JB Minireview—Quality Control of the Cellular Protein System

J. Biochem. 2009;146(5)609–616
doi:10.1093/jb/mvp139

Quality Control Against Misfolded Proteins in the Cytosol: A Network for Cell Survival

Hiroshi Kubota*

Department of Life Science, Faculty of Engineering and Resource Science, Akita University, Akita 010-8502, Japan

Received July 7, 2009; accepted August 5, 2009; published online September 7, 2009

Misfolded proteins are toxic to cells and the accumulation of toxic species can lead to protein misfolding diseases, such as neurodegenerative disorders. The toxicity of misfolded proteins is thought to result from the presence of exposed hydrophobic surfaces, which mediate unnecessary binding to normal proteins, interrupting essential interactions between cellular proteins. To prevent toxicity, quality control systems monitor protein folding and remove misfolded species in the cytosol. Molecular chaperones recognize and mask hydrophobic surfaces of misfolded monomers, and transfer them to the ubiquitin–proteasome system and chaperone-mediated autophagy. To eliminate soluble aggregates of misfolded proteins, the macroautophagy–lysosome system is thought to degrade proteasome-resistant toxic species. In addition, the microtubule-dependent transport system sequesters soluble oligomers/aggregates into inclusion bodies. These systems are regulated by stress-inducible transcription factors, cochaperones and other cofactors for the effective removal of toxic monomers and oligomers. This review explores the roles of protein quality control pathways and networks that control quality control activities in the cytosol, particularly focusing on recent progress in this field.

Key words: autophagy–lysosome system, molecular chaperone, protein misfolding, protein quality control, ubiquitin–proteasome system.

Abbreviations: ALS, amyotrophic lateral sclerosis; BAG, BCL2-associated athanogene; CCT, chaperonin containing *t*-complex polypeptide 1; CHIP, carboxy terminus of HSP70-binding protein; CMA, chaperone-mediated autophagy; FKBP, FK506-binding protein; HOP, HSP70/HSP90 organizing protein; HSC70, cognate of HSP70; HSE, heat shock element; HSF, heat shock factor; HSP, heat shock protein; MEF2D, myocyte enhancer factor 2D; NBR1, neighbour of BRCA1 gene 1; TPR, tetratricopeptide repeat; SOD1, superoxide dismutase 1.

PRODUCTIVE FOLDING AND PREVENTION OF AGGREGATE FORMATION OF MISFOLDED PROTEINS BY CYTOSOLIC MOLECULAR CHAPERONES

Newly translated polypeptides spontaneously adopt their secondary structure by interaction of their side chains. However, the efficiency of correct folding into a functional tertiary structure is often low because of unnecessary interactions between partially folded structures. For example, the structural complexity of multidomain proteins and the sticky nature of hydrophobic β-sheets can cause misfolding. A highly crowded intracellular environment can further increase the risk of non-productive protein folding by undesirable interactions with other proteins. Proteins synthesized in the cell, therefore, require assistance from molecular chaperones during their folding and assembly process to acquire their functional conformation (*1*–*3*). Molecular chaperones are also required for the refolding of proteins denatured under stress conditions, such as higher temperatures. The most important function of molecular chaperones is to prevent protein aggregation by trapping the hydrophobic surfaces of folding intermediates and facilitating correct folding. These two activities depend on each other and are often indistinguishable. If the functions of chaperons are inhibited, misfolded proteins accumulate and aggregate in the cell. Accumulation of misfolded proteins is highly toxic to the cell and often causes neuronal cell death, which leads to neurodegenerative diseases. Thus, molecular chaperones are essential for protecting the cells against the toxicity of misfolded proteins.

There are several groups of molecular chaperones in the cytosol of mammalian cells. These chaperones include the 90-kDa heat shock protein (HSP90), 70-kDa heat shock protein (HSP70), chaperonin containing *t*-complex polypeptide 1 (CCT, also called TRiC) and small HSP family proteins (*4*, *5*). HSP90 is the most abundant molecular chaperone in the cytosol and a dimer of the highly homologous 90-kDa subunits (HSP90α and HSP90β) (*6*, *7*). Although HSP90 is stress inducible, it is strongly expressed even under non-stress conditions. The function of HSP90 is exerted by conformational changes upon ATP binding and hydrolysis, and its activity is required for maturation of steroid hormone receptors and protein kinases.

*To whom correspondence should be addressed.
Tel/Fax: +81 18 889 3053, E-mail: hkubota@ipc.akita-u.ac.jp

There are six HSP70 family members expressed in the cytosol of mammalian cells (*8*). HSP70 and the cognate of HSP70 (HSC70) are major cytosolic HSP70 family members. Although expression of HSP70 is induced by stress, HSC70 is expressed under non-stress conditions. HSP70 and HSC70 (HSP70s) are highly homologous and almost indistinguishable in their chaperone activity. They recognize the hydrophobic surfaces of unfolded proteins and partially folded intermediates, and the binding and release of substrate proteins are controlled by their ATP-binding and hydrolysis activities (*9*). The HSP70 family proteins inhibit protein aggregation, thus facilitating productive folding of substrate proteins.

CCT is a chaperonin that localizes in the eukaryotic cytosol (*10*, *11*) and has a double ring-like structure composed of 16 subunits of ~60 kDa, similar to bacterial chaperonin GroEL and mitochondrial chaperonin HSP60 (*12*). Substrate proteins are trapped and folded in the central cavity of the CCT protein. CCT uses a built-in lid called helical protrusions to encapsulate substrate proteins, whereas GroEL and HSP60 use cochaperonin as a lid for this purpose. By encapsulation, CCT protects substrate proteins from aggregation, allows them to undergo conformational changes under the control of ATP binding and hydrolysis to ensure correct folding and then substrate proteins are released.

