Protein Folding

Specific learning objectives

1. Proteostasis pathways
2. Denaturation and renaturation of proteins
3. Steps of Protein Folding
4. Assisted Protein Folding by Chaperons
5. Enzymes involved in Folding Pathways
6. Protein Misfolding and related Diseases

Introduction

- 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 which is stable.
- Proteins must maintain conformational flexibility to function
- Continual maintenance of active set of cellular proteins required under given set of conditions is called proteostasis

Proteostasis pathways

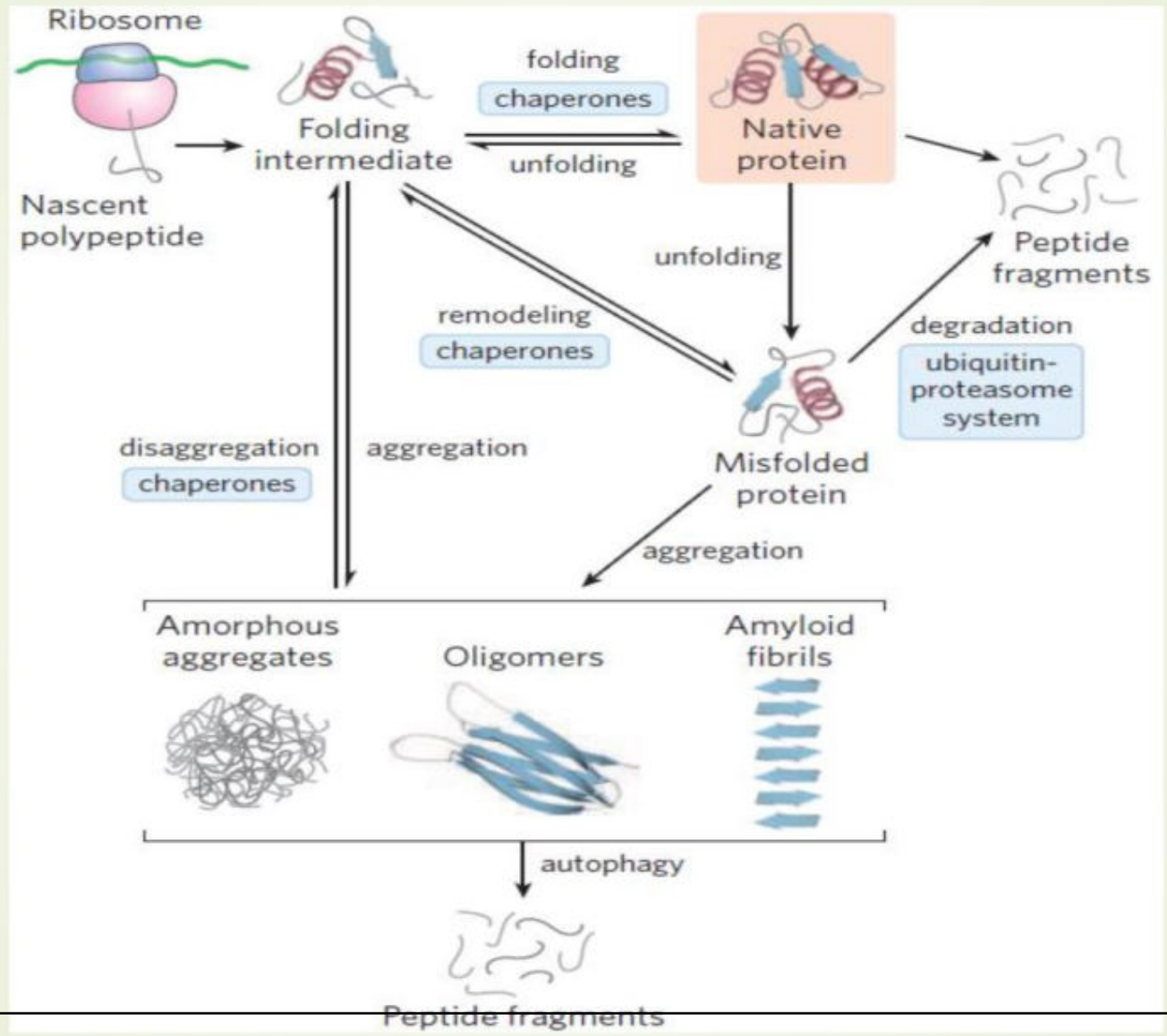

Fig.4.25. Lehninger Principles of Biochemistry, 6th Ed.

