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# Multifaceted Role of Heat Shock Protein 70 in Neurons

**Tom Z. Lu · Yi Quan · Zhong-Ping Feng**

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**Abstract** Heat shock protein 70 (Hsp70) plays important roles in neural protection from stress by assisting cellular protein folding. In this review we discuss the current understanding of inducible and constitutive Hsp70 in maintaining and protecting neuronal synaptic function under normal and stressed conditions.

**Keywords** Hsc70 · Hsp40 · HSPA · Synapse · Calcium · Neuronal protection

## Introduction

Heat shock protein (Hsp) is a family of proteins that were originally found to respond to heat shock stimuli [1] but also associated with other cellular stressors such as alcohol [2–4], oxidants [4–7], heavy metals [8–10], amino acid analogues [11, 12], hypoxia [13, 14] and glucose deprivation [15, 16]. Hsp family proteins are in general molecular chaperones that serve cytoprotective roles by maintaining or assisting cellular protein folding. Although Hsp proteins come from diverse families and differ in their structures and cellular functions [17], they all contain a highly conserved heat shock element (HSE) in the promoter region of their corresponding genes [18]. Hsp proteins are subgrouped based on their molecular weights. The structural features and functional roles are highly conserved within the members of each subfamily but not observed among members of different subfamilies [19].

Hsp70, a subgroup of heat shock proteins with the molecular weight of 70 kDa, is the most ubiquitous Hsp subgroups, and structurally and functionally highly conserved across species. Under physiological conditions, Hsp70s serve as molecular chaperones. The major constitutive isoform of Hsp70, heat shock cognate 70 (Hsc70), has the ability to bind to exposed hydrophobic surfaces of various substrates and prevent nonproductive protein–protein interactions that would lead to aggregation, and it promotes protein refolding.

Under stressed conditions, upregulation of Hsps, such as the inducible Hsp70, confers protection at all levels from the whole organism [20], specific organs [21], cells [22] to specific organelles [23]. Increasing Hsp expression level by overexpression [24], drug induction [21], genetic manipulation [25] and preconditioning [20, 26–28] protects the cells/organisms from lethal conditions. The mechanisms underlying the cytoprotective effects of heat shock proteins, specifically the Hsp70s, have been well delineated. Hsp70 expression reduces protein aggregation, maintains mitochondria physiology, inhibits apoptosis/necrosis and suppresses inflammatory responses [29].

Of great importance is the role of neuronal Hsps at the synapse, a site that is sensitive to stress and prone to stress-induced damage. This minireview highlights the distinct functional roles of inducible and constitutive isoforms of the 70-kDa Hsp proteins at the neuronal synapse both under physiological conditions and in response to stress.

## Structural Features of Hsp70s

Similar to other *Hsp*s [30, 31], *Hsp*70s genes encode the HSE in their promoter region. The HSE consists of three

Tom Z. Lu and Yi Quan contributed equally to the manuscript.

T. Z. Lu · Y. Quan · Z.-P. Feng (✉)
Department of Physiology, University of Toronto,
3306 MSB, 1 King's College Circle,
Toronto, Ontario, Canada M5S 1A8
e-mail: zp.feng@utoronto.ca

contiguous inverted repeats of the A/GGAAn sequence; each repeat interacts with one of the trimeric heat shock transcription factor (HSF) [32]. The number of pentameric repeats varies greatly among different heat shock proteins. For example, the promoter of *Hsp*40 has eight contiguous sequences [33], *Hsp*70 has five and *Hsp*90 has six such sequences [34]. Additional pentameric sequences results in greater affinity interactions between HSFs and HSE [35]. Hsp70s have three structural domains (Fig. 1a); a 44-kDa N-terminal domain with ATPase activity (NBD), an 18-kDa substrate binding domain (SBD) and a 19-kDa C-terminal domain (CTD) that assists with substrate binding [36–38]. The concerted actions of all three domains are required for Hsp70 function. For example, the binding affinity of the SBD is dependent on the state of the NBD, such that there is increased substrate affinity when Hsp70 is adenosine diphosphate (ADP)-bound and decreased affinity when it is adenosine triphosphate (ATP)-bound [39].

The gene sequences of Hsp70s are highly conserved from bacteria to human. The human hsp70-gene family is evolutionarily diverse [40]. There are at least 14 isoforms of Hsp70 in humans encoded by 17 different genes [40, 41] (Table 1). There are another 30 sequences coded as hsp70 pseudogenes, structurally characterized with variations including frame shifts and in-frame stop codons [40]. The hsp70 genes are located on 18 chromosomes except 15–17, 19 and 22 [40, 41]. The classification and localization of the identified Hsp70s are summarized in Table 1. The mitochondrial Hsp70 (Hsp70-9; GRP75) is encoded by a gene at chromosome segment 5q31.1 [42, 43]. The gene for ER homologue of Hsp70 (GRP78) [44] is localized to human chromosome 9q34 [45]. The major constitutively active form of Hsp70, Hsc70, is encoded by a gene located on chromosome segment 11q24 [46]. Hsc70 is abundantly expressed under physiological conditions and is not significantly heat inducible. Its major functions include protein folding and translocation, clathrin uncoating and peptide binding to prevent aggregation [47]. A number of Hsp70s are stress-inducible (Table 1). The major inducible Hsp70s (or Hsp72) are encoded by HSP1A and HSP1B [48, 49], which are located on chromosome segment 6p21.3 [50, 51]. The expression level of these Hsp70s is minimal under normal conditions but is greatly upregulated after exposure to stress such as hypoxia, ischemia, hyperthermia and toxins [52]. Most genes encoding Hsp70 do not contain introns; an exception is found in the case of Hsc70 and Grp78, offering the possibility of alternative splicing [41].

## Cofactors of Hsp70s

Two main cofactors have been identified for Hsp70s, namely, Hsp40s [33] and nucleotide exchange factors (NEFs) [53]. Hsp40s are homologues of the bacterial DnaJ [33], which generally contains three distinct domains

