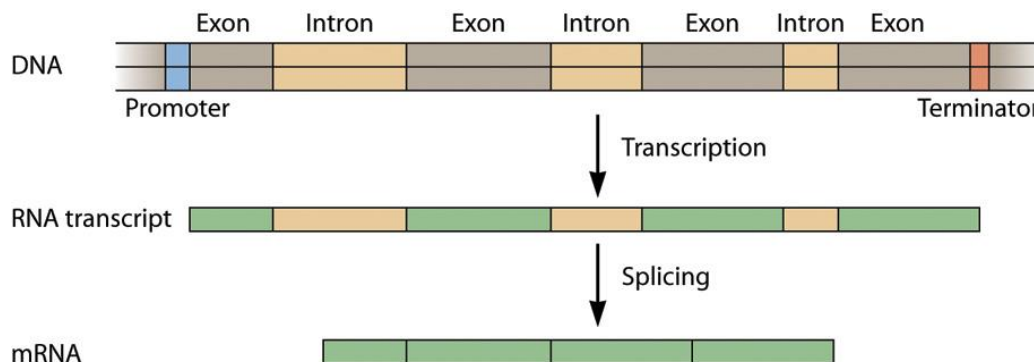
Transcription

- RNA synthesis using only one strand as a template
 - mRNA → encode protein
 - Ribosomal RNA (rRNA) and tRNA → no translation
- RNA polymerase
 - mRNA synthesis

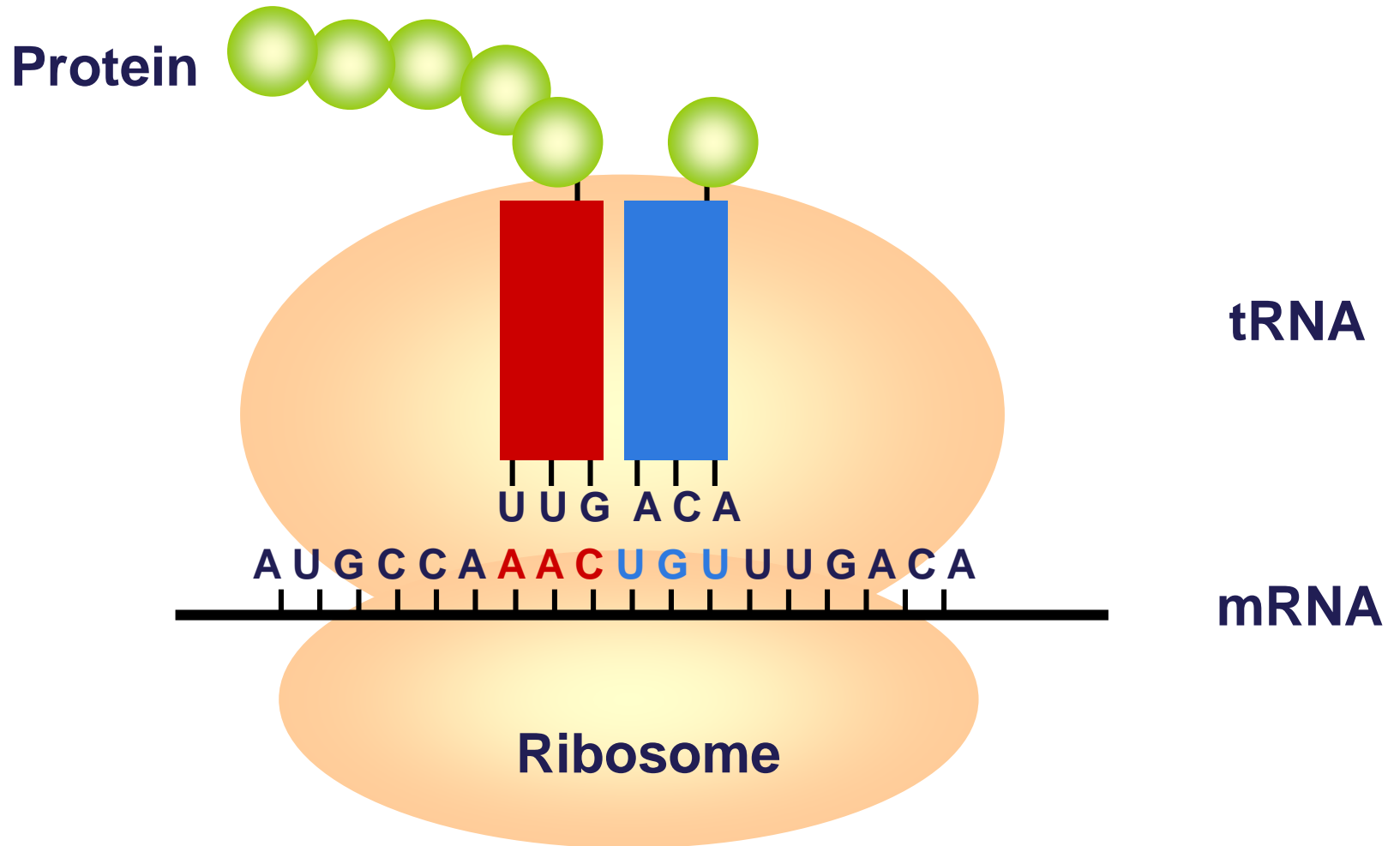


Regulation of Transcription

- Promoter
 - Binding site of RNA polymerase and regulatory proteins (transcriptional regulator; activator or repressor)
- Terminator
 - The site where transcription stops
- Processing of eukaryotic RNA
 - Splicing : joining of exons



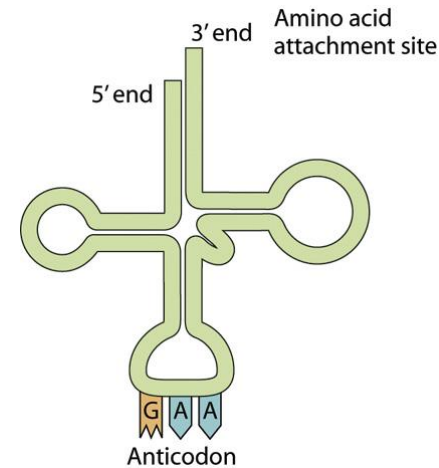
Translation



Translation

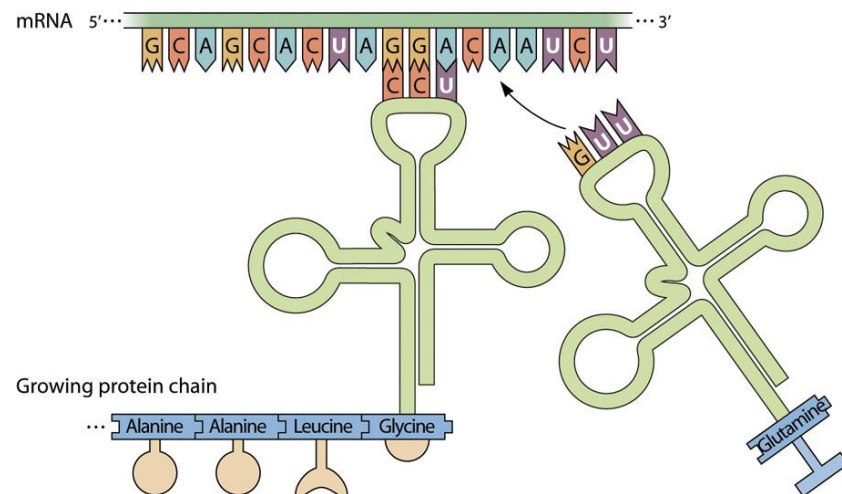
■ tRNA

- Cloverleaves shaped folding
- Anticodon: complementary to codon
- 3' end: amino acid attachment



