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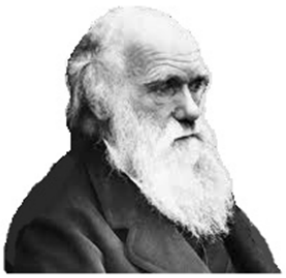

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Mechanism of Action of Chaperones in Protein Function

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ABSTRACT

The building blocks of living cells, proteins are enormous collections of nitrogenous organic molecules that are polymers of the amino acids that animals must consume to grow and repair their tissues. ATP-dependent proteins known as chaperones serve as foldases (protein folding assistants), holdases (bind folding intermediates), and disaggregates (convert aberrant protein to monomers). Chaperones include, but are not limited to, DnaJ, DnaK, GrpE, and Hsp33. The majority of chaperones have a cleft containing the nucleotide-binding site that divides the ATPase domain into two subdomains. The features of the C-terminal domain depend on the kind of bound nucleotide. In the presence of ATP, peptides bind and dissociate quickly and with low affinity. In contrast, the affinity increases significantly while the rate of peptide binding reduces when neither ADP nor nucleotide are connected to the N-terminal domain. Hsp90 is a homodimer with a 60 n dissociation constant. In reaction to high temperature or other types of cellular stress that prevent protein folding, several chaperones turn on their activity. Neurodegenerative, Parkinson's, and polyQ diseases, among others, can all be treated with chaperones. This is possible when a protein prevents the accumulation of protein species with improper folding. The suppression of dangerous protein oligomers by clustering, illness response related to protein aggregation, and cancer maintenance are a few new functions for chaperones that are still being discovered.

***Keywords*:** Proteins, Chaperones, Polypeptides, GroEL, Hsp-70

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1. INTRODUCTION

Chaperones safeguard protein homeostasis. Despite being created as a straight line of amino acids connected by peptide bonds, proteins need a particular three-dimensional shape in order to work. The final three-dimensional structure of the protein is subsequently reached by folding these amino acids to produce the correct spatial arrangement of these residues. Despite the fact that even a tiny protein has a tremendous number of potential conformations, the information needed to adopt a native three-dimensional conformation is encoded in the core amino acid sequence. When a protein folds on its own, it frequently does so via a process known as co-translation, in which the protein's N terminus starts to fold while the C terminus is still being translated. Proteins' hydrophobic amino acids promote folding by avoiding the watery cellular environment. Once folded, some proteins can interact with other proteins non-covalently to form higher order functional complexes. Because of the congested intracellular environment during this phase, protein aggregation and misfolding should be encouraged (Ellis and Hartl, 1999; Ellis 2001).

A class of proteins called chaperones facilitates the folding of proteins (Mayer,2010). By avoiding protein aggregation and misfolding during de novo folding and controlling later phases of protein translocation and complex formation through brief non-covalent contacts with proteins, molecular chaperones operate as catalysts in the physiological folding process. The role of molecular chaperones in protein folding rises under unnatural or demanding circumstances. Heat, oxidation, and chemicals are just a few of the stimuli that can lead to cellular stress. Protein function loss brought on by stress-induced protein unfolding and aggregation is the fundamental biological effect of cellular stress. Any cell that is unable to recover from this loss could suffer a terrible loss. The phrases protein, polypeptide, and peptide are a little hazy and might mean different things. In contrast to peptide, which is used to describe small amino acid oligomers that typically lack a solid three-dimensional structure, protein refers to the full biological molecule in a stable conformation. However, the distinction between the two is often between 20 and 30 residues and is not clearly defined (Lodish et al., 2004). Regardless of length, a polypeptide can be any single linear chain of amino acids, however it typically indicates the lack of a well-defined shape.

Proteins

Proteins are large biomolecules, or macromolecules, made of one or more extended chains of amino acid residues. Within animals, proteins play a wide range of functions, including as activating metabolic processes, reproducing DNA, reacting to stimuli, and transporting materials. Proteins differ primarily in their amino acid sequence, which is defined by the nucleotide sequence of their genes. This process usually results in the protein folding into a certain three-dimensional structure, which controls the function of the protein (Beck et al., 2011). A multiprotein replicase transcriptase complex is formed when a number of non-structural proteins come together (Ridwan et al., 2022). A polypeptide is an amino acid residue chain that is linear in nature. At least one polypeptide chain is present in every protein. Peptides, called oligopeptides on occasion, are short polypeptides with fewer than 20–30 residues that are occasionally thought to make up proteins. The various amino acid residues are connected by peptide bonds and nearby amino acid residues. The sequence of amino acid residues of a protein is determined by the sequence of a gene, which is encoded in the genetic code. The

genetic code generally specifies 20 conventional amino acids, but in some species and archaea, it can also include selenocysteine and pyrrolysine.

