Role Of Molecular Chaperons In Protein Folding

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Abstract:

Gene functions are manifested in the form of proteins. They are central for the various biological activities. For the cell to properly function, proteins must be properly expressed at specific location where they are required and at proper time. Though the proteins are expressed properly, they should acquire a proper stable 3D structure to perform its activity. This is brought about by proper protein folding. Protein folding is a process by which a string of amino acids which is formed by a process translation of mRNA, interact among themselves to form a stable three dimensional structure during production of protein within the cell. This folding must be fast, proper and accurate. But in the cellular environment, newly synthesized proteins are prone to misfolding resulting in adverse effects. So cells have evolved a complex mechanism which enables proteins to fold properly and avoid unwanted aggregation and also the misfolded proteins are eliminated by assistance of chaperones and ubiquitin proteasome degradation system respectively to ensure high accuracy in protein expression. Under certain circumstances, misfolded proteins escape the degradation process resulting in the arose of large number of neurodegenerative disorders in humans. These neurodegenerative disorders in humans include Alzheimer's disease, Parkinson's disease, Huntington's disease, Cystic Fibrosis, Gaucher's disease and many more. Cellular Molecular Chaperones which are naturally found in body and newly synthesised chemical and pharmacological chaperones are being used to decrease the severity of several neurodegenerative disorders by preventing the misfolding of proteins. They also help in the treatment of various disorders like parkinson's disease, huntington's disease, Alzhimer's disease, oral ulcers, gastric carcinoma, prion diseases, cardio vascular diseases and many more. They can be induced by creating stress to the organism and thus in the production of heat shock proteins that help in the proper folding of the proteins and reduce the damage of the tissue.

Key Words: Alzheimers, Chaperones, Huntington's disease, Heat shock proteins, Protein folding, Protein misfolding, Parkinson's disease, Neurogenerative diseases,

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Introduction:

Cell is dynamic. Every second several biochemical reactions, which comprise to form metabolic reactions (both catabolic and anabolic) occur inside the cell for proper functioning of the cell. The information for all these metabolic activities is present in DNA which is transcribed and translated to give a protein as final product. An organism's proteins make it alive and work. Several diseases can be attributed to imbalance of proteins in the cell. Imbalance of the proteins in the cell is due to either too little of a particular protein being present, or too much of a protein, or a protein being produced is dysfunctional, or produced at a wrong place or at the wrong time. In order to be functionally active, a protein has to acquire a unique 3D confirmation by a folding pathway, which is described by the primary aminoacid sequence and also depends on local cellular environment and the state in which cells are present and according to the requirements of the cell. Proper folding of the proteins is a vital mechanism for the proper functioning of the living organism. When a protein is completely folded or unfolded it acts chemically neutral. If its in between, in actual process of folding or unfolding,it becomes sticky and reacts non specifically with other proteins. Even crowded environment of the cytosol is one of the reasons for the production of uncorrectly folded proteins by increasing protein aggregation.

A small error during the folding results in the misfolded protein which leads to many adverse diseases. During evolution, mechanisms are evolved which ensure folding of proteins properly in such a way that necessary reactive elements are exposed and unwanted reactive elements are not exposed. Within the cellular environment which is crowded, many proteins fold properly by themselves with the assistance of Molecular Chaperones probably within milliseconds of production of linear chain of aminoacids from the translation, resulting the 3D structures which are quite stable with a biological functions. As the protein molecules highly dynamic, constant chaperone surveillance is required to ensure Proteostatis.

The properties of the peptide bond and the amino acid side chains confer a high degree of confirmational flexibility to the protein confirmation, resulting in tremendous possible confirmations from a single polypeptide chain(Fresht and Daggett, (2002)). Out of these, only one confirmation that is thermodynamically the most stable state generally corresponds to the native state, which is determined by the primary sequences. Protein folding has recently been described in terms of a 'folding funnel' (Dill and Chan, (1997)). The bottom of the funnel represents the native state of the protein. At top of the funnel, the protein exists in random states with large number of confirmations of high free energy. Progresss down the funnel is accompanied by an increase in native like structure as folding proceeds.

Protein Folding in the Cell:

Folding process depends on the environment in which folding takes place. When polypeptides are synthesised in the cells, they fold in the cytoplasm after the release from the ribosome or in other subcellular compartments such as ER or mitochondria after they are translocated through membranes (Hartl and Hayer- Hartl, (2002); Dobson, (2004)). Protein folding can also begin cotranslationally while carboxy – terminal segment of a nascent chain inside the exit channel of ribosome (Hardesty and Kramer, (2001); Baram and Yonath, (2005)). Within the cells, proteins in the process of folding encounters challenges imposed by crowed environment of the cell. Incompletely folded chains expose hydrophobic regions which cause further complications as they tend to react non specifically and then to aggregate. Situations are more problematic because aggregation process follows second order kinetics and therefore surpasses the first order folding process as their concentration increases (Fresht, (1999)). Systems which evolved to prevent protein aggregation include chaperones and ubiquitin proteasome system.

