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Control of Cell Fate by Hsp70: More than an Evanescent Meeting

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During their lifetime, proteins inevitably expose hydrophobic segments within the polypeptide chains on a molecule's surface, which may be otherwise buried inside the molecules in the proper conformation. This potentially dangerous situation is managed with the aid of the 70-kDa heat shock proteins (Hsp70s) and other molecular chaperones. Although a major function of Hsp70 is assisting in efficient folding of anonymous proteins in unfolded states, recent studies have revealed that Hsp70 plays a variety of specific roles, sometimes deciding the cell fate. These multiple activities are based on the specific binding of Hsp70 to proteins in native states, which regulate cell growth and/or death. It is now well recognized that unfolding of some proteins may cause serious diseases, especially those associated with neurodegeneration, such as Alzheimer's disease. It is suggested that Hsp70 might be a potential drug against these diseases, but caution should be taken because Hsp70 exerts multiple effects by binding to specific proteins.

Key words: apoptosis, chaperone, Hsp70, neurodegeneration, *Sce*I.

The 70-kDa heat shock proteins, or Hsp70s, are highly conserved in all organisms, from bacteria to humans. Although this family of proteins were initially identified as inducible proteins whose transcription were up-regulated especially in response to heat shock, later it became apparent that many members of this family are present in cells under normal conditions. Eukaryotic cells contain multiple Hsp70s, which are localized in a variety of cellular compartments including the cytosol (*e.g.*, Hsc70 and the inducible Hsp70 of higher organisms), mitochondria (Hsp75), and the endoplasmic reticulum (BiP/Grp78). Classical functions of Hsp70 are the prevention of protein aggregation and assistance in protein folding, thereby chaperoning other proteins in multiple processes, such as translation, translocation, folding, and quality control under both normal and stress conditions (*1*, *2*). These functions are based on transient associations of Hsp70 with substrates. In this review, the basics of Hsp70s are summarized, and the recent progress in the cellular function studies of Hsp70, which sometimes play decisive roles in the cell's life, is described. This article also touches on a novel function of Hsp70 in regulating enzymes in DNA metabolism. These newly found roles of Hsp70 are based on rather stable binding of Hsp70 with functional proteins in their native conformation.

Classical activities of Hsp70

Hsp70s are mostly conserved in the first ~530 amino acid residues, with substantially less conservation in the range of residues 530–600, followed by highly variable sequences in the carboxy-terminal 30–50 amino acids (Fig. 1). The amino-terminal region of about 44 kDa (380–390 residues) is an ATPase domain (*3*), which is followed by a central substrate-binding domain (*4*). Although the function of the C-terminus variable region has not yet been fully revealed (*5*, *6*), the region is assigned to the binding site for co-chaperones, such as Hop (*7*). The extreme carboxy-terminal acidic motif (EEVD) found in mammalian cytosolic Hsp70s (both the constitutive Hsc70 and the inducible Hsp70) affects ATPase activity, substrate binding, and interactions with co-chaperones (*8*). In addition, recent studies show that the motif is also recognized by a component of translocation machinery on the mitochondrial outer membrane (*9*), suggesting that multiple proteins recognize the motif depending on the location in the cell. Interestingly, Hsp90, another major chaperone protein, has the identical EEVD motif at its C-terminus, indicating that the cytosolic Hsp70 and Hsp90 can be physically linked *via* Hop (*10*).

Hsp70 transiently holds unfolded substrates in an intermediately folded state, preventing irreversible aggregation and catalyzing the refolding of unfolded substrates in an ATP-hydrolysis- and co-chaperone-dependent reactions. Exposure of hydrophobic stretches that, in most cases, are assumed to be buried inside protein molecules, inevitably occurs during nascent protein synthesis and upon translocation through *e.g.*, the mitochondrial membrane (*11*). With assistance of Hsp70, the unfolded protein molecule is eventually folded into its native structure, or, if the proper folding is not achieved, is transferred to the intracellular degradation system (*12*).

