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Review

Nucleocytoplasmic transport under stress conditions and its role in HSP70 chaperone systems

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ABSTRACT

Background: In eukaryotic cells, molecular trafficking between the nucleus and cytoplasm is a highly regulated process related to cellular homeostasis and cellular signaling. However, various cellular stresses induce the perturbation of conventional nucleocytoplasmic transport pathways, resulting in the nucleocytoplasmic redistribution of many functional proteins.
Scope of review: We describe the recent insights into the mechanism and functions of nuclear import of cytosolic chaperone HSP70 under stress conditions and the cellular distribution and functions of its co-chaperones.
Major conclusions: Hikeshi mediates the nuclear import of the molecular chaperone HSP70. A few of the regulators of the HSP70 chaperone system also accumulate in the nucleus under heat stress conditions. These proteins function collaboratively to protect cells from stress-induced damage and aid in the recovery of cells from stress.
General significance: Studies on the regulation of nucleocytoplasmic transport under several cellular stresses should provide new insights into the fundamental principles of protein homeostasis (proteostasis) in both compartments, the nucleus and cytoplasm.

1. Basic mechanisms of nucleocytoplasmic transport

1.1. The nuclear pore complex (NPC) acts as a gate between the nucleus and cytoplasm

The regulation of nucleocytoplasmic transport through nuclear pore complexes (NPCs) is crucial for various cellular functions in eukaryotic cells. Small molecules (<approximately 40 kDa), ions, and metabolites can diffuse passively through the NPC following a concentration gradient, whereas larger macromolecules are generally transported by specific transport carriers. The NPC is embedded into the double lipid membrane of the nuclear envelope and is composed of multiple copies of about 30 different proteins called nucleoporins (Nups) [1–5]. The vertebrate NPC is a huge structure of approximately 125 MDa and provides a large aqueous channel (40–50 nm in diameter) between the nucleus and cytoplasm [6,7]. The central channel of the NPC is filled by FG-nucleoporins (FG-Nups), which contain distinct domains with many phenylalanine–glycine (FG) repeats separated by charged or polar spacer amino acids. The FG repeat domains are unstructured or natively unfolded [8] and are thought to contribute to the highly selective permeability of the NPC (reviewed in [9]). In fact, facilitated selective transport requires specific interactions between the import or export complexes and these hydrophobic clusters for rapid NPC passage (reviewed in [10]).

1.2. The Importin β family: nucleocytoplasmic transport carriers

The selective nucleocytoplasmic transport of proteins is mediated by specific amino acid sequences, which are referred to as the nuclear localization signal (NLS) and the nuclear export signal (NES). These NLS or NES-containing proteins are recognized by carrier molecules and are translocated through the NPC. Members of the Importin β family, referred to as importins, transportins, exportins, or karyopherins, are the best-characterized nucleocytoplasmic carrier molecules and are thought to mediate most of the selective nucleocytoplasmic protein transport (reviewed in [10–12]). The classical NLS contains one or two clusters of basic amino acids and was first characterized as a signal for nuclear import; proteins containing an NLS are transported into the nucleus by an Importin α/β heterodimer [13–18]. The leucine-rich NES was first identified as a signal for nuclear export, and proteins containing an NES are transported into the cytoplasm by Exportin 1 (Crm1) [19–21]. However, the classical NLS- or leucine-rich NES-mediated pathway is not the only route for nucleocytoplasmic transport in the cell. For example, more than 20 members of the Importin β family carrier proteins (reviewed in [22–24]) and 7 members of the Importin α family in human cells [25] have been identified, showing that human

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cells contain diverse multiple transport pathways that mediate the exchange of macromolecules between the nucleus and the cytoplasm.

Importin β family carriers interact with their specific cargos, FG-Nups, and the small GTPase Ran. Importin β family carriers allow NPC transit via specific interactions with Nups (mainly FG-Nups) and are able to shuttle continuously between the cytoplasm and the nucleus [26–31]. All of the transport pathways mediated by the Importin β family carriers are coupled with the GTPase cycle of Ran [32,33]. Cargo-bound Importins translocate through the NPC from the cytoplasm to the nucleus and bind to GTP-bound Ran (RanGTP) in the nucleus, resulting in the release of cargo from the Importins (carriers). The Importins, bound to RanGTP, then return to the cytoplasm and dissociate from Ran when RanGTP is hydrolyzed into GDP form. In contrast, Exportins are complexed with cargo and RanGTP in the nucleus and then translocated through the NPC to the cytoplasm. This trimeric Exportin/RanGTP/cargo complex dissociates in the cytoplasm upon GTP-hydrolysis of Ran.

