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Regulation of protein homeostasis in neurodegenerative diseases: the role of coding and non-coding genes

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Abstract Protein homeostasis is fundamental for cell function and survival, because proteins are involved in all aspects of cellular function, ranging from cell metabolism and cell division to the cell's response to environmental challenges. Protein homeostasis is tightly regulated by the synthesis, folding, trafficking and clearance of proteins, all of which act in an orchestrated manner to ensure proteome stability. The protein quality control system is enhanced by stress response pathways, which take action whenever the proteome is challenged by environmental or physiological stress. Aging, however, damages the proteome, and such proteome damage is thought to be associated with aging-related diseases. In this review, we discuss the different cellular processes that define the protein quality control system and focus on their role in protein conformational diseases. We highlight the power of using small organisms to model neurodegenerative diseases and how these models can be exploited to discover genetic modulators of protein aggregation and toxicity. We also link findings from small model organisms to the situation in higher organisms and describe how some of the genetic modifiers discovered in organisms such as worms are functionally conserved throughout evolution. Finally, we demonstrate that the non-coding genome also plays a role in maintaining protein homeostasis. In all, this review highlights the importance

of protein and RNA homeostasis in neurodegenerative diseases.

Keywords Protein homeostasis · Genetic modifiers · Non-coding RNA · Protein aggregation · Neurodegeneration · Alzheimer's disease · Parkinson's disease · Huntington's disease · *C. elegans* · Proteotoxicity · Protein quality control · Proteostasis · tRNA · iPOD · JunQ · Aggresome · Chaperone · miRNA

Protein homeostasis

Protein folding

Maintaining a healthy proteome is important to ensure cell survival and function. The cell maintains a healthy proteome through a series of complex and tightly regulated surveillance systems (Fig. 1). These systems ensure that each protein is properly folded or assembled in a state that is required for it to perform its function in the cell.

After the synthesis of a nascent polypeptide chain, the protein's amino acid sequence determines whether or not the protein becomes folded, and whether or not chaperone proteins are required for its folding (Fig. 1a, b). Some proteins are thought to exist in a predominantly “unfolded”, “disordered” or “intrinsically unstructured” state ([1], also reviewed in [2, 3]). Such proteins are typically involved in transcription, in signaling pathways and in protein networks ([4], also reviewed in [5, 6]). In mammals, about half of all possible proteins are predicted to have long disorganized regions and about 25 % are estimated as being intrinsically unstructured [2]. Other proteins have domains within their amino acid sequence

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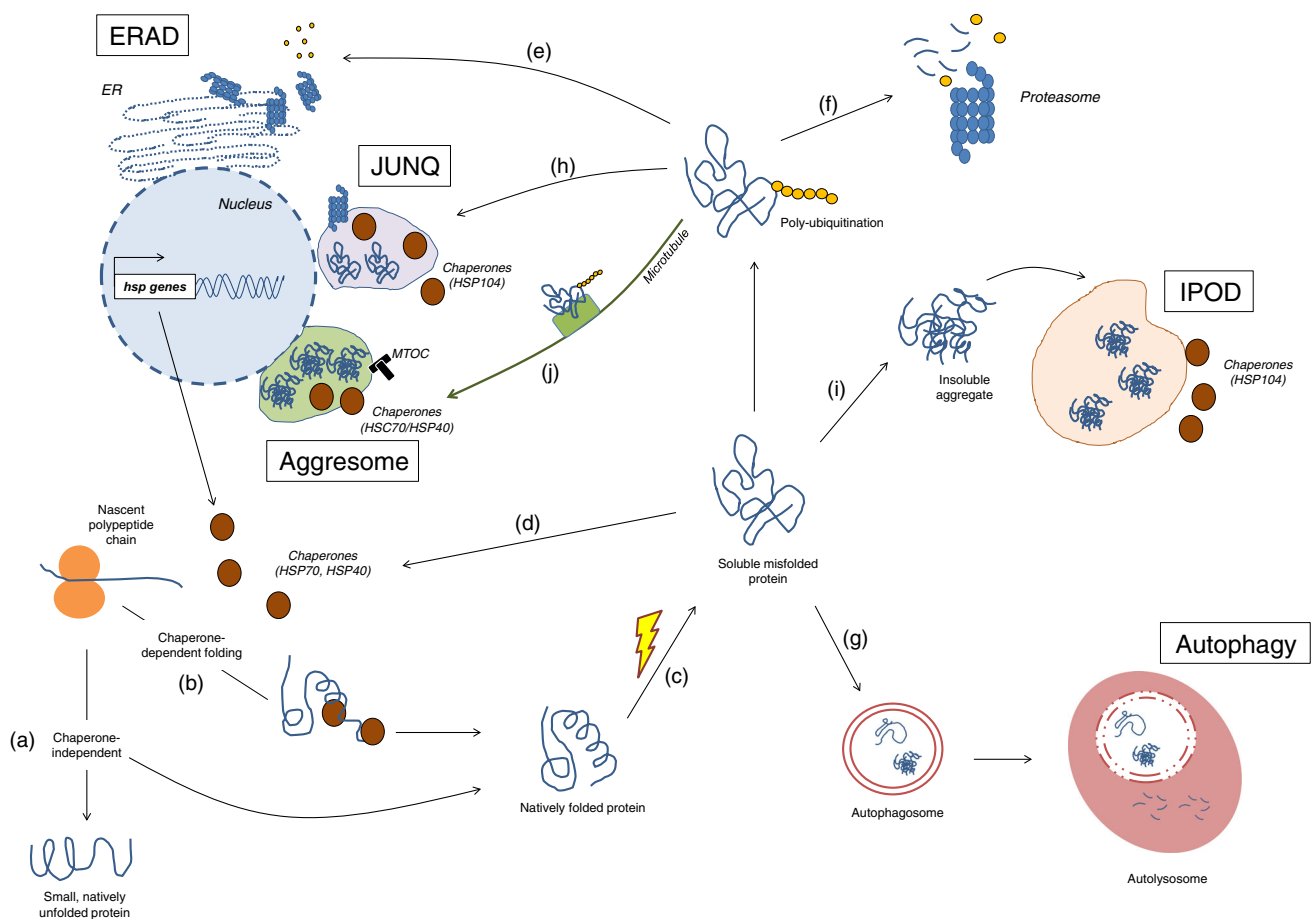


Fig. 1 Quality control of cellular proteins. When a protein is synthesized, it can acquire its native state in a chaperone-independent (a) or dependent (b) manner. Upon environmental stress or mutations, the protein may either not acquire its native state or lose it, both leading to misfolding (c). Here, the misfolded protein can be refolded

back to its functional conformation with the aid of chaperones (d); or sent to degradation via the ERAD (e), the ubiquitin–proteasome system (f) or autophagy (g). Alternatively, it can be redirected to the JUNQ for posterior refolding or degradation by the proteasome (h) or it can be permanently sequestered in the IPOD (i) or aggresome (j)

that can fold spontaneously, whereas other large, multi-subunit proteins require molecular chaperones to assist in folding to their native state, as shown in *in vitro* studies [7–11].