Denaturation at protein levels

- At primary structure: Sequence of aa held together by covalent peptide bonds, is not disrupted by denaturation.
- At secondary structure: Proteins lose all regular repeating patterns such as α-helices and β-pleated sheets and adopt a random coil shape.

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At tertiary structure: Disruption of covalent interactions between aa side chains (such as disulfide-bridges bet cysteine groups), non-covalent interactions between polar aa side-chains, van-der waals interactions between non-polar aa side chains.

At quaternary structure: Protein sub-units are dissociated and/or spatial arrangement of protein subunits is disrupted.

Loss of protein structure results in loss of function

- Native Protein denaturate in presence of reducing agent, alter pH, temp, ionic strength, and solubility.

Various denaturants are:

1. Heat disrupts hydrogen bonds and hydrophobic interactions bet non-polar residues. Ex. Albumin in egg denature and coagulate during cooking.

2. Strong acids and bases disrupts salt bridges formed in a protein structure. Ex. In digestive system, acidic gastric juices causes coagulation of milk by proteolytic enzyme renin.

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3. Reducing agents like guanidine hydrochloride (GdnHCl) or β-mercaptoethanol reduces disulphide bonds to sulfhydryl group and breaks intra and interchain disulphide bonds

4. Organic solvents, urea and detergents (SDS) disrupts hydrophobic interactions that stabilize core of globular proteins

- Urea disrupts stabilizing hydrophobic interactions, thus freeing entire polypeptide from its folded conformation.

5. Extremes of pH alter net charge on protein, causing electrostatic repulsion and disruption of hydrogen bonding

Amino acid sequence determines tertiary structure

- 3° structure of globular protein determined by its aa sequence. Proof of this came from experiments showed that denaturation of some proteins is reversible

- Certain globular proteins denatured by denaturing reagents will regain their stable native structure and biological activity. This process is called renaturation.

- Denaturation and renaturation of ribonuclease A, demonstrated by Christian B Anfinsen got Nobel prize in chemistry 1972

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- He provided first evidence that amino acid sequence of a polypeptide chain contains all information required to fold chain into its native, 3-D structure.

- 3-D structure and function of proteins destroyed by denaturation, which demonstrate relationship between structure and function.

- Some denatured proteins can renature spontaneously to form biologically active protein.

