



Review

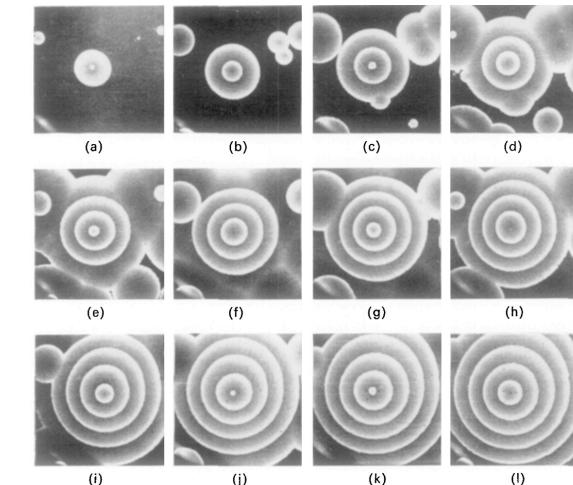
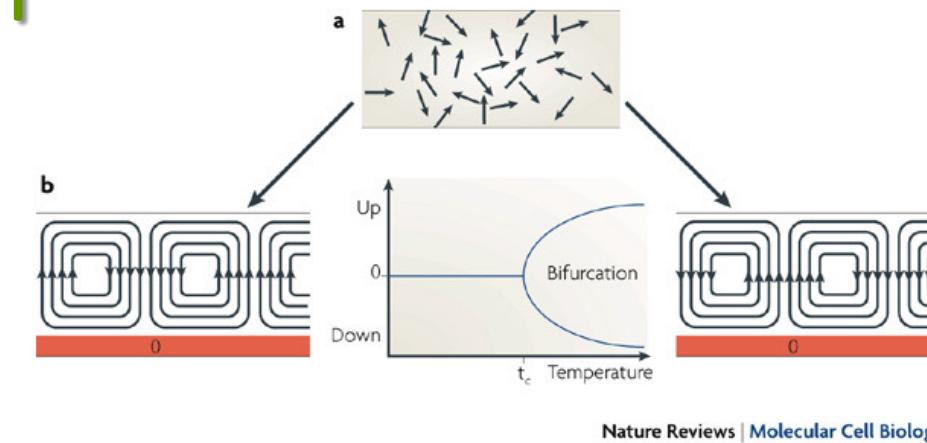
HIGHLIGHTS OF THE IMPORTANT TOPICS OF THE COURSE

Lecture 11

We discussed how principles of self-assembly leads to the notion that it is possible that a living cell could be created under the conditions existing in primordial earth

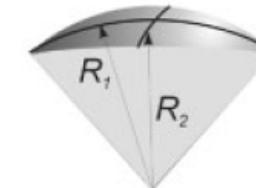
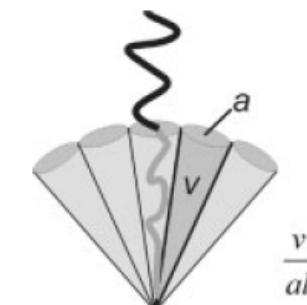
In-animate Systems

- ❖ Physical systems
- ❖ Chemical systems
- ❖ Biological self-assembled systems

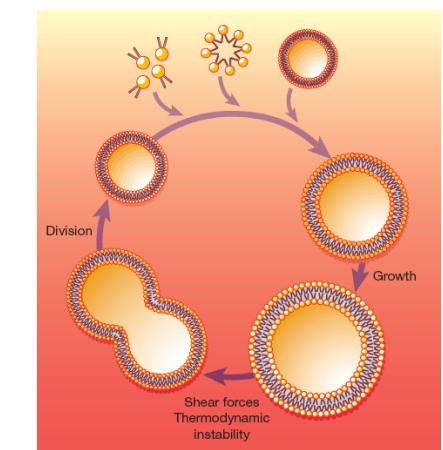


Living Systems

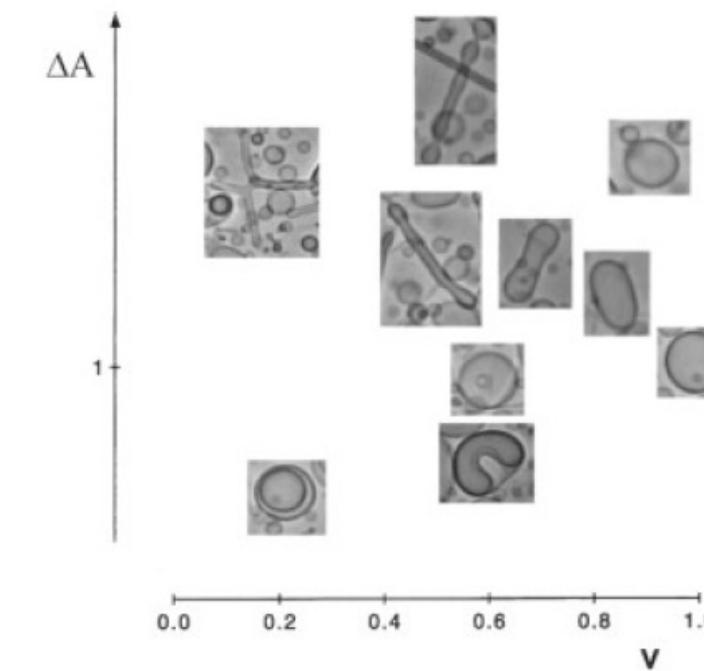
- ❖ The cell



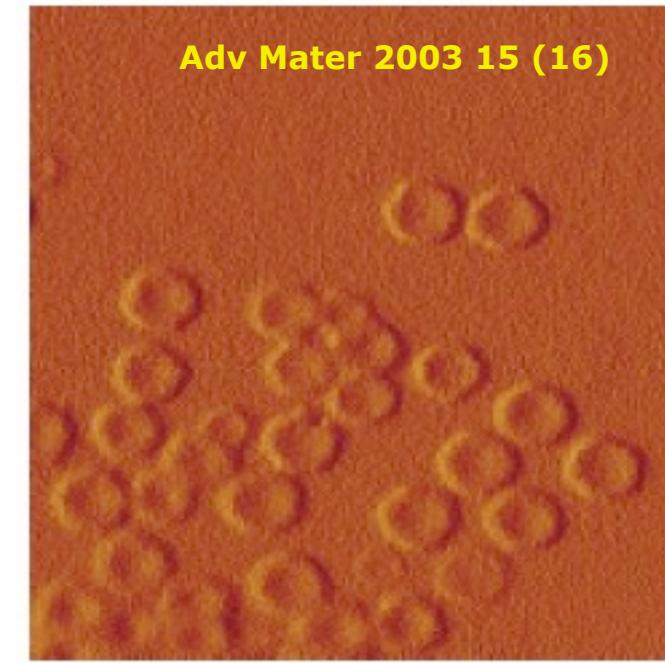
$$H = \frac{1}{2} \left(\frac{1}{R_1} + \frac{1}{R_2} \right)$$
$$K = \frac{1}{R_1 R_2}$$



Vesicle of any desired size and shape can be synthesized under lab conditions



- ❖ Depends on mixing entropies (pull towards many assemblies) and molar bending energies (tends towards a smaller number of vesicles)
- ❖ ΔA is the difference in area, v is the dimensionless volume to area ratio

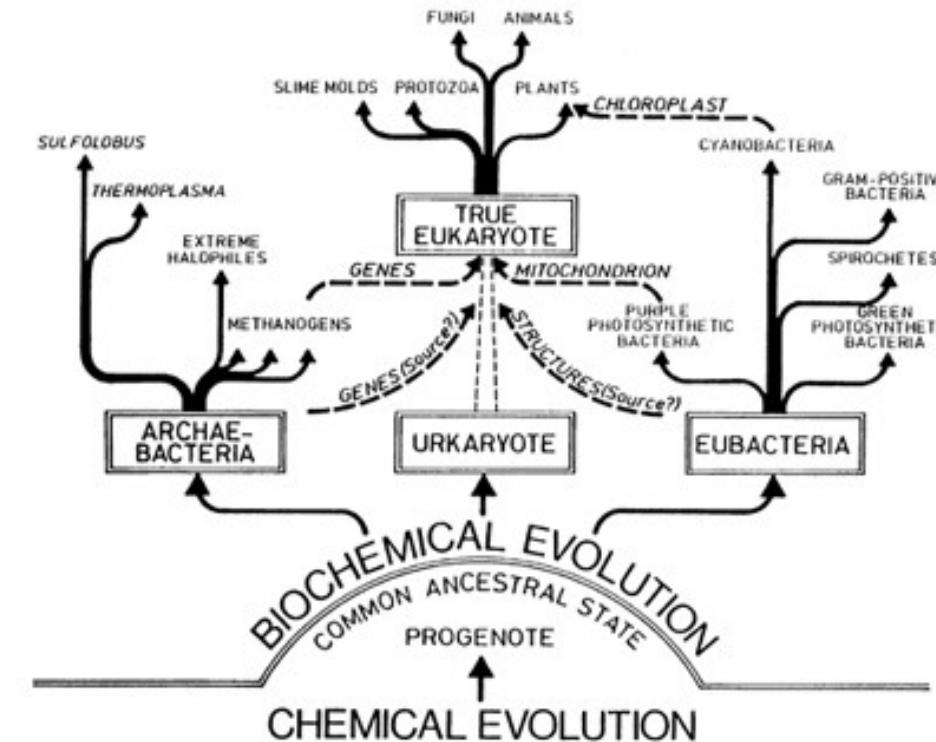


AFM image of a polymer vesicle. The erythrocyte-like shape arises from the evaporation of water from the vesicle interior, leaving the prominent rim

Last Universal Common Ancestor

- ❖ The evolution of the translation apparatus occurred in a series of increasingly complex stages, rather than all at once,
- ❖ The stages subsequent to the establishment of the basic mechanism were concerned by and large with increasing the mechanism's accuracy, and possibly speed as well

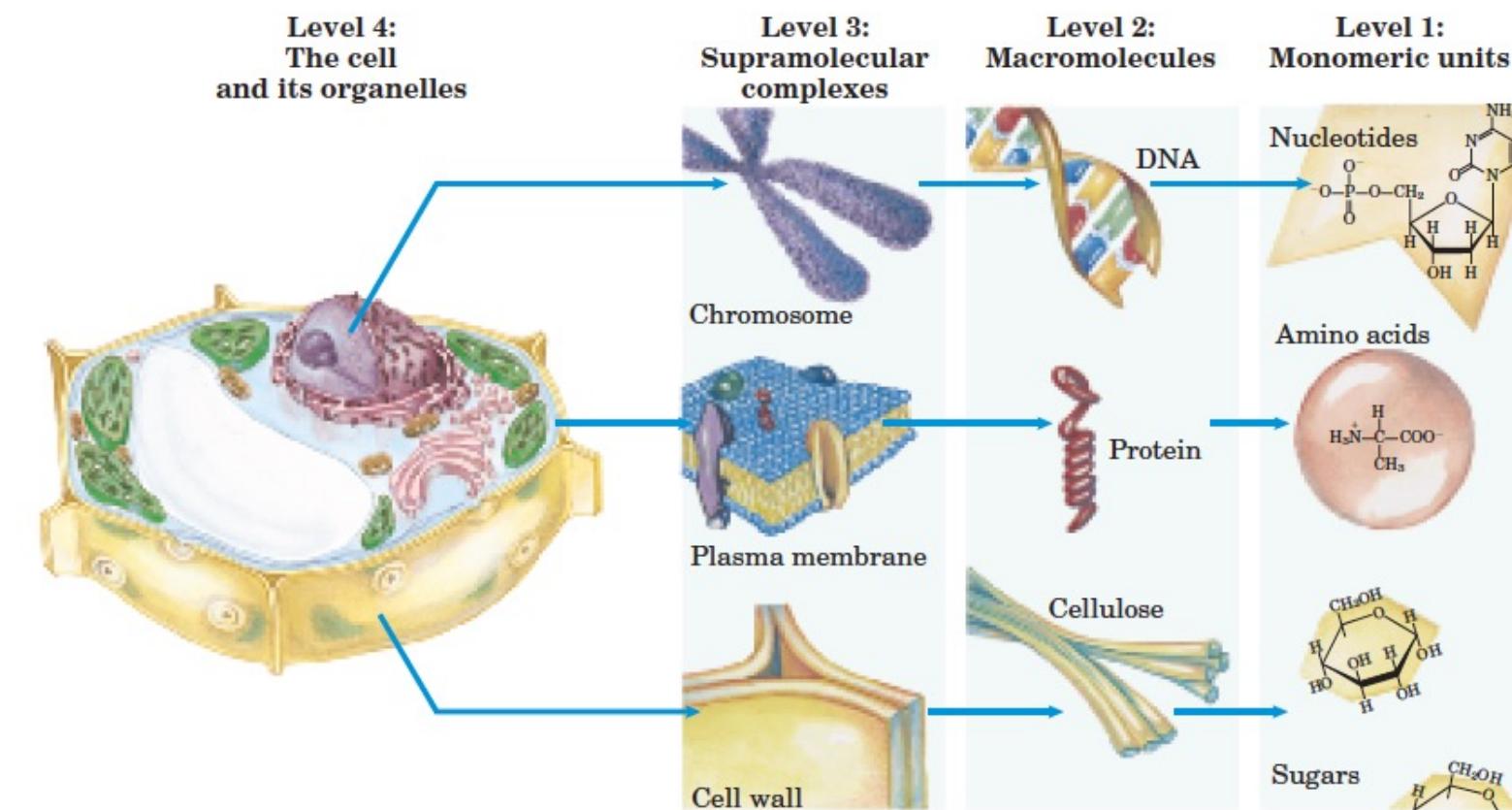
J. Mol Evol 10, 1-6, 1977



Fundamental Units of Life

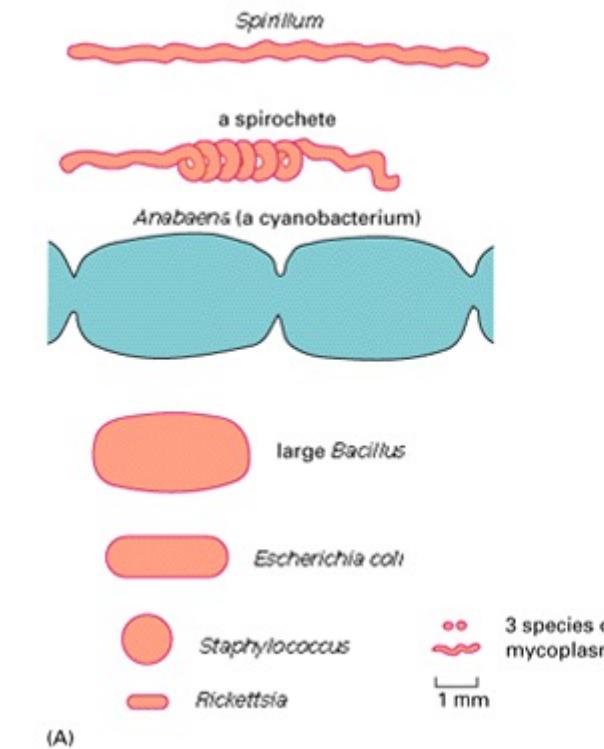
The main biomolecules that are part of the building blocks of cells

- ❖ Carbohydrates and Lipids – cell structure
- ❖ Amino acids – proteins
- ❖ Nucleotides – DNA and RNA



Prokaryotic Cells Are Structurally Simple but Biochemically Diverse

- ❖ Bacteria are the simplest organisms found in most natural environments.
- ❖ Spherical or rod-shaped cells, commonly several micrometers in linear dimension Possess a tough protective coat, cell wall, beneath which a plasma membrane encloses a single cytoplasmic compartment containing DNA, RNA, proteins, and small molecules.



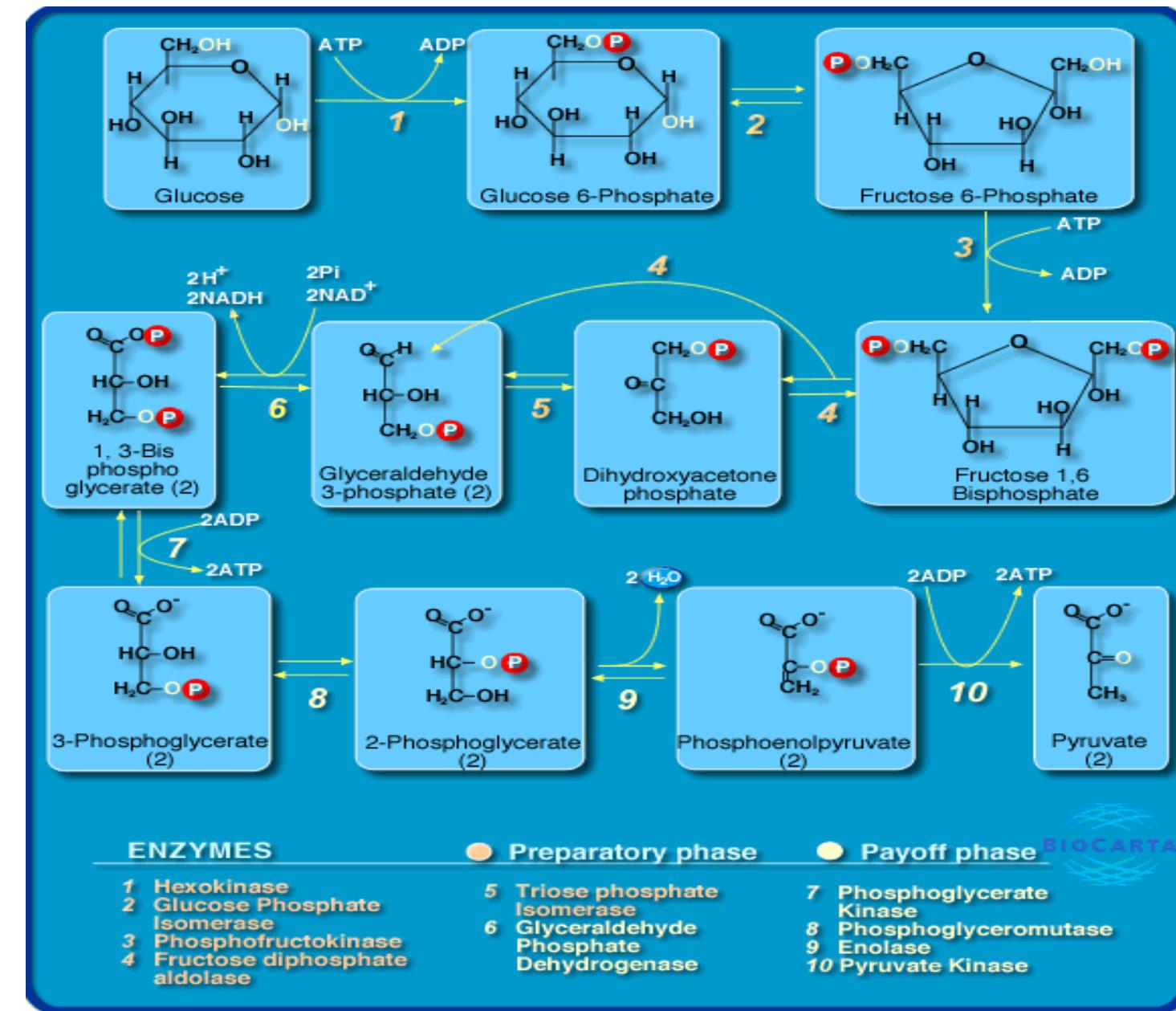
Prokaryote sizes and structures. (A) Some prokaryotic cells drawn to scale. (B) Electron micrograph of a longitudinal section through a bacterium (*Escherichia coli*); the cell's DNA is concentrated in the palely stained region. (Courtesy of E. Kellenberger.)

