Chapter 2
The Functions of HSP70 in Normal Cells

Abstract In normal cells, the HSP70 ATPase cycle performs several fundamental functions: (1) together with co-chaperones, HSP70 forms a protein folding mechanism and provides protein transportation into organelles; (2) assisted by HSP40, HSP70 recognizes irreversibly damaged proteins and, assisted by CHIP, Bag-1 and HSJ1 ubiquitinates these proteins, thereby targeting them for degradation via proteasomes; and (3) together with the co-chaperones HSP90, HSP40, Hip, Hop and Bag-1, HSP70 recognizes normal proteins containing the marker sequence KFPRQ and sends these proteins for degradation in lysosomes. Thus, the HSP70 ATPase cycle forms a protein quality control system or the FOlding Refolding Degradation machinery (FORD) and, depending on the state of the protein, sends the protein either for re-folding or for degradation. Because of the FORD machinery, a cell maintains protein homeostasis. The HSP70 ATPase cycle also controls the activity of key signalling proteins by maintaining these proteins in an inactive or active state by regulating their levels and by intracellular transport.

Keywords HSP70 • Protein folding • HSP90 • CHIP • Proteasomal degradation • Lysosomal degradation

In the first chapter, I introduced an important class of proteins that exist in cells—heat shock proteins. Synthesis of these proteins, primarily HSP70, dramatically increases when a cell falls into adverse conditions. HSP70 can quickly receive the signal that damaged proteins and abnormal protein formations have appeared in a cell and repair the damage. In this sense, HSP70 acts as an intra-cellular "911" service!

As soon as the first hints about the protective role of HSP70 appeared in scientific journals, scientists started asking: what is the structure of these proteins? How do they act? Significant efforts went into answering these questions. As a result, we now know that HSP70 contains three domains: the ATPase N-domain that hydrolyzes ATP, the substrate domain that binds proteins, and the C-domain that provides a "lid" for the substrate domain. We know now that, because of its three-domain structure, HSP70 forms a single ATPase cycle that involves the association and disassociation of the client protein. We also know that the "team" of

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HSP70 cycle regulators include: **HSP40**, which delivers the clients to HSP70 and stimulates ATP hydrolysis; **Hip**, which assists HSP70 in retaining the client, and **Bag-1** and **HspBP1**, which increase the rate of ADP dissociation and the release of the client protein.

The idea of the ATPase cycle of HSP70 provided an explanation as to how HSP70 can protect a cell from heat damage. According to the Pelham hypothesis, HSP70 binds to denatured protein aggregates, restricts their aggregation and breaks protein aggregates using the energy generated from the hydrolysis of ATP. A fairly logical system of ideas on HSP70 was built: these ideas regarded HSP70 as a protective protein which is rapidly synthesized "on demand" in response to cell damage.

However, HSP70s are also found in normal cells. Consequently, what would be the role of HSP70 in this situation? How does the ATPase cycle assist HSP70 in performing its functions in a normal cell? This is the subject of the second chapter.

It is impossible to define who began thinking about the role of HSP70 in a normal cell. However, the one who started addressing this problem in the right direction certainly knew two things. This "Mister X" knew, first of all, that HSP70 could bind to the hydrophobic part of proteins and delay protein folding. Second, he knew the problem in explaining the protein folding phenomenon. In fact, in vitro most proteins fold spontaneously, confirming the Anfinsen principle implying that a polypeptide chain sequence contains all the necessary information to define the exact three-dimensional protein fold (Anfinsen 1973). However, the situation in a cell is much more complicated. The cytoplasm is full of various molecules and organelles, and the problems arise already during the translation: growing polypeptide chains may begin aggregating with each other. (Dobson 2003). Such events happen because the hydrophobic patches in these chains stay exposed until the entire polypeptide chain comes off the ribosome. It is only after the synthesis is completed that the polypeptide chain can fold, burying all hydrophobic regions of the polypeptide in the interior of the protein.

Having compared these two points, it was easy to propose that HSP70 may be the best candidate for playing a role in assisting protein folding, because it can bind and shield hydrophobic zones of the polypeptides being synthesized, thus preventing unwanted protein aggregation. Once the entire chain is synthesized, HSP70 can release the chain, allowing it to fold properly and to reach its native three-dimensional state.

However, even after a proper folding event, proteins tend to aggregate, since small physical and chemical changes within the cell or just normal functioning may expose hydrophobic regions thereby leading to protein aggregation. Here, HSP70 may play a substantial role in preventing the aggregation of normal proteins.

Therefore, by analyzing the role of HSP70 in a normal cell, researchers have noted first of all that, in terms of functionality, HSP70 was well suited to solving problems that arise with the appearance of hydrophobic regions, both in the process of translation and protein folding, and during the normal functioning of mature proteins.

2.1 The Role of HSP70 in Protein Folding and the Prevention of Protein Aggregation

So how does HSP70 guarantee correct protein folding in normal cells? In eukaryotes, HSP70, in cooperation with its co-factors, interact with at least 30 % of all synthesized proteins to ensure that the correct native conformation is achieved. It all starts at the point where HSP40 assists each emerging hydrophobic area of a protein synthesized on the ribosome to bind to the substrate domain of HSP70 (Fig. 2.1.). The events we discussed in the previous chapter then occur (Hartl and Hayer-Hartl 2009). HSP40 initiates ATP hydrolysis within the N-domain. This increases the protein affinity of the substrate domain; at the same time the flexible C-domain "covers" the bound protein from the top. Now we understand why it is important that HSP70 binds to hydrophobic areas of the polypeptide chains! Shielding of the hydrophobic areas by HSP70 prevents unwanted aggregation of protein chains. Removing HSP70 gives rise to the situation where the cell will have a significant population of proteins that will not form functionally active tertiary structures. During polypeptide chain synthesis, factors such as Hip keep hydrophobic areas bound to HSP70. Further, when the whole polypeptide chain emerges from the ribosome and is ready