Protein Structural Organization

Proteins typically fold into recognizable 3-dimensional forms. The term "native conformation" refers to how a protein folds naturally (Dobson, 2000). Because of the chemical properties of their amino acids, many proteins can fold freely, but some others need the help of molecular chaperones to acquire their native shapes (Kent, 2009; Schwarzer and Cole, 2005)

- The amino acid sequence is the basic structure. A polyamide is a protein.
- Secondary structure is the term for locally repeated structures that are held together by hydrogen bonds. The three most prevalent examples are the -helix, -sheet, and twists. A protein molecule can have several areas with different secondary structures since secondary structures are local.
- The overall shape of a protein molecule and the arrangement of secondary structures in relation to one another are examples of tertiary structure. Tertiary structures are typically stabilized by nonlocal interactions, including the formation of a hydrophobic core, salt bridges, hydrogen bonds, disulfide bonds, and even posttranslational modifications. It is common to use the terms "fold" and "tertiary structure" interchangeably. The tertiary structure is in charge of the protein's basic functionality.
- Quaternary structure: a protein complex is a collection of different protein molecules (polypeptide chains), which are typically referred to as protein subunits in this context.

Molecules made by proteins are not entirely rigid. Proteins can transition between a variety of related configurations in addition to these levels of structure as they perform their functions. In the context of these functional rearrangements, these tertiary or quaternary forms are often referred to as "conformations," and transitions between them are referred to as conformational shifts.

Such alterations often follow the binding of a substrate molecule to an enzyme's active site, or the region of the protein engaged in chemical catalysis. In a solution, heat vibration and molecular collisions also cause structural changes in proteins (Bruckdorfer et al., 2004).

Types of Protein

Proteins can be roughly categorized into three basic groups that correspond to typical tertiary structures. These groups are referred to as membrane proteins, fibrous proteins, and globular proteins. Numerous globular proteins are enzymes, and almost all of them are soluble. Like collagen, the main component of connective tissue, and keratin, a protein that is present in hair, nails, and skin, many fibrous proteins serve a structural purpose. When membrane proteins act as receptors or channeling proteins, polar or charged molecules can pass across the cell membrane (Standley et al., 2008).

Dehydrons are a subtype of intramolecular hydrogen bonds that are found in proteins and are not well protected from the corrosive effects of water, according to Fernández and Scott (2003). Dehydrons thereby encourage the dehydration of themselves.

Functions of Protein

Proteins are regarded to be the main actors in a cell, carrying out the tasks specified by the instructions found in DNA. Except for particular RNA forms, the majority of other biological components serve as mostly inert substrates for protein function. Proteins make approximately half of an Escherichia coli cell's dry weight, but other macromolecules like DNA and RNA make up only 3 percent and 20 percent, respectively. The proteome is a group of proteins that are expressed in a certain cell type or kind of cell (Herges and Wenzel, 2005). The ability of proteins to securely and selectively bind other molecules is their primary characteristic, which also enables a wide range of jobs. The region of the protein where another molecule will engage is known as the binding site, which is typically a depression or "pocket" on the molecular surface. The side chains of the surrounding amino acids and the tertiary structure of the protein, which creates the binding site pocket, both contribute to moderating this binding capacity. The ribonuclease inhibitor protein, for example, binds to human angiogenin with a sub-femtomolar dissociation constant (1015 M), but not to its amphibian homolog onconase (>1 M), despite having a higher dissociation constant. This is an example of how protein binding can be extremely selective and tight. Sometimes, the addition of a single methyl group to a binding partner is sufficient to virtually entirely abolish binding. For instance, the very identical side chain of the amino acid isoleucine is discriminated against by the aminoacyl tRNA synthetase specific to the amino acid valine (Hoffman et al., 2006).

Proteins can interact with other proteins as well as small molecule substrates. When proteins specifically bind to other copies of the same molecule, they can oligomerize to form fibrils. This process typically occurs in structural proteins, which are composed of globular monomers that self-associate to form rigid fibres. Enzymatic activity, cell cycle progression, and the building of large protein complexes that carry out a range of intricately related biological processes are all regulated by protein-protein interactions. Proteins have the ability to cling to and even incorporate themselves into cell membranes. The capacity of binding partners to alter protein conformation enables the construction of extraordinarily complex signaling networks. Because interactions between proteins are reversible and heavily rely on the availability of different groups of partner proteins to form aggregates that can carry out discrete sets of function, studying the interactions between specific proteins is crucial to understanding key aspects of cellular function and, ultimately, the traits that distinguish different cell types (Brosnan, 2003).

Functional Properties of Chaperones

By encouraging refolding after stress and inhibiting aggregation, chaperones aid in the survival of cells. This allegedly stress response is shared and conserved by all living things. A class of proteins known as heat shock proteins (HSP) primarily regulate chaperone-assisted protein folding in cells (Bukauet al. 2006).

Chaperones are a number of families of multidomain proteins that have evolved to prevent protein aggregation, promote targeted unfolding and disassembly, and shield developing proteins from heat shock during complex formation. Their increased expression in response to stress has a substantial impact on the health of the cell and the longevity of an organism. Chaperones are potent molecular machines that can work on a multitude of substrates, in contrast to enzymes that have unique active sites that are carefully controlled.

Molecular chaperones are a class of proteins that have related roles. Based on their molecular weight, molecular chaperones are divided into various groups or families. A cell may express many members of the same chaperone family. For instance, the yeast S. cerevisiae produces 14 distinct versions of the chaperone Hsp70 (Craig et al., 1999). Although proteins within the same class of molecular chaperones usually exhibit high levels of sequence homology and are structurally and functionally linked, there is practically any sequence homology between molecular chaperones from other families. Despite this diversity, most molecular chaperones share the same functional traits (Colon et al., 1997).