Small HSP family proteins are 10–40 kDa in size and share amino acid sequence homology and structural similarity. There are at least 11 genes encoding small HSP proteins in the human genome (*HSPB1–11*). HSP27 (HSPB1), αB crystalline (HSPB5) and HSP22 (HSPB8) are known to inhibit protein aggregation without the aid of ATP (*13*), and many small HSP proteins play important roles in the maintenance of muscles (*14*). There is an equilibrium between dimers (or very small oligomers) and large oligomers (*e.g.* 20- to 50-mers) (*15*). An increasing amount of evidence suggests that dimers inhibit protein aggregation much more effectively than large oligomers (*16*). Since small HSPs are ATP-independent chaperones, they may have evolved a disassembly dependent activation system to control the amount of active chaperones.

COCHAPERONES

Molecular chaperone activity is controlled by chaperone-interacting proteins called cochaperones (*9*, *17*) (Fig. 1). HSP40/DnaJ family proteins contain a J-domain that binds to the ATPase binding domain of HSP70s and stimulates their ATPase activity (*18*). Since these proteins also bind substrate proteins, they can modulate the chaperone activity of HSP70s in

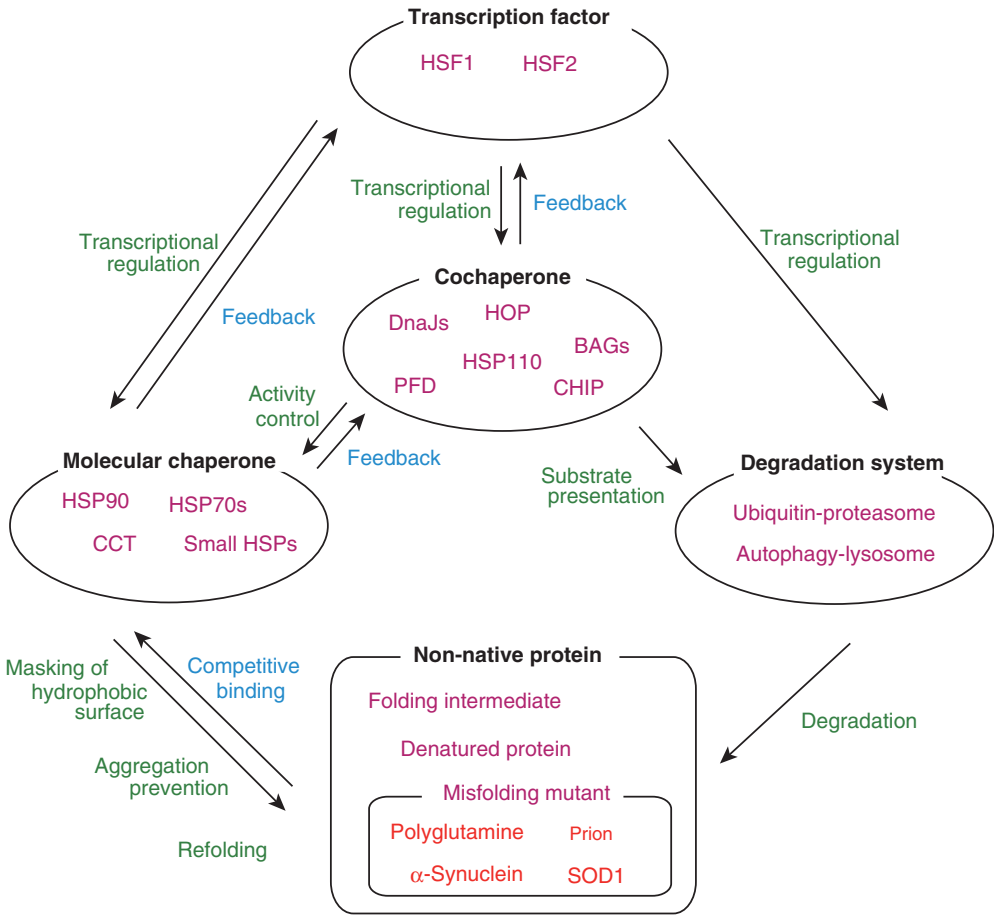

Fig. 1. **Network of quality control systems that protect against misfolded and other non-native proteins in the cytosol.**

a substrate-specific manner. More than 40 genes in this family are found in the human genome, and they are classified into three categories: types I (*DnaJA*), II (*DnaJB*) and III (*DnaJC*) (*19*). These gene products play diverse functions in protein folding, assembly, translocation and degradation *in vivo*. BCL2-associated athanogene (BAG) family proteins (BAG1–6) share an evolutionarily conserved BAG domain and bind to the ATPase domain of HSP70s. These proteins affect nucleotide exchange and substrate binding/release by HSP70s and exert various *in vivo* functions *via* non-BAG domains (*e.g*. ubiquitin-like domains in BAG1 and BAG6). For example, BAG1 and BAG3 were recently reported to be regulators of proteasomal and autophagic pathways, respectively (*20*), indicating a link between molecular chaperones and degradation systems mediated by cochaperones. HSP110 (also called HSP105) is also a nucleotide exchange factor of HSP70s and regulates the chaperone activity of HSP70s (*21*, *22*).

