Cell. Mol. Life Sci. 65 (2008) 2385 – 2396
1420-682X/08/152385-12
DOI 10.1007/s00018-008-8089-z

Cellular and Molecular Life Sciences

Review

Biological Roles of Neural J Proteins

X. Zhao[a], A. P. Braun[b] and J. E. A. Braun[a,] *

[a] Hotchkiss Brain Institute, Department of Physiology and Biophysics, The University of Calgary, Calgary, Alberta, T2N 4N1 (Canada), Fax (403) 210 7446, e-mail: braunj@ucalgary.ca
[b] Libin Cardiovascular Institute of Alberta, Department of Pharmacology and Therapeutics, University of Calgary, Calgary, Alberta, T2N 4N1 (Canada)

Received 20 February 2008; received after revision 14 March 2008; accepted 18 March 2008
Online First 26 April 2008

Abstract. J proteins are chief regulators of the Hsp70 family, a highly conserved family of ATPases that mediate conformational changes in a broad range of proteins. The J protein family has been the central focus of numerous prokaryote and eukaryote biologists. Common questions that arise include: How does the J protein/Hsp70 machinery support protein folding? What role do J proteins play in protein misfolding and neurodegenerative disorders? Can the J protein/Hsp70 machinery be harnessed to provide a rational basis for recombinant protein production? The current progress that has resulted from the convergence of biochemistry with *Escherichia coli* and *Saccharomyces cerevisiae* genetics has accelerated the pace at which these questions are being elucidated. We are beginning to gain some insights into the neuronal network of J proteins. Here, we highlight recent advances in our understanding of how select J proteins harness Hsp70 s for fundamentally important conformational work in neurons.

Keywords. J proteins, Hsp70 s, chaperones, auxilin, GAK, CSPα, Rdj2, Rme-8, Hsp40, Mrj, Hsj1.

Introduction

Conformational changes in proteins mediate a wide array of cellular responses that range from synaptic transmission, to signal transduction, to intracellular transport. Molecular chaperones are the cellular machinery that has evolved to stabilize protein structure and eliminate misfolded proteins inside cells. The molecular chaperone machinery is comprised of several diverse groups of proteins that collectively maintain a critical balance between protein folding and protein triage. Hence, chaperones intersect with almost every facet of cell function to maintain a complete set of functionally competent proteins.

The dominance of the amino acid sequence in determining the functionally active structure of a protein is well established. In particular, some proteins, such as RNaseA, refold spontaneously from a denatured state *in vitro* with high efficiency [1]. However, most proteins exist in more than one conformation and many proteins must change conformation (and activity) regularly. Molecular chaperones are 'folding catalysts' that regulate the conformation and activities of other proteins but are not themselves components of the final structure. Given their pivotal roles in protein conformation, molecular chaperones have become the subject of intense interest. In a few short years, we have come a long way from the notion of protein conformation as an unregulated default process to appreciate a full array of chaperone-mediated structural processes.

* Corresponding author.

J protein:Hsp70 units

The 70 kDa heat shock protein (Hsp70) family is a central component of the cellular folding processes in all three kingdoms. All Hsp70 family members feature a conserved architecture consisting of an amino-terminal ATPase domain and a carboxy-terminal substrate binding domain that is well documented and extensively reviewed [2–8]. The ATPase activity is coupled to a broad range of folding processes that include the folding of newly synthesized proteins, the transport of proteins across membranes, the refolding of misfolded proteins, the disassembly of protein complexes, as well as conformational changes of components in signal transduction, cell cycle, transcription, and apoptotic pathways. Substrates of Hsp70 s do not share homology but typically demonstrate significant conformational flexibility. Precisely how Hsp70 machines facilitate folding remains to be solved. However, the common mechanism underlying these diverse functions appears to be the ability of Hsp70 s to transiently bind and sequester unfolded regions of substrate proteins, thereby preventing unproductive folding and aggregation. Hsp70 s' protein binding and release kinetics are governed by their ATPase activity (Fig. 1). ATP binding to the ATPase domain triggers the substrate binding pocket to open and increases substrate association and dissociation rates (weaker affinity). In the presence of ADP, the substrate binding pocket closes and reduces substrate dissociation (higher affinity).

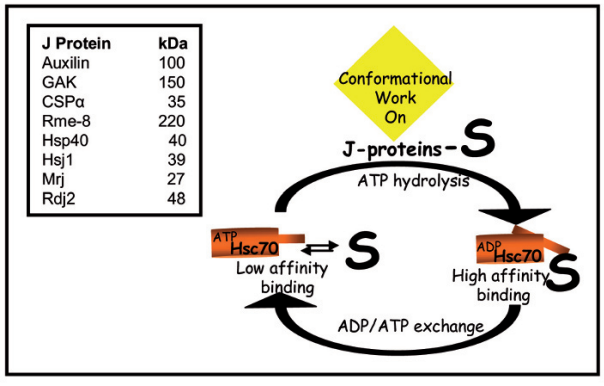

Figure 1. Model of the Hsc70 (70 kDa heat shock cognate protein) chaperone cycle. Upon ATP binding, Hsc70 exhibits low affinity and a fast exchange rate for its substrate (S). After ATP hydrolysis, Hsc70 exhibits high affinity and a low exchange rate for its substrate (S). Conformational work on proteins is associated with cycles of binding and release. J proteins increase ATP hydrolysis and substrate loading.

Genome sequencing projects have revealed that bacterial Hsp70 s share approximately 50% amino acid identity with eukaryotic homologues, indicating that nature has maintained the Hsp70 chaperone units throughout evolution. Hence, Hsp70 s have been extensively utilized as important phylogenetic nominators in the field of evolution. Multiple Hsp70 family members are found in most organisms, creating an elaborate chaperone network. In humans, there are at least eight Hsp70 s [5]. Three of the Hsp70 s in humans are rapidly induced in response to a conditioning (non-lethal) heat shock, while others are constitutively expressed (eg. the constitutively expressed and cytosolic Hsp70 family member is called Hsc70; heat shock cognate protein of 70 kDa). Induction of the 'heat shock response' protects cells from a variety of subsequent, more severe cell stresses by rapidly upregulating a number of distinct chaperones (see Hsp40 below). The importance of the Hsp70 function is underscored by the multitude of regulatory mechanisms to which it is subjected. J proteins (sometimes referred to as Hsp40 s) stimulate Hsp70 ATPase activity (Fig. 1). Other cofactors, such as NEFs (nucleotide exchange factors) stimulate the release of bound ADP for ATP, and HOP (Hsc70-organizing protein) couples Hsp70 to the ATPase Hsp90.

The discovery that over 40 human proteins contain a J domain, a domain found in the bacterial J protein, DnaJ, that is required for bacteriophage λ DNA replication in *Escherichia coli,* provided the evidence that regulation of protein conformation in mammalian cells is a specialized version of general protein folding [9;10]. J proteins interact with and activate the ATPase of Hsp70 s, thereby harnessing ATPase activity for conformational work on target proteins. The realization that protein structure is regulated by these conserved families initially fueled the search for mammalian J proteins. We are now beginning to understand how the Hsp70:J protein machines have been adapted for specialized folding tasks in neurons. While ongoing research continues to decipher the rules by which a multitude of prokaryote and eukaryote proteins utilize the Hsp70:J protein machinery to regulate protein conformation, an increasing amount of work has focused on the role that Hsp70:J protein units play in neural function and neurodegenerative disorders. Here, we will examine recent advances in our understanding of eight neural J proteins: auxilin, GAK, CSPα, Rme-8, Hsp40, Hsj1, Mrj and Rdj2. Our review is intended to complement the following excellent reviews on the J protein family [10–16].