Renaturation of unfolded, denatured Ribonuclease

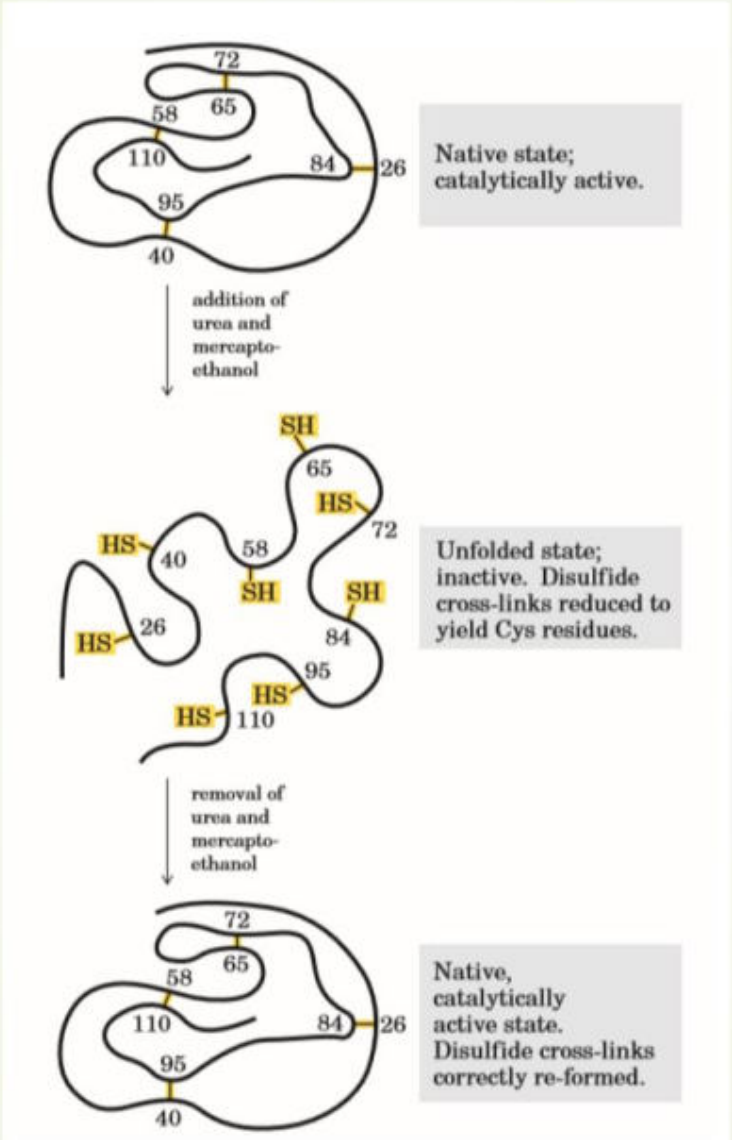
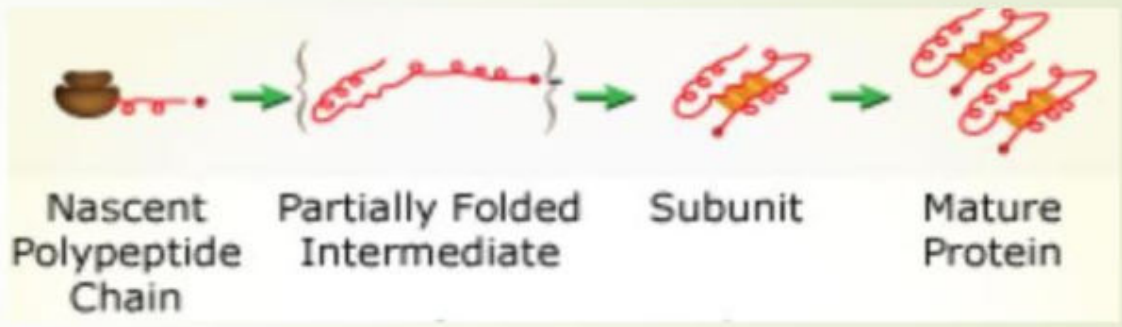


FIGURE 4–27 Renaturation of unfolded, denatured ribonuclease. Urea is used to denature ribonuclease, and mercaptoethanol ($HOCH_2CH_2SH$) to reduce and thus cleave the disulfide bonds to yield eight Cys residues. Renaturation involves reestablishment of the correct disulfide cross-links. Lehninger Principles of Biochemistry by David L Nelson

Protein folding in sequential manner

1. Newly synthesized polypeptide chain emerges from 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 hydrophobic residues lead to formation of a compact arrangement (molten globule state)
- Hydrophobic residues of proteins gather inside collapsed forms within core
- Collapsed state favors formation of 2° structure & encourages 3° interaction among residues

Assisted Protein Folding

- Most Proteins fold spontaneously to their native but some proteins undergo assisted folding
- Folding of some proteins require chaperons, these are specialized proteins which interact with partially folded or improper folded polypeptides, ensure correct folding pathways. Ex. Chaperones i.e, Hsp 70 family and chaperonins
- Finally, folding pathways of some proteins require two enzymes (Protein disulfide isomerase and Peptide prolyl cis-trans isomerase)

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Chaperones: Hsp70 family of chaperones more abundant in cells stressed by elevated temperatures:

- It binds to regions of unfolded polypeptides rich in hydrophobic residues may break up protein aggregate or to prevent formation of new aggregate
- It protect both proteins by heat denaturation
- It blocks folding of certain proteins that remains unfolded until they translocated across a membrane
- It assisted folding and assembly

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2. Chaperonins: Chaperones provide microenvironments in which folding occur:

- They are elaborate protein complexes required for folding of some cellular proteins that do not fold spontaneously
- Hsp60 acts as chaperonins in folding process, together with an Hsp70 chaperone
- Central cavity of Hsp60 chaperone provides a sheltered environment in which a polypeptide fold until all hydrophobic regions are buried in its interior, thus prevent protein aggregation.