The molecular chaperones that cooperate in the *de novo* folding or refolding process are subdivided into different classes, which include the Hsp70 system, the small chaperones, the chaperonins and the Hsp90 system [11–14]. In the case of *de novo* synthesis, chaperones protect the nascent polypeptide chain from aberrant contacts with other domains of the same proteins and from aggregation with other proteins (Fig. 1b) ([13, 14], also reviewed in [12, 15]). As a protein is synthesized, it is transiently unfolded and its hydrophobic regions are exposed. Hsp70 is able to recognize these regions and it binds to the protein substrate via its peptide-binding site in an ATP-dependent manner (reviewed in [12, 15, 16]). Hsp70 holds the substrate in an extended conformation, stabilizing it and preventing premature misfolding and aggregation. Next, the substrate can

be transferred to another chaperone system, such as the chaperonins, where folding takes place and a three-dimensional structure is acquired (reviewed in [12, 16, 17]).

When misfolded proteins accumulate, unfolded protein responses can increase the levels of chaperones, which are then able to restore the proteins to their properly folded form (Fig. 1c, d, reviewed in [16, 18–21]). Such an accumulation of misfolded protein is just one of the types of stress that can trigger unfolded protein responses. Unfolded protein responses are mechanisms that are highly conserved from yeast to humans and that are induced upon environmental and physiological stress, such as thermal or oxidative stress (reviewed in [22–24]). In one of these pathways thought to respond to the accumulation misfolded proteins in the cytosol, heat shock factor 1 (HSF-1) acts as a master transcriptional regulator. HSF-1 is activated upon phosphorylation, after which it translocates from the cytosol to the nucleus to bind to the so-called heat shock elements, thereby upregulating the transcription of

heat shock genes. These genes are then translated into proteins that assist in the refolding of misfolded proteins into functionally active proteins, in preventing unspecific interactions, or in mediating their degradation (Fig. 1d) (reviewed in [19, 22]).

Another strategy used by the cell to restore protein homeostasis is the unfolded protein response that is associated with the endoplasmic reticulum (ER) (Fig. 1e, also reviewed in [18, 25, 26]). The ER is the organelle where proteins enter the secretory pathway to acquire post-translational modifications, after which they are delivered to their corresponding organelle, fixed in the plasma membrane or shuttled outside of the cell to perform their function [27]. If misfolded proteins accumulate, the ER-associated degradation (ERAD) pathway is activated through signal transduction pathways that are mediated by three upstream effectors: inositol-requiring protein 1 (IRE1), activating transcription factor (ATF)-6 and PKR-like endoplasmic reticulum kinase (PERK).

IRE1, ATF-6 and PERK mediate three distinct pathways. Firstly, IRE1 is a transmembrane protein kinase that activates itself by auto-phosphorylation and mediates splicing of Hac1 in yeast and XBP-1 in eukaryotes [28–32]. IRE1 is known to promote the transcription of three groups of genes: stress-responsive genes including molecular chaperones and folding enzymes, genes involved in ERAD and genes involved in ER trafficking [33–35]. Secondly, ATF-6 is a transmembrane protein with a transcription factor domain (leucine zipper) that translocates from the ER lumen to the Golgi apparatus to be cleaved by proteases [36, 37]. This proteolysis releases the ATF-6 cytosolic fragment, which then enters the nucleus to induce the transcription of ER-resident chaperones and the transcription factor XBP-1, thereby increasing ER protein quality control capacity [29, 37–39]. Thirdly, PERK is a transmembrane kinase protein that phosphorylates the alpha-subunit of the eukaryotic translation initiation factor 2a (eIF2a), thus preventing the binding of the initiator tRNA(Met) to the ribosomal complex, necessary for translation initiation [40–42]. This results in an overall reduction in protein synthesis, thereby attenuating the accumulation of misfolded proteins at the ER.

Protein degradation

If an aberrant protein cannot be folded back into its native state by the molecular chaperones, then it can be eliminated by two proteolytic systems, the proteasome and autophagy (Fig. 1f, g). In the degradation via the ERAD pathway, the ER cooperates tightly with the ubiquitin–proteasome system (UPS) to recognize, mark and traffic the misfolded proteins to the cytosol for degradation (Fig. 1e, reviewed in [18, 43–45]). The exact mechanisms that allow the cell to

discriminate misfolded proteins from correctly folded proteins are not fully understood (reviewed in [44, 46, 47]). However, the current notion is that misfolded proteins can be recognized by molecular chaperones (the HSP70 family of proteins) and co-chaperones (the DnaJ/HSP40 family of proteins) [48–51].

An example that illustrates this recognition is the immunoglobulin binding protein (BiP), an HSP70 chaperone that recognizes and binds to the hydrophobic regions of misfolded proteins, thereby preventing their aggregation [49–53]. The binding of the ERAD substrate to BiP and its subsequent release depends on the conversion of ADP to ATP, a process regulated by ERdj proteins, which are part of the DnaJ/Hsp40 family of co-chaperones, and the nucleotide exchange factors GRP170 and BAP/Sil1 [48, 52]. These factors stimulate the ATPase activity of BiP and stabilize its binding to the misfolded protein [54–58]. The ERdj co-chaperones have also been shown to bind directly to unfolded proteins, thus maintaining them in a soluble state to be later recruited by BiP [48, 59]. After the misfolded protein has been identified, it is poly-ubiquitinated to be subsequently targeted for degradation [60–62].

Ubiquitination is a sequential three-step process that marks proteins destined for the proteasome (Fig. 1f). It starts with the activation of ubiquitin (a small 76 amino acid protein) by the activating enzyme E1, followed by binding of ubiquitin to the active site of the ubiquitin-carrier protein E2 and, finally, transfer of the ubiquitin molecule to the substrate in a reaction catalyzed by the ubiquitin protein ligase E3. At least four ubiquitin molecules must be bound to the ERAD substrate for it to be later recognized by the proteasomal machinery [63, 64]. Following this step, the misfolded proteins are delivered to the proteasome (a process called retrotranslocation) and the ubiquitin molecules are removed from the substrate prior to degradation by the deubiquitinating enzymes and recycled [65–67]. The proteasome is a barrel-shaped, multicatalytic proteinase where proteolysis occurs and proteins are cleaved into peptides 2–30 amino acid long [68].