J proteins recruit Hsc70 for distinct cellular tasks

The mechanistic diversity of Hsp70:J protein-mediated protein folding in neurons is fascinating. Shown schematically in Figures 2 and 3, different neural J proteins harness Hsp70 s for non-overlapping cellular tasks (outlined in detail below). Auxilin and GAK

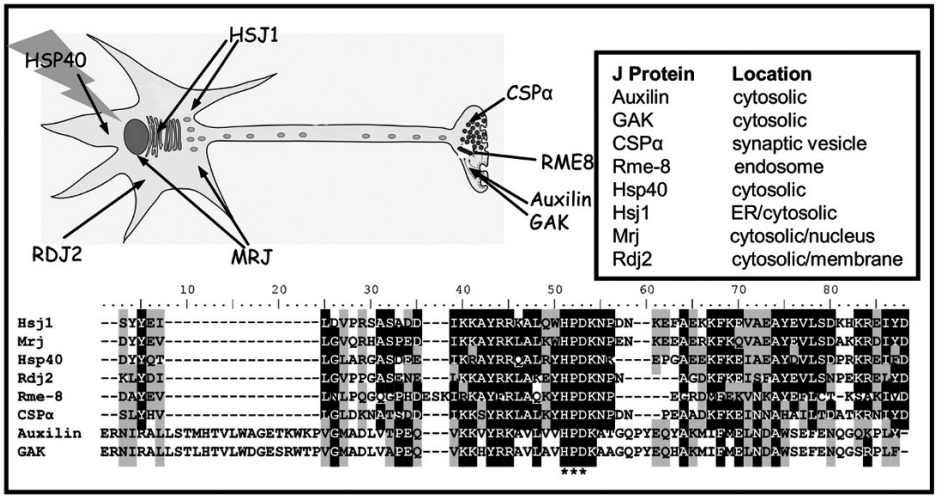

Figure 2. The subcellular locations of J proteins: auxilin, GAK, CSPα, Rme-8, Hsp40, Hsj1, Mrj and Rdj2, and a comparison of their amino acid sequences within the J domains. Hsj1a is cytoplasmic, while Hsj1b is located on the cytoplasmic face of the endoplasmic reticulum. Rdj2 is found in both cytosolic and membrane fractions. Mrj is cytosolic, but translocates to the nucleus. Rme-8 is endosomal; its schematic subcellular location in the synapse is speculative. Alignment was performed using CLUSTALW with default settings in place.
* indicates the highly conserved "HPD" motif; conserved regions are indicated by the black background.

mediate dynamic conformational re-arrangements of clathrin during clathrin-mediated endocytosis, Mrj facilitates conformational work on keratin/nuclear factor of activated T cells, and Hsj1 aids substrate ubiquitination and sorting to the proteosome for degradation. Rme-8 regulates endosomal and CSPα synaptic vesicle conformational work by the Hsp70 family. Each of these J proteins contains a signature domain called a 'J domain' which is shown in alignment in Figure 2. The J domain is, on average, a 70-amino acid region of homology comprised of four helices with a highly conserved tripeptide of histidine, proline, and aspartic acid (HPD motif) located between helices II and III. The common mechanism underlying these diverse activities is the transient J domain activation of the Hsp70 s' ATPase, thereby increasing the substrate-binding affinity. At face value, there is a range in the amino acid identity of these eight J proteins with *Escherichia coli* DnaJ (31%-1% amino acid identity, highest for Rdj2 and lowest for GAK), the first member of the J protein to be identified. Within the J domain itself, the amino acid identity between *Escherichia coli* DnaJ and the mammalian protein is: Mrj 58%, Hsj1 56%, Hsp40 52%, Rdj2 52%, CSPα 49%, Rme-8 42%, GAK 19%, and auxilin 18%. Furthermore, there are some differences in the length of linker regions between the helices I-II (7–18 amino acids; shortest for Rdj2/Mrj, longest for auxilin), II-III (6–10 amino acids shortest for Mrj, longest for auxilin/GAK). Moreover, auxilin (as well as its analog, GAK) is missing helix IV. Outside of the J domain, auxilin, GAK, CSPα, Rme-8, Hsp40, Hsj1, Mrj and Rdj2 are dissimilar (Fig. 4).

Within the cell J protein:Hsp70 folding units ar highly regulated. For example, clathrin is removed from clathrin-coated vesicles, but not from clathrin-coated pits, by the J proteins auxilin and GAK in conjunction with Hsc70. A burst of auxilin/GAK recruitment from the cytosol to the clathrin-coated pits occurs during membrane constriction and precedes the onset of clathrin uncoating [17;18]. The wave of auxilin recruitment depends on the inositol phospholipid binding motif of auxilin, PTEN, suggesting that a localized accumulation of specific lipids may serve as the auxilin/GAK recruiting signal. Importantly, the precision and accuracy of these chaperone-mediated clathrin rearrangements occur within the time constraints of the synaptic vesicle cycle. Precisely how various J protein:Hsp70 units are regulated intracellularly is unclear; however, three key aspects have emerged. First, J proteins are located in various places within the cell (Fig. 2). CSPα is anchored to the synaptic vesicle, Rme-8 is endosomal, Hsj1b is an endoplasmic reticulum protein, Rdj2 is found in cytosolic and membrane fractions, Mrj is both cytoplasmic and nuclear, Hsj1a, auxilin, GAK, and Hsp40 are cytosolic. Second, J protein:Hsp70 complexes have been found as components of larger multimeric chaperone complexes that, in some cases, include the Hsp90 ATPase in addition to a multitude of protein cofactors (Hsp70 interacting protein (HIP); Hsp70 organizing protein (HOP)). Within the cell, the assembly/disassembly of diverse regulatory factors with J protein:Hsp70 units most certainly underlie differences in chaperone activity. Third, client proteins are targeted to Hsp70 s through the intercession

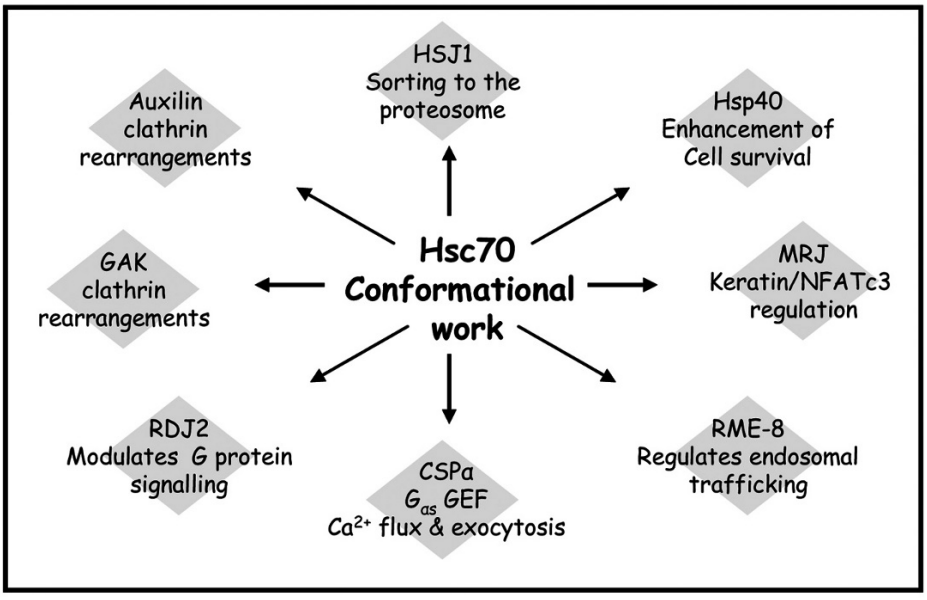

Figure 3. Proposed roles for several neural J proteins.

of J proteins. J proteins may serve as recruiting factors for work on one specific protein or are capable of structural work on multiple proteins [11]. J proteins are divergent outside of the J domain. Figure 4 shows the domain alignments and secondary structure prediction [19] of the eight J proteins; helix, sheet, mixed (i.e. weak "helix" and "sheet" predictions within the J domain) are indicated. The J domain is found within the N terminus of CSPα, Hsp40, Hsj1, Mrj, Rdj2, the middle region of Rme-8, and the C terminus of auxilin and GAK. The divergent regions most certainly underlie intracellular targeting, control by regulatory cofactors, and substrate capture and loading onto Hsp70 s. Thus, regions outside the J domain are thought to underlie the functional diversity of different J protein:Hsp70 units. That being said, while it is clear that different chaperones utilize distinct folding strategies, the precise distinctions among the intrinsic mechanisms still remain elusive.

