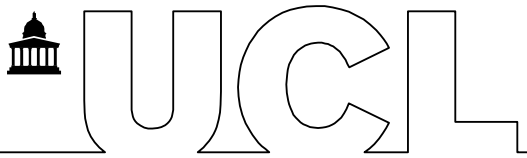

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Review

Molecular chaperones and neuronal proteostasis

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Abbreviations

17-AAG 17-Allylamino-17-demethoxygeldanamycin; 17-DMAG 17-Dimethylamino-ethylamino-17-demethoxygeldanamycin; Aβ amyloid beta; AD Alzheimer's disease; ALS amyotrophic lateral sclerosis; ARSACS Autosomal recessive spastic ataxia of Charlevoix-Saguenay; α-syn α-synuclein; CMT2 Charcot-Marie-Tooth type 2; dHMN distal hereditary motor neuropathies; DRP1 dynamin-related protein 1; FTDP-17 Frontotemporal dementia with parkinsonism linked to chromosome 17; GF glycine-phenylalanine; HD Huntington's disease; HPD histidine-proline-aspartate; Hsps Heat shock proteins; Htt huntingtin; MPTP 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NBD Nucleotide binding domain; NEFs nucleotide exchange factors; PD Parkinson's disease; polyQ polyglutamine; p-tau hyperphosphorylated tau; Rho rhodopsin; SBD substrate binding domain; SOD1 superoxide dismutase; UIMs ubiquitin interacting motifs

Abstract

Protein homeostasis (proteostasis) is essential for maintaining the functionality of the proteome. The disruption of proteostasis, due to genetic mutations or an age-related decline, leads to aberrantly folded proteins that typically lose their function. The accumulation of misfolded and aggregated protein is also cytotoxic and has been implicated in the pathogenesis of neurodegenerative diseases. Neurons have developed an intrinsic protein quality control network, of which molecular chaperones are an essential component. Molecular chaperones function to promote efficient folding and target misfolded proteins for refolding or degradation. Increasing molecular chaperone expression can suppress protein

aggregation and toxicity in numerous models of neurodegenerative disease; therefore, molecular chaperones are considered exciting therapeutic targets. Furthermore, mutations in several chaperones cause inherited neurodegenerative diseases. In this review, we focus on the importance of molecular chaperones in neurodegenerative diseases, and discuss the advances in understanding their protective mechanisms.

Introduction

Proteins must fold to their native state in order to achieve functionality. However, in the crowded cellular environment, and under environmental and physiological stress conditions such as heat, oxidative stress and inflammation, proteins are susceptible to the formation of non-native interactions that can lead to protein misfolding and aggregation. The accumulation of misfolded and aggregated protein is considered toxic to the cell and is implicated in numerous diseases such as type 2 diabetes, cardiovascular disease and neurodegenerative diseases. Cells have therefore developed an intrinsic network of protein quality control machinery that functions to balance protein folding, misfolding, aggregation and degradation, thereby maintaining protein homeostasis (proteostasis) and the functionality of the proteome. The protein quality control machinery includes molecular chaperones, which act as the first line of defence and participate in the refolding or, alternatively, the degradation of misfolded proteins. Their role in maintaining proteostasis in neurons, which are post-mitotic cells that are particularly vulnerable to protein aggregation, is the subject of this review.

Molecular chaperones

Molecular chaperones are defined as proteins that interact with, stabilise or assist another protein to gain its native and functionally active conformation, without being present in the final structure [1]. Members of the molecular chaperone family are often referred to as heat-shock proteins (Hsps) due to their upregulation under stress conditions that typically destabilise proteins, such as elevated temperature and oxidative stress. Molecular chaperones are often classified according to their molecular weight and members include Hsp90, Hsp70, Hsp60, Hsp40 (DnaJ) and the small Hsps. Molecular chaperones display large functional diversity and in addition to their fundamental roles in *de novo* folding, and the refolding of misfolded protein, chaperones also regulate critical cellular processes such as protein trafficking, protein degradation and macromolecular complex assembly [2].

Hsp90 (HSPC) family

Hsp90 is an ATP-dependent chaperone that functions in the activation and stabilisation of client proteins; including protein kinases, cell cycle regulators, cell surface receptors and transcription factors. Therefore, Hsp90 plays a critical role in cellular processes including signal transduction, cell cycle progression, apoptosis and protein degradation [3]. The activation of Hsp90 clients is driven by a cycle of substrate binding and release mediated by a series of conformational changes in the chaperone and an ATP-induced transition between an open and a closed conformation. Hsp90 exists as a homodimer, with each subunit consisting of three domains; an N-terminal ATP-binding domain (N-domain), a middle domain that binds the substrate (M-domain) and a C-terminal dimerisation domain (C-domain). In the absence of nucleotide Hsp90 adopts a V-shaped open conformation. The binding of ATP to the N-domain induces a conformational change that closes a lid over the nucleotide binding pocket. Following lid closure, the N-domains dimerise, forming a compact structure with a closed conformation. The formation of the closed dimer induces ATP hydrolysis, subsequently promoting the N-domains to dissociate and the return of Hsp90 to the open conformation, with the release of the substrate [4].

The reaction cycle of Hsp90 is regulated by various co-chaperones. The co-chaperones exhibit specific binding preferences for different Hsp90 conformations and affect different stages of the cycle, such as client binding and ATP hydrolysis. Co-chaperones therefore usually co-operate in a sequential cycle to facilitate the maturation of Hsp90 clients.