The ability of a molecular chaperone to bind unfolded or partially folded polypeptides is unquestionably one of its most crucial properties because hydrophobic residues in a protein become partially solvent accessible during the early stages of folding or when misfolding occurs, making the protein susceptible to aggregation.

When used with molecular chaperones, these hydrophobic protein species successfully inhibit aggregation. Because of the hydrophobic interaction's lack of specificity and the flexibility of folding intermediates' conformation, chaperones exhibit promiscuous behavior. A vast variety of polypeptides with different amino acid sequences and conformations can attach to them. But because many late folding stages and most native proteins lack hydrophobic patches, they are no longer substrates for molecular chaperones (Jaenicke and Angew, 1984).

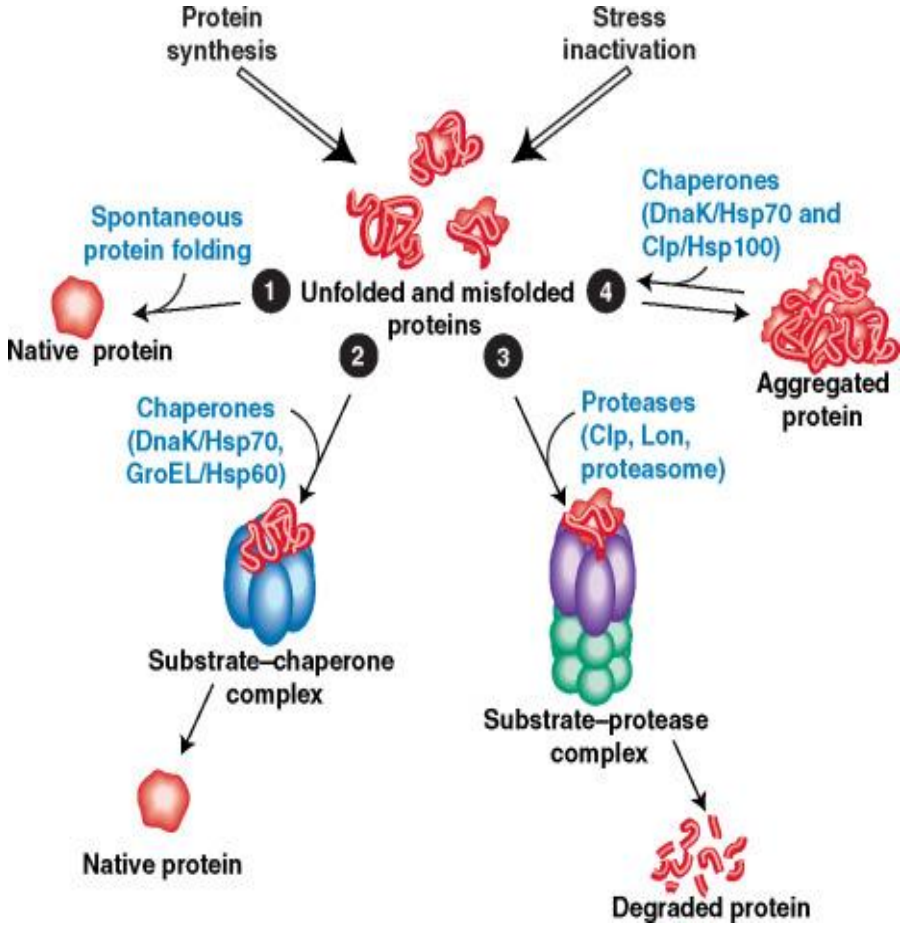

Figure 1. Chaperone-assisted protein folding (Creighton, 1990)

The Hsp60/Chaperonin Family

The Hsp60 (HSPD) family is extensively described and has a high conservation rate. The chaperonin-like Hsp60 protein family includes the GroEL protein found in prokaryotes as well as mitochondrial Hsp60, plastid Rubisco subunit binding protein, and the archaea group. The GroE proteins of the bacteria E. coli have been the subject of the most extensive research on molecular chaperones (Fenton and Horwich, 1997). For E. coli to survive, the groEL and groES genes create proteins with molecular weights of 57 kDa and 10 kDa, respectively (Fayet, 1989). Thus, at least one necessary E. coli protein must fold in the presence of the GroE chaperone.

Structure of the GroE Chaperone

GroEL's quaternary structure, which resembles a barrel with its two ends chopped off, is what sets it apart from other materials the most (Braig et al., 1994). Two seven-membered rings, each with a diameter of 45 mm, are built from a total of fourteen subunits to create two separate cavities. The GroEL subunits can be separated into three different domains. The central region of the middle barrel is composed of the equatorial domains. The majority of the interactions between the subunits of the same ring as well as all contacts between the two rings are mediated by them.

Additionally, they bind ATP and break it down. The co-chaperone GroES and the protein substrates are bound by the apical domains. The barrel's outside rims contain these domains. The intermediary domains that connect the equatorial and apical domains during the GroE functional cycle. These intermediate domains serve as moveable hinges, enabling substantial structural reorganizations. A dome-shaped ring construction with a 75 mm diameter and seven parts is the GroES co-chaperone (Hunt et al., 1996). A crucial part of the protein is the so-called mobile loop on GroES, which has a stretch of 16 amino acids and mediates binding to GroEL (Landry et al., 1993). Nucleotides are required for GroES to attach to the GroEL barrel's extremities, more precisely, ADP or ATP must be joined to the equatorial domains of the GroEL ring that corresponds to the GroES being employed (Saibil et al., 1991).