The functions of numerous J proteins have yet to be determined. In humans, over 40 proteins with J domains, classified historically as type I, II, and III, have been identified. Figure 5 shows the domain alignment of 41 human J proteins and two mouse J proteins (DnaJB3 and DnaJB10) (names from Qui et al, 2006.). The domains of four Type I (designated DnaJA), 14 Type II (DnaJB), 25 Type III (DnaJC) were identified with Interproscan. Analysis reveals that the structural organization is more diverse than hitherto appreciated. The length of the J domains ranges from 37 (DnaJB1b) to 84 amino acids (auxilin and GAK). In class I and II, the J domains are located at the N terminus. In humans, the J domain of DnaJA1, DnaJA2 and DnaJA4 is followed by a glycine/phenylalanine-rich linker. However, the glycine/phenylalanine linker region is not present in DnaJA3. All proteins of class I designation have a DnaJ central region that includes the CXXCXGXG motif and a chaperone DnaJ C-terminal. In class II, 10 of 14 family members have glycine/phenylalanine linkers (absent in DnaJB1b, DnaJB9, DnaJB10 and DnaJB11). In class III, J domains are located at the C terminus (e.g. auxilin), the center region (e.g. Rme-8) or the N terminus (e.g. CSPα). In addition, the DnaJ central region and DnaJ C-terminal region are absent in the type III J proteins. Hence, class III J proteins are the most diverse class, with only the ancient J domain in common. The high degree of conservation of the J domain throughout evolution makes a strong case for an important role by J proteins. It also raises the possibility that J proteins from *Escherichia coli*, *Saccharomyces cerevisiae*, *Plasmodium falciparum* (malaria), Simian virus 40, and polyomavirus may invade the mammalian J protein:Hsp70 network and recruit mammalian Hsp70 s. An unequivocal demonstration of such an invasion, however, is still lacking. In a given organism, J proteins are present in larger numbers than Hsp70 s. Furthermore, degenerate J proteins, with similarities to J proteins but lacking the conserved HPD tripeptide, have been identified as additional regulators of J protein function [11]. This current progress raises a number of intriguing questions. Why have so many J proteins evolved? How do these J protein:Hsp70 units coexist? Are Hsp70:J protein pairs coupled? How have neurons evolved to exploit J protein:Hsc70-induced conformational work?

Are J Proteins Neuroprotective?

As our understanding of the J protein machinery emerges, attention has shifted to
evidence that, in experimental models, J proteins are powerful inhibitors of neurodegeneration [20–22]. We now realize that disruption of chaperones (e.g. J protein, Hsp70, Hsp90) has severe consequences to cell survival [23–25]. In neurons, there are significant demands on cellular folding events. Complex interactions between multiple cellular components underlie synaptic transmission, a process that occurs with speed, precision, and plasticity. Synapses, therefore, are critical points of information transfer between neurons that are vulnerable to the risk of protein misfolding due to the conformational flexibility and speed required of the synaptic machinery. The cellular milieu is crowded and can favor off-pathway reactions that lead to protein misfolding, the generation of toxic aggregates, and ultimately cell death. Many disease states are linked to aberrant protein conformations, underscoring the importance of effective quality control for cell survival. Post-mitotic cells, such as neurons, can not dilute aggregated proteins through cell division, thereby further promoting the accumulation of misfolded proteins. Huntington's, Alzheimer's, Parkinson's, and prion diseases are caused by defects in protein folding, underlining the biological importance of the problem of aberrant protein folding in neurons. Numerous studies have evaluated the protective effect of high cellular levels of J proteins (e.g. Hsp40, Mrj, Hsj1) on the toxicity of mutant huntingtin with an expanded polyglutamine region [21;22;26–31]. Furthermore, it has been shown that high levels of misfolded huntingtin region blocks CSPα's chaperone activity, emphasizing the importance of the cellular balance between chaperones and misfolded proteins [32].

The J protein:Hsp70 network has emerged as a highly conserved and sophisticated quality-control system that supports the structural changes in proteins required in a wide array of cellular responses and protects the cell from dangerous misfolded species. Despite their obviously important role in enforcing the rapid and efficient regulation of protein conformation required for synaptic transmission, many unanswered questions remain regarding the J protein family's role in the prevention of neurodegeneration. We do not fully understand how J protein-driven conformational changes are optimized for different cellular roles. It is not yet known what prevents Hsp70:J protein units from disposing of potentially functional (remediable) proteins. An obstacle to identifying the kinetics of J protein folding and refolding pathways is the transience of the J protein:Hsp70:client protein species. Some of the better characterized J proteins (auxilin, GAK, CSPα, Rme-8, Hsp40, Hsj1, Mrj and Rdj2) are outlined below.

Auxilin

In their seminal study, Ungewickell et al. (1995) [33] demonstrated that the 100kDa cytosolic nerve-specific type III J protein auxilin (DnaJC6) potently stimulated the removal of clathrin coats from synaptic vesicles. Auxilin is the best understood mammalian J protein, and its critical role in uncoating clathrin from clathrin-coated vesicles during endocytosis has been well documented (reviewed: [34;35]). Not only does auxilin bind clathrin, but association with AP2, dynamin monophosphoinositol derivatives, and phosphatidylinositol bisphosphate are essential for auxilin function. During endocytosis, adaptor proteins and clathrin are recruited to the intracellular plasma membrane. Reversible clathrin exchange occurs between the membrane and the cytosol during the growth phase of clathrin-coated pits [36]. The clathrin coat recruits the molecular machinery that drives membrane curvature and the formation of invaginated pits. Like SNARE-induced membrane fusion in exocytosis, clathrin-induced membrane curvature in endocytosis requires energy only to dissociate protein complexes. Membrane fission releases the clathrin-coated vesicle. A burst of auxilin or GAK (see below) recruitment from the cytosol to the clathrin-coated pits occurs during the membrane constriction [17;18]. Clathrin is removed from a clathrin-coated vesicle either by the auxilin:Hsp70 or the GAK:Hsp70 (see below) chaperone unit. The auxilin recruitment that preceedes uncoating depends on an inositol phospholipid binding motif in auxilin and GAK, suggesting that a localized accumulation of specific lipids underlies auxilin/GAK recruitment. Deletion of auxilin in *Drosophila* is lethal, emphasizing the essential nature of clathrin exchange. The structure of auxilin's J domain has been determined by X-ray crystallography as well as nuclear magnetic resonance studies [37;38]. Auxilin has the characteristic J domain and HPD motif that interacts with Hsc70; however, it is unusual in that its J domain is located at its C terminus, helix IV is absent, and the loops between helices I-II and II-III are longer (Figs. 4 and 5).

GAK

GAK (Cyclin G-associated kinase) is a cytosolic J protein of 150kDa. Like auxilin, GAK is found in association with clathrin-coated vesicles [39;40] and is essential in clathrin exchange during endocytosis. However, unlike the neural specific auxilin, expression of GAK is ubiquitous. There is a very high homology between auxilin and GAK (35% amino acid identity whole protein; 84% J domain) but they