More about metabolic reactions

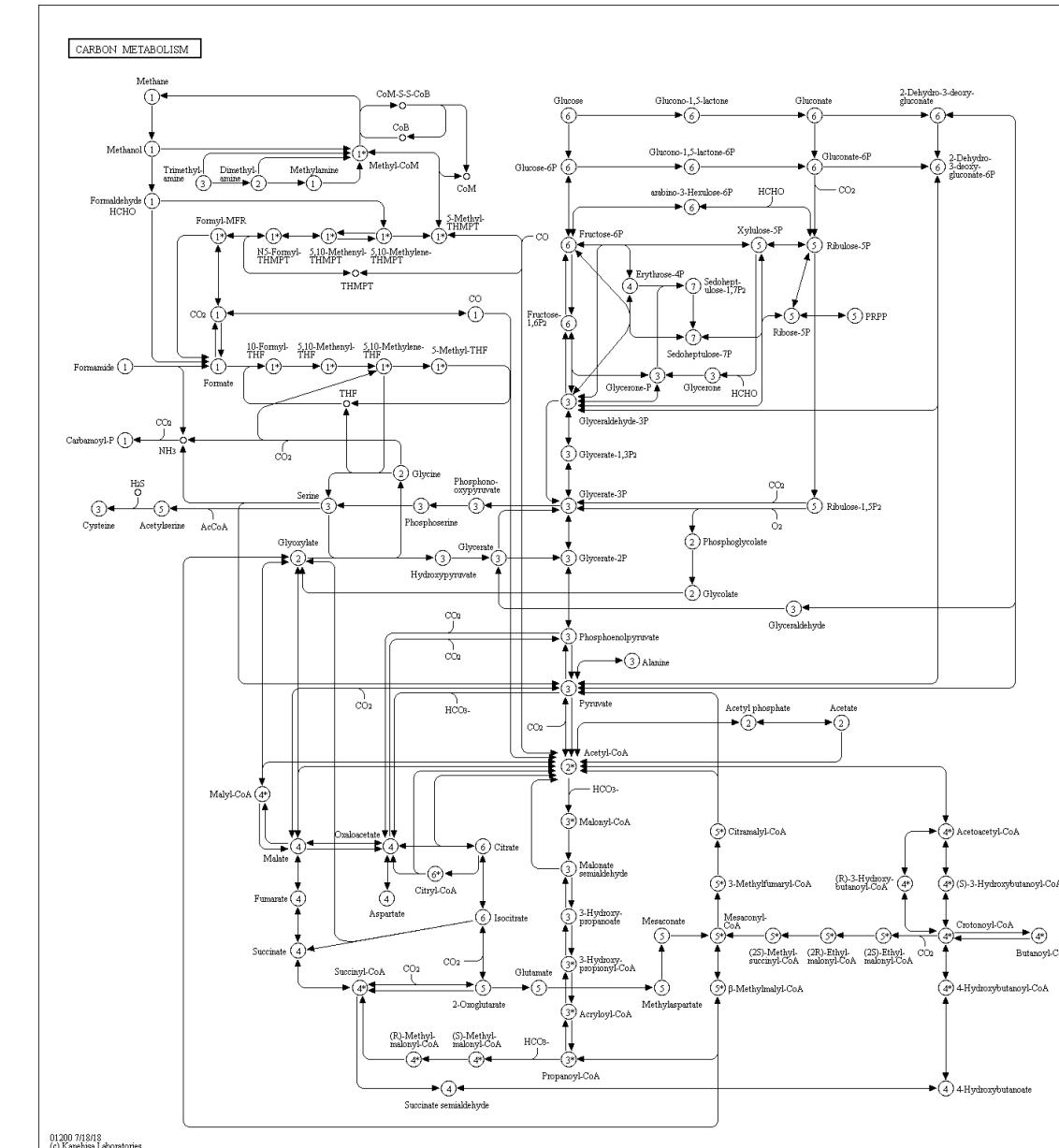
❖ KEGG



- ❖ Similarity in all kinds of organisms, suggesting an extremely ancient origin
- ❖ Linked to core reactions of glycolysis are hundreds of other chemical processes
 - ❖ Generation of energy in ATP-ADP currency
 - ❖ Synthesis of small molecules
 - ❖ Make large polymers specific to the organism
 - ❖ Degrade complex molecules, taken in as food, into simpler chemical units



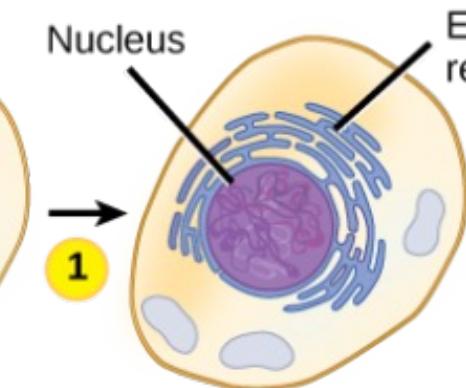
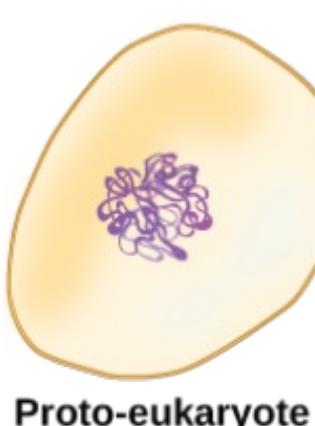
Carbon Metabolism



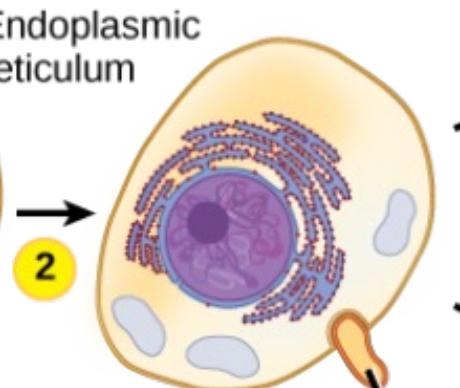
The creation of the eukaryote cell

The ENDOSYMBIOTIC THEORY

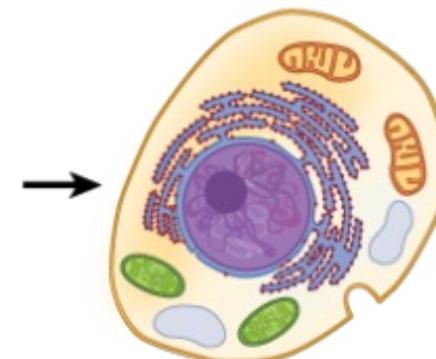
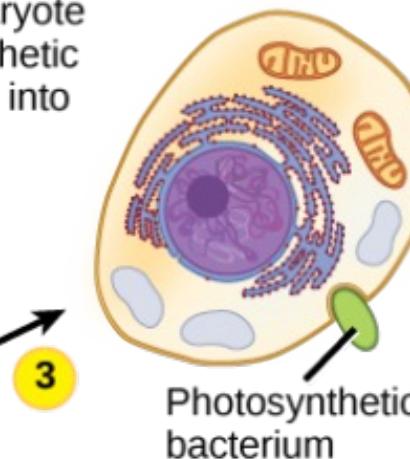
1 Infoldings in the plasma membrane of an ancestral prokaryote gave rise to endomembrane components, including a nucleus and endoplasmic reticulum.



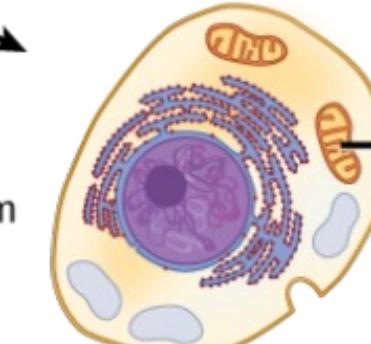
2 In a first endosymbiotic event, the ancestral eukaryote consumed aerobic bacteria that evolved into mitochondria.