The second proteolytic system, autophagy (“self-eating”), is a cellular degradation mechanism that eliminates cytosolic components, organelles and pathogens via lysosomes (Fig. 1g, [69–72]). It is the part of the cell that ensures protein and organelle turnover, where old cellular components are degraded and recycled molecules become available for cell metabolism [70, 71, 73]. For the purpose of this review, we discuss only the role of autophagy as a protein quality control system.

Autophagy can be classified into three categories: macroautophagy, microautophagy and chaperone-mediated autophagy (CMA). In macroautophagy, a newly formed double membrane vesicle engulfs the cytosolic material, forming the autophagosome. The autophagosome then

fuses with an endosome or lysosome, giving rise to the autolysosome where degradation takes place through the action of hydrolytic enzymes (Fig. 1g) [71]. The double membrane that surrounds the autophagosome is derived from the ER, the mitochondria or the plasma membrane [74–78]. In yeast, autophagy is a multi-step process that requires at least 37 autophagy-related (ATG) genes [79–89]. The majority of the ATG genes have shown to be functionally conserved in mammals [90, 91]. In microautophagy, small molecules from the cytoplasm are internalized by the lysosome through invagination of its own membrane [70, 73]. In contrast to autophagy and CMA, much less is known about microautophagy [92].

CMA differs from the former two forms of autophagy in that it does not involve membrane reorganization. Instead, substrates with a KFERQ amino acid motif are recognized by an HSP70 cytosolic chaperone, Hsc70, that binds and delivers them to the CMA receptor at the lysosome [93–96]. Here, the substrate is unfolded before it is translocated into the lumen of the lysosome for degradation, which is assisted by Hsc73, an intralysosomal HSP70 chaperone [97, 98].

Crosstalk exists between the UPS and autophagy. Chronic low-level proteasomal inhibition is known to be sufficient to activate autophagy, and it has been suggested that ubiquitinated proteins may also be eliminated through this pathway [99–101]. It has also been proposed that macroautophagy may occur as a compensatory mechanism when either the UPS or CMA is impaired [102, 103].

Protein compartmentalization

An alternative pathway for misfolded proteins is the sequestration into specialized protein quality control compartments where they can be either recovered or permanently sequestered (Fig. 1h, i, j) ([104–109], also reviewed in [110, 111]). Distinct quality control compartments harbor different species of misfolded proteins and are evolutionary conserved from yeast to mammals [105, 107–109, 112, 113]. Ubiquitinated misfolded cytosolic proteins are assigned to the juxtannuclear quality control compartment (JUNQ, Fig. 1h). These soluble, mobile misfolded proteins can subsequently be recovered by the molecular chaperone Hsp104 and either refolded back into functionally active proteins or degraded by the proteasomes localized nearby (Fig. 1h) [108, 112]. Non-ubiquitinated misfolded proteins—comprising amyloidogenic proteins—are redistributed to the insoluble protein deposit (IPOD, Fig. 1i). This compartment is localized at the cell periphery and is known to contain insoluble and immobile species, which are not recoverable and seem to remain terminally sequestered there (Fig. 1i) [108]. More recently, it has been proposed that there are no pre-existing

compartments in the cell, and that soluble ubiquitinated misfolded proteins (but not the non-ubiquitinated amyloidogenic type) may coalesce and form transient structures termed ‘Q bodies’ that eventually mature into the JUNQ compartments [104].

Much research has focused on finding out whether the redistribution of misfolded proteins to these spatial cytosolic compartments is a random event or whether it depends on the concerted action of sorting factors. Evidence suggests that the latter is the case, and that sorting factors interact with chaperones to deliver misfolded proteins to each compartment [105]. For example, upon physiological stress, Btn2 (a Hook family protein involved in linking organelles to microtubules) was shown to associate either with the yeast small heat shock protein Hsp42 to assign misfolded proteins to the IPOD or with the chaperone Sis1 to guide misfolded proteins to the JUNQ [105, 107].

Another type of cytosolic compartment—the aggresome—is localized at the microtubule organizing center (MTOC) and is formed when the proteasome is unable to clear misfolded proteins properly (Fig. 1j) [114]. Aggresome formation is accompanied by redistribution of vimentin, an intermediary filament that acquires a cage-like structure in the aggresome. Ubiquitinated misfolded proteins depend on microtubules to be transported to the aggresome, this being done by the dynein/dynactin complex (Fig. 1j) [115]. Interestingly, the JUNQ shares several properties with the aggresome, including its perinuclear localization, and the presence of chaperones and ubiquitinated misfolded proteins [108, 114]. It has also recently been shown to functionally associate with the MTOC and vimentin [112]. Indeed, continuous accumulation of misfolded proteins in the JUNQ is thought to turn it in an aggresome over time [112].

Similar structures to aggresomes are the so-called aggresome-like induced structures (ALIS), which were originally discovered in dendritic cells but were later also found in other type of cells [109, 116]. The ALIS is a transient structure with peripheral and juxtannuclear localization. It is induced under a wide variety of stress conditions (e.g., heat shock, starvation, oxidative stress, inflammation) and clusters newly synthesized, ubiquitinated misfolded proteins [106, 109]. ALIS substrates can also be cleared by the proteasome and lysosome [106].

Cell division could be considered as yet another protein quality control system that sequesters misfolded, aggregated proteins (reviewed in [117, 118]). Studies in bacteria and yeast have shown that accumulation of protein aggregates reduces the fitness of these cells, a problem partially resolved by asymmetric division: these protein deposits are retained in the aging mother cell while the daughter cells are freed from damaged proteins, a process also known as

replicative rejuvenation [119–123]. In budding yeast, it has been shown that misfolded proteins sorted either to the JUNQ or IPOD remain in the mother cell after asymmetric cell division, thus avoiding passage of these species onto the daughter cells [124]. Follow-up work from the same group extended this observation to mammalian cells, where the JUNQ (but not the IPOD) continues to be inherited asymmetrically, thereby always freeing one of the two daughter cells from proteotoxicity [112].