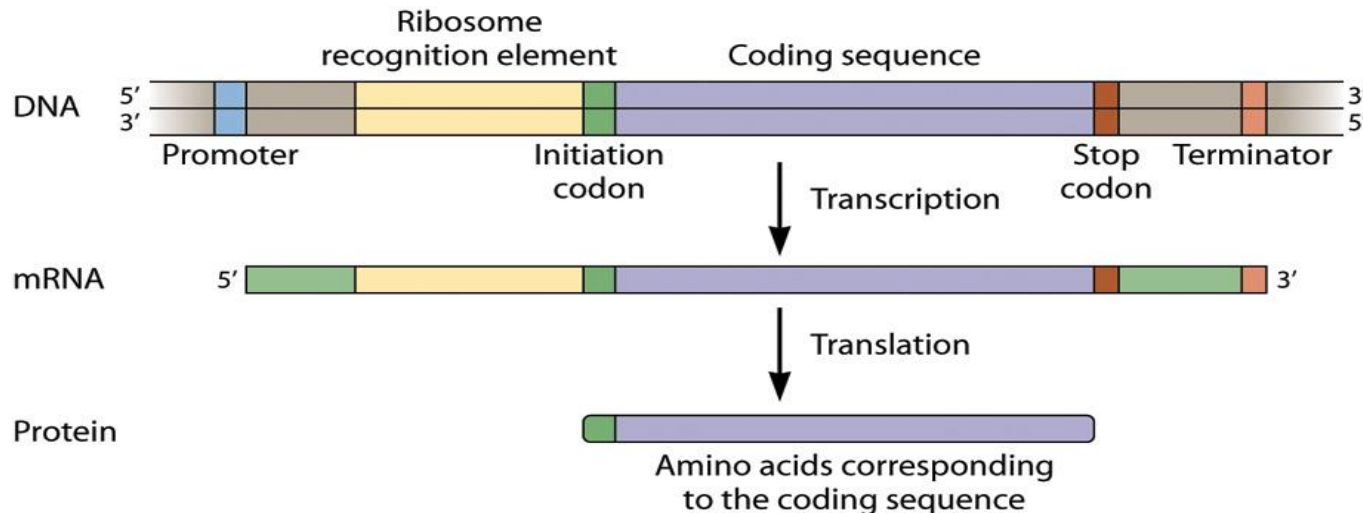
■ Translation

- Assembly of ribosome and mRNA
- Binding of tRNA
- Peptide bond formation
- Release of tRNA



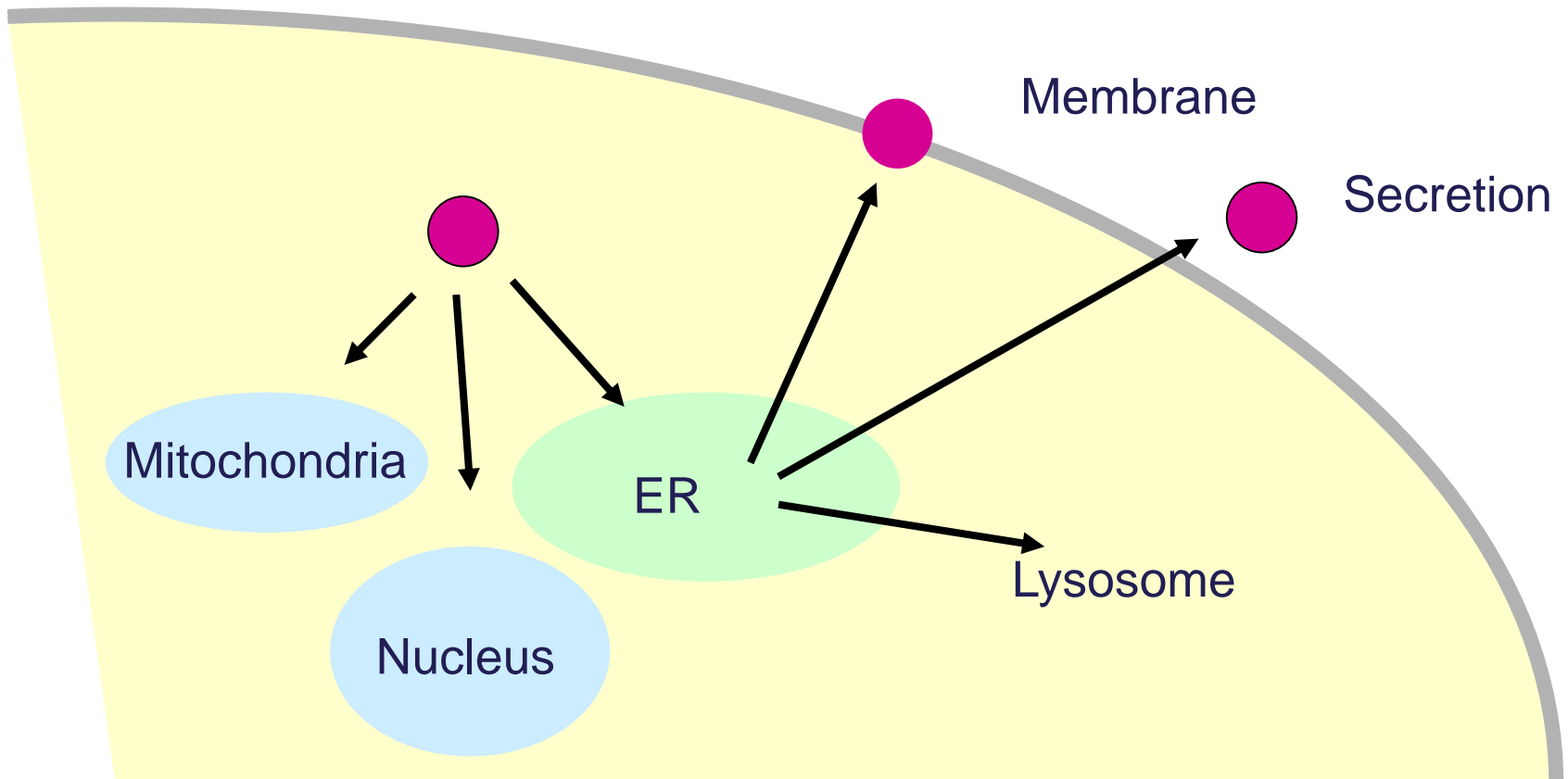
Signals for Transcription and Translation

- Ribosome binding site in mRNA
 - Shine-Dalgarno Sequence
- Initiation codon
 - AUG for methionine
- Stop codon
 - UGA, UAA, UAG : no corresponding tRNA