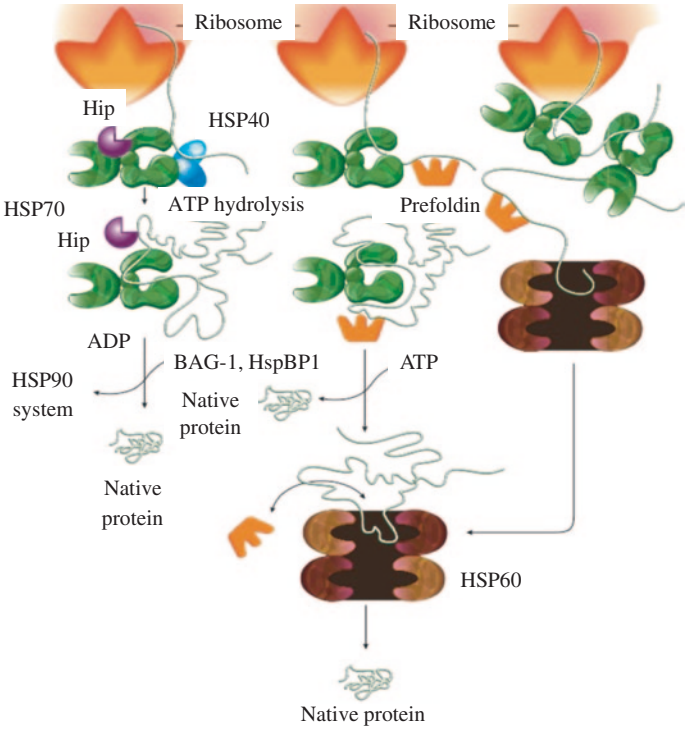


Fig. 2.1 How HSP70s guarantee correct protein folding in the cytoplasme of normal cells

to fold into a native tertiary structure, the nucleotide exchange factors exchange the N-domain ADP for ATP. As you remember from the first chapter, this leads to a reduction in protein affinity toward the substrate domain, opening of the "lid" and eventually to the release of the entire polypeptide chain and its folding.

In addition to HSP70, HSP40 and nucleotide exchange factors, in some cases, the process of protein folding may involve HSP60—barrel-shaped particles, inside which the protein folding process takes place. Protein barrels further isolate synthesized polypeptides or denatured proteins to ensure favourable folding or refolding of proteins. HSP60 are involved in the folding of ~10 % of all proteins.

Some proteins do not fold in the cytoplasm, but in the endoplasmic reticulum (Fig. 2.2.). For this process, the protein synthesized on a ribosome should be transported into the reticulum through a translocon, and the first to meet the "guest" protein is HSP70, which is called BiP in the endoplasmic reticulum. BiP binds to the first protein hydrophobic area to appear in the ER and thus prevents the polypeptide chain from returning back into the cytoplasm. In addition, with the help of repeated ATPase cycles, BiP binds every time to the next hydrophobic area of the protein which has just appeared in the reticulum lumen, and pulls the polypeptide chain into the lumen of the reticulum like a ratchet. Interestingly, the function of HSP40 in attracting BiP and regulating its ATPase activity is performed by translocon proteins located on the endoplasmic reticulum membrane.

Proteins destined for mitochondria also bind to HSP70 rather than folding in the cytoplasm. The function of cytoplasmic HSP70 is to maintain a linear state of the protein chain, so that the protein can penetrate through the narrow translocons of the mitochondrial membranes (Fig. 2.3.). As soon as the beginning of the

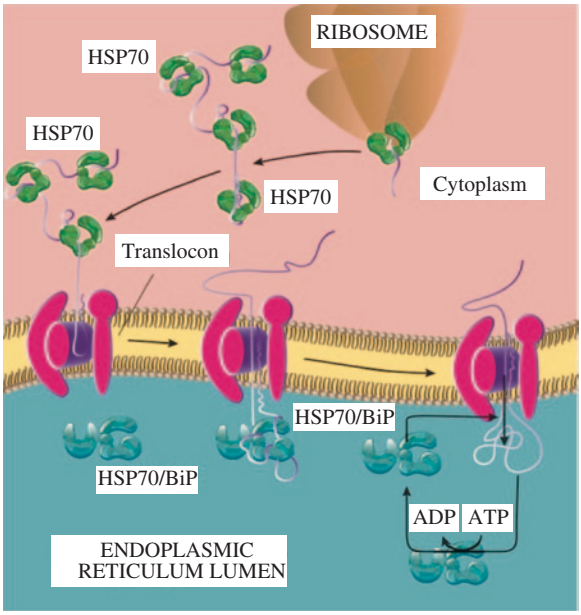


Fig. 2.2 How HSP70/BiP guarantee correct protein folding in the endoplasmic reticulum of normal cells