In this way, the GTPase cycle of Ran plays a crucial role in ensuring the directionality and driving force of Importin β-mediated trafficking. Therefore, the guanine nucleotide state of Ran in the cell is strictly regulated by various interacting proteins, mainly the guanine nucleotide exchange factor of Ran (RanGEF) and the GTPase-activating protein of Ran (RanGAP). Regulator of chromosome condensation 1 (RCC1), RanGEF, is a chromatin-binding protein and localized in the nucleus and promotes the conversion of RanGDP to RanGTP [34,35]. Mammalin RanGAP is modified with the small ubiquitin-like protein, SUMO-1, and predominantly localized at nuclear envelope. Modification of RanGAP with SUMO-1 is required for association with Ran-binding protein 2 (RanBP2), which localizes to the cytoplasmic fibrils of the NPC [36,37]. In addition, RanBP1, which enhances the GTPase activity of Ran mediated by RanGAP [38], is localized to the cytoplasm because RanBP1 possesses a nuclear export signal [39]. Therefore, RanGTP is rapidly hydrolyzed in the cytoplasm. Cytoplasmic RanGDP is efficiently recycled back into the nucleus by another carrier protein called p10/NTF2 [40,41]. Based on these results, RanGTP exists mainly in the nucleus, and a sharp concentration gradient of RanGTP is formed at the boundary of nuclear envelope, allowing cargo to accumulate in one compartment against the chemical concentration gradient.

2. Environmental stresses affect nucleocytoplasmic transport

A variety of cellular stresses induce the nucleocytoplasmic redistribution of various functional proteins and the perturbation of conventional nucleocytoplasmic transport pathways. Down-regulation of the classical Importin α/β-mediated pathway under stress conditions is well characterized. Cellular stresses, such as heat shock, oxidative stress, and UV irradiation, induce nuclear retention and inhibit the nuclear export of Importin α, the classical nuclear localization signal (NLS) receptor, resulting in the suppression of the Importin α- and β-dependent classical import pathway [42–44]. More critically, these cellular stresses have been reported to induce the perturbation of the RanGTP gradient between the nucleus and the cytoplasm [42,43,45–47]. Ran is localized primarily in the nucleus. However, a significant proportion of endogenous Ran is observed in the cytoplasm in response to cellular stresses. Hydrogen peroxide induces the activation of mitogen-activated protein (MAP) kinase [45] and also decreases the intracellular level of ATP [48], resulting in the alteration of the cellular distribution of Ran. The use of a fluorescence resonance energy transfer (FRET) biosensor of Ran revealed that the hyperosmotic stress signaling to the nucleus disrupts the intracellular Ran gradient and the production of RanGTP [47]. Since all members of the Importin β family are RanGTP-binding proteins, the perturbation of endogenous Ran distribution and biogenesis induced by cellular stresses might lead to the strong down-regulation of all Importin β family-mediated nucleocytoplasmic pathways. However, it is important to note that even nuclear import of the classical NLS-mediated pathway is not completely arrested under stress conditions. The issues of how the other Importin β family mediated-nucleocytoplasmic transport pathways are regulated under stress conditions should be investigated more intensively.

The physical changes of the NPC should significantly affect the trafficking of molecules between the cytoplasm and the nucleus. Cellular stress often activates the extracellular signal-regulated kinase (ERK) mitogen-activated protein (MAP) kinase pathway and also induces changes in the cellular distribution of transport factors and the efficiency of nucleocytoplasmic transport as a result of protein post-translational modification [45,47,49,50], although most of the target molecules and the precise mechanisms underlying these effects are currently unknown. Recently, phosphoproteomics with a combination of the estrogen receptor fusion system revealed that ERK MAP kinase directly phosphorylates FG-Nups, such as Nup50, Nup153, and Nup214 [50]. The phosphorylation of FG-Nups reduces the affinity of these factors for the Importin β family molecules, such as Importin β and Transportin, and results in the impairment of nuclear migration of Importin β and Transportin. Further, it was revealed that oxidative stress induces the formation of intermolecular disulfide bonds between nucleoporins [51]. This disulfide bond formation within the NPC affects the Importin β-mediated nuclear import pathway directly. The modification of the Nup proteins may be an important regulatory process for nucleocytoplasmic traffic, affecting both carrier-mediated transport and passive diffusion.