Hsp70 (HSPA) family

Hsp70 functions in a wide array of cellular processes including the folding of newly synthesised protein, the refolding of misfolded and aggregated protein, transport of proteins across membranes, and protein degradation [3]. These functions rely on the ability of Hsp70 to interact with hydrophobic stretches exposed in client proteins and subsequently undergo an ATP-dependent cycle of substrate binding and release. Hsp70 is composed of a N-terminal ATPase domain (NBD) and a C-terminal substrate binding domain (SBD), divided into subdomains that form a hydrophobic binding pocket and a lid [2]. The NBD and SBD are connected by a flexible linker that enables the NBD to allosterically control the conformation of the SBD. In the ATP-bound state the binding pocket and lid are in an open conformation. The SBD has a low substrate affinity and fast substrate exchange rates. The hydrolysis of ATP to ADP drives the SBD into a high affinity state for substrate binding through the closing of the lid, enabling stable binding of the client protein. The release of ADP and the rebinding of ATP triggers the opening of the lid and the subsequent unloading of the bound substrate.

The Hsp70 reaction cycle contains two rate limiting steps, ATP hydrolysis, due to low basal ATPase activity, and ADP dissociation, due to the high levels of cytoplasmic ATP under physiological conditions. Therefore, Hsp70 requires the action of co-chaperones to facilitate the reaction cycle between ATP and ADP bound states. The ATPase activity of Hsp70 is stimulated by members of the DnaJ (Hsp40) family via their conserved J domain [5]. The dissociation of ADP requires the opening of the nucleotide binding cleft, a process catalysed by a range of nucleotide exchange factors (NEFs), including the Hsp70-like Hsp110 proteins, HspBP1, SIL1 and BAG family [6].

DnaJ (Hsp40) family

As mentioned above, members of the DnaJ family (also referred to as Hsp40) are important regulators of Hsp70 activity by stimulating ATP hydrolysis. All DnaJ proteins contain a J domain, a conserved region of 70 amino acids that folds into four α-helices. Helix II and helix III form an antiparallel two-helix bundle, with a loop connecting the two helices containing a histidine-proline-aspartate (HPD) motif. The HPD motif is critical for lowering the activation energy of ATP hydrolysis [7].

The human genome encodes 49 DnaJ proteins, which can be divided into three classes according to their domain composition [8]. Class I (DNAJA) proteins share all domains present in the *Escherichia coli* DnaJ protein, with an N-terminal J domain, a glycine-phenylalanine (GF) rich region, a zinc binding domain and a C-terminal domain. Class II (DNAJB) proteins contain an N-terminal J domain and a GF rich region, whereas class III (DNAJC) proteins share only the J domain. The diversification of domains outside the J domain has enabled DnaJ proteins to adopt specialised functions. For example, domains that target DnaJ proteins to precise intracellular locations to promote the interaction of Hsp70 with specific clients. Furthermore, client binding domains enable some DnaJ proteins to deliver clients to the SBD of Hsp70. Specialised domains also facilitate the targeting of clients for degradation, thus providing an important link between Hsp70, misfolded proteins and degradation [7,8].

Hsp60 (HSPD/E and CCT) family

Hsp60, also called chaperonins, are large double ring complexes that enclose a central cavity. Substrate proteins, typically folding intermediates, are encapsulated in the central cavity, thereby shielding exposed hydrophobic residues from aggregation and allowing the substrate to fold in a protected environment. Chaperonins can be divided to two subgroups. Group I (HSPD) chaperonins are present in bacteria (GroEL) and the mitochondrial matrix (Hsp60). They contain 7 subunits per ring and require co-chaperones (HSPE) that act as lids over the complex (GroES in bacteria and Hsp10 in mitochondria). Group II chaperonins are found in archaea (thermosome) and in the cytosol of eukaryotes (CCT or TRiC). They typically have 8 subunits per ring and do not require co-chaperones.

The GroEL-GroES chaperonin in *E. coli* has been the most extensively studied [9]. Each subunit consists of an equatorial ATP binding domain, a hinge domain and an apical substrate binding domain. The apical domain forms the entrance to the GroEL cavity and exposes hydrophobic residues to mediate substrate binding. Following substrate binding to one of the rings, ATP binds to each of the 7 subunits, inducing conformational changes, enabling the association of GroES and the subsequent encapsulation of the substrate. Upon GroES binding, the GroEL cavity enlarges, creating an environment for productive protein folding. The hydrolysis of ATP to ADP triggers the dissociation of the GroES lid and the release of the substrate. Multiple folding cycles may be required before the substrate reaches its native state [2].

Small HSP (HSPB) family

Small heat shock proteins differ from the other major molecular chaperones in that they are ATP independent. The human genome encodes 10 small Hsps and they range in size from 12-42 kDa. They share a conserved 100 amino acid α-crystallin domain that is flanked by variable N-terminal and C-terminal extensions. These extensions mediate substrate recognition as well as enabling the formation of oligomers. Small Hsps are able to bind to unfolded or misfolded protein and prevent their aggregation until the protein can be transferred to other cellular systems, such as the Hsp70-Hsp40 system [10].

Protein aggregates are a hallmark of neurodegenerative diseases

Neurodegenerative diseases are caused by the progressive degeneration of neurons in the brain, eye and spinal cord. The loss of specific populations of neurons defines each disease and ultimately determines the clinical manifestations of the disease. The diseases can present as sporadic or familial, which typically share common symptoms and disease progression, thus the modes of neuronal dysfunction and death are likely to be similar in the sporadic and inherited cases.