While much is now known about the sophisticated quality control mechanisms that the cell has evolved to ensure proper protein homeostasis, several questions remain to be answered. We know that the cell relies on the concerted action of chaperones to prevent an unfolded or misfolded protein interacting aberrantly with other proteins until it can be refolded back into its native state. In case this is not possible, the aberrant protein is sent to be degraded via the ubiquitin–proteasome system or by autophagy. However, it is still not known how the cell chooses one mechanism of degradation over the other or whether the two mechanisms occur simultaneously. Another unknown relates to protein compartmentalization—yet another strategy for putting away proteins that need to be degraded or permanently sequestered. It has not yet been established how the cell can differentiate between degradable and non-degradable proteins and shuttle them to different subcellular compartments. Finally, another important question is how protein quality control changes during aging. Aging itself may be the contributing factor for progressive deterioration of protein homeostasis, impairing the ability of the protein quality control system to handle the equilibrium between protein folding and degradation.

Protein misfolding and aggregation in neurodegenerative diseases

The effects of progressive deterioration of protein homeostasis are thought to play a role in age-related neurodegenerative diseases. The presence of protein aggregates in the brain is namely a hallmark shared by several neurodegenerative diseases, including Parkinson's (PD), Alzheimer's (AD) and Huntington's disease (HD) (reviewed in [125, 126]). In these diseases, it is not yet clear why proteins accumulate into aggregates and how this relates to pathogenesis.

Protein aggregation and its relationship to aging and neurodegeneration have also been widely studied in animal models. Evidence from several animal models suggests that, as the animal ages, the cell's stress response systems become less efficient and less capable of maintaining a balanced proteome [127–133]. This could lead to the

progressive accumulation of cytotoxic aggregation-prone disease proteins that cannot be cleared, ultimately resulting in toxicity and cell death [100, 134–137]. In the roundworm *Caenorhabditis elegans*, a model organism much used to study aging, protein aggregation has been shown to occur during aging and to affect the lifespan of the organism [138–140]. As previously discussed, when a protein misfolds it exposes its aggregation-prone domains to the cellular environment—domains that would otherwise be structurally concealed—thereby facilitating the likelihood of aberrant interactions with other proteins, potentially leading to proteotoxicity. Such proteotoxicity is proposed to play a role in protein conformational diseases in humans, including PD, AD and HD.

The type of aggregates that are formed varies for different neurodegenerative diseases. Frontotemporal lobar degeneration with fused in sarcoma is an example of a neurodegenerative disease that is characterized by the presence of amorphous, non-amyloidogenic aggregates ([141, 142], also reviewed in [143]). On the other hand, the common neuropathological feature of PD, AD and HD is the presence of an aggregation-prone disease protein that acquires amyloidogenic properties, causing it to form intracellular amyloid aggregates or extracellular amyloid plaques in the brains of patients (reviewed in [125, 126, 144]). The amyloids present in these neurodegenerative diseases can be distinguished from other amorphous, unstructured aggregates because they are organized, insoluble fibrils with a cross-beta structure and because they can be detected by specific amyloid-binding dyes, namely Congo red and thioflavin T (reviewed in [145, 146]). It is interesting to note that—despite their differences in amino acid sequence and function—several unrelated aggregation-prone disease proteins have one thing in common: in disease they are present as amyloid. This suggests that their ability to form amyloid is related to disease and that they may cause proteotoxicity in a similar manner.

In vitro studies have made clear that virtually any protein can form amyloid fibrils under certain conditions. Such conditions include low pH, high temperature and high pressure [147–154]. Native proteins are known to exist in equilibrium with their partially unfolded state, and when they are destabilized by certain conditions or mutations, the equilibrium shifts towards amyloid formation. Predicting aggregation-prone regions in proteins is now possible using bioinformatic tools. Examples of such tools are TANGO, which can specifically identify regions prone to form beta sheets, and Waltz, which can distinguish between amyloid sequences and amorphous beta-sheet aggregates [155, 156].

A proposed mechanism for amyloid formation is depicted in Fig. 2. Most of our understanding of this pathway has come not only from *in vitro* studies of

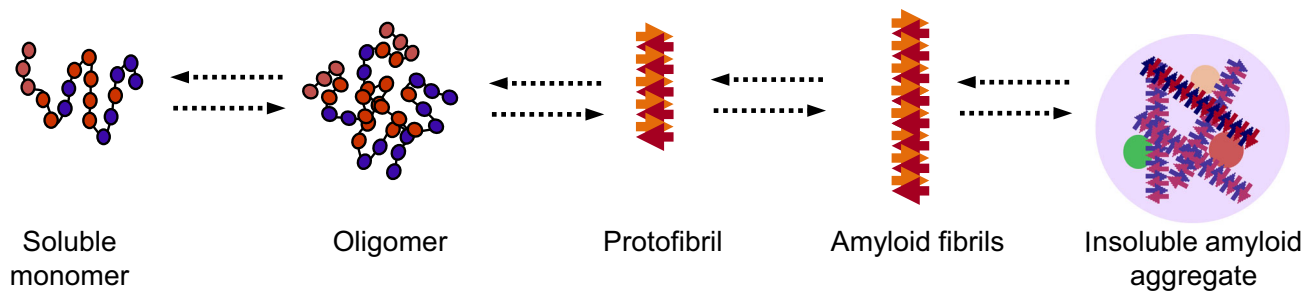


Fig. 2 Proposed mechanism for amyloid formation. A protein loses its monomeric native state by conversion into an oligomer which can grow further into amyloidogenic fibrils and ultimately into insoluble amyloid aggregates

aggregation-prone proteins, including amyloid-beta (seen in AD) and alpha-synuclein (seen in PD) but also from studies of globular proteins, including human lysozyme, superoxide dismutase 1, transthyretin and the acylphosphatase from the archaea *Sulfolobus solfataricus* (reviewed in [17, 125, 126, 146, 147]). One common step of amyloid formation appears to be the conversion of the monomeric, native state protein into an oligomeric intermediate state (Fig. 2). An oligomer is a small and transient cluster of protein molecules that has no fibrillar structure and is of low molecular weight [157–159]. These oligomers can then form protofibrils, which are fibrils 6 to 8 nm in diameter, about 200 nm in length and known to contain beta sheets detectable by Congo red and thioflavin T staining (Fig. 2) [160, 161]. Protofibrils can then convert into amyloid fibrils (Fig. 2) [160]. Of all these aggregation intermediates, it is currently thought that the early ones are cytotoxic and that aggregation may be a neuroprotective response to permanently sequester these intermediates, thereby preventing potentially toxic interactions with other proteins in the cellular milieu [162–165]. In support of this hypothesis, it has been shown that proteins rich in beta-sheet structures aggregate with newly synthesized proteins that have not yet become folded or with intrinsically unfolded proteins, thereby reducing the availability of these proteins to perform their normal function [135]. Further evidence demonstrating that oligomeric or protofibrillar forms of aggregation-prone disease proteins contribute to cell toxicity and death is reviewed elsewhere [144, 146, 166–168].