Proteins that are not able to achieve the native state, due to an unwanted mutation in their aminoacid sequence or simply due to an error in folding process, are recognised as misfolded and subsequently targeted to degration pathway. This is referred to as a protein 'Quality Control" (QC)

system and is composed of two components: Molecular chaperones and the Ubiquitin Proteasome System (UPS). The QC system plays a critical role in cell function and survival. Calnexin chaperone forms a part of QS system. It recognises and target abnormally folded proteins for rapid degradation. One important system of cell is UP System. Studies suggest that disturbance in or impairment of the UPS, which may be induced by accumulation of misfolded proteins leads to altered UPS function, which enhances the formation of aggregates. Aggregated proteins often inside the cell lead to the formation of an amyloid like structure, which eventually cause disorders.

Molecular Chaperones are the proteins that assist the non covalent folding or unfolding and the assembly or disassembly of other macromolecular structures but do not be a part of these structures when the structures perform their normal biological functions (Ellis RJ (1996).). They stabilize non-native conformation and facilitate correct folding of protein subunits. They do not interact with native proteins, nor do they form part of the final folded structures. Some chaperones are non-specific, and interact with a wide variety of polypeptide chains, but others are very specific and interact with specific protein targets. They prevent inappropriate association or aggregation of exposed hydrophobic surfaces and direct their substrates into productive folding resulting in a useful protein and its transport or degradation pathways. In short chaperons play regulatory role in different ways, which include: folding of proteins in the cytosol, endoplasmic reticulum and mitochondria; intracellular transport of proteins; repair or degradation of proteins partially denatured by exposure to various environmental stresses; control of regulatory proteins; and refolding of misfolded proteins. They are also called as cell stress proteins (CSP) and are highly conserved proteins (Kaijser B (1995)).

Molecular chaperons are on the surface of or are secreted from a range of cells including myeloid, lymphoid, epithelial and mesenchymal cells have been appearing over the past 20 years (Gabai VL *et al.,* (2000)). Though there are several reports, the hypothesis that they are secreted was not accepted widely as the mechanism is not well understood.

Specifically, the molecular chaperones generally do not have a signal sequence which is involved in one pathway of the secretion of proteins from eukaryotic cells. Many HSPs form multimolecular complexes that act as molecular chaperones binding other proteins, denoted as client proteins. Chaperones not only concerned with protein folding, but are also involved in the assembly of nucleosomes from folded histones and DNA. Chaperones are members of diverse protein families capable of binding so as to stabilize non native confirmations of other proteins. The binding prevents aggregation of the intermediates and facilitates the correct folding and assembly through controlled binding and release cycles (Frydman and Hartl, (1996); Ellis (2001a); Hartl and Hayer-Hartl (2002)). Other function of chaperones is to prevent both newly synthesised polypeptides and assembled subunits from aggregating.

Today there are more than 25 families of molecular chaperones, with more than 100 proteins and individual genes within families which differ with respect to sequence and expression patterns, as well as the function and subcellular localization of the respective gene products. Chaperones are found in all types of cells from archea to eukarya and even in eukaryotic cell components like mitochondria. They are located at various points in the cell. They are secreted from a range of cells including myeloid, lymphoid, epithelial and mesenchymal cells (Gabai VL *et al.,* (2000)). Major Hsp families, named to reflect the approximate molecular size (in kilodaltons). Some are Hsp100, Hsp90, Hsp70, Hsp60, Hsp40, and the small Hsp family (typically 20 to 25 kDa) (Hartl FU, Hayer-Hartl M, (2002)). The heat shock response was first described in 1962 (Hartl FU, Hayer-Hartl M, (2002)), and heat shock proteins (HSPs) are named for their increased synthesis after heat shock that is contrary to the reduced synthesis of most cellular proteins under these conditions. In addition to heat, these proteins are seen under conditions such as less nutrients, elevated temperature, anoxia, and exposure to ethanol, heavy metals, metabolic poisons, ischemia/reperfusion, free radicals or other chemical denaturants and oxidative and other stresses.

Chaperonins are a group of chaperones with molecular weight of about 60 kDa. Members include bacterial GroEL, Hsp60 of mitochondria and chloroplasts, and the TriC in eukaryotic cytosol (Bukau and Horwich, (1998)). They are characterised by the barrel shaped double ring structure (Braig *et al.*, (1994)). GroEL seems to be the best studied chaperonin with regard to folding mechanism (Grantcharova *et al.*,(2001)). GroEL works with GroES, a cofactor of Hsp 10 family. Inside of the ring structure of GroEL, a central cavity is formed, in which an incompletely folded polypeptide is sequestered by hydrophobic interactions. The first role of GroEL is providing a protected environment to prevent the folding by preventing sticking to one another (Ellis, (2001b)). Confirmational changes of GroEL subunits driven by the ATP hydrolysis includes enlargement of the cavity and a shift in its surface to a more hydrophilic lining (Barreal *et al.*, (2004)). As a consequence, the polypeptide molecule can proceed on its folding pathway. GroEL has also unfolding activity. Unfolding the misfolded protein will allow the protein to try further attempts at productive folding (Grantcharova *et al.*, (2001)). TriC constitutes a different subgroup of chaperonin, because it functions independent of Hsp10 cofactor. TriC play role in the folding of distinct protein classes (Siegers *et al.*, (2003)).