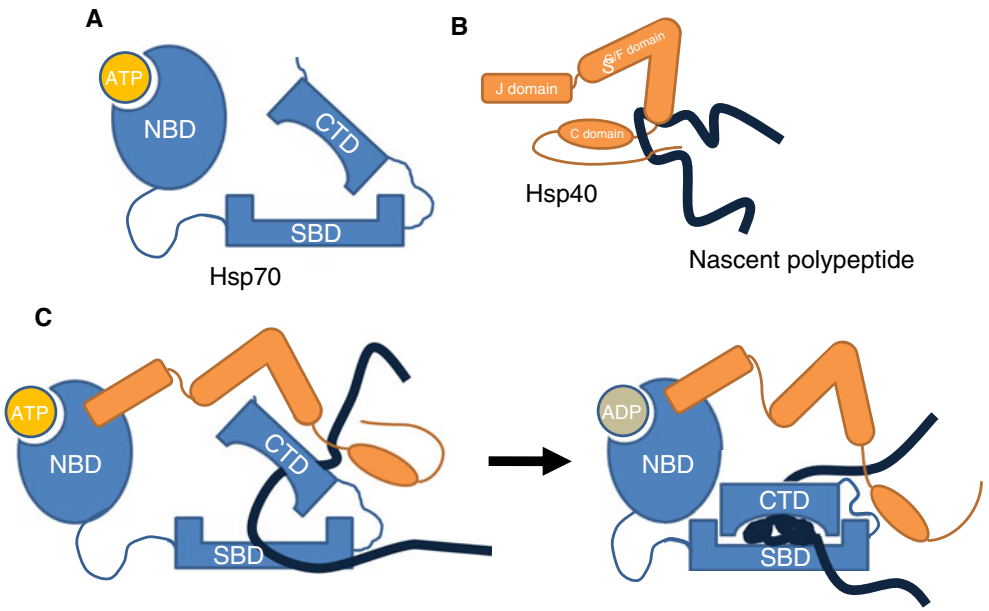


**Fig. 1** Schematic illustration of Hsp70 and Hsp40 complex. **a** Hsp70 has three major functional domains. The NBD has ATPase activity and drives the conformational change that allows its interaction with the two other domains. The SBD contains the substrate affinity site and CTD forms the lid on top of the SBD. **b** Three structural domains of Hsp40: the J domain consisting of histidine, proline and aspartic acid motif, the glycine/phenylalanine-rich G/F domain, and cysteine-rich region of the C domain. Hsp40 interacts with nascent peptide via a peptide-binding site located on the C-terminal. **c** The peptide-bound Hsp40 is anchored to Hsp70 through interaction between the G/F domain of Hsp40 with a C-terminus sequence of the CTD of Hsp70. The J domain of Hsp40 interacts with the NBD domain of Hsp70 to increase ATPase activity

**Table 1** Classification of human heat shock proteins

| Gene | Approved names | Alternative/synonyms | Localization | Stress inducible | References |
|---|---|---|---|---|---|
| HSPA1A | Heat shock 70 kDa protein 1A | Hsp70-1 | Nucleus, cytoplasm, liposome | Y | Wu et al [49] Mol Cell Biol 5:330-341; Milner and Campbell [48] Immunogenetics 32:242-251. |
| HSPA1B | Heat shock 70 kDa protein 1B | Hsp70-2 | Nucleus, cytoplasm, lysosome | Y | Wu et al [49] Mol Cell Biol 5:330-341; Milner and Campbell [48] Immunogenetics 32:242-251. |
| HSPA1L | Heat shock 70 kDa protein 1-like | Hsp70-HOM, Hum70t | Nucleus, cytoplasm | N | Goate et al. [50] Hum Genet 75:123-128. |
| HSPA2 | Heat shock 70 kDa protein 2 | | Nucleus, cytoplasm | N | Bonnycastle et al. (1994) Genomics 23: 85-93. |
| HSPA4 | Heat shock 70 kDa protein 4 | Hsp70, RY | Cytoplasm | Y | Kang CM et al (2002) Radiat Res 157: 650-655. |
| HSPA4L | Heat shock 70 kDa protein 4 L | APG-1, Osp94 | Cytoplasm | Y | Held et al. (2006). Mol Cell Biol 26: 8099-8108. |
| HSPA5 | Heat shock 70 kDa protein 5 | BiP, GRP78 | ER | N | Munro and Pelham [44] Cell 46: 291-300. |
| HSPA6 | Heat shock 70 kDa protein 6 | HSP70B' | Nucleus, cytoplasm | Y | Leung et al. [5] Biochem J 267: 125-132. |
| HSPA7 | Heat shock 70 kDa protein 7 | HSP70B | Nucleus cytoplasm | Y | Leung et al. [5] Biochem J 267: 125-132; Siddiqui et al. (2008) Cancer Invest 26: 553-561. |
| HSPA8 | Heat shock 70 kDa protein 8 | Hsc71, Hsc70, Hsc73 | Nucleus, cytoplasm | N | Dworniczak and Mirault (1987) Nucleic Acids Res 15: 5181-5197. |
| HSPA9 | Heat shock 70 kDa protein 9 | GRP75, PBP74, mot-2, mtHSP75 | Mitochondria | N | Domanico et al. [43] Mol Cell Biol 13: 3598-3610; Bhattacharyya et al. [42] J Biol Chem 270:1705-1710. |
| HSPA12A | Heat shock 70 kDa protein 12A | FLJ13874, KIAA0417 | Nucleus, cytoplasm | – | Pongrac et al (2004) Biol Psych 56:943-950 |
| HSPA13 | Heat shock 70 kDa family, member 13 | STCH | ER | N | Yamagata et al (2008) Biochem Biophys Res Commun 376:499-503. |
| HSPA14 | Heat shock 70 kDa protein 14 | Hsp70-4, Hsp70L1 | Cytoplasm | Y | Wan et al (2004) Blood 103:1747-1754; Otto et al (2005) Proc Natl Acad Sci U S A 102:10064-10069. |

(Fig. 1b). (1) A highly conserved 70-amino-acid J domain near the N-terminus. The J domain consists of a highly conserved sequence of histidine, proline and aspartic acid (HPD motif); it mediates the interaction between Hsp40 and Hsp70 and stimulates the ATPase activity of Hsp70. (2) A glycine/phenylalanine (G/F) rich region acting as a flexible linker. (3) A cysteine-rich region near the C-domain containing four CXXCXGXG motifs. All DnaJ homologues contain the N-terminal J domain and either one or both the other two domains [54]. Based on this variability, DnaJ homologues are classified into three groups. Type I homologues contain all three domains. Type II homologues have the J and G/F domain but lack the C domain. Type III Hsp40s contain only the J domain. The human Hsp40 is a type II homologue [54].

There are 41 DnaJ homologues identified in mammals, which greatly outnumbers the amount of Hsp70s [55]. Thus, it is suggested that each Hsp70 chaperone complex consists of multiple Hsp40s [33]. Functionally, Hsp40s have no chaperone activity, but their intracellular localization may determine the functional specificity of each Hsp70/Hsp40 chaperone complex [55]. The substrate polypeptides first bind to Hsp40, and this complex then interacts with Hsp70/ATP [53]. The substrate is transferred to the open peptide-binding cleft of Hsp70 and locked in by subsequent Hsp40 stimulated hydrolysis of ATP (Fig. 1c). For example, the simian virus 40 virus large T antigen protein contains a J domain that recruits Hsp70 to drive mitosis [56]. Similarly, the Hsp40 homologue in yeast Sec63 recruits Hsp70 to the ER through its J domain to assist in protein unfolding prior to membrane transport [57]. NEFs [53] regulate the rate ADP/ATP exchange and serve as an important regulatory point for Hsp70 function [52]. Depletion of NEFs Fes1 or Sse 1 in *Saccharomyces cerevisiae* impairs prion propagation. The prolonged binding between Hsp70 and its substrate in the absence of NEFs prevents the protein from reverting back to its prion state [52].

## Hsc70 at the Synapse

Under physiological conditions, the Hsc70/Hsp40 complex plays a prominent role in maintaining and regulating various components of the synapse. Presynaptically, Hsc70 regulates the N-type calcium channels, the rate of synaptic vesicle recycling and the rate of neurotransmitter synthesis and packaging. The potential mechanisms of Hsc70 involvement at the synapse are proposed in Fig. 2 (endocytotic mechanisms) and Fig. 3 (exocytotic mechanisms).

