Non-cell Autonomous Maintenance of Proteostasis by Molecular Chaperones and Its Molecular Mechanism

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Molecular chaperones have essential roles in cell survival, to prevent misfolding, aggregation, and aberrant accumulation of cellular proteins, and thus to maintain protein homeostasis (proteostasis). However, recent studies using animal models suggest that transcriptional upregulation of molecular chaperones in response to various types of stresses does not ubiquitously occur in all cells and tissues, but is a cell type-specific event. The imbalanced response to stresses between cells and tissues has been pointed out since more than 30 years ago, but the molecular basis as to how organisms maintain proteostasis in all cells, especially cells deficient for chaperone induction, remains unknown. In this review, I introduce the non-cell autonomous function of molecular chaperones that has been suggested in animal studies, especially focusing on our recent findings, and discuss the possibility that the non-cell autonomous function might provide a potential explanation as to how organisms would maintain proteostasis despite the imbalanced stress response between cells and tissues. Further elucidation of the molecular basis underlying the non-cell autonomous function of molecular chaperones would provide not only better understanding as to how organisms maintain proteostasis but also important insights into the potential development of therapies and diagnostics for the currently intractable neurodegenerative diseases that are associated with protein misfolding and aggregation.

Key words molecular chaperone; proteostasis; exosome; non-cell autonomous; polyglutamine

1. INTRODUCTION

Proteins are one of the major components in living organisms, and thus quality control of cellular proteins over synthesis, folding, and degradation must be tightly regulated. It is known, however, that not all newly synthesized proteins in ribosomes spontaneously fold into correct conformations, and that, even if correctly folded, mature proteins are likely to become unfolded under stressful environments. Proteins with abnormal conformations not only may lose their cellular functions, but also are likely to assemble into insoluble aggregates, causing deleterious consequences including several diseases that are associated with protein misfolding and aggregation, such as Alzheimer’s disease, Parkinson’s disease, and the polyglutamine (polyQ) diseases.

For quality control of proteins, cells have an integrated protective machinery to prevent misfolding, aggregation, and aberrant accumulation of cellular proteins, and thus to maintain protein homeostasis (proteostasis). This machinery includes molecular chaperones, autophagy, and ubiquitin-proteasome systems, all of which are highly conserved in a wide range of eukaryotic organisms from yeast to mammals. Among them, molecular chaperones have essential roles not only in assisting folding of the newly synthesized or misfolded proteins, but also in mediating degradation of proteins with aberrant conformations through autophagy and proteasome systems.1) Most molecular chaperones are proteins that are inducible under stressful environments, where cellular proteins would likely be misfolded; in response to various types of stresses including heat, oxidative stress, and accumulation of misfolded proteins, expression of molecular chaperones such as heat shock protein 70 (Hsp70), Hsp90, and Hsp40 is transiently upregulated by activation of heat shock transcription factors (HSFs).2) These chaperones bind to unfolded or denatured conformations of cellular proteins to assist refolding to the native conformations, leading to prevention of aggregation and aberrant accumulation of proteins in cells. Deficiency for transcriptional upregulation of chaperone expression has been demonstrated directly linked to enhanced vulnerability to various types of stresses.

Although molecular chaperones are essential for maintenance of cellular proteostasis, there is accumulating evidence that stress-induced transcriptional upregulation of molecular chaperones in multicellular organisms does not ubiquitously occur in all cells and tissues, but is a cell type-specific event; some cells are apparently less or almost lacking such response under stressed conditions. The imbalanced response to stresses between cells and tissues has been pointed out since more than 30 years ago, but the molecular basis as to how organisms maintain proteostasis in all cells, especially cells deficient for chaperone induction, remains unknown. An alternative mechanism to compensate the absence of the protective response against proteotoxic challenges in a subpopulation of cells might exist at the multicellular organismal level.

In the following sections, I briefly summarize several studies on the imbalanced response of chaperone induction. Then I introduce our recent findings that molecular chaperones show the non-cell autonomous suppressive effect on protein ag-
This novel function of chaperones might provide a potential explanation, at least in part, as to how organisms maintain proteostasis despite the imbalanced stress response between cells and tissues, which will be discussed at the end of this article.