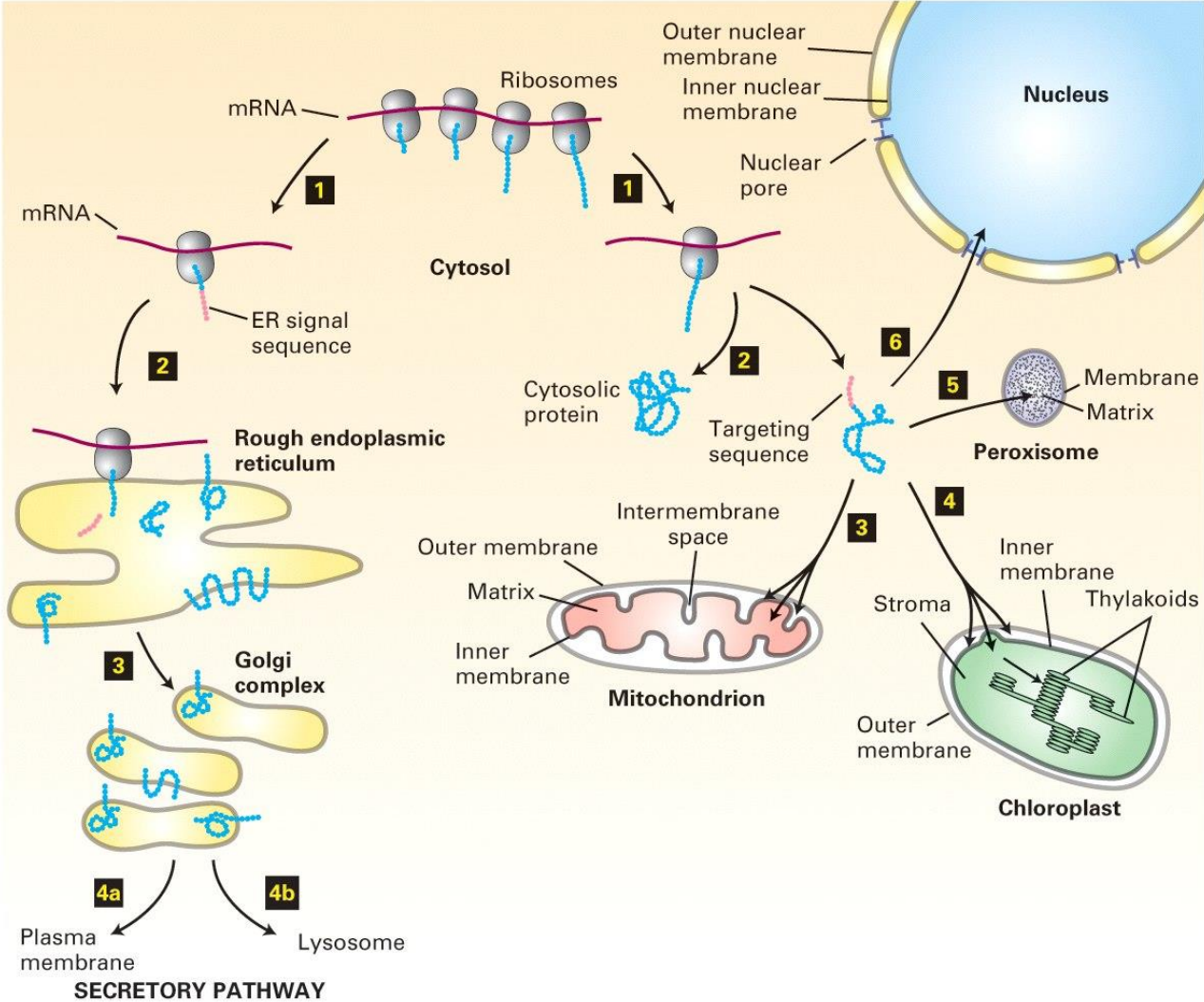


Cellular Fate of Proteins

- Protein Targeting to specific compartment (ER, Nucleus, Mitochondria) is guided by **signal peptide (tags)**

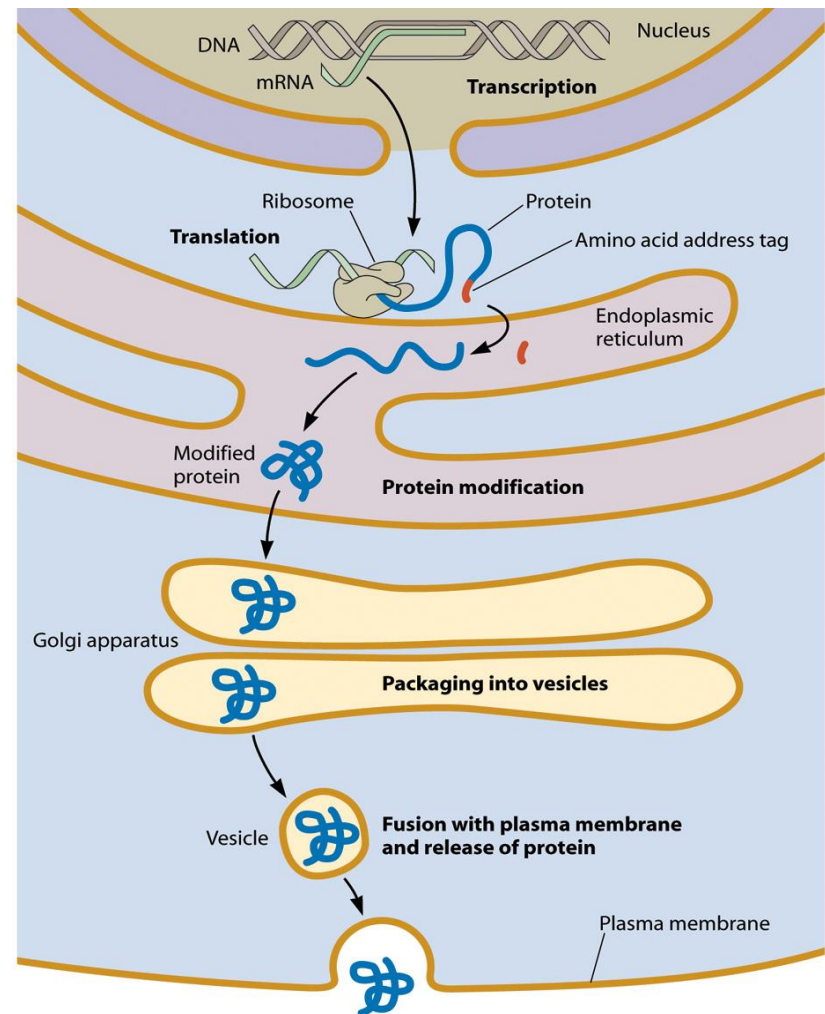


Overview of Protein Sorting Pathway



Protein Targeting to ER

- Proteins with signal peptide
 - Secretory proteins
 - Membrane proteins
 - Proteins in ER, Golgi, and lysosome
- Modification during transport from ER to Golgi apparatus
 - Glycosylation



Mutations

■ Mutation

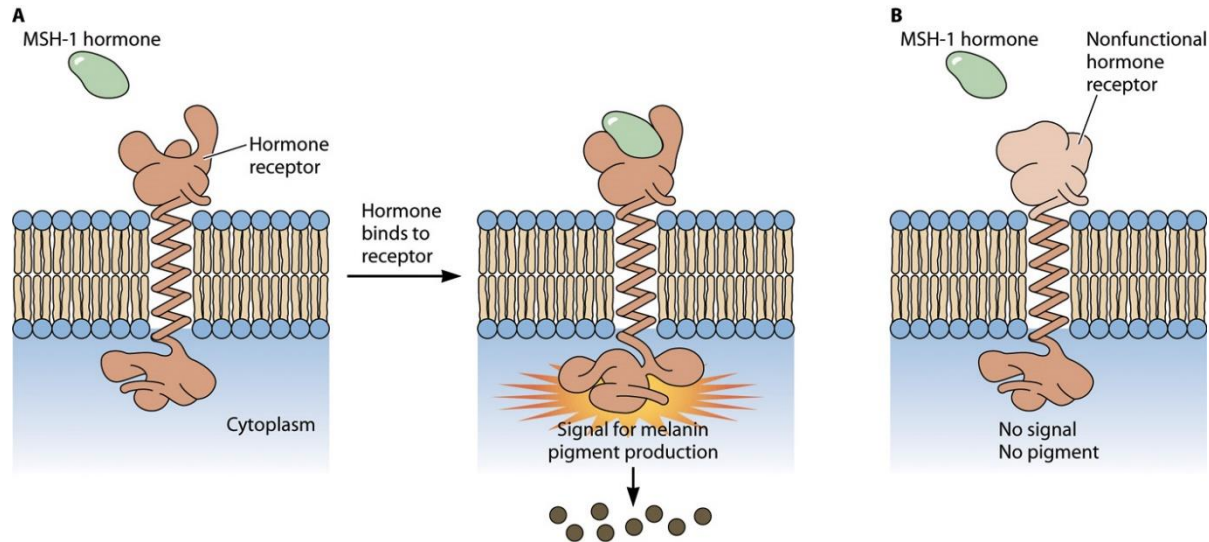
- Any change in a DNA sequence
 - During normal cellular processes
 - Error of DNA polymerase
 - Transposition (Chapter 13)
 - Environmental factors
 - DNA damage by UV or chemicals
- Source of genetic variation and evolution