3 In a second endosymbiotic event, the early eukaryote consumed photosynthetic bacteria that evolved into chloroplasts.

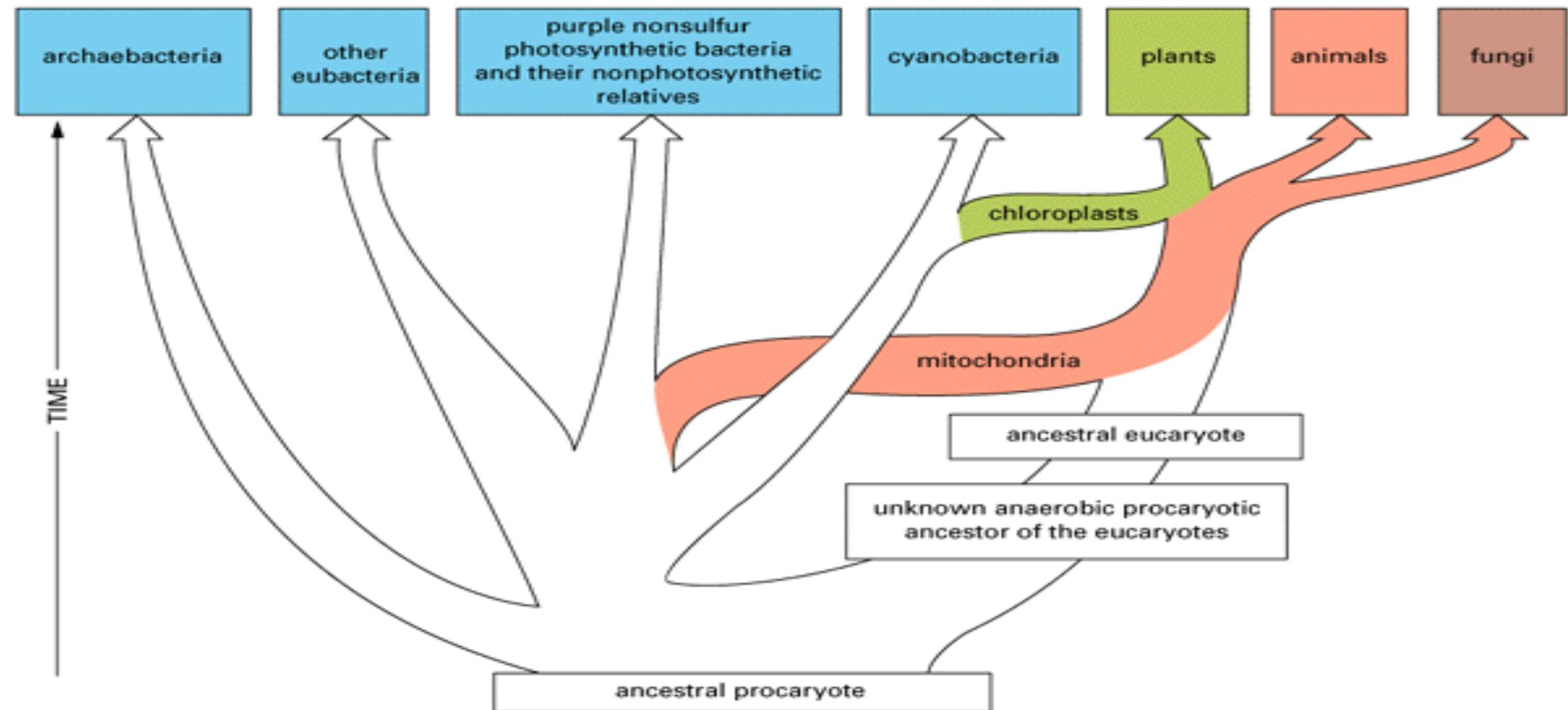


Modern photosynthetic eukaryote



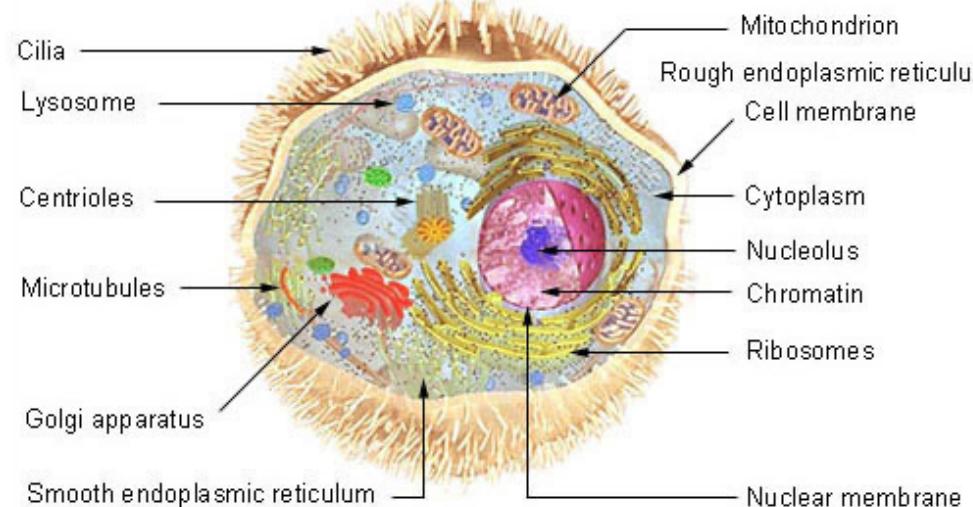
Modern heterotrophic eukaryote

Postulated origin of the eukaryotic cell



Self Assembly and self organization of internal cellular structures

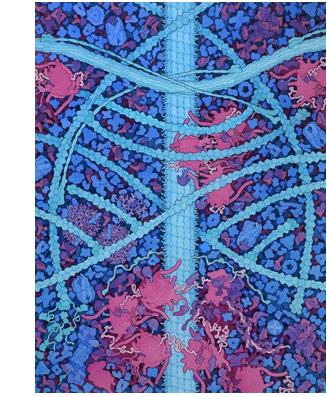
Cell Structure



Flocks of Starlings



Autophagy



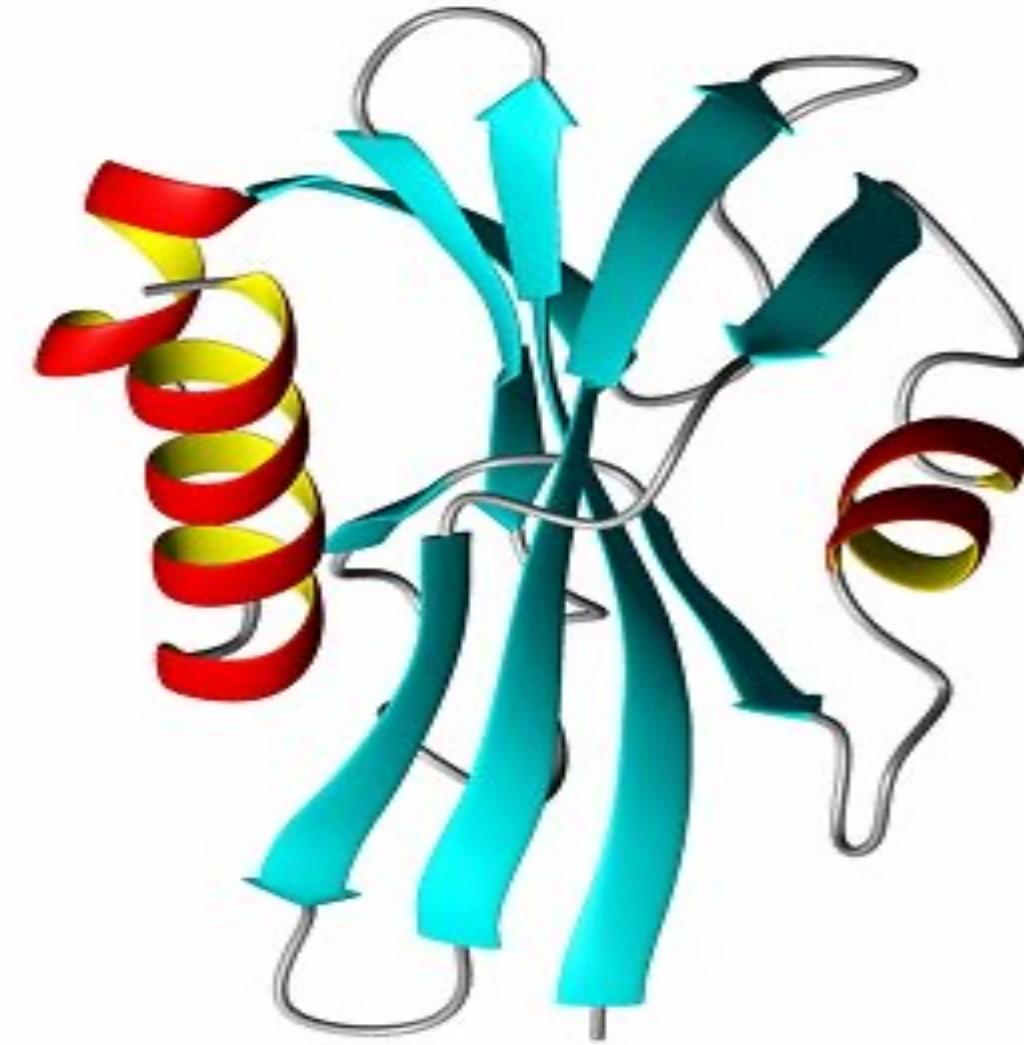
Cytoskeleton

Images from the RCSB site

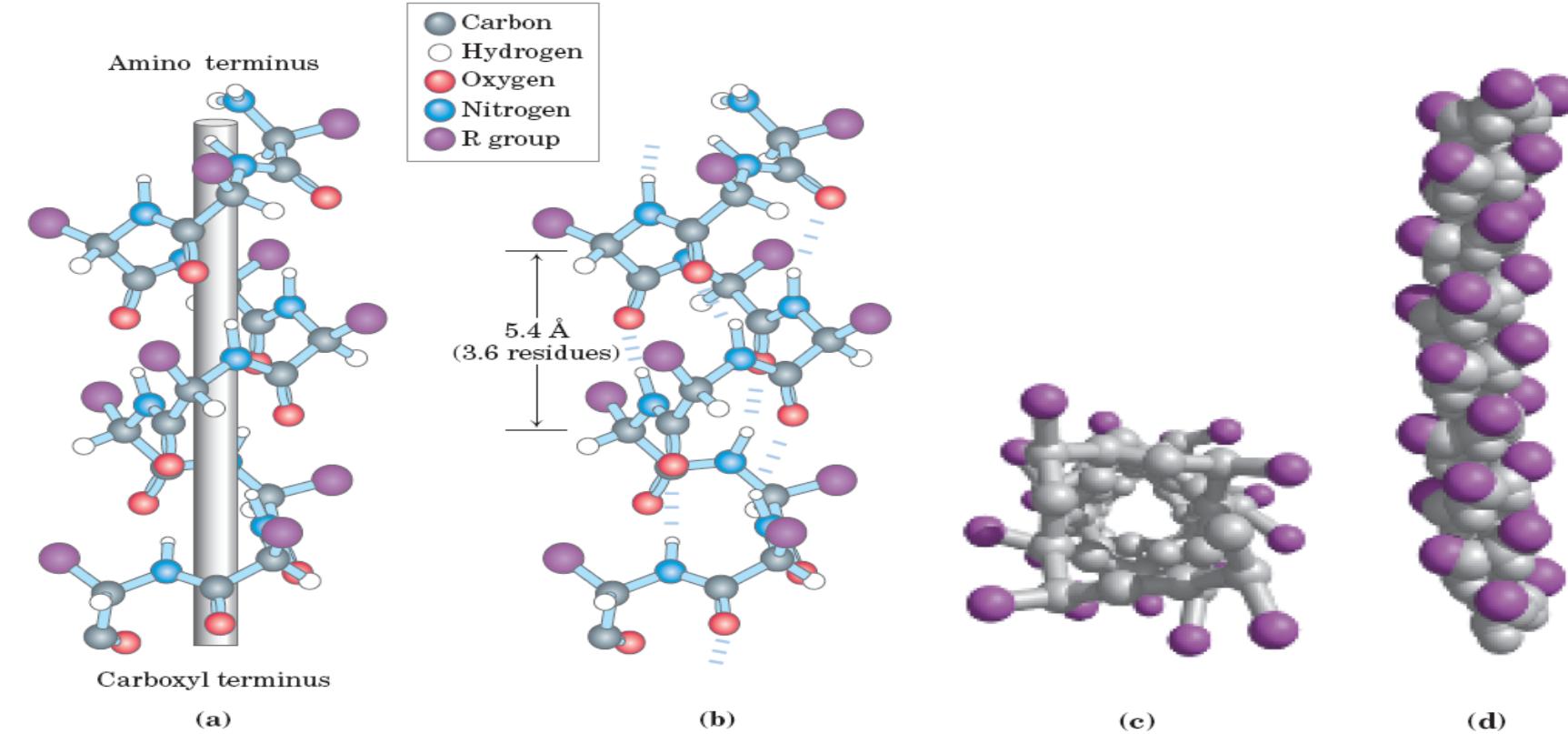
<https://ccsb.scripps.edu/goodsell/>

Protein Folding

- ❖ Secondary Structures
 - ❖ Alpha Helix
 - ❖ Beta Sheet
- ❖ Tertiary Structure (3-D conformation)
- ❖ Quarternary Structure

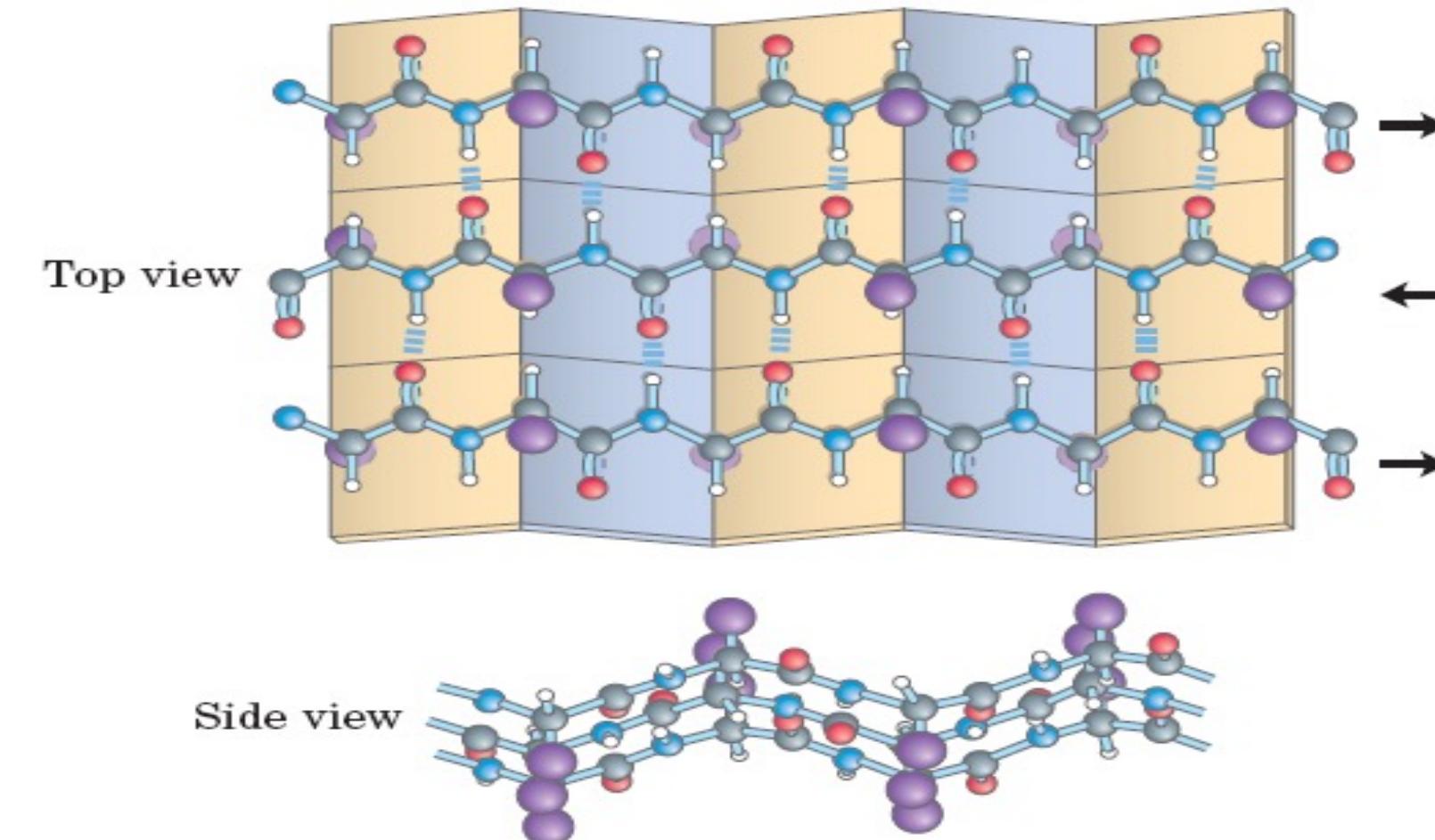


N
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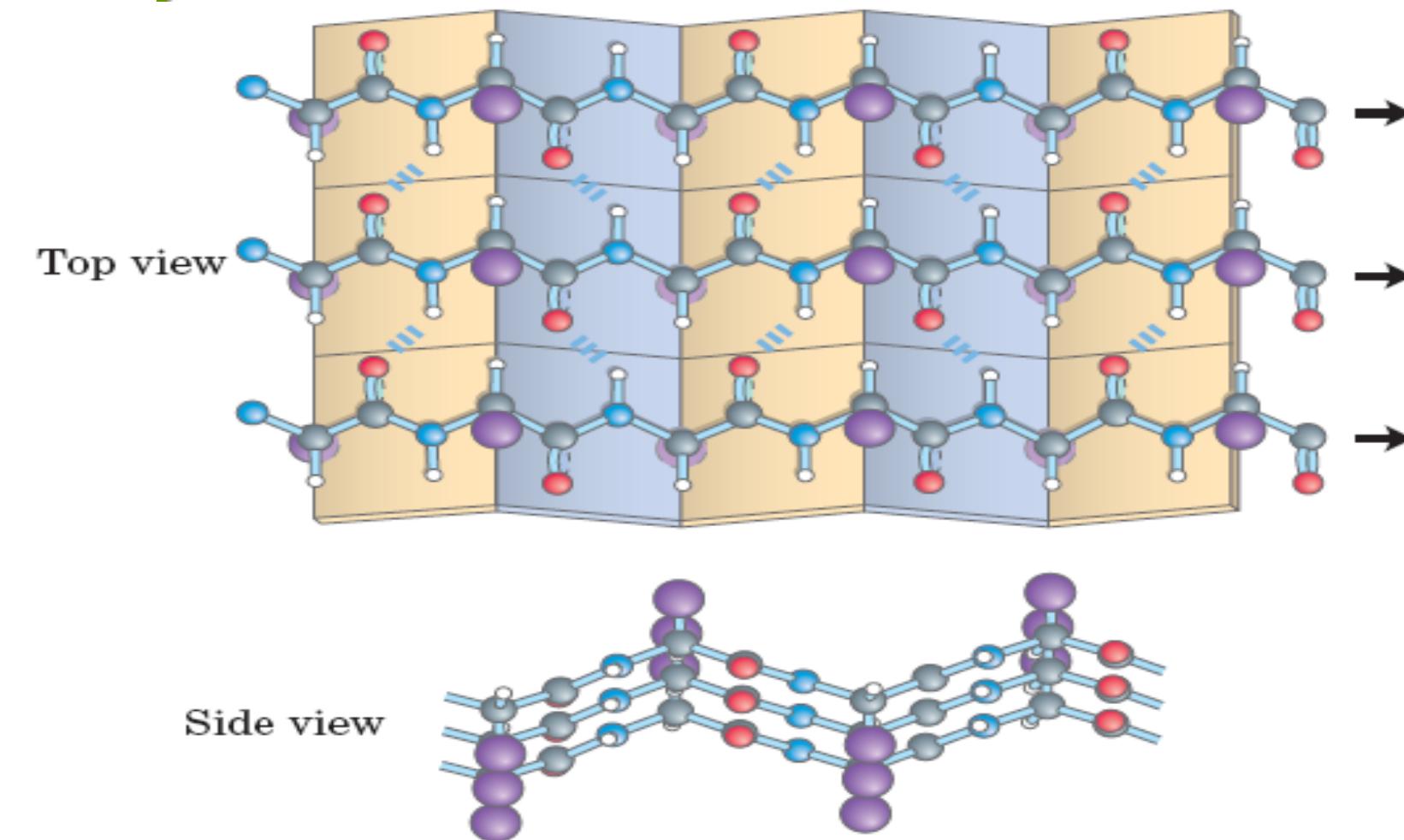


- Formation of a right-handed α -helix. The planes of the rigid peptide bonds are parallel to the long axis of the helix, depicted here as a vertical rod
- Ball-and-stick model of a right-handed α -helix, showing the intrachain hydrogen bonds. The repeat unit is a single turn of the helix, 3.6 residues
- The α -helix as viewed from one end, looking down the longitudinal axis
- Atoms in the center of the α -helix are in very close contact

Antiparallel β -Sheet Conformation



Parallel β -Sheet Conformation



Stability of protein structures

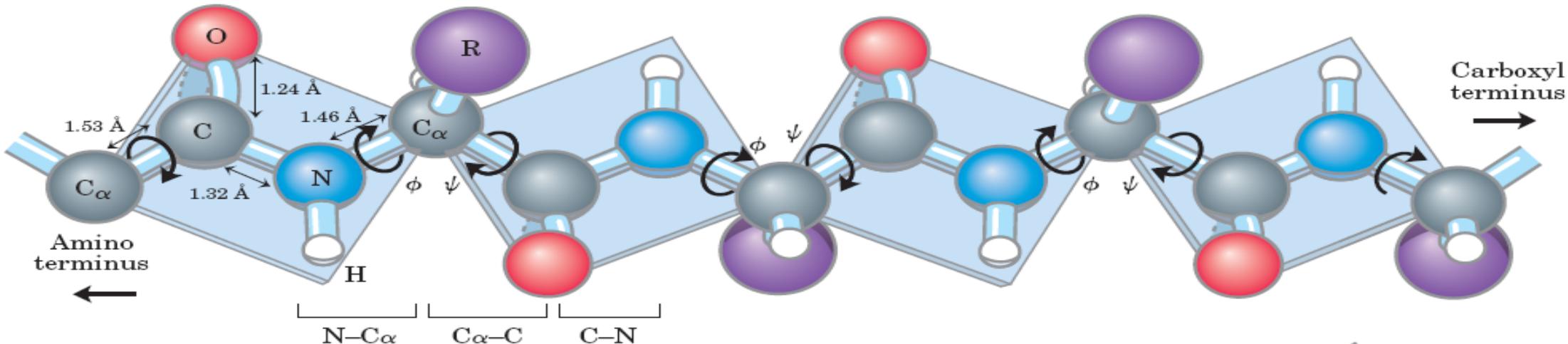
1. The stability of protein structures depends on weak interactions
2. It requires 200-460 kJ/mol to break a single covalent bond, compared to 4-30kJ/mol for weak interactions
 - a) The weak interactions predominate because they are numerous
3. The free energies of the folded and un-folded states are similar

Governing Equations

$$\Delta G = \Delta H - T \Delta S$$

$$\Delta G = -RT\ln k$$

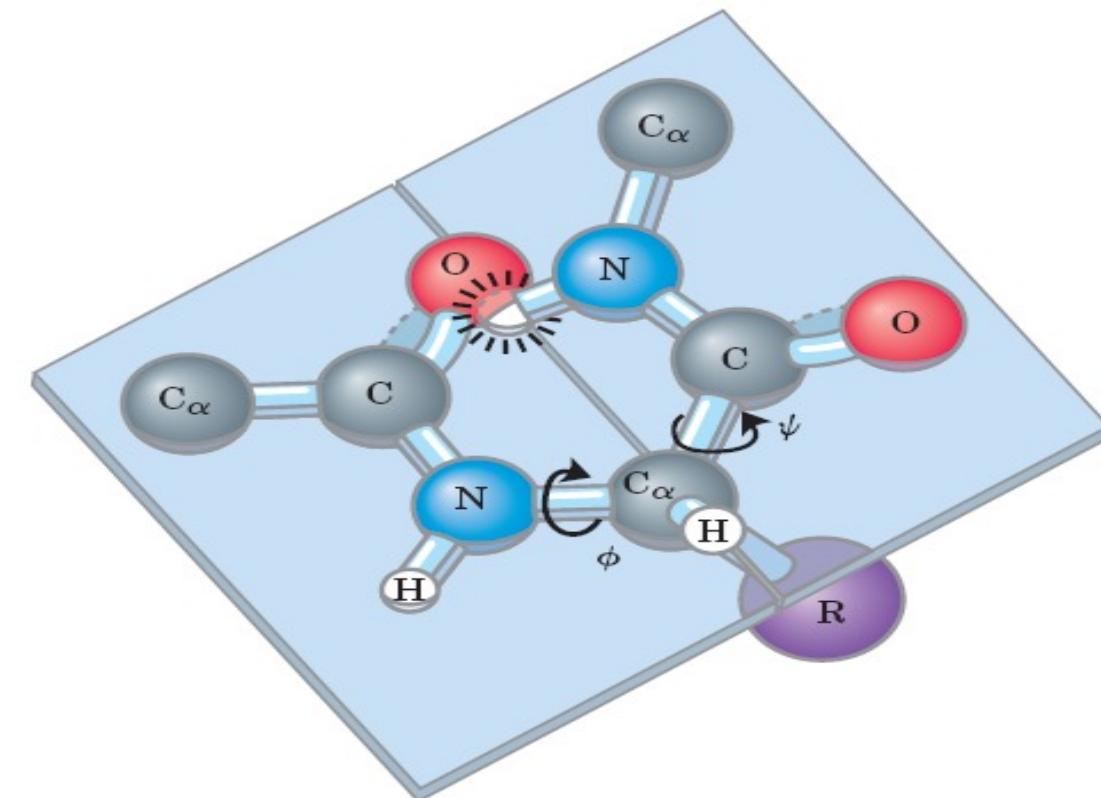
The phi and psi angles



- ❖ Three bonds separate sequential α carbons in a polypeptide chain. The $N-C_{\alpha}$ and $C-C_{\alpha}$ bonds can rotate, with bond angles designated ϕ and ψ , respectively
- ❖ The peptide $C-N$ bond is not free to rotate
- ❖ Other single bonds in the backbone may also be rotationally hindered, depending on the size and charge of the R groups. In the conformation shown, ϕ and ψ are 180 deg (or -180 deg).
- ❖ As one looks out from the α -carbon, the ϕ and ψ angles increase as the carbonyl or amide nitrogens (respectively) rotate clockwise

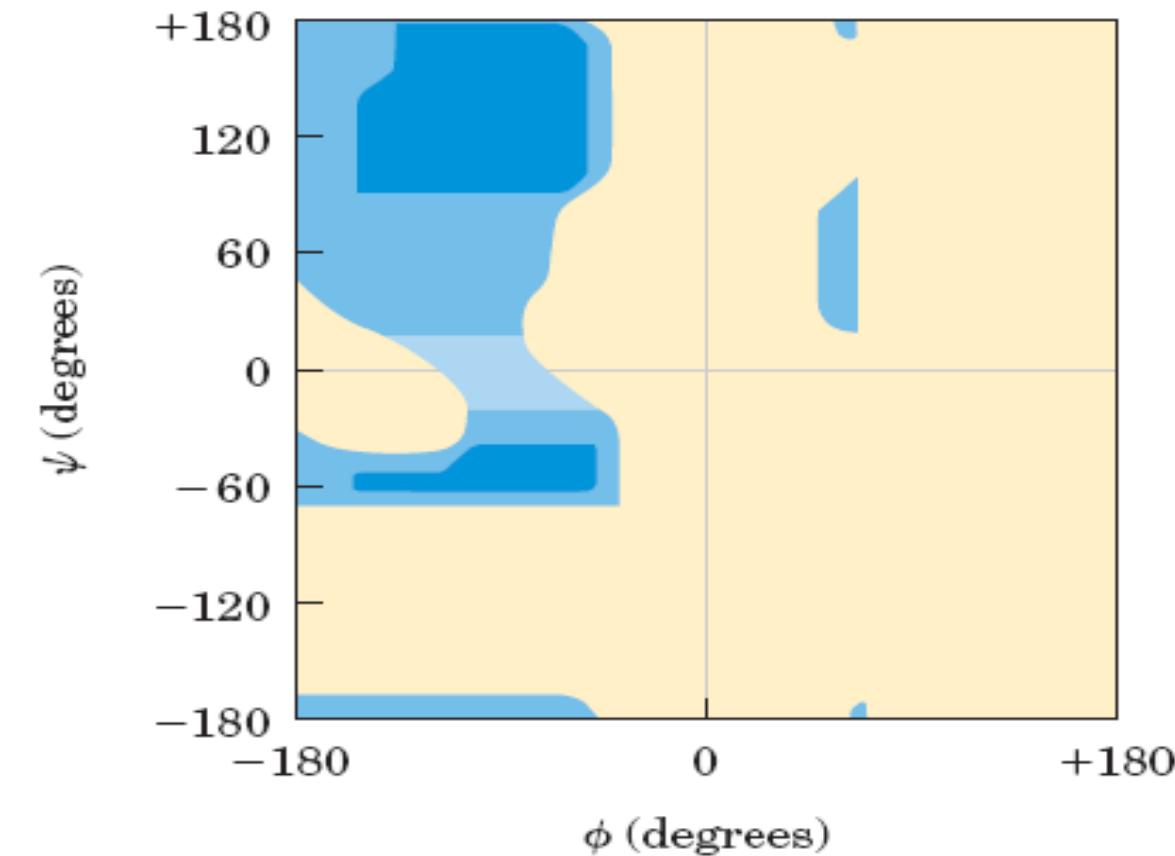
The zero phi & psi angles

- ❖ By convention, both ϕ and ψ are defined as 0 deg when the two peptide bonds flanking that carbon are in the same plane and positioned as shown.
- ❖ In a protein this conformation is prohibited by steric overlap between an – carbonyl oxygen and an – amino hydrogen atom
- ❖ To illustrate the bonds between atoms, the balls representing each atom are smaller than the van der Waals radii for this scale. $1 \text{ \AA} = 0.1 \text{ nm}$.