2. IMBALANCED UPREGULATION OF MOLECULAR CHAPERONES BY PROTEOTOXIC STRESSES

As mentioned above, transcriptional upregulation of molecular chaperones such as Hsp70 and Hsp40 is essential for cell survival, because they prevent misfolding and aggregation of cellular proteins that would be otherwise accumulated under stressed conditions. In 1987, Sprang and Brown reported, however, that hippocampal neurons in the brains of the heat-shocked rabbits show almost no increase in the level of Hsp70 mRNA, whereas fiber tracts and cerebellar neurons show vigorous expression of the same gene, demonstrating large differences in the extent of the heat stress-induced transcriptional upregulation between cells. Differential induction of Hsp70 and Hsp27 was also observed between tissues such as brain, liver, lung, and skin of rats exposed to elevated ambient temperatures, suggesting that the transcriptional induction of heat shock proteins in response to stresses differs not only at the cellular level, but also at the tissue level. Interestingly, Pardue et al. found that the level of Hsp70 expression in granule cells of dentate gyrus and pyramidal neurons of the heat-shocked rats, but not in glial cells, significantly decreases in aged rats, demonstrating that the imbalanced chaperone upregulation in heat-stressed animals is highly affected during the aging process. These studies suggest that transcriptional activation of chaperone expression in multicellular organisms is not a universal event, but largely dependent on cell types and tissues, and that some cells are almost lacking such response (Table 1).

| Tissues/cell lines | Hsp70 induction upon heat stress | Reference |
|-------------------|-------------------------------|-----------|
| Fiber tracts      | Highly activated              | 5         |
| Cerebellar neurons| Highly activated              |           |
| Hippocampal neurons| No induction                 |           |
| Dentate gyrus granule cells | Several-fold reduced  | 7         |
| Hippocampal pyramidal cells | Reduced               |           |
| Hippocampal glia  | Activated                     |           |
| Y79 retinoblastoma cells | No induction               | 8         |
| Mouse cell lines (SN6.1b, CL8c4.7, NSC34.6, B2A, C2C12) | Highly activated | 9         |
| Rat cell lines (PC12, C-6, L3) | Highly activated |           |
| Human cell lines (NB-1, GOTO, IMR-32, HeLa) | Highly activated |           |
| Mouse neuroblastoma cell line N18TG2 | No induction | 10        |
| Differentiated PC12 cells | No induction |           |
| Most astrocytes and microglia | Activated | 11        |
| Most oligodendrocytes and neurons | Low or no induction |           |
| Primary cultured rat hippocampal neurons | No induction | 48        |
| Secondary cultured rat glial cells | Highly activated |           |

Cell type-dependent chaperone induction has been observed not only in animal models, but also in primary cultures and cell lines. Satoh and Kim examined the cell type specificity of transcriptional response of heat shock genes using various types of cells isolated from fetal human brains, and found that Hsp70 expression upon heat stress is induced predominantly in astrocytes and glial cells, whereas oligodendrocytes and neurons show less or almost no response. A diminished expression of Hsp70 and other heat shock proteins has also been reported on primary cultures of cortical neurons and cerebellar granule cells, which may account for selective vulnerability of neuronal cells to stresses.

### Biography

Toshihide Takeuchi received his Ph.D. from Graduate School of Pharmaceutical Sciences, Kyoto University, under the supervision of Professor Shiroh Futaki, in 2008. After postdoctoral stays in the group of Professor Stefan Matile at University of Geneva, Switzerland, and in the group of Director Keiji Wada and Section Chief Yoshitaka Nagai at National Center of Neurology and Psychiatry, Japan, he became an Assistant Professor at Institute for Chemical Research, Kyoto University, in 2013, and then an Associate Professor at Osaka University Graduate School of Medicine in 2016. In 2017, he became a PRESTO researcher, Japan Science and Technology Agency (JST). His current research interest is to understand organismal regulation of protein homeostasis at the molecular level toward development of therapeutics and diagnostics for protein-misfolding neurodegenerative diseases.
Typical coordination of transcriptional upregulation among heat-inducible chaperones is apparently affected in some cells. In cultured cells, HSF1 activates the expression of most heat shock proteins simultaneously, leading to coordinate transcriptional activation of heat shock proteins under the stressed conditions. However, Blake et al. found that Hsp70 and Hsp27, which are typical heat-inducible chaperones, show different kinetics and expression levels between tissues of the same animal, and even between cells within the same tissue. This observation indicates that heat-induced response in vivo lacks coordinate control of chaperone expression that is characteristic of cell cultures in vitro, and implies an additional regulatory mechanism for chaperone induction at the organismal level. Mathur et al. reported that Y79 human retinoblastoma cells show almost no upregulation of Hsp70 expression upon exposure to heat stress, whereas HSF1 still activates expression of another heat shock protein Hsp90α. The selective absence of heat-induced upregulation of Hsp70 in Y79 cells is likely regulated at the level of chromatin structure; HSF1 and other transcription factors are not bound to the hsp70 promoter in Y79 cells, whereas an exogenous hsp70 promoter of a transfected vector is highly activated upon heat shock.