Substrate binding and release: ATPase cycle

Binding of exposed stretches of hydrophobic residues in unfolded or partially unfolded proteins is regulated by ATP-hydrolysis-induced conformational changes of Hsp70 (*5*). The ATP-regulated cycle of substrate binding and release is best understood for the bacterial Hsp70, DnaK (Fig. 2). In the ATP-bound state, DnaK binds and

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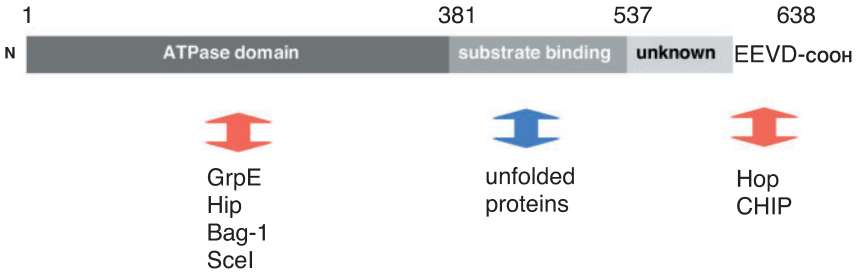

Fig. 1. **Schematic representation of the domain structure and cofactor binding sites of Hsp70.** The amino acid residue numbers refer to human Hsp70. Only the cytosolic Hsp70 of eukaryotes have the carboxy terminal sequence EEVD (E, Glu; V, Val; D, Asp), involved in binding tetratricopeptide repeat cofactors. Red arrows indicate specific interactions between Hsp70 and proteins, while a blue arrow depicts interaction with anonymous proteins in unfolded states.

rapidly releases substrates. Hydrolysis of ATP to ADP catalyzed by intramolecular ATPase results in the stabilization of the chaperone-substrate complex. The cycling of DnaK between the ATP- and ADP-bound states is regulated by the co-factors, DnaJ and GrpE. DnaJ stimulates the ATPase activity of DnaK and thus facilitates peptide capture and retention. DnaJ can also recognize hydrophobic peptides and thereby may present unfolded polypeptides to DnaK. GrpE, a specific nucleotide exchange factor for DnaK, induces the dissociation of ADP from DnaK, enhancing substrate release. Release of substrates requires the binding of ATP to Hsp70, after which the substrates either enter a new cycle of binding and release or fold into their native conformation (*8*, *13*, *14*).

Co-chaperones of eukaryotes

In the cytosols of eukaryotes, Hsp40 (Hdj-1), the homolog of DnaJ, stimulates the ATPase activity of Hsp70 (*8*). *In vitro* experiments clearly demonstrate that Hsp70 cooperates with Hsp40 in efficient folding of unfolded proteins (*15*, *16*). Although the GrpE homolog mitochondria version has been found in eukaryotes (*17*), there are no GrpE homologs in the cytosols of eukaryotes, which functions as a nucleotide exchange factor. Instead, non-GrpE type nucleotide-exchange factors (*e.g.* Hip and Bag-1) have been identified.

Hip (Hsc70 interacting protein) binds to the ATPase domain and increases the chaperone activity of Hsp70 by stabilizing the ADP state (*18*). In contrast, Bag-1 (Bcl-2 associated athanogene 1) inhibits the chaperone activity of Hsp70, in part, by facilitating premature release of the unfolded substrate by accelerating nucleotide exchange (*19*, *20*). However, Bag-1 does not appear to be a major co-chaperone which directly regulates the Hsp70 chaperone activity because the level of Bag-1 is only approximately 1% of that of Hsp70. Nevertheless, more interestingly about this co-chaperone, Bag-1 contains a ubiquitin-like domain and cooperates functionally with CHIP (carboxy terminus of Hsc70-interacting protein) (*20*). CHIP is a tetratricopeptide repeat-containing co-chaperone of Hsp70 (and Hsp90, too) inhibiting the chaperone function (*21*). CHIP promotes ubiquitination and proteasomal degradation of proteins captured by Hsp70 (*22*, *23*). Thus, CHIP together with Bag-1 may serve as a link between chaperone-assisted folding and proteolytic degradation, the two main components of protein quality control in the cytosol (*24*).