Many heritable and acquired diseases result solely from the loss of wild-type protein activity, because of a mutation that disrupts a critical function of the gene product. On the other hand, some disease states result from production of a mutant protein that misfolds and acquires a novel activity (for example, a tendency to form aggregates) that has pathological consequences. Several human diseases involving protein misfolding have been putforward in many reviews (Welch and Brown, (1996)). Here we address few types of heat schok proteins, a few representative disease states, discuss how protein misfolding and chaperones participate in the disease process, and then consider potential treatments that target chaperone activity.

Types of Chaperones:

There are many different families of chaperones. Each family acts to aid protein folding in a different way. In bacteria like E.coli, many of these proteins are highly expressed under conditions of high stress, for example when placed in high temperatures. For this reason, the term Heat Shock Protein has historically been used to name these chaperones. Some chaperones are involved in folding newly made proteins as they are extruded from the ribosome. Although most newly synthesised proteins can fold in absence of chaperones, a minority strictly require them for the folding. Some chaperones act to repair the potential damage caused by misfolding. Other types of chaperones are involved in transport across membranes, for example membranes of the mitochondria and ER in eukaryotes. New functions for chaperones continue to be discovered such as assistance in protein degration. Bacterial adhesin activity and in responding to diseases linked to protein aggreagation. Hsp70, Hsp40, and Hsp60 family members play important roles in nascent chain folding. Hsp70 members are also major components in membrane translocation processes. The small Hsps have important functions in disaggregation or degradation of misfolded complexes (Gething, (1997)), but it is not clear how important they are for nascent chain folding. Hsp90 is notable for its numerous associations with important regulatory proteins. Hsp100 family members, such as Hsp104, play an important role in thermotolerance in yeast. An overall increase in Hsp levels correlates with the acquisition of thermotolerance, but increased Hsp104 levels appear to be the critical factor.

Hsp70:

Hsp70s are well conserved chaperone family comprising central components of the cellular network of molecular chaperones (Mayer and Bukau, (2005)). They are among the first chaperones that bind newly synthesised polypeptides and are intimately involved in the translocation of unfolded polypeptides across intracellular membranes (Hartl and Hayer- Hartl, (2002)). Accordingly, Hsp70 family members are found associated with ribosomes and are present in Eukaryotic organelles, such as mitochondria and ER (Munro and Pelham, (1986)).

In contrast to the chaperonins, Hsp70 chaperones are monomeric consisting of two large globular subdomains, amino terminal ATPase domain and a carboxy terminal peptide binding domain (Flaherty *et al*., (1991); Zhu el al., (1996)). Peptide binding domain recognises short segments of substrate polypeptidein an extended confirmation (Zhu *et al*., (1996)), which can easily found on nascent polypeptide during translation, on the unfolded polypeptided during translocation across membranes and on misfolded and damaged proteins (Thulasiraman *et al*., (1999)). The ATPase cycle of Hsp70 consists of an alteration between the ATP state with low affinity and fast exchange rate for substrates, and the ADP state with high affinity and low exchange rate of substrates. Hsp70 chaperone activity is enhanced by the co- chaperone Hsp40 that binds to the C- terminus of Hsp70, stimulates its ATPase activity thereby stabilises the substrate bound form (Freeman *et al*., (1995); Minami *et al*., (1996)). Other co- chaperones including Hop and CHIP also bind to C- terminal end of Hsp70. Hop is a tetratricopeptide repeat(TRP) domain containing protein that couples the Hsp70 and Hsp90 chaperone machines via association through C- terminus of Hsp90. Hop binding inhibits the ATPase activity of Hsp90 that stabilises its interaction with client proteins. CHIP, another TRP containing protein has been suggested to reduce the ability of Hsp70 to refold certain denatured substrates (Ballinger *et al*., (1999)).These results contrast in vitro observations that over expression of CHIP increases the ability of Hsp70 to refold heat denatures firefly luciferase (kampinga *et al*., (2003)). It was seen that over expression of Hsp70 can protect cells from the adverse effects of protein damaging stresses and also supresses heat induced inactivation of firefly luciferase and facilitates its reactivation during recovery from heat shock (Michels *et al*., (1997)). The observation that thermotolerant cells resist stress induced apoptosis suggests that, rather than simply preventing cellular inactivation, the heat shock proteins have specific roles in the supressions of selective components of cell determined death pathways (Mosser and Martin, (1992)). Inhibition of stress induced apoptosis by Hsp70 requires chaperone function, since deletion of the ATPase domain or the C-terminal EEVD sequence or replacement of EEVD sequence with alanine residues