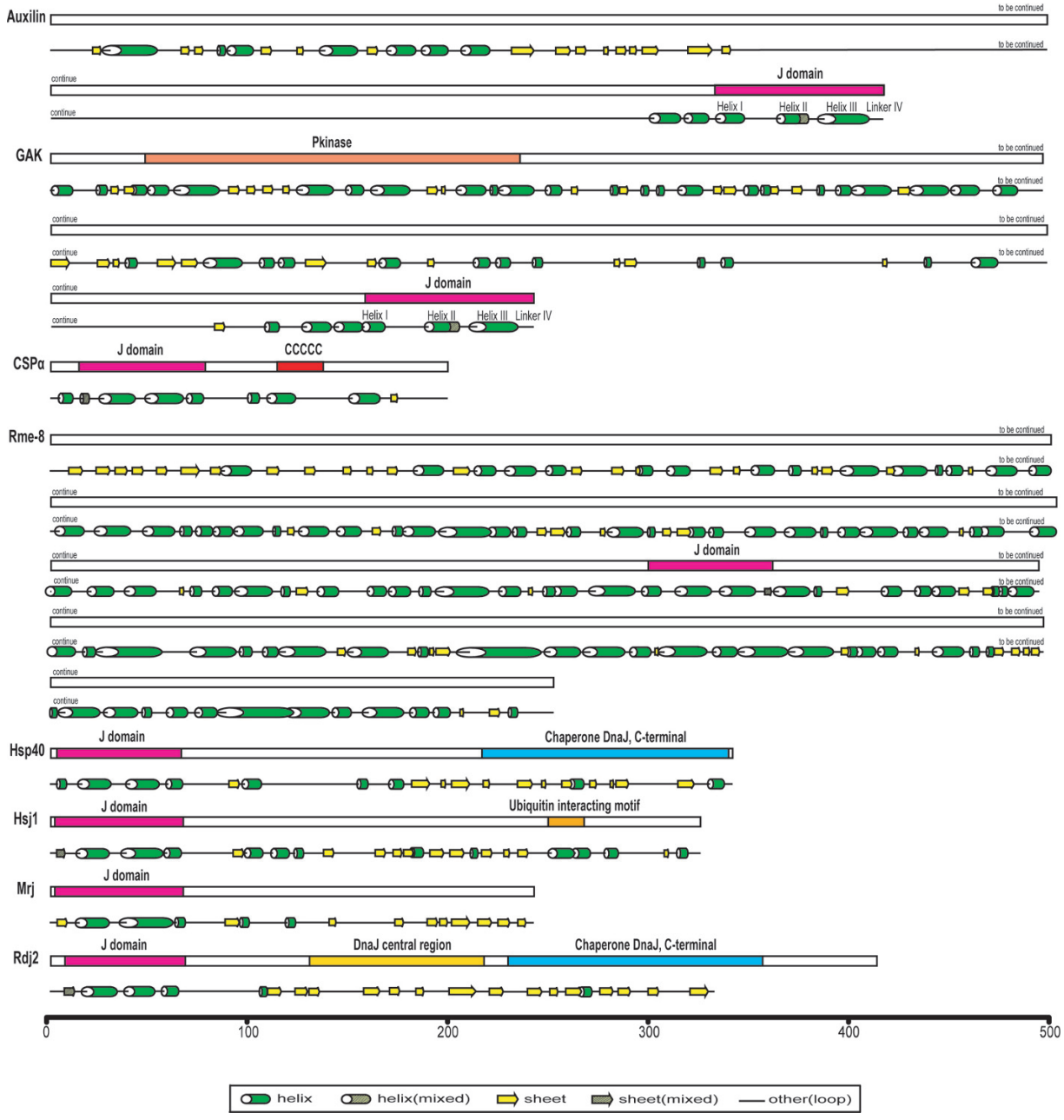

Figure 4. Domain alignments and secondary structure prediction of auxilin, GAK, CSPα, Rme-8, Hsp40, Hsj1, Mrj and Rdj2. InterProScan, and Predict Protein have been used to identify the domains and secondary structures, respectively. J domain of auxilin and GAK were selected based on [37]. Weak "helix" and "sheet" predictions within the J domain are marked as "mixed". Scale bar marks the length measured by amino acids.

differ in that GAK has an N-terminal kinase domain that has been shown to phosphorylate clathrin adaptor proteins AP1 and AP2 (Fig. 5). Deletion of GAK in mice, yeast, *C. elegans* and *Drosophila* is lethal [34], emphasizing the essential nature of clathrin exchange and its regulation by chaperones.

CSPα

CSPα (Cysteine string protein; DnaJC5), is a type III, 35kDa synaptic vesicle anchored J protein. The structure of the J domain of CSPα (mouse) has been reported (nuclear magnetic resonance study reported on the PDBsum website) [41]. The CSPα's J domain appears to be the classical signature J domain. Like auxilin and GAK, CSPα is important for synaptic vesicle function. In addition to the signature 'J domain', CSPα contains a distinctive cysteine string region, after which it is named. Most of the cysteine residues are palmitoylated, which anchors CSPα and CSPα-forced folding to the synaptic vesicle mem-

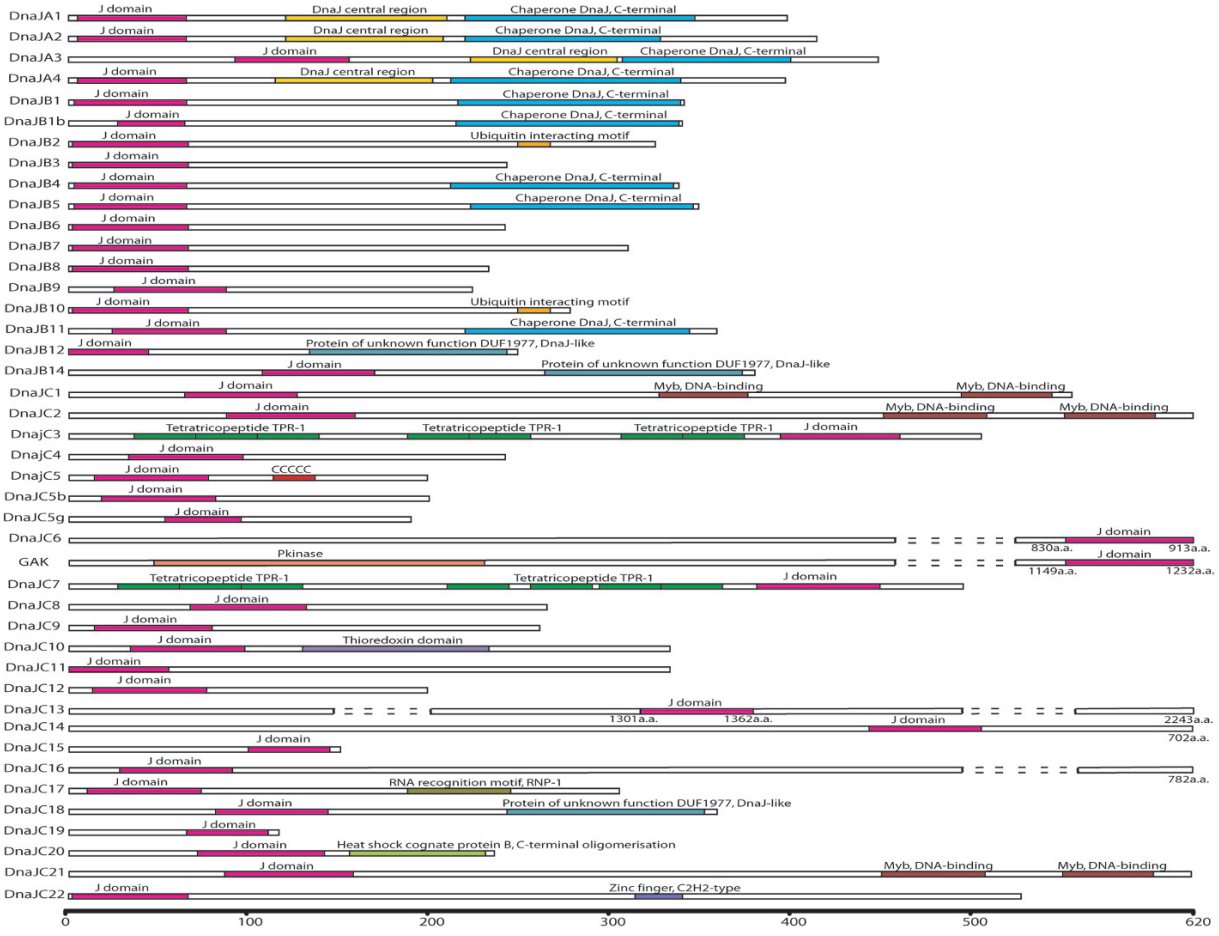

Figure 5. Domain alignments of 43 J proteins from *Homo sapiens* (DnaJB3 and DnaJB10 from *Mus musculus*). Interproscan has been used to identify the domains. J domain of auxilin and GAK were selected based on reference. Scale bar marks the length measured by amino acids. Thirteen domains were identified as follows: J domain; DnaJ central region; chaperone DnaJ, C-terminal; Zinc finger, C2H2-type; Ubiquitin interacting motif; Protein of unknown function DUF1977, DnaJ-like; Myb, DNA-binding; Thioredoxin domain; RNA recognition motif, RNP-1; Heat shock cognate protein B, C-terminal oligomerisation; Tetratricopeptide TPR-1; Pkinase protein kinase; and CCCC cysteine string region. J proteins are named according to [10], with the exception of GAK (Cyclin G-associated kinase) and DnaJC22 (previously DnaJA5). See http://www.ucalgary.ca/~braunj/index.html for accession numbers. We have reclassified the J protein originally identified as DnaJA5 [94], to DnaJC22. Domain analysis revealed that DnaJA5/DnaJC22 does not have a glycine/phenylalanine linker, a DnaJ central region or a chaperone DnaJ C-terminal like other J proteins of the type I designation.

brane [42–44]. CSPα interacts with Hsc70 and enhances its ATPase activity via its J domain [45;46]. Furthermore, together with Hsc70 and SGT (small glutamine-rich tetratricopeptide repeat domain protein), CSPα assembles into an enzymatically active chaperone complex [47;48]. HIP, a protein cofactor suggested to stabilize the interaction of J proteins with their target proteins, has also been found in association with the CSPα complex (not to be confused with huntingtin-interacting protein 14 (HIP14)). Exactly what the authentic substrate(s) is (are) for the CSPα chaperone system has been controversial, and separate schools of thought have emerged. Our lab has demonstrated that the CSPα chaperone system is a GEF (Guanine Nucelotide Exchange Factor) for $G_{\alpha s}$ [32;47;49;50] which, in turn, regulates Ca^{2+} channels [32;49;50]. $CSP\alpha_{1-198}$ is an inactive GEF on its own but is activated in the presence of SGT and Hsc70. Other reports conclude that CSPα has a direct role in the regulation of Ca^{2+} channel activity [51–53;53–57]. Finally, a number of studies deduce that CSPα is directly involved in exocytosis, independent of Ca^{2+} entry [58–63]. Specifically, it has been suggested that CSPα serves as a 'SNARE-repair chaperone', thereby modulating SNARE-induced membrane fusion [20]. Taken together, although the synaptic vesicle location of CSPα argues for highly specialized conformational work, current experimental evidence implicates more than one client protein.