3. NON-CELL AUTONOMOUS EFFECT OF HEAT SHOCK PROTEINS IN VIVO

Previously, we explored the possibility of gene therapy using molecular chaperones for the polyglutamine (polyQ) diseases, a group of neurodegenerative diseases including Huntington’s disease (HD) and several types of spinocerebellar ataxias (SCA). Patients with polyQ diseases express proteins with an abnormally expanded polyQ tract, which are likely to undergo misfolding and assembling into insoluble aggregates, leading to accumulation as inclusion bodies in neurons and eventual neurodegeneration. Since molecular chaperones have essential roles in suppression of misfolding and aggregation of cellular proteins, activation of such protective machinery is expected to be an effective approach for development of disease-modifying therapies for the polyQ diseases. In cellular and animal models of polyQ diseases, overexpression of molecular chaperones including heat shock proteins (e.g., Hsp70 and Hsp40), co-chaperones (e.g., carboxy terminus of Hsp70-interacting protein; CHIP), and small heat shock proteins (e.g., HspB1, HspB7, and HspB8) has been shown to suppress inclusion body formation and improve typical disease-associated phenotypes including motor disturbance and shortened life span. In addition, pharmacological induction of multiple chaperones through activation of HSF1, a transcriptional factor that regulates expression of most heat shock proteins, using geldanamycin, radicicol, geranylgeranylacetone, and 17-(allylamo)-17-demethoxygeldanamycin (17-AAG), has also been demonstrated to inhibit polyQ aggregation, leading to therapeutic effects in vivo.

Fig. 1. Non-cell Autonomous Effect of Molecular Chaperones Observed in Drosophila

(A, B) When heat shock proteins such as Hsp40 and Hsp70 are expressed tissue-specifically in muscle or body fat of the fly models of polyglutamine (polyQ) diseases, polyQ protein-induced neurodegeneration in remote tissues (i.e., compound eyes) is suppressed in a non-cell autonomous manner.
and expression of molecular chaperones such as Hsp40 and Hsp70 in the same tissues leads to suppression of eye degeneration.\textsuperscript{19,36} Surprisingly, eye degeneration in this fly model was significantly improved when Hsp40 was ectopically expressed in muscle and fat body, which are different tissues from those expressing the polyQ proteins. Similarly, tissue-specific expression of Hsp70 in muscle and fat body led to improvement of eye degeneration, suggesting that expression of molecular chaperones in one tissue leads to the non-cell autonomous therapeutic effect on the other tissues. Since a deletion mutant of Hsp70 deficient for chaperoning activity did not show such improvement, the non-cell autonomous effect seems to require the chaperoning activity of heat shock proteins expressed in remote tissues.

The non-cell autonomous beneficial effect on organismal proteostasis caused by expression of molecular chaperones has recently been suggested by several groups. Morimoto and colleagues found that tissue-specific expression of Hsp90 in Caenorhabditis elegans results in suppression of protein misfolding in other tissue sites.\textsuperscript{37} The same group also reported that expression of expanded polyQ proteins in specific tissues of worms such as intestine and muscle results in local accumulation of misfolded polyQ proteins, which causes induction of molecular chaperones not only in the corresponding tissues, but also in the whole body of the animals. Dillin and colleagues reported that induction of mitochondrial stress by inhibiting a component of the respiratory chain in neurons of C. elegans results in systemic induction of mitochondrial chaperones.\textsuperscript{38} These data collectively indicate that local expression of molecular chaperones would have impact on proteostasis not only in the local areas, but also at the systemic level. It is noted that different groups have independently reported the non-cell autonomous effect of molecular chaperones using different species of animals, implying the possible existence of molecular mechanisms shared among multicellular organisms for the non-cell autonomous regulation of organismal proteostasis.