prevented its ability to block caspase activation which inturns prevents apoptosis in heat shocked cells (Mosser *et al*., (2000)). Hsp70 has also been reported to prevent the clevage of a caspase-3 target (focal adhesion kinase) by directly interacting with the protein (Mao *et al*., (2003)). It is thought that many Hsp70s crowd an unfolded substrate, stabilising it and preventing aggregation until the unfolded molecule folds properly. Once the protein is folded properly, Hsp70s loose affinity for the molecule and diffuse away. Hsp70 also acts as a mitocondrial and chloroplastic molecular chaperone in eukaryotes.
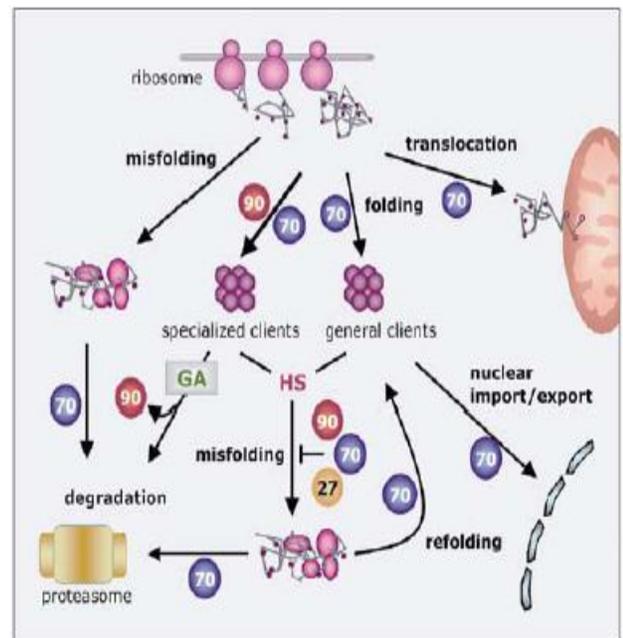

Fig: 1. Chaperone assisted folding, translocation and mediating to proteosome (Dick D Mosser and Richard I Morimoto, Molecular chaperones and the stress of oncogenesis *Oncogene* (2004). 23, 2907- 2918.)

Hsp90:

Hsp90(HtpG in E.coli) is necessary for the viability of the eukaryotes. Heat shock protein 90 is a molecular chaperone essential for the activating

many signalling proteins in the eukaryotoic cell. Each Hsp90 has an ATP binding domain, a middle domain and a dimerization domain. Hsp90 may also require co- chaperones like immunophilins, Sti, p50, and Aha1 and also cooperates with the Hsp70 chaperone system. The chaperone activities of Hsp90 are also regulated by ATP binding and hydrolysis. Unlike Hsp70, which has ability to both hold unfolded polypeptides and refold protein substrates to the native state, Hsp90 appears to be more specific to capture and hold client proteins in intermediate confirmations with refolding of denatured substrates requiring transfer to the Hsp70/Hsp40 chaperone ((Freeman and Morimoto, (1996)). The discovery that the benzoquinone ansamycin drug geldanamycin (GA) binds to the ATP binding site of Hsp90 causing disruption of Hsp90 stabilised complexes has provided key insights into the roles of Hsp90 on signaling cascades and cell cycle regulation. Other roles for Hsp90 in the stress induced apoptotic pathway have been suggested by the observation that Hsp90, like Hsp70, binds to Apaf- 1 and prevents the cytochrome c/dATP- mediated oligomerisation of Apaf- 1 (Pandey et al., 2000b). One suggestion is that Hsp90 appears to compete with cytochrome c for binding to Apaf- 1, consequently during stress, the amount of Hsp90 associated with Apaf- 1 decreases as cytoplasmin levels of cytochrome c increase. This suggests that Hsp90 could prevent premature activation of Apaf-1, ensuring that formation of apoptosome occurs only when Hsp90 is preoccupied by misfolded proteins and the cytosol is with cytochrome c.

Hsp27:

Hsp27, in contrast to the other major chaperons, is ATP independent, yet can effeciently associate with unfolded proteins and maintain them in a folding – competent state. The chaperone activity of Hsp27 is regulated by heat induced changes in phosphorylation and oligomerization (Haslbeck and Buchner, (2002)). Hsp27 has been shown to interact with and inhibit components of both stress and death receptor induced apoptotic pathways. In cells expressing higher levels of Hsp27, caspase activation was blocked (Garrido et al., (1999)). Hsp27 has been

shown to interact with released cytochrome c, thus providing an additional mechanism to interfere with binding of cytochrome c to Apaf-1 (Bruey et al., (2000)). Hsp27 is also found to inhibit apoptosis.