There are cochaperones that interact with two different chaperones. HSP70/HSP90 organizing protein (Hop, also called Sti1) binds HSP90 and HSC70/HSP70 *via* distinct tetratricopeptide repeat (TPR) domains and organizes their actions, including transferring substrates between them (*6*, *23*). The organized action of the two chaperones facilitates productive folding of proteins and formation of functional protein complexes. Another TPR domain-containing cochaperone, the carboxy terminus of HSP70-binding protein (CHIP), interacts with HSP70 and HSP90, and plays crucial roles in the quality control of ubiquitination of misfolded proteins (see Chapter 4). Immunophilins (FKBP51 and FKBP52, and Cyp40) are TPR-domain-containing proteins required for HSP90-assisted maturation of steroid hormone receptors, and the steroid aporeceptor-associated protein, p23, stabilizes HSP90–substrate complexes (*23*).

Prefoldin (also called GimC) assists in CCT-dependent folding of actin and tubulin by transferring the folding intermediates to CCT. Prefoldin has a jellyfish-like structure with six tentacles, by which it traps unfolded substrates (*24*).

ORGANIZATION OF CYTOSOLIC PROTEIN QUALITY CONTROL *VIA* TRANSCRIPTIONAL REGULATION

The rate of misfolding is significantly increased under stress conditions (*e.g*. heat stress, oxidative stress, heavy metals and proteasome inhibition) and many chaperone genes are induced under these conditions to protect cells against the potential toxicity of misfolded proteins (*25*). The major transcription factor controlling the cytosolic stress response is heat shock factor 1 (HSF1). This transcription factor binds to the heat shock response element (HSE), a specific *cis*-acting element in the promoter region of stress-responsive genes. The HSE–HSF1 system also induces ubiquitin expression, indicating a role in the regulation of the degradation system (see Chapter 4). HSF1 is distributed in the cytosol as monomers under non-stress conditions and is transferred into the nucleus as trimers in response to stress. In the cytosol, association of HSP90 with the HSF1 monomer inhibits its activation (*26*). Under stress conditions, HSF1 is released from HSP90 by competitive binding of denatured proteins to HSP90. The activation of HSF1 is transient and rapidly attenuated because the presence of excess molecular chaperones is toxic to cells due to increased non-essential interactions. HSP70 is considered to play a role in the attenuation of chaperone induction by interacting with HSF1 (*27*). These observations indicate a feedback system in the stress response. Recently, sirtuin 1 (SIRT1), a sirtuin family deacetylase, was shown to maintain HSE-binding ability by deacetylating a specific residue in HSF1, indicating a new pathway to control the stress response (*28*). How HSF1 is acetylated at the site that was deacetylated by SIRT1 is of interest.

THE ROLE OF CYTOSOLIC MOLECULAR CHAPERONES IN PROTECTION AGAINST NEURODEGENERATIVE DISEASE

Accumulation of misfolded proteins causes a number of neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, prion disease, polyglutamine disease and amyotrophic lateral sclerosis (ALS) (*29*, *30*). In these diseases, specific misfolded proteins exert toxicity leading to neuronal cell dysfunction and death, although the exact molecular mechanisms are still poorly understood. Insoluble aggregates (inclusions) containing disease-causing proteins are often observed in the neuronal cells of patients. For example, α-synuclein aggregates are found in neuronal cells of Parkinson's disease patients, while amyloid precursor protein Aβ forms amyloid fibrils in extracellular spaces in the brain of Alzheimer's disease patients. In prion disease, misfolded prion proteins propagate by altering correctly folded prion proteins to misfolded ones during the aggregation process. In polyglutamine disease, genetic mutation causes expansion of polyglutamine tracts in specific proteins (*e.g*. huntingtin protein for Huntington's disease). The polyglutamine-expanded proteins tend to form aggregates due to instability of the long polyglutamine tract. Point mutations of specific genes also cause neurodegenerative diseases. For example, mutations in superoxide dismutase 1 (SOD1) cause ALS, where motor neurons are progressively killed due to the toxicity of misfolded mutant proteins.

A possible molecular mechanism for the toxicity of misfolded proteins is the unnecessary interactions of misfolded proteins with normal cellular proteins. These interactions may disturb the maturation process (*e.g*. folding and assembly) and activities (*e.g*. signal transduction and transcriptional regulation) of normal cellular proteins. In addition, misfolded monomers and oligomers may exhibit toxicity by trapping normal proteins during their aggregation process, although large inclusions are formed by microtubule-dependent forced aggregation to sequester oligomers (see Chapter 6). These observations suggest that the cytotoxicity of misfolded proteins is determined by the total area of exposed hydrophobic surfaces on misfolded proteins accumulated in the cell. Thus, small species, like misfolded monomers and small oligomers, are probably highly toxic to cells.