Deletion of the CSPα gene severely impairs central and presynaptic transmission in *Drosophila melanogaster* [64–66]. *Drosophila* CSPα null mutants exhibit temperature sensitive paralysis and die as

larvae or within days of adulthood [64]. The small number of flies that survive to adulthood is characterized by spasmodic jumping, intense shaking, temperature sensitive paralysis, and premature death. Deletion of CSPα in mice causes no significant change at birth, but blindness and progressive neurodegeneration ensues, with no survival beyond four months [67;68]. A surprising finding by Chandra et al. demonstrated that the transgenic expression of α-synuclein, a protein implicated in Parkinson's disease pathology, abolished the neurodegeneration and lethality caused by CSPα deletion in mice [20]. The mechanistic basis by which synuclein abrogates the progressive neurodegeneration of CSPα null mutants is not yet known. CSPα is not restricted to neurons. In addition to its presence on synaptic vesicles [69] and clathrin-coated vesicles [40], CSPα is found on exocrine [70], endocrine [58] and neuroendocrine secretory granules [71;72], suggesting a conserved role in the secretory pathway.

Rdj2

In a recent study, Rdj2 (also called DjA2, Dj3, Dnj3, Cpr3, Hirip4 and DnaJA2), a widely expressed 48kDa type I J protein [73], was shown to enhance isoproterenol-mediated G protein signaling pathways (Rosales and Braun unpublished results). It is not yet known if Rdj2, like CSPα, has GEF activity for $G_{\alpha s}$ or if it chaperones a distinct aspect of the signaling pathway. In addition to its association with G proteins, Rdj2 has been shown to interact with PrP^c, the native nontoxic prion, suggesting that Rdj2:Hsc70 may have a role in maintaining PrP^c conformation [74]. Located in both cytosolic and membrane fractions, Rdj2 has been reported to undergo posttranslational lipid modification leading to membrane association [73].

Analysis reveals that Rdj2 has high homology with *Escherichia coli* DnaJ (38% amino acid identity) and *Saccharomyces cerevisae* Ydj1 (49% amino acid identity) along the length of the entire protein. The amino acid identity between the J domain of DnaJ-Rdj2 and Ydj1-Rdj2 is even higher, at 52% and 66% respectively. The J domain of Rdj2 is followed by a glycine/phenylaline linker region, a DnaJ-central, and DnaJ C-terminal region (Figs. 4 and 5). Rdj2 is found to assemble into a large, highly conserved multimeric chaperone complex with Hsc70 as well as Hsp110, Hsp90, HIP and HOP. It has been proposed that some J proteins are generalists capable of conformational work on many proteins, while other J proteins are specialists, dedicated to specific protein targets [11]. General chaperone activity by Rdj2:Hsp70 s is supported by the extensive DnaJ,Ydj1,Rdj2 amino acid identity; nonetheless, this is not a forgone conclusion and further experimentation is required to establish the role of mammalian Rdj2.

Rme-8

Rme-8 (receptor mediated endocytosis-8; DnaJC13), a 220kDa type III endosomal J protein, is one of the largest mammalian J proteins identified [75;76] (Figs. 4 and 5). It is ubiquitously expressed but not especially abundant in brain [76] and has been studied in *Caenorhabditis elegans*, *Drosophila melanogaster*, mammals, and plants. Rme-8 is required for endocytosis and endosomal trafficking [75–78]; however, the precise protein target for the Rme-8:Hsp70 unit remains unknown. Rme-8 localizes to endosomes in plants, *Drosophila melanogaster*, *Caenorhabditis elegans* and mammals. Deletion in *Caenorhabditis elegans* causes defects in endocytosis of yolk proteins [78], while deletion in *Arabidopsis* causes gravitropism defects [79]. In mammals, Rme-8 regulates cellular levels of epidermal growth factor receptor by influencing its trafficking through early endosomes [76]. Reduction in cellular levels of Rme-8 decreases epidermal growth factor receptor, implicating Rme-8 in some types of cancer [80]. Unlike most J proteins, the signature J domain is located in the center of Rme-8. Domain analysis predicts a unique J domain with three helices (I-III), the secondary structure of helix IV is a negotiable sheet/linker, suggesting that, like auxilin and GAK, Rme-8 could have a three-helix J domain. Thus, multiple J proteins (auxilin, GAK and Rme-8) operate in the endocytotic pathway; however, unlike auxilin and GAK, the mechanism by which Rme-8 alters endosomal trafficking is not currently understood.

Hsj1

Hsj1a (amino acids 1–277) and Hsj1b (amino acids 1–351) (DnaJB2, HSPF3) are neuronally-enriched type II 39kDa cytosolic J proteins that have different C termini generated by alternate splicing [81]. Hsj1 was first identified by screening neurofibrillar tangles associated with Alzheimer's disease [81]. In addition to the N-terminal signature J domain, both proteins contain ubiquitin-interacting motifs that bind ubiquitylated proteins [30] (Fig. 5). Hsj1 proteins have distinct intracellular localizations: Hsj1a is cytoplasmic with proteosome association, whereas the larger Hsj1b isoform is targeted to the cytoplasmic face of the endoplasmic reticulum through postranslational modification of the C terminus [82]. The Hsj1:Hsp70 pair is suggested to prevent client aggregation as well as the trimming of ubiquitin, thereby stimulating sorting of polyubiquitylated proteins to the proteosome for degradation. Ultimately, it is the mechanistic diversity among J proteins that provides the frame-

work for Hsp70 s to facilitate either protein re-folding (e.g. auxilin) or protein-degradation (e.g. Hsj1).

Mrj

Mrj (mammalian relative of DnaJ; DnaJB6, HHDj1, HSJ-2, MSJ-1) is a 26kDa widely expressed type II cytosolic J protein that is abundant in brain [22;83]. Compelling evidence suggests that the target substrate for Mrj:Hsp70 s are keratin-intermediate filaments, including both K8/K18 [84;85]. Deletion of Mrj in mice is lethal; the keratin cytoskeleton is disrupted and chorioallantoic attachment during placental development fails [83;85]. Keratin homeostasis is essential for a normal keratin cytoskeleton and deletion of Mrj prevents proteasomal degradation of keratin, resulting in the formation of keratin inclusion bodies and cell death [85]. In an independent study, Dai et al. [86] demonstrated that Mrj interacts with nuclear factor of activated T cells (NFATc3) to mediate transcriptional repression. It was further shown that, following heat shock (described below), Mrj translocates from the cytosol to the nucleus [86] and that Mrj is important for neural stem cell renewal (Watson and Cross, unpublished results). In addition to its roles in cellular keratin homeostasis and transcription, Mrj, like the J proteins Hsj1, Hsp40 and Rdj2, has been shown to reduce the toxicity of mutant huntingtin aggregates [22]. Thus, current evidence indicates that Mrj:Hsp70 units function in both the cytoplasm and nucleus to prevent cytotoxic intracellular inclusion bodies and gene expression.

Hsp40 and the Neural Heat Shock Response

Hsp40 (40kda heat shock protein) (DnaJB1, HSPF1, HDJ1, DJ1) is a widely expressed type II cytosolic J protein that is up-regulated in response to stress in most cell types. The structure of the signature J domain of Hsp40 has been determined by nuclear magnetic resonance [87], and the crystal structure of the putative peptide binding domain (residues 158–340) has been reported [88]. In Hsp40, the J domain is followed by a glycine/phenylaline linker region, and a chaperone DnaJ C-terminal region is located at the C terminus (Figs. 4 and 5). Cells that have been subjected to a conditioning stress (including, but not exclusive to, heat shock) become less receptive to subsequent stress. This transient stress-tolerant state is due to the activation of an ancient, evolutionarily conserved cellular program called the heat-shock response. After a conditioning stress, the expression of several chaperones is induced, which then enhances cell survival to subsequent insults (reviewed: [89]). Although the mechanistic basis of the stress-induced chaperone-cytoprotection is unknown, anti-apoptotic activity is widely thought to be due to up-regulation of the cellular machinery that rids the cell of misfolded proteins. In neurons, the heat shock response involves the translocation of molecular chaperones [90] and, in some cases, the association of heat shock proteins with lipid rafts [91] as well as the synthesis of stress-inducible chaperones. In addition to the fundamentally important constitutive J proteins outlined above, heat shock response rapidly induces stress chaperones. The transient, stress-induced chaperones include members of the J protein:Hsp70 network: Hsp70, which is 85% homologous to the constitutively expressed Hsc70, and Hsp40. Hsp40 is ubiquitous and is often expressed at low levels prior to heat shock. Although the severity of the stress required to trigger the transient stress-tolerant state varies among neurons, in *Drosophila*, a conditioning heat shock maintains synaptic function at temperatures that would normally be disruptive [92]. Although transient up-regulation of molecular chaperones is critical for cell survival, chronically high Hsp70 levels have been reported to correlate with poor prognosis in some types of cancer [3;4]. On a final note, manipulation of stress-induced chaperones offers potential strategies to protect brain cells from ischemia and neurodegenerative disease. In support of this possibility, Hsp40 has been shown to suppress polyglutamine pathogenicity of toxic mutant huntingtin in *Drosophila* [29] and prion toxicity in cell culture [93].