In the literature, complexes between GroES and GroEL have been classified into two separate categories. These complexes, which have the fitting names bullets and footballs, differ in their stoichiometry (Langer et al., 1992). In footballs, both of the GroEL rings are capped with GroES to create what looks to be a symmetrical particle, whereas only one of the GroEL rings is connected with GroES in bullets. When ADP is present, it appears that bullets are the most prevalent species, however when ATP is present, both footballs and bullets can be seen. GroEL belongs to the chaperonein family, also known as the Hsp60 chaperone family. Based on their roles and commonalities, the members of this class can be divided into two separate groups. GroES is a co-chaperone that is required for the effective operation of Group I chaperonins like GroEL, which are composed of seven distinct subunits called perrings. They are found in eukaryotic cells' mitochondria and chloroplasts as well as in eubacteria. They are also present in eukaryotic cells. Group II chaperonins do not cooperate with other chaperones and have either eight or nine subunits per ring. They are found in the cytoplasm of both archaea and eukaryotic cells. Compared to group I chaperonins, our understanding of the molecular mechanisms of group II chaperonins is limited. Whether or not their substrate specificity is as broad as what has been discovered for GroEL is one of the many questions that still need to be solved (Willison et al., 1993).

Polypeptide Binding to GroEL

Due to the hydrophobic surfaces that are exposed in unfolded and misfolded proteins, GroEL can recognize polypeptides as possible substrates. It has been demonstrated through analysis of the thermodynamics of the binding reaction that hydrophobic interactions prevail in polypeptide binding (Lin, 1995).

The GroEL protein-binding site was discovered by mutational analysis (Fenton, 1994) and more recent X-ray crystal structures of the isolated apical domain and of a complex between GroEL and a hydrophobic peptide (Buckle et al., 1997). The peptide binds to a hydrophobic groove that encircles the opening of the central cavity. Each substrate can have a binding surface that is appropriate for it because of the binding site's extraordinary flexibility, which permits it to undergo minor structural modifications. The partially folded substrate is also flexible, and it is very likely that upon attachment, both the substrate and the apical domains will undergo structural modifications (Mayhew et al., 1996). This explains why numerous proteins that are only partially folded can bind to GroEL. Several substrate proteins' structures have been described when they are coupled to GroEL. From polypeptides that are completely unfolded to highly structured stable folding stages, GroEL appears to be able to interact with a number of different conformations (Roseman et al., 1996).

There is a thermodynamic competition between the two processes for the protein substrate because the hydrophobic effect, which is what drives both binding to GroEL and folding (i.e., the creation of structure) of proteins, is what drives both of these processes. The hydrophobic residues build a hydrophobic core that shields them from the solvent as a protein folds. In the case of binding, shielding is performed by coming into touch with the hydrophobic groove of the GroEL apical domains. For a single residue in a polypeptide substrate, these options are mutually exclusive, but not necessarily for the complete protein. The structure(s) of the bound polypeptide may reflect an energy minimum that is determined by the relative sizes of two G values, one for folding and one for binding. In essence, GroEL does not restrict the shape of the bound protein as long as there are enough hydrophobic surfaces to interact with. In contrast, Hsp70's channel-like binding site requires that the bound polypeptide take on a locally stretched form (Jackson et al., 1993).

The Functional Cycle of GroE

Capture, folding, and release are the three stages in the functional cycle of GroE-assisted protein folding (Thirumalai and Lorimer, 2001). During the capture phase, the apical domains of one GroEL ring bind a polypeptide substrate. Binding of Mg/ATP and GroES to the same ring in the second phase results in the production of a folding-active cis complex by inducing substantial conformational changes in the GroEL tetradecamer. The safe refuge of the cis cavity is where the polypeptide is freed and starts to fold. After 15–30 seconds, a second conformational change binds the ATP that had been linked to the trans ring, priming GroEL for the release of GroES. The polypeptide is released from the cavity when it is folded, regardless of its condition. The energy supplied by ATP hydrolysis is used to maintain the balance sheet of the chaperone cycle. Because each individual step in the cycle—the binding of the polypeptide, GroES, and ATP—is an exergonic and thus irreversible process, the cycle only ever proceeds in one way. There must be an energy source to make up for this energy loss since a cycle's start and end are the same (Chen et al., 1995).

Unfolding of Polypeptides by GroE

It is crucial to understand how GroEL helps proteins locked in abnormal conformations, often known as misfolding. GroEL may have the ability to partially unfold these proteins, returning them to their initial state. Data from numerous laboratories indicate that GroEL could unfold a protein via a variety of potential mechanisms. The competition between binding and folding that was previously addressed serves as the foundation for the most basic model, known as thermodynamic coupling. GroEL preferentially binds to unfolded protein conformations because binding to GroEL requires a polypeptide to reveal hydrophobic surfaces, and the amount of exposed hydrophobic surface often decreases with the degree of folding. If the various conformations of the polypeptide reach an equilibrium quickly, GroEL will successfully unfold the protein. This ability of GroEL has been demonstrated in numerous comparatively small proteins.