A pathological hallmark of many neurodegenerative diseases is the presence of ubiquitylated protein aggregates, indicating disturbances in proteostasis. Indeed, many of the causative mutations identified in familial cases lead to the misfolding and aggregation of the disease-related protein, for example, superoxide dismutase 1 (SOD1) in amyotrophic lateral sclerosis (ALS), huntingtin in Huntington's disease (HD), α-synuclein in Parkinson's disease (PD), amyloid beta (Aβ) in Alzheimer's disease (AD), and tau in FTDP-17. Aging is considered the most significant risk factor for sporadic cases of ALS, AD and PD. It is well established that aging results in a decline in the efficiency of the protein quality control machinery [11], although the cause for this decline is currently unknown. The age-associated decline in protein quality control has been suggested to lead to an imbalance in the production of misfolded protein, which slowly overwhelms the protective capacity of the

molecular chaperone machinery, ultimately leading to a vicious cycle which results in the collapse of proteostasis and causes neurodegeneration [2].

The microscopically visible inclusions seen pathologically are thought to represent the end point of the protein aggregation process, which begins with the formation of small oligomers, protofibrils and mature fibrils. The exact species that exerts cytotoxicity is unknown; however, accumulating evidence suggests that soluble oligomers and protofibrils pose the most toxic potential to the cell. In support of this hypothesis, in vivo studies have shown that the injection of rats with soluble oligomers of α-synuclein or Aβ showed increased neuronal loss compared to rats injected with fibril forms of the proteins [12,13]. The formation of large aggregates is thought to be a protective defence mechanism adopted by the cell in order to sequester the toxic oligomers and protofibrils and prevent their interaction with other proteins. Although not considered the most toxic entity, large aggregates are still likely to contribute to cytotoxicity and disease progression in neurons due to their ability to recruit other proteins and physically obstruct axonal transport and other cellular processes. Neurons are highly polarised cells and rely heavily on axonal transport between the cell body and the synaptic terminal in order to effectively sustain their function. Neurons are therefore vulnerable to protein aggregation and thus place a high demand on the molecular chaperone machinery in order to maintain proteostasis.

The upregulation of molecular chaperones is neuroprotective

Considering the protective nature of molecular chaperones, their upregulation is a promising therapeutic approach to combat the progression of neurodegenerative disease. Proof of principle studies have suggested this is a potential strategy for the treatment of many neurodegenerative diseases by increasing the expression of individual chaperones (Table 1) or groups of chaperones (Table 2). Some of these studies are discussed in detail below.

PD

PD is caused by the progressive loss of dopaminergic neurons in the substantia pars compacta. The disease affects around 1% of the population aged 65 or over and is characterised by bradykinesia, rigidity and tremor [14]. A pathological hallmark of PD is the presence of intracellular protein aggregates called Lewy bodies that are primarily composed of ubiquitylated α-synuclein [15]. Hsp70 is the most widely studied molecular chaperone in relation to α-synuclein aggregation, and the overexpression of Hsp70 has been shown to regulate α-synuclein aggregation both in vitro and in vivo (Table 1). In a cell culture model, the overexpression of Hsp70 reduced high molecular weight and detergent insoluble α-synuclein, as well as reducing total α-synuclein levels. This corresponded with a reduction in α-synuclein-induced toxicity [16]. Consistent with these findings, the expression of Hsp70 in an α-synuclein transgenic mouse model was also able to reduce high molecular weight and detergent insoluble species [16]. Further in vivo studies in both *Drosophila melanogaster* and mice showed that the Hsp70-mediated reduction in α-synuclein aggregation corresponded with an increase in dopaminergic neuron survival, enabling the preservation of striatal dopamine levels [17,18]. The mechanism by which Hsp70 suppresses α-synuclein aggregation is proposed to rely on the inhibition of fibril formation. Huang *et al.* (2006) showed that Hsp70 retarded the formation of α-synuclein prefibrils by binding to these species to inhibit nuclei formation [19]. Furthermore, Hsp70 was also found to bind to nuclei already present on the prefibrils to prevent their elongation.

AD

AD is the most common neurodegenerative disease, affecting over 500,000 people in the UK. The disease primarily affects the hippocampus and cerebral cortex regions of the brain, causing impairments in memory, cognitive function and difficulties with language. AD is

characterised by the presence of extracellular Aβ plaques and intracellular neurofibrillary tangles of hyperphosphorylated tau. Several chaperones have been shown to modulate Aβ aggregation in vitro. Both αβ-crystallin (HSPB5), a member of the small Hsp family, and DnaJB6 bind to Aβ fibrils, inhibiting their elongation and growth [20,21]. Hsp70, Hsp40 and Hsp90 have also been shown to interact with Aβ peptides, this time acting on Aβ oligomers [22]. Recombinant Hsp70/Hsp40 and Hsp90 significantly slowed the rate of Aβ aggregation, re-directing Aβ into soluble circular structures instead of forming fibrils. However, Hsp70/40 or Hsp90 had little effect on aggregation when added to pre-formed Aβ oligomers or fibrils. Only the combination of all three chaperones was able to significantly alter the structure of pre-formed oligomers. The data supported the concept that the upregulation of a combination of chaperones, rather than individual chaperones, may be more effective in combating Aβ aggregation (Table 2). This concept has been explored through the use of the Hsp90 inhibitor, 17-AAG, which activates the transcription factor HSF1, promoting the expression of a range of chaperones including Hsp70, Hsp40 and Hsp60. The upregulation of these chaperones protected neurons against Aβ toxicity both in vitro and in vivo. In neuronal cultures, 17-AAG restored Aβ-induced damage to dendritic structures [23]. Consistent with the in vitro data, the treatment of mice with 17-AAG protected neurons against Aβ-induced synaptic damage, enhancing long term potentiation and leading to an improvement in memory function [23].