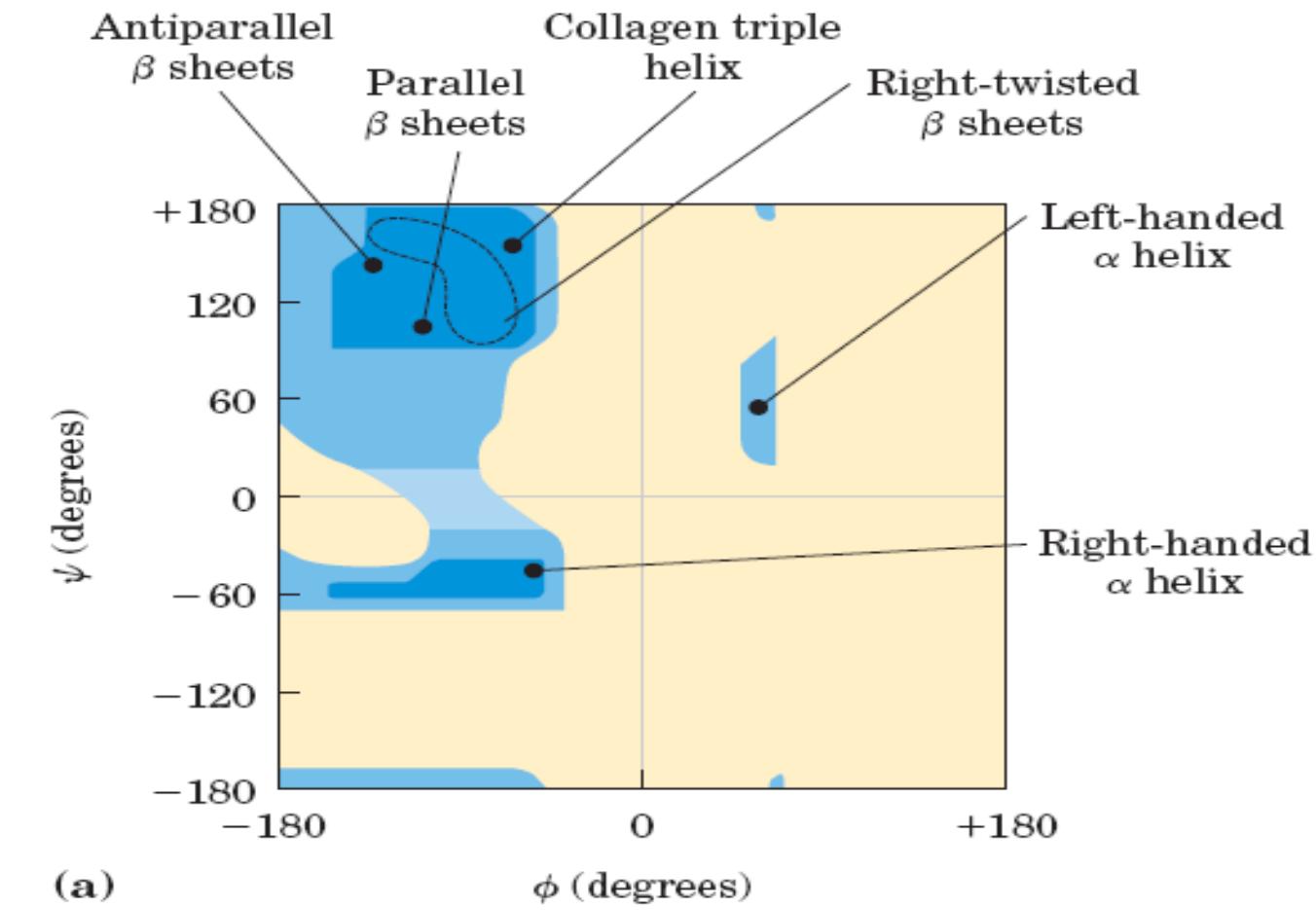
The Ramachandran Plot for L-Ala

- ❖ Conformations deemed possible are those that involve little or no steric interference, based on calculations using known van der Waals radii and bond angles.
 - ❖ The areas shaded dark blue reflect conformations that involve no steric overlap and thus are fully allowed
 - ❖ medium blue indicates conformations allowed at the extreme limits for unfavorable atomic contacts
 - ❖ the lightest blue area reflects conformations that are permissible if a little flexibility is allowed in the bond angles
- ❖ The asymmetry of the plot results from the L stereochemistry of the amino acid residues

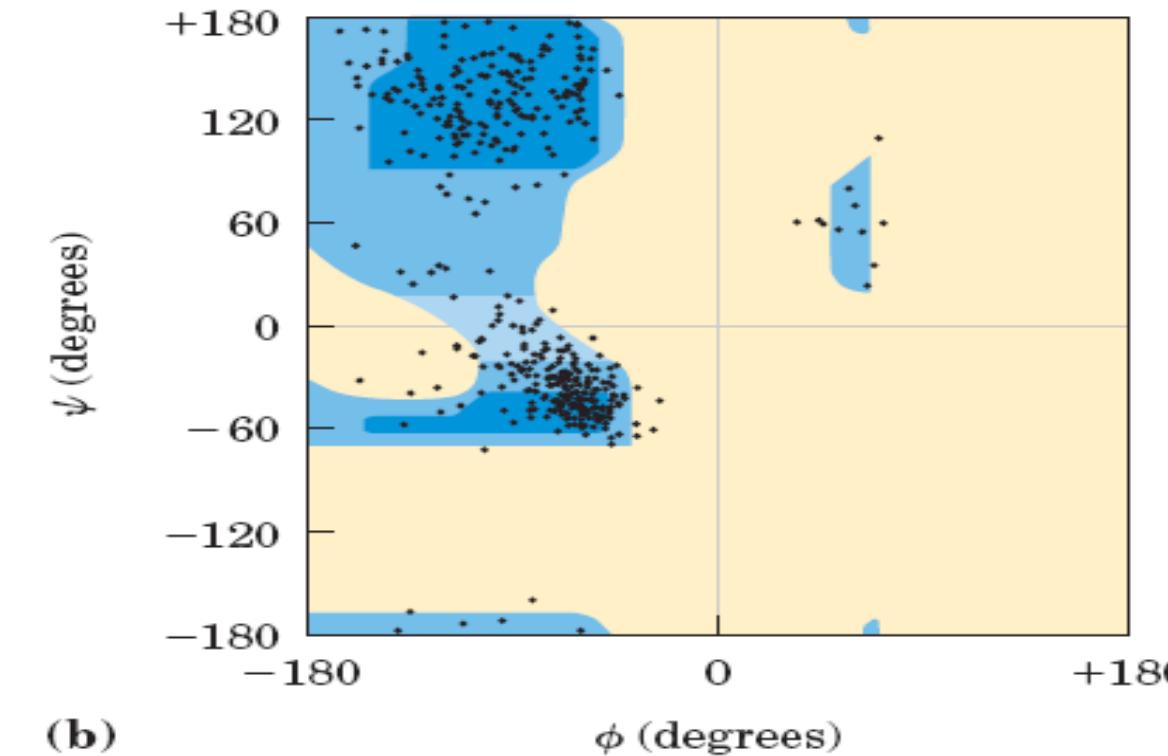


Secondary Protein Structure

- ❖ Secondary structure is the regular arrangement of amino acid residues in a segment of a polypeptide chain, in which each residue is spatially related to its neighbors in the same way
- ❖ The most common secondary structures are the alpha helix, the beta conformation, and beta turns
- ❖ The secondary structure of a polypeptide segment can be completely defined if the phi and psi angles are known for all amino acid residues in that segment



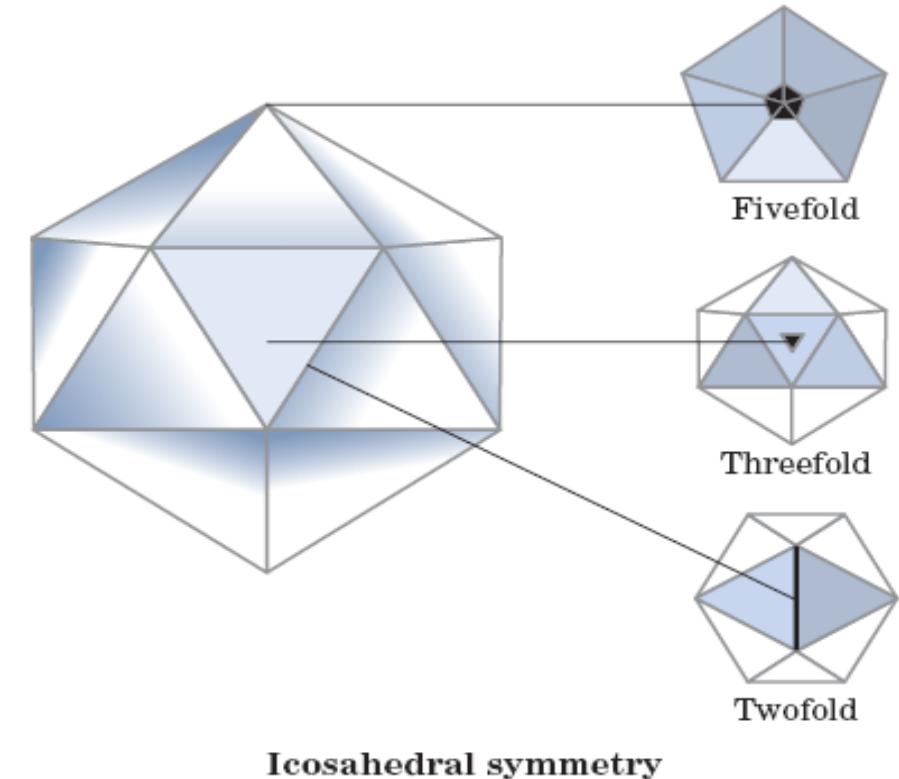
- ❖ The values of ϕ and ψ for various allowed secondary structures are overlaid
- ❖ Although left-handed helices extending over several amino acid residues are theoretically possible, they have not been observed in proteins



- ❖ All the amino acid residues except Gly in the enzyme pyruvate kinase (isolated from rabbit) are overlaid on the plot of theoretically allowed conformations. The small, flexible Gly residues were excluded because they frequently fall outside the expected ranges

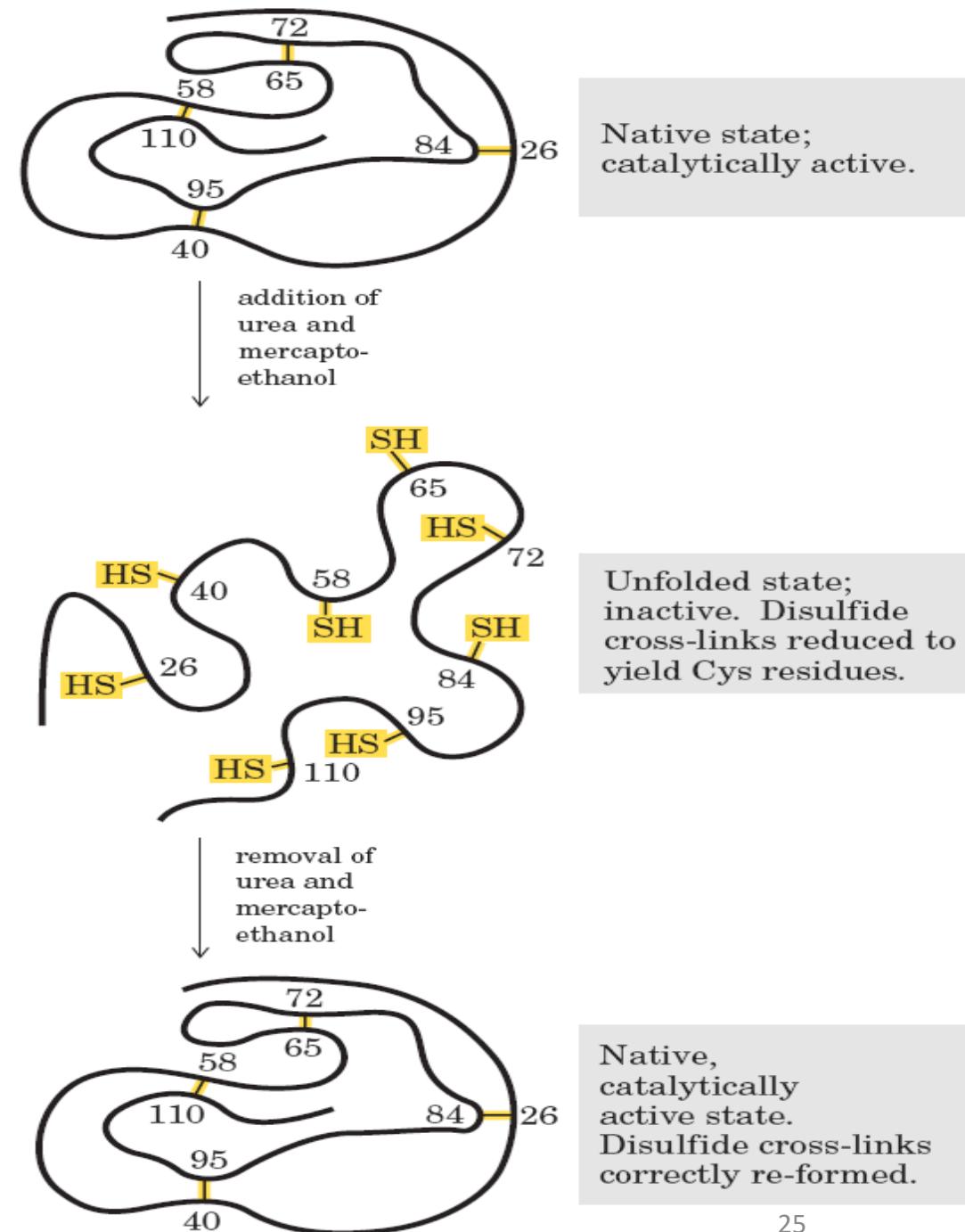
Symmetry in Tertiary Structures: Icosahedral Symmetry

- ❖ Icosahedral symmetry. Relating all 20 triangular faces of an icosahedron requires rotation about one or more of three separate rotational axes: twofold, threefold, and fivefold.

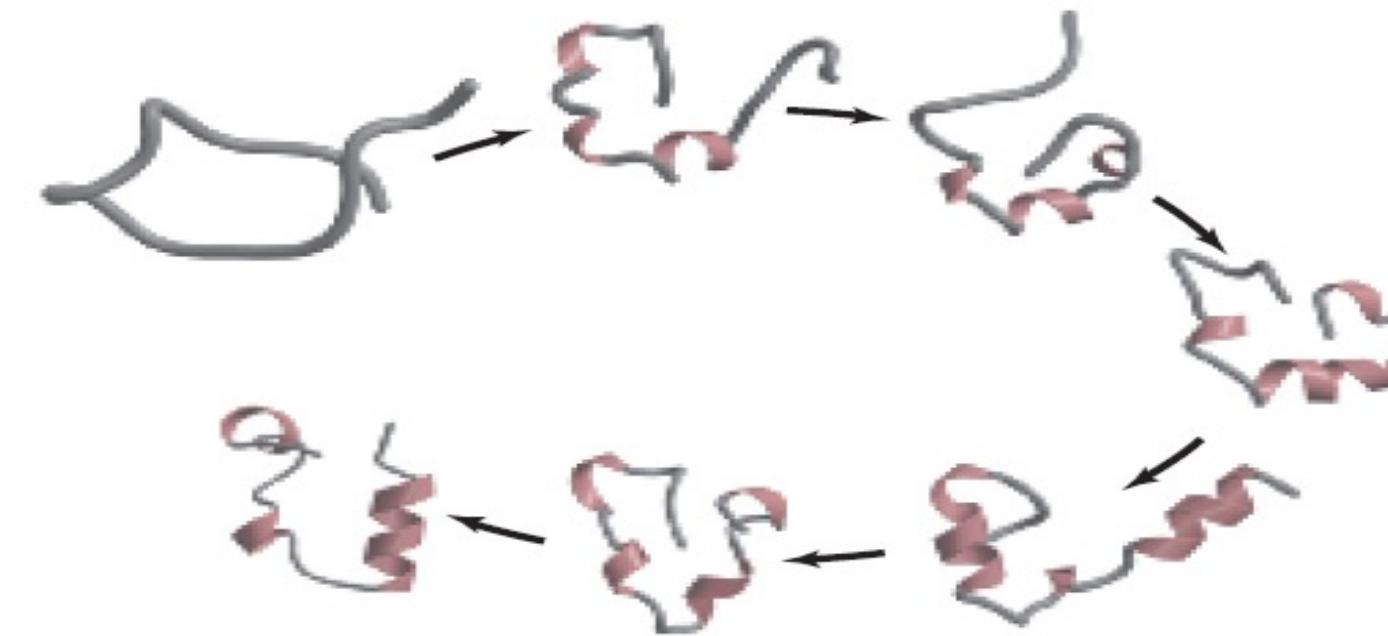


Renaturation of unfolded Protein

- ❖ Urea is used to denature ribonuclease, and mercaptoethanol ($\text{HOCH}_2\text{CH}_2\text{SH}$) to reduce and thus cleave the disulfide bonds to yield eight Cys residues. Renaturation involves reestablishment of the correct disulfide cross-links.