Hsp70 as a regulator of cell survival and apoptosis

Recent studies have revealed that the chaperoning activity is not the sole function of Hsp70. As clearly indicated by the fact that Hsp70 has specific co-chaperones as described above, Hsp70 recognizes and binds to unfolded substrates and specific partner proteins in their native states. These partner proteins confer Hsp70 versatility, which may require the cell to respond to either proliferation signals or apoptotic signals. Bag-1 interacts with and influences the function of many key components of cell death and signal transduction pathways (*25*), including the anti-apoptotic protein Bcl-2 and the growth

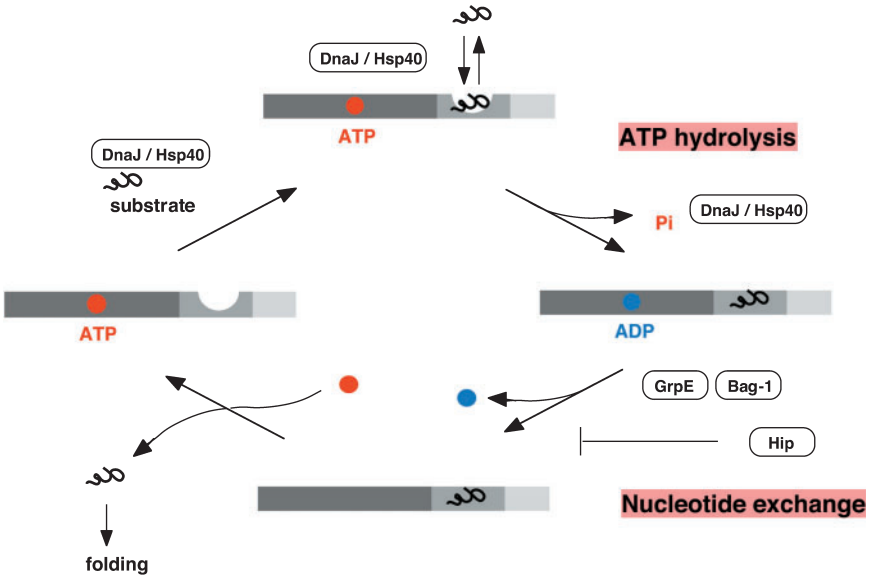

Fig. 2. **The Hsp70 ATPase cycle.** Rapid peptide binding occurs in the ATP-bound form of Hsp70 in which the peptide binding pocket is open. ATP hydrolysis, enhanced by DnaJ/Hsp40, causes the pocket to close. Subsequent nucleotide release and reloading of ATP induces dissociation of the peptide from Hsp70. GrpE and Bag-1 stimulate the dissociation reactions, whereas Hip stabilizes the ADP form.

regulator Raf-1 (*20*). Bag-1 activates Raf-1 kinase, while Hsp70 competitively inhibits the Bag-1–Raf-1 interaction due to the Bag-1–Hsp70 interaction, especially when levels of Hsp70 are elevated after heat shock (*26*). This observation suggests that the regulation of Bag-1 functions by Hsp70 serves as a checkpoint to regulate cell growth and death, and that Hsp70 functions as a sensor in stress signaling.

Increasing evidence indicates that Hsp70 is more directly involved in "to live or let die" decisions of the cell. Apoptosis is an active process of cellular suicide, which is executed by a family of cysteine proteases, caspases (*27*). Since all the members of the caspase family are synthesized as precursors, activation of them requires processing. Various apoptotic signals converge in mitochondria from which cytochrome *c* is released in response to mitochondrial damage. Released cytochrome *c* binds to, and triggers oligomerization of Apaf-1 (apoptosis protease activating factor-1), which specifically activates caspase-9 (*28*, *29*), a central player of caspase activation. Several groups have shown that Hsp70 associates with Apaf-1, thereby preventing Apaf-1 from activating caspase-9 (*30*–*32*). The Hsp70-mediated inhibition of caspase-9 activation requires the substrate binding domain (*32*) and the EEVD motif (*30*). Although the precise mechanisms for the inhibitory effects remains unclear (*33*), these studies suggest that Hsp70 suppresses apoptosis by directly interacting with the main machinery of apoptosis. The regulatory function of Hsp70 in apoptosis may explain cell resistance to apoptosis exhibited by some tumors, which constitutively express high levels of Hsp70 (*34*).

