Protein Folding

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Specific learning objectives

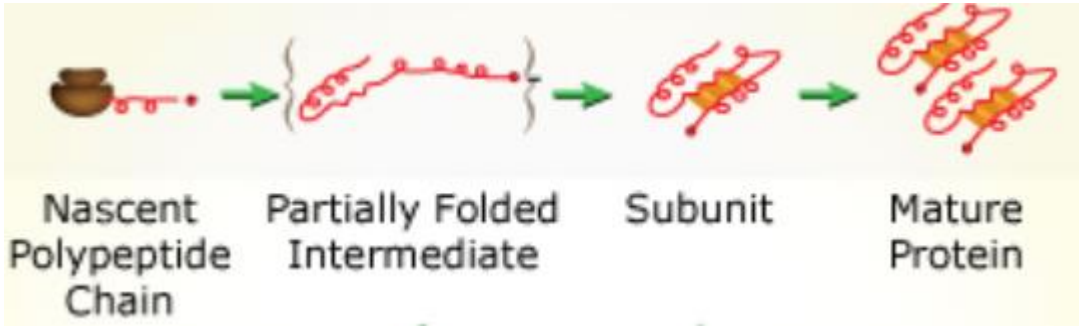
1. Steps of Protein Folding
2. Assisted Protein Folding by Chaperons
3. Enzymes involved in Folding Pathways
4. Protein Misfolding and related Diseases

Introduction

- Proteins are synthesized in ribosome as a linear sequence of amino acids (aa).
- Newly synthesized polypeptide folds into its characteristic and functional 3-D structure via a physical process known as protein folding.
- Interactions among aa lead to formation of a folded 3-D structure known as native protein.
- 3-D structure is determined by its aa sequence.

Protein folding in sequential manner

1. Newly synthesized polypeptide chain emerges from the ribosome, short segments fold into secondary structural units.



(Image by MIT Open Course Ware, adapted from image by Professor Jonathan King)

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2. Folding proceeds via an initial clustering among side chains of hydrophobic residue which prefer to be aloof from an aqueous environment.

- Clustering due to non-specific interaction among the hydrophobic residues that lead to the formation of a compact arrangement (molten globule state).

- Hydrophobic residues of proteins gather inside the collapsed forms within the core.

- Collapsed state favors the formation of secondary structure and encourages tertiary interaction among the residues.

Assisted Protein Folding

- Most Proteins fold spontaneously to their native.
- Cells have a variety of mechanism to ensure proteins are folded correctly by specialized proteins.
- Molecular chaperones interact with partially folded or improperly folded polypeptides, facilitating correct folding pathways.
- Chaperones provide microenvironments in which folding can occur.

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Chaperones: Hsp70 family of chaperones binds short sequences of hydrophobic aa that emerge while a new polypeptide is being synthesized, shielding them from solvent.

- **Protein chaperones aid protein folding in two basic ways:**

a) Assisted folding and assembly.

b) Binding of unfolded proteins to prevent aggregation.

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2. **Chaperonins:** Hsp60 acts as chaperonins in the folding process, together with an Hsp70 chaperone.

- Central cavity of the Hsp60 chaperone provides a sheltered environment in which a polypeptide fold until all hydrophobic regions are buried in its interior, thus prevent protein aggregation.

Chaperone-assisted protein folding

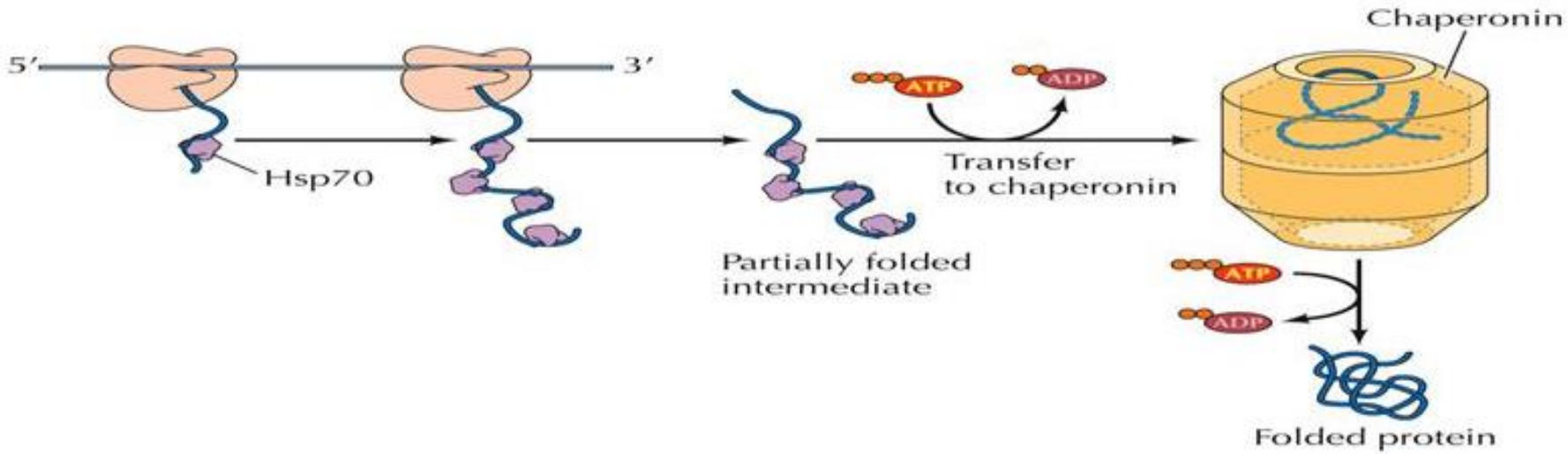

Fig.8.23: The Cell, 4th ediiton

Enzymes involved in Protein Folding Pathways

1) **Protein disulfide isomerase (PDI):**

- Protein-suflhydryl oxidase catalyzes oxidation of cysteine residues to form disulfide bonds.