Protein folding simulation

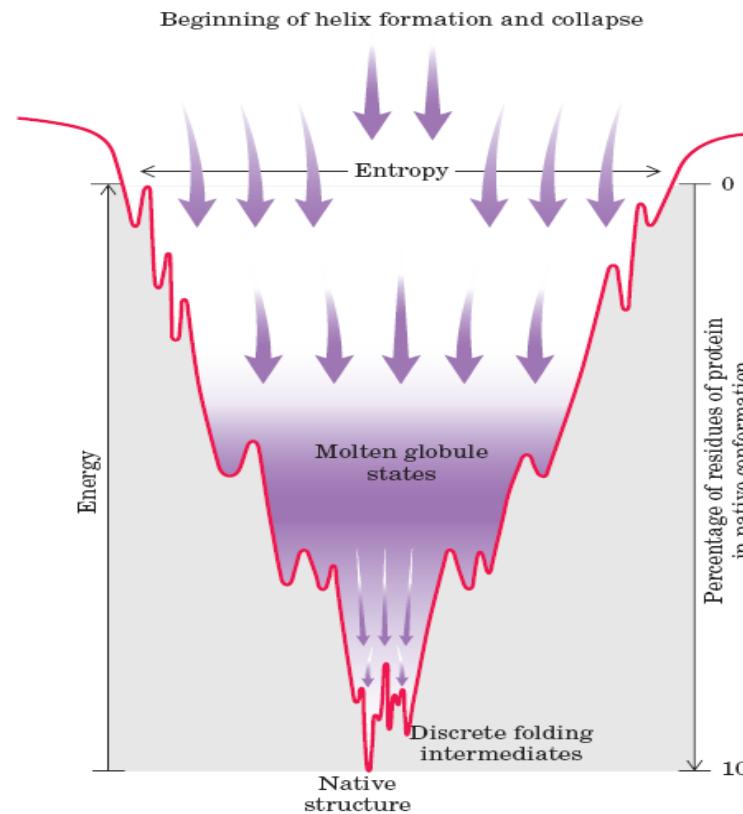


- ❖ The process started with the randomly coiled peptide and 3,000 surrounding water molecules in a virtual “water box.” The molecular motions of the peptide and the effects of the water molecules were taken into account in mapping the most likely paths to the final structure among the countless alternatives.
- ❖ 1 ms simulation time took half a billion integration steps on 2 Cray Computers

Thermodynamics of Protein Folding

WHAT IS THE LEVINTHAL'S PARADOX?

2 MODELS OF FOLDING



1. The number of conformations, and hence the conformational entropy, is large. Only a small fraction of the intramolecular interactions that will exist in the native conformation are present.
2. As folding progresses, the thermodynamic path down the funnel reduces the number of states present (decreases entropy), increases the amount of protein in the native conformation, and decreases the free energy.
3. Depressions on the sides of the funnel represent semistable folding intermediates, which may, in some cases, slow the folding process.

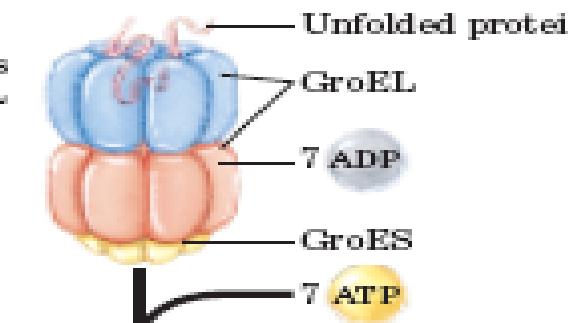
PROTEIN MISFOLDING IS THE CAUSE OF MANY FATAL DISEASES

- ❖ Spongiform encephalopathies – brain gets riddled with holes
 - ❖ Creutzfeldt-Jacob disease – fatal illness; symptoms – dementia, loss of coordination
- ❖ Stanley Prusiner traced the cause to PRION (*proteinaceous infectious only*) protein (PrP)
 - ❖ Two form – normal cellular form (PrP or PrP^C) and altered conformation "scrapie" form (PrP^{Sc})

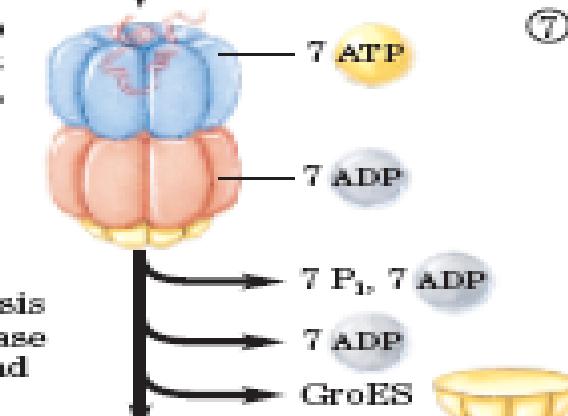
Cells have “Chaperone” proteins to assist proteins in folding correctly or refold proteins that have been denatured by heat or other conditions

GroEL-GroES – member of HSP 60 family

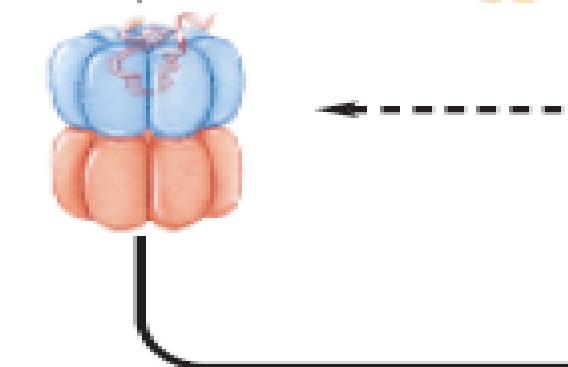
① Unfolded protein binds to the GroEL pocket not blocked by GroES.



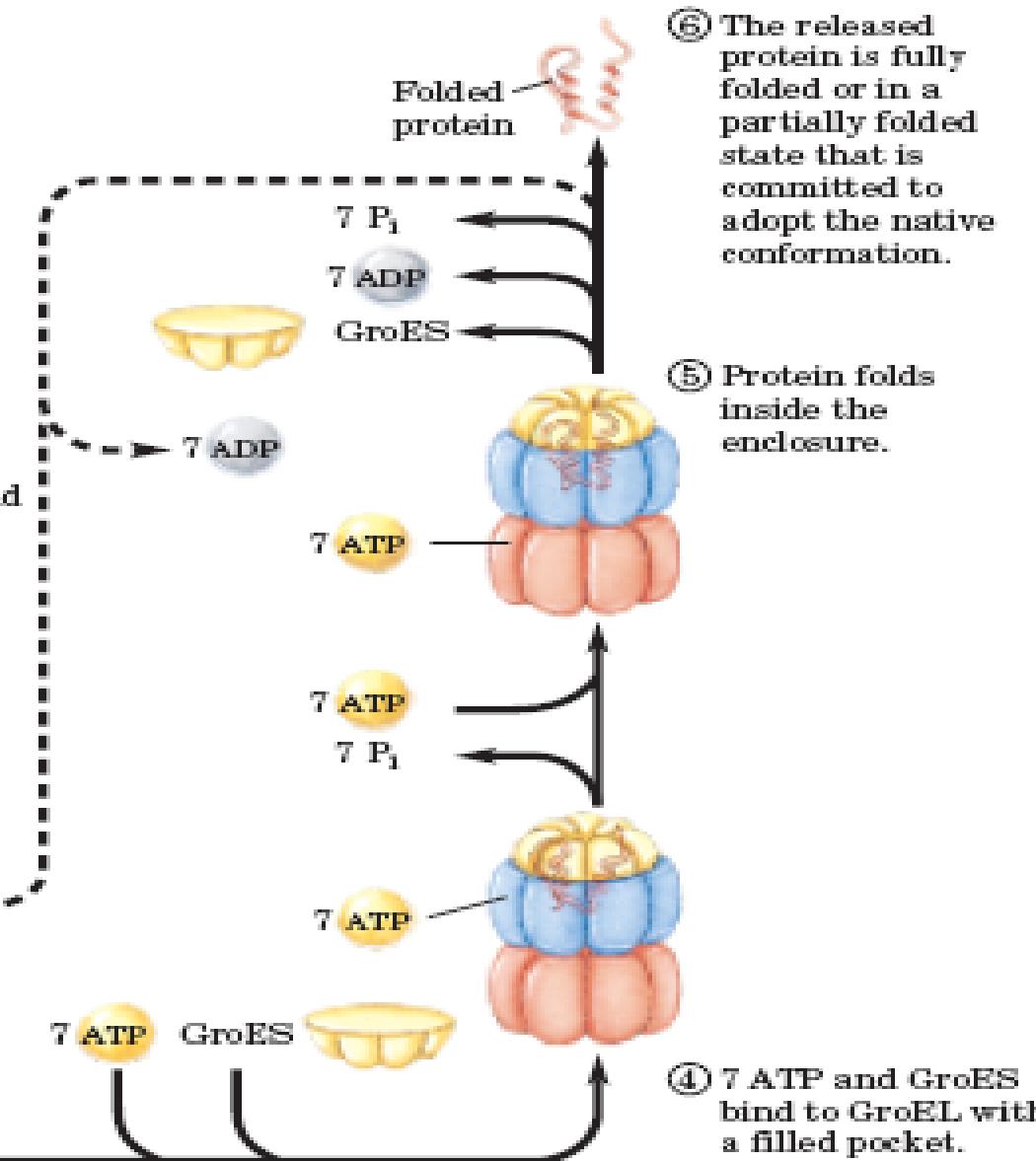
② ATP binds to each subunit of the GroEL heptamer.



③ ATP hydrolysis leads to release of 14 ADP and GroES.



⑦ Proteins not folded when released are rapidly bound again.



(a)

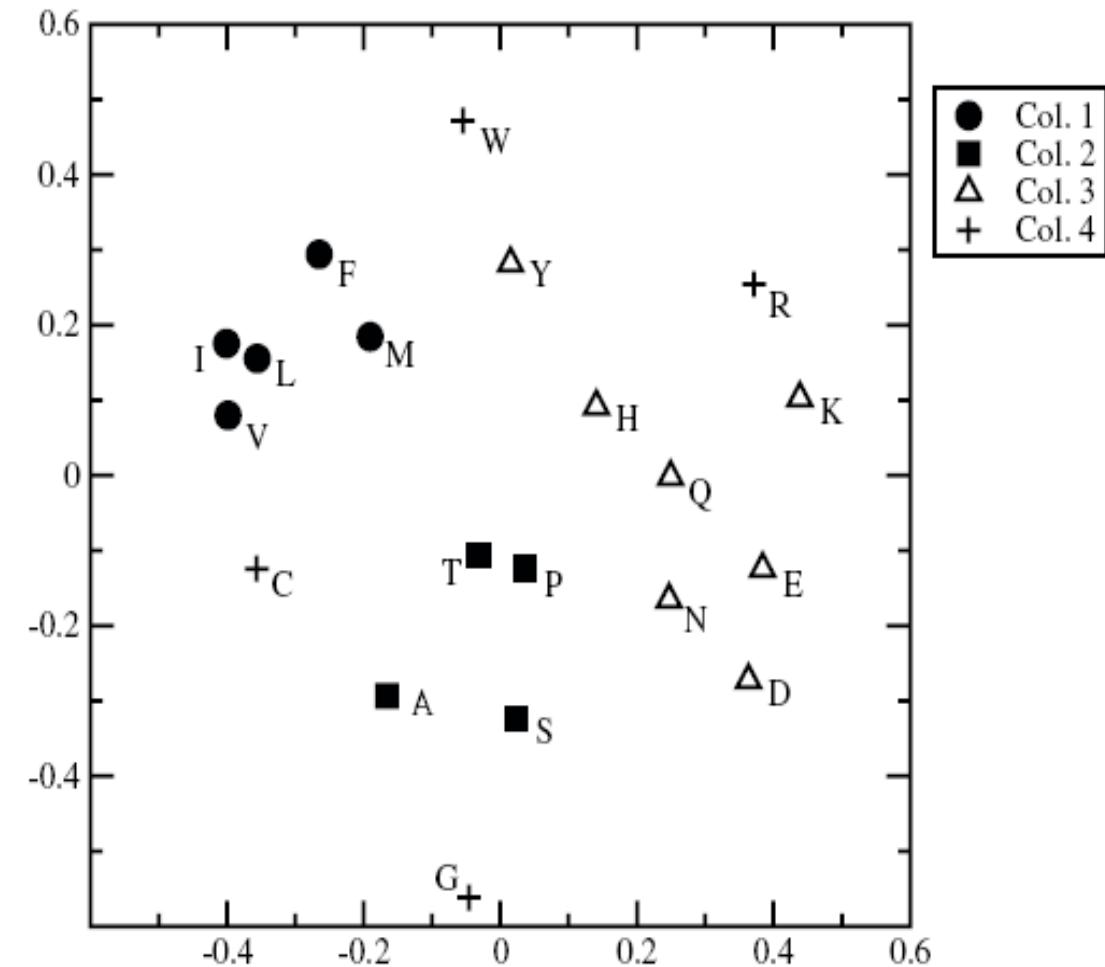
The Genetic Code

The genetic code is a map of Codons “C” to Amino Acids “A”
g: C → A

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

Grouping by Physical Properties of Amino Acids Best Explains the Genetic Code Table

		2nd base					
		U	C	A	G		
1st base	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 A G	3rd base
	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 GGC Arg CGA Arg GGG Arg	U C A G	
	A	AUU Ae AUC Ae AUA Ae AUG Met	ACU Thr ACC Thr ACA Thr ACG Thr	AAU Asn AAC Asn AAA Lys AAG Lys	AGU Ser AGC Ser AGA Arg AGG Arg	U C A G	
	G	GUU Val GUC Val GUA Val GUG Val	GCU Ala GCC Ala GCA Ala GCG Ala	GAU Asp GAC Asp GAA Glu GAG Glu	GGU Gy GGC Gy GGA Gy GGG Gy	U C A G	



Important points related to translation

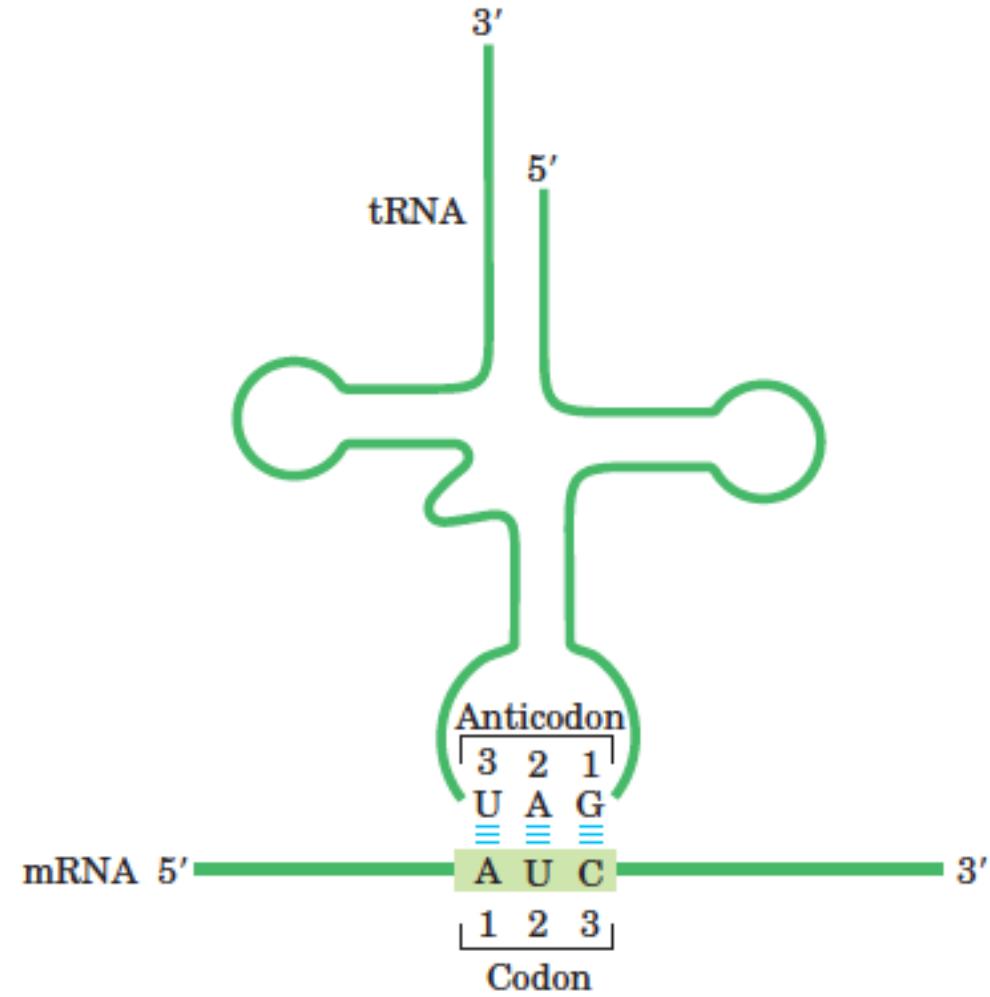
- ❖ The particular amino acid sequence of a protein is constructed through the translation of information encoded in mRNA. This process is carried out by ribosomes.
- ❖ Amino acids are specified by mRNA codons consisting of nucleotide triplets. Translation requires adaptor molecules, the tRNAs, that recognize codons and insert amino acids into their appropriate sequential positions in the polypeptide.
- ❖ The base sequences of the codons were deduced from experiments using synthetic mRNAs of known composition and sequence.
- ❖ The codon AUG signals initiation of translation. The triplets UAA, UAG, and UGA are signals for termination.