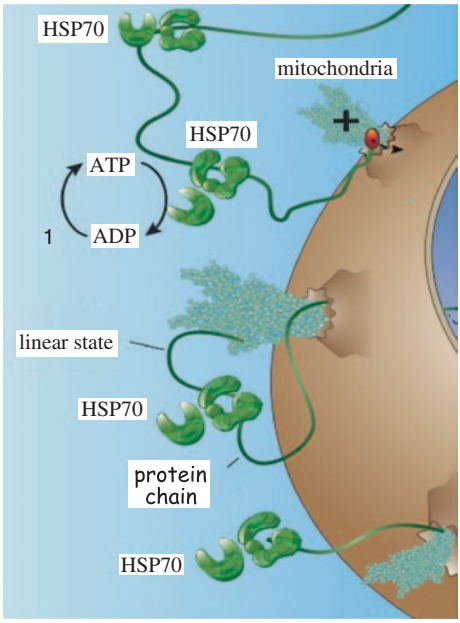


Fig. 2.3 The role of HSP70 in protein folding in the mitochondria of normal cells

polypeptide chain appears in the matrix, other mitochondrial HSP70 would, by the energy of ATP, draw the protein chain into the mitochondrial matrix. In the same location, within the matrix there are regulators required for the cycle: the nucleotide exchange factor and translocon proteins accelerate the ATPase activity of mitochondrial HSP70. Retraction of the protein into the mitochondria occurs in the same way as explained above for the events occurring in the endoplasmic reticulum.

Therefore HSP70 carefully escorts polypeptide chains from the moment of its birth on a ribosome until the final formation of the protein spatial structure. *Since HSP70 largely does what a nanny does for children, HSP70 proteins were named "chaperones"* (Bukau et al. 2006). *Chaperone, according the Oxford dictionary, means an older married or widowed woman who accompanies a young woman to her first ball (or a person who accompanies and looks after another person or group of people or an older woman responsible for the decorous behaviour of a young unmarried girl at social occasions).*Proteins that help chaperones to perform their functions, such as HSP40, were named co-chaperones.

2.2 The Role of Hsp70 in Protein Degradation: Welcome to an Execution!

Unfortunately, errors sometimes occur during protein folding and re-folding. This can lead to the emergence of non-functional proteins or to proteins that are harmful to the cell. Folding of mutant proteins leads to the same consequences;

however, even proteins that have folded properly have a limited functional lifespan from a few hours to a few days. Old dysfunctional proteins can even become dangerous to the cell. Fortunately, nature provided several ways of disposing such old, mutant or poorly folded proteins; heat shock proteins participate in two of them, namely proteasomal and lysosomal degradation.

The "password" for an unwanted protein entering into a proteasome is a ubiquitin chain (Fig. 2.4.). Protein ubiquitination consists of three stages (Glickman and Ciechanover 2002). In the first stage the ubiquitin-activating enzyme E1 binds to ubiquitin and transfers it to the enzyme-carrier E2. In the second stage E2 delivers ubiquitin to the protein designated for degradation, and then in the third stage a special E3 ligase transports ubiquitin from E2 to the "wrong" protein. After these three events, proteasomes recognize and degrade the proteins "labelled" with ubiquitin.

However, for a long time it was unclear how damaged and old proteins were recognized to be labelled by ubiquitin (Hershko and Ciechanover 1998). Only in the mid-1990s was an answer to this question found. Goldberg and Ciechanover laboratories clearly showed that the ubiquitin–proteasome system needs HSP70 and HSP40 for degradation of short-lived, mutant or old proteins (Lee et al. 1996; Bercovich et al. 1997).

It turned out that HSP70, assisted by its co-chaperone HSP40, recognizes damaged and irreversibly denatured proteins and sends them to proteosomes (Cyr et al. 2002) with the help of co-chaperones such as CHIP, Bag-1 and HSJ1 (Lüders et al. 2006; Petrucelli et al. 2004).

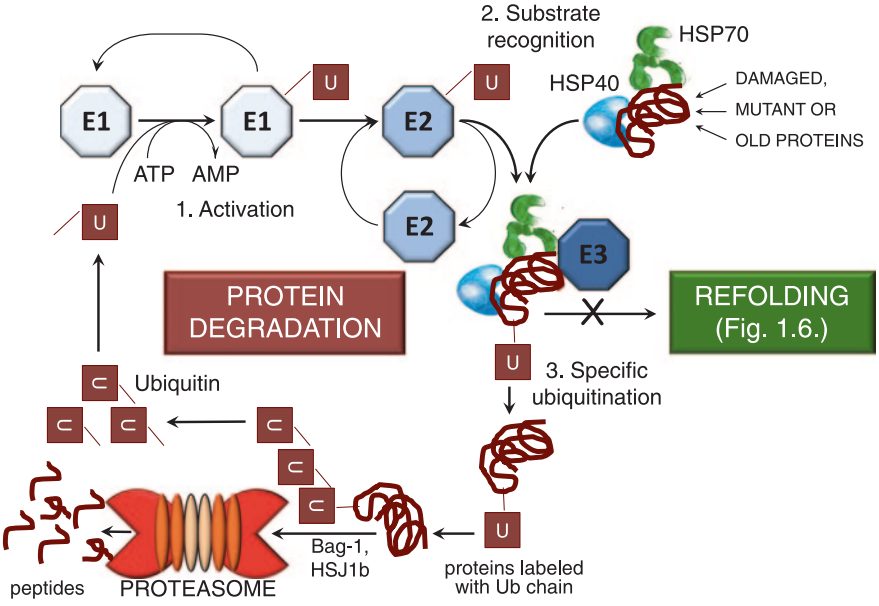


Fig. 2.4 The role of Hsp70 in protein degradation into proteasomes

The exact mechanism remains unclear; however, when HSP70 binds an irreversibly denatured or abnormal protein, the HSP70 C-domain exposes the sites for binding the co-chaperone **CHIP** (C terminus of HSP70 interacting protein) (Ballinger et al. 1999; Connell et al. 2001). This is a key moment in the switching of the effector function of the HSP70 ATPase cycle from the folding function to the proteolytic function (Fig. 2.4.). This is where the fate of the client protein is decided: whether there is a refolding process or whether the denatured protein is sent for proteasomal degradation. So why does CHIP play such an important role?