- Cysteine form a disulfide bond with cysteinyl residue.

- By catalyzing disulfide exchange, the rupture of an S-S bond and its reformation with a different cysteine.

- Protein disulfide isomerase facilitates the formation of disulfide bonds that stabilize a protein’s native conformation.

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- Eukaryotic sulfhydryl oxidases are flavin-dependent, dietary riboflavin deficiency accompanied by an increased incidence of improper folding of disulfide-containing proteins.

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2) **Peptide prolyl cis-trans isomerase (PPI):**

- All X-Pro peptide bonds—where X represents any residue— are synthesized in the trans configuration.

- X-Pro bonds of mature proteins, ~6% are cis. Cis configuration is common in β turns.

- Interconversion of the cis and trans-isomers of peptide bonds with the proline by Cyclophilins.

- Cyclophilins participate in the folding of proteins expressed by viral invaders.
- Cyclophilins are pursued as targets for development of drugs such as cyclosporine.
- Alisporivir for the treatment of HIV, hepatitis C and other virally transmitted diseases.

Protein Misfolding and Diseases

Incompletely and incorrectly folded proteins present two serious problems to the cells:

1. **Loss of function due to absence of correctly folded protein:**

- Cystic fibrosis

Cystic Fibrosis (CFTR)

- Cystic fibrosis transmembrane conductance regulator (CFTR) is a membrane protein and encoded by CFTR gene.

- CFTR gene codes for an ABC transporter-class ion channel protein that conducts chloride ions cross epithelial cell membranes.

- Mutations of CFTR gene affect chloride ion channel function leads to dysregulation of epithelial fluid transport in lung, pancreas resulting in cystic fibrosis.

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- Caused by deletion of a 3 nucleotides which results in a loss of aa (Phe) residue at 508^{th} position, causes improper protein folding.

- Improved understanding of protein folding may lead to new therapies for CFTR.

2. Aggregation of incorrectly folded proteins:

- Beta-Thalassemias
- Alzheimer’s disease (Amyloid beta)
- Mad Cow Disease (Prion)

Alzheimer’s disease (Amyloid beta)

- Refolding or misfolding of β-amyloid protein endogenous to human brain tissue.
- Senile plaques and neurofibrillary bundles contain aggregates of the protein β-amyloid.
- A 4.3-kDa polypeptide produced by proteolytic cleavage of a larger protein known as amyloid precursor protein.

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- Levels of β-amyloid become elevated.
- This protein undergoes a conformational transformation from a soluble α-helix to β-sheet and prone to self-aggregation.
- Apolipoprotein E is a potential mediator of this conformational transformation.

Mad Cow Disease (Prion)

- Prion diseases, are fatal neurodegenerative diseases characterized by spongiform changes, astrocytic gliomas, and neuronal loss resulting from the deposition of insoluble protein aggregates in neural cells.

- They include Creutzfeldt-Jakob disease in humans, scrapie in sheep, and bovine spongiform encephalopathy (mad cow disease) in cattle.

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- Human prion-related protein (PrP), a glycoprotein encoded on the short arm of chromosome 20, monomeric and rich in α-helix.
- Normal form of protein is PrP^{C}, while the infectious form is PrP^{Sc}.
- PrP^{Sc} has a higher proportion of β-sheet in place of α-helix structure.
- Aggregation of these PrP^{Sc} form highly structures fibers, accumulates to form plaques.

Beta-Thalassemias

- Caused by genetic defects that impair synthesis of one of the polypeptide subunits of hemoglobin (Hb).

- During the burst of Hb synthesis, a specific chaperone called α-hemoglobin-stabilizing protein (AHSP) binds to free Hb α-subunits awaiting incorporation into the Hb multimer.

- In absence of AHSP, free α-Hb subunits aggregate, and resulting precipitate has cytotoxic effects on the developing erythrocyte.

Summary

- In protein folding steps, regions of secondary structure may form, followed by folding into supersecondary structures.
- Large ensembles of folding intermediates are rapidly brought to a single native conformation.
- For many proteins, folding is facilitated by Hsp70 chaperones and by chaperonins.

- Disulfide bond formation and the cis-trans isomerization of Pro peptide bonds are catalyzed by specific enzymes.

Group Discussion

- Subtopics of previous class discussed in group discussion.

Reference Books

1) Harper's Illustrated Biochemistry-30th edition
2) Biochemistry 7th edition by Jeremy M. Berg, John L. Tymoczko and Lubert Stryer
3) Lehninger Principles of Biochemistry

Thank you