Hsp90 inhibition also protects against tau aggregation (Table 2). Tau is a client of Hsp90, binding via its aggregation prone microtubule binding repeat region [24]. The inhibition of Hsp90 prevents the stabilisation and maturation of tau, resulting in its degradation. In vitro studies have demonstrated that Hsp90 inhibition reduces the steady state levels of tau in addition to decreasing the levels of insoluble aggregated tau [25,26]. The reduction in tau aggregation was abolished in the presence of MG132, confirming that Hsp90 inhibition facilitates the proteasomal degradation of tau [27]. These findings were confirmed in vivo, with 17-AAG treatment reducing insoluble and hyperphoshorylated tau in a mouse model of tauopathy [27].

HD and polyQ diseases

HD is an autosomal dominant, genetic disease that leads to progressive degeneration in the striatum and cortex of the brain, resulting in impairments in motor, cognitive and psychiatric abilities. HD is caused by the expansion of a CAG triplet repeat region in the gene *Huntingtin*. The translated protein contains a long stretch of polyglutamines (polyQ), driving huntingtin aggregation. Other CAG expansion diseases also have aggregation prone polyQ expanded polypeptides and show similarities in disease pathogenesis.

The first ever report that molecular chaperones could act as potent modulators of protein aggregation in neurodegeneration was demonstrated by Cummings *et al.* (1998) in a cellular model of polyQ disease [28]. The overexpression of the DnaJ protein, DnaJA1, reduced the aggregation of polyglutamine expanded ataxin-1. Since this report the role of molecular chaperones in polyQ diseases has been extensively studied. Hsp70 and DnaJ proteins have been shown to suppress polyQ aggregation both in cells and in *Drosophila*. The expression of Hsp70 and DnaJB1 reduced the assembly of detergent insoluble polyQ fibrils, whilst increasing the formation of detergent soluble aggregates [29,30]. However, these results did not translate to a R6/2 mouse model of HD; Hsp70 overexpression had no significant effect on disease progression [31]. Increasing evidence suggests that DnaJ proteins and small Hsps are more potent suppressors of polyQ aggregation than Hsp70. Four members of the small Hsp family, HSPB6, HSPB7, HSPB8 and HSPB9, were found to significantly reduce polyQ aggregation in vitro [32]. Their protective effect was linked to stimulating the clearance of polyQ aggregates via the UPS or autophagy. For example, Hsp22 (HSPB8) forms a complex with Hsp70 and its co-chaperone Bag3 [33]. Bag3 interacts with the

autophagosome receptor p62 [34], enabling the recruitment of Hsp70-Hsp22-Bag3 bound cargo into the autophagosome for degradation. The protective effect of Hsp22 overexpression was suppressed in cells in which Bag3 had been reduced, or alternatively where autophagy was inhibited, implying that Hsp22 reduces neurotoxicity by targeting polyQ for autophagy-mediated degradation [33].

In the case of DnaJ proteins, a subfamily of Type II DnaJ proteins, including DnaJB2, DnaJB6 and DnaJB8, appear to be particularly effective at suppressing polyQ aggregation [35]. The overexpression of the neuronal DnaJ protein HSJ1 (DnaJB2) counteracts polyQ aggregation both in vitro and in vivo (Fig. 1). HSJ1 contains an N-terminal J domain, a client binding domain and two ubiquitin interacting motifs (UIMs) [36,37]. The combination of these domains enables HSJ1 to target some client proteins for proteasomal degradation. HSJ1 is alternatively spliced to produce two isoforms, HSJ1a and HSJ1b, with distinct intracellular localisations mediated by the C-terminal prenylation of the longer HSJ1b isoform [38]. Only the cytosolic and nuclear HSJ1a isoform was able to significantly reduce aggregation in a cellular model expressing polyQ huntingtin, highlighting that the localisation of chaperones is critical for their protective function [37]. The overexpression of HSJ1a was also shown to be neuroprotective in a R6/2 mouse model of HD [39]. HSJ1a expression significantly reduced the aggregation of mutant huntingtin in brain, whilst increasing the level of soluble protein. The mechanism of action was dependent on the association of HSJ1a with K63 ubiquitylated, higher order, detergent insoluble species preventing their ability to nucleate further aggregation (Fig. 1). These changes led to an improvement in neurological performance of the R6/2 mice at a late stage of the disease.

ALS

ALS is the most common form of motor neuron disease, affecting around 2 in every 100,000 in the UK. The disease is caused by the progressive loss of upper and lower motor neurons in the brain, the brainstem and the spinal cord, and is characterised by muscle weakness and atrophy leading to progressive paralysis. Around 20% of familial cases of ALS are caused by mutations in SOD1. Transgenic mice expressing mutant SOD1 develop an ALS-like phenotype, characterised by intracellular SOD1 aggregates, and is the most widely used model to study ALS.