Ballinger et al. (Ballinger et al. 1999) discovered and described properties of CHIP that allowed the understanding of the mechanism of chaperone-dependent ubiquitination. CHIP was found to be an E3 ligase (Ballinger et al. 1999; Connell et al. 2001) and it has binding sites not only for HSP70 or HSP90, but also for the E2 enzyme-carrier (Jiang et al. 2001). CHIP binds to HSP70 and thereby interferes with the ATPase cycle and suppresses the HSP70 capacity for protein refolding (Ballinger et al. 1999); however, at the same time it builds the «HSP70-HSP40-protein-client-CHIP-E2» complex. When this complex is built, CHIP ubiquitinates the client protein bound to HSP70, thereby labelling the protein for proteasomal degradation (Connell et al. 2001; Glover and Lindquist 1998; Meacham et al. 2001).

Ubiquitin labelling is sufficient for the protein to enter the proteasome and be degraded. There are, however, two more co-chaperones, Bag-1 and HSJ1b, which until the very end control the execution of the ubiquitinated protein.

You know already that **Bag-1** contains a BAG domain, which is necessary for the interaction with HSP70. You also know that Bag-1 acts as a nucleotide exchange factor for HSP70, causing the release of the client protein. Finally, by now you also know that this Bag-1 activity is necessary for normal folding of the client protein. Since the ATPase cycle can only function in one of the two alternative states, it seems obvious that the folding activity of the cycle should inhibit its proteasomal activity. Indeed it was demonstrated that Bag-1 does inhibit the proteasomal degradation of the Tau protein[1] because of an increase in the refolding activity of the HSP70 cycle (Elliott et al. 2007).

However, nothing is that simple. The “double game” of Bag-1 is that in addition to the BAG domain it also contains an ubiquitin-like domain, through which it can interact with proteasomes (Lüders et al. 2000) and transport the ubiquitinated protein into proteasomes (Alberti et al. 2003). This is, for example, how the glucocorticoid receptor gets degenerated (Demand et al. 2001).

While the ubiquitinated client protein is Bag-1-escorted to its proteasome “scaffold”, there is always a danger that ubiquitin hydrolases would cleave the ubiquitin chain off the client protein, and the protein would no longer be recognized for proteasomal degradation. The system of the “sentenced execution” for the disposal of unwanted proteins takes care of this danger too. To make sure it

[1] Tau proteins stabilize microtubules in neurons of the central nervous system. When tau proteins are defective, they can result in Alzheimer's disease.

does not happen, another co-chaperone, **HSJ1b**, which contains ubiquitin-binding sites, binds to ubiquitinated proteins and protects them from ubiquitin hydrolases, thus ensuring that the ubiquitinated proteins are delivered to proteasomes. (Westhoff et al. 2005).

Membrane proteins and proteins of the endoplasmic reticulum lumen are directed for proteasomal degradation through a mechanism called endoplasmic reticulum-associated degradation (ERAD). In this mechanism, the reticular HSP70, Bip, recognizes some poorly folded proteins in the reticulum (Molinari et al. 2002) and binds directly to these mis-folded proteins (Nishikawa et al. 2005; Zhang et al. 2001). BiP, assisted by other proteins, then transports the denatured protein to the inner side of the reticulum membrane and transfers it through the membrane to cytoplasmatic HSP70. HSP70 then delivers these proteins to proteasomes for degradation.

An alternative way of degrading intracellular proteins—the one assisted by lysosomes (Fig. 2.5.)—comes to the foreground when a cell exists in a state of prolonged fasting.

Using the culture of human fibroblasts Chiang (Chiang et al. 1989) showed that HSP70 recognizes the protein destined for degradation, binds to it and transports it to the lysosome.

Majeski and Dice (Majeski and Dice 2004) helped to establish details of this process. When a cell is starving interesting things happen: normal proteins start degrading. This apparently "strange" cell behaviour is caused by the necessity to supply the protein biosynthesis process with amino acids to produce proteins essential to the survival of the cell. It turns out that up to 30 % of normal proteins sacrificed by a cell and sent to lysosomes contain a marker amino acid sequence known as **KFPRQ**. This degradation "black mark" is recognized by the

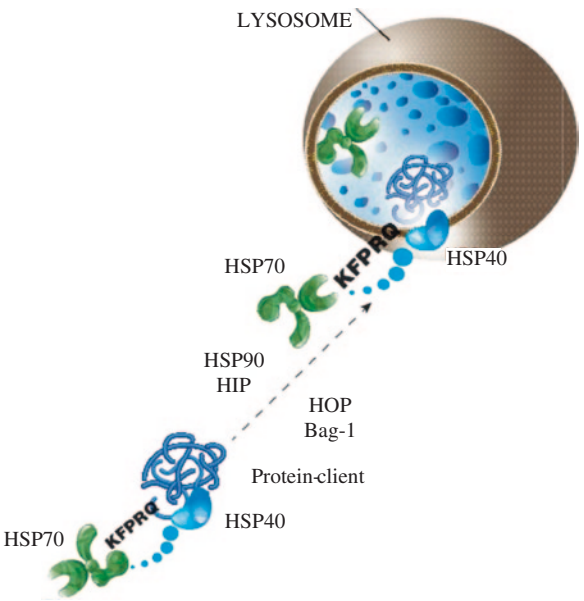


Fig. 2.5 The role of Hsp70 in protein degradation into lysosomes

HSP70/HSP90 chaperone complex that also contains co-chaperones Hsp40, Hip, Hop and Bag-1. This complex then binds to the lysosome membrane and imports the client protein to the lysosome to be degraded (Salvador et al. 2000).