Hsc70 is not a native protein at the synapse but is recruited there by several DnaJ homologues (Hsp40s) that are natural residents of the presynaptic terminal. One such protein is the cysteine string protein (CSP) [58]. CSP is mainly expressed in the presynaptic terminal of neurons and other secretory cells, where it is involved in exocytosis of neurotransmitters, hormones and enzyme precursors. CSP knockout (KO) flies show impaired presynaptic neuromuscular transmission with loss of synaptic vesicles [59]. CSP is a type III Hsp40 protein and is composed of three domains [58]; it has an N-terminus containing a J domain (residues 1–82) that allows binding to Hsc70, a 30-residue (83–112) linker region followed by the C-terminus (residues 113–198) region. The C-terminus is the least conserved region among all three CSP isoforms and is believed to be responsible for its flexibility and diversity in cellular processes. The Hsc70/CSP complex forms a trimer with a third protein, the small glutamine-rich tetratricopeptide repeat protein (SGT) that binds to the C-terminus of CSP [60]. SGT contains three tandem tetratricopeptide repeat domains (TPRs) with a degenerate 34-amino-acid sequence [58]. The complex is further stabilized by the interactions between Hsc70 and SGT, mediated by the binding between the TPR domain of SGT with the C-terminus of Hsc70 [60]. Binding of the protein complex is dependent on the presence of ADP, while ATP promotes the disassembly of the complex. This complex formation stimulates the ATPase activity of Hsc70 by 19 folds and thus greatly enhances its protein refolding abilities on the synaptic vesicle surface [58].

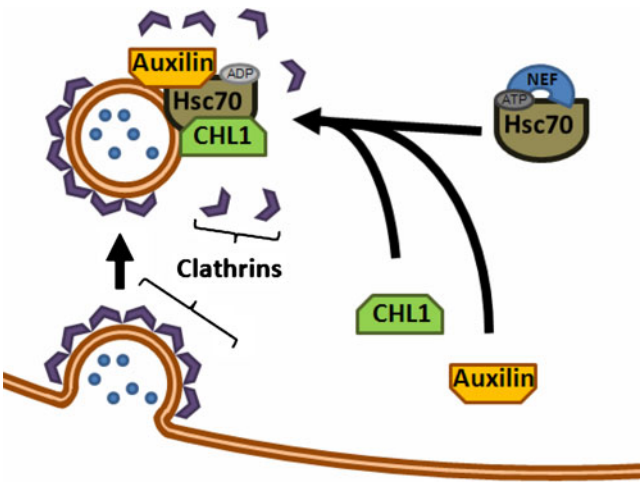


**Fig. 2** Schematic of Hsc70 associated proteins in endocytosis. Cytosolic Hsc70 is targeted to the synapse by CHL1 and auxilin. Upon binding, the CHL1/Hsc70 or the auxilin/Hsc70 complex stimulates the clathrin decoating activity of Hsc70, greatly enhancing synaptic recycling and thus increasing the number of available synaptic vesicles for subsequent exocytotic events. *ADP* adenosine diphosphate; *ATP* adenosine triphosphate; *CHL1* close homolog of L1; *Hsc70* heat shock protein cognate 70; *NEF* nucleotide exchange factor

### *Inhibition of N-Type Calcium Channel*

G-proteins are heterotrimeric GTP-binding proteins composed of α, β and γ subunits [61]. Activation by exogenous ligand results in exchange of GDP for GTP on $G_\alpha$ subunit and dissociation of $G_{\beta\gamma}$ subunit from the trimeric G-protein complex. This step is regulated by the Hsc70/CSP/SGT trimer ([61]; see also [62]). Full-length CSP protein interacts with $G_\alpha$ and increases the steady-state GTP hydrolysis, and the rate of hydrolysis is further increased in the presence of the Hsc70/SGT. The N-terminal (residues of CSP1–112) of CSP alone can bind to the GDP-bound inactive form of $G_\alpha$ and stimulate GDP/GTP exchange in a manner that is unaffected by the presence or absence of Hsc70/SGT. Thus, it was proposed that the C-terminal of CSP (residues 112–192) is a regulatory domain that inhibits the activity of CSP and this inhibition is abolished by the binding of Hsc70/SGT [61].

The two activated subcomplexes, $G_\alpha$ and $G_{\beta\gamma}$, have distinct cellular functions [63]. The $G_\alpha$ subunit binds to the N-terminal J domain of CSP in an ATP-dependent manner, whereas the $G_{\beta\gamma}$ subunits associate with the C-terminus of CSP independently of ATP. The complex of CSP and $G_{\beta\gamma}$ subunits binds to the $\alpha_{1B}$ subunit of N-type calcium channels, resulting in tonic inhibition of calcium influx. The association of either $G_\alpha$ or $G_{\beta\gamma}$ to CSP can cause N-type calcium channel inhibition via different mechanisms. Inhibition of calcium channel by CSP requires direct physical binding of $G_{\beta\gamma}$ subunit to CSP C-terminus. Interaction between $G_\alpha$ subunit and CSP J domain, on the other hand, does not require this direct interaction. This association is thought to inhibit the assembly of the trimeric G-protein, freeing the $G_{\beta\gamma}$ subunits to interact with CSP C-terminus, thus leading to inhibition of N-type calcium channel activity [63]. Hsc70, in conjunction with CSP and SGT, promotes the activity of G proteins with the end effect of tonic inhibition of N-type channels. In the presence of Hsc70 and SGT, CSP is activated and promotes the GDP/GTP exchange on $G_\alpha$ subunit, releasing the $G_{\beta\gamma}$ subunit to inhibit N-type calcium channels.