In a nutshell, the amyloid pathway has only just started to be described and it is not fully understood how protein aggregation correlates with disease. At the clinicopathological level, it is striking that there are individuals with high AD pathology (i.e., abundant amyloid deposits and neurofibrillary tangles) that yet do not display any cognitive impairment (reviewed in [169]). This fact makes it difficult to discern what are the boundaries between normal aging and disease. At the cellular and molecular level, what structural properties do aggregation-prone proteins acquire that make them toxic? This question is further complicated

by the fact that aggregation-prone proteins such as amyloid-beta, huntingtin or alpha-synuclein do not share sequence, structure or function. A second question is that of how long neuronal cells can deal with these aggregation-prone proteins. And is their slow accumulation in the brain a reflection of an impaired protein quality control system? Finally, the majority of our knowledge about aggregation intermediates has come from in vitro studies. It remains to be shown whether oligomeric and fibrillar species exist in vivo and what their relevance to pathogenesis is.

Genetic modifiers of proteotoxicity

Genetic screens in small model organisms for protein aggregation in disease

The current understanding of how protein misfolding and aggregation contributes to neurodegeneration is far from complete. Molecular and cellular mechanisms that may regulate neurodegenerative disorders have been discovered in small organisms, the major ones being yeast (*Saccharomyces cerevisiae*), fly (*Drosophila melanogaster*) and nematode (*C. elegans*) (Table 1). In general, these small organisms are easy to grow and manipulate; their genomes are fully sequenced and accessible in public databases; and they provide information relatively quickly due to their short life cycle. Moreover, the availability of resources such as genome-wide mutant libraries (deletion, overexpression or RNAi-based) further adds to the attraction of using these organisms as powerful genetic tools. Indeed, the well-established models of several neurodegenerative diseases, including AD, PD and polyglutamine diseases, have now been generated in each of these small organisms [170]. Of note is that expression of an exogenous aggregation-prone protein typically exclusive to mammals can faithfully mimic some neuropathological features, namely the protein aggregation and toxicity phenotype seen in the diseased brain [170, 171]. And it is this that makes models in small organisms so attractive in the search for

Table 1 Overview of a selection of genetic modifiers of protein aggregation and toxicity that are conserved between small model organisms, mammalian cells and animal models

Disease	Organism	Transgene	Tissue	Modifier	Mammalian ortholog	Cellular process	Transposed to	References
PD	<i>S. cerevisiae</i>	Alpha-synuclein	na	YPT1P	RAB1	ER-to-Golgi vesicular trafficking	<i>D. melanogaster</i> , <i>C. elegans</i> and rat midbrain primary neurons	[176]
	<i>S. cerevisiae</i>	Alpha-synuclein	na	YPK9	ATP13A	Manganese homeostasis	<i>C. elegans</i> and rat midbrain primary neurons	[177, 178]
	<i>C. elegans</i>	Alpha-synuclein	Body wall muscle and dopaminergic neurons	ATGR7	ATG7	Autophagy	Mouse	[181, 183]
AD	<i>C. elegans</i>	Amyloid-beta	Body wall muscle	DAF-2	IGFRI	Insulin/IGF-1 signaling pathway	Mouse	[195, 197]
	<i>S. cerevisiae</i>	Amyloid-beta	na	YAP1802	PICALM	Endocytosis	<i>C. elegans</i> , rat cortical neurons, human	[198]
PolyQ	<i>D. melanogaster</i>	Tau	Eye	GLUT1	SLC2A14	Glucose metabolism	Human	[199]
	<i>C. elegans</i>	Polyglutamine	Body wall muscle	MOAG-4	SERF1A and SERF2	Unknown	Human HEK293 and SK-N-SH cells	[203]
	<i>C. elegans</i>	Polyglutamine	Touch receptor neurons	662 modifiers	239 genes, of which 49 differentially expressed in HD mouse brain	Diverse	CHL2 and R6/2 mouse models	[205]
	<i>C. elegans</i>	Polyglutamine	Body wall muscle	CCT	TRiC	<i>De novo</i> protein folding	<i>In vitro</i> , <i>S. cerevisiae</i> , mouse N2A neurons, human HeLa cells	[202, 206, 207]
	<i>S. cerevisiae</i>	<i>huntingtin</i> fragment with expanded polyglutamine	na	BNA4	KMO	Kynurenine pathway of tryptophan degradation	<i>D. melanogaster</i> , mouse	[208–210]

PD Parkinson's disease, PolyQ polyglutamine diseases, AD Alzheimer's disease, na not applicable

evolutionary conserved modifiers of proteotoxicity. These modifiers will provide insight into disease pathology and can be further explored as targets for therapy.

Finding modifiers of proteotoxicity in such models can be relatively quick: researchers can take advantage of high-throughput screening techniques using genome-wide overexpression, deletion, or RNAi libraries or using chemical mutagenesis. These resources are unbiased methods that can be used to screen for genes that—when mutated, overexpressed or suppressed—contribute to an increase or decrease of protein aggregation and toxicity. Some of the hits that result from these screens may very well be genes that have already been associated with disease in humans. On the other hand, it is also a way of identifying previously unknown regulators of proteotoxicity—such findings may provide mechanistic insights into that particular disease. It should be noted, however, that genes shown to strongly suppress or enhance aggregation in one model do not always have a similar effect in other models, possibly due to the inherent differences between species or between the methods employed. Nevertheless, functionally conserved genetic modifiers of aggregation and toxicity have been identified across species.

In the end, to establish the value of genes discovered to be involved in aggregation and toxicity in small organisms, the results will have to be reproduced in human neurons and in mammalian animal models. If the function of modifiers of proteotoxicity identified in small organisms is evolutionarily conserved, their mammalian counterparts may become therapeutic targets worthy of future pharmacological investigation (Table 1). At the same time, small model organisms provide a simple platform that can be used not only to understand the basic mechanisms underlying the causal gene of disease but also as a pharmacological screening tool. Below we describe examples of genetic modifiers that have been studied in different model organisms for PD, AD and polyglutamine diseases.