4. SECRETION OF HEAT SHOCK PROTEINS VIA EXOSOMES

Although the non-cell autonomous effect of molecular chaperones has been suggested, the molecular mechanism underlying such effect is almost unknown. Morimoto and colleagues proposed that the non-cell autonomous effect of Hsp90 expression in C. elegans occurs independently of neural activity and would be communicated to the other cells through unknown factors, which they call transcellular stress factors (TSFs), enabling non-cell autonomous regulation of proteostasis at the multicellular organismal level.\textsuperscript{\textcopyright{}2,39} Independently, we found that the non-cell autonomous effect of molecular chaperones can be observed even in in vitro co-culture experiments, where different cells are incubated separately across cell culture inserts.\textsuperscript{40} This observation led us to hypothesize that the non-cell autonomous effect of molecular chaperones is attributable to cell–cell communication without direct contact and therefore might be mediated by certain secretory factors released from chaperone-expressing cells.

We then explored the secretory factors responsible for the non-cell autonomous effect of molecular chaperones. Comprehensive analysis of cell culture media revealed that the chaperone-expressing cells actively release some heat shock proteins, such as Hsp70, Hsp90, and Hsp40, into the surrounding medium. This finding is in good agreement with previous studies that showed the potential release and transmission of a subset of stress-inducible proteins in response to stresses.\textsuperscript{41–43} Although an energy-dependent process is suggested involved in the extracellular secretion of these chaperones, the conventional secretory pathway through endoplasmic reticulum (ER)/Golgi apparatus seems not involved; these chaperones lack the signal sequence needed for conventional secretion, and treatment of brefeldin A, an inhibitor for such secretion pathway, did not affect chaperone secretion. On the other hand, an unconventional secretion pathway using exosomes, one of the extracellular vesicles secreted from cells, has been recently reported.\textsuperscript{44} Analysis of the exosome fractions, which are isolated from the cell culture media by ultracentrifugation, revealed that those fractions contain heat shock proteins such as Hsp70, Hsp40, and Hsp90, indicating that chaperone-expressing cells actively secrete heat shock proteins via the exosome-mediated secretion pathway (Fig. 2). It is noted that not all heat shock proteins are secreted via exosomes. As for Hsp40 family proteins, the proteins that are localized in cytosol were detected in the exosome fractions, whereas other proteins localized in ER and mitochondria were not detected in such fractions. This observation suggests that among heat shock proteins, at least cytosolic ones share the characteristic of exosome-mediated secretion, implying that there should be

Fig. 2. Non-cell Autonomous Suppression of Protein Aggregation via Exosome-Mediated Transmission of Molecular Chaperones

(A, B) Molecular chaperones are secreted from cells via exosomes and transmitted to other cells, where they suppress aggregation formation of misfolding proteins in a non-cell autonomous manner.
a molecular machinery that enables selective secretion of a subset of molecular chaperones via exosomes.

The molecular basis as to how heat shock proteins are secreted via exosomes remains unclear. Functional analysis focusing on the domain structure of Hsp40 demonstrates that secretion of Hsp40 requires its amino-terminus domain called J domain, which is a highly conserved domain shared among Hsp40 family proteins. It has been reported that Hsp40 interacts with Hsp70 through J domain, which facilitates chaperoning activity of Hsp70 machinery.43 Interestingly, RNA interference (RNAi)-mediated knockdown of Hsc70, a Hsp70 family protein that is constitutively expressed, significantly reduces the amounts of extracellularly secreted Hsp40, suggesting that Hsp40 secretion is regulated by Hsc70, possibly through interaction via J domain.