Hsp70 in diseases

In recent years, the process of protein folding has been recognized as relevant to medicine and thus it is more often discussed from a clinical point of view. Many inherited human diseases are caused by specific mutations within a gene, which fold the protein product or its substrates into aberrant conformations. For instance, a group of highly serious neurodegenerative diseases [*e.g.* Alzheimer's disease, the prion disease, and the polyglutamine (polyQ)-expansion disease] are associated with brain lesions where either intra- or extracellular depositions of protein aggregates, some of which are called amyloids, are formed. These disorders are considered to be the result of an increase of toxic functions by aberrantly folded proteins, which are often accompanied by Hsp70 and other chaperones (*35*). The association of aggregates or misfolded proteins with Hsp70 implies that the chaperone system including those involving Hsp70 acts as a defense mechanism for the prevention of misfolded protein accumulation, although the attempt seems to have ended without success.

The exact mechanism by which protein misfolding and aggregation are linked to disease is still unclear, but accumulating evidence suggests that the main toxic agents are unfolded, but still soluble species of the disease proteins, rather than their end products, the insoluble fibrils (*35*). Misfolded proteins may sequestrate components of the chaperone, reducing the ability of the cell to manage unfolded proteins. Reduction in the chaperone activity would be very harmful to cells because it affects both the folding system and the intracellular degradation systems. Note that Hsp70 promotes folding when associated with Hsp40, but it could promote proteasomal degradation of substrates when associated with Bag-1 and CHIP (*36*). No matter how toxicity is generated, either by soluble forms or insoluble fibrils of the disease proteins, the identification of protein aggregates, including Hsp70, inside or around dead cells has tempted many researchers to manipulate the level of Hsp70 to examine whether overexpression of chaperones would reduce the extent of aberrant aggregation, thereby suppressing disease phenotypes or delaying the onset of the disease.

A major success of such attempts has so far come from experiments using the fruit fly. Conservation of genes between humans and fruit flies enables us to use *Drosophila* as a model organism for the study of human neurodegenerative diseases. Ectopic expression of genes associated with neurodegeneration in the fruit fly causes phenotypes similar to those of the counterpart human diseases. Such *Drosophila* models of neurodegeneration have proven that Hsp70 is a potent suppressor of both polyQ disease and Parkinson's disease in the fly (*37*). Interestingly, but perhaps unexpectedly, the potent suppression of abnormal cell death by Hsp70 occurs without suppression of the aggregate formation (*38*). The pathogenic protein aggregates isolated from the degenerative region in the fruit fly was found to be insoluble in SDS, while those co-expressed with the exogenous Hsp70 was SDS soluble. It is thus possible that the mutant protein is maintained in a more normal conformation, with less toxicity. These studies indicate that Hsp70 may provide a means of treating neurodegenerative disorders associated with aberrant folding of proteins. Remaining questions include the reason why the endogenous chaperone system represented by Hsp70 fails in patients suffering from neurodegenerative disorders such as Alzheimer's disease. It is likely that a gene mutation responsible for a disease causes an imbalance between potential protein toxicity and the defense capacity determined by the activity of chaperone proteins, eventually resulting in the onset of disease.

Hsp70 in alteration of mitochondrial genotypes

All the representative functions of Hsp70 described above may be achieved by transient associations of Hsp70 with either substrates in an unfolded state or by signaling molecules in cells under specific conditions. Among the binding partners of Hsp70, a mitochondrial endonuclease *Sce*I encoded by the mitochondrial genome of the budding yeast (*39*) is unique because of its stable binding to the mitochondrial Hsp70 (*40*). The binding site for the endonuclease is located within the Hsp70 ATPase domain, as in the case of other co-chaperones such as Bag-1. *In vitro*, the *Sce*I protein forms a stable dimer with ADP-bound Hsp70 without any need for denaturation of the endonuclease protein (*41*). It is currently unknown, however, whether *Sce*I has a co-chaperone activity modulating the Hsp70 chaperone function.

Although *Sce*I monomers show sequence specific endonuclease activity, which recognizes a unique sequence of 26 bp, the binding of Hsp70 to *Sce*I results in broadening of the sequence specificity as well as enhancement and stabilization of the activity (*41*). The heterodimer can cleave more than 30 sites within different 26 bp-