This is quite incredible! When the cell was only being formed and experienced no starvation, it was already defined at the genetic level (by the **KFPRQ** motif) what proteins would be sacrificed in the situation of possible starvation.

Another intriguing fact was that HSP70 contains two KFERQ sequence motifs. Thanks to these motifs HSP70 penetrates into the lumen of lysosomes, where it is probably involved in protein transportation. However, lysosomes do not contain ATP; therefore the lysosomal HSP70 cannot retract proteins into the lysosome lumen using the ATP-dependent ratchet, similar to what mitochondrial HSP70 and Bip do in the lumen of the endoplasmic reticulum. The type of mechanism the lysosomal HSP70 use remains unresolved.

So we have examined *the role of HSP70 in proteasomal and lysosomal protein degradation*. A careful comparison of these two mechanisms of proteolysis shows something important: *in both cases HSP70 together with its co-chaperones performs similar functions, namely, it recognizes the "unwanted" protein and delivers it to the disposal site: to either proteasomes or lysosomes.* It appears also that the choice of the disposal site is determined genetically by specific amino acid motifs, such as KFPRQ.

Another important conclusion we can make is that the HSP70 ATPase cycle—together with chaperones and co-chaperones which ensure folding and re-folding, and the proteasomes or lysosomes that ensure degradation of "irreparable" proteins—form the protein "quality control" mechanism (Fig. 2.6.). To make it easier to remember the effector function of the molecular chaperone machine, one can use the abbreviation **FORD-machinery** to define the whole protein quality control system. It is made of the first letters of the words ***Fo****lding–****R****efolding–****D****egradation*, namely the **FORD machinery**.

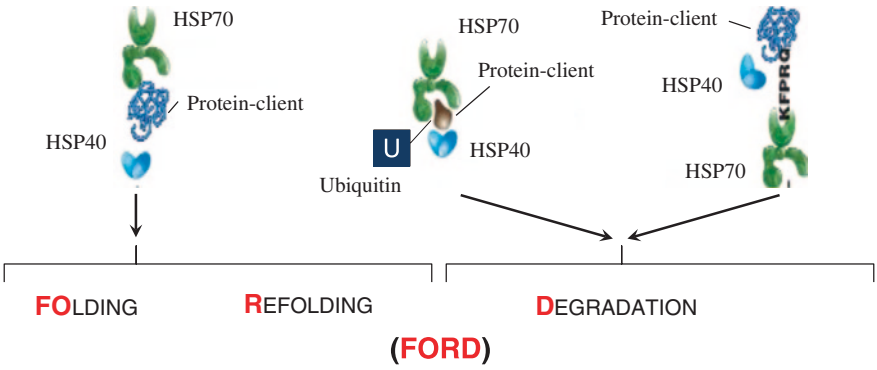


Fig. 2.6 The HSP70 ATPase cycle which ensures folding and re-folding, and the proteasomes or lysosomes that ensure degradation of "irreparable" proteins form the protein "quality control" mechanism (FORD-machinery)

2.3 Folding–Refolding–Degradation: The Molecular Protein Quality Control Machinery. The One that Decides the Fate of a Protein

Since HSP70 interacts with a number of intercellular proteins, its participation in folding or degrading should be carefully controlled. An important question arises then. Who switches the FORD-machinery on (or off)? At least two mechanisms play a potential role in forming a switch.

The first switch mechanism is formed as a result of competition between folding-stimulating co-chaperone and the factors that stimulate protein degradation for HSP70 binding sites. Normally the concentration of the folding-stimulating co-chaperones is 5–10 times higher than the concentration of the degradation stimulating factors. (Connell et al. 2001; Demand et al. 1998; Höhfeld and Jentsch 1997) Therefore under normal conditions, the main role of HSP70 will be protein folding. However, under various influences on a cell, the ratio of different co-factors may change, and that can switch the cycle from the folding-refolding pathway to degradation.

We know the examples of both co-factors. CHIP, and sometimes Bag-1 and HSJ1 can stimulate degradation. In humans, the nucleotide-exchange factor HSPBP1 is the activator of HSP70-mediated protein folding and, consequently, the degradation inhibitor. (Esser et al. 2004). HSPBP1 can bind HSP70 and CHIP to hamper protein ubiquitination (Alberti et al. 2004). As a result, HSPBP1 can switch the HSP70 cycle from degradation to folding. It was shown, for instance, that HSPBP1 inhibits the CHIP-mediated Cystic fibrosis transmembrane conductance regulator (CFTR)[2] protein degradation (Alberti et al. 2004; Arndt et al. 2005).

Another mechanism of switching the functional activity of the HSP70 ATPase cycle arises from chaperone HSP90. Whitesell and coworkers were the first to conduct research that made scientists think about the role of HSP90 in regulating the functions of HSP70 (Whitesell et al. 1994) and Sepp-Lorenzino et al. (Sepp-Lorenzino et al. 1995). These two groups showed that inhibition of HSP90 by the antibiotics herbimycin A and geldanamycin significantly increased the ubiquitination and proteasomal degradation of certain proteins. The simplest explanation is that HSP90 inhibits the CHIP-dependent ubiquitination of the client protein and can switch the HSP70 cycle from degradation to folding.

In this way, HSP70 manifests itself as a sort of molecular transformer: when certain factors get attached, the HSP70 cycle ensures folding of the newly produced or denatured and damaged protein; when other factors are attached, the cycle does the opposite; the protein is targeted for degradation.