■ Types of mutation

- Silent mutation: : mutation with no effect on a protein (the codon encoding the same amino acid)
- Mutations having slight effect : mutation in non-functional domain of a protein
- Mutations affecting protein function
 - Promoter or ribosome binding sequence : no protein synthesis
 - Essential protein sequence

Mutations

- No effect on survival
 - e.g. hair color



Mutations

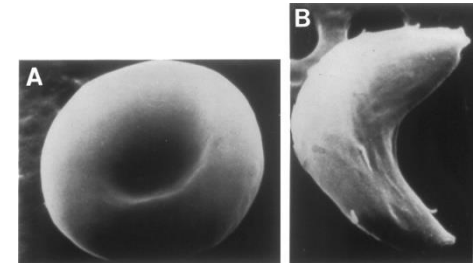
- Harmful

- e.g. sickle-cell anemia

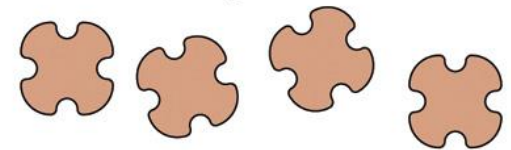
- A to T mutation of hemoglobin

→ 6th amino acid change from
glu to val

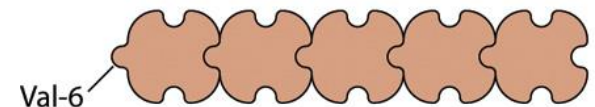
→ hydrophobic aggregation of
hemoglobin



A. Normal hemoglobin



B. Sickle-cell hemoglobin

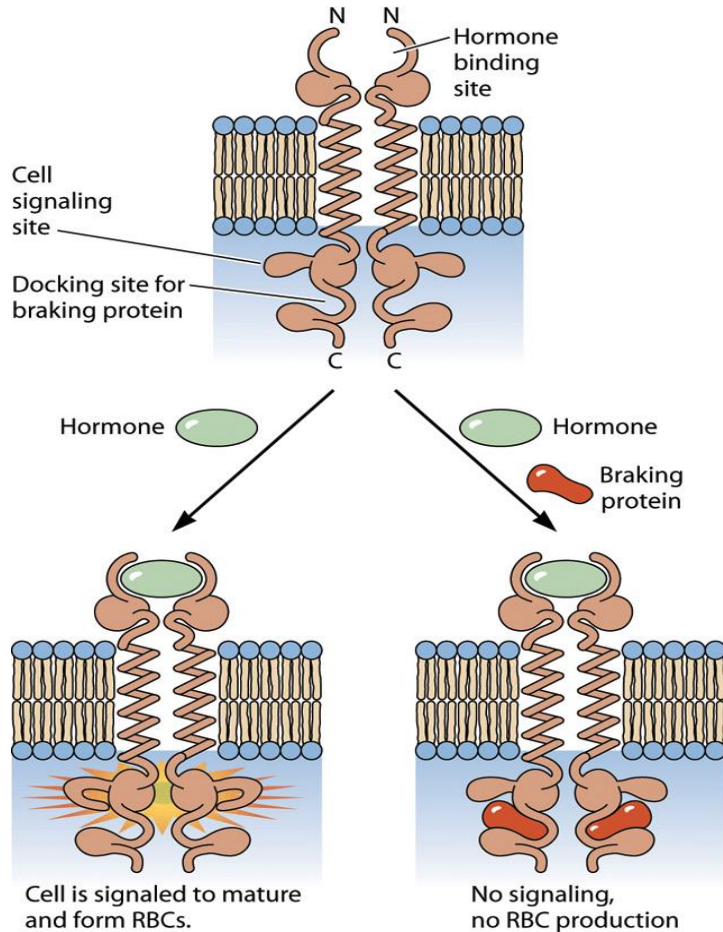


Mutations

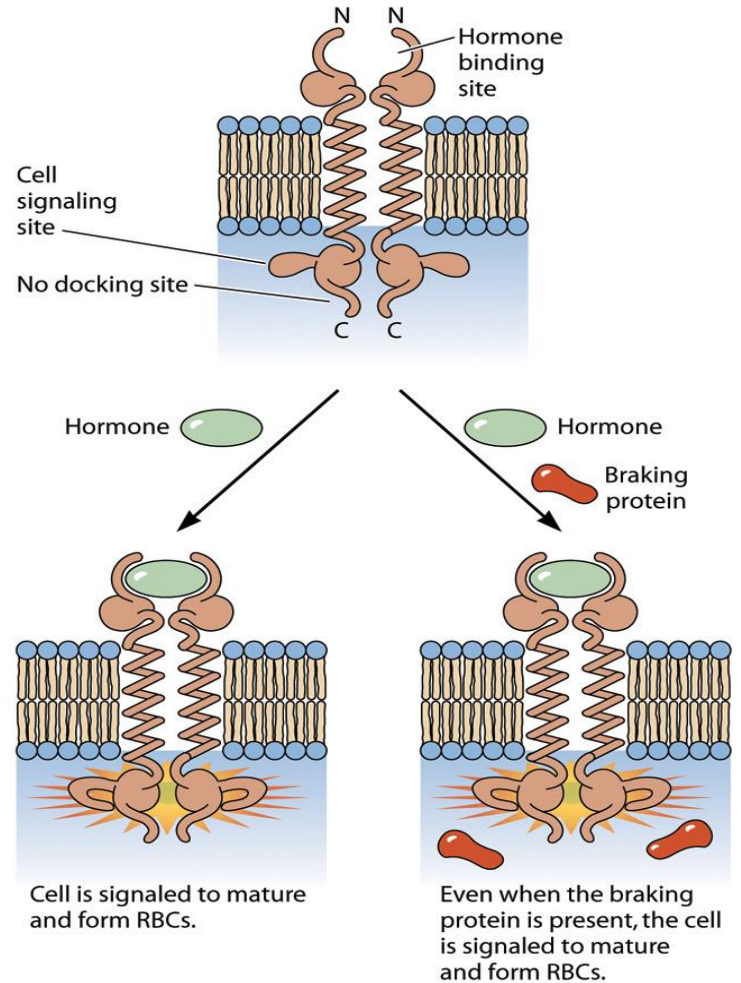
- Benign erythrocytosis
 - Elevated levels of RBC
 - Mutation in erythropoietin receptor
 - 481 TGG to TAG (stop codon)
 - Deletion of 70 amino acids for repression of signal transduction
 - More RBC production from bone marrow stem cells
 - Greatly enhanced stamina
 - (Finnish athlete Eero Mäntyranta won three gold medals for cross-country skiing in the 1964 Winter Olympics)