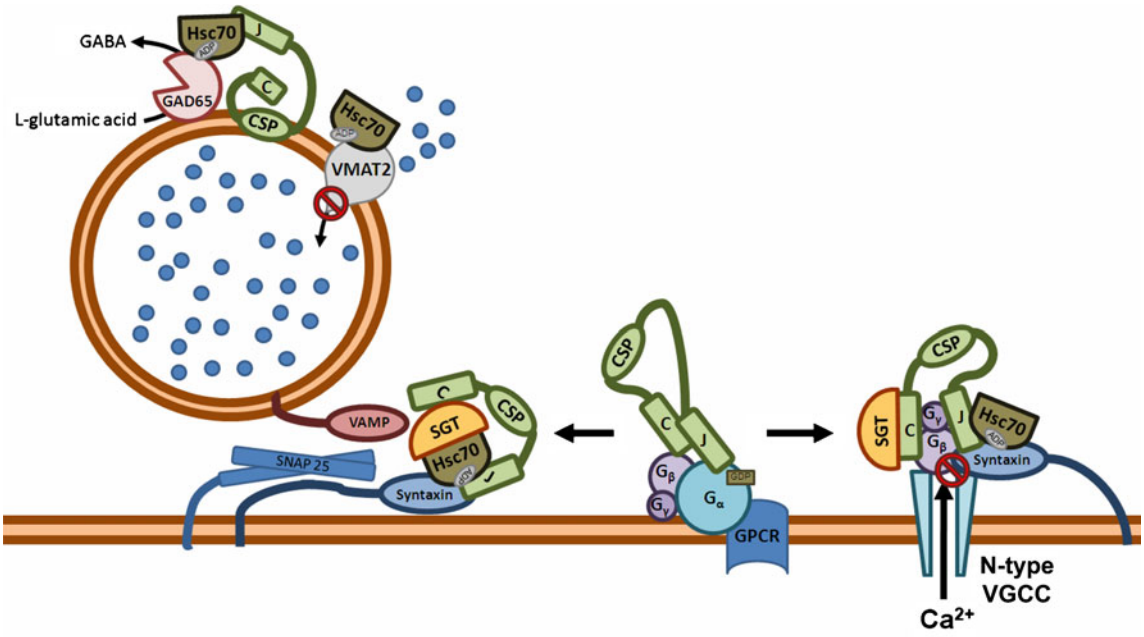


**Fig. 3** Schematic of Hsc70-associated proteins in exocytosis. Hsc70/CSP/SGT trimer catalyzes the steady-state GTP hydrolysis on $G_{\alpha}$, thereby promoting the dissociation of the trimeric G-protein complex into the $G_{\alpha}$ and $G_{\beta\gamma}$ subunits. Subsequent association of $G_{\beta\gamma}$ with CSP tonically inhibits calcium influx through N-type calcium channels. Hsc70/CSP complex also anchors GAD65 to the synaptic vesicle and stimulates GAD65 activity, thereby promoting synthesis of GABA. Lastly, Hsc70 interacts with and inhibits the activity VMAT2. *ADP* adenosine diphosphate; *ATP* adenosine triphosphate; *C* C-terminus on CSP; *CSP* cysteine string protein; *GDP* guanosine diphosphate; *GPCR* G-protein coupled receptor; *GTP* guanosine triphosphate; *Hsc70* heat shock protein cognate; *J* J domain on CSP; *N-type VGCC* N-type voltage gated calcium channel; *SGT* small glutamine-rich tetratricopeptide; *VAMP* vesicle-associated membrane protein (synaptobrevin); *VMAT2* vesicle monoamine transporter 2; *GAD65* L-glutamic acid decarboxylase 65

Syntaxin 1A, a tSNARE protein, is critical for neurotransmitter release. Syntaxin 1A incorporation into the SNARE complex is required for vesicle fusion and exocytosis. Syntaxin 1A associates with Hsc70, CSP and N-type calcium channels [64] and binds to the $\alpha_{1B}$ subunit of N-type calcium channels, in the same region that CSP binds. Its specific function in this complex has yet been determined. The association between syntaxin and Hsc70 has been shown to promote syntaxin incorporation into the SNARE complex [64]. Thus, it appears that the Hsc70/syntaxin complex performs antagonistic roles at the synapse. Syntaxin and Hsc70, as part of the multimeric complex with CSP, SGT and G proteins, inhibit calcium influx through N-type calcium channels [64] and thus may prevent exocytosis. This may serve as a negative feedback mechanism to control synaptic transmission.

*Synaptic Recycling—CHL1 and Auxilin*

Hsc70 at the synapse is involved in clathrin uncoating during endocytosis [65]. Synaptic vesicles, upon releasing their content at the synaptic cleft, is internalized via formation of clathrin-coated pits on the synaptic plasma membrane, which leads to formation of clathrin-coated vesicles (CCVs). Removal of clathrin from vesicles is an essential process for repackaging synaptic vesicles and for subsequent exocytotic events [66]. Hsc70 binds to clathrin molecules and catalyze uncoating of clathrin from CCVs, but this proceeds at a low rate. *CHL1* is a cell adhesion molecule of the immunoglobulin superfamily. It normally accumulates in presynaptic membranes and contains an HPD tripeptide sequence (J domain) in its intracellular C-terminal, which allows interaction with Hsc70 [67]. It is proposed that upon synaptic activation (depolarization), CHL1 recruits Hsc70 to the synaptic membrane from the cytosol and forms a dimeric complex in an ADP-dependent manner. Binding with CHL1 enhances the uncoating ability of Hsc70 [67] and thus aids in synaptic recycling. Neurons from CHL1 KO mice show decreased level of Hsc70 at the synapse, an increase in CCVs and decreased number of available synaptic vesicles in subsequent exocytotic events [67]. CHL1 KO mice show reduced synaptic plasticity and mental retardation [68]. In humans, this gene has been linked to schizophrenia, which is characterized by abnormal synaptic neurocircuits [69]. In addition to CHL1, CSP has been proposed as an alternate for Hsc70 targeting to the synapse, and may play similar roles as CHL1 in endocytosis, as it has been found to localize on CCVs [67, 70].

Synaptic recycling also involves the association between auxilin and Hsc70. Auxilin is a type III member of DnaJ

chaperone family and a native resident of CCVs [71]. It contains a J domain that allows it to bind to Hsc70s [71] and to recruit Hsc70 to the synaptic vesicle membrane. ATP-dependent binding of auxilin stimulates the ATPase activity of Hsc70, which in turn catalyzes clathrin decoating. Blocking of binding between auxilin and Hsc70 inhibits synaptic transmission in the giant squid axon [72].

There are several similarities between auxilin and CHL1. They both reside mainly in the presynaptic terminal and are critical for successful synaptic vesicle recycling. They recruit Hsc70 to the synapse with their J domain, in a manner similar to that of Hsp40. Thus, it is likely that Hsc70 interacts with both auxilin and CHL1 in a functional synapse at the synapse, although the exact relationship between CHL1 and auxilin has yet been determined.

### *Neurotransmitter Packaging*

*Hsc70 and VMAT2* Vesicle monoamine transporter (VMAT) is responsible for storing monoamines such as dopamine, norepinephrine and serotonin into synaptic vesicles [73, 74]. Hsc70 binds to the N-terminus of VMAT2 with its own SBD and CTD domains. This interaction results in a decrease in VMAT2 activity [75] and thus may result in regulation of neurotransmitter packaging into synaptic vesicles.

*Hsc70 and GABA synthesis* γ-Aminobutyric acid, GABA, is one of the major inhibitory neurotransmitters in the central nervous system [76]. It is synthesized from L-glutamate via the enzyme L-glutamic acid decarboxylase (GAD) [77]. There are two forms of GAD, GAD65 and GAD67, so named because of their molecular weights. The membrane-associated GAD (MGAD) is concentrated on synaptic vesicle membranes and is composed mainly of GAD65. The soluble GAD pool is concentrated in the cytosol and is composed mainly of GAD67. MGAD is the major contributor for GABA synthesis, and the activity of MGAD is dependent on its association with the synaptic membrane. GAD65 proteins lack the integral membrane stretch that is characteristic of most membrane proteins [78–80]. Binding to Hsc70 and CSP, GAD65 can become associated with the synaptic vesicle membrane, thus increasing its ability to synthesize GABA [76]. After exocytosis, these synaptic vesicles are recycled through clathrin-mediated endocytosis, whose activity is regulated by the Hsc70/CSP/SGT trimer previously described.

## Hsp70 in the Stressed Synapse

Stressed cells display several distinct characteristics, including an increase in resting calcium concentration, decrease in pH, generation of reactive oxygen species and alteration of membrane potential. At the synapses, there is impairment in both the presynaptic and postsynaptic function. Induced expression of Hsp70 is vital for counteracting the effects of stress-induced abnormalities at the synapse.

Study by Karunanithi et al. [27] first showed that in *Drosophila melanogaster*, induction of Hsp70 prior to heat shock protects individual synapses from subsequent hyperthermic insult. Specifically, heat shock preconditioning prevents the reduction of the quantal content (number of vesicles liberating neurotransmitter per stimulus) and stabilizes presynaptic functions. It also maintains the postsynaptic currents at the neuromuscular junction at a constant amplitude in heat-shocked flies as temperature increased [27]. These synaptic changes correlate well with the time course of elevation of Hsp70 after heat shock. Thus, synaptic protective effects of thermal preconditioning are partly attributable to the upregulation of Hsp70 [81, 82].