Protein Folding:

When polypeptides are synthesised in the cells, they fold in the cytoplasm after release from the ribosome or in other subcellular compartments such as endoplasmic reticultum or mitochondria after they are translocated through membranes (Hartl and Hayer- Hartl, (2002); Dobson, (2004)). Eukaryotic cell has a specialised compartment , endoplasmic reticulum where folding occurs for the proteins that are destined for secretion to an extracellular environment or to the plasma membrane and other organelles in the secretory pathway. The ER lumen is more likely to the extracellular space than the cytosol with respect to high calcium concentration and high oxidising potential. It contains various types of chaperones and folding factors not only to assist efficient folding of proteins but also to play a quality-control role (Sitia and Braakaman, (2003); Welch, (2004)). A variety of quality contraol mechanisms operate in the ER and in compartments of the secretory pathway to ensure the fidelity and regulation of protein expression. Only proteins that fold and mature properly pass a stringent selection process and go their own ways to target compartments. If proper maturation fails, a complex degradation machinery eliminates misfolded or unassembled secretory proteins from the ER (Plemper and Wolf, (1999)). The proteins are retained in an ER/pre- Golgi compartment and then hydrolysed by the cytosolic ubiquitin- proteosome system.

The ER quality control pathway operates at several levels and by multiple mechanisms. All newly synthesised proteins entering the ER are subjected to a primary quality control that monitors their architectural design through ubiquitous folding sensors. Cheperones of the Hsp70 and Hsp90 families recognize redundant structural features of unfolded polypeptides and assist in their folding and assembly. BiP(Hsp70) and GRP94 (Hsp90) with

other folding factors, seem to form an ER network that can bind to unfolded protein substrates instead of existing as free pools that assemble onto substrate proteins (Meunier *et al.*, (2002)). Secondary quality control mechanisms rely on cell- specific factors and facilitate export of individual proteins. Like the primary quality control factors, secondary quality control factors promote folding, maturation and assembly of their substrates. Cellular machinery regulating synthesis, translocation, folding and degradation of protein seems to operate in a very complex, cooperative and stringent manner to ensure protein aggregation is minimised. Nevertheless, in a certain disease condition, misfolded protein will escape such elaborate system forming protein aggregates.

Protein Misfolding:

On one level, life may be thought of as the co- ordinated activity of proteins and disease as an imbalance of proteins that adversely affects the quality of life. Protein misfolding gives rise to the malfunctioning of living systems. Protein misfolding and its pathogenic consequences have become an important issue over the last two decades. Accoding to the Prion researcher Susan Lindquist. "Protein misfolding could be involved in up to half of all human diseases". Even misfolding of proteins is responsible for many p53- mediated cancers. Csermely suggested a 'Chaperone overload' hypothesis, which explains that with aging, there is an overburden of accumulated misfolded protein that prevents molecular chaperones from repairing phenotypically silent mutations which might cause disease. It has been shown that the yield of correctly folded protein obtained from in vitro refolding is low due to the formation of thermodynamically stable folding intermediates. These confirmations are called 'dead end' confirmations and are 'off pathway' intermediates, they generally lead to formation of aggregates that may eventually causes different degenerative diseases. A common feature of almost all protein confirmational diseased is the formation of aggregates caused by the destabilisation of the alpha- helical structure and the simultaneous formation of a Beta sheet.

It is not clear whether misfolding triggers protein aggregation or protein oligomerisation induces confirmational changes. Based on the kinetic modeling of protein aggregation, it has been proposed that the critical event in Protein Confirmational Disease is the formation of protein oligomers that can then act as seeds to induce protein misfolding. In this model, misfolding occurs as a conseequence of aggregation (polymerisation hypothesis), which follows a crystallisation like process and nucleus formation. The alternative model suggests that the underlying protein is stable in both the folded and misfolded forms in solution (confirmational hypothesis). This hypothesis proposes that spontaneous or induced confirmational changes result in formation of the misfolded protein, which may or may not form an aggregate. In the third hypothesis, the native protein confirmation is changed to an amyloidogenic intermediate, which is not stable in the cellular environment. This intermediate has many exposed hydrophobic regions and therefore develops small oligomers, mainly composd of Beta sheets, via intermolecular interactions. This small oligomers form an ordered fibril like struture called amyloid via an intermolecular interaction.

Many protein misfolding diseases are characterised not by disappearance of a protein but by its deposition in insoluble aggregates within cell. The diagnostic feature common to the protein aggregation diseases is the deposition of insoluble protein aggregates called amyloid fibrils, hence the generic term amyloidosis. The fibrils may themselves be assembled into discrete bodies, termed plaques. In each disease the misfolded protein is unique and a unique set of tissue is affected but the diposited amyloid fibrils produced by aggregation of the misfolded protein appear structurally very similar. Protein aggregation is facilitated by partial unfolding during thermal and oxidative stress and by alterations in the primary struture caused by mutation. Protein aggregates can either structured or amorphous. In either case, they are insoluble and metabolically stable in the physiological environment. For various diseases associated with protein misfolding, one or more proteins are converted from the native structure to an aggregated mass, which is commonly called an amyloid. The net accumulation of toxic protein

aggregates in the cell depends on the stability, compactness and hydrophobic exposure of the aggregates as well as on the rate of protein synthesis in the cell. The accumulation of toxic aggregates in the cell depends on chaperone expression and protease network. Environmental stress may induce the synthesis of higher levels of chaperones and proteases in the cell, which can better remove toxic aggregates.