The coupling method does have a serious problem, though. This model assumes that all unfolding reactions happen in free solution at their intrinsic speeds, which would make it impossible for a polypeptide to get out of a kinetic trap on its folding pathway. This problem might be resolved by a different GroEL-mediated unfolding process. It was found that GroEL may attach to a stable, compact folding intermediate of the enzyme Rubisco without incurring significant structural alterations. When ATP and GroES were added, this intermediate briefly unfolded, most likely as a result of the apical domains migrating upon GroES binding. The connected protein might experience a mechanical force as a result, losing its shape. It's important to note that this approach necessitates the polypeptide's simultaneous binding to several apical domains. Additional GroE substrate proteins have not yet been associated with active unfolding, though. No structural alterations are observed in malate dehydrogenase (MDH) upon dissociation of the MDH/GroEL complex upon binding of GroES (Chen et al., 2001).

The Hsp70 System

The majority of eukaryotic cell compartments, eubacteria, and many archaea all contain a ubiquitous chaperone system that is centered on the Hsp70 proteins. The member of this chaperone family that has been the subject of the most research, similar to GroE proteins, is the E. coli DnaK protein. Hsp70 proteins take part in a wide range of biological functions, such as the folding of proteins and the destruction of unstable proteins. The binding of short hydrophobic segments in partially folded polypeptides, which prevents aggregation and halts the folding process, appears to be the common function of Hsp70 in these processes. DnaK and countless other Hsp70 chaperones interact in vivo with two classes of partner proteins that control crucial parts of its functional cycle, Hsp40 and GrpE proteins, in a manner similar to the collaboration between GroEL and GroES. Furthermore, more partners have been discovered recently, especially in eukaryotic cells; some of these partners connect Hsp70 to other chaperone systems (Flaherty et al., 1990).

Structural and Functional Properties of Hsp70

An ATPase domain at the N-terminus and a smaller peptide-binding domain at the C-terminus make up the two functional components of Hsp70. The crystal structures of DnaK's

ATPase domain and peptide-binding domain have both been determined. The ATPase domain of Hsp70 is composed of two subdomains that are divided by a cleft that houses the nucleotide-binding site. The type of bound nucleotide determines the C-terminal domain peptide's binding characteristics. In the ATP state, peptide substrates bind and dissociate quickly but with low affinity. The binding and dissociation rates of peptides fall by more than two orders of magnitude and the affinity increases noticeably in the absence of nucleotide or ADP binding to the N-terminal region. Therefore, the molecular transition between the two states of Hsp70—high dynamics/low affinity and low dynamics/high affinity—is provided by ATP hydrolysis. We lack direct knowledge of the direct molecular interactions between nucleotide binding and peptide binding since the full-length structure of Hsp70 has not yet been established. But how this communication might work is shown by the X-ray crystal structure of the DnaK peptide binding domain that was co-crystallized with a hepta peptide coupled to its active site (Zhu et al., 1996).

The Hsp90 Chaperone System

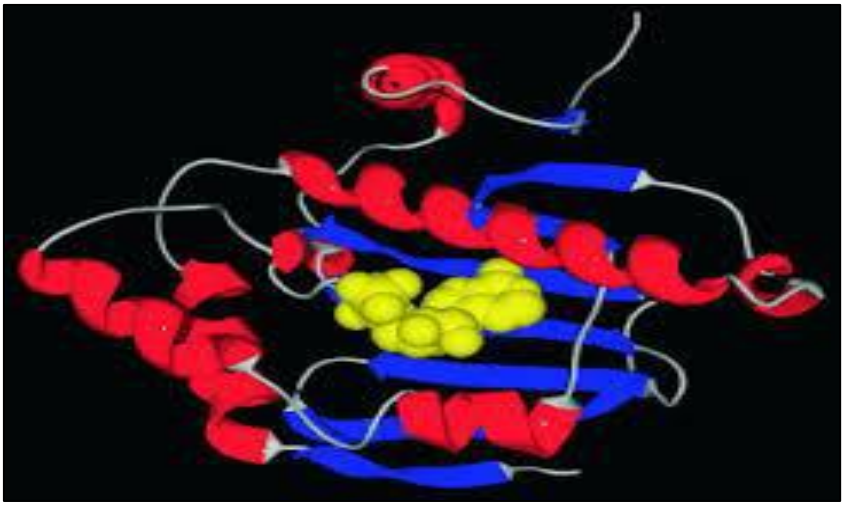

Figure 2. Structure of the N-terminal ATP-binding domain of yeast Hsp90 complexed with ADP (Grallert et al., 2000)