3. The cytosolic HSP70 molecular chaperone system

Cellular protein homeostasis (proteostasis) systems, also known as protein quality control (PQC), play an essential role in all stages of cells. A variety of cellular stresses induce protein misfolding, protein dysfunction, and non-specific protein aggregation (reviewed in [52–54]). This protein damage occurs in various cellular compartments, leading to lethal effects. To maintain and restore proteostasis, many molecular chaperones are rapidly expressed in response to environmental stresses. The 70-kDa heat shock protein and the constitutive heat shock cognate proteins Hsp70/Hsc70 (HSP70) belong to a major family of molecular chaperones involved in highly conserved protective systems (reviewed in [55,56]). HSP70 proteins interact with unfolded or misfolded proteins and function in de novo protein folding and the suppression of protein aggregation under several stress conditions. The human genome contains 13 Hsp70 (HSPA family) proteins, most of which localize primarily to the cytoplasm under normal conditions (Table 1) [57,58].

HSP70 consists of an N-terminal nucleotide-binding domain (NBD) and a C-terminal polypeptide substrate-binding domain (SBD), which are connected by a conserved linker domain (reviewed in [59]). The nucleotide state of HSP70 is crucial for the binding and release of their polypeptide-substrates. ATP-bound form of HSP70 has low affinity with substrate, and ATP hydrolysis of HSP70 accelerates the strong binding of substrate to ADP-bound form of HSP70. Because the intrinsic ATPase hydrolysis rates of HSP70 are low, HSP70 proteins generally do not work alone as chaperones. Two of the most important co-chaperones, the J domain-containing proteins (the HSP40 family) and the nucleotide exchange factors (NEFs), are generally required for the function of the HSP70 chaperone system. The hydrolysis of ATP-bound HSP70 is strongly accelerated by HSP40, and HSP70 can then bind tightly to its substrates. Further, the release of ADP and substrates from HSP70 is accelerated by various NEFs (such as the HSP110/HSPH family and the BAG family), which catalyze the ADP–ATP exchange of HSP70. Recent studies on the structure of the *Escherichia coli* Hsp70 homolog DnaK have shown that ATP-bound HSP70 induces an allosteric open conformation of the C-terminal polypeptide substrate-binding domain (SBD), resulting in a low-affinity of HSP70 for polypeptide substrate and substrate-release [60,61].

In addition to the 13 HSPA proteins, approximately 50 HSP40 (DNAJA, DNAJB, DNAJC) family proteins, 4 HSPH proteins, and 7 BAG proteins have been reported in humans (reviewed in [62–64]). Most

Table 1
Cytosolic and nuclear HSP70s and its co-chaperones (J domain proteins and NEFs).

					Under heat stress condition	
Gene name [62][63]	Human ID	also known as	MW (kDa)	localization [57][64]	localization change	heat-inducible expression [112]
HSP70s						
HSPA1A	3303	HSP70-1; HSP72; HSPA1	70.0	Cytoplasm	→ Nucleus [77-79]	
HSPA1B	3304	HSP70-2	70.0	Cytoplasm	→ Nucleus [77-79]	
HSPA1L	3305	hum70t; hum70t; Hsp-hom	70.4	Cytoplasm		
HSPA2	3306	Heat-shock 70kD protein-2	70.0	Cytoplasm		
HSPA6	3310	Heat-shock 70kD protein 6 (HSP70B′)	71.0	Cytoplasm/Nucleus		
HSPA8	3312	HSC70; HSC71; HSP71; HSP73	70.9	Cytoplasm	→ Nucleus [77-79]	
HSPA14	51182	HSP70-4; HSP70L1; MGC131990	54.8	Cytoplasm		
J domain proteins						
DNAJA1	3301	DJ-2; DjA1; HDJ2; HSDJ; HSJ2; HSPF4; hDJ-2	44.9	Cytoplasm		
DNAJA2	10294	DNJ3; mDj3; Dnaj3; HIRIP4	45.7	Cytoplasm		
DNAJA4	55466	Dj4; Hsj4	44.7	Cytoplasm		
DNAJB1	3337	HSPF1; HSP40	38.2	Cytoplasm/Nucleus	→ Nucleus [92-94]	
DNAJB2	3300	HSJ1; HSPF3; Dnajb10; MDJ8	35.6/30.6	Cytoplasm		
DNAJB4	11080	Hsc40	37.8	Cytoplasm/Nucleus		
DNAJB5	25822	Hsc40; HSP40-3	39.1/26.9	Cytoplasm		
DNAJB6	10049	Mrj; mDj4	36.1	Cytoplasm		
DNAJB7	150353	Dj5; mDj5	35.4	Nucleus		
DNAJB8	165721	mDj6	25.7	Cytoplasm		
DNAJC2	27000	Zrf1; Zrf2; MIDA1; MPP11; zuotin; ZUO1	72.0	Cytoplasm/Nucleus		
DNAJC7	7266	Ttc2; mDj11; mTpr2	56.4	Cytoplasm		
DNAJC14	85406	HDJ3; LIP6; DRIP78	78.6	Nucleus		
DNAJC28	54943	Orf28 open reading frame 28; C21orf55, oculomedin	45.8	Nucleus		
DNAJC29	26278	Sacsin; SACS	521.1	Nucleus		
NEFs (HSP110&BAG)						
HSPH1	10808	HSP105	96.9	Cytoplasm/Nucleus	→ Nucleus (HSPH1β) [95][97]	
HSPH2	3308	HSPA4, APG-2, HSP110	94.3	Cytoplasm/Nucleus		
HSPH3	22824	HSPA4L, APG-1	94.5	Cytoplasm/Nucleus		
BAG1	573	RAP46, HAP46, HAP50	38.9	Nucleus (Bag1L)		
BAG2	9532		23.8	Cytoplasm	→ Nucleus [108]	
BAG3	29810	CAIR-1, Bis	61.6	Cytoplasm		
BAG4	9530	Sodd	49.6	Cytoplasm	→ Nucleus [107]	
BAG6	7917	Scythe, BAT3	118.7	Nucleus		