Mutations

A. Normal receptor protein



B. Mutant receptor protein



Chapter 5

Protein Structure and Function



Amino Acids and Primary Structure

■ Amino acids

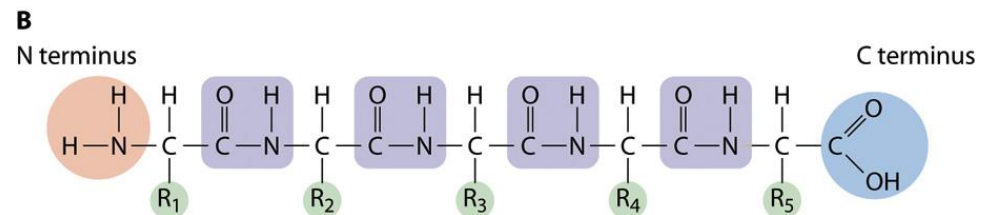
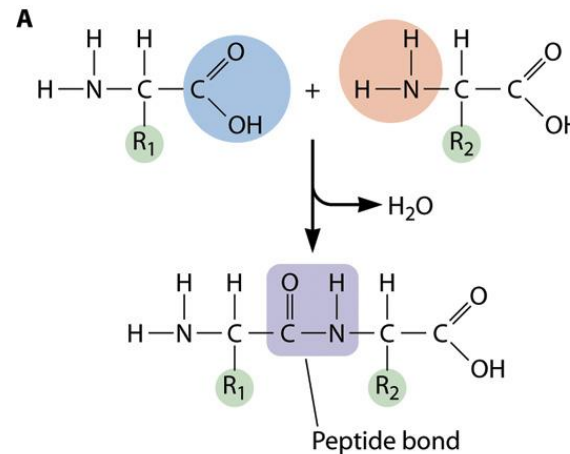
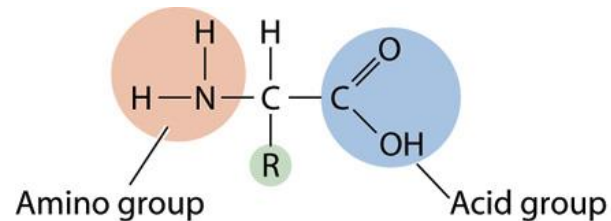
- Amino group
- Carboxyl group
- R group; 20 Side chains

■ Peptide bond

- Between NH_2 and COOH

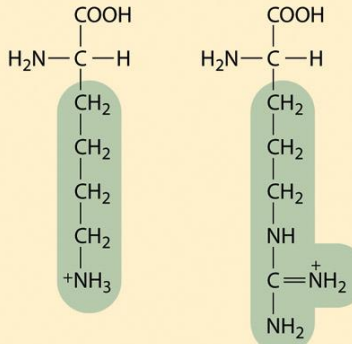
■ Polypeptide

- A chain of amino acids
- N terminus and C terminus



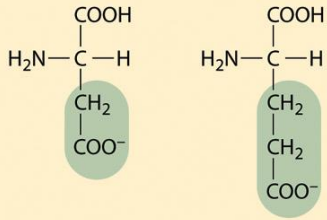
Amino Acids

Charged R groups



Lysine (Lys)

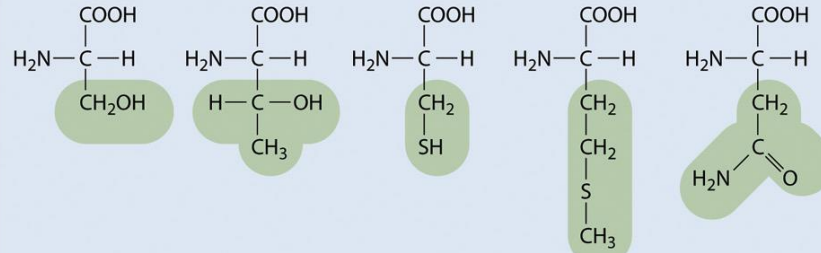
Arginine (Arg)



Aspartate (Asp)

Glutamate (Glu)

Polar R groups



Serine (Ser)

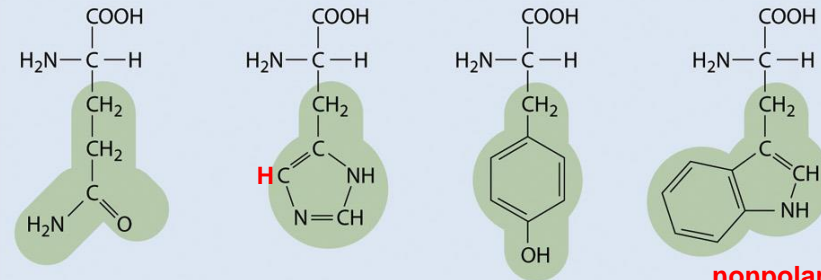
Threonine (Thr)

Cysteine (Cys)

Methionine (Met)

Asparagine (Asn)

nonpolar



Glutamine (Gln)

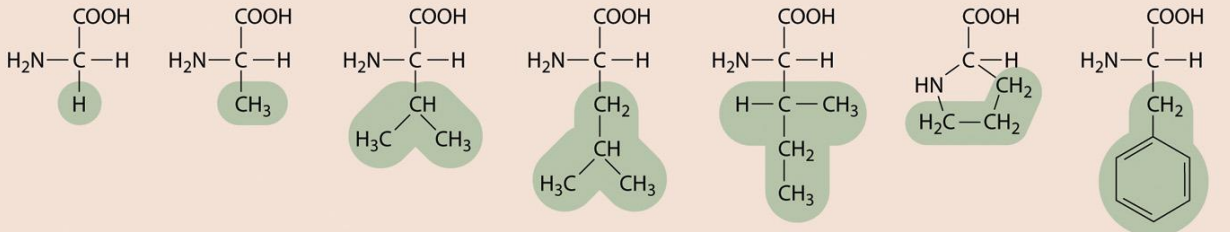
Histidine (His)

Tyrosine (Tyr)

Tryptophan (Trp)

nonpolar

Nonpolar R groups



Glycine (Gly)

Alanine (Ala)

Valine (Val)

Leucine (Leu)

Isoleucine (Ile)

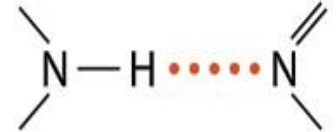
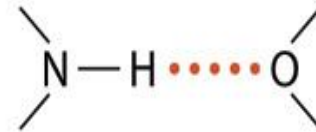
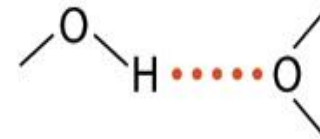
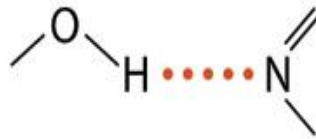
Proline (Pro)

Phenylalanine (Phe)

Primary and Secondary Structure

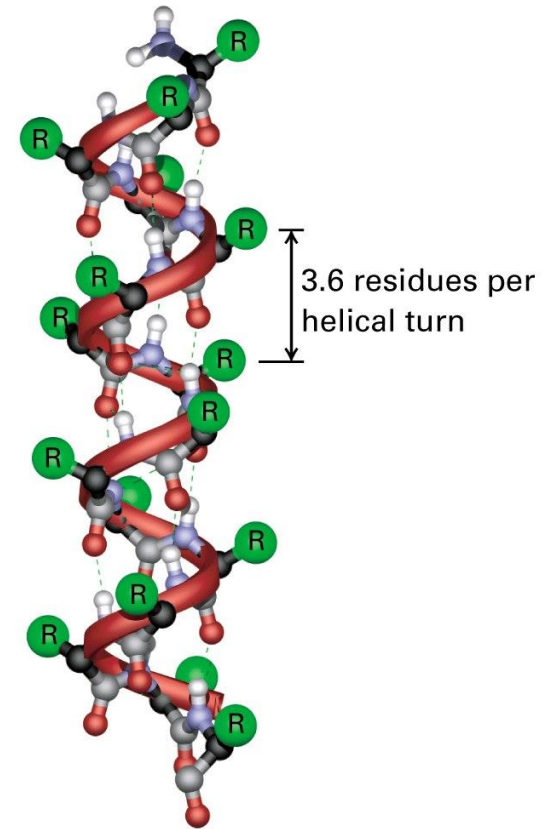
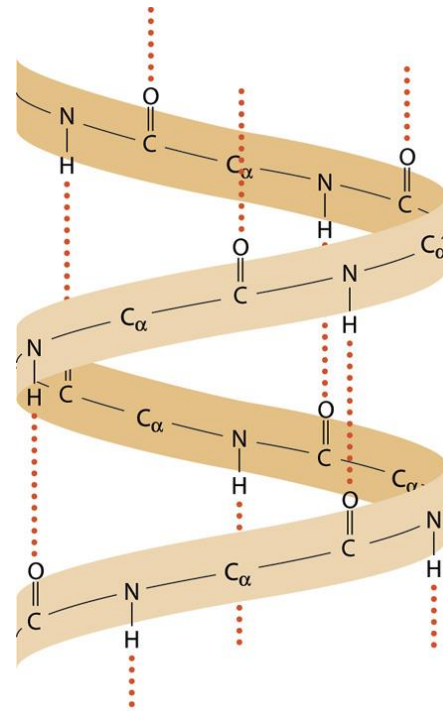
- Primary structure
 - Linear arrangement (sequence) of amino acids
- Secondary structure
 - Core elements of protein architecture
 - Neutralization of partial charges of the peptide backbone by hydrogen bonding
 - Local folding of polypeptide chain
 - α helix, β sheet : 60% of the polypeptide chain
 - Random coils and U-shaped turn