GroE and Hsp70 are more basic chaperones than Hsp90, an additional chaperone component that has only developed in eukaryotic organisms. The Hsp70 system is present, at least for part of the chaperone cycle, and it also contains a sizable number of cofactors. These two elements are necessary for it to perform properly. Up till now, more than a dozen of them have been identified. What sets this chaperone system apart from others is the fact that, in contrast to the other chaperones, a significant number of client proteins have previously been discovered as being dependent on Hsp90 in order to achieve their functional conformation under physiological settings. This suggests that Hsp90 and its client proteins form stable complexes that enable the separation of the client proteins. Src Kinase serves as a particularly convincing illustration of how Hsp90 can significantly affect protein folding. This protein offers a compelling prospect for therapeutic intervention since it plays such a critical role in the process of controlling the cell cycle. Following searches for naturally occurring compounds,

the putative src activity in vivo inhibitors kanamycin and geldanamycin were identified. However, further investigation showed that this potential Src inhibitor interacts with Hsp90 with a very high level of specificity and significant affinity. Due to this interaction, Hsp90 is made inactive, which lowers the amount of src kinase that is active. More and more proteins are becoming dependent on the Hsp90 system to achieve their functional shape. At this moment, it is unclear if their interaction with Hsp90 is caused by a shared trait, a sequence motif, or a specific structural component.

Regulatory proteins or proteins with crucial roles in the process of proliferation make up a sizeable fraction of the Hsp90 substrate proteins. One can speculate that the fact that Hsp90 is required for their action might provide for an extra level of control. The target polypeptide appears to pass through a variety of different Hsp90 complexes, each of which has a distinct set of partner proteins, according to the results of in vivo studies. Whether or not the target protein experiences any structural changes during this passage is completely unclear. The acquisition of the target protein's functional conformation appears to be facilitated by interactions with different Hsp90 complexes in an unexplained way (Fischer and Angew, 1994).

Protein Folding and Molecular Chaperones

The situation is not as advantageous within a living cell as it is in vitro, where many proteins may be refolded under ideal circumstances with high yields. When high protein concentration and temperature are present, an unexpected side reaction called aggregation takes place. Aggregation competes with efficient folding (Fink, 1998). Aggregation is the disordered, non-specific association of polypeptide chains that results in the formation of heterogeneous protein particles that are devoid of any biological function, as opposed to protein assembly, which describes the ordered association of several polypeptide chains into a defined functional oligomer.

Protein assembly, which defines the orderly connection of many polypeptide chains into a specific functional oligomer, is the opposite of aggregate formation. Given the amount of energy the cell has already used on the synthesis of a new polypeptide, it should not be surprising that methods have developed to encourage the productive folding of a protein into its active configuration. Taking into account the energy that the cell has already expended on a new polypeptide's synthesis Polypeptide sequences were probably chosen during the course of molecular evolution based on their biological properties as well as whether or not they fold successfully. Molecular chaperones, which are collections of proteins that bind with unfolded polypeptides to avoid aggregation and encourage productive folding in an ATP-dependent manner, are a product of the development of cells. Cells could enhance the amount of conformation space available by doing this (Buchner, 1996).

Role of Chaperone in neurodegenerative disease

Due to the accumulation of protein species that are improperly folded, many neurodegenerative illnesses are regarded as "conformational" in nature (Selkoe, 2004; Muchowski and Wacker, 2005; Brown 2007). It has been proposed that because neurons are a terminally developed, post-mitotic cell type, they are particularly vulnerable to the cumulative effects of misfolded proteins because they are unable to reduce the load of dangerous intermediates through repeated rounds of mitosis. Therefore, preserving the integrity of neurons

depends on the ability of neuronal chaperones to reduce the amount of improperly folded proteins.

Moreover, misfolded protein aggregates that closely associate with molecular chaperones are a hallmark of the majority of neurodegenerative diseases. Recent research compared the levels of Hsc70 expression in neuronal subtypes that are typically vulnerable in neurodegenerative diseases, such as spinal motoneurons (vulnerable in amyotrophic lateral sclerosis (ALS)), neurons of the hippocampus or entorhinal cortices (vulnerable in Alzheimer's disease (AD), and tyrosine hydroxylase positive neurons of the substantia nigra (vulnerable in Parkinson's (chen, Brown 2007). For instance, spinal moto- and substantia nigra neurons express far more Hsc70 than hippocampal or entorhinal neurons, although the prevalence of Alzheimer's disease in the US population is four times that of Parkinson's disease and 133 times that of amyotrophic lateral sclerosis. This demonstrates that certain subpopulations of neurons may be more susceptible due to their different chaperone pools (e.g., less ability to buffer misfolded proteins) (Brown, 2007).

Parkinson's disease (PD)

The most common neurological movement disorder, Parkinson's disease affects more than 0.1 percent of those over 40. (Sliderowf, Stern, 2003). Parkinson's disease patients suffer motor impairments that include sluggish movement, rest tremors, rigidity, and imbalance problems. Lewy bodies, inclusion bodies that represent PD and are the result of aberrant protein misfolding and aggregation (Berke and Paulson, 2003).

Chaperones colocalize with Lewy bodies that contain -synuclein (SN), such as Hsp70 and Torsin A. (Mclean et al., 2002). Additionally, it was shown that Hsp70 alters the toxicity of SN aggregates and binds to prefibrillar oligomers to prevent SN fibril formation (Dedmon et al., 2005). DJ-1, a novel oncogene, has been shown to contribute to familial Parkinson's disease by causing oxidative stress, mitochondrial degeneration, protein aggregation, and neuronal cell death (Le and Appel, 2004). H2O2 treatment of cells enhances the interaction between DJ-1 and its mutations and Hsp70, CHIP (chaperone interacting protein), and mtHSP70/Grp75 (Li et al., 2005). It is critical to emphasize that the amount of HSPs decreases significantly with age, which causes a breakdown in cellular protein homeostasis and ultimately causes or contributes to such age-related illnesses because stress chaperones are so important in the genesis of Parkinson's disease (Merlin and Sherman, 2005).