The protective effects of Hsp27 (HSPB1) have been investigated in both cell and animal models of ALS. The overexpression of Hsp27 in neuronal cells stably expressing either SOD1 G93A or G93R had a protective effect against mutant SOD1-induced cell death [40]. Interestingly, this anti-apoptotic effect was enhanced by the overexpression of Hsp70 in conjunction with Hsp27 [40]. In vitro studies suggest that Hsp27 directly interacts with mutant SOD1, reducing aggregation by inhibiting fibril elongation, rather than by inhibiting the formation of fibril nuclei [41]. However, it is argued that, in addition to its chaperone function, the protective effect of Hsp27 may also be attributed to its roles in negatively regulating apoptosis and maintaining redox homeostasis. This is supported by in vitro studies on primary motor neuron cultures that show the overexpression of Hsp27 makes the cells more resistant to pro-apoptotic and oxidative insults [42]. Unfortunately, the role of Hsp27 in these two processes remains poorly characterised so the exact mechanism by which Hsp27 reduces neurotoxicity is not clear. In vivo, the overexpression of Hsp27 protected motor neurons from cell death induced by nerve crush [43]. Furthermore, transgenic mice overexpressing Hsp27 and $SOD1^{G93A}$ showed an improvement in muscle force, reflected by an increase in motor unit numbers and an increase in motor neuron survival in the spinal cord [44]. However, these improvements were only evident at an early stage of the disease and were not sustained over the long term.

In contrast to Hsp27, the overexpression of HSJ1a was able to ameliorate late stage disease in a $SOD1^{G93A}$ mouse model [45]. Overexpression of HSJ1a led to a significant improvement

in neuromuscular function, with a preservation of muscle force, an increase in motor unit number and enhanced motor neuron survival. HSJ1a was present in a complex of $SOD1^{G93A}$ and reduced its aggregation at late stages of the disease. In a cell model, HSJ1a stimulated $SOD1^{G93A}$ ubiquitylation and proteasomal degradation in a J domain and UIM dependent manner (Fig. 1). Altered ubiquitin immunoreactivity was observed in the double transgenic mice, suggesting this process may also be occurring in vivo.

Overall the genetic manipulation of molecular chaperones, in particular Hsp70 and Hsp27, has shown limited success in combating ALS disease progression in vivo. However, a different approach that utilises pharmacological agents to modulate the proteostasis network, rather than just one specific chaperone, was more effective in targeting protein aggregation and restoring proteostasis (Table 2). Arimoclomol, a hydroxylamine derivate, amplifies Hsp expression by stabilising the binding of HSF1 to heat shock elements, leading to the upregulation of chaperones including Hsp90, Hsp70 and Hsp60. The treatment of $SOD1^{G93A}$ mice with arimoclomol at pre-symptomatic or early symptomatic stages of the disease delayed disease progression, with a decrease in ubiquitin positive aggregates in the spinal cord and an improvement in muscle function and motor neuron survival at late stages of the disease [46]. This was accompanied by a 22% increase in lifespan. In addition to potentiating the heat shock response, arimoclomol can also enhance the unfolded protein response, potentially expanding the range of applicable diseases [47].

Mutations in molecular chaperones cause neurodegenerative disease

The importance of molecular chaperones in maintaining neuronal proteostasis is further highlighted by the identification of mutations in molecular chaperones in familial cases of neurodegenerative disease (Table 3). Interestingly, mutations have been identified in chaperones that are also protective against neurotoxicity when overexpressed in models of disease, such as HSJ1, Hsp22 and Hsp27.

HSJ1 (DnaJB2)

Distal hereditary motor neuropathies (dHMN) are a clinically and genetically heterogeneous group of disorders caused by the progressive degeneration of the lower motor neurons in the spinal cord. The disease is characterised by muscle weakness and atrophy of the lower limbs. Symptom onset typically occurs in the first two decades of life, and patients typically present with gait abnormalities, paralysis of foot and toe extension and lack of ankle and knee-jerk reflexes. In 2012, Blumen *et al.* reported mutations in HSJ1 in a consanguineous Moroccan family with dHMN [48]. Recently, a second mutation was identified in a Turkish family also with dHMN [49]. Both mutations are homozygous and are located at donor splice sites, leading to the loss of HSJ1 protein expression. A third mutation in HSJ1 has also been identified recently, this time in a family with Charcot-Marie-Tooth type 2 (CMT2) [49]. CMT2 closely resembles dHMN but patients have sensory abnormalities in addition to motor involvement. The mutation is a substitution of a tyrosine for a cysteine at reside 5 (Y5C). Residue 5 of HSJ1 is located in the J domain and is highly conserved in HSJ1 orthologs across multiple species. Our lab has confirmed the pathogenicity of the HSJ1 Y5C mutation (Heather Smith, unpublished observations). All three mutations identified thus far lead to a loss of HSJ1 function, suggesting that HSJ1 is important for motor neuron survival. Further investigations are required to reveal how the loss of HSJ1 chaperone function leads to motor neuron degeneration.

Hsp27 (HSPB1) and Hsp22 (HSPB8)

Mutations in Hsp27 (HSPB1) and Hsp22 (HSPB8) have been reported in families with both dHMN and CMT2. 16 mutations in Hsp27 have been identified to date and the majority of mutations identified are missense mutations in the α-crystallin domain [50-57]. The mutations share the common property of inducing protein instability and aggregation. All mutations reported, apart from one, were inherited in an autosomal dominant manner. The dominant inheritance might result from a gain of toxic function, but it is also likely that the loss of chaperone function contributes to the disease mechanism, possibly through dominant negative effects on chaperone oligomers. In addition to their chaperone function, some HSPB proteins can modulate the dynamics of the cytoskeleton by regulating the stabilisation of microtubules and intermediate filaments. Investigations suggest that this regulatory function is impaired in Hsp27 mutants. The expression of mutant Hsp27 in cells was found to result in the sequestration of intermediate filaments into Hsp27 aggregates, leading to the destabilisation of the cytoskeletal network [50,58]. Furthermore, transgenic mice expressing either Hsp27 S135F or P182L exhibit impaired axonal transport of mitochondria, with the mice developing a distal motor neuropathy [59]. The evidence therefore suggests that Hsp27-mediated disruption of axonal transport is an important underlying mechanism of motor neuron degeneration.