Common Hydrogen Bonds in Biological Systems



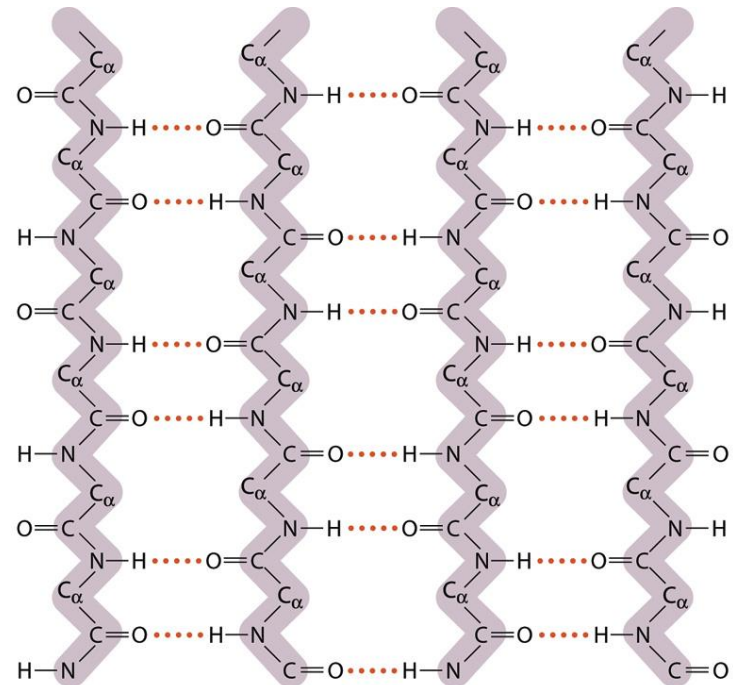
α -Helix

- Hydrogen bond between carbonyl O (n) and amid H (n+4)
- Directionality on the helix : The same orientation of H bond donor
- Side chains point outward : Determine hydrophobic or hydrophilic quality



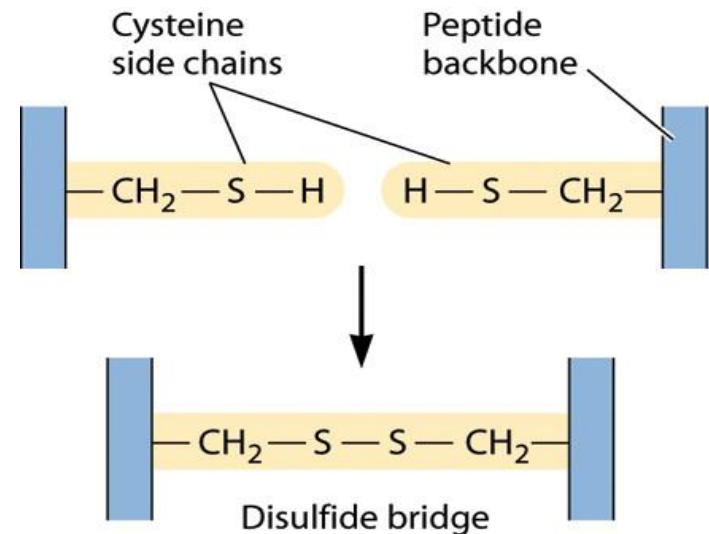
β -Sheet

- Hydrogen bonding between β strands \rightarrow β sheet, pleated sheet
- Usually not flat, but twisted



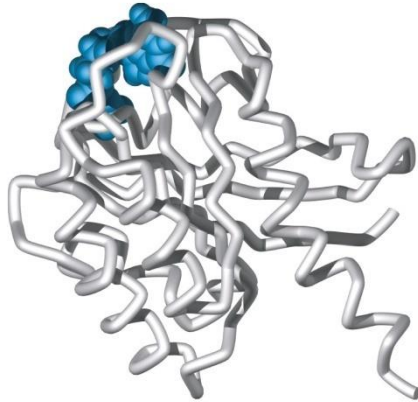
Tertiary Structure

- Overall folding of a polypeptide chain
- Stabilization
 - weak interaction
 - Hydrophobic interaction between nonpolar side chains
 - Hydrogen bond between polar side chains and peptide bonds
 - Disulfide bond formation

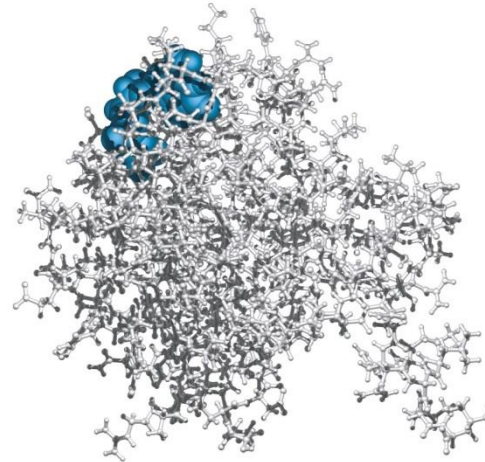


Graphical Representation of the Protein

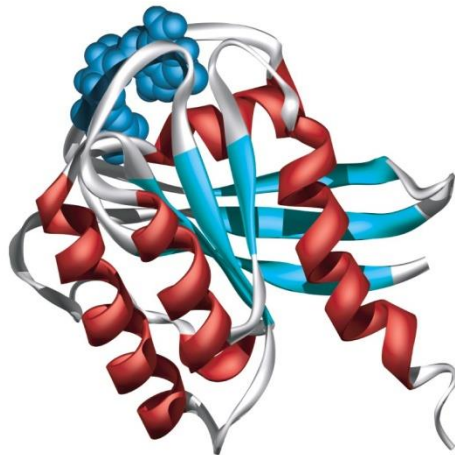
(a) C_{α} backbone trace



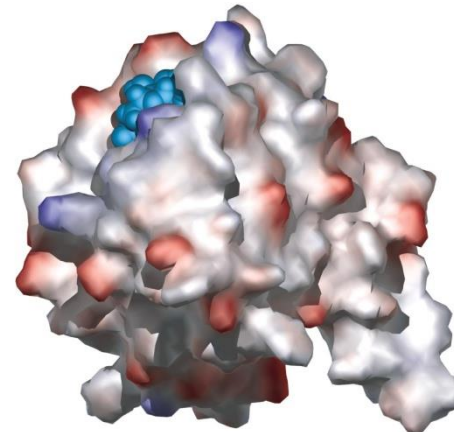
(b) Ball and stick



(c) Ribbons



(d) Solvent-accessible surface



Higher Levels of Structure

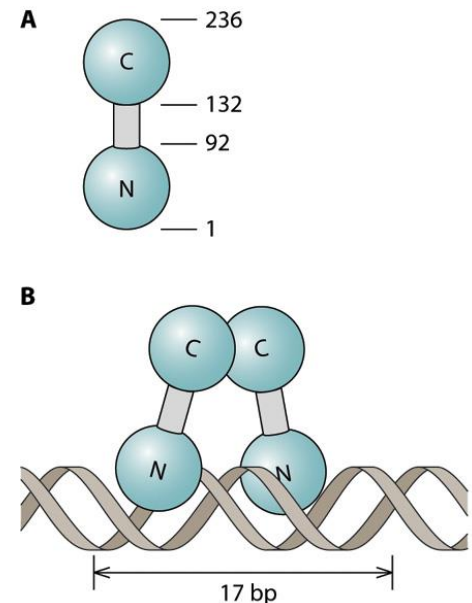
■ Domains

- One stable, compact, three-dimensional shape
- Fundamental units of protein structure and function
 - DNA binding domain, transmembrane domain