The aggregates into which they can convert, share a common structural feature. Allmost all the aggregations result in the Beta linkages formed by hydrogen bonding between peptide loops and sheets. Some point mutations destabilise central Beta sheet to allow incorporation of a loop of another molecule into the Beta sheet resulting in chains of polymers. Results from the experiments by X- ray fibre diffraction, cryoelectron microscopy and solid state NMR have shown that amyloi fibrils are long, unbranched and often twisted structures a few nanometers in diameter and the organised core structure is composed of Beta sheets whose strands run perpendicular to the firbril axis (Sunde and Blake, (1997); Jimenz *et al*., (1999); Petkova *et al*., (2002)).

Under appropriate circumstances, misfolded monomers may oligomerise into prefibrillar assembles. Such circumstances can be satisfied by extreme conditions of pH and temperature or partial proteolysis for non- disease proteins. Generally, the aggregation occurs in two steps: the first step is characterised by a slow lag phase seems to involve the formatioin of soluble oligomers as a result of relatively nonspecific interactions. The oligomeric nucleus then rapidly grows. The lag phase can be minimised or eliminated by seedling preformed nucleus (Caughey and Lansbury, (2003)). The accumulation of toxic misfolded protein may overload the quality control system. Unfolding of substrate proteins is the prerequisite for the degradation by the proteasome and Beta structure is more difficult to unravel than the alpha helix or surface loop. This explains why amyloid deposits are not easily cleared away by the proteasome system (Lee *et al*., (2001)). The undegradable deposits may in turn sequestrate components of the chaperone and degradation systems, reducing the activity of assisted folding and proteolysis. The whole process will lead to cellular toxicity.

Pathways that control the fate of misfolded proteins, for example molecular chaperones or proteolytic systems, may become interesting novel targets for therapy of neurodegenerative disorders. Chaperones facilitate refolding or degradation of misfolded polypeptides, prevent protein aggregation and play a role in formation of aggresome, a centrosome-associated body to which small cytoplasmic aggregates are transported. The ubiquitin-proteasome proteolytic system is critical for reducing the levels of soluble abnormal proteins, while autophagy plays the major role in clearing of cells from protein aggregates. Accumulation of the aggregation prone proteins activates signal transduction pathways that control cell death, including JNK pathway. The major chaperone Hsp72 can interfere with this signalling pathway, thus promoting survival. A very important consequence of a build-up and aggregation of misfolded proteins is impairment of the ubiquitin-proteasome degradation system and suppression of the heat shock response. Such an inhibition of the major cell defense systems may play a critical role in neurodegeneration.

Alzheimer's disease:

Alzheimer's, is the most common form of dementia. Many major neurodegenerative diseases like Alzheimer's disease are associated with degeneration and death of specific neuronal populations due to accumulation of certain abnormal polypeptides (a common marker) (reviewed by Checler, (1995); Dickson, (1997); Selkoe, (1994, 1996,1997)). These misfolded species aggregate and form amyloid plaques inclusion bodies and their neurotoxicity is associated with the aggregation. To handle a build-up of abnormal proteins cells employ a complicated machinery of molecular chaperones and various proteolytic systems. The amyloid precursor protein (APP), whose normal cellular function is unresolved, is a membrane protein and, distinctively, an intramembranous substrate for proteases that generate a characteristic set of

fragments. Aβ is one of these and consists of the amino-terminal 40 to 43 amino acids of APP.

Several factors that predispose individuals to the development of Alzheimer's disease also lead to increased generation of Aβ 42- and 43-amino acid peptides. Mutations in APP associated with Alzheimer's disease all map within or adjacent to the Aβ sequence. The carboxyl-terminal region of Aβ exists in a β-sheet conformation, whereas a region toward the amino terminus can exist in either an α-helical or β-sheet conformation. Various factors, such as Aβ mutations, can shift the conformational equilibrium of the amino-terminal region toward a β-sheet conformation that favors aggregation of Aβ peptides and plaque formation. Once a seed aggregate has formed, equilibrium might be shifted further toward β-sheet conformations as monomers in the transient β-strand conformation are trapped by addition to preexisting aggregates. A conformational shift toward β-sheets is a factor in other diseases involving protein aggregation.

In addition to promoting amyloid plaque formation, accumulation of Aβ may be involved in other neurotoxic mechanisms. In one recent study, a cellular protein termed endoplasmic reticulum-associated amyloid β-peptide binding protein, which is related to hydroxysteroid dehydrogenases, was shown to bind specifically to Aβ; this protein's abundance and interactions with Aβ correlated positively with Aβ-induced neurotoxicity. In another study recombinant Aβ was found to interact specifically with glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a key enzyme in glycolysis. Conceivably, sequestration of GAPDH and a deficit in GAPDH activity could result in cellular metabolic deficiencies and contribute to neuropathological changes, but this remains to be demonstrated.

Huntington's disease:

Huntington's disease is one of a class of neurodegenerative conditions caused by aggregation of a poly-glutamine-containing protein (reviewed by Bates *et al.*, (1997); Lunkes and Mandel, (1997)).