Degeneracy of the Genetic Code

<i>Amino acid</i>	<i>Number of codons</i>	<i>Amino acid</i>	<i>Number of codons</i>
Met	1	Tyr	2
Trp	1	Ile	3
Asn	2	Ala	4
Asp	2	Gly	4
Cys	2	Pro	4
Gln	2	Thr	4
Glu	2	Val	4
His	2	Arg	6
Lys	2	Leu	6
Phe	2	Ser	6

The Wobble Hypothesis

- ❖ Alignment of the two RNAs is antiparallel. The tRNA is shown in the traditional cloverleaf configuration
- ❖ Three different codon pairing relationships are possible when the tRNA anticodon contains inosinate.



	3 2 1	3 2 1	3 2 1
Anticodon	(3') G-C-I	G-C-I	G-C-I (5')
Codon	(5') C-G-A	C-G-U	C-G-C (3')
	1 2 3	1 2 3	1 2 3

1. One codon recognized:

1. Anticodon

(3') X-Y-C (5')

(3') X-Y-A (5')

Codon

(5') Y-X-G (3')

(5') Y-X-U (3')

2. Two codons recognized:

1. Anticodon

(3') X-Y-U (5')

(3') X-Y-G (5')

Codon

(5') Y-X-A (3')

(5') Y-X-C (3')

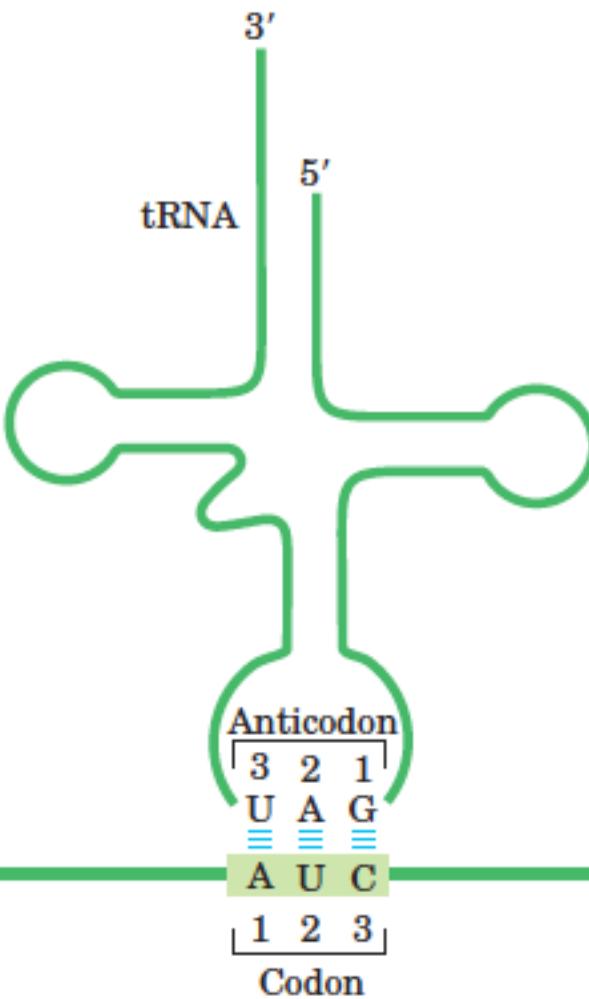
3. Three codons recognized:

1. Anticodon

(3') X-Y-I (5')

Codon

(5') Y-X-A (3')



mRNA 5' —————— A U C —————— 3'
1 2 3
Codon

G	GUU GUC GUA GUG	Val	GCU GCC GCA GCG	Ala	GAU GAC GAA GAG	Asp	GGU GGC GGA GGG	Gly	U C A G
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C	CUU CUC CUA CUG	Leu	CCU CCC CCA CCG	Pro	CAU CAC CAA CAG	His	CGU CGC CGA CGG	Arg	U C A G
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Reading Frames

Reading frame 1 5'---[U U C] [U C G] [G A C] [C U G] [G A G] [A U U] [C A C] [A G U] --- 3'

Reading frame 2 --- **U** **U C U** **C G G** **A C C** **U G G** **A G A** **U U C** **A C A** **G U** ---

Reading frame 3 ---U U C U C G G A C C U G G A G A U U C A C A G U---

Nonoverlapping code A U A C G A G U C _____
 1 2 3

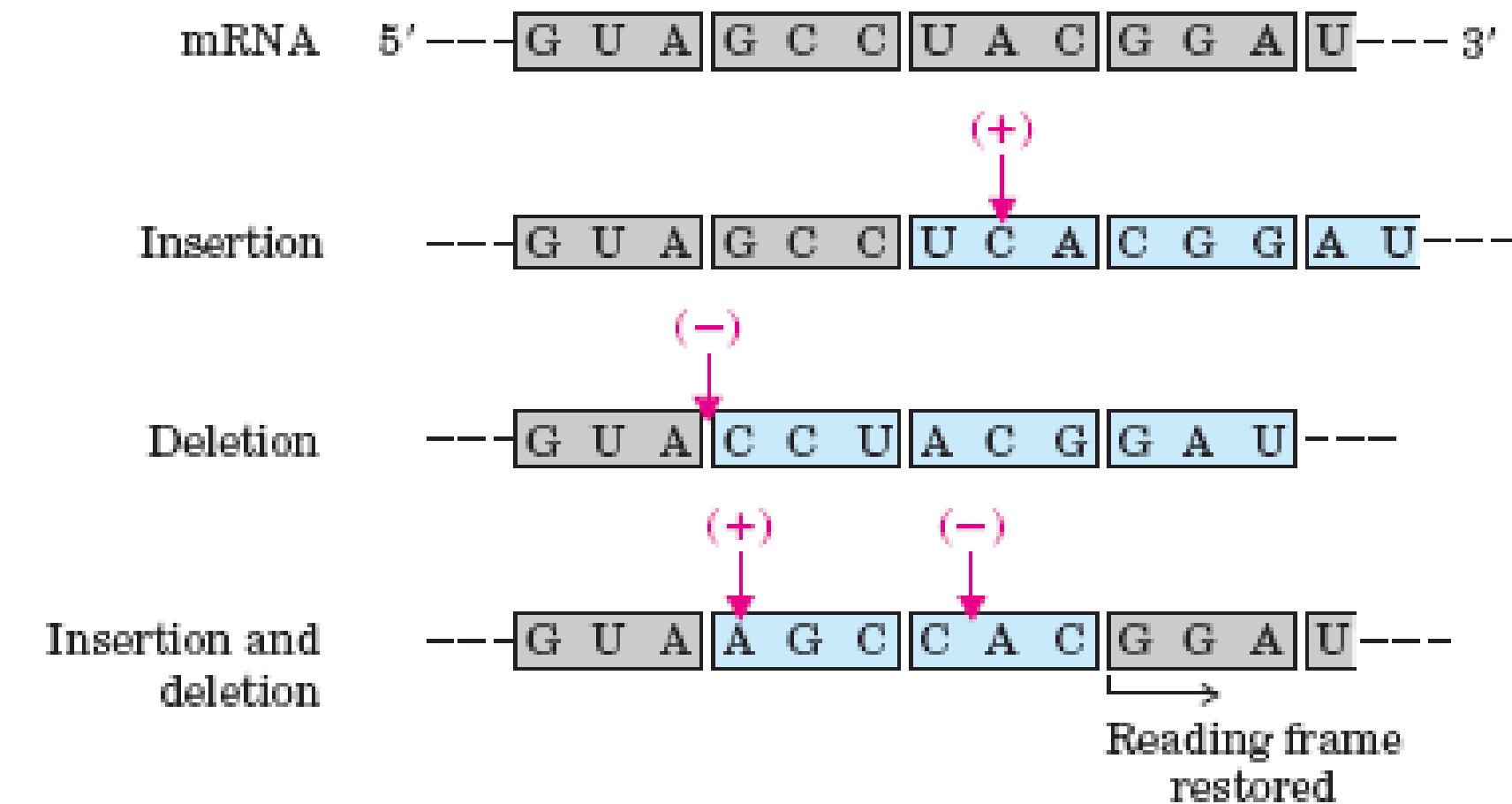
Overlapping code A U A C G A G U C
 1

3

Reading frame 1 5' --- G U A | A G U | A A G | U A A | G U A | A G U | A A --- 3'

Reading frame 2 --- G **U A A** **G U A A** **A G U A** **A G U A** **G U A A** **G U A A** ---

The Triplet Non-overlapping Code



Five Stage of Protein Synthesis in *E. coli*

1. Activation of Amino Acids
2. Initiation
3. Elongation
4. Termination
5. Folding and post translational processing

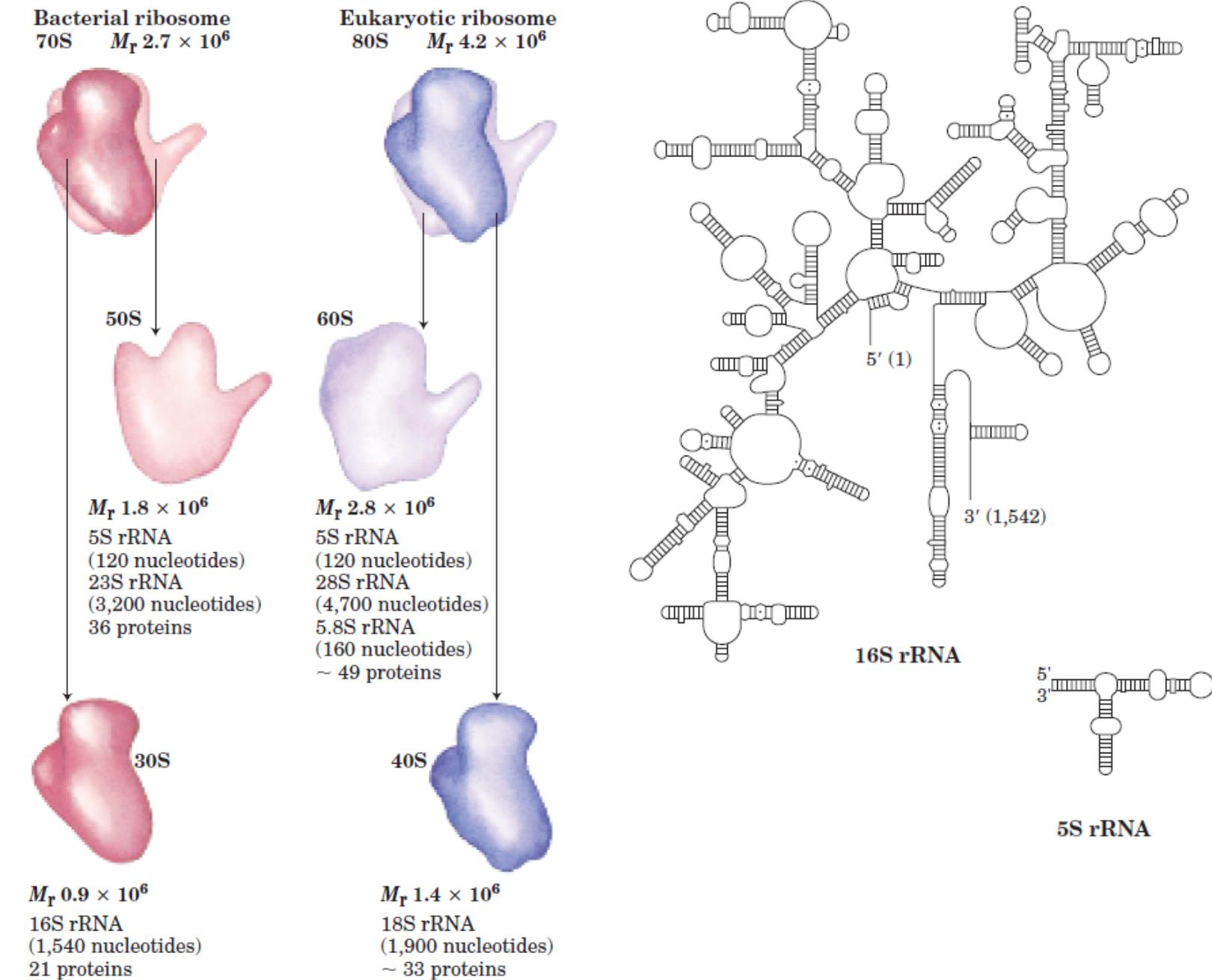
Ribosome is a Complex Supramolecular Machine

1. Each *E. coli* contains 15000 or more ribosomes (1/4 cell weight)
2. 18 nm is size
3. Two subunits (i) 30S (ii) 50S combined 70S (S is the sedimentation coefficient)
4. Subunits are made of many proteins and at least one large rRNA
5. Bacterial ribosomes have 55 proteins with molecular weights varying from 6000 to 75000

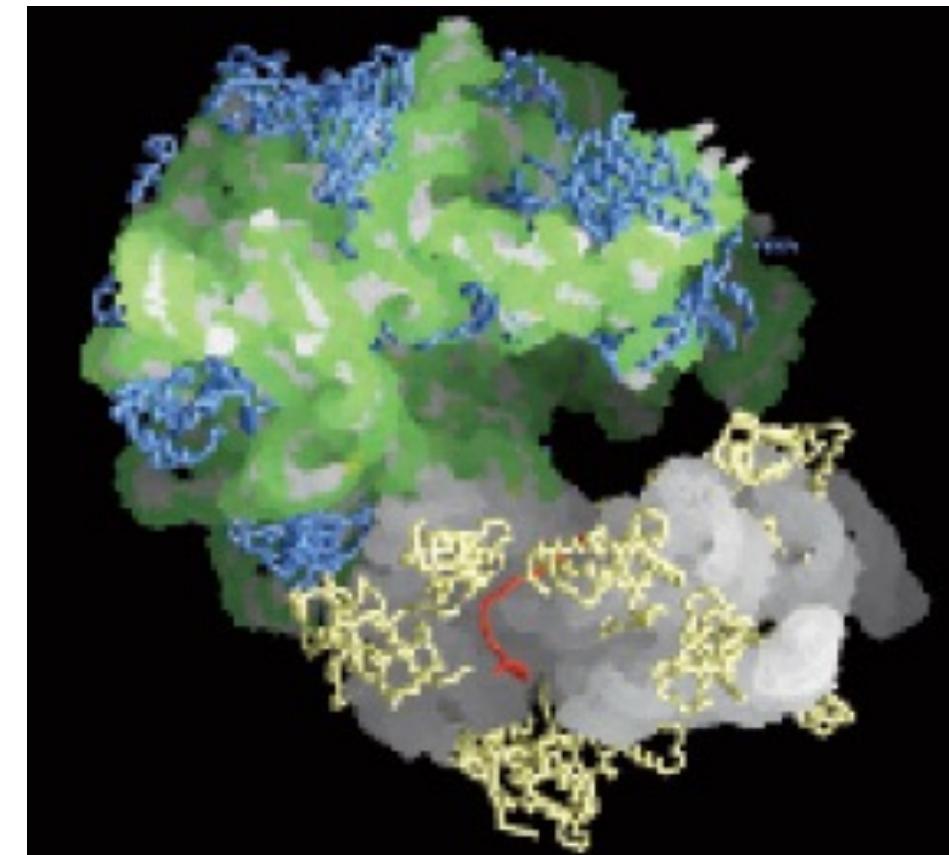
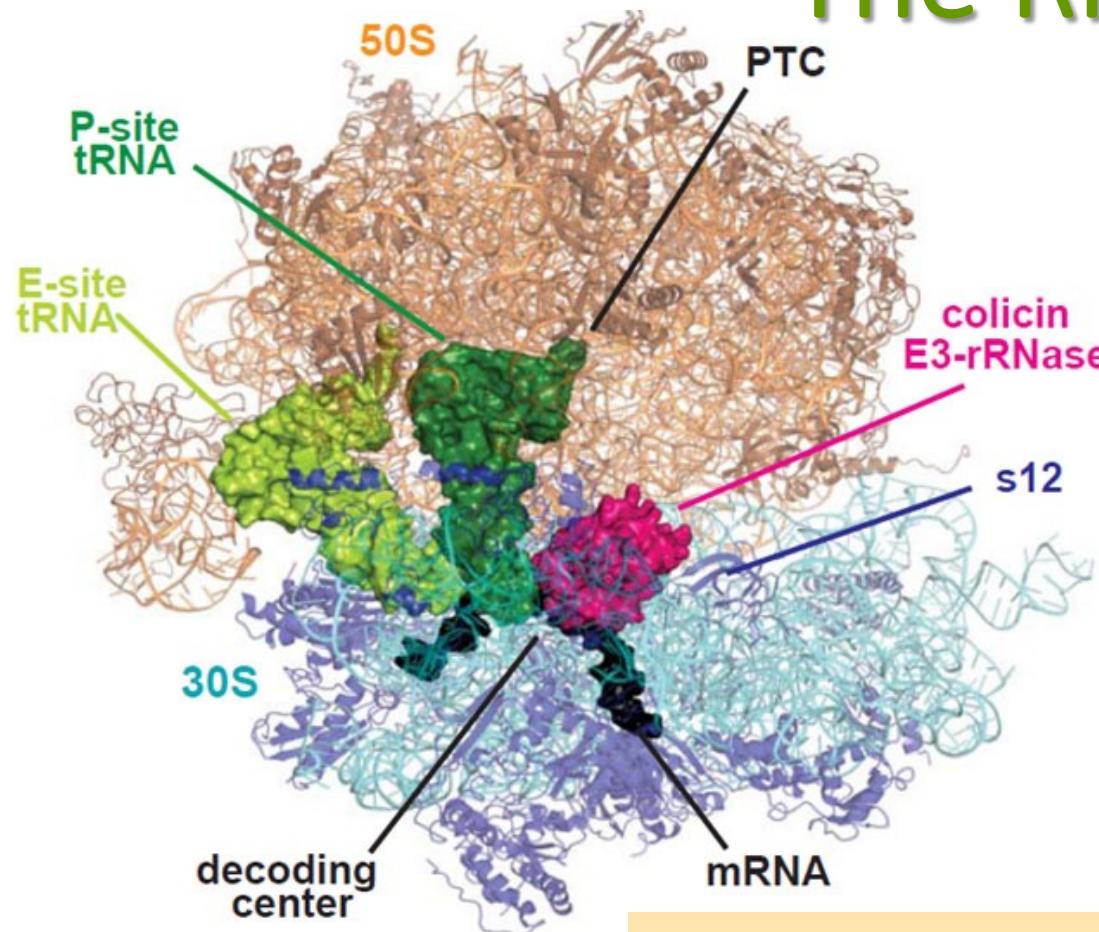
PDB ID **1JJ2** and **1GIY**

Bacterial rRNA

Secondary structure of *E. coli* 16S and 5S rRNAs. The first (5' end) and final (3' end) ribonucleotide residues of the 16S rRNA are numbered.