Taken together, this allows **HSP70** with its co-chaperones and the chaperone HSP90 to build an intracellular system of protein quality control. In this system, HSP70 acts as a "judge" together with its "jury" of co-chaperones to decide the

[2] CFTR is an ion channel that transports chloride ions across epithelial cell membranes. Mutations of the CFTR gene affect functioning of the chloride ion channels, leading to cystic fibrosis.

fate of a damaged or denatured protein. Here, a protein will be either sent for "re-education", i.e., refolding, or the protein will be sent for degradation to the proteasomal or lysosomal "scaffold".

2.4 The Role of HSP70 in Signal Transduction

The concept of HSP70 and its co-chaperones' role in coupling mechanisms of protein folding and degradation also made researchers revise or supplement their understanding of the mechanisms for intracellular signal transduction. Each year additional data show that normal intracellular activity of signalling proteins is controlled by HSP70. These proteins include, for instance, steroid hormone receptors, Raf kinase, eIF2α-kinase, CyclinB1/Cdk1 and heat shock transcription factors–1 (HSF-1), c-Myc and pRb.

Through its interaction with such signalling molecules, HSP70 is involved in cell cycle regulation, differentiation and transduction of hormonal signals. It is not surprising therefore that HSP70 and its co-chaperones play an important role in the process of normal development as well as in pathological processes, such as carcinogenesis, aging and neurodegenerative dysfunctions (Jäättelä 1999; Kregel 2002).

A few general principles of the effect of HSP70 on signal transduction mechanisms were discovered.

First, HSP70 complexes with a signalling client protein also involve various co-chaperones, such as HSP40, CHIP, chaperone HSP90 and some other proteins (Pratt 1997).

Second, generally in such complexes the intracellular transduction proteins remain in an inactive state from which they can be quickly activated (Pratt and Toft 2003). For instance, HSP70 detachment from the complex, when denatured or damaged proteins appear in a cell, can lead to activation of signalling proteins, because HSP70 has a high affinity towards denatured proteins. Therefore adverse environmental conditions can activate intracellular transduction pathways. This is how activation of the HSF-1 transcription factor occurs.

In a second example, HSP70 together with HSP90 keeps the intracellular steroid receptor in an inactive form that is still ready to bind a ligand. When the steroid hormone binds to the receptor, the chaperone complex disassociates, and the active hormone-receptor complex is directed to the nucleus and activates gene transcription.

Third, HSP70 and its co-factors can interact with components of signalling pathways to protect them also from degradation and to ensure their continued active state. HSP70 employs this "set of tactics" with respect to the retinoblastoma protein (pRB). pRB is a major regulatory molecule involved in the initial stages of the cell cycle. HSP70 protects pRB from degradation and allows active pRB to restrain the cell from entry into the cell cycle.

Fourth, through rapid switching of its folding and degradation activities the ATPase cycle can effectively regulate the quantitative and qualitative composition of a certain part of the pool of signalling molecules. This can also ensure effective

regulation of signalling pathways. For instance, hypoxia-induced factor I (HIF-I) is a transcription factor that mediates the cell adaptation to hypoxic conditions. Under normal conditions, HSP70 and CHIP interact with HIF-1 and thus ubiquitinate this protein and direct it for proteasomal degradation (Luo et al. 2010). Under hypoxia, denatured proteins appear in a cell. This leads to HSP70 detachment from HIF-1 and attachment to the damaged proteins, to which it has a higher affinity. As a result, HSP70 stops sending HIF-1 for degradation and HIF-1 can penetrate into the nucleus and activate genes responsible for adaptation to hypoxia.

Finally, HSP70 can be involved in the intracellular trafficking of signalling proteins. This was clearly shown in the example of G protein-coupled receptors (GPCRs). In order to transduce an extracellular signal into the cell GPCRs have to be properly folded, transported to the cell surface and subsequently degraded once they have performed their function. The HSP70 chaperone FORD-machinery plays the major role in organizing all these processes (Ancevska-Taneva et al. 2006; Chapple and Cheetham 2003; Lanctôt et al. 2006; Neuhaus et al. 2006).

These receptors are of particular relevance to human health. Since most hormones transmit a signal through GPCRs, many diseases associated with mutations in the GPCRs disrupt the endocrine system. In these diseases there is usually a violation of receptor trafficking to the surface of the plasma membrane (Conn et al. 2007). This happens because in the endoplasmic reticulum the resident HSP70 Bip recognizes the mutant GPCR as a mis-folded protein and sends this GPCR for proteasomal degradation (Conn et al. 2007; Anelli and Sitia 2008).

2.4.1 What New we did Learn from this Chapter (Summary) and P.S.

We have examined only the most important part of what is known about HSP70 functions in normal cells. We have learnt that:

1. The HSP70 ATPase cycle together with co-chaperones forms the protein folding mechanism and provides protein transportation into organelles.
2. The HSP70 ATPase cycle assisted by HSP40 recognizes irreversibly damaged proteins and, assisted by CHIP, Bag-1 and HSJ1 ubiquitinates these proteins and sends them for proteasomal degradation.
3. The HSP70/HSP90 complex, together with the co-chaperones Hsp40, Hip, Hop and Bag-1, recognizes normal proteins containing the marker sequence KFPRQ and sends these proteins to the lysosome for degradation.
4. The HSP70 ATPase cycle forms the protein quality control system (FORD machinery) and, depending on the state of the protein, sends the protein either for re-folding or for degradation. Thus, the FORD machinery maintains protein homeostasis.
5. Transitions between the “folding” and “degradation” states of the cycle are carefully monitored by HSP90 and by the ratio of co-chaperones, folding stimulators and degradation stimulators.

6. The HSP70 ATPase cycle controls the activity of key signalling proteins by keeping them in an inactive or active state, by regulation of their levels and by intracellular transport.