Concluding Remarks

Protein folding is the final step in the information transfer from gene to functional protein. As such, it is one of the most important processes of biology. J proteins are a central component of cellular folding processes that have become specialized in all three kingdoms over the course of evolution. Figure 6 shows the amino acid sequence conservation within the J domains of *Escherichia coli*, *Saccharomyces cerevisiae*, *Homo sapiens* and *Caenorhabditis elegans*. The height of symbols within the stack indicates the relative frequency of each amino acid at that position and emphasizes the conserved HPD tripeptide. While some J proteins are implicated in folding of newly synthesized proteins, others are implicated in regulating required conformational changes and, finally, others are implicated in protein triage. Only recently have we begun to appreciate the full array of Hsc70 conformational work in neurons. *Escherichia coli* and *Saccharomyces cerevisiae* Hsp70 s are better understood than their mammalian counterparts and, given the high degree of conservation, have provided valuable clues to both brain function and neurodegeneration.

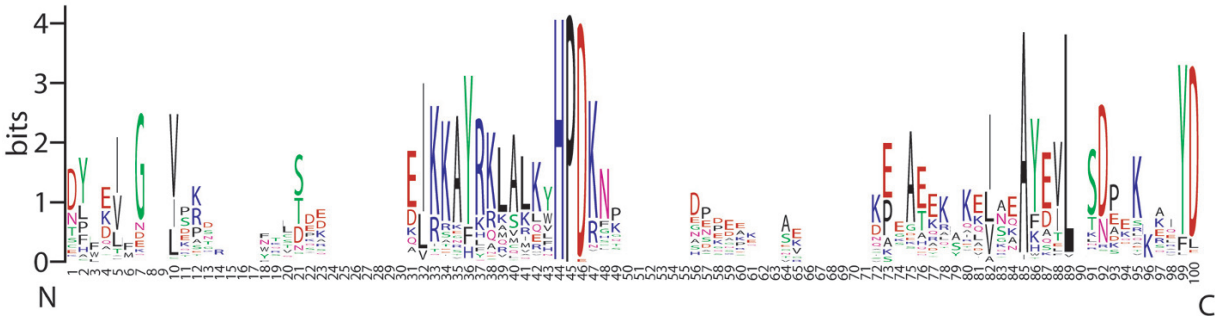

Figure 6. Alignment of J domains or J-like domains in 6 DnaJ proteins from *Escherichia coli*, 22 from *Saccharomyces cerevisiae*, 41 from *Homo sapiens* and 28 from *Caenorhabditis elegans*. CLUSTALW and WebLogo [95] were used for sequence alignment. The height of symbols within the stack indicates the relative frequency (conservation) of each amino acid at that position. The J domain is a ~70-amino acid sequence consisting of four helices and a loop region between helices II and III that contains a highly conserved tripeptide of histidine, proline, and aspartic acid (the HPD motif). See http://www.ucalgary.ca/~braunj/index.html for accession numbers.

A striking feature of the synapse is the highly dynamic nature of the protein-protein interactions. Synaptic proteins undergo conformational changes, while binding partners of a given synaptic protein are known to change frequently. Elaborate networks of signaling proteins trigger changes in synaptic strength. An important and poorly understood issue is how neurons maintain a balance between protecting proteins and preventing accumulation of misfolded proteins, ensuring integration of the many components underlying synaptic transmission. What cellular machinery underlies these surveillance, protection, and triage decisions? J protein:Hsp70 chaperone units are prime candidates. The molecular pathways that underlie the specific features of these J proteins remain under investigation, and may provide insights that eventually facilitate therapeutic manipulation of chaperones for neurodegenerative diesases.

Acknowledgements. JEAB and APB are Alberta Heritage Foundation for Medical Research Senior Scholars. We thank Dr. James Cross, University of Calgary, for the critical review of this manuscript.

1 Anfinsen C. B. (1973) Principles that govern the folding of protein chains. Science 181, 223–230.
2 Mayer M. P. and Bukau B. (2005) Hsp70 chaperones: cellular functions and molecular mechanism. Cell Mol. Life Sci 62, 670–684.
3 Morano K. A. (2007) New tricks for an old dog: the evolving world of Hsp70. Ann. N. Y. Acad Sci.
4 Brodsky J. L. and Chiosis G. (2006) Hsp70 molecular chaperones: emerging roles in human disease and identification of small molecule modulators. Curr. Top. Med. Chem 6, 1215–1225.
5 Daugaard M., Rohde M. and Jaattela M. (2007) The heat shock protein 70 family: Highly homologous proteins with overlapping and distinct functions. FEBS Lett.
6 Hartl F. U. (1996) Molecular chaperones in cellular protein folding. Nature 381, 571–579.
7 Sousa R. and Lafer E. M. (2006) Keep the traffic moving: mechanism of the Hsp70 motor. Traffic 7, 1596–1603.
8 Genevaux P., Georgopoulos C. and Kelley W. L. (2007) The Hsp70 chaperone machines of Escherichia coli: a paradigm for the repartition of chaperone functions. Mol. Microbiol. 66, 840–857.
9 Georgopoulos C. P., Lundquist-Heil A., Yochem J. and Feiss M. (1980) Identification of the E. coli dnaJ gene product. Mol. Gen. Genet 178, 583–588.
10 Qiu X. B., Shao Y. M., Miao S. and Wang L. (2006) The diversity of the DnaJ/Hsp40 family, the crucial partners for Hsp70 chaperones. Cell Mol. Life Sci.
11 Craig E. A., Huang P., Aron R. and Andrew A. (2006) The diverse roles of J-proteins, the obligate Hsp70 co-chaperone. Rev Physiol Biochem. Pharmacol 156, 1–21.
12 Cyr D. M., Langer T. and Douglas M. G. (1994) DnaJ like proteins: molecular chaperones and specific regulators of hsp70. TIBS 19, 176–181.
13 Cheetham M. E. and Caplan A. J. (1998) Structure, function and evolution of DnaJ: conservation and adaptation of chaperone function. Cell Stress. Chaperones. 3, 28–36.
14 Walsh P., Bursac D., Law Y. C., Cyr D. and Lithgow T. (2004) The J-protein family: modulating protein assembly, disassembly and translocation. EMBO Rep. 5, 567–571.
15 Ohtsuka K. and Hata M. (2000) Mammalian HSP40/DNAJ homologs: cloning of novel cDNAs and a proposal for their classification and nomenclature. Cell Stress. Chaperones. 5, 98–112.
16 Hennessy F., Nicoll W. S., Zimmermann R., Cheetham M. E. and Blatch G. L. (2005) Not all J domains are created equal: implications for the specificity of Hsp40-Hsp70 interactions. Protein Sci 14, 1697–1709.
17 Lee D. W., Wu X., Eisenberg E. and Greene L. E. (2006) Recruitment dynamics of GAK and auxilin to clathrin-coated pits during endocytosis. J Cell Sci 119, 3502–3512.
18 Massol R. H., Boll W., Griffin A. M. and Kirchhausen T. (2006) A burst of auxilin recruitment determines the onset of clathrin-coated vesicle uncoating. Proc Natl Acad Sci U S A 103, 10265–10270.
19 Rost B., Yachdav G. and Liu J. (2004) The PredictProtein server. Nucleic Acids Res 32, W321-W326.
20 Chandra S., Gallardo G., Fernandez-Chacon R., Schluter O. M. and Sudhof T. C. (2005) Alpha-synuclein cooperates with CSPalpha in preventing neurodegeneration. Cell 123, 383–396.
21 Muchowski P. J., Schaffar G., Sittler A., Wanker E. E., Hayer-Hartl M. J. and Hartl F. U. (2000) Hsp70 and Hsp40 chaperones