Alzheimer's disease (AD)

The most common type of irreversible dementia, AD, is characterized by a rapid progression from episodic memory issues to a decline in overall cognitive functions, impairing patients' capacity to carry out activities of daily living (ADL), and typically leading to death nine years after diagnosis. AD is characterized by these symptoms: (Davis and Samuel 1998). Extracellular amyloid- (A), also known as senile plaques, and intraneuronal inculusions, also known as neurofibrillary tangles (NFTs), formed by the accumulation of abnormal tau filaments, are two features of Alzheimer's disease that are primarily present in areas of the brain associated with memory and learning (McGeer, 2007). Post mortem expression analysis of Alzheimer's disease (AD) brain tissue revealed that gliosis and stressed neurons elevated a number of chaperones, including HSP27 and HSP70 (Renkawek et al, 1994; Yoo et al., 1999).

Chaperones have a crucial role in the pathogenesis of AD. The ER resident chaperone BiP/Grp78 (ER isoform of Hsp70) interacts with the amyloid precursor protein (APP) during its normal processing in the ER-Golgi pathway (Yang et al., 1998). Increased Grp78 may therefore help with the proper processing of APP, lowering the production of amyloid (Renkawek et al., 1994). Additionally, it has been shown that high concentrations of cytosolic Hsp70 and Hsp90 inhibit the initial stages of amyloid aggregation (Evans et al., 2006). It has been demonstrated that small HSPs like Hsp22 and Hsp27 bind to fibrillar amyloid plaques and prevent fibrillarization (Wilhelmus et al., 2006).

Additionally, C. elegans overexpressing the tiny Hsp16.2 protein is very protective against the toxicity brought on by A. (Fronte et al., 2008). The role of chaperones in Alzheimer's disease with regard to tau aggregation and fibrilization has been widely studied. Hsp27, Hsp70, and CHIP overexpression promoted the clearance of misfolded tau and lowered hyperphosphorylation (Petrucelli et al., 2004; Shimura et al., 2004). These chaperones were found to bind more strongly to hyperphosphorylated tau and paired helical filamentous tau than to nonphosphorylated tau.

Additionally, increased levels of Hsp70 and Hsp90 improved tau binding to microtubules and increased tau solubility in a variety of cell types (Dou et al., 2003). These results demonstrate the critical role of chaperones in keeping tau in its normal condition, linked to microtubules, and avoiding tau aggregation owing to hyperphosphorylation.

PolyQ disease

A group of dominant neurodegenerative diseases known as PolyQ illnesses are brought on by proteins with tandem polyglutamine repeats. Currently, six different types of spinocerebellar ataxia, Huntington's disease, dentatorubropallidoluysian atrophy, and spinal bulbar muscular atrophy have all been recognized as polyQ-related diseases (SCA1, 2, 3, 6, 7, and 17) (Bauer and Nukina, 2009). One of the earliest protein misfolding disorders for which neuroprotective chaperone properties have been identified is polyQ disease. Hsp40 and Hsp70 overexpression has been shown to decrease polyQ toxicity and inclusion body development in many animal systems (Jana et al., 2000; Chai et al., 1999)

Similarly, it was shown that overexpression of Hsp27 reduces oxidative stress without affecting the formation of inclusion bodies, alleviating polyQ toxicity (Wyttenbach et al., 2002). Ydj1 (an Hsp40 homolog) and Ssa1 (Hsp70) overexpression in yeast significantly reduces the generation of large, detergent-insoluble inclusion bodies and increases the accumulation of smaller aggregates (Muchowski et al., 2000). In a Celegans model of polyQ sickness, inhibiting two Hsp70 isoforms consistently worsens the disease (Hsu et al., 2003). When human Hsp70 is coexpressed with a shorter form of ataxin 3 with a polyQ expansion (MJDtr-Q78), the lethality it causes in the Drosophila nervous system is reversed (Warrick et al., 1998). The size of inclusion bodies was not significantly affected by Hsp70 expression, indicating that these aggregates might not be the dangerous species. Hsc70 overexpression also improves neuronal transport and significantly reduces cell death brought on by polyQ pathology (Gunawardena et al., 2003).

The behavioral and pathological traits of spinocerebellar ataxia 1 homolog (SCA1) transgenic mice (Ataxin 1 with 82 polyQ repeats) were significantly improved by overexpressing Hsp70, without influencing the generation of inclusion bodies (Cummings et al., 2001).