Heat-inducible expression in HeLa cells after 1 h at 43 °C was measured by RNA-seq. Red, more than 10-fold expression; orange, 3–10-fold; and green, less than 3-fold [112].

of these co-chaperones also localize to the cytosol and nucleus (Table 1) [58,65].

Tetratricopeptide repeat (TRP) domain-containing proteins bind to the highly conserved C-terminal EEVD motif of HSP70 and HSP90 and function as co-chaperones [66–68]. HOP (Hsp70 and Hsp90 organizing protein) contains three TRP domains that interact with the C-terminal EEVD motif of HSP70 and HSP90; through these interactions, HOP links these two chaperones and modulates their activity [69] (reviewed in [70]). HOP is predominantly a cytoplasmic protein, but a small nuclear fraction and leptomycin B-sensitive nuclear accumulation has been observed [71,72], suggesting that the subcellular distribution of HOP is regulated by the balance of nucleocytoplasmic import and export.

CHIP (the C-terminus of Hsc70-interacting protein) is a dimeric protein containing an N-terminal TRP domain and a C-terminal U box domain (reviewed in [73]). CHIP functions as an E3 ligase that interacts with HSP70 and HSP90 via its TRP domain, facilitating the ubiquitylation of substrate proteins bound to chaperones. Therefore, CHIP provides a direct link between the chaperone and ubiquitin–proteasome systems. Furthermore, CHIP regulates the amount of HSP70 proteins by targeting this chaperone to the ubiquitin–proteasome system during the process of recovery from stress, when the unfolding of HSP70 substrates is decreased in the cells [74]. The C-terminal phosphorylation of HSP70 and HSP90 causes reduced binding of HSP70 and HSP90 to CHIP and enhanced binding to HOP [75]. Therefore, posttranslational modifications of chaperones may be critical for the regulation of the balance between protein folding and degradation. Although CHIP is localized to the cytoplasm and the cytoplasmic surface region of the endoplasmic reticulum under normal conditions [76], transient accumulation and punctate foci formation of CHIP in the nucleus have been observed under heat stress conditions [77].

4. Nuclear import of HSP70 under stress conditions

4.1. The Hikeshi-mediated nuclear import pathway

In response to heat shock, Hsc70 and Hsp70 (HSPA8/HSPA1) are rapidly and transiently relocated from the cytoplasm into the nucleus and nucleolus [78–80]. Recently, we identified a new transport pathway that functions under heat shock stress conditions and is mediated by a novel carrier protein named Hikeshi [81] (reviewed in [82]) (Fig. 1). Hikeshi does not belong to the Importin β family but is evolutionarily conserved from yeast to mammals.