Not all organisms show upregulation of Hsp70 in response to stress. For example, populations of the *Rana temporaria* tadpoles from southern climates show less induction of Hsp70 expression following heat shock than those from a northern population [83]. Also, the thermotolerant species of hydra, *Hydra attennata,* is able to synthesize large amounts of Hsp60 after heat shock, whereas the thermosensitive *Hydra oligactis* cannot [84]. Furthermore, the desert species of fruit flies *Drosophila arizonae* show increased thermotolerance compared to the standard *D. melanogaster* fruit flies despite lower amount of upregulation of Hsp70 [85]. It was suggested that as *D. arizonae* flies had higher basal expression of Hsp70, this was able to afford heat shock protection [85]. Recent data suggest that a different sets of heat shock proteins are activated in thermosensitive species of *D. melanogaster* ($hsf^4$ mutant) [86]. Upon heat shock, there is significant upregulation of Hsp83 and Dna-J 1 protein without significant changes in expression of Hsp70. The function of Hsp83 is vague. The homologue of Dna-J1, Hsp40, is involved in repairing damaged proteins in a complex with Hsc70/Hsp70 [86]. Furthermore, in response to heat shock, Hsp40 forms a complex with CSP at the synapse, disrupting CSP dimerization and promoting CSP-mediated GTP hydrolysis on $G_\alpha$ subunit, in a manner that is similar to that of Hsc70 [87]. Given the expression level and functions of Hsp83 and Dna-J/Hsp40, these heat shock proteins can provide significant thermoprotection in the absence of inducible Hsp70.

Presynaptically, elevation of Hsp70 may stabilize presynaptic protein expression. Work from our laboratory show that early induction of Hsp70 stabilizes presynaptic protein expression and prevents hypoxia-induced motor and sensory depression in *Lynmaea stagnalis*, fresh pond water snails.

The snails exposed to chronic hypoxia show slowed sensory reaction to light stimuli and reduced motor movements [13, 14, 88]. The neural suppression of the snail coincides with an increase in Hsp70 expression and decreased expression of several proteins critical for exocytosis, including syntaxin I, synaptic vesicle protein 2 (SV2) and synaptotagmin [13, 14, 88]. Time-course analysis shows an early induction of Hsp70 within 6 hours, downregulation of syntaxin in the first 24 hours, a delayed reduction of synaptotagmin after 4 days and a biphasic response in SV2. Prevention of upregulation of Hsp70 with RNAi leads to further suppression of these presynaptic proteins and exacerbation of neurobehavioural dysfunction. Coimmunoprecipitation assay shows that Hsp70 directly interacts with syntaxin [13]. Consistent to the previous findings in neuromuscular junction in *D. melanogaster* [27], our results indicate that early induction of Hsp70 during hypoxic stress is critical in maintaining the expression level of presynaptic proteins, thus preventing aggravation hypoxic-induced neurobehavioural dysfunction [13, 14, 88].

Hsp70 may also confer protection through association with calcium channels. In addition to the N-type calcium channel, Hsp70s interact with the cytosolic loop of $Ca_V2.3$ R-type voltage-gated calcium channel [89]. The interaction may be important in decreasing the intracellular calcium overload that accompanies hyperthermic insults [25]. Prior to heat shock or induced elevation, Hsp70 at the *D. melanogaster* neuromuscular junction stabilizes the intracellular calcium concentration by maintaining near-resting calcium level and promoting calcium clearance in subsequent hyperthermic episodes. Furthermore, stabilization of the intracellular calcium level is correlated with better synaptic transmission and locomotory behaviour [25].

Hsp70 confers protection to the presynaptic terminal by maintaining the normal expression level of presynaptic proteins and by regulating calcium fluxes through interaction with presynaptic calcium channels. The protective role of Hsp70 at the postsynaptic terminal is unclear. Recent research shows that other heat shock proteins (e.g., Hsp90) associate with postsynaptic potassium [90, 91] and chloride channels [22] to produce hyperpolarization that may counteract the stress-induced depolarization. Thus, in response to stress, the actions of heat shock proteins strive to return the physiological environment of the neuronal synapse to a resting condition, thereby stabilizing both presynaptic and postsynaptic functions.

### Hsp70 in Neuroprotection

The protective role of Hsp70 extends in neurons beyond the confines of the synaptic terminal. The detailed evidence on the neuroprotective role of Hsp70 has been thoroughly reviewed preciously [92, 93]. Hsp70 exerts anti-inflammatory actions [94], upregulates anti-apoptotic molecules and downpregulates proapoptotic molecules [95] in ischemic cells. It also prevents protein aggregation and facilitates protein trafficking, and thus leads to its potential applications in "protein misfolding disorders" such as Parkinson's and Alzheimer's diseases [95–97]. The neuroprotective effects of Hsp70 are not limited to its role in neurons. In astrocytes, overexpression of Hsp70 reduces apoptosis and necrosis induced by glucose deprivation and oxygen glucose deprivation [95, 98]. In mice cocultures of microglia and astrocytes, Hsp70 suppresses activation of the inflammatory transcription factor nuclear factor κB (NFκB) via direct binding, and reduces the expression level of NFκB regulated genes, resulting in higher survival of neighbouring astrocytes [94]. This interplay between different cell populations of the CNS is also observed between astrocytes and neurons [92]. Neurons internalize the extracellular Hsp70s secreted by neighbouring glial cells during cell stress. The released Hsp70s during stress may function as a physiological alarm signal for cell trauma; however, whether the endogenous Hsp70s play a similar role remains unclear [92]. Thus, in the stressed CNS, Hsp70 may transcend its classic role as a molecular chaperone and emerge as a multifaceted protector for the specialized synapse, the damage-prone neuronal protoplasm and the supporting astrocytes.

## Perspectives

The current understanding is that Hsp70 acts as the major protective molecule against stress. However, Hsc70 is mostly expressed in the cytosol, and thus must be targeted to the specific sites such as synapse, by local proteins in order to perform specific functions. That is, the functional role of Hsc70 is largely determined by its essential cofactors, such as the Hsp40s [33]. At the synapse, Hsc70 participates in both exocytosis and endocytosis through association with different sets of proteins, although the overall mechanisms share great similarity. In all cases, Hsc70 is recruited to the synaptic site by native synaptic proteins containing a J domain. Binding of Hsc70 to these proteins is ATP/ADP dependent. Therefore, manipulation of the levels of native synaptic proteins, their Hsc70 binding abilities or the levels of ATP/ADP can alter the levels of Hsc70 at the synapse, and consequently promote or hinder Hsc70-mediated processes (e.g., clathrin uncoating and synaptic protein refolding). Thus, identification the targets and/or binding partners of Hsp70 is critical for our understanding of the role of Hsp70 in neuronal functions.

There are several differences between Hsc70 and Hsp70s. First, Hsc70 contains several introns, whereas Hsp70 is intronless [41]. The structural simplicity of Hsp70 may enable its quick induction in response to stress. However, the functional and perhaps structural consequence of this genetic difference is unclear. Next, with regard to the synapse, Hsc70 mainly participates in the maintenance of normal synaptic functions such as exocytosis and endocytosis. Hsp70, however, functions to counteract the effects of stress so that the processes involving Hsc70 can proceed, thus illustrating a functional distinction between Hsc70 and Hsp70. This distinction is further exemplified by the fact that, currently, there is no common target observed (at the synapse). Also, study has shown direct antagonistic effects of Hsc70 and Hsp70, such as in the case of the epithelial sodium channels [99]. Currently, it is premature to conclude whether Hsc and Hsp70 share more differences or similarities. Further research thus is warranted to elucidate this matter.