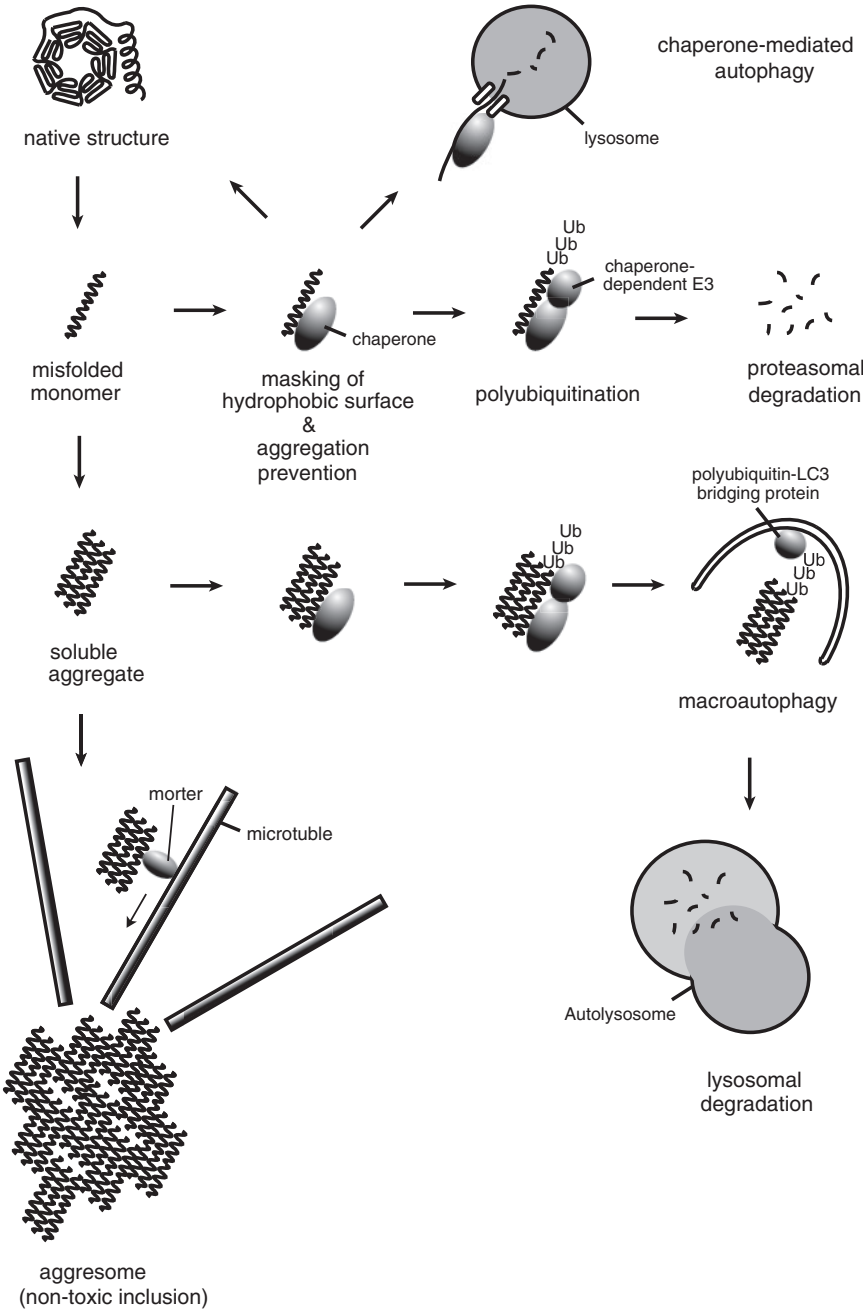

Fig. 2. **Pathways for preventing the toxicity of misfolded proteins.**

Several chaperones are known to prevent aggregation and toxicity of misfolded proteins in the cytosol (*31*). To block the potential toxicity of exposed hydrophobic surfaces of misfolded monomers and oligomers, molecular chaperones trap them by hydrophobic interactions (Fig. 2). A major chaperone that interacts with misfolded proteins inhibiting their toxicity is HSP70/HSC70. HSP70s dynamically interact with polyglutamine-expanded proteins and prevent toxicity by modulating aggregation, particularly in the presence of HSP40 (*32–35*). HSP70 suppresses the toxicity of α-synuclein mutants that cause Parkinson's disease (*36*), and HSC70 binds ALS-linked mutant SOD1 in a soluble state (*37*). Small HSPs also prevent toxicity of polyglutamine-expanded huntingtin (*38*) and α-synuclein (*39*).

Inhibition of HSP90 is known to stimulate degradation of polyglutamine-expanded androgen receptor, which causes spinal and bulbar muscular atrophy, through alteration of partner chaperones/cochaperones in the HSP90-containing complex (*40*). In this case, however, chaperone is a stimulating factor of disease.

CCT alters the aggregation state of polyglutamine-expanded huntingtin and prevents the cytotoxicity of this protein (*41–44*). Intriguingly, CCT appears to alter

the process of aggregation both *in vivo* (*43*) and *in vitro* (*42*), as detected by fluorescent correlation spectroscopy (or confocal single-particle analysis). Since overexpression of all eight subunits of CCT suppresses the aggregation and toxicity of huntingtin in yeast and neuronal cells, modulation of CCT activity may be useful as a possible therapy in the treatment of Huntington's disease.

DEGRADATION OF MISFOLDED PROTEINS BY THE UBIQUITIN–PROTEASOME SYSTEM IN THE CYTOSOL

Molecular chaperones prevent toxicity of misfolded proteins by masking their exposed hydrophobic surfaces. However, the amount of molecular chaperones within a cell is limited. The rate of protein misfolding is significantly increased and overcomes chaperone activities under strong stress conditions. Moreover, structurally unstable proteins, like neurodegenerative disease-causing mutants, are never correctly folded even though chaperones assist their folding. If the accumulation of misfolded proteins exceeds the cellular chaperoning capacity, these toxic species directly damage essential cellular systems by interacting with normal proteins. Thus, a degradation system for the misfolded species is essential for cells to overcome this toxicity.

The major system to degrade misfolded proteins in the cytosol is the ubiquitin–proteasome system (*45*). Misfolded proteins are specifically ubiquitinated by ubiquitin-conjugating enzymes and degraded by the proteasome through recognition of polyubiquitin as a degradation tag. Substantial amounts of misfolded proteins are degraded after protein synthesis even under non-stress conditions, as inhibition of proteasome activity results in the formation of aggregates. The ubiquitin–proteasome system is critical in removing misfolded mutants since they cannot acquire properly folded structures. A major enzyme that polyubiquitinates misfolded proteins is CHIP, a chaperone dependent E3 protein (*46*). CHIP binds HSP70/HSC70 *via* its TPR domain and uses this chaperone for substrate recognition. HSP70 recognizes misfolded proteins and maintains them in a soluble state. When HSP70 fails to fold a misfolded protein into its native structure, the HSP70-substrate complex is recognized by CHIP and the substrate is polyubiquitinated. Further investigations are required to determine how the HSP70–CHIP system discriminates between substrates to be refolded or degraded.