Like all known nucleocytoplasmic carrier molecules, Hikeshi must possess the ability to translocate through the NPC on its own to be a nucleocytoplasmic carrier. When examined in the permeabilized semi-intact cells [83], GFP-fused Hikeshi accumulated in the nucleus on its own in the absence of soluble factors and ATP. Furthermore, Bead Halo and pull-down assays showed that Hikeshi interacts weakly, but significantly, with FG-Nups, such as Nup62 and Nup153. These results suggest that Hikeshi indeed possess the ability of nucleocytoplasmic carrier protein.

However, unlike the Importin β family molecules, Hikeshi does not bind to Ran. Therefore, the interactions between Hikeshi and cargo molecules must be regulated by another system. Hikeshi-mediated transport is coupled to the ATPase cycle of the molecular chaperone Hsp70. It is conceivable that this ATPase cycle serves as the driving

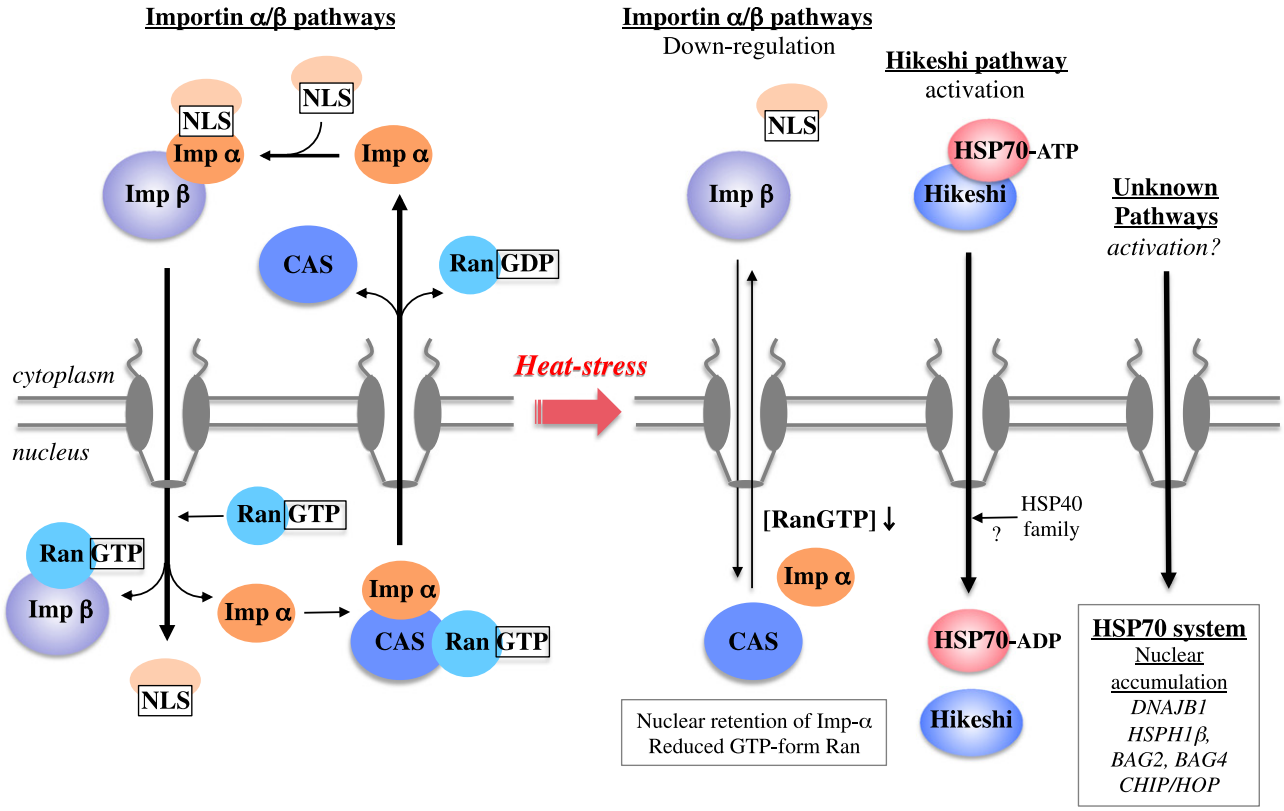

Fig. 1. Nucleocytoplasmic transport under normal and heat stress conditions. Among the Importin β-family-mediated nucleocytoplasmic transport, the Importin α/β-mediated nuclear protein transport is best-characterized. Proteins containing the classical nuclear localization signal (NLS) are transported into the nucleus by an Importin α/β heterodimer. Nuclear RanGTP binds to Importin β and then promotes the dissociation of NLS-substrate from an Importin α/β heterodimer. Importin β bound RanGTP returns to the cytoplasm, and dissociates from Ran, coupled with GTP-hydrolysis of Ran. Importin α is complexed with RanGTP and CAS, which is an export carrier in the Importin β family, in the nucleus, and then returns to the cytoplasm. This export complex also dissociates in the cytoplasm upon GTP-hydrolysis of Ran. Cellular stresses, such as heat shock, induce nuclear retention of Importin α and the perturbation of the RanGTP gradient between the nucleus and cytoplasm, resulting in the suppression of the Importin α/β-dependent pathway. On the other hand, Hikeshi-mediated nuclear import pathway for molecular chaperone HSP70 is up-regulated under heat stress conditions, and then HSP70 is accumulated in the nucleus, although cytosolic HSP70 is mainly localized in the cytoplasm under normal conditions.

force for Hikeshi-mediated transport. Hikeshi has a higher affinity for the ATP-bound HSP70 than the ADP-bound HSP70. In fact, Hsp40 molecules (DNAJB1, DNAJA1), that accelerate the ATP hydrolysis of HSP70 inhibit the interaction between Hikeshi and HSP70. Therefore, Hikeshi supports the nuclear import of ATP-bound HSP70 more efficiently than that of ADP-bound HSP70. Although the detailed mechanism of the interaction between Hikeshi and HSP70 is still unresolved, Hikeshi does not bind to HSP70s fragment containing either the NBD or the SBD alone, suggesting that Hikeshi may recognize the whole allosteric conformation change resulting from ATP/ADP exchange of HSP70 (Fig. 2).