- e.g. lambda repressor (236 aa)

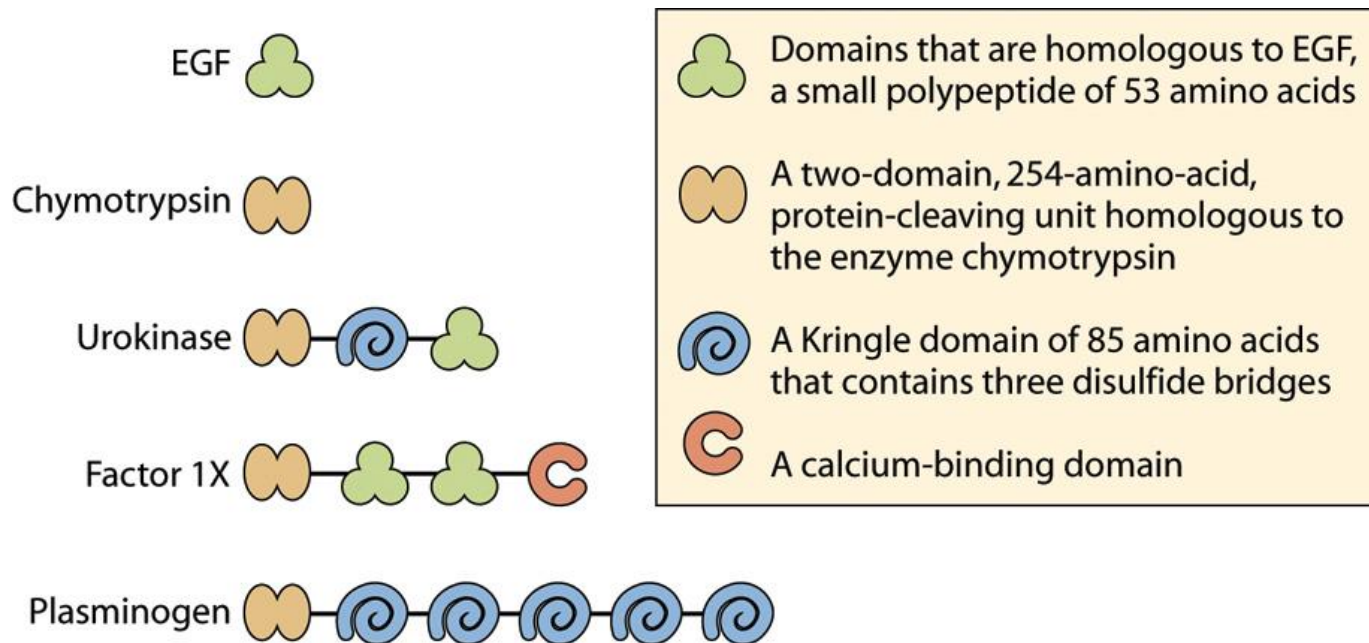
- N terminal domain : DNA binding
- C terminal domain :

Interaction with C terminal domain
of another molecule (dimerization)



Modular Proteins

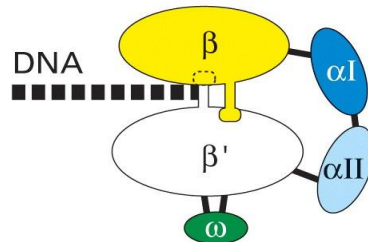
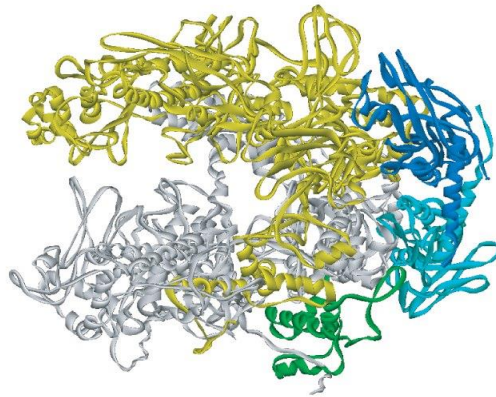
- New proteins by combination of functional domains
- Biotechnological application using recombinant DNA technology



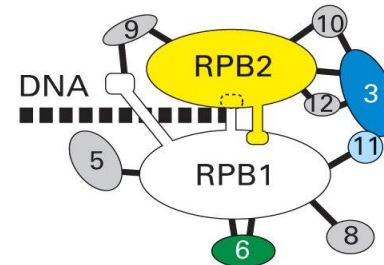
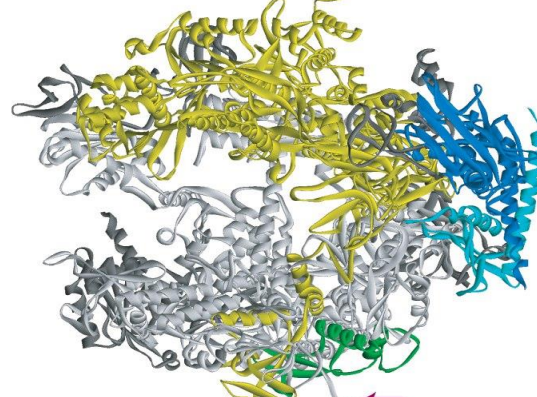
Quaternary Structure

- Association of multiple polypeptide chains
 - Lambda repressor : dimer
 - *E. coli* RNA polymerase : Five polypeptide chains

(a) Bacterial RNA polymerase



(b) Yeast RNA polymerase II



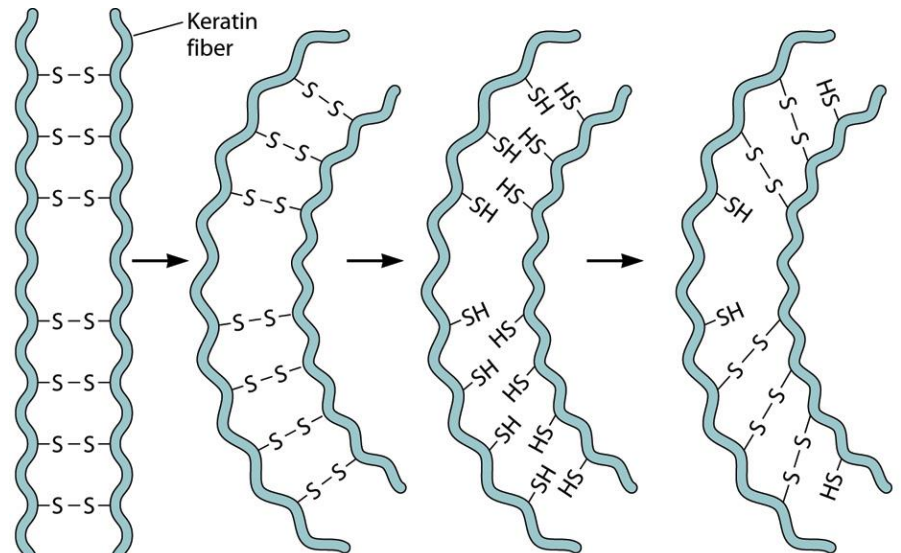
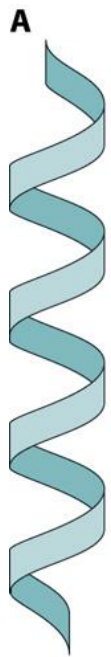
Disruption of Protein Structure