CHIP is required for the induction of HSPs by positively regulating HSF1 under stress conditions (*47*), but the same enzyme stimulates HSP70 degradation by ubiquitination during attenuation of the stress response (*48*). These observations indicate another link between the stress response and ubiquitination-dependent degradation systems.

ROLES OF THE AUTOPHAGY–LYSOSOME SYSTEM IN PROTEIN QUALITY CONTROL

The autophagy–lysosome system plays a crucial role in the degradation of cytosolic proteins (*49*, *50*). Autophagy (macroautophagy) is a series of cytoplasmic events including sequestration of molecules or organelles by double-membrane vesicles and vesicular fusion to lysosomes, where the sequestered molecules are finally degraded by lysosomal proteases. Although the autophagy–lysosome pathway is well known as a system to produce free amino acids and other materials required for survival during fasting, this pathway also plays a significant role in the clearance of cytotoxic misfolded proteins (*51*). For example, autophagy is required for effective elimination of polyglutamine-expansion proteins, in addition to proteasomal degradation (*52*, *53*). Mice deficient for autophagy show neurodegeneration in the brain (*54*, *55*). Recently, two proteins were shown to link polyubiquitination and autophagy: p62 (also called sequestome 1) (*56*) and the neighbour of BRCA1 gene 1 (NBR1) (*57*). Each of these proteins bridges between polyubiquitin, a degradation tag and LC3, an essential component of autophagy. The ubiquitin–proteasome system degrades soluble monomers of misfolded proteins, but not irreversibly oligomerized ones. Thus, the autophagy–lysosome system probably degrades soluble aggregates through recognition of polyubiquitin by p62 and NBR1. By these aggregation phase-dependent systems, toxic misfolded species are effectively eliminated from the cytosol, although little is known about how small oligomers (trimers, tetramers, . . .) are degraded.

Another pathway could direct cytosolic proteins to degradation in the lysosome. This pathway, chaperone-mediated autophagy (CMA), does not require sequestration of target proteins into autophagosomes (*58*). In the CMA pathway, a specific motif (KFERQ and related sequences) in target proteins is first recognized by HSC70 that forms a complex with HSP40, Hip and Hop. The KFERQ-like sequences are found in 30% of cytosolic proteins, suggesting an abundance of potential targets of CMA-mediated degradation. Substrate proteins are transferred from the chaperone complex to the lysosomal lumen through the lysosome-associated membrane protein type 2A. CMA contributes to the degradation of α-synuclein, and Parkinson's disease-associated mutations in α-synuclein block the degradation pathway (*59*). Recently, CMA was found to degrade myocyte enhancer factor 2D (MEF2D), a transcription factor required for neuronal cell survival (*60*). Although the amount of MEF2D in the cytosolic fraction and total cell extract was increased by CMA inhibition, the level of MEF2D in the nucleus and the DNA-binding activity of the transcription factor decreased at the same time. Overexpression of α-synuclein reduced transcriptional activity of MEF2D and resulted in enhanced cell death. Thus, CMA contributes to the quality control of MEF2D and plays a role in the pathology of Parkinson's disease by linking α-synuclein degradation to MEF2D activity.

SEQUESTRATION INTO LARGE AGGREGATES OR INCLUSION BODIES

Many cytotoxic misfolded proteins form a large perinuclear aggregate (inclusion) called an aggresome (*61*). The formation of an aggresome is dependent on microtubules and dynein motors and occurs under the control of histone deacetylase 6 (*62*, *63*). There are two types of inclusion-like compartments that are distinct from each

other in their ubiquitination and aggregation state, which in turn suggests that the compartments have different roles in cytosolic protein quality control (*64*). A number of studies suggest that formation of aggresome or aggresome-like inclusion bodies has cytoprotective effects against the toxicity of misfolded proteins (*65*, *66*). Since misfolded proteins are cytotoxic due to their exposed hydrophobic surfaces, small species like misfolded monomers and small oligomers are considered to be more toxic than large aggregates or inclusions as described earlier. The highly toxic small species are thus transported to a perinuclear compartment for sequestration. To avoid confusion, aggregation should be divided into two categories: spontaneous aggregation from misfolded monomers to small soluble aggregates, and forced aggregation from small soluble aggregates to aggresome or inclusion bodies. The former is cytotoxic, but the latter is cytoprotective. Thus, the microtubule-dependent sequestration of misfolded soluble species is considered to be a protein quality control system for cell survival, although the fate of the sequestered proteins is poorly understood. The exact roles of the sequestration compartments should be revealed by future studies.

CONCLUSIONS

I have reviewed here the roles of molecular chaperones, co-chaperones, stress-sensing transcription factors, proteasomal degradation, autophagy-mediated lysosomal degradation and forced aggregation into inclusions in the quality control system operating in the cytosol. These systems each play complementary roles in dealing with toxic misfolded species at distinctive phases of the misfolding–aggregation pathway. Furthermore, these systems form a network for critically monitoring and preventing toxicity arising from protein misfolding. By the interaction and complementation of each of these individual systems, the cytosolic quality control system exerts maximal protection of cells against the toxicity of misfolded proteins.