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## References

1. Lindquist S (1986) The heat-shock response. Annu Rev Biochem 55:1151–1191
2. Pignataro L, Miller AN, Ma L, Midha S, Protiva P, Herrera DG, Harrison NL (2007) Alcohol regulates gene expression in neurons via activation of heat shock factor 1. J Neurosci 27:12957–12966
3. Sanchez-Moreno C, Paniagua M, Madrid A, Martin A (2003) Protective effect of vitamin C against the ethanol mediated toxic effects on human brain glial cells. J Nutr Biochem 14:606–613
4. Su CY, Chong KY, Owen OE, Dillmann WH, Chang C, Lai CC (1998) Constitutive and inducible hsp70s are involved in oxidative resistance evoked by heat shock or ethanol. J Mol Cell Cardiol 30:587–598
5. Leung TK, Rajendran MY, Monfries C, Hall C, Lim L (1990) The human heat-shock protein family. Expression of a novel heat-inducible HSP70 (HSP70B') and isolation of its cDNA and genomic DNA. Biochem J 267:125–132
6. Turner CP, Panter SS, Sharp FR (1999) Anti-oxidants prevent focal rat brain injury as assessed by induction of heat shock proteins (HSP70, HO-1/HSP32, HSP47) following subarachnoid injections of lysed blood. Brain Res Mol Brain Res 65:87–102
7. Wallen ES, Buettner GR, Moseley PL (1997) Oxidants differentially regulate the heat shock response. Int J Hyperthermia 13:517–524
8. Misra S, Zafarullah M, Price-Haughey J, Gedamu L (1989) Analysis of stress-induced gene expression in fish cell lines exposed to heavy meals and heat shock. Biochim Biophys Acta 1007:325–333
9. Mutwakil MH, Reader JP, Holdich DM, Smithurst PR, Candido EPM, Jones D, Stringham EG, de Pomerai DI (1997) Use of stress-inducible transgenic nematodes as biomarkers of heavy metal pollution in water samples from an English river system. Arch Environ Contam Toxicol 32:146–153
10. Wagner M, Hermanns I, Bittinger F, Kirkpatrick CJ (1999) Induction of stress proteins in human endothelial cells by heavy metal ions and heat shock. Am J Physiol 277:L1026–L1033
11. Nissim I, Hardy M, Pleasure J, Nissim I, States B (1992) A mechanism of glycine and alanine cytoprotective action: stimulation of stress-induced HSP70 mRNA. Kidney Int 42: 775–782
12. Stephen DW, Jamieson DJ (1997) Amino acid-dependent regulation of the *Saccharomyces cerevisiae* GSH1 gene by hydrogen peroxide. Mol Microbiol 23:203–210
13. Fei G, Guo C, Sun HS, Feng ZP (2007) Chronic hypoxia stress-induced differential modulation of heat-shock protein 70 and presynaptic proteins. J Neurochem 100:50–61
14. Fei G, Guo C, Sun HS, Feng ZP (2008) HSP70 reduces chronic hypoxia-induced neural suppression via regulating expression of syntaxin. Adv Exp Med Biol 605:35–40
15. Bergeron M, Mivechi NF, Giaccia AJ, Giffard RG (1996) Mechanism of heat shock protein 72 induction in primary cultured astrocytes after oxygen-glucose deprivation. Neurol Res 18:64–72
16. Xu L, Lee JE, Giffard RG (1999) Overexpression of bcl-2, bcl-XL or hsp70 in murine cortical astrocytes reduces injury of co-cultured neurons. Neurosci Lett 277:193–197
17. Benjamin IJ, McMillan DR (1998) Stress (heat shock) proteins: molecular chaperones in cardiovascular biology and disease. Circ Res 83:117–132
18. Schlesinger MJ (1990) Heat shock proteins. J Biol Chem 265:12111–12114
19. Walter S, Buchner J (2002) Molecular chaperones—cellular machines for protein folding. Angew Chem Int Ed Engl 41:1098–1113
20. Azad P, Zhou D, Russo E, Haddad GG (2009) Distinct mechanisms underlying tolerance to intermittent and constant hypoxia in *Drosophila melanogaster*. PLoS ONE 4:e5371
21. Zhang K, Zhao T, Huang X, Liu ZH, Xiong L, Li MM, Wu LY, Zhao YQ, Zhu LL, Fan M (2009) Preinduction of HSP70 promotes hypoxic tolerance and facilitates acclimatization to acute hypobaric hypoxia in mouse brain. Cell Stress Chaperones 14:407–415
22. Hinzpeter A, Lipecka J, Brouillard F, Baudoin-Legros M, Dadlez M, Edelman A, Fritsch J (2006) Association between Hsp90 and the ClC-2 chloride channel upregulates channel function. Am J Physiol Cell Physiol 290:C45–C56
23. Williamson CL, Dabkowski ER, Dillmann WH, Hollander JM (2008) Mitochondria protection from hypoxia/reoxygenation injury with mitochondria heat shock protein 70 overexpression. Am J Physiol Heart Circ Physiol 294:H249–H256
24. Chang W, Song BW, Lim S, Song H, Shim CY, Cha MJ, Ahn DH, Jung YG, Lee DH, Chung JH, Choi KD, Lee SK, Chung N, Lee SK, Jang Y, Hwang KC (2009) Mesenchymal stem cells pretreated with delivered Hph-1-Hsp70 protein are protected from hypoxia-mediated cell death and rescue heart functions from myocardial injury. Stem Cells 27:2283–2292
25. Klose MK, Atwood HL, Robertson RM (2008) Hyperthermic preconditioning of presynaptic calcium regulation in *Drosophila*. J Neurophysiol 99:2420–2430
26. Kang MJ, Jung SM, Kim MJ, Bae JH, Kim HB, Kim JY, Park SJ, Song HS, Kim DW, Kang CD, Kim SH (2008) DNA-dependent protein kinase is involved in heat shock protein-mediated accumulation of hypoxia-inducible factor-1alpha in hypoxic preconditioned HepG2 cells. FEBS J 275:5969–5981