Two mutations in Hsp22 have been identified in five separate families with dominant inheritance. The mutations (K141E and K141N) affect lysine residue 141 in the α-crystallin domain and cause aggregation [57]. Interestingly, when expressed in cells, mutant Hsp22 was found to sequester Hsp27 in its aggregates [57]. The loss of Hsp27 may contribute to the degeneration process. It has also been proposed that mutations in Hsp22 cause motor neuron death due to dysfunctions in the removal of misfolded proteins by autophagy [33], a reduction in the ability to remove misfolded protein could be a critical determinant in neuronal toxicity.

Sacsin

Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is an early onset recessive neurodegenerative disease caused by the progressive loss of Purkinje cells in the cerebellum, followed by motor neurons in the spinal cord [60]. The disease is caused by mutations in the DnaJ protein sacsin (DnaJC29), with over 170 mutations identified throughout the 520 kDa protein. Sacsin is composed of a N-terminal ubiquitin-like domain that binds to the proteasome [61], followed by three large sacsin repeat regions, suggested to have Hsp90-like chaperone function [62,63], a XPCB domain that binds to protein ligases [64], a C-terminal J domain and a HEPN domain that mediates sacsin dimerisation [61,62,65]. The combination of these domains implies a role in protein quality control. The mutations identified are proposed to lead to loss of sacsin function, however, a full understanding of sacsin function is currently lacking. Sacsin is predominantly localised in the cytoplasm but also shows localisation to the mitochondria. Sacsin interacts with dynamin-related protein 1 (DRP1), a GTPase that mediates mitochondrial fission [66]. Interestingly, in both patient fibroblasts and a sacsin knockout mouse, the mitochondria appear overly fused and show a reduction in mobility [66]. This suggests that sacsin may participate, either directly or indirectly, in mitochondrial fission. Mitochondrial dysfunction is a common feature in many neurodegenerative diseases and is likely to be a key mechanism underlying ARSACS.

Conclusions

Neurodegenerative diseases are characterised by disturbances in neuronal proteostasis caused by genetic mutations or alternatively an age-related decline in the proteostasis network. The upregulation of molecular chaperones has been demonstrated to suppress the neurotoxicity associated with protein misfolding and restore proteostasis both in vitro and in vivo. Therefore, molecular chaperones represent important therapeutic targets and their manipulation could potentially slow disease progression. A better understanding of specialised chaperone functions in neurons will assist in the design of new potential therapies based on restoring proteostasis. In addition, it is likely that the study of chaperone mutations in disease will provide important insights into how chaperones function in neurons and will be important in understanding the specific challenges of neuronal proteostasis.

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Table 1: Chaperones that combat neurodegeneration related protein misfolding.

Chaperone Family	Chaperone	Disease/ Protein(s)	Comments
HSP110	HSP110 (HSPH2)	ALS/SOD1	Improved vesicle transport deficit in $SOD1^{G85R}$ squid axoplasm [67]; with DNAJB1 suppressed polyQ toxicity in flies [68].
		HD/Htt	
	HSP105 (HSPH1)	AD/Tau	HSP105 knock out mouse had increased p-tau and Aβ [69]; suppressed aggregation of $SOD1^{G93A}$ in cells [70].
		ALS/SOD1	
HSP90	HSP86 (HSPC1)	AD/ Aβ	Reduced Aβ aggregation in vitro [22]; with Hsp60 and Hsp70 reduced Aβ mitochondrial dysfunction in cells [71].
HSP70	BiP (HSPA5)	AD/ Aβ	Bound APP and reduced Aβ secretion [72]; reduced polyQ aggregation and toxicity in cells [73]; reduced α-synuclein toxicity in rat [74]; reduced P23H rhodopsin aggregation and photoreceptor cell death [75] [76].
		HD/Htt	
		PD/α-syn	
		RP/Rho	
	Hsc70 (HSPA8)	AD/Tau	Binds tau and facilitates microtubule polymerization reducing insoluble tau [77] [78] [25]; QBP1 fusion reduced polyQ aggregation and toxicity in cells and mice [79], ATPase mutant reduced large polyQ aggregates but no effect on toxicity [80], reduced axonal transport defect in polyQ fly [81]; binds α-synuclein and reduced toxicity of fibrils [82] [83]; binding to mutant SOD1 [84].
		HD/Htt	
		PD/α-syn	
		ALS/SOD1	
	Hsp70 (HSPA1A*)	AD/ Aβ	Reduced Aβ aggregation in vitro [22] and in transgenic mice [85]; modest effect on R6/2 Htt mice [31] but increased aggregation on knock-down in HD flies [86]; suppression of α-synuclein toxicity in flies [17] cells and mice [16], but another report found no effect in mice [87]; reduced mutant SOD1 aggregation in cells [88] but had no effect in mice [89]; suppressed TDP-43 toxicity in fly [90].
		AD/Tau	
		HD/Htt	
		PD/α-syn	
		ALS/SOD1	
		ALS/TDP43	
HSP60	HSP60 (HSPD1)	AD/ Aβ	Reduced Aβ aggregation in vitro [22] and improved mitochondria function in a cell model [71].
HSP40/ DNAJ	DNAJA1	AD/Tau	Antagonized protective effect of Hsp70 on tau [91]; increased polyQ aggregation in some cell models [92]; increase binding of Hsp70 to α-synuclein [83].
		HD/Htt	
		PD/α-syn	
	DNAJB1 (Hsp40 or	AD/ Aβ	Reduced Aβ aggregation in vitro with Hsp70 [22]; suppressed Htt inclusion formation but did not affect toxicity in cells [93] but protective with Hsp110 in flies [68]; increase binding of Hsp70 to
		HD/Htt	