Parkinson's disease models

Alpha-synuclein is the major constituent of the protein aggregates found in the brains of PD patients, which are also known as Lewy bodies [172]. It is a 140-amino acid protein that is mostly expressed in the brain and is thought to have a function at the synapse (reviewed in [126, 173]).

The aggregation phenotype is successfully recapitulated in the budding yeast *S. cerevisiae*, where heterologous expression of alpha-synuclein induces toxicity in a concentration-dependent manner and is associated with the formation of cytoplasmic protein aggregates similarly to those observed in the human brain [174]. The characteristics that make yeast a powerful genetic tool for studying

neurodegenerative disorders are reviewed elsewhere [171, 175].

In yeast, Cooper et al. demonstrated that overexpression and subsequent accumulation of alpha-synuclein impairs vesicle transport from the ER to the Golgi (Table 1) [176]. In the same study, a genome-wide overexpression screen identified the small GTPase Ypt1 as a modifier of alpha-synuclein toxicity. Overexpression of Ypt1p was sufficient to prevent alpha-synuclein toxicity, by enabling forward trafficking from the ER to the Golgi. This observation was further extended to *Drosophila* and *C. elegans* models of PD as well as in rat midbrain primary neurons, where Rab1—the functionally conserved ortholog of Ypt1p—suppressed dopaminergic neuron loss (Table 1) [176].

Another modifier of proteotoxicity identified from the same original yeast screen was YPK9, an ortholog of the human lysosomal P-type ATPase ATP13A2 (also known as PARK9), an enzyme known to be associated with early onset parkinsonism (Table 1). YPK9 overexpression prevented alpha-synuclein-induced toxicity by reducing intracellular aggregation and restoring alpha-synuclein localization to the plasma membrane [177]. The same study showed that the *C. elegans* ortholog CATP-6 partially prevented dopaminergic neuron loss, and that knockdown of CATP-6 increased alpha-synuclein misfolding in an age-dependent manner. Finally, in rat primary neuron cultures transduced with a lentivirus carrying the familial alpha-synuclein A53T mutation, heterologous expression of human ATP13A2 prevented neuronal loss (specifically dopaminergic neurons). Notably, this study was the first to show a link between environmental and genetic causes of PD, since YPK9 protected against manganese toxicity in yeast, a heavy metal thought to be risk factor for PD. Indeed, YPK9 was later shown to regulate manganese tolerance via diverse cellular processes, such as vesicle transport, vacuolar organization and chromatin remodeling in yeast (Table 1) [178].

The important role of vesicle-mediated transport in alpha-synuclein toxicity has also been demonstrated by other studies [179, 180]. In a screen performed by Kuwahara et al., the authors discovered ten neuroprotective genes, four of which were involved in endocytosis. Knockdown of two of these genes (*apa-2* and *aps-2*, encoding two different subunits of the AP-2 adaptor protein which mediates clathrin-dependent endocytosis) revealed that deficiencies at synaptic vesicles led to alpha-synuclein neurotoxicity [179].

Several modifiers of proteotoxicity have also been identified using RNAi screens in *C. elegans* models of PD [179–181]. Follow-up on this work has revealed *tdo-2* as a general regulator of proteotoxicity and lifespan [182].

Genetic screens not only help us to identify novel modifiers of proteotoxicity, but they can also be useful for

rediscovering genes that were previously known to be associated with disease. Such an example comes from work by Hamamichi et al., where an RNAi screen identified the autophagy-related gene *Atgr7* as protecting against alpha-synuclein-induced toxicity in *C. elegans* dopaminergic neurons [181]. The mammalian ortholog of *Atgr7* has previously been implicated in neurodegeneration in mice, where it was found to cause axonal degeneration and dystrophy when ablated, thereby highlighting the importance of neuronal autophagy in preventing degeneration (Table 1) [183].

Alzheimer's disease models

The brains of patients with AD are characterized by the presence of amyloid plaques and neurofibrillary tangles, which develop as a result of an accumulation of extracellular deposition of two different proteins: amyloid-beta in the plaques and intracellular hyperphosphorylated tau in the tangles (reviewed in [184, 185]). The disease can be caused by a mutation in the gene for amyloid precursor protein (APP), or in presenilin 1 or presenilin 2, all of which alter amyloid production (reviewed in [184, 186]).

C. elegans has been a fundamental tool for dissecting the pathways that link lifespan to AD (Table 1). Specifically, one of the major pathways that regulates lifespan is the insulin/IGF-1 signaling (IIS) pathway—a pathway that has been validated in nematodes, flies and mice and strongly implicated in humans [187–193]. In one of the models that recapitulates AD, *C. elegans* expresses a human amyloid-beta protein fragment (peptide 3–42) in the body wall muscle and progressive paralysis is used as readout for amyloid-beta toxicity [194]. In this model, knockdown of the insulin/IGF-1 receptor DAF-2 not only significantly extended lifespan but also prevented amyloid-beta toxicity by delaying the onset of paralysis, identifying a link between the mechanisms of aging and proteotoxicity [195]. Modulation of lifespan by DAF-2 was also found to be highly dependent on HSF-1 and DAF-16, two transcription factors known to drive the expression of longevity genes [196]. Curiously, while both blocked proteotoxicity, they did so through opposing effects: while HSF-1 promoted disaggregation, DAF-16 pushed aggregation forward, possibly as a means of sequestering the amyloidogenic protein from the cellular milieu [195].

The observation that inhibition of the IIS pathway protects against proteotoxicity was further confirmed in an AD mouse model with haploinsufficiency of IGFR-1, the mammalian ortholog of DAF-2 (Table 1) [197]. Here, reducing only half the expression of IGFR-1 (and thereby the IIS pathway) was sufficient to prevent amyloid-beta toxicity, namely by reducing inflammation and neuron loss. The AD mice with reduced IGFR-1 also performed better

in memory and learning tasks than their age-matched AD controls did and this was found to be correlated with the formation of densely packed aggregates in the brain. This supports the idea that aggregation is a protective mechanism to permanently sequester smaller, soluble oligomeric amyloid-beta species that are proteotoxic.