5. NON-CELL AUTONOMOUS EFFECT OF HEAT SHOCK PROTEINS VIA EXOSOME-MEDIATED INTERCELLULAR TRANSMISSION

It has been suggested that exosomes that are secreted from cells contain various cellular proteins, nucleic acids, and metabolites, and deliver these molecules to other cells through intercellular transmission, leading to the non-cell autonomous regulation of cellular functions.44,45 The exosome-mediated secretion of molecular chaperones led us to hypothesize that extracellularly secreted chaperones would be transferred to other cells, where they may exert chaperoning activity to improve the folding environment in a non-cell autonomous manner. We confirmed intercellular transmission of exosomal chaperones in cell culture experiments, where they were internalized into the recipient cells and distributed around the nucleus, which is suggestive of endocytosis-dependent internalization. Furthermore, addition of exosomes to cell culture models of polyQ diseases expressing the aggregation-prone polyQ proteins led to significant suppression of aggregation formation of the polyQ proteins, demonstrating that exosome transmission indeed impacts on proteostasis in the recipient cells.46 Since our data show that the extent of suppressive effect on polyQ aggregation in the recipient cells is positively correlated with the amounts of heat shock proteins in the exosomes, molecular chaperones would conceivably be one of the responsible factors for exosome-mediated suppression of aggregation formation among molecules associated with exosome vesicles. We also examined whether exosomal transmission would be involved in the non-cell autonomous effect of molecular chaperones in vivo. As described above (section 3), in Drosophila flies expressing polyQ proteins in the compound eyes, tissue-specific expression of heat shock proteins such as Hsp40 and Hsp70 in muscle and fat body suppressed eye degeneration. We found that RNAi-mediated knockdown of Ykt6, a R-SNARE protein necessary for exosome secretion in Drosophila, dramatically cancels this non-cell autonomous improvement of the eye phenotype.47 Therefore Ykt6-dependent exosomal transmission may be at least one of the molecular bases for non-cell autonomous suppression of eye degeneration by remote tissue-specific expression of Hsp40 in Drosophila. These observations collectively indicate that molecular chaperones are transmitted from cells to cells via exosomes and suppress aggregation formation of aggregation-prone proteins in other cells, leading to non-cell autonomous maintenance of organismal proteostasis (Fig. 2).

6. PERSPECTIVES

Here we reviewed the imbalanced response of chaperone expression, and introduced current understanding of the non-cell autonomous effect of molecular chaperones, especially based on our recent studies. Although intercellular transmission of molecular chaperones via the exosome-mediated pathway may be the mechanistic basis for the non-cell autonomous beneficial effect of chaperones in multicellular organisms, several key questions remain elusive: how is a subset of chaperones selectively sorted and loaded on exosomes? Are there any targeting mechanisms that would regulate delivery of chaperone-containing exosomes to specific tissues? How do the exosomal chaperones become functional in recipient cells after endocytosis-mediated internalization? Further elucidation of the molecular mechanism underlying exosome-mediated chaperone transmission would lead to better understanding as to how organisms maintain proteostasis using exosomes.

Stress-induced transcriptional upregulation of molecular chaperones such as heat shock response is in general believed a protective response that would be initiated independently by cells exposed to proteotoxic stresses. However, our data show that exosome-mediated secretion of molecular chaperones is significantly activated by heat shock, and increased secretion of chaperones from donor cells leads to enhanced suppressive effect on aggregation formation in recipient cells.43 This indicates that heat stress-induced response at the multicellular organismal level is not simply transcriptional upregulation of molecular chaperones in individual cells; cells also activate exosome-mediated secretion of chaperones for the non-cell autonomous improvement of protein-folding environment in neighboring recipient cells. Surprisingly, we found that addition of exosomes isolated from other chaperone-expressing cells results in suppression of inclusion body formation even in HSF1-knockout cells, which are deficient for chaperone induction.44 This indicates that cells lacking chaperone induction, which are otherwise quite vulnerable to stresses, can be protected by exosome-mediated transmission of molecular chaperones from other heat-responsive cells. We propose that exosome-mediated chaperone transmission may be one of the compensatory mechanisms for imbalanced transcriptional upregulation of molecular chaperones between cells and tissues in multicellular organisms, although further studies are needed better to understand this unique mode of organismal regulation for proteostasis maintenance.

Since molecular chaperones suppress misfolding and aggregation of cellular proteins, much attention has been paid to their therapeutic potential for neurodegenerative diseases that are associated with protein misfolding, such as Alzheimer’s disease, Parkinson’s disease, and polyQ diseases. Our data show that remote tissue-specific expression of heat shock proteins such as muscle and fat body suppresses neurodegeneration in the compound eyes in Drosophila.45 This result encourages us to consider that activation of exosome-mediated secretion of molecular chaperones in peripheral tissues might improve the protein-folding environment at the systemic level, even in brains, which provides a novel therapeutic approach for neurodegenerative diseases; the permeability of exosomes through blood–brain barrier is, however, still debated. On the
other hand, exosomes and extracellular vesicles have recently attracted much attention for their potential applications to diagnostic biomarkers for diseases such as cancers and immune disorders. Since significant alteration in expression levels of molecular chaperones has been reported not only in animal models of neurodegenerative diseases but also in patient brains, the levels of exosomal chaperones are expected to be affected in such diseases. Comprehensive analysis of exosome-associated molecules, especially focusing on molecular chaperones, would provide a platform for potential development of diagnostic markers for protein-misfolding neurodegenerative diseases.

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Conflict of Interest The author declares no conflict of interest.

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