- Factors disrupting protein structure
 - Heat, extreme pH, organic solvent, detergent
- Denaturation
 - Complete unfolding of amino acid chain
 - Sometimes irreversible : e.g. boiled egg
 - Melting temperature (T_m)
 - Denaturation temperature for a given protein
 - Depending on protein structure
 - Proteins from organisms living at high temperature
 - Disulfide bridge increases protein stability

Examples of Protein Structure and Function

- Keratin
 - Structural protein for hair, wool, feathers, nails, scales, hooves, horns, skin
 - Very strong and water insoluble
 - Hydrophobic alpha helices
 - Long α -helix with hydrophobic amino acids
 - Forming fibers by hydrophobic interactions
 - Disulfide bonds
 - The more S-S bonds the harder the structure
 - Permanent hair wave
 - » Reducing of disulfide bond \rightarrow Generation of new disulfide bond

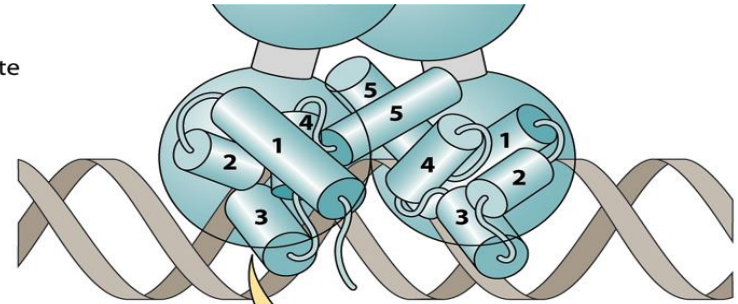
Examples of Protein Structure and Function



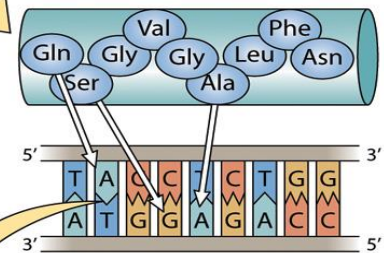
Lambda Repressor

- Binding of N-terminal domain helix 3 to specific bases within the DNA sequence

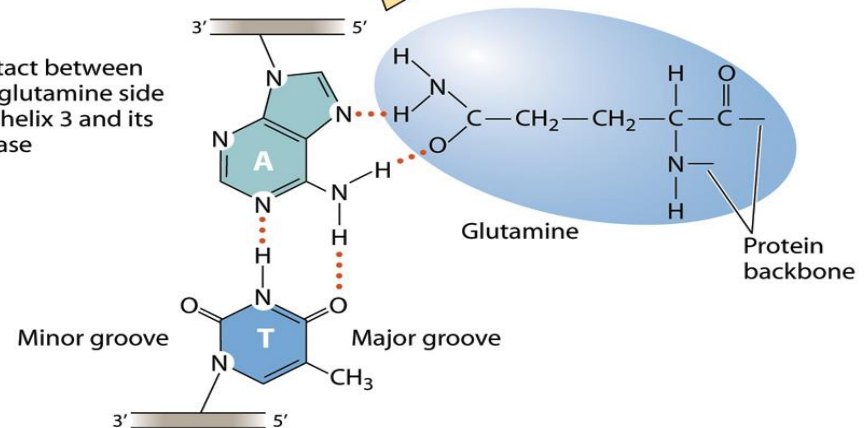
A. The orientation of protein helices on the DNA-binding site



B. The amino acid sequence of helix 3. The specific bases in the DNA sequence that are contacted by amino acid side chains are indicated by arrows.

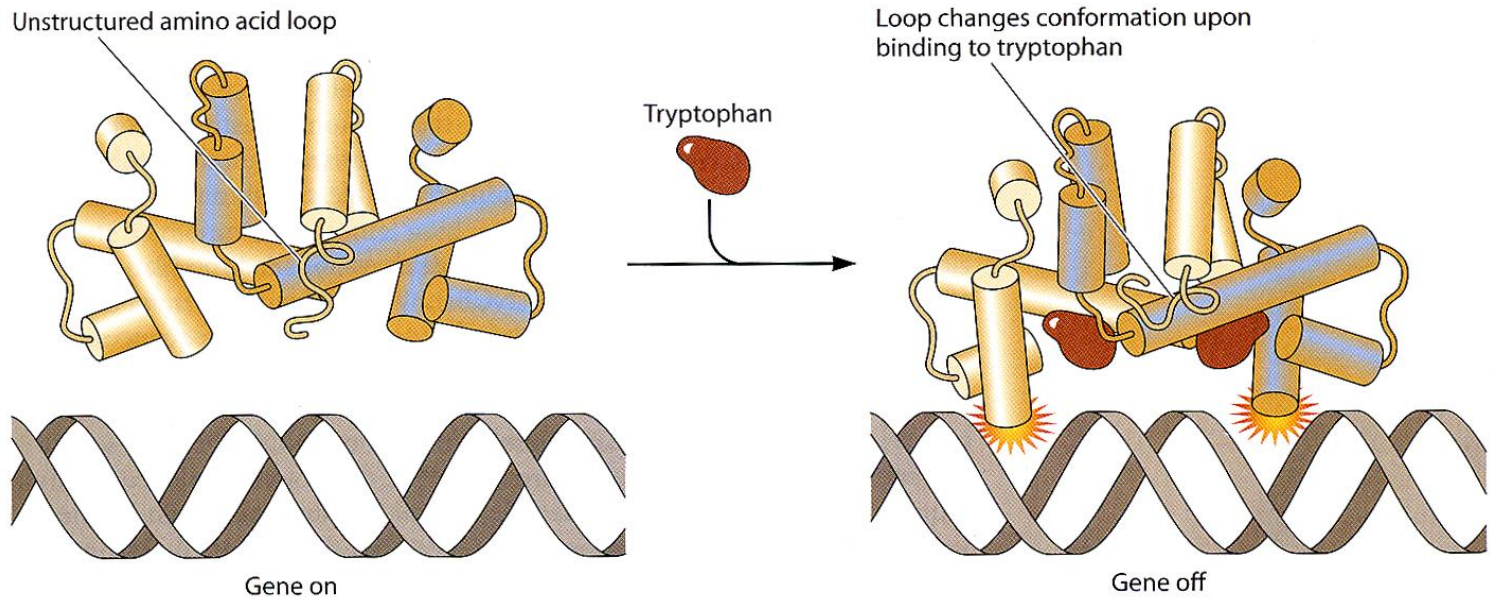


C. The contact between the first glutamine side chain in helix 3 and its target base



Trp Repressor

- If plenty of Trp in the cytosol
- Binding of Trp into Trp Repressor and change the conformation
- Trp repressor binds to DNA and represses the expression of genes involved in Trp synthesis



Predicting Protein Structure

- It is difficult to predict three dimensional structure from the amino acid sequence
- Compare to other proteins with known function or structure
 - Easy access of information through public database
 - NCBI (National center for Biotechnology Information) run by National Institutes of Health (NIH)
 - <http://www.ncbi.nlm.nih.gov>
 - Testing structure-function prediction
 - Using molecular biological tools
 - Introduction of mutations into protein
 - change of protein function

Protein Engineering

- Manipulation of protein's amino acid sequence to change its function or properties
- Chemical manufacturing
 - Develop enzymes more suitable for industrial applications
 - Increasing enzyme stability
 - e.g. bacteriophage lysozyme: introduce S-S bond to increase heat resistance
 - Proteases in detergent