Classical Roles Of Heat Shock Proteins Expression

Living things respond at the cellular level to unfavorable circumstances, such as heat shock, and other stressful events of various causes by quickening the expression of particular genes, the heat shock genes. Stress proteins or heat shock proteins are common names for the byproducts of these genes (HSPs). Stress also inhibits the expression of the vast majority of other genes in addition to activating heat shock genes. As a result, depending on how severe and long the stress is, normal gene expression is disrupted, which, if it persists, can have a disastrous impact on cell and system homeostasis (Morimoto, 1993). In both eukaryotic and prokaryotic organisms, HSPs are highly conserved proteins that are expressed in a wide range of cell types, including striated skeletal muscle. According to their molecular sizes, HSPs are really divided into families, which include the subclasses HSP110, HSP100, HSP90, HSP70, HSP60, HSP30, and HSP10. The function of HSPs in muscle will be discussed in this chapter in terms of the most studied (because to their blatantly high expression in mammalian cells under stress) and conserved.

Recognized as intracellular molecular chaperones, HSP70s prevent protein aggregation during folding, promote protein transport, and protect newly synthesized polypeptide chains from misfolding and protein denaturation. Such proteins serve as molecular chaperones, facilitating the non-covalent assembly and disassembly of other macromolecular structures without becoming a permanent component of those structures. In addition, molecular chaperones assist the unfolded protein in achieving its single right three-dimensional configuration (by an as-yet-unidentified process that has evolved to generate this folded form), without becoming a part of the final folded protein (Hu et al., 2006). Most proteins that are meant for organelles are made in the cytosol and must travel through one or more organelle membranes to get there. For instance, 95 percent of the proteins found in mitochondria are precursor proteins that are made in the cytosol and are mostly transported into the mitochondrial subcompartments post-translationally. Cytosolic HSP70 are essential in this situation for maintaining precursor proteins in a transport-competent form. Precursor proteins are first transported in an unfolded state, but eventually they are refolded, sorted to their destination, and assembled into functional complexes. The inhibition of nascent polypeptide synthesis is one of HSP70's chaperone functions. Within the range of 0.1 to 0.4 nmoles of HSP70, this inhibition is dose-dependent, and the effect is stronger for larger polypeptides.

All of these results suggest that a high concentration of HSP70 can interfere with nascent protein folding, prevent cell growth, and decrease cell viability. So, these could be the reasons that careful autoregulation of HSP70 levels in human cells is necessary. Skeletal muscle is one of the body's most adaptable tissues, therefore HSP70's molecular chaperone function may be necessary for every structural component of the muscle that may change in response to a stimulus challenge (or its absence). The up- or down-regulation of HSP70 causes significant alterations in the skeletal muscle, including changes in fiber type distribution, fiber diameter, myosin heavy chain profile, and mitochondrial distribution (Maresca and Lindquist, 1991).

The Negative Effect of Chaperone

In microbial cell factories, recombinant protein misfolding and subsequent cell response activation are frequent occurrences (Gasser et al., 2008). It is commonly accepted that the soluble protein version is the preferred form of a protein production process's end result, despite

the potential of soluble aggregates and the presence of functional protein species in protein aggregates (Martinez-Alonso et al., 2009). This is accurate despite the fact that there is a lot of disagreement over what constitutes high-quality protein. By slowing down production (e.g., by lowering temperature), reducing the dosage of recombinant genes or the strength of the promoter, or increasing host chaperones—all of which are thought to be limiting during the overproduction of misfolding-prone protein species—it has traditionally been possible to increase solubility (Sorensen et al., 2005). Chaperones, important participants in the quality control system, may be over-titrated in the environment of recombinant cells with a high substrate load. As a result, their protein targets may be prevented from following folding routes that lead to the native shape, resulting in the accumulation of inclusion bodies (IBs), which are refractory particles (Villaverde et al., 2003). As a result, several different individual chaperones or chaperone sets have been selected for overproduction along with the target recombinant protein. Most of these techniques have been used to DnaK and GroEL, the two main cytosolic chaperones in Escherichia coli (E. coli), as well as to a number of their co-chaperones (de Marco, 2007). According to a careful research of physiological reactions to protein production in bacteria and other microorganisms, chaperone co-generation, as a quality-addressed approach, may eventually show detrimental side effects regarding protein quantity and quality (Rinas et al., 2007). Here, we emphasize the pertinent information demonstrating the detrimental effects of chaperones, concentrating on DnaK, GroEL, and their related folding modulators (Gragerov et al., 1992).

2. CONCLUSIONS

The majority of the highly conserved protein families that make up the chaperone are heat shock protein families. Chaperones are essential for many physiological processes, including protein translocation, refolding or destruction of proteins after cell stress, and protein folding or refolding. Their primary role is to support the folding or refolding of proteins. The function of molecular chaperones in promoting proper protein folding is heightened in non-physiological or stressful circumstances. Cellular stress can be brought on by a variety of stressors, such as heat, oxidation, and chemicals. The loss of protein function brought on by unfolding and protein aggregation is the main physiologic impact of cellular stress. Any cell that is unable to recover from this loss risk dying. Stress, which has been shown to colocalize strongly with molecular chaperones, is regularly exposed to cells. Protein misfolding and aggregation are caused by a variety of stressors. HSP70, HSP60 (chaperonin), HSP90, HSP40, and small HSPs are just a few of the many Chaperone classes. The most well studied form of chaperone is GroEL, which is produced by bacteria. Misfolded protein aggregates are a hallmark of neurodegenerative diseases, therefore neuronal chaperones' capacity to reduce misfolded proteins is essential for maintaining neuronal integrity.

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