P.S. The multitude of HSP70 functions and cellular processes is astounding! This protein pokes its nose everywhere! Now we understand that there is safety in numbers and that the HSP70 multifunctional uniqueness is connected both with its three-domain structure and, primarily, with a large number of its co-chaperone "assistants". First HSP70 attracts co-chaperones, and then, with their help, it builds a multifunctional regulatory network that is used by the cell for many purposes: folding, re-folding, degradation or regulation of signalling transduction events. Truly, first the "the king makes the suite" and then "the suite makes the king"!

Despite the significant progress in understanding how this network works, many questions still remain unanswered. We do not understand how the co-chaperones direct HSP70 to substrates; how HSP70 knows that this particular protein is to be folded, but the other one should be sent for degradation; what determines the substrate specificity of HSP70 to signalling proteins? There are many more other questions that remain unanswered.

Therefore, many more discoveries about cell processes that depend on the activity of HSP70 will undoubtedly be found in the future. In relation to the study of HSP70 effects it would be appropriate now to recall the words of the great British Prime Minister Winston Churchill, "Now this is not the end. It is not even the beginning of the end. But it is, perhaps, the end of the beginning" (Fig. 2.7).

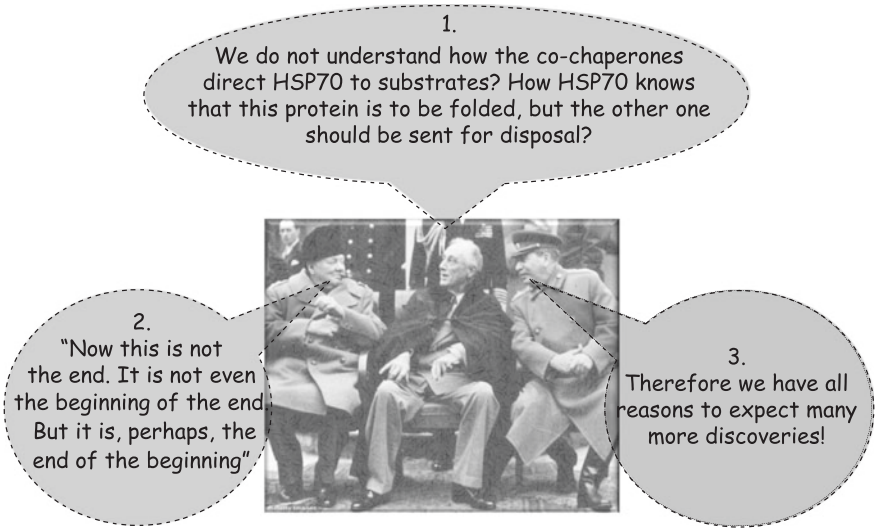


Fig. 2.7 Discussion of the important problems

References

Alberti S, Esser C, Höhfeld J (2003) BAG-1–a nucleotide exchange factor of Hsc70 with multiple cellular functions. Cell Stress Chaperon 8(3):225–231

Alberti S, Böhse K, Arndt V et al (2004) The cochaperone HspBP1 inhibits the CHIP ubiquitin ligase and stimulates the maturation of the cystic fibrosis transmembrane conductance regulator. Mol Biol Cell 15(9):4003–4010

Ancevska-Taneva N, Onoprishvili I, Andria ML et al (2006) A member of the heat shock protein 40 family, hlj1, binds to the carboxyl tail of the human mu opioid receptor. Brain Res 1081(1):28–33

Anelli T, Sitia R (2008) Protein quality control in the early secretory pathway. EMBO J 27(2):315–327

Anfinsen CB (1973) Principles that govern the folding of protein chains. Science 181:223–230

Arndt V, Daniel C, Nastainczyk W et al (2005) BAG-2 acts as an inhibitor of the chaperone-associated ubiquitin ligase CHIP. Mol Biol Cell 16(12):5891–5900

Ballinger CA, Connell P, Wu Y et al (1999) Identification of CHIP, a novel tetratricopeptide repeat-containing protein that interacts with heat shock proteins and negatively regulates chaperone functions. Mol Cell Biol 19(6):4535–4545

Bercovich B, Stancovski I, Mayer A et al (1997) Ubiquitin-dependent degradation of certain protein substrates in vitro requires the molecular chaperone Hsc70. J Biol Chem 272(14):9002–9010

Bukau B, Weissman J, Horwich A (2006) Molecular chaperones and protein quality control. Cell 125(3):443–451

Chapple JP, Cheetham ME (2003) The chaperone environment at the cytoplasmic face of the endoplasmic reticulum can modulate rhodopsin processing and inclusion formation. J Biol Chem 278(21):19087–19094

Chiang HL, Terlecky SR, Plant CP, Dice JF (1989) A role for a 70-kilodalton heat shock protein in lysosomal degradation of intracellular proteins. Science 246(4928):382–385

Conn PM, Ulloa-Aguirre A, Ito J, Janovick JA (2007) G protein-coupled receptor trafficking in health and disease: lessons learned to prepare for therapeutic mutant rescue in vivo. Pharmacol Rev 59(3):225–250

Connell P, Ballinger CA, Jiang J et al (2001) The co-chaperone CHIP regulates protein triage decisions mediated by heat-shock proteins. Nat Cell Biol 3(1):93–96

Cyr DM, Höhfeld J, Patterson C (2002) Protein quality control: U-box-containing E3 ubiquitin ligases join the fold. Trends Biochem Sci 27(7):368–375

Demand J, Lüders J, Höhfeld J (1998) The carboxy-terminal domain of Hsc70 provides binding sites for a distinct set of chaperone cofactors. Mol Cell Biol 18(4):2023–2028