The identification of this new nuclear import pathway mediated by Hikeshi provides a significant advance in our understanding of the regulatory mechanisms for nucleocytoplasmic transport. However, how the Hikeshi-mediated pathway is activated under stress conditions remains unclear. It has been reported that phosphorylation at tyrosine-524 of HSP70 influences the nuclear accumulation of HSP70 and cell viability under heat stress [84]. A pseudo-phosphorylated mutant of HSP70 accumulates more efficiently in the nucleus under heat stress than wild type HSP70 and suppresses cell death from heat damage. Our preliminary analysis showed that kinase inhibitors, such as staurosporine, do not affect the Hikeshi-mediated nuclear import of HSP70, at least in an in vitro reconstituted transport assay. However, there is the possibility that various posttranslational protein modifications of HSP70 and Hikeshi (including phosphorylation) are involved in the activation of Hikeshi-mediated nuclear import under stresses. Further, HSP70 possesses a putative leucine-rich NES sequence, which is likely to be recognized by Exportin-1/Crm1 [85], although the functional details of this NES-like sequence on subcellular localization of HSP70 remains obscure. The mechanism of HSP70 for nuclear export may be functional for cytoplasmic localization or re-localization of HSP70 during recovery to stresses. How the balance between conventional and stress-dependent nucleocytoplasmic transport and the dynamics of this transport are maintained in cells in response to several stimuli is an important question for future investigation.

4.2. The physiological significance of the Hikeshi-mediated nuclear import pathway

Hikeshi is a nuclear import carrier that operates in response to heat shock, which is the survival of cells after heat shock stress. Hikeshi-depleted cells have decreased cell viability after heat shock stress and show a slower recovery from heat shock-induced nuclear phenotypes [81].

The expression of HSP genes in eukaryotes is regulated mainly by the activation of heat shock factor 1 (HSF1) (reviewed in [86,87]). During recovery from stress, the activity of HSF1 is down-regulated. HSP70 also interacts directly with the transactivation domain of HSF1 and represses heat shock gene transcription [88]. However, in Hikeshi-depleted cells, although HSF1 is rapidly activated in response to heat stress, the activity of HSF1 and the formation of nuclear stress granules (nSGs) [89,90] are sustained for a significantly longer time compared to normal cells. The nucleolus shows dynamic changes in its organization and composition during cellular stresses, and these changes affect ribosome biogenesis and DNA metabolism (reviewed in [91]). Nucleolar-localized proteins such as nucleolin disperse transiently in the nucleus during heat stress. However, in Hikeshi-depleted