can inhibit self-assembly of polyglutamine proteins into amyloid fibrils. Proc Natl Acad Sci U S A 97, 7841–7846.
22 Chuang J. Z., Zhou H., Zhu M., Li S. H., Li X. J. and Sung C. H. (2002) Characterization of a brain-enriched chaperone, MRJ, that inhibits huntingtin aggregation and toxicity independently. J Biol Chem 277, 19831–19838.
23 Barral J. M., Broadley S. A., Schaffar G. and Hartl F. U. (2004) Roles of molecular chaperones in protein misfolding diseases. Semin. Cell Dev. Biol 15, 17–29.
24 Muchowski P. J. and Wacker J. L. (2005) Modulation of neurodegeneration by molecular chaperones. Nat Rev Neurosci 6, 11–22.
25 Macario A. J. and Conway dM. (2005) Sick chaperones, cellular stress, and disease. N. Engl. J Med. 353, 1489–1501.
26 Wyttenbach A., Carmichael J., Swartz J., Furlong R. A., Narain Y., Rankin J. and Rubinsztein D. C. (2000) Effects of heat shock, heat shock protein 40 (HDJ-2) and proteasome inhibition on protein aggregation in cellular models of Huntington's disease. Proc Natl Acad Sci U S A 97, 2898–2903.
27 Minami Y., Hohfeld J., Ohtsuka K. and Hartl F. U. (1996) Regulation of the heat-shock protein 70 reaction cycle by the mammalian DnaJ homolog, Hsp40. J Biol Chem 271, 19617–19624.
28 Zhou H., Li S. H. and Li X. J. (2001) Chaperone suppression of cellular toxicity of huntingtin is independent of polyglutamine aggregation. J Biol Chem 276, 48417–48424.
29 Bonini N. M. (2002) Chaperoning brain degeneration. Proc Natl Acad Sci U S A 99 Suppl 4, 16407–16411.
30 Howarth J. L., Kelly S., Keasey M. P., Glover C. P., Lee Y. B., Mitrophanous K., Chapple J. P., Gallo J. M., Cheetham M. E. and Uney J. B. (2007) Hsp40 molecules that target to the ubiquitin-proteasome system decrease inclusion formation in models of polyglutamine disease. Mol. Ther. 15, 1100–1105.
31 Jana N. R., Tanaka M., Wang G. and Nukina N. (2000) Polyglutamine length-dependent interaction of Hsp40 and Hsp70 family chaperones with truncated N-terminal huntingtin: their role in suppression of aggregation and cellular toxicity. Hum. Mol. Genet 9, 2009–2018.
32 Miller L. C., Swayne L. A., Chen L., Feng Z. P., Wacker J. L., Muchowski P. J., Zamponi G. W. and Braun J. E. A. (2003) Cysteine String Protein (CSP) inhibition of N-type calcium channels is blocked by mutant huntingtin. J Biol Chem 278, 53072–53081.
33 Ungewickell E., Ungewickell H., Holstein S. E.H., Linder R., Prasad K., Barouch W., Martin B., Greene L. E. and Eisenberg E. (1995) Role of auxilin in uncoating clathrin-coated vesicles. Nature 378, 632–635.
34 Eisenberg E. and Greene L. E. (2007) Multiple roles of auxilin and hsc70 in clathrin-mediated endocytosis. Traffic 8, 640–646.
35 Ungewickell E. J. and Hinrichsen L. (2007) Endocytosis: clathrin-mediated membrane budding. Curr. Opin Cell Biol 19, 417–425.
36 Wu X., Zhao X., Puertollano R., Bonifacino J. S., Eisenberg E. and Greene L. E. (2003) Adaptor and clathrin exchange at the plasma membrane and trans-Golgi network. Mol. Biol Cell 14, 516–528.
37 Jiang J., Taylor A. B., Prasad K., Ishikawa-Brush Y., Hart P. J., Lafer E. M. and Sousa R. (2003) Structure-function analysis of the auxilin J-domain reveals an extended Hsc70 interaction interface. Biochemistry 42, 5748–5753.
38 Gruschus J. M., Han C. J., Greener T., Ferretti J. A., Greene L. E. and Eisenberg E. (2004) Structure of the functional fragment of auxilin required for catalytic uncoating of clathrin-coated vesicles. Biochemistry 43, 3111–3119.
39 Greener T., Zhao X., Nojima H., Eisenberg E. and Greene L. E. (2000) Role of cyclin G-associated kinase in uncoating clathrin-coated vesicles from non-neuronal cells. J Biol Chem 275, 1365–1370.
40 Blondeau F., Ritter B., Allaire P. D., Wasiak S., Girard M., Hussain N. K., Angers A., Legendre-Guillemin V., Roy L., Boismenu D., Kearney R. E., Bell A. W., Bergeron J. J. and McPherson P. S. (2004) Tandem MS analysis of brain clathrin-coated vesicles reveals their critical involvement in synaptic vesicle recycling. Proc Natl Acad Sci U S A 101, 3833–3838.
41 kobayashi n, tomizawa t, koshiba s, inoue m, kigawa t and Yokoyama S. Solution structure of J-domain from mouse dnaj subfamily c member 5. 2008. Ref Type: Unpublished Work
42 Gundersen C. B., Mastrogiacomo A., Faull K. and Umbach J. A. (1994) Extensive lipidation of a Torpedo cysteine string protein. Journal of Biological Chemistry 269, 19197–19199.
43 Chamberlain L. H. and Burgoyne R. D. (1998) The cysteine-string domain of the secretory vesicle cysteine-string protein is required for membrane targeting. Biochem J 335 (Pt 2), 205–209.
44 Greaves J. and Chamberlain L. H. (2006) Dual Role of the Cysteine-String Domain in Membrane Binding and Palmitoylation-dependent Sorting of the Molecular Chaperone Cysteine-String Protein. Mol. Biol Cell.
45 Braun J. E., Wilbanks S. M. and Scheller R. H. (1996) The cysteine string secretory vesicle protein activates Hsc70 ATPase. J Biol Chem 271, 25989–25993.
46 Chamberlain L. H. and Burgoyne R. D. (1997) Activation of the ATPase activity of heat-shock proteins Hsc70/Hsp70 by cysteine-string protein. Biochem J 322 (Pt 3), 853–858.
47 Natochin M., Campbell T. N., Barren B., Miller L. C., Hameed S., Artemyev N. O. and Braun J. E. (2005) Characterization of the G alpha(s) regulator cysteine string protein. J Biol Chem 280, 30236–30241.
48 Tobaben S., Thakur P., Fernandez-Chacon R., Sudhof T. C., Rettig J. and Stahl B. (2001) A trimeric protein complex functions as a synaptic chaperone machine. Neuron 31, 987–999.
49 Magga J. M., Jarvis S. E., Arnot M. I., Zamponi G. W. and Braun J. E. (2000) Cysteine string protein regulates G-protein modulation of N-type calcium channels. Neuron 28, 195–204.
50 Miller L. C., Swayne L. A., Kay J. G., Feng Z. P., Jarvis S. E., Zamponi G. W. and Braun J.E.A. (2003) Molecular Detrminants of Cysteine String Protien Modulation of N-type Calcium Channels. Journal of Cell Science 116, 2967–2974.
51 Gundersen C. B. and Umbach J. A. (1992) Suppression cloning of the cDNA for a candidate subunit of a presynaptic calcium channel. Neuron 9, 527–537.
52 Buchner E. and Gundersen C. B. (1997) The DnaJ-like cysteine string protein and exocytotic neurotransmitter release. Trends Neurosci 20, 223–227.
53 Chen S., Zheng X., Schulze K. L., Morris T., Bellen H. and Stanley E. F. (2002) Enhancement of presynaptic calcium current by cysteine string protein. J Physiol 538, 383–389.
54 Umbach J. A. and Gundersen C. B. (1997) Evidence that cysteine string proteins regulate an early step in the Ca2+-dependent secretion of neurotransmitter at Drosophila neuromuscular junctions. J Neurosci 17, 7203–7209.
55 Ranjan R., Bronk P. and Zinsmaier K. E. (1998) Cysteine string protein is required for calcium secretion coupling of evoked neurotransmission in drosophila but not for vesicle recycling. J Neurosci 18, 956–964.
56 Leveque C., Pupier S., Marqueze B., Geslin L., Kataoka M., Takahashi M., De Waard M. and Seagar M. (1998) Interaction of cysteine string proteins with the alpha1A subunit of the P/Q-type calcium channel. Journal of Biological Chemistry 273, 13488–13492.
57 Umbach J. A., Saitoe M., Kidokoro Y. and Gundersen C. B. (1998) Attenuated influx of calcium ions at nerve endings of csp and shibire mutant Drosophila. J Neurosci 18, 3233–3240.
58 Brown H., Larsson O., Branstrom R., Yang S., Leibiger B., Leibiger I., Fried G., Moede T., Deeney J. T., Brown G. R., Jacobsson G., Rhodes C. J., Braun J. E., Scheller R. H., Corkey B. E., Berggren P. and Meister B. (1998) Cysteine string protein (CSP) is an insulin secretory granule-associated protein regulating beta-cell exocytosis. EMBO J 17, 5048–5058.
59 Chamberlain L. H. and Burgoyne R. D. (1998) Cysteine string protein functions directly in regulated exocytosis. Mol Cell Biol 9, 2259–2267.

