# Chaperones and protein folding

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*ALTHOUGH THE HUMAN BODY HAS MANY DIFFERENT COORDINATED MECHANISMS DESIGNED TO ENSURE PROPER PROTEIN SYNTHESIS, MISTAKES SOMETIMES OCCUR THAT CAN LEAD TO SERIOUS HEALTH PROBLEMS. THE MISFOLDING OF PROTEINS, FOR EXAMPLE, CAN CAUSE A NUMBER OF DISORDERS INCLUDING MAD COW DISEASE, CYSTIC FIBROSIS, AND ALZHEIMER'S DISEASE. MOLECULAR CHAPERONE SYSTEMS ARE SOMETIMES ABLE TO RECTIFY SOME OF THESE PARTIAL UNFOLDING MISTAKES THAT OCCUR IN PROTEIN SYNTHESIS. RESEARCHERS IN THIS FIELD CLAIM THAT INCREASED KNOWLEDGE ABOUT CHAPERONE MECHANISMS MAY LEAD TO NEW TARGETS FOR ANTI-CANCER THERAPIES.*

Proteins are the most important macromolecules and play a pivotal role in performing and regulating the functions of the cell. For a protein to be fully functional, it should have a three dimensional structure with proper folding. Chaperones, also known as heat shock proteins, are a class of protein found in all the organisms starting from bacteria to humans and are located in every cellular compartment. Chaperones have a very important role as they are largely responsible for efficient folding of proteins in most cells. Chaperones have non-specific binding, which means that they can bind to a wide range of proteins (Lodish et al., 2004).
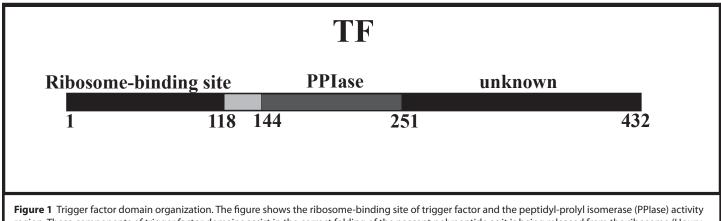
## TYPES OF CHAPERONES

There are various chaperone systems that differentiate between targets based on the conformation or the sequence of the protein substrate (Houry, 2001). The four main chaperone systems found in Escherichia coli cytoplasm are ribosome-associated trigger factor, the Hsp70 system, the Hsp60 system and the Clp ATPases. Each of these systems carry out a unique function to ensure the proper folding of target proteins (Houry, 2001).

Trigger factors assist in the folding of newly synthesized nascent proteins by binding next to the exit site on the larger subunit of the ribosome. This ensures the effective association between the trigger factor and nascent polypeptide chain and is important for cell survival. It has been found that trigger factors have peptidyl-prolyl isomerase (PPIase) activity. PPIase mediate rotation about peptide bonds and directs protein folding (Figure 1) (Houry, 2001).
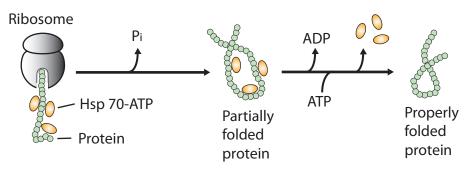
Molecular chaperones are another chaperone system involved with protein maturation (Georgopoulos,



**Figure 1** Trigger factor domain organization. The figure shows the ribosome-binding site of trigger factor and the peptidyl-prolyl isomerase (PPIase) activity region. These components of trigger factor domains assist in the correct folding of the nascent polypeptide as it is being released from the ribosome (Houry, 2001).