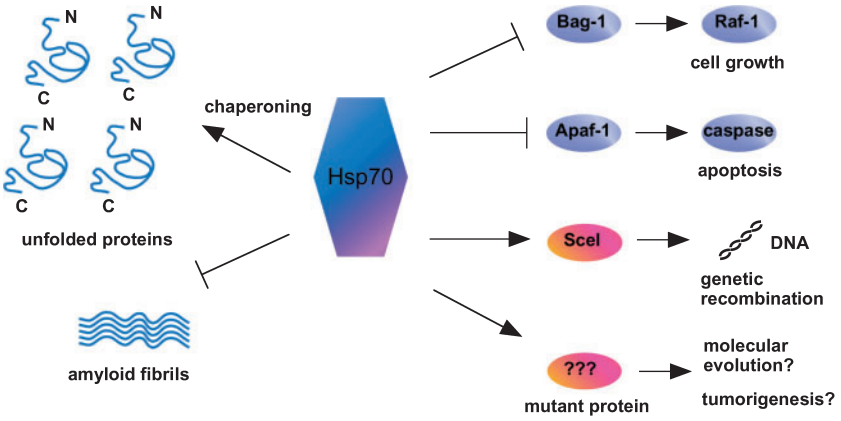

Fig. 3. **The involvement of Hsp70 in the regulation of various cellular functions.** As a molecular chaperone, Hsp70 prevents many proteins from misfolding and aggregating. The chaperone activity may provide a basis for the development of therapeutic reagents against protein misfolding diseases. On the other hand, through the interaction with specific proteins in the native form, Hsp70 is involved in regulating various processes within the cells including cell proliferation, apoptosis, and genetic recombination.

sequences in the mitochondrial DNA (*41*, *42*). Mitochondrial fusion, which occurs when haploid cells mate to form zygotic cells, provides the biological venue for this endonuclease to function. During mitochondrial fusion the Hsp70-*Sce*I heterodimer-mediated cleavage induces genetic recombination between the heterogeneous mitochondrial DNAs inherited from each parent (*42*). Since the cleavage of the mitochondrial genome by the *Sce*I-Hsp70 heterodimer determines the initiation sites for recombination reaction (*42*, *43*), the stable binding of Hsp70 with *Sce*I is relevant to the biological function of *Sce*I.

In addition to the roles of Hsp70 found in the current enzyme, Hsp70 seems to have facilitated molecular evolution of *Sce*I and its ortholog, *Suv*I (*39*). Two amino acid substitutions found between *Sce*I and *Suv*I are silent mutations in terms of the unique sequence specificity of the monomeric endonuclease. However, binding of Hsp70 to the monomers results in divergence in the multiple sequence specificity between the two enzymes (*39*, *44*). Manifestation of "mutant" phenotypes found in the *Sce*I-related enzyme may be an example where chaperone molecules have played a role in molecular evolution. Interestingly, Hsp90, a sister of Hsp70, is assumed to have been involved in molecular evolution in an opposite manner. Hsp90 is believed to maintain the "normal" behavior of mutated proteins whose altered phenotype becomes manifest only when Hsp90 function is comprised. Hsp90, thus acts as a capacitor for morphological evolution by buffering cryptic genetic variation (*45*, *46*). In either case of Hsp70 or Hsp90, molecular chaperones act as modulators of protein function and stability through stable association.

Conclusion

The name, molecular chaperone, may imply that Hsp70 behaves like a part time worker, acting only transiently on anonymous substrates such as nascent polypeptide chains, organellar proteins during translocation, and unfolded proteins under stress conditions. In these classical examples, after proper folding, Hsp70 does not seem to be involved in the actions of its substrate proteins. However, growing evidence has demonstrated that Hsp70 can act as a modulator of cellular functions *via* diverse interactions to specific proteins, some of which require Hsp70 as a stable subunit (Fig. 3). Structural analysis, by X-ray crystallography and NMR spectroscopy, should be applied to solve the mechanism of these specific interactions. Alterations in protein interaction combinations within cells would occur when cells undergo considerable changes in their function and structure *e.g.* during cell cycle progression, cell differentiation, or apoptosis. Therefore, it may be no wonder to see that the list of specific partner proteins of Hsp70 is growing in the future. Identification of such partners could be achieved through a comprehensive search by proteomics analysis. Also important is the consideration of possible Hsp70 involvement in tumorigenesis for which multiple mutations are responsible, because Hsp70 could assist mutant proteins to be stable and functional with aberrant activity. Furthermore, caution should be considered in using Hsp70 as a therapeutic- or prophylactic reagent against protein misfolding diseases. As summarized in this article, Hsp70 may modulate functions of normal proteins when used at a non-physiologically high dose.

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