The Ribosome



RNA and Protein Components of the *E. coli* Ribosome

Subunit	Number of different proteins	Total number of proteins	Protein designations	Number and type of rRNAs
30S	21	21	S1-S21	1 (16S rRNA)
50S	33	36	L1-L36*	2 (5S and 23S rRNAs)

Nobel Prize in Chemistry 2009



The Nobel Prize in Chemistry 2009 was awarded jointly to Venkatraman Ramakrishnan, Thomas A. Steitz and Ada E. Yonath "for studies of the structure and function of the ribosome"

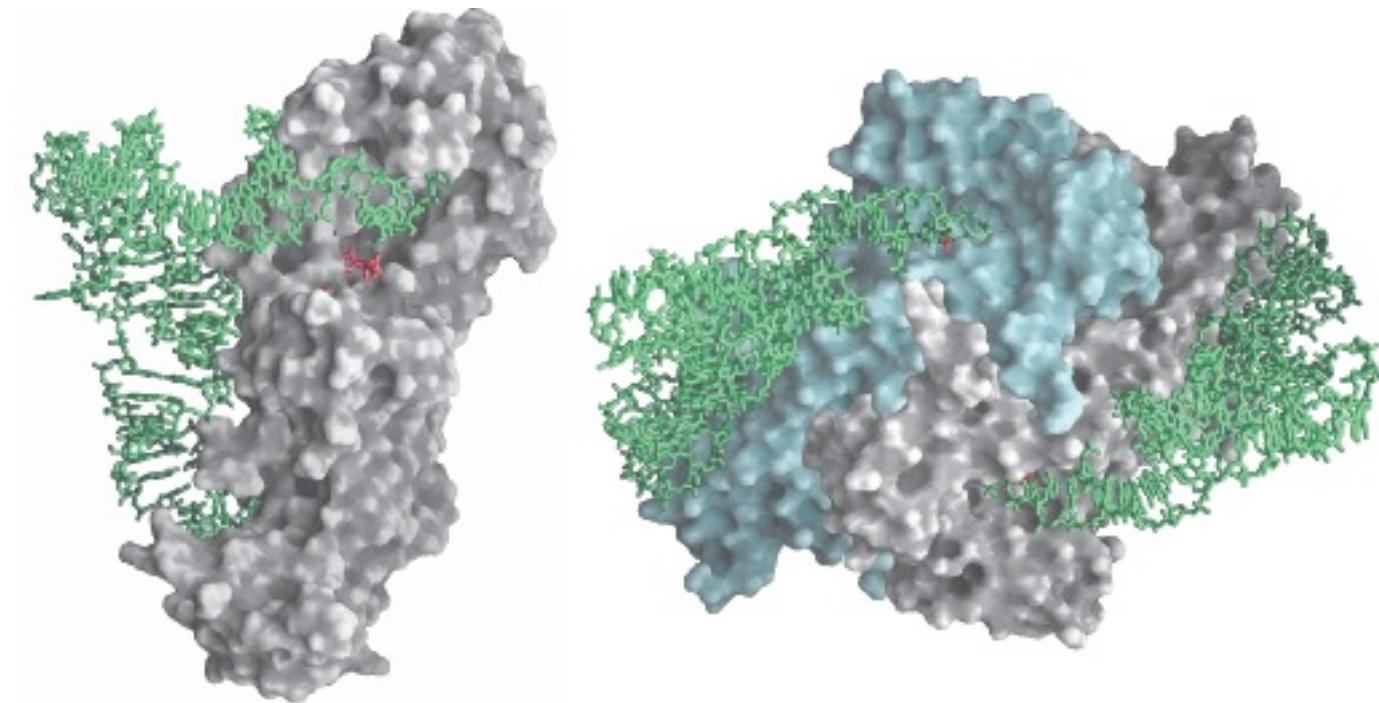
Visit to KSBS Aug 2020

<https://www.nobelprize.org/prizes/chemistry/2009/summary/>

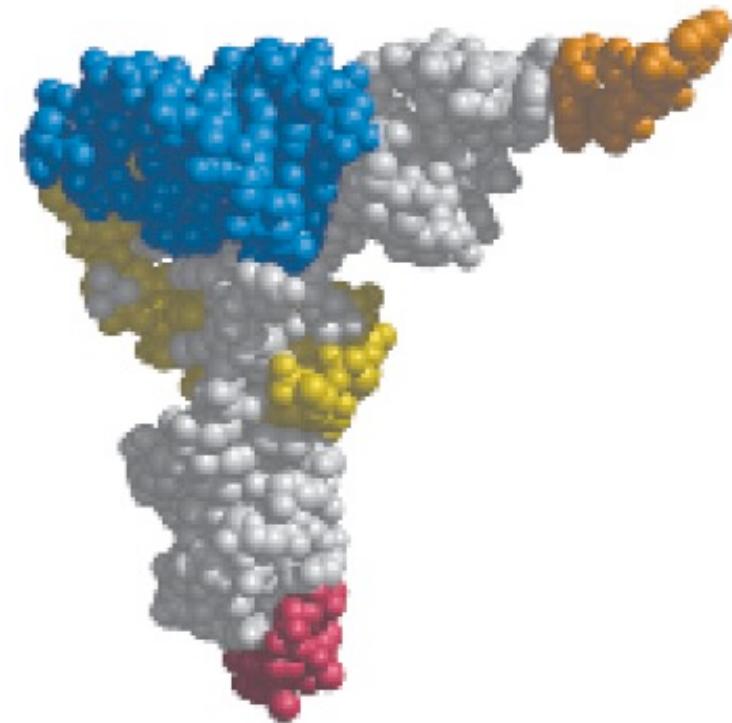
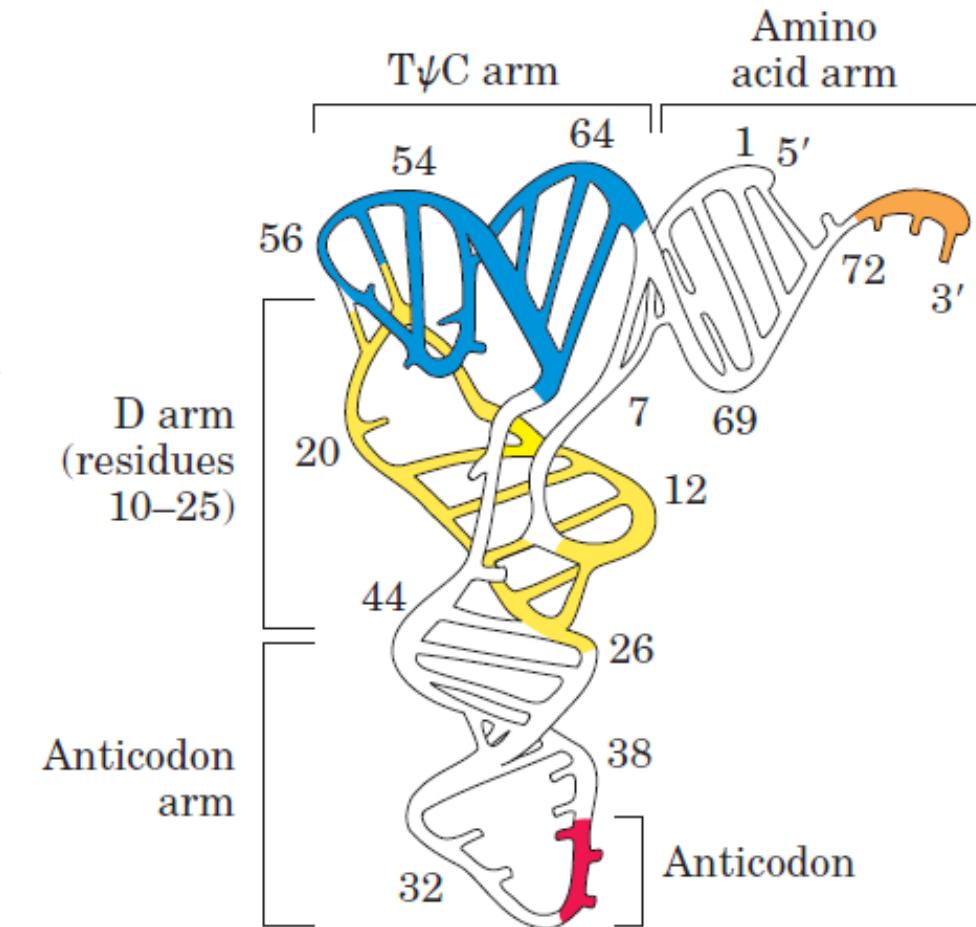
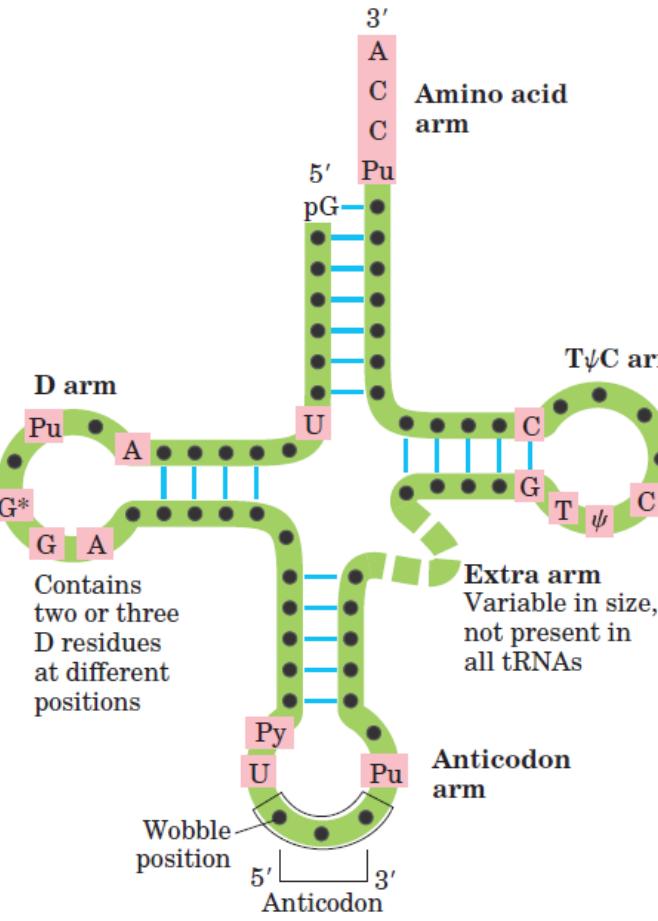
Aminoacyl t-RNA Synthetase

**Aminoacyl-tRNA Synthetases
Attach the Correct Amino Acids to
Their tRNAs**

- ❖ aminoacyl-tRNA synthetases esterify the 20 amino acids to their corresponding tRNAs. Each enzyme is specific for one amino acid and one or more corresponding tRNAs
- ❖ Proofreading by Aminoacyl-tRNA Synthetases
- ❖ Interaction between an Aminoacyl-tRNA Synthetase and a tRNA



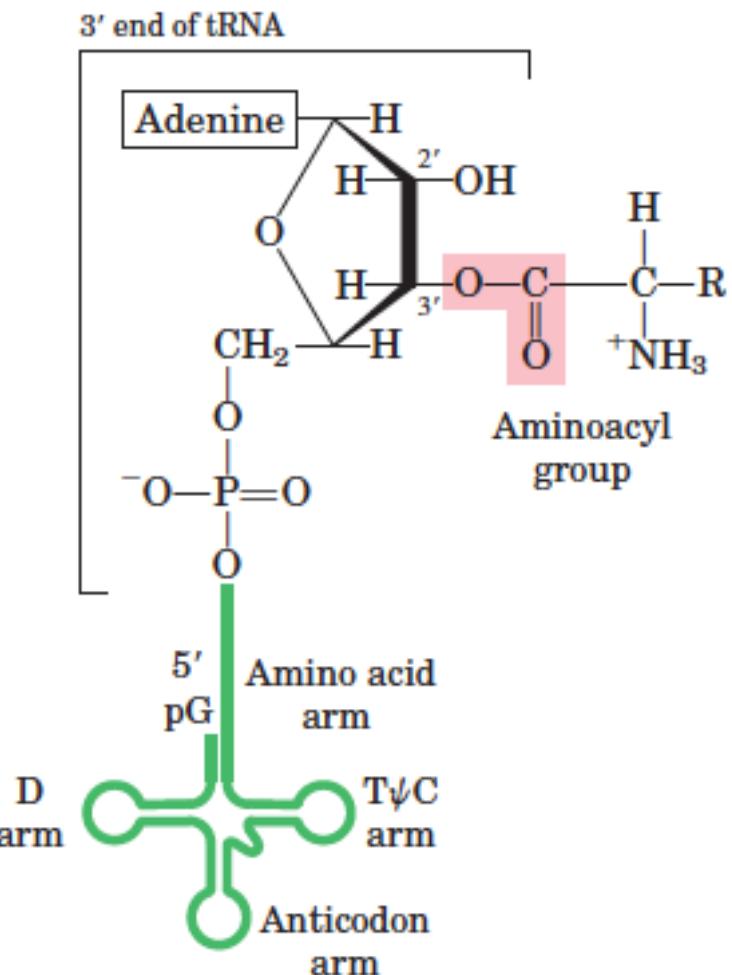
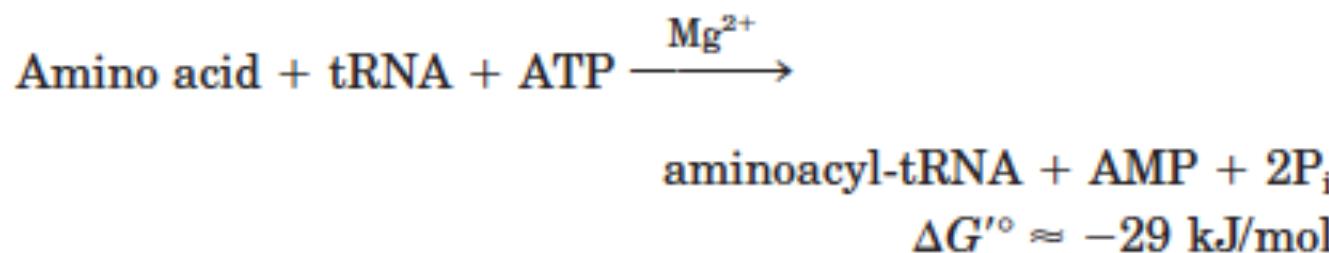
N
D G K N Q L P Y N Q L P Y D G K D G



Stage 1: Attaching the correct amino acid to the correct tRNA

in the cytosol, aminoacyl-tRNA synthetases esterify the 20 amino acids to their corresponding tRNAs.

- ❖ Each enzyme is specific for one amino acid and one or more corresponding tRNAs
- ❖ Most organisms have one aminoacyl-tRNA synthetase for each amino acid
- ❖ For amino acids with two or more corresponding tRNAs, the same enzyme usually aminoacylates all of them



Proof Reading by Aminoacyl tRNA Synthetases

Aminoacylation of tRNA accomplishes

1. Activation of amino acid for peptide bond formation
2. attachment of amino acid to an adaptor tRNA for placement of amino acid

The amino acid attached is not checked on the ribosome!

How is the fidelity assured?

Consider Valine and Isoleucine - different by only – CH_2 –

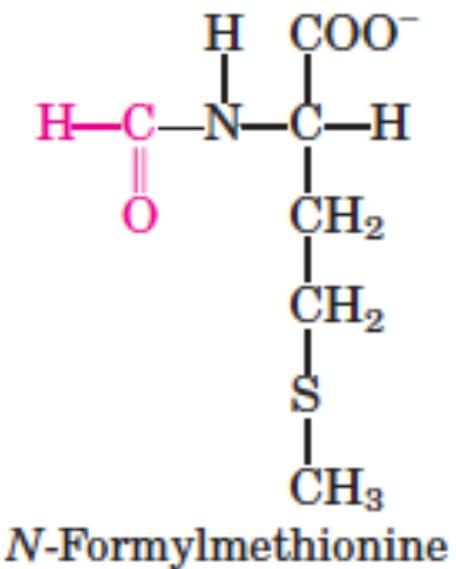
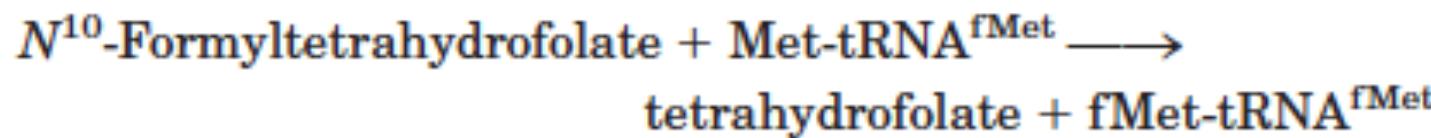
In the case of Ile-tRNA synthetase

1. Activation of Ile is favored by a factor of 200
2. Binding is carried out in 2 steps (acts as filter)
3. Incorrect binding occurs at a second site that has a higher hydrolytic rate
4. In this case, overall process is 1:3000 in favor of the correct amino acid Ile