The importance of modeling neurodegenerative diseases in small organisms has been further reinforced by Treusch et al., who have identified modifiers of amyloid-beta toxicity that are conserved from yeast to humans (Table 1) [198]. Taking advantage of a yeast model of AD, they performed an unbiased genetic screen for modifiers of amyloid-beta toxicity. Of the identified modifiers, six were found to be risk factors for AD in humans—either validated or potential—that had been previously identified from family-based genome-wide association studies (GWAS). These modifiers were specific to amyloid-beta, in that in yeast they did not prevent toxicity induced by another aggregation-prone protein, alpha-synuclein. Another modifier of amyloid-beta toxicity identified by Treusch et al. is YAP1802, a suppressor of amyloid-beta proteotoxicity that is involved in clathrin-mediated endocytosis. Its human homolog PICALM is also involved in endocytosis and has been validated as a high-risk factor for AD (Table 1). YAP1802 prevents amyloid-beta toxicity in yeast and the human homolog PICALM prevents amyloid-beta toxicity in rat cortical neurons. Notably, this study identifies a causal gene for susceptibility to AD and proposes defective endocytosis as a contributing factor in AD pathology, with a possible role for PICALM.

In another independent study, GWAS data for AD were combined with a functional screen in *Drosophila* (Table 1) [199]. From a set of GWAS variants obtained from patients with AD, Shulman et al. found 19 evolutionarily conserved orthologs in the fly that either enhanced or suppressed neurotoxicity associated with tau. Six of these interacted with tau in vivo, including the glucose transporter GLUT1, found to be functionally conserved in the human ortholog SLC2A14, further supporting a role for this risk factor as a disease modifying factor (Table 1) [199].

Polyglutamine disease models

In addition to models for PD and AD, there are several other models for aggregation-prone proteins, which include those for human polyglutamine diseases such as Huntington's disease. In polyglutamine diseases, trinucleotide repeats cause expanded tracts of the amino acid glutamine in the encoded protein. In one *C. elegans* model, the animals express expanded glutamine repeats fused to a fluorescent protein in the body wall muscle. Expression of 35–40 glutamines is sufficient to cause aggregation, which increases with aging and is correlated with toxicity [200].

This model has been used in at least two genome-wide RNAi screens performed to search for suppressors and enhancers of proteotoxicity [201, 202]. These screens identified genes involved in RNA metabolism, as well as in protein synthesis, folding, trafficking and degradation as polyglutamine modifiers. In a subsequent screen to look for more modifiers, it was found that polyglutamine aggregation is not always coupled with proteotoxicity [201].

In an EMS screen to find genes that drive aggregation, van Ham et al. identified MOAG-4 (modifier of aggregation) as an aggregation-promoting factor in disease models expressing polyglutamine, alpha-synuclein and amyloid-beta, establishing MOAG-4 as a general regulator of proteotoxicity (Table 1) [203]. MOAG-4 is thought to be active during the early steps of the aggregation process, where it drives the formation of compact aggregation intermediates [203]. MOAG-4 is functionally conserved in two human orthologs, SERF1A and SERF2, which have the same aggregation-promoting function in human cell-based models of polyglutamine diseases (Table 1) [203]. Recent insights into the function of one of these proteins, SERF1A, suggest that it acts as an amyloid-promoting factor [204]. In this study, SERF1A recognized a broad range of aggregation-prone proteins (alpha-synuclein, huntingtin, amyloid-beta, prion protein) and mediated their conversion into amyloid in vitro [204]. It was further demonstrated that, to do this, SERF1A interacted directly with the monomeric form of the protein to seed amyloid growth, therefore supporting the hypothesis that MOAG-4/SERF1A acts on the early intermediates of the amyloid pathway [204]. SERF1A did not promote aggregation of non-amyloidogenic proteins.

An RNAi screen performed by Lejeune et al. identified 662 modifiers that regulate polyglutamine-induced proteotoxicity in *C. elegans* touch receptor neurons, 49 of which were found to be differentially expressed in two mouse models of HD (Table 1) [205].

Another protein originally identified as a suppressor of polyglutamine aggregation in a *C. elegans* model is the chaperonin CCT [202]. It is composed of eight subunits and, together with HSP70, is involved in *de novo* folding of newly synthesized proteins [12]. Its ortholog, TRiC (also known as TCP), was shown to cooperate with HSP70 to prevent proteotoxicity by promoting the formation of non-toxic, soluble polyglutamine oligomers in a yeast model [206]. TRiC also modulated proteotoxicity in mouse and human cell models (Table 1) [207]. The subunit CCT1 was also shown to physically interact with polyglutamine to suppress aggregation in vitro, supporting the hypothesis that TRiC binds to polyglutamine to prevent it from acquiring a potentially toxic conformation [207].

Finally, a modifier identified in yeast is the kynurenine 3-monooxygenase BNA4, whose deletion prevented

proteotoxicity induced by mutant huntingtin [208]. Follow-up work showed that genetic ablation or pharmacological inhibition of the ortholog KMO prevented toxicity in a fly and mouse model for HD (Table 1) [209, 210].

In summary, small model organisms including yeast, flies and nematodes are powerful tools for identifying genes involved in protein aggregation and toxicity. Several examples where small animal organisms complement findings from human cell models or mouse models further validate the importance of using these small animal models.

Non-coding RNA in neurodegeneration

When the Human Genome Project started in 1990, it was estimated that 30,000–40,000 protein coding genes would be found in the human genome [211]. When the project was completed in 2001, researchers were surprised to find far fewer protein coding genes than expected, namely 21,000, representing only about 2 % of the total genome—with the remaining 98 % being considered as “junk DNA” [212, 213]. However, it soon became clear that this “junk DNA” actually contained regulatory elements such as non-coding RNA (ncRNA), transcription factor binding sites or certain chromatin structures that govern gene expression. These conserved functional elements in the human genome were subsequently comprehensively identified and characterized [214]. Within these conserved functional elements, many classes of ncRNA were identified and the list has been growing ever since ([215, 216], also reviewed in [217]). Indeed, the number of ncRNA transcripts is far greater than those coding for proteins and the list of all existing ncRNAs is not yet complete [218]. What we do know is that there are different classes of ncRNA with essential functions in gene transcription, RNA processing and translation, a selection of which is presented in Table 2 (a more complete list can be found in [217]). Indeed, impaired RNA metabolism has been correlated with several neurodegenerative diseases. For example, abnormal repeat expansions in the non-coding regions of disease-related genes induce toxic gain-of-function of RNA in myotonic dystrophy, amyotrophic lateral sclerosis, and frontotemporal dementia [219, 220]. For the purpose of this review, we focus on a few examples of ncRNAs that have been directly implicated in neurological or neurodegenerative diseases (Table 2).

microRNAs

Over the past few years, it has become evident that ncRNAs are key players in the development and maintenance of the nervous system. Of all classes of ncRNAs