Although different gene products are involved in different disorders, a common genetic feature is that each locus contains CAG tandem repeats that have been expanded by a poorly understood mechanism. Because CAG is a glutamine codon, an in-frame CAG expansion results in a protein product with a corresponding extension of its polyglutamine (polyGln) tract. Beyond a critical length of approximately 40 glutamine residues, protein aggregation occurs that leads to pathological changes.

In Huntington's disease, the responsible gene (Huntington's Disease Collaborative Research Group, (1993)) codes for huntingtin (Htg), a 350-kDa protein. Htg appears to be expressed in all tissues, not just the brain, and embryonic lethality is observed in mice nullizygous for the Htg gene. In normal individuals, the amino-terminal region of Htg contains a tract of 6 to 39 glutamine residues; in Huntington's disease, the tract is 35 to 180 residues long, but 40 to 55 units are found in most cases. Recent evidence suggests that Htg is required for neurogenesis but that pathological changes associated with expanded polyGln tracts in Htg are the result of a gain of function rather than loss of normal Htg function.

In one recent study, Htg fragments containing a range of polyGln lengths were generated. Fragments with 20 or 30 glutamines failed to aggregate in an in vitro assay, but fragments with 51 or more glutamines formed fibrillar aggregates. As with Aβ, the aggregating form of Htg is conformationally distinct from nonaggregating protein and appears to be enriched for β-sheet structure. Expression of only the amino-terminal portion of Htg, containing a 100- to 150-glutamine tract, generated Huntington-like pathological markers and symptoms in transgenic mice. Neuronal intranuclear inclusions were formed that contained the Htg fragment as well as ubiquitin, suggesting that there might be a defect in ubiquitin-mediated degradation of the fragment. Interestingly, Htg is a substrate for apopain, a protease involved in apoptotic pathways, and cleavage increases with the length of the polyGln tract. Apopain cleavage generates an amino-terminal, polyGln fragment similar to the transgene product observed in a study

therefore, generation of an amino-terminal Htg fragment may be pathogenic in Huntington's disease.

Prion diseases:

Sheep scrapie, bovine spongiform encephalopathy, and human Cruetzfeldt-Jakob disease are transmissible neurodegenerative diseases; the infectious agent for each is thought to be a prion vector termed prion protein (PrP) (Prusiner and Scott, (1998)). Although findings are still controversial, prion vectors are thought to lack nucleic acids and to consist solely of an alternately folded form of a protein that is normally expressed in the host. Strong genetic evidence for a heritable prion-like protein has come from studies of certain non-Mendelian inheritance patterns in yeast.

The mammalian PrP has complex biological characteristics (reviewed byHorwich and Weissman, (1997)). The normal function of the noninfectious cellular form of PrP (PrP^C) is poorly defined. PrP^C is a membrane protein found in many tissues, not only in brain. PrP^C has a half-life of 3 to 6 hours in cells and is sensitive to proteases in vitro. The infectious form of PrP (termed PrP^{Sc} to reflect its role in scrapie) undergoes little or no turnover in cells and is resistant to proteolysis in vitro. PrP^{Sc}, but not PrP^C, forms amyloid fibrils associated with scrapie, bovine spongiform encephalopathy, and Cruetzfeldt-Jakob disease. Similar to Aβ and the disease-associated form of Htg, PrP^{Sc} has a much higher content of β-sheet structure than does the normal conformation in PrP^C, and a recombinant amino-terminal fragment of PrP (142 amino acids) has been shown to switch between α-helical and β-sheet conformations in a pH-dependent manner. PrP^{Sc} is thought to propagate itself by inducing PrP^C to change its conformation to the protease-resistant form, which then participates in amyloid formation. PrP^{Sc} appears to be required for infectivity and formation of amyloid fibers, but it may not be required for PrP-dependent neuropathological changes. Several years ago, an observation was made that PrP synthesized in a cell-free system can exist in alternate topological forms in ER membranes. Recently, it was shown that transgenic mice expressing a PrP mutant that favors a greater cytoplasmic orientation of PrP domains developed a neurodegenerative phenotype in the absence of protease-resistant PrP^{Sc}.

As recently discussed , several questions remain regarding the mechanism by which PrP^C is converted to PrP^{Sc}, as well as whether PrP^{Sc} is the infectious agent or instead serves as a highly protective reservoir for an unidentified virus. There are some suggestions that conversion may be mediated by an additional cellular protein, perhaps one of the molecular chaperones . Interestingly, the chaperone Hsp104 has been genetically and biochemically linked to the function of prion-like proteins in yeast. In addition, Hsp104 was recently found to interact in vitro with the β-sheet conformer of a PrP fragment (not the α-helical conformer) and with the Alzheimer's protein Aβ. Hsp104 and the bacterial chaperone GroEL were found to enhance PrP^{Sc}-dependent conversion of PrP^C in vitro, whereas other protein chaperones had no effect and chemical chaperones inhibited conversion. Still, the biological role of chaperone interactions in the pathogenesis of mammalian prion-based diseases remains to be defined.