Stage 2: A Specific Amino Acid Initiates Protein Synthesis

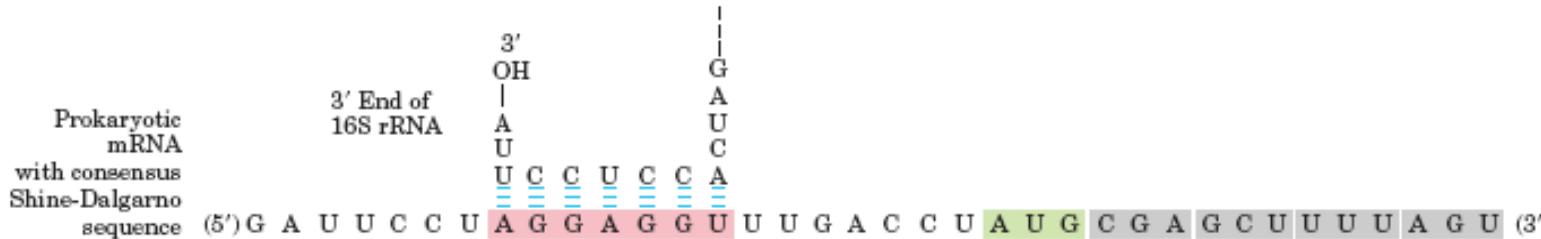
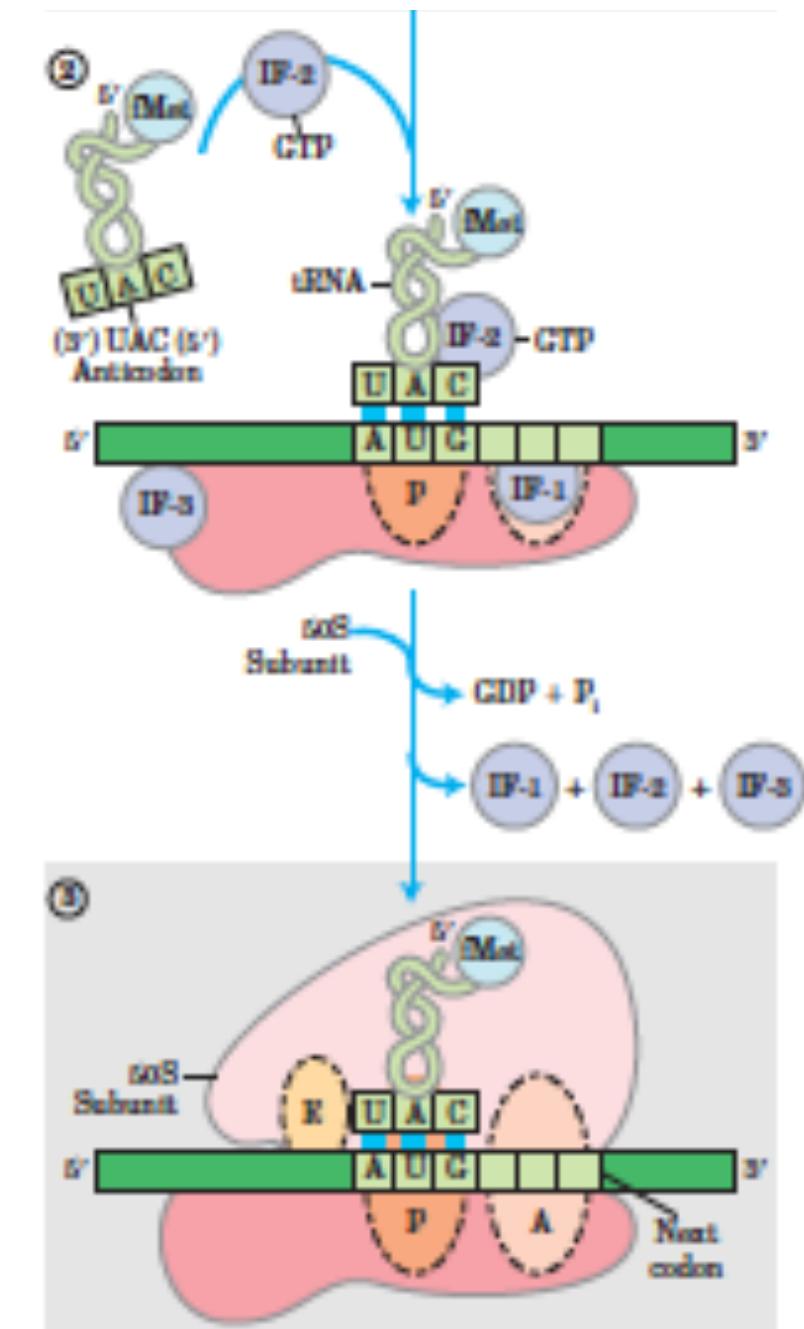
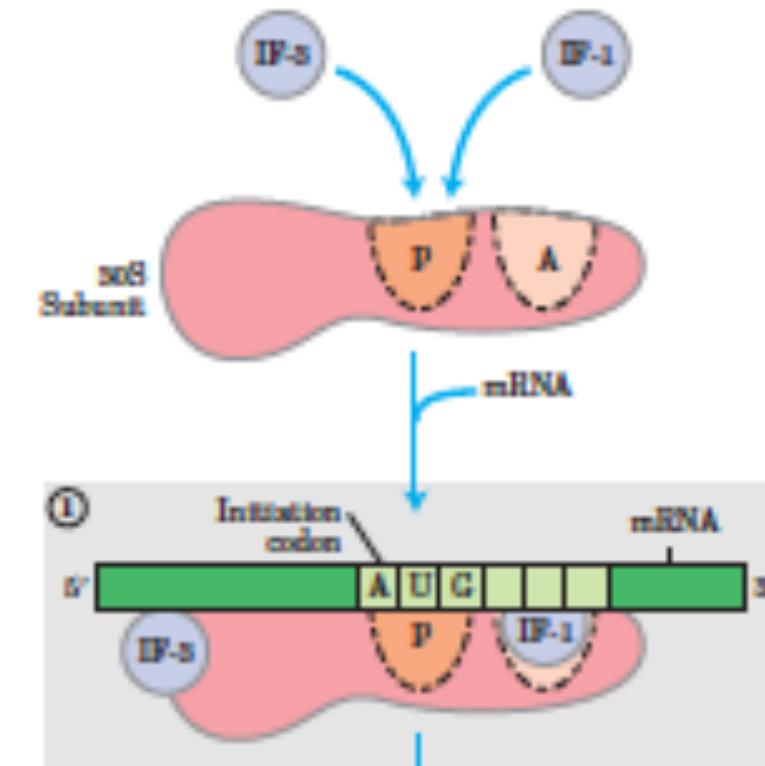
Although methionine has only one codon, (5')AUG, all organisms have two tRNAs for methionine

- ❖ One is used exclusively when (5')AUG is the initiation codon for protein synthesis
- ❖ The other is used to code for a Met residue in an internal position in a polypeptide
- ❖ The amino acid incorporated in response to the (5')AUG initiation codon is N-formylmethionine (fMet)



Formation of the Initiation Complex

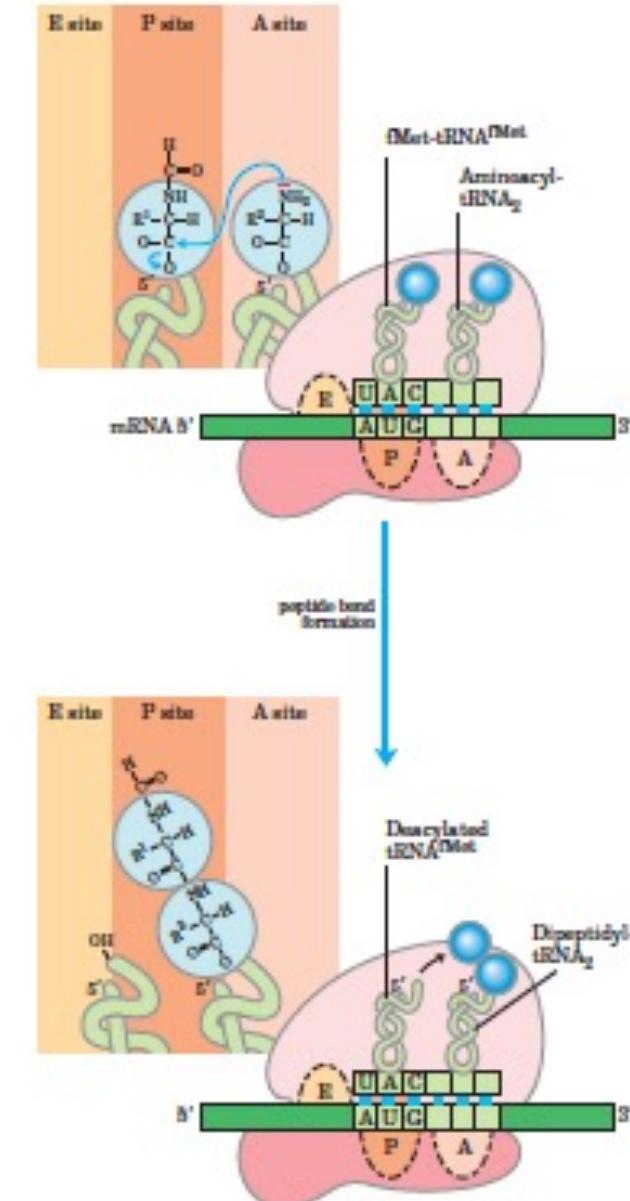
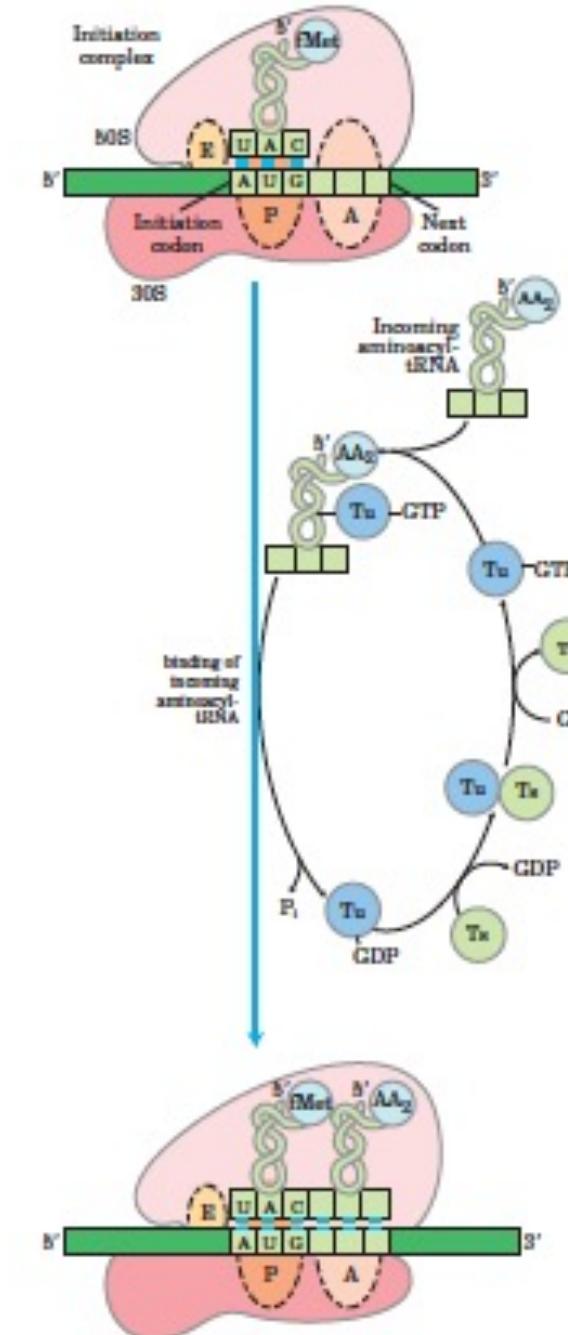
1. 30S ribosomal subunit
2. mRNA coding for the polypeptide to be made
3. Initiating fMet-tRNA_fMet
4. A set of three proteins called initiation factors (IF-1, IF-2, and IF-3)
5. GTP
6. 50S ribosomal subunit
7. Mg²⁺.



Stage 3: Elongation of the Peptide Chain

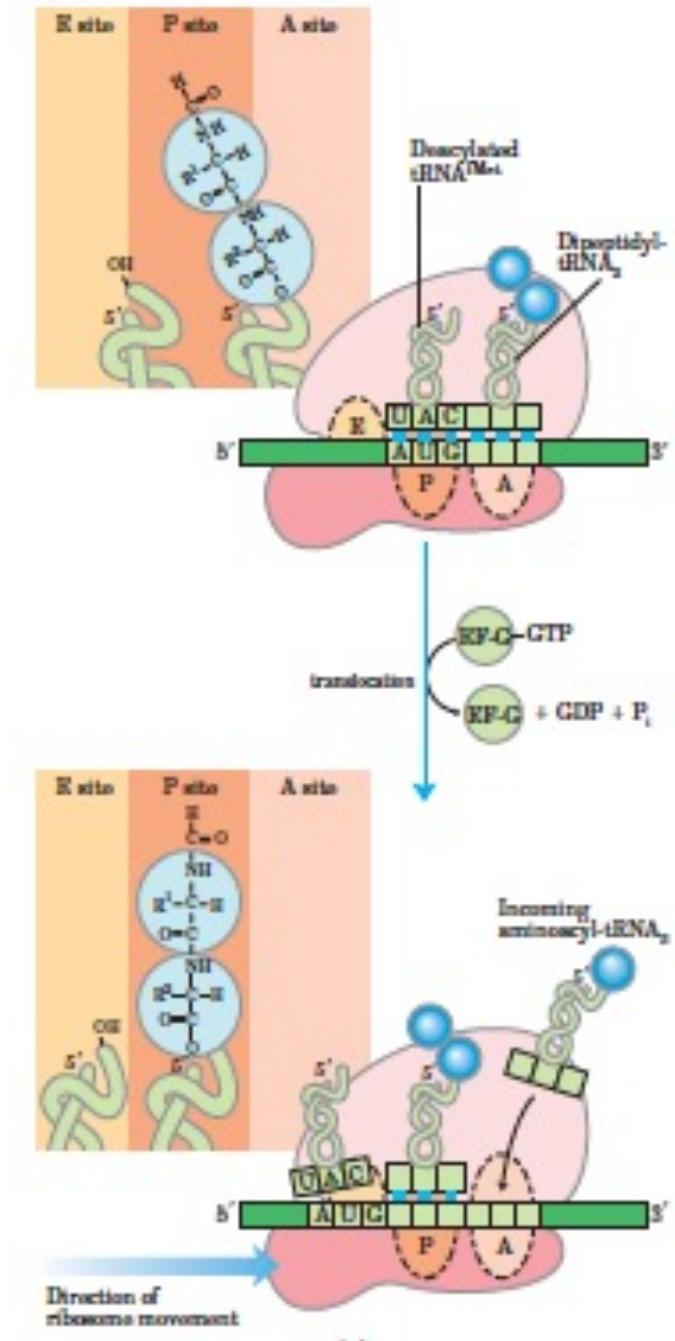
Elongation requires

1. the initiation complex
2. aminoacyl-tRNAs
3. a set of three soluble cytosolic proteins called elongation factors (EF-Tu, EF-Ts, and in bacteria)
4. GTP



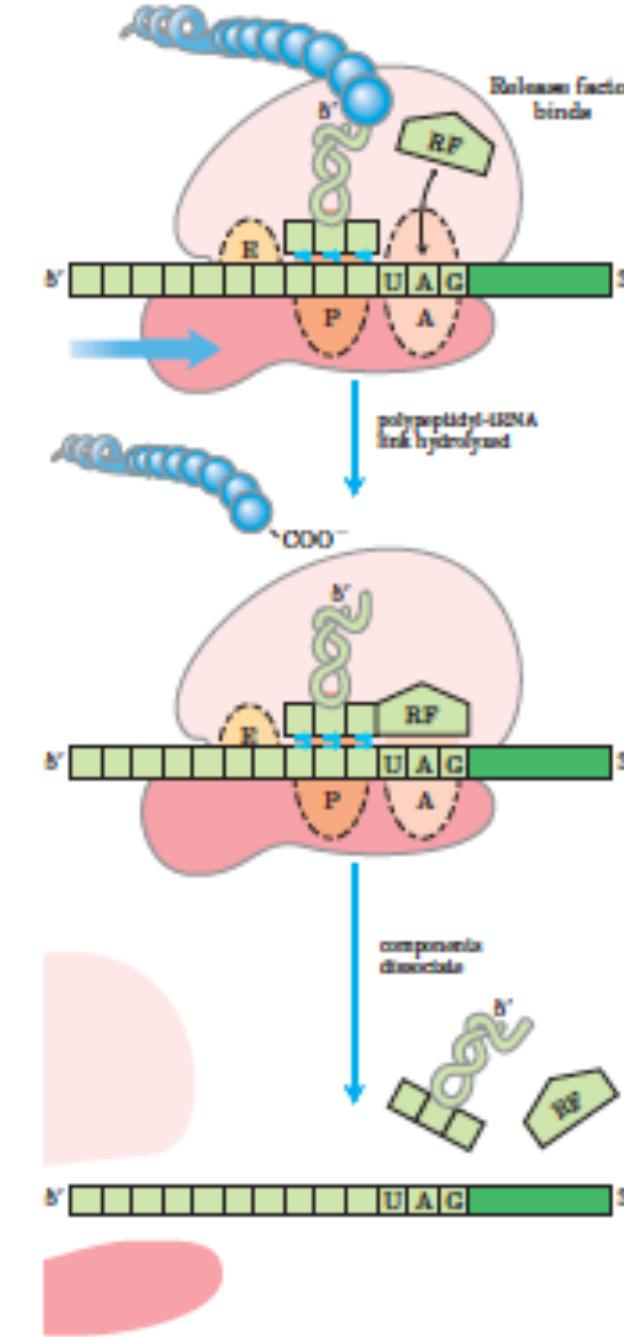
Stage 3 : Translocation

1. The ribosome moves one codon toward the 3' end of the mRNA
This shifts the anticodon of the tRNA attached to the 2nd codon from the A→P site and the deacylated tRNA from P→ E site
2. This movement required EF-G (translocase) and the energy is provided by the hydrolysis GTP→GDP+Pi
3. The uncharged tRNA is dislocated from the E-site and a peptide bond is formed between the growing chain and the new amino acid carried by the tRNA at the A-site



Stage 4: Termination of Synthesis

1. Elongation continues until the last amino acid in the sequence
2. Termination is signaled by the presence of one of the 3 stop codons UAA, UAG or UGA immediately following the final coded amino acid
3. Once the terminal codon occupies A-site, three termination or release factors RF1, RF2 and RF3 contribute to
 - i. Hydrolysis of terminal peptidyl tRNA bond
 - ii. Release of polypeptide from P-site
 - iii. Dissociation of the 70S ribosome into the 30S and 50S subunits



Summary of the 5 stages of protein synthesis

Stage	Essential components
1. Activation of amino acids	20 amino acids 20 aminoacyl-tRNA synthetases 32 or more tRNAs ATP Mg^{2+}
2. Initiation	mRNA <i>N</i> -Formylmethionyl-tRNA ^{fmet} Initiation codon in mRNA (AUG) 30S ribosomal subunit 50S ribosomal subunit Initiation factors (IF-1, IF-2, IF-3) GTP Mg^{2+}
3. Elongation	Functional 70S ribosome (initiation complex) Aminoacyl-tRNAs specified by codons Elongation factors (EF-Tu, EF-Ts, EF-G) GTP Mg^{2+}
4. Termination and release	Termination codon in mRNA Release factors (RF-1, RF-2, RF-3)
5. Folding and posttranslational processing	Specific enzymes, cofactors, and other components for removal of initiating residues and signal sequences, additional proteolytic processing, modification of terminal residues, and attachment of phosphate, methyl, carboxyl, carbohydrate, or prosthetic groups