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Two enzymes involved in Protein Folding Pathways

1) Protein disulfide isomerase (PDI):

- Catalyzes interchange, shuffling of disulfide bonds until bonds of native conformation are formed

- Catalyzes elimination of folding intermediates with inappropriate disulfide cross-links

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

- Catalyzes interconversion of cis and trans isomers of pro residue peptide bonds, which can be slow step in protein folding that contains some pro peptide bonds in cis conformation

Chaperone-assisted protein folding

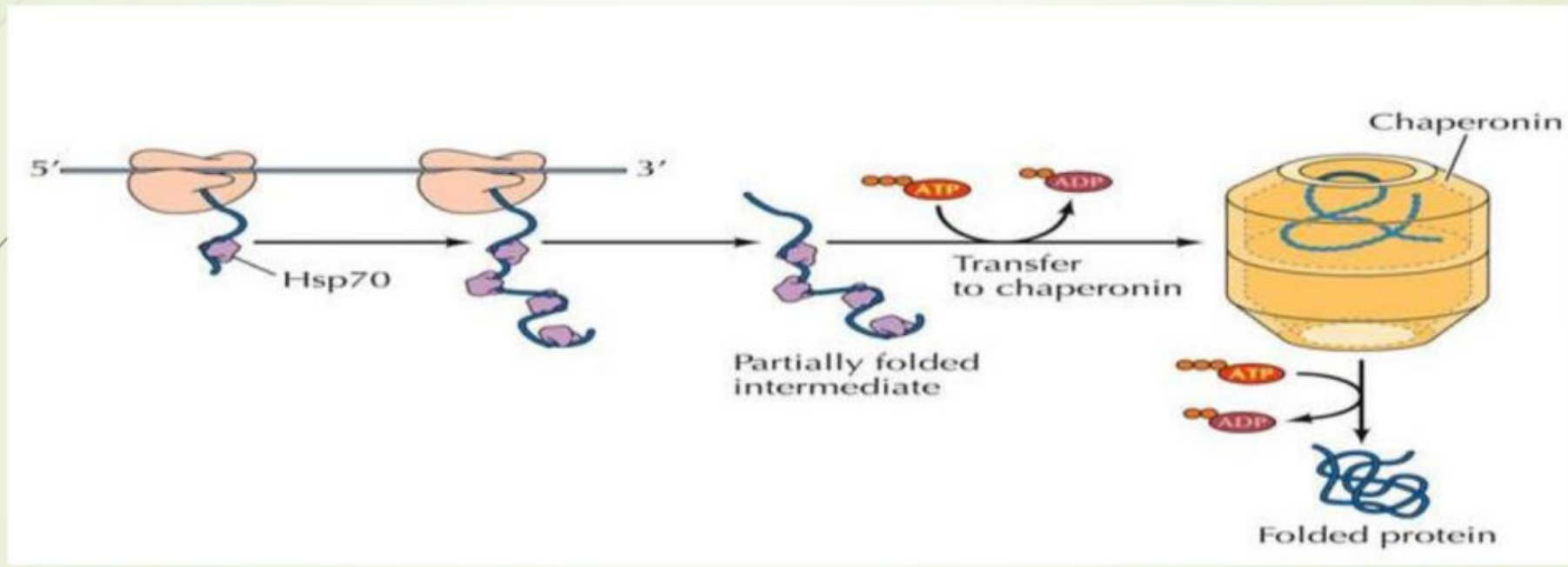

Fig.8.23: The Cell, 4th Ed

Protein Misfolding and Diseases

Incompletely and incorrectly folded proteins leads two serious problems to cells:

1. Loss of function due to absence of correctly folded protein:
- Cystic fibrosis

2. Aggregation of incorrectly folded proteins:
- Alzheimer's disease (Amyloid beta)

Cystic Fibrosis (CFTR)

- Cystic fibrosis transmembrane conductance regulator (CFTR) is a membrane protein and encoded by CFTR gene.
- CFTR gene codes for an ABC (ATP binding cassette) 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 508th position, causes improper protein folding.
- Improved understanding of protein folding may lead to new therapies for CFTR.

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 protein β-amyloid.
- A 4.3 KDa polypeptide produced by proteolytic cleavage of a larger protein known as amyloid precursor protein (APP).

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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.

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.

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- The 3-D structure and the function of proteins destroyed by denaturation, which demonstrate the relationship between structure and function.
- Some denatured proteins can renature spontaneously to form biologically active protein.
- Disulfide bond formation and cis-trans isomerization of Pro peptide bonds are catalyzed by specific enzymes.

Reference Books

1) Harper's Illustrated Biochemistry-30th Ed
2) Biochemistry 7th Ed by Jeremy M. Berg, John L. Tymoczko and Lubert Stryer
3) Lehninger Principles of Biochemistry, 6th Ed.

Thank you