60 Morales M., Ferrus A. and Martinez-Padron M. (1999) Presynaptic calcium-channel currents in normal and csp mutant Drosophila peptidergic terminals. Eur J Neurosci 11, 1818–1826.
61 Dawson-Scully K., Bronk P., Atwood H. L. and Zinsmaier K. E. (2000) Cysteine-string protein increases the calcium sensitivity of neurotransmitter exocytosis in Drosophila. J Neurosci 20, 6039–6047.
62 Graham M. E. and Burgoyne R. D. (2000) Comparison of cysteine string protein (CSP) and mutant alpha-SNAP overexpression reveals a role for CSP in late steps of membrane fusion in dense-core granule exocytosis in adrenal chromaffin cells. J Neurosci 20, 1281–1289.
63 Swayne L. A., Beck K. E. and Braun J. E. (2006) The cysteine string protein multimeric complex. Biochem. Biophys. Res Commun. 348, 83–91.
64 Zinsmaier K. E., Eberle K. K., Buchner E., Walter N. and Benzer S. (1994) Paralysis and early death in cysteine string protein mutants of Drosophila. Science 263, 977–980.
65 Umbach J. A., Zinsmaier K. E., Eberle K. K., Buchner E., Benzer S. and Gundersen C. B. (1994) Presynaptic dysfunction in Drosophila csp mutants. Neuron 13, 899–907.
66 Barclay J. W., Atwood H. L. and Robertson R. M. (2002) Impairment of central pattern generation in Drosophila cysteine string protein mutants. J Comp Physiol A Neuroethol. Sens. Neural Behav. Physiol 188, 71–78.
67 Chandra S., Gallardo G., Fernandez-Chacon R., Schluter O. M. and Sudhof T. C. (2005) alpha-Synuclein Cooperates with CSPalpha in Preventing Neurodegeneration. Cell 123, 383–396.
68 Fernandez-Chacon R., Wolfel M., Nishimune H., Tabares L., Schmitz F., Castellano-Munoz M., Rosenmund C., Montesinos M. L., Sanes J. R., Schneggenburger R. and Sudhof T. C. (2004) The synaptic vesicle protein CSP alpha prevents presynaptic degeneration. Neuron 42, 237–251.
69 Mastrogiacomo A., Parsons S. M., Zampighi G. A., Jenden D. J., Umbach J. A. and Gundersen C. B. (1994) Cysteine string proteins: a potential link between synaptic vesicles and presynaptic Ca2+ channels. Science 263, 981–982.
70 Braun J. E. and Scheller R. H. (1995) Cysteine string protein, a DnaJ family member, is present on diverse secretory vesicles. Neuropharmacology 34, 1361–1369.
71 Kohan S. A., Pescatori M., Brecha N. C., Mastrogiacomo A., Umbach J. A. and Gundersen C. B. (1995) Cysteine string protein immunoreactivity in the nervous system and adrenal gland of rat. J Neurosci 15, 6230–6238.
72 Chamberlain L. H., Henry J. and Burgoyne R. D. (1996) Cysteine string proteins are associated with chromaffin granules. Journal of Biological Chemistry 271, 19514–19517.
73 Andres D. A., Shao H., Crick D. C. and Finlin B. S. (1997) Expression cloning of a novel farnesylated protein, RDJ2, encoding a DnaJ protein homologue. Arch. Biochem. Biophys. 346, 113–124.
74 Beck K. E., Kay J. G. and Braun J. E. (2006) Rdj2, a J protein family member, interacts with cellular prion PrP(C). Biochem. Biophys. Res Commun. 346, 866–871.
75 Chang H. C., Hull M. and Mellman I. (2004) The J-domain protein Rme-8 interacts with Hsc70 to control clathrin-dependent endocytosis in Drosophila. J Cell Biol 164, 1055–1064.
76 Girard M., Poupon V., Blondeau F. and McPherson P. S. (2005) The DnaJ-domain protein RME-8 functions in endosomal trafficking. J Biol Chem 280, 40135–40143.
77 Silady R. A., Ehrhardt D. W., Jackson K., Faulkner C., Oparka K. and Somerville C. R. (2008) The GRV2/RME-8 protein of Arabidopsis functions in the late endocytic pathway and is required for vacuolar membrane flow. Plant J 53, 29–41.
78 Zhang Y., Grant B. and Hirsh D. (2001) RME-8, a conserved J-domain protein, is required for endocytosis in Caenorhabditis elegans. Mol. Biol Cell 12, 2011–2021.
79 Silady R. A., Kato T., Lukowitz W., Sieber P., Tasaka M. and Somerville C. R. (2004) The gravitropism defective 2 mutants of Arabidopsis are deficient in a protein implicated in endocytosis in Caenorhabditis elegans. Plant Physiol 136, 3095–3103.
80 Girard M. and McPherson P. S. (2008) RME-8 regulates trafficking of the epidermal growth factor receptor. FEBS Lett. 582, 961–966.
81 Cheetham M. E., Brion J. P. and Anderton B. H. (1992) Human homologues of the bacterial heat-shock protein DnaJ are preferentially expressed in neurons. Biochem. J 284 (Pt 2), 469–476.
82 Chapple J. P. and Cheetham M. E. (2003) The chaperone environment at the cytoplasmic face of the endoplasmic reticulum can modulate rhodopsin processing and inclusion formation. J Biol Chem 278, 19087–19094.
83 Hunter P. J., Swanson B. J., Haendel M. A., Lyons G. E. and Cross J. C. (1999) Mrj encodes a DnaJ-related co-chaperone that is essential for murine placental development. Development 126, 1247–1258.
84 Izawa I., Nishizawa M., Ohtakara K., Ohtsuka K., Inada H. and Inagaki M. (2000) Identification of Mrj, a DnaJ/Hsp40 family protein, as a keratin 8/18 filament regulatory protein. J Biol Chem 275, 34521–34527.
85 Watson E. D., Geary-Joo C., Hughes M. and Cross J. C. (2007) The Mrj co-chaperone mediates keratin turnover and prevents the formation of toxic inclusion bodies in trophoblast cells of the placenta. Development 134, 1809–1817.
86 Dai Y. S., Xu J. and Molkentin J. D. (2005) The DnaJ-related factor Mrj interacts with nuclear factor of activated T cells c3 and mediates transcriptional repression through class II histone deacetylase recruitment. Mol. Cell Biol 25, 9936–9948.
87 Qian Y. Q., Patel D., Hartl F. U. and McColl D. J. (1996) Nuclear magnetic resonance solution structure of the human Hsp40 (HDJ-1) J-domain. J Mol. Biol 260, 224–235.
88 Hu J., Wu Y., Li J., Qian X., Fu Z. and Sha B. (2008) The crystal structure of the putative peptide-binding fragment from the human Hsp40 protein Hdj1. BMC. Struct. Biol 8, 3.
89 Brown I. R. (2007) Heat shock proteins and protection of the nervous system. Ann. N. Y. Acad Sci.
90 Manzerra P. and Brown I. R. (1996) The neuronal stress response: nuclear translocation of heat shock proteins as an indicator of hyperthermic stress. Exp Cell Res 229, 35–47.
91 Chen S., Bawa D., Besshoh S., Gurd J. W. and Brown I. R. (2005) Association of heat shock proteins and neuronal membrane components with lipid rafts from the rat brain. J Neurosci Res 81, 522–529.
92 Karunanithi S., Barclay J. W., Brown I. R., Robertson R. M. and Atwood H. L. (2002) Enhancement of presynaptic performance in transgenic Drosophila overexpressing heat shock protein Hsp70. Synapse 44, 8–14.
93 Rambold A. S., Miesbauer M., Rapaport D., Bartke T., Baier M., Winklhofer K. F. and Tatzelt J. (2006) Association of Bcl-2 with misfolded prion protein is linked to the toxic potential of cytosolic PrP. Mol. Biol Cell 17, 3356–3368.
94 Chen J., Yin G., Lu Y., Lou M., Cheng H., Ni X., Hu G., Luo C., Ying K., Xie Y. and Mao Y. (2004) Cloning and characterization of a novel human cDNA encoding a J-domain protein (DNAJA5) from the fetal brain. Int. J Mol. Med. 13, 735–740.
95 Crooks G. E., Hon G., Chandonia J. M. and Brenner S. E. (2004) WebLogo: a sequence logo generator. Genome Res 14, 1188–1190.