	Hdj1)	PD/α-syn	α-synuclein [83].
	DNAJB2a (HSJ1a)	HD/Htt	Reduce polyQ aggregation in vitro, in cells, in mice [37] [39] and rats [94]; reduced mutant SOD1 aggregation in cells [48] [45] and mice [45]; suppress mutant parkin aggregation and promote functional refolding in cells [95]
		ALS/SOD1	
		PD/Parkin	
	DNAJB6	AD/ Aβ	Efficient block of Aβ aggregation in vitro [21]; block polyQ aggregation and toxicity in cells and frogs [35] [96].
		HD/Htt	
	DNAJB8	HD/Htt	block polyQ aggregation and toxicity in cells and frogs [35] [96].
	DNAJC10	RP/Rho	Reduced P23H rhodopsin aggregation in cells [97].
small HSP	HSP27 (HSPB1)	AD/ Aβ	Reduced Aβ aggregation in vitro and toxicity on cells [98] and in mice [99]; alters tau dynamics in mice [100]; reduced polyQ aggregation and toxicity in cells [101] and by viral delivery in rats [102] but not transgenic mice [103]; reduced α-synuclein fibril formation in vitro [104] and toxicity in cells [105]; reduced SOD1 aggregation in vitro [41] but small effects in mice [106] [44].
		AD/Tau	
		HD/Htt	
		PD/α-syn	
		ALS/SOD1	
	HSP22 (HSPB8)	AD/ Aβ	Reduced Aβ aggregation in vitro and toxicity on cells [98]; reduced polyQ aggregation [32]; Most effective small Hsp at reducing α-synuclein fibril formation in vitro [104]; enhanced autophagic clearance of SOD1 and TDP-43 [107].
		HD/Htt	
		PD/α-syn	
		ALS/SOD1	
		ALS/TDP43	
	αB-crystallin (HSPB5)	AD/ Aβ	Reduced Aβ aggregation in vitro and toxicity on cells [98] [108]; reduced toxicity of α-synuclein in cells [105]; reduced α-synuclein fibril formation in vitro [104]; SOD1 aggregation in vitro [41] but does not protect in mice [109].
		PD/α-syn	
		ALS/SOD1	
	HSP20 (HSPB6)	AD/ Aβ	Reduced Aβ aggregation in vitro and toxicity on cells [98] [110] and in worms [111]; reduced α-synuclein fibril formation in vitro [104].
		PD/α-syn	
	cvHSP (HSPB7)	HD/Htt	Most potent small Hsp against polyQ in cell model [32].
Co-chaperone	CHIP	AD/Tau	Reduced tau aggregation in cell [26] and in mice [112]; reduced polyQ (ataxin-1) aggregation and toxicity in cells [113]; enhanced ubiquitylation of α-synuclein [114]; degradation of mutant SOD1 [84].
		PD/α-syn	
		ALS/SOD1	
	Cdc37	AD/Tau	Regulates tau stability with Bag5 [115]; with Hsp90 in enhanced authophagic clearance of TDP-43 [116].
		ALS/TDP43	

	Bag-1	AD/Tau	With Hsc70 to target degradation of tau [117]; protects against α-synuclein in cells and MPTP in mice [118].
		PD/α-syn	
	Bag-3	ALS/SOD1	Reduction in polyQ (SCA3) with Hspb8 in cells and flies [119]; With HspB8 to stimulate autophagy of SOD1 in cells [107] [34].
	Bag-5	PD/α-syn	Enhanced ubiquitylation of α-synuclein [114].

Table 2: Manipulations involving networks of chaperones

Target	Method	Animal models showing benefit
HSF-1	Overexpression or constitutively active HSF-1	AD deficits in mice [120]; polyQ in mice [121]; mutant SOD1 in mice [122].
	Knock down or dominant negative HSF-1	Enhanced neurodegeneration in polyQ flies [123]; enhanced TDP-43 toxicity in worms [124].
	Other gene manipulation e.g. SIRT1	SIRT1 mediated suppression of α-synuclein aggregation in mice [125].
Hsp90	Hsp90 inhibitors e.g. Geldanamycin,17-AAG, 17-DMAG, HSP990, PU-H71	Reduction of tau pathology and tau phosphorylation in mice [126, 27]; polyQ in flies [123] [127] but in mice effect was only temporary due to epigenetic changes [128]; SBMA mouse model [129]; SCA3 mouse model [130] effective on mutant SOD1 cells but effect in mice questionable [131]; α-synuclein toxicity in flies [132] [17]; rhodopsin RP in rats [133].
Not defined	Arimoclomol	SOD1 mouse [134] [135] [46]; SBMA mouse model [136]; rhodopsin RP [47].
	Celastrol	Mouse model of Aβ [137]; SOD1 mice [138].
	Geranylgeranylacetone	SBMA mice[139]; AD model mice[140]; traumatic brain injury[141]

Table 3: Chaperones mutations identified in neurodegenerative diseases.