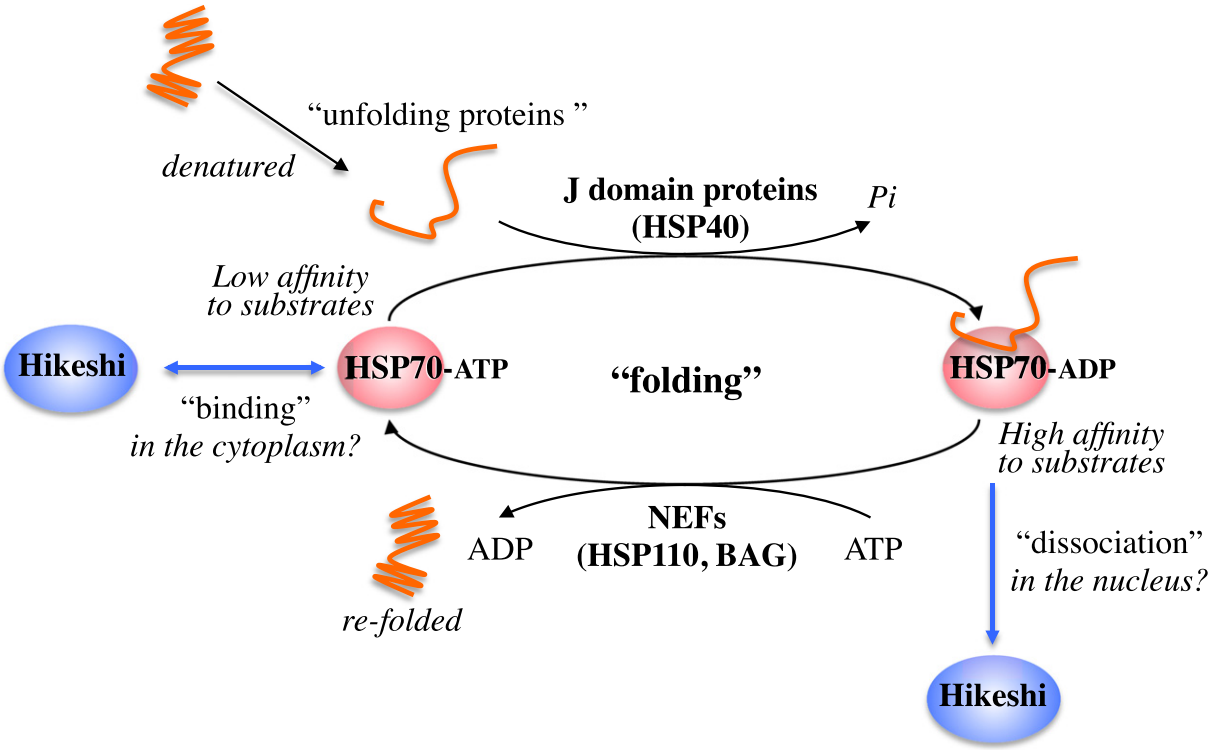

Fig. 2. The HSP70 chaperone system and the regulated interaction between HSP70 and Hikeshi. Protein re-folding process by HSP70 system generally requires from HSP70, J-domain proteins (HSP40) and nucleotide exchange factors (NEFs) (HSP110/BAG). J-domain proteins associate unfolding proteins and stimulate the ATPase activity of HSP70, resulting that HSP70 binds to unfolding substrates tightly. NEFs induce the nucleotide exchange of HSP70, and then the substrate is released from HSP70, presumably as a re-folded form. Interaction between Hikeshi and HSP70 is also regulated by J-domain proteins and NEFs. Hikeshi binds to ATP-form HSP70 with high affinity, and ADP-form HSP70 with low affinity, possibly dissociating from HSP70.

cells, the relocation of nucleolin from the nucleus to the nucleolus is also strongly inhibited during recovery from stress. A dynamics analysis of heat-unfolded nuclear luciferase showed that HSP70 is involved in the binding and refolding of heat-unfolded nuclear proteins and translocates these proteins to the nucleolus during stress [92], suggesting that HSP70 may prevent the random expansion of unfolded protein aggregates within the nucleus. Such a role of HSP70 may also be inhibited in the Hikeshi-depleted cells under heat stress conditions.

These phenomena show that Hikeshi is required to protect cells from heat shock damages and is required for the attenuation and reversion of multiple heat-shock-induced nuclear phenotypes. Because the exogenous expression of nuclear HSP70 suppresses, at least in part, the lethal damage due to heat stress in the Hikeshi-depleted cells, the nuclear function of both Hikeshi and HSP70 is likely to be important for cells to survive after heat stress.