# Hsp70 Family (Heat-shock protein)



**Figure 2** Molecular chaperone-mediated protein folding. The Hsp70-ATP complex binds to the polypeptide as it emerges from a ribosome. ATP hydrolysis results in conformational change, causing the target protein to fold partially. When ATP rebinds to Hsp70, the protein is release and is properly folded (Lodish et al., 2004).
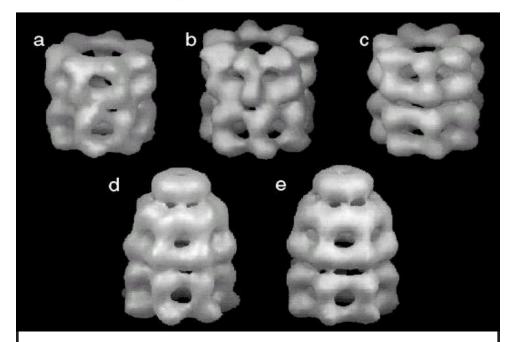
1993). These chaperones consist of the Hsp70 family and its homologues which are all conserved ATPases (Lodish et al., 2004). Members of the Hsp70 family play an important role in protein translocation and in high order protein assembly (Georgopoulos, 1993). Hsp70 bound to ATP assumes an open conformation and the exposed hydrophobic pocket of Hsp70 binds to the exposed hydrophobic regions of the unfolded target protein. The hydrolysis of ATP to ADP causes a conformational change of Hsp70 that allows it to encapsulate the protein. In this position, the target protein can now begin to undergo folding, which continues until an exchange of ATP for ADP occurs, releasing the target protein. The target protein is now properly folded and is functional (Figure 2) (Lodish et al., 2004). Thus, molecular chaperone proteins bind to the polypeptide chain as it emerges from the ribosome (site of protein synthesis), stabilize it and allow it to fold properly.

Another chaperone system which assists in the protein folding is the GroES/GroEL system, also known as chaperonins. GroEL and its cofactor GroES represent the

Hsp60 families and the Hsp 10 families, respectively (Houry, 2001). These heat shock proteins are large oligomeric proteins that fold proteins by forming an isolation chamber and consist of macromolecular assemblies, which have

a cylinder-like appearance (Lodish et al., 2004). GroEL's mechanism includes forming a barrel-shaped complex using its hydrophobic rim and together with its co-protein GroES, facilitates protein refolding in an ATP-dependent manner (Figure 3) (Ranson, White & Saibil, 1998). The hydrophobic portion of the misfolded target protein binds with the GroEL rim, resulting in the capture of the misfolded protein. Once the protein has been trapped, the GroES cap and ATP bind to the GroEL subunit. The binding of the GroES cap and ATP produces conformational changes in GroEL which stretches, encloses, and partly folds the protein. The last step is the hydrolysis of ATP to ADP which shifts GroEL to the open, relaxed state and releases the folded protein (Figure 4) (Lodish et al., 2004).

The last major chaperone



**Figure 3**  Various conformations of GroEL subunit.

The structures shown are: (a) the tight conformation where the GroEL subunit is not bound to anything; (b) the GroEL-ADP complex; (c) the GroEL-ATP complex; (d) the GroEL-GroES-ADP complex; (e) the GroEL-GroES-ATP complex. Note that ATP from (c) is the most asymmetric. The GroEL-GroES-ATP complex results in the partial folding of a protein. The complexes in (d) and (e) are similar to each other but the overall orientation of the rings is different between the two complexes. In the presence of ADP, GroES binding is asymmetrical (d) whereas in the presence of ATP, the structure appears to be more symmetrical (e) (Ranson, White & Saibil, 1998).

system are the Clp ATPases. These are the ATPase-dependent chaperones responsible for the assembly and disassembly of protein complexes. Clp ATPases act like specificity factors which help to present the various protein substrates to catalytic proteases for degradation by unfolding or disaggregating them. There are different classes of Clp ATPases found in Eschericia coli cytoplasm, each of which can function as a molecular chaperone as well as ATP-dependent regulatory components for various proteases. However, there are still many questions surrounding Clp ATPases that remain unanswered (Houry, 2001).

## Significance of Proper Protein Folding

Proteins are synthesized as a linear array of amino acids and they become functional when folded into their native state. Chaperones help other proteins in their initial folding and rescue them during partial misfolding due to aging or environmental cues. It is important for the cell to have a collection of chaperones in order to recover or eliminate misfolded proteins, as they tend to accumulate as large aggregates that compromise cell survival. Accumulation of protein aggregates is the fingerprint for amyloid misfolding disorders such as Alzheimer's Disease, as well as prion diseases that include bovine spongiform encephalopathy and scrapie.