Chaperone family	Chaperone	Inheritance	Disease	Reference
Hsp70	HSPA9 (Mortalin)	Dominant	Parkinson's disease	[142,143]
Hsp60	HSP60 (HSPD)	Dominant	Spastic paraplegia	[144,145]
		Recessive	Hypomyelinating leukodystrophy	[146]
	CCT4	Recessive	Hereditary sensory neuropathy	[147]
	CCT5	Recessive	Sensory neuropathy with spastic paraplegia	[148]
Hsp40 (DnaJ)	DNAJB2 (HSJ1)	Recessive	Distal hereditary motor neuropathy, Charcot Marie Tooth disease	[48,49]
	DNAJB6 (Mrj)	Dominant	Limb-girdle muscular dystrophy	[149-151]
	DNAJC3 (ERdj6)	Recessive	Diabetes and multisystemic neurodegeneration	[152]
	DNAJC5 (CSPα)	Dominant	Neuronal ceroid lipofusinosis	[153,154]
	DNAJC6 (Auxilin)	Recessive	Juvenile Parkinsonism	[155,156]
	DNAJC13 (RME-8)	Dominant	Parkinson's disease	[157]
	DNAJC19 (TIM14)	Recessive	Dilated cardiomyopathy and cerebellar ataxia	[158,159]
	DNAJC29 (Sacsin)	Recessive	Spastic ataxia of Charlevoix-Saguenay	[160,161]
Small Hsp	HSPB1 (Hsp27)	Dominant and recessive	Distal hereditary motor neuropathy, Charcot Marie Tooth type 2	[50-56]
	HSPB3 (HspL27)	Dominant	Hereditary motor neuropathy	[162]
	HSPB5 (αβ-crystallin)	Recessive	Infantile muscular dystrophy	[163]
	HSPB8 (Hsp22)	Dominant	Distal hereditary motor neuropathy, Charcot Marie Tooth disease	[57]
Chaperone co-factors	SIL1	Recessive	Cerebellar ataxia	[164]
	VCP	Dominant	Amyotrophic lateral sclerosis	[165,166]
	BAG3	Dominant	Muscular dystrophy. Giant axonal neuropathy	[167,168]

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Figures

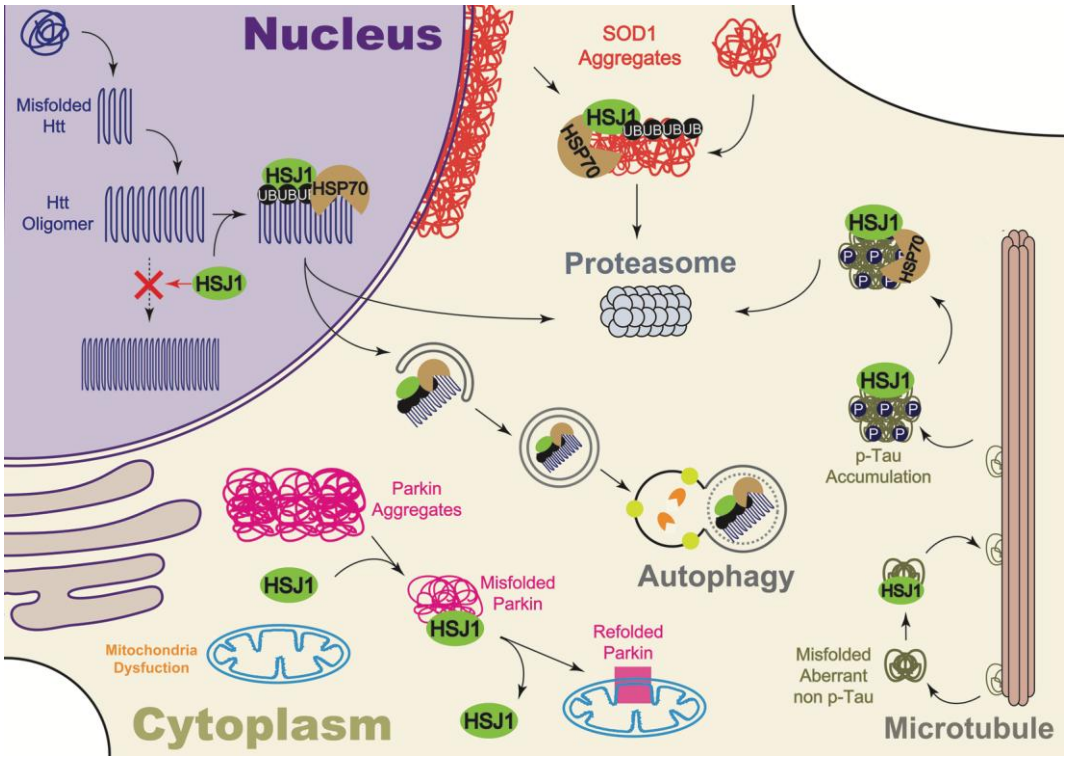

Fig. 1. HSJ1a acts restore proteostasis for several neurodegeneration proteins. Schematic showing the effect of HSJ1a on Htt, SOD1, parkin and tau in neurons. HSJ1a can bind to ubiquitylated oligomers of Htt in the nucleus blocking the recruitment of more misfolded Htt and further aggregation, leading to increases in soluble Htt and potential autophagic clearance of cytoplasmic Htt oligomers [39]. HSJ1 also facilitates proteasomal degradation of Htt [37]. HSJ1a blocks the aggregation of mutant parkin and stimulates its refolding, so that parkin can function in mitochondrial quality control [95]. In ALS, HSJ1a reduces the aggregation of mutant SOD1 and promotes the degradation by proteasome [45]. HSJ1a can bind tau, reduce tau phosphorylation and aggregation (Novoselov and Cheetham unpublished observations).