FUNDING

Ministry of Education, Culture, Sports, Science and Technology of Japan (Grant-in-Aid for Scientific Research Programs); Japanese Society for the Promotion of Science.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Bukau, B., Weissman, J., and Horwich, A. (2006) Molecular chaperones and protein quality control. *Cell* **125**, 443–451
2. Saibil, H.R. (2008) Chaperone machines in action. *Curr. Opin. Struct. Biol.* **18**, 35–42
3. Hartl, F.U. and Hayer-Hartl, M. (2009) Converging concepts of protein folding in vitro and in vivo. *Nat. Struct. Mol. Biol.* **16**, 574–581
4. Vos, M.J., Hageman, J., Carra, S., and Kampinga, H.H. (2008) Structural and functional diversities between members of the human HSPB, HSPH, HSPA, and DNAJ chaperone families. *Biochemistry* **47**, 7001–7011
5. Kampinga, H.H., Hageman, J., Vos, M.J., Kubota, H., Tanguay, R.M., Bruford, E.A., Cheetham, M.E., Chen, B., and Hightower, L.E. (2009) Guidelines for the nomenclature of the human heat shock proteins. *Cell Stress Chaperones* **14**, 105–111
6. Wegele, H., Muller, L., and Buchner, J. (2004) Hsp70 and Hsp90–a relay team for protein folding. *Rev. Physiol. Biochem. Pharmacol.* **151**, 1–44
7. Pearl, L.H. and Prodromou, C. (2006) Structure and mechanism of the Hsp90 molecular chaperone machinery. *Annu. Rev. Biochem.* **75**, 271–294
8. Daugaard, M., Rohde, M., and Jaattela, M. (2007) The heat shock protein 70 family: Highly homologous proteins with overlapping and distinct functions. *FEBS Lett.* **581**, 3702–3710
9. Mayer, M.P. and Bukau, B. (2005) Hsp70 chaperones: cellular functions and molecular mechanism. *Cell Mol. Life Sci.* **62**, 670–684
10. Kubota, H. (2002) Function and regulation of cytosolic molecular chaperone CCT. *Vitam. Horm.* **65**, 313–331
11. Spiess, C., Meyer, A.S., Reissmann, S., and Frydman, J. (2004) Mechanism of the eukaryotic chaperonin: protein folding in the chamber of secrets. *Trends Cell Biol.* **14**, 598–604
12. Horwich, A.L., Fenton, W.A., Chapman, E., and Farr, G.W. (2007) Two families of chaperonin: physiology and mechanism. *Annu. Rev. Cell. Dev. Biol.* **23**, 115–145
13. Ecroyd, H. and Carver, J.A. (2009) Crystallin proteins and amyloid fibrils. *Cell Mol. Life Sci.* **66**, 62–81
14. Arrigo, A.P., Simon, S., Gibert, B., Kretz-Remy, C., Nivon, M., Czekalla, A., Guillet, D., Moulin, M., Diaz-Latoud, C., and Vicart, P. (2007) Hsp27 (HspB1) and alphaB-crystallin (HspB5) as therapeutic targets. *FEBS Lett.* **581**, 3665–3674
15. Haslbeck, M., Franzmann, T., Weinfurtner, D., and Buchner, J. (2005) Some like it hot: the structure and function of small heat-shock proteins. *Nat. Struct. Mol. Biol.* **12**, 842–846
16. Hayes, D., Napoli, V., Mazurkie, A., Stafford, W. F., and Graceffa, P. (2009) HSP27: phosphorylation dependence of its multimeric size and molecular chaperone function. *J. Biol. Chem.* **284**, 18801–18807
17. Nollen, E.A. and Morimoto, R.I. (2002) Chaperoning signaling pathways: molecular chaperones as stress-sensing 'heat shock' proteins. *J. Cell Sci.* **115**, 2809–2816
18. Summers, D.W., Douglas, P.M., Ramos, C.H., and Cyr, D.M. (2009) Polypeptide transfer from Hsp40 to Hsp70 molecular chaperones. *Trends Biochem. Sci.* **34**, 230–233
19. Qiu, X.B., Shao, Y.M., Miao, S., and Wang, L. (2006) The diversity of the DnaJ/Hsp40 family, the crucial partners for Hsp70 chaperones. *Cell Mol. Life Sci.* **63**, 2560–2570
20. Gamerdinger, M., Hajieva, P., Kaya, A.M., Wolfrum, U., Hartl, F.U., and Behl, C. (2009) Protein quality control during aging involves recruitment of the macroautophagy pathway by BAG3. *EMBO J.* **28**, 889–901
21. Polier, S., Dragovic, Z., Hartl, F.U., and Bracher, A. (2008) Structural basis for the cooperation of Hsp70 and Hsp110 chaperones in protein folding. *Cell* **133**, 1068–1079
22. Andreasson, C., Fiaux, J., Rampelt, H., Mayer, M.P., and Bukau, B. (2008) Hsp110 is a nucleotide-activated exchange factor for Hsp70. *J. Biol. Chem.* **283**, 8877–8884
23. Pratt, W.B. and Toft, D.O. (2003) Regulation of signaling protein function and trafficking by the hsp90/hsp70-based chaperone machinery. *Exp. Biol. Med. (Maywood)* **228**, 111–133
24. Lundin, V.F., Stirling, P.C., Gomez-Reino, J., Mwenifumbo, J.C., Obst, J.M., Valpuesta, J.M., and Leroux, M.R. (2004) Molecular clamp mechanism of substrate binding by hydrophobic coiled-coil residues of the