27. Karunanithi S, Barclay JW, Robertson RM, Brown IR, Atwood HL (1999) Neuroprotection at *Drosophila* synapses conferred by prior heat shock. J Neurosci 19:4360–4369
28. Lin YW, Yang HW, Min MY, Chiu TH (2004) Heat-shock pretreatment prevents suppression of long-term potentiation induced by scopolamine in rat hippocampal CA1 synapses. Brain Res 999:222–226
29. Obrenovitch TP (2008) Molecular physiology of preconditioning-induced brain tolerance to ischemia. Physiol Rev 88:211–247
30. Amin J, Ananthan J, Voellmy R (1988) Key features of heat shock regulatory elements. Mol Cell Biol 8:3761–3769
31. Perisic O, Xiao H, Lis JT (1989) Stable binding of *Drosophila* heat shock factor to head-to-head and tail-to-tail repeats of a conserved 5 bp recognition unit. Cell 59:797–806
32. Peteranderl R, Nelson HC (1992) Trimerization of the heat shock transcription factor by a triple-stranded alpha-helical coiled-coil. Biochemistry 31:12272–12276
33. Ohtsuka K, Hata M (2000) Molecular chaperone function of mammalian Hsp70 and Hsp40—a review. Int J Hyperthermia 16:231–245
34. Williams GT, Morimoto RI (1990) Maximal stress-induced transcription from the human HSP70 promoter requires interactions with the basal promoter elements independent of rotational alignment. Mol Cell Biol 10:3125–3136
35. Santoro MG (2000) Heat shock factors and the control of the stress response. Biochem Pharmacol 59:55–63
36. Flaherty KM, DeLuca-Flaherty C, McKay DB (1990) Three-dimensional structure of the ATPase fragment of a 70K heat-shock cognate protein. Nature 346:623–628
37. Scheufler C, Brinker A, Bourenkov G, Pegoraro S, Moroder L, Bartunik H, Hartl FU, Moarefi I (2000) Structure of TPR domain-peptide complexes: critical elements in the assembly of the Hsp70–Hsp90 multichaperone machine. Cell 101:199–210
38. Zhu X, Zhao X, Burkholder WF, Gragerov A, Ogata CM, Gottesman ME, Hendrickson WA (1996) Structural analysis of substrate binding by the molecular chaperone DnaK. Science 272:1606–1614
39. Han W, Christen P (2003) Interdomain communication in the molecular chaperone DnaK. Biochem J 369:627–634
40. Brocchieri L, Conway dM, Macario AJ (2008) hsp70 genes in the human genome: conservation and differentiation patterns predict a wide array of overlapping and specialized functions. BMC Evol Biol 8:19
41. Tavaria M, Gabriele T, Kola I, Anderson RL (1996) A hitchhiker's guide to the human Hsp70 family. Cell Stress Chaperones 1:23–28
42. Bhattacharyya T, Karnezis AN, Murphy SP, Hoang T, Freeman BC, Phillips B, Morimoto RI (1995) Cloning and subcellular localization of human mitochondrial hsp70. J Biol Chem 270:1705–1710
43. Domanico SZ, DeNagel DC, Dahlseid JN, Green JM, Pierce SK (1993) Cloning of the gene encoding peptide-binding protein 74 shows that it is a new member of the heat shock protein 70 family. Mol Cell Biol 13:3598–3610
44. Munro S, Pelham HR (1986) An Hsp70-like protein in the ER: identity with the 78 kd glucose-regulated protein and immunoglobulin heavy chain binding protein. Cell 46:291–300
45. Hendershot LM, Valentine VA, Lee AS, Morris SW, Shapiro DN (1994) Localization of the gene encoding human BiP/GRP78, the endoplasmic reticulum cognate of the HSP70 family, to chromosome 9q34. Genomics 20:281–284
46. Tavaria M, Gabriele T, Anderson RL, Mirault ME, Baker E, Sutherland G, Kola I (1995) Localization of the gene encoding the human heat shock cognate protein, HSP73, to chromosome 11. Genomics 29:266–268
47. Tononi G, Cirelli C (2001) Modulation of brain gene expression during sleep and wakefulness: a review of recent findings. Neuropsychopharmacology 25:S28–S35
48. Milner CM, Campbell RD (1990) Structure and expression of the three MHC-linked HSP70 genes. Immunogenetics 32:242–251
49. Wu B, Hunt C, Morimoto R (1985) Structure and expression of the human gene encoding major heat shock protein HSP70. Mol Cell Biol 5:330–341
50. Goate AM, Cooper DN, Hall C, Leung TK, Solomon E, Lim L (1987) Localization of a human heat-shock HSP 70 gene sequence to chromosome 6 and detection of two other loci by somatic-cell hybrid and restriction fragment length polymorphism analysis. Hum Genet 75:123–128
51. Harrison GS, Drabkin HA, Kao FT, Hartz J, Hart IM, Chu EH, Wu BJ, Morimoto RI (1987) Chromosomal location of human genes encoding major heat-shock protein HSP70. Somat Cell Mol Genet 13:119–130
52. Sharma D, Masison DC (2009) Hsp70 structure, function, regulation and influence on yeast prions. Protein Pept Lett 16:571–581
53. Morano KA, Thiele DJ (1999) Heat shock factor function and regulation in response to cellular stress, growth, and differentiation signals. Gene Expr 7:271–282
54. Cheetham ME, Caplan AJ (1998) Structure, function and evolution of DnaJ: conservation and adaptation of chaperone function. Cell Stress Chaperones 3:28–36
55. Qiu XB, Shao YM, Miao S, Wang L (2006) The diversity of the DnaJ/Hsp40 family, the crucial partners for Hsp70 chaperones. Cell Mol Life Sci 63:2560–2570
56. Fewell SW, Pipas JM, Brodsky JL (2002) Mutagenesis of a functional chimeric gene in yeast identifies mutations in the simian virus 40 large T antigen J domain. Proc Natl Acad Sci U S A 99:2002–2007
57. Feldheim D, Rothblatt J, Schekman R (1992) Topology and functional domains of Sec63p, an endoplasmic reticulum membrane protein required for secretory protein translocation. Mol Cell Biol 12:3288–3296
58. Tobaben S, Thakur P, Fernandez-Chacon R, Sudhof TC, Rettig J, Stahl B (2001) A trimeric protein complex functions as a synaptic chaperone machine. Neuron 31:987–999
59. Zinsmaier KE, Eberle KK, Buchner E, Walter N, Benzer S (1994) Paralysis and early death in cysteine string protein mutants of *Drosophila*. Science 263:977–980
60. Tobaben S, Varoqueaux F, Brose N, Stahl B, Meyer G (2003) A brain-specific isoform of small glutamine-rich tetratricopeptide repeat-containing protein binds to Hsc70 and the cysteine string protein. J Biol Chem 278:38376–38383
61. Natochin M, Campbell TN, Barren B, Miller LC, Hameed S, Artemyev NO, Braun JE (2005) Characterization of the G alpha(s) regulator cysteine string protein. J Biol Chem 280: 30236–30241
62. Zhao X, Braun AP, Braun JE (2008) Biological roles of neural J proteins. Cell Mol Life Sci 65:2385–2396
63. Miller LC, Swayne LA, Kay JG, Feng ZP, Jarvis SE, Zamponi GW, Braun JE (2003) Molecular determinants of cysteine string protein modulation of N-type calcium channels. J Cell Sci 116:2967–2974
64. Swayne LA, Beck KE, Braun JE (2006) The cysteine string protein multimeric complex. Biochem Biophys Res Commun 348:83–91
65. Newmyer SL, Schmid SL (2001) Dominant-interfering Hsc70 mutants disrupt multiple stages of the clathrin-coated vesicle cycle in vivo. J Cell Biol 152:607–20
66. Takei K, Mundigl O, Daniell L, De Camilli P (1996) The synaptic vesicle cycle: a single vesicle budding step involving clathrin and dynamin. J Cell Biol 133:1237–1250