Parkinson's Disease:

Parkinson's disease is a disorder of the brain that leads to shaking (tremors) and difficulty with walking, movement, and coordination, Causes, incidence, and risk factors (Witt SN. Department of Biochemistry and Molecular Biology). Parkinson's disease most often develops after age 50. It is one of the most common nervous system disorders of the elderly. Sometimes Parkinson's disease occurs in younger adults. It affects both men and women. Nerve cells use a brain chemical called dopamine to help control muscle movement. Parkinson's disease occurs when the nerve cells in the brain that make dopamine are slowly destroyed. Without dopamine, the nerve cells in that part of the brain cannot properly send messages. This leads to the loss of muscle function. The damage gets worse with time. Exactly why these brain cells waste away is unknown. Parkinson's disease affects movement, producing motor symptoms. Non-motor symptoms,

which include autonomic dysfunction, neuropsychiatric problems (mood, cognition, behavior or thought alterations), and sensory and sleep difficulties, are also common (Molecular chaperones as rational drug targets for Parkinson's disease therapeutics.), (Kalia SK, Kalia LV, McLean PJ)

Cardio vascular disease:

Some of the earliest studies of chaperones in the cardiovascular field suggested an intracellular role as cardioprotectants. (Delogu G, Signore M, Mechelli A, Famularo G. (2002)) (Yellon DM, *et al.,* .(1992)) (Marber MS *et al.,* .(1995)) These studies suggested that Hsp70 played a major role in cardioprotection.(Pockley AG.(2002)) To show that Hsp70 is cardioprotective, transgenic mice overexpressing Hsp70 were generated and these animals were, indeed, found to be protected against ischemic injury (Craig EA.(1985)) Two molecular cheperons have been extensively studied. It is believed that Hsp70 inhibits caspase-dependent and caspaseindependent apoptotic stimuli (DillmannWH *et al.,* (1986)). It has also been shown that Hsp27 inhibits cytochrome c-dependent activation of procaspase (Garrido C *et al.,* 1999)) (Currie RW, J Mol Cell Cardiol (1987)) and stabilises cytoskeletal structures (DillmannWH *et al.,* (1986)). exposure of isolated hearts to ischemia or elevated perfusion temperature caused the induction of Hsp70 (Currie RW *et al.,* (1988)). Experimental occlusion of the coronary artery to induce myocardial ischemia also resulted in elevation of Hsp70 in heart tissues (Yellon DM *et al.,* (1992)). By these experiments it was concluded that elevation of temperature results in the induction of HSP 70 which reduced tissue damage and exhibited quicker recovery from the ischemic episode (Marber MS *et al.,* (1995)). Similar results were found in the rabbit (Ooie T *et al*., (2001)). These studies suggested that Hsp70 played a major role in cardioprotection. However, other CSPs would also be upregulated. To show that Hsp70 is cardioprotective, transgenic mice overexpressing Hsp70 were generated and these animals were, indeed, found to be protected against ischemic injury (Soti C *et al.,* (2005)).

Gene transfer of Hsp70 has been shown to reduce post-ischemic infarct size in the rabbit heart (Pockley AG, Bulmer J, Hanks BM, Wright BH, (1999)). Another strategy being investigated is the use of agents that enhance the cellular expression of CSPs. Such agents include the antiulcer compound genanyl–geranyl–acetone which has been shown to inhibit post-ischemic heart damage (Ritossa F, (1962)) and a hydroxylamine derivative, bimoclomol whose cardioprotective actions are closely approximated with increased expression of Hsp70 (Morimoto RI, Kline MP, Bimston DN, Cotto JJ., (1997)). The over-expression of Hsp70 molecular chaperones suppresses the toxicity of aberrantly folded proteins that occur in Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis, and various polyQ-diseases (Huntington's disease and ataxias), Hsp70 is gaining attention as a possible therapeutic agent for these various diseases.

Conclusion:

Proper folding of proteins is very much essential for the proper functioning of the cell and inturn the functioning of an organism. Studies reveal that a nascent polypeptide chain become misfolded due to specific gene mutation which takes place or a matured native protein can also be misfolded. The fates of these misfolded proteins are different in different diseases. In some diseases, misfolded proteins interact with other misfolded proteins to form aggregates gaining toxicity. In some cases, these misfolded proteins are directed to UPP with the help of chaperones and ubiquitins and are degraded by Proteasome. Hence the absence of these prteins cause disease. Whatever be the reason for a protein not achieving its functional form leads to disease which is lethal sometimes. Insight into the mechanisms of protein folding and the molecular pathway to confirmational diseases and about the chaperons assisting the folding helps to find out cure for many neurogenerative diseases caused by the protein misfolding. It is very clear that chaperones play a critical role in protein folding and also in controlling protein misfolding and thus reducing the threat of resulting disorders. To have chaperone treatment in use, it is worth knowing the mechansims of folding, misfolding and aggregate fomation.

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