archaeal chaperone prefoldin. *Proc. Natl Acad. Sci. USA* **101**, 4367–4372
25. Morimoto, R.I. (2008) Proteotoxic stress and inducible chaperone networks in neurodegenerative disease and aging. *Genes Dev.* **22**, 1427–1438
26. Zou, J., Guo, Y., Guettouche, T., Smith, D.F., and Voellmy, R. (1998) Repression of heat shock transcription factor HSF1 activation by HSP90 (HSP90 complex) that forms a stress-sensitive complex with HSF1. *Cell* **94**, 471–480
27. Shi, Y., Mosser, D.D., and Morimoto, R.I. (1998) Molecular chaperones as HSF1-specific transcriptional repressors. *Genes Dev.* **12**, 654–666
28. Westerheide, S.D., Anckar, J., Stevens, S.M. Jr, Sistonen, L., and Morimoto, R.I. (2009) Stress-inducible regulation of heat shock factor 1 by the deacetylase SIRT1. *Science* **323**, 1063–1066
29. Ross, C.A. and Poirier, M.A. (2004) Protein aggregation and neurodegenerative disease. *Nat. Med.* **10 (Suppl)**, S10–S17
30. Chiti, F. and Dobson, C.M. (2006) Protein misfolding, functional amyloid, and human disease. *Annu. Rev. Biochem.* **75**, 333–366
31. Muchowski, P.J. and Wacker, J.L. (2005) Modulation of neurodegeneration by molecular chaperones. *Nat. Rev. Neurosci.* **6**, 11–22
32. Muchowski, P.J., Schaffar, G., Sittler, A., Wanker, E.E., Hayer-Hartl, M.K., and Hartl, F.U. (2000) Hsp70 and hsp40 chaperones can inhibit self-assembly of polyglutamine proteins into amyloid-like fibrils. *Proc. Natl Acad. Sci. USA* **97**, 7841–7846
33. Cummings, C.J., Sun, Y., Opal, P., Antalffy, B., Mestril, R., Orr, H.T., Dillmann, W. H., and Zoghbi, H.Y. (2001) Over-expression of inducible HSP70 chaperone suppresses neuropathology and improves motor function in SCA1 mice. *Hum. Mol. Genet.* **10**, 1511–1518
34. Kim, S., Nollen, E.A., Kitagawa, K., Bindokas, V.P., and Morimoto, R.I. (2002) Polyglutamine protein aggregates are dynamic. *Nat. Cell Biol.* **4**, 826–831
35. Schaffar, G., Breuer, P., Boteva, R., Behrends, C., Tzvetkov, N., Strippel, N., Sakahira, H., Siegers, K., Hayer-Hartl, M., and Hartl, F.U. (2004) Cellular toxicity of polyglutamine expansion proteins: mechanism of transcription factor deactivation. *Mol. Cell* **15**, 95–105
36. Auluck, P.K., Chan, H.Y., Trojanowski, J.Q., Lee, V.M., and Bonini, N.M. (2002) Chaperone suppression of alpha-synuclein toxicity in a Drosophila model for Parkinson's disease. *Science* **295**, 865–868
37. Wang, J., Farr, G.W., Zeiss, C.J., Rodriguez-Gil, D.J., Wilson, J.H., Furtak, K., Rutkowski, D.T., Kaufman, R.J., Ruse, C.I., Yates, J.R. 3rd, Perrin, S., Feany, M.B., and Horwich, A.L. (2009) Progressive aggregation despite chaperone associations of a mutant SOD1-YFP in transgenic mice that develop ALS. *Proc. Natl Acad. Sci. USA* **106**, 1392–1397
38. Wyttenbach, A., Sauvageot, O., Carmichael, J., Diaz-Latoud, C., Arrigo, A.P., and Rubinsztein, D.C. (2002) Heat shock protein 27 prevents cellular polyglutamine toxicity and suppresses the increase of reactive oxygen species caused by huntingtin. *Hum. Mol. Genet.* **11**, 1137–1151
39. Outeiro, T.F., Klucken, J., Strathearn, K.E., Liu, F., Nguyen, P., Rochet, J.C., Hyman, B.T., and McLean, P.J. (2006) Small heat shock proteins protect against alpha-synuclein-induced toxicity and aggregation. *Biochem. Biophys. Res. Commun.* **351**, 631–638
40. Waza, M., Adachi, H., Katsuno, M., Minamiyama, M., Tanaka, F., Doyu, M., and Sobue, G. (2006) Modulation of Hsp90 function in neurodegenerative disorders: a molecular-targeted therapy against disease-causing protein. *J. Mol. Med.* **84**, 635–646
41. Nollen, E.A., Garcia, S.M., van Haaften, G., Kim, S., Chavez, A., Morimoto, R.I., and Plasterk, R.H. (2004) Genome-wide RNA interference screen identifies previously undescribed regulators of polyglutamine aggregation. *Proc. Natl Acad. Sci. USA* **101**, 6403–6408
42. Behrends, C., Langer, C.A., Boteva, R., Bottcher, U.M., Stemp, M.J., Schaffar, G., Rao, B.V., Giese, A., Kretzschmar, H., Siegers, K., and Hartl, F.U. (2006) Chaperonin TRiC promotes the assembly of polyQ expansion proteins into nontoxic oligomers. *Mol. Cell* **23**, 887–897
43. Kitamura, A., Kubota, H., Pack, C.G., Matsumoto, G., Hirayama, S., Takahashi, Y., Kimura, H., Kinjo, M., Morimoto, R.I., and Nagata, K. (2006) Cytosolic chaperonin prevents polyglutamine toxicity with altering the aggregation state. *Nat. Cell Biol.* **8**, 1163–1170
44. Tam, S., Geller, R., Spiess, C., and Frydman, J. (2006) The chaperonin TRiC controls polyglutamine aggregation and toxicity through subunit-specific interactions. *Nat. Cell Biol.* **8**, 1155–1162
45. Ciechanover, A. and Brundin, P. (2003) The ubiquitin proteasome system in neurodegenerative diseases: sometimes the chicken, sometimes the egg. *Neuron* **40**, 427–446
46. Cyr, D.M., Hohfeld, J., and Patterson, C. (2002) Protein quality control: U-box-containing E3 ubiquitin ligases join the fold. *Trends Biochem. Sci.* **27**, 368–375
47. Dai, Q., Zhang, C., Wu, Y., McDonough, H., Whaley, R.A., Godfrey, V., Li, H.H., Madamanchi, N., Xu, W., Neckers, L., Cyr, D., and Patterson, C. (2003) CHIP activates HSF1 and confers protection against apoptosis and cellular stress. *EMBO J.* **22**, 5446–5458
48. Qian, S.B., McDonough, H., Boellmann, F., Cyr, D.M., and Patterson, C. (2006) CHIP-mediated stress recovery by sequential ubiquitination of substrates and Hsp70. *Nature* **440**, 551–555
49. Mizushima, N., Levine, B., Cuervo, A.M., and Klionsky, D.J. (2008) Autophagy fights disease through cellular self-digestion. *Nature* **451**, 1069–1075
50. Nakatogawa, H., Suzuki, K., Kamada, Y., and Ohsumi, Y. (2009) Dynamics and diversity in autophagy mechanisms: lessons from yeast. *Nat. Rev. Mol. Cell Biol.* **10**, 458–467
51. Rubinsztein, D.C. (2006) The roles of intracellular protein-degradation pathways in neurodegeneration. *Nature* **443**, 780–786
52. Ravikumar, B., Duden, R., and Rubinsztein, D.C. (2002) Aggregate-prone proteins with polyglutamine and polyalanine expansions are degraded by autophagy. *Hum. Mol. Genet.* **11**, 1107–1117
53. Ravikumar, B., Vacher, C., Berger, Z., Davies, J.E., Luo, S., Oroz, L.G., Scaravilli, F., Easton, D.F., Duden, R., O'Kane, C.J., and Rubinsztein, D.C. (2004) Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease. *Nat. Genet.* **36**, 585–595
54. Komatsu, M., Waguri, S., Chiba, T., Murata, S., Iwata, J., Tanida, I., Ueno, T., Koike, M., Uchiyama, Y., Kominami, E., and Tanaka, K. (2006) Loss of autophagy in the central nervous system causes neurodegeneration in mice. *Nature* **441**, 880–884
55. Hara, T., Nakamura, K., Matsui, M., Yamamoto, A., Nakahara, Y., Suzuki-Migishima, R., Yokoyama, M., Mishima, K., Saito, I., Okano, H., and Mizushima, N. (2006) Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. *Nature* **441**, 885–889
56. Komatsu, M., Waguri, S., Koike, M., Sou, Y.S., Ueno, T., Hara, T., Mizushima, N., Iwata, J., Ezaki, J., Murata, S., Hamazaki, J., Nishito, Y., Iemura, S., Natsume, T., Yanagawa, T., Uwayama, J., Warabi, E., Yoshida, H., Ishii, T., Kobayashi, A., Yamamoto, M., Yue, Z., Uchiyama, Y., Kominami, E., and Tanaka, K. (2007) Homeostatic levels of p62 control cytoplasmic inclusion body formation in autophagy-deficient mice. *Cell* **131**, 1149–1163