Demand J, Alberti S, Patterson C, Höhfeld J (2001) Cooperation of a ubiquitin domain protein and an E3 ubiquitin ligase during chaperone/proteasome coupling. Curr Biol 11(20):1569–1577

Dobson CM (2003) Protein folding and misfolding. Nature 426(6968):884–890

Elliott E, Tsvetkov P, Ginzburg I (2007) BAG-1 associates with Hsc70. Tau complex and regulates the proteasomal degradation of Tau protein. J Biol Chem 282(51):37276–37284

Esser C, Alberti S, Höhfeld J (2004) Cooperation of molecular chaperones with the ubiquitin/proteasome system. Biochim Biophys Acta 1695(1–3):171–188

Glickman MH, Ciechanover A (2002) The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. Physiol Rev 82(2):373–428

Glover JR, Lindquist S (1998) Hsp104, Hsp70, and Hsp40: a novel chaperone system that rescues previously aggregated proteins. Cell 94(1):73–82

Hartl FU, Hayer-Hartl M (2009) Converging concepts of protein folding in vitro and in vivo. Nat Struct Mol Biol. doi:10.1038/nsmb.1591

Hershko A, Ciechanover A (1998) The ubiquitin system. Annu Rev Biochem 67:425–479

Höhfeld J, Jentsch S (1997) GrpE-like regulation of the hsc70 chaperone by the anti-apoptotic protein BAG-1. EMBO J 16(20):6209–6216

Jäättelä M (1999) Escaping cell death: survival proteins in cancer. Exp Cell Res 248(1):30–43

Jiang J, Ballinger CA, Wu Y et al (2001) CHIP is a U-box-dependent E3 ubiquitin ligase: identification of Hsc70 as a target for ubiquitylation. J Biol Chem 276(46):42938–42944

Kregel KC (2002) Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. J Appl Physiol 92(5):2177–2186

Lanctôt PM, Leclerc PC, Escher E et al (2006) Role of N-glycan-dependent quality control in the cell-surface expression of the AT1 receptor. Biochem Biophys Res Commun 340(2):395–402

Lee DH, Sherman MY, Goldberg AL (1996) Involvement of the molecular chaperone Ydj1 in the ubiquitin-dependent degradation of short-lived and abnormal proteins in *Saccharomyces cerevisiae*. Mol Cell Biol 16(9):4773–4781

Lüders J, Demand J, Höhfeld J (2006) The ubiquitin-related BAG-1 provides a link between the molecular chaperones Hsc70/Hsp70 and the proteasome. J Biol Chem 275(7):4613–4617

Luo W, Zhong J, Chang R et al (2010) Hsp70 and CHIP selectively mediate ubiquitination and degradation of hypoxia-inducible factor (HIF)-1alpha but Not HIF-2alpha. J Biol Chem 285(6):3651–3663

Majeski AE, Dice JF (2004) Mechanisms of chaperone-mediated autophagy. Int J Biochem Cell Biol 36(12):2435–2444

Meacham GC, Patterson C, Zhang W et al (2001) The Hsc70 co-chaperone CHIP targets immature CFTR for proteasomal degradation. Nat Cell Biol 3(1):100–105

Molinari M, Galli C, Piccaluga V et al (2002) Sequential assistance of molecular chaperones and transient formation of covalent complexes during protein degradation from the ER. J Cell Biol 158(2):247–257

Neuhaus EM, Mashukova A, Zhang W et al (2006) A specific heat shock protein enhances the expression of mammalian olfactory receptor proteins. Chem Senses 31(5):445–452

Nishikawa S, Brodsky JL, Nakatsukasa K (2005) Roles of molecular chaperones in endoplasmic reticulum (ER) quality control and ER-associated degradation (ERAD). J Biochem 137(5):551–555

Petrucelli L, Dickson D, Kehoe K et al (2004) CHIP and Hsp70 regulate tau ubiquitination, degradation and aggregation. Hum Mol Genet 13(7):703–714

Pratt WB (1997) The role of the hsp90-based chaperone system in signal transduction by nuclear receptors and receptors signaling via MAP kinase. Annu Rev Pharmacol Toxicol 37:297–326

Pratt WB, Toft DO (2003) Regulation of signaling protein function and trafficking by the hsp90/hsp70-based chaperone machinery. Exp Biol Med (Maywood) 228(2):111–133

Salvador N, Aguado C, Horst M, Knecht E (2000) Import of a cytosolic protein into lysosomes by chaperone-mediated autophagy depends on its folding state. J Biol Chem 275(35):27447–27456

Sepp-Lorenzino L, Ma Z, Lebwohl DE et al (1995) Herbimycin A induces the 20 S proteasome- and ubiquitin-dependent degradation of receptor tyrosine kinases. J Biol Chem 270(28):16580–16587

Westhoff B, Chapple JP, van der Spuy J et al (2005) HSJ1 is a neuronal shuttling factor for the sorting of chaperone clients to the proteasome. Curr Biol 15(11):1058–1064

Whitesell L, Mimnaugh EG, De Costa B et al (1994) Inhibition of heat shock protein HSP90-pp 60v-src heteroprotein complex formation by benzoquinone ansamycins: essential role for stress proteins in oncogenic transformation. Proc Natl Acad Sci USA 91(18):8324–8328

Zhang Y, Nijbroek G, Sullivan ML et al (2001) Hsp70 molecular chaperone facilitates endoplasmic reticulum-associated protein degradation of cystic fibrosis transmembrane conductance regulator in yeast. Mol Biol Cell 12(5):1303–1314

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