Cystic Fibrosis is caused by a defective chloride channel (CFTR) in epithelial cells which leads to excessive mucous production in the lungs. The most common mutation associated with this disorder is the deletion of phenylalanine at residue position 508. CFTR genes containing this mutation cannot fold properly and fail to mature

to a fully glycosylated form. The nascent protein folds incorrectly, is recognized as abnormal by cellular mechanisms, and is degraded rather than transported to the plasma membrane. Other causes of this disorder include mutations in the nucleotide-binding domain and membrane-spanning domain. The

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symptoms of Cystic Fibrosis include difficulty in breathing and it often results in various lung infections (Weish & Smith, 1993).
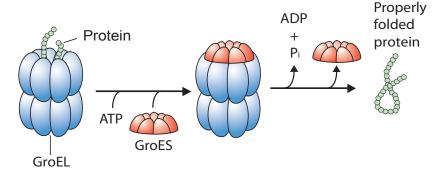
Bovine spongiform encephalopathy (BSE), also known as Mad Cow Disease, is one of the most commonly known prion-misfolding disorders. When these prions become associated with a normal protein, they convert the appropriately folded protein into an abnormal conformation. This proves to be fatal for the host organism and results in the formation

of further aggregates of the misfolded protein. BSE is marked by the formation of tangled filamentous plaques. There are other neurodegenerative disorders which are also caused by the aggregation of proteins that are stably folded in a pathological conformation (Lodish et al., 2004). Although the etiology of various neurodegenerative disorders may be different, the common factor is that during the aggregate formation, the alpha-helical domains start disappearing and there is an increase in the beta-dominated secondary structures (Figure 5) (Chaudari & Paul, 2006). It has also been found that during the protein aggregation, amyloid fibrils polymerize to cross beta sheet structures in such a way that the beta strands are arranged perpendicular to the long axis of the fiber (Figure 6) (Chaudari & Paul, 2006).
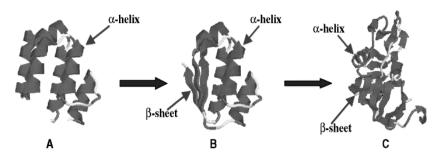
## Concluding Remarks

From the above discussion, it is clear that a normally folded protein is essential for proper function. The misfolding of proteins can be caused by mutations, inappropriate post-translational mechanisms and other environmental
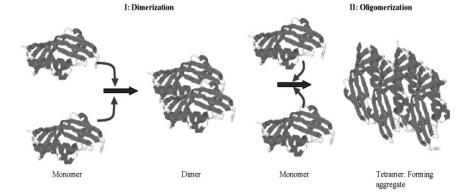
## Chaperonins



**Figure 4** The GroES/GroEL functional cycle. In the absence of ATP or in the presence of ADP, GroEL is in its tight state which is capable of binding to the misfolded protein. When ATP binds to GroEL, it assumes the open, relaxed state which results in the release of folded protein. (Lodish et al., 2004).

**Figure 5** During amyloid formation in various neurodegenerative disorders, the alpha-helical structures are replaced by beta-pleated sheets, resulting in a change in the overall secondary structure of a native protein. (A) Native protein contains alpha-helical secondary structure. (B) The alpha-helical structure is converted in a beta-pleated sheet due to misfolding. (C) Misfolded protein which contains mainly beta-pleated secondary structure (Chaudari & Paul, 2006).



**Figure 6** A diagrammatic representation of how aggregate formation takes place. Misfolded monomers, which consist of mainly beta-pleated secondary structure, interact with each other through hydrogen bonding resulting in the formation of long beta sheets (Chaudari & Paul, 2006).

cues which are still being researched. Misfolding also marks the protein for proteolytic degradation, and the accumulation of protein fragments during this process contributes to the development of certain diseases. Therefore, chaperones are extremely important for effective protein folding and prevention of deleterious disorders. Recent advancements in understanding how chaperones control protein folding, and how cells regulate the chaperone's activities, has provided insight into the signal pathway alterations involved in cancer development. Hopefully, this new information will help identify new targets for anti-cancer therapies (Chaudari & Paul, 2006). Chaperones are currently the subject of a great deal of research, and the scientific community is exploring the importance of chaperones in other cellular processes. Ⓜ

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