67. Leshchyns'ka I, Sytnyk V, Richter M, Andreyeva A, Puchkov D, Schachner M (2006) The adhesion molecule CHL1 regulates uncoating of clathrin-coated synaptic vesicles. Neuron 52:1011–1025
68. Frints SG, Marynen P, Hartmann D, Fryns JP, Steyaert J, Schachner M, Rolf B, Craessaerts K, Snellinx A, Hollanders K, D'Hooge R, De Deyn PP, Froyen G (2003) CALL interrupted in a patient with non-specific mental retardation: gene dosage-dependent alteration of murine brain development and behavior. Hum Mol Genet 12:1463–1474
69. Chen QY, Chen Q, Feng GY, Lindpaintner K, Chen Y, Sun X, Chen Z, Gao Z, Tang J, He L (2005) Case-control association study of the close homologue of L1 (CHL1) gene and schizophrenia in the Chinese population. Schizophr Res 73:269–274
70. Bai L, Swayne LA, Braun JE (2007) The CSPalpha/G protein complex in PC12 cells. Biochem Biophys Res Commun 352:123–129
71. Ungewickell E, Ungewickell H, Holstein SE, Lindner R, Prasad K, Barouch W, Martin B, Greene LE, Eisenberg E (1995) Role of auxilin in uncoating clathrin-coated vesicles. Nature 378:632–635
72. Morgan JR, Prasad K, Jin S, Augustine GJ, Lafer EM (2001) Uncoating of clathrin-coated vesicles in presynaptic terminals: roles for Hsc70 and auxilin. Neuron 32:289–300
73. Ahnert-Hilger G, Holtje M, Pahner I, Winter S, Brunk I (2003) Regulation of vesicular neurotransmitter transporters. Rev Physiol Biochem Pharmacol 150:140–160
74. Eiden LE, Schafer MK, Weihe E, Schutz B (2004) The vesicular amine transporter family (SLC18): amine/proton antiporters required for vesicular accumulation and regulated exocytotic secretion of monoamines and acetylcholine. Pflugers Arch 447:636–640
75. Requena DF, Parra LA, Baust TB, Quiroz M, Leak RK, Garcia-Olivares J, Torres GE (2009) The molecular chaperone Hsc70 interacts with the vesicular monoamine transporter-2. J Neurochem 110:581–594
76. Hsu CC, Davis KM, Jin H, Foos T, Floor E, Chen W, Tyburski JB, Yang CY, Schloss JV, Wu JY (2000) Association of L-glutamic acid decarboxylase to the 70-kDa heat shock protein as a potential anchoring mechanism to synaptic vesicles. J Biol Chem 275:20822–20828
77. Roberts E, Kuriyama K (1968) Biochemical–physiological correlations in studies of the gamma-aminobutyric acid system. Brain Res 8:1–35
78. Christgau S, Schierbeck H, Aanstoot HJ, Aagaard L, Begley K, Kofod H, Hejnaes K, Baekkeskov S (1991) Pancreatic beta cells express two autoantigenic forms of glutamic acid decarboxylase, a 65-kDa hydrophilic form and a 64-kDa amphiphilic form which can be both membrane-bound and soluble. J Biol Chem 266:23516
79. Hsu CC, Thomas C, Chen W, Davis KM, Foos T, Chen JL, Wu E, Floor E, Schloss JV, Wu JY (1999) Role of synaptic vesicle proton gradient and protein phosphorylation on ATP-mediated activation of membrane-associated brain glutamate decarboxylase. J Biol Chem 274:24366–24371
80. Kaufman DL, Houser CR, Tobin AJ (1991) Two forms of the gamma-aminobutyric acid synthetic enzyme glutamate decarboxylase have distinct intraneuronal distributions and cofactor interactions. J Neurochem 56:720–723
81. Kelty JD, Noseworthy PA, Feder ME, Robertson RM, Ramirez JM (2002) Thermal preconditioning and heat-shock protein 72 preserve synaptic transmission during thermal stress. J Neurosci 22:RC193
82. Xiao C, Mileva-Seitz V, Seroude L, Robertson RM (2007) Targeting HSP70 to motoneurons protects locomotor activity from hyperthermia in *Drosophila*. Dev Neurobiol 67:438–455
83. Nikinmaa M, Leveelahti L, Dahl E, Rissanen E, Rytkonen KT, Laurila A (2008) Population origin, development and temperature of development affect the amounts of HSP70, HSP90 and the putative hypoxia-inducible factor in the tadpoles of the common frog *Rana temporaria*. J Exp Biol 211:1999–2004
84. Bosch TC, Krylow SM, Bode HR, Steele RE (1988) Thermotolerance and synthesis of heat shock proteins: these responses are present in *Hydra attenuata* but absent in *Hydra oligactis*. Proc Natl Acad Sci U S A 85:7927–7931
85. Newman AE, Xiao C, Robertson RM (2005) Synaptic thermoprotection in a desert-dwelling *Drosophila* species. J Neurobiol 64:170–180
86. Neal SJ, Karunanithi S, Best A, So AK, Tanguay RM, Atwood HL, Westwood JT (2006) Thermoprotection of synaptic transmission in a *Drosophila* heat shock factor mutant is accompanied by increased expression of Hsp83 and DnaJ-1. Physiol Genomics 25:493–501
87. Gibbs SJ, Barren B, Beck KE, Proft J, Zhao X, Noskova T, Braun AP, Artemyev NO, Braun JE (2009) Hsp40 couples with the CSPalpha chaperone complex upon induction of the heat shock response. PLoS ONE 4:e4595
88. Fei GH, Feng ZP (2008) Chronic hypoxia-induced alteration of presynaptic protein profiles and neurobehavioral dysfunction are averted by supplemental oxygen in *Lymnaea stagnalis*. Neuroscience 153:318–328
89. Krieger A, Radhakrishnan K, Pereverzev A, Siapich SA, Banat M, Kamp MA, Leroy J, Klockner U, Hescheler J, Weiergraber M, Schneider T (2006) The molecular chaperone hsp70 interacts with the cytosolic II–III loop of the Cav2.3 E-type voltage-gated Ca2+ channel. Cell Physiol Biochem 17:97–110
90. Jiao JD, Garg V, Yang B, Hu K (2008) Novel functional role of heat shock protein 90 in ATP-sensitive K+ channel-mediated hypoxic preconditioning. Cardiovasc Res 77:126–133
91. Sun HS, Feng ZP, Barber PA, Buchan AM, French RJ (2007) Kir6.2-containing ATP-sensitive potassium channels protect cortical neurons from ischemic/anoxic injury in vitro and in vivo. Neuroscience 144:1509–1515
92. Calderwood SK, Mambula SS, Gray PJ Jr, Theriault JR (2007) Extracellular heat shock proteins in cell signaling. FEBS Lett 581:3689–3694
93. Yenari MA, Liu J, Zheng Z, Vexler ZS, Lee JE, Giffard RG (2005) Antiapoptotic and anti-inflammatory mechanisms of heat-shock protein protection. Ann N Y Acad Sci 1053:74–83
94. Zheng Z, Kim JY, Ma H, Lee JE, Yenari MA (2008) Anti-inflammatory effects of the 70 kDa heat shock protein in experimental stroke. J Cereb Blood Flow Metab 28:53–63
95. Giffard RG, Xu L, Zhao H, Carrico W, Ouyang Y, Qiao Y, Sapolsky R, Steinberg G, Hu B, Yenari MA (2004) Chaperones, protein aggregation, and brain protection from hypoxic/ischemic injury. J Exp Biol 207:3213–3220
96. Brown IR (2007) Heat shock proteins and protection of the nervous system. Ann N Y Acad Sci 1113:147–158
97. Fujikake N, Nagai Y, Popiel HA, Okamoto Y, Yamaguchi M, Toda T (2008) Heat shock transcription factor 1-activating compounds suppress polyglutamine-induced neurodegeneration through induction of multiple molecular chaperones. J Biol Chem 283:26188–26197
98. Lee JE, Yenari MA, Sun GH, Xu L, Emond MR, Cheng D, Steinberg GK, Giffard RG (2001) Differential neuroprotection from human heat shock protein 70 overexpression in in vitro and in vivo models of ischemia and ischemia-like conditions. Exp Neurol 170:129–139
99. Goldfarb SB, Kashlan OB, Watkins JN, Suaud L, Yan W, Kleyman TR, Rubenstein RC (2006) Differential effects of Hsc70 and Hsp70 on the intracellular trafficking and functional expression of epithelial sodium channels. Proc Natl Acad Sci U S A 103:5817–5822