5. The subcellular distribution and function of the HSP70 chaperone systems

Heat stress apparently affects multiple cellular biogenesis pathways localized to the cytoplasm and cytoplasmic cell organelles and to the nucleus and nucleolus. Experiments using subcellular targeting of luciferase suggested that the nucleus is the most sensitive organelle to heat-induced protein unfolding [93]. After the rapid translocation of HSP70 from the cytoplasm to the nucleus under heat stress, HSP70 is thought to function in the folding and refolding of denatured and aggregated proteins. Mammalian cells contain the highly diverse members of the HSP70 chaperone system, HSP70 and its co-chaperones, most of which are localized to the cytoplasm and/or nucleus. However, in contrast to the cytosolic function of HSP70, the nuclear functions and detailed molecular mechanisms of the HSP70 chaperone system in the nucleus are still unknown.

Among the J domain-containing proteins, DNAJA1 protein mainly localizes to the cytoplasm under both normal and heat-stress conditions [94]. However, the DNAJB1 protein is translocated to and accumulates in the nucleus and nucleolus under heat-stress conditions [94-96], and some members of the DNAJB family are also localized to the nucleus [58].

The NEFs of HSP70, HSPH1 and HSPH2 are localized to both the nucleus and cytoplasm [58,97,98]. Additionally, one splicing variant (Hsp110β) of HSPH1/Hsp110α is induced under mild heat shock and accumulates in the nucleus [97,99]. The conserved Bag domain is sufficient for HSP70-binding and regulation [100-106]. BAG1L, the longest form of BAG1, contains a nuclear localization signal (NLS) [107]. BAG6 is reported to contain a functional NLS and is localized to the nucleus [108]. Furthermore, BAG2, as well as CHIP (carboxyl terminus of Hsc70-interacting protein), and BAG4 are translocated into the nucleus under heat stress (Table 1) [109,110].

As described above, many of the regulators on the HSP70 chaperone system are localized to the nucleus under normal conditions, and a few of the regulators of this system are known to accumulate in the nucleus under heat stress. After HSP70 accumulates in the nucleus through the action of Hikeshi, HSP70 is likely to function as a molecular chaperone in the nucleus and nucleolus for cell survival. The molecular mechanisms of ubiquitylation and proteasomal proteolysis in the nucleus of mammalian cells remain to be clarified. Intriguingly, the cytosolic E3 ligase CHIP and the Hop (Hsp70/Hsp90 organizing) protein, both of which are HSP70 co-chaperones, also accumulate in the nucleus in response to heat stress [72]. In addition to the refolding of unfolded and aggregated proteins, proteasomal proteolysis coupled with the HSP70 chaperone system is likely to be important in the restoration of normal cellular conditions and cell viability. Protein aggregates interfere with the clearance of unfolded proteins and disturb cellular protein quality control. Recently, it was reported that Sis1p in yeast and its homolog DNAJB1 in mammalian cells mediate the delivery of polyQ

aggregates into the nucleus for proteasomal degradation [111]. Further functional analyses of chaperones and proteolysis coupled with nucleocytoplasmic transport systems will contribute to the discovery of new important aspects in the protein quality control network.

6. Perspectives

An understanding of the basic mechanism for nucleocytoplasmic transport and the identification of Hikeshi mediated-nuclear import of HSP70 under stress conditions provides new insights into the regulation of nucleocytoplasmic transport and the nuclear function of the HSP70 chaperone system. The discovery of the nuclear import pathway of HSP70 provided a significant advance in our understanding of the nuclear functions and targets of HSP70 for cell recovery from thermal stress damage. Nevertheless, there are still many unresolved questions. How the Hikeshi-mediated import pathway is activated under stress conditions and how the HSP70 chaperone system functions efficiently in the nucleus are still unanswered. How the Ran-GTP/GDP and HSP70-ATP/ADP ratios are changed and regulated in heat-shocked cells is also an important issue for understanding nucleocytoplasmic transport switching and HSP70 functions. Why is HSP70 absent from the nucleus under normal conditions, and what distinguishes the function of a great variety of co-chaperones in the HSP70-assisted refolding or proteolysis process? It will be important to analyze the molecular mechanism underlying the cellular distribution of the diverse members of the HSP70 system and other chaperone systems in more detail. Further studies on the regulation of nucleocytoplasmic transport under several cellular stresses should provide new insights into the fundamental principles of proteostasis in each subcellular compartment.

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