57. Kirkin, V., Lamark, T., Sou, Y.S., Bjorkoy, G., Nunn, J.L., Bruun, J.A., Shvets, E., McEwan, D.G., Clausen, T.H., Wild, P., Bilusic, I., Theurillat, J.P., Overvatn, A., Ishii, T., Elazar, Z., Komatsu, M., Dikic, I., and Johansen, T. (2009) A role for NBR1 in autophagosomal degradation of ubiquitinated substrates. *Mol. Cell* **33**, 505–516
58. Dice, J.F. (2007) Chaperone-mediated autophagy. *Autophagy* **3**, 295–299
59. Cuervo, A.M., Stefanis, L., Fredenburg, R., Lansbury, P.T., and Sulzer, D. (2004) Impaired degradation of mutant alpha-synuclein by chaperone-mediated autophagy. *Science* **305**, 1292–1295
60. Yang, Q., She, H., Gearing, M., Colla, E., Lee, M., Shacka, J.J., and Mao, Z. (2009) Regulation of neuronal survival factor MEF2D by chaperone-mediated autophagy. *Science* **323**, 124–127
61. Kopito, R.R. (2000) Aggresomes, inclusion bodies and protein aggregation. *Trends Cell Biol.* **10**, 524–530
62. Johnston, J.A., Illing, M.E., and Kopito, R.R. (2002) Cytoplasmic dynein/dynactin mediates the assembly of aggresomes. *Cell Motil. Cytoskeleton* **53**, 26–38
63. Kawaguchi, Y., Kovacs, J.J., McLaurin, A., Vance, J.M., Ito, A., and Yao, T.P. (2003) The deacetylase HDAC6 regulates aggresome formation and cell viability in response to misfolded protein stress. *Cell* **115**, 727–738
64. Kaganovich, D., Kopito, R., and Frydman, J. (2008) Misfolded proteins partition between two distinct quality control compartments. *Nature* **454**, 1088–1095
65. Arrasate, M., Mitra, S., Schweitzer, E.S., Segal, M.R., and Finkbeiner, S. (2004) Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. *Nature* **431**, 805–810
66. Tanaka, M., Kim, Y.M., Lee, G., Junn, E., Iwatsubo, T., and Mouradian, M.M. (2004) Aggresomes formed by alpha-synuclein and synphilin-1 are cytoprotective. *J. Biol. Chem.* **279**, 4625–4631