Table 2 Classes of RNAs and their localization, size and function

Class	Localization	Transcribed by	Function	Size	Disease
Messenger RNA (mRNA)	Nucleus and cytoplasm	Pol II	Gene expression into protein	2–5 kb	na
Ribosomal RNA (rRNA)	Cytoplasm	Pol I	Translation, is part of the small or large subunit of the ribosome	70S (prokaryotic) or 80S (eukaryotic)	Unknown
Micro RNA (miRNA)	Nucleus and cytoplasm	Pol II or Pol III	Inhibition of translation or degradation of mRNA	21–23 bp	AD, PD, HD
Transfer RNA (tRNA)	Cytoplasm	Pol II or Pol III	Translation, brings cognate amino acid of mRNA to nascent polypeptide chain	70–90 bp	PHC-2, PHC-4, neurodevelopmental and neurological disease
Long non-coding RNA (lncRNA)	Nucleus and cytoplasm	Pol II or Pol III	Diverse regulatory roles of other RNAs and proteins, chromatin remodeling	>200 bp	AD, SCA7
Small nuclear RNA (snRNA)	Nucleus	Pol II or Pol III	Splicing of pre-mRNA	100–300 bp	SMA

AD Alzheimer's disease, PD Parkinson's disease, HD Huntington's disease, PHC pontocerebellar hypoplasia, SCA spinocerebellar ataxia, SMA spinal muscular atrophy, na not applicable

identified so far, microRNAs (miRNAs) are those that have been most extensively studied and documented. The function of miRNAs is to bind to the 3'-untranslated region (3' UTR) of messenger RNA and inhibit its translation or target it for degradation (Table 2) [221]. *In situ* hybridization studies in mouse and zebrafish have revealed miRNA to be expressed throughout the brain; these studies have also demonstrated that miRNA expression is spatiotemporally controlled, supporting a biological function for miRNAs in the central nervous system [222–224]. Indeed, several hundreds of miRNAs are involved in brain development [225–229]. miRNAs play a role in virtually every aspect of brain function including neurogenesis, neural differentiation and maintenance, and synaptic plasticity, all of which are described extensively elsewhere [230–232].

miRNAs have also been associated with various aspects of aging and neurodegenerative diseases (Table 2) [233–237]. For example, Northern blotting experiments in the hippocampi of fetuses, adults and AD patients have shown that miRNA expression changes during development and during aging [237]. In these experiments, miR-9 and miR-128 were upregulated in the AD hippocampus relative to age-matched controls, hinting that these miRNAs may be regulating the expression of genes required for pathogenesis. At least two other human studies have shown an association between miRNAs and the beta-site APP cleaving enzyme 1 (BACE-1), which is responsible for cleaving APP into the amyloid-beta 1-42 toxic species [235, 236]. In these studies, the expression of miR-107, miR-29a and miR-29b-1 was decreased in the AD brain while expression of BACE-1 was increased (Fig. 4a) [235, 236]. Since these miRNAs target the 3' UTR of BACE-1, it follows that miRNAs can reduce BACE-1 mRNA levels and, therefore, amyloid-beta 1-42 generation in the brain, an effect that is lost in the diseased brain due to the reduced expression of these miRNAs (Fig. 4a) [235, 236]. In a mouse model for AD, miR-34a is thought to inhibit *bcl-2*, an anti-apoptotic gene that prevents cell death provoked by amyloidogenic species (Fig. 4b) [234]. Additionally, miR-124 has been found to regulate APP alternative splicing in neurons [233].

In PD, downregulation of the miR-34b/c cluster is correlated with downregulation of DJ-1 and Parkin, two genes implicated in the pathogenesis of PD, although a causal link has yet to be determined [238]. It has recently been shown in a cell model that this same cluster directly represses alpha-synuclein mRNA levels and consequently aggregate formation, establishing that miRNAs can have a direct effect on the expression of an aggregation-prone protein [239].

Several miRNAs have also been found to be dysregulated in polyglutamine diseases (Table 2) [240–244]. In

HD, REST is a transcription factor that negatively regulates neuronal gene expression and has been found to repress brain-specific miRNAs in mouse and human brains [241, 242]. Two of these miRNAs, miR-9 and miR-9*, have been identified as targeting the REST complex in a negative feedback loop [244]. In a cell model of spinocerebellar ataxia type 1, miR-19, miR-101 and miR-130 cooperatively regulate ataxin-1 expression levels by binding to its 3' UTR [243]. Inhibition of these miRNAs leads to ataxin-1 accumulation in cells and subsequent cell death [243].

tRNAs

Transfer RNAs are essential for mRNA translation into a protein, as they are responsible for transporting the cognate amino acid to the nascent polypeptide chain (Table 2) [245, 246]. Due to the degeneracy of the genetic code, there can be up to five tRNAs per amino acid—termed isoacceptors—that have distinct anticodons for recognizing the same amino acid [245, 246]. On the other hand, tRNAs that share the same anticodon but have distinct body sequences are termed isodecoders, and their number vary greatly [246].

Growing evidence suggests that mutations in individual tRNAs—or in the enzymes involved in their biosynthesis—are a contributing factor in neurodegeneration (Fig. 3)

[247–252]. For example, a point mutation (4274T>C) in the mitochondrial tRNA for isoleucine was identified in a patient suffering from motor neuron disease, although the mechanism by which this mutation might lead to disease is unknown (Fig. 3a) [252]. In a recent study, loss of function of one of the brain-specific tRNA isodecoders for arginine was found to be correlated with neurodegeneration in mice (Fig. 3a) [247]. Specifically, a point mutation (50C>T) in the T loop of the arginine tRNA provoked ribosome stalling, which is normally offset by GTPBP2. However, simultaneous impairment of GTPBP2 in these mice disabled its function as a so-called rescue factor, subsequently resulting in neurodegeneration [247].

Other impairments in the tRNAs biosynthesis pathway are seen in pontocerebellar hypoplasia (PHC). PHC is an autosomal recessive neurodegenerative disorder that has six subtypes (PHC1-6) and is generally characterized by hypoplasia and atrophy of the cerebellum and pons [253]. PHC2 and PHC4 arise from impaired tRNA splicing endonuclease (TSEN) activity. TSEN is composed of two catalytic subunits (TSEN 2 and TSEN34) and two non-catalytic subunits (TSEN54 and TSEN15) (Fig. 3c) [250, 254]. It is thought that mutations in both catalytic subunits and in TSEN54 may prevent proper complex formation, leading to missplicing of premature tRNAs (pre-tRNAs) into their mature form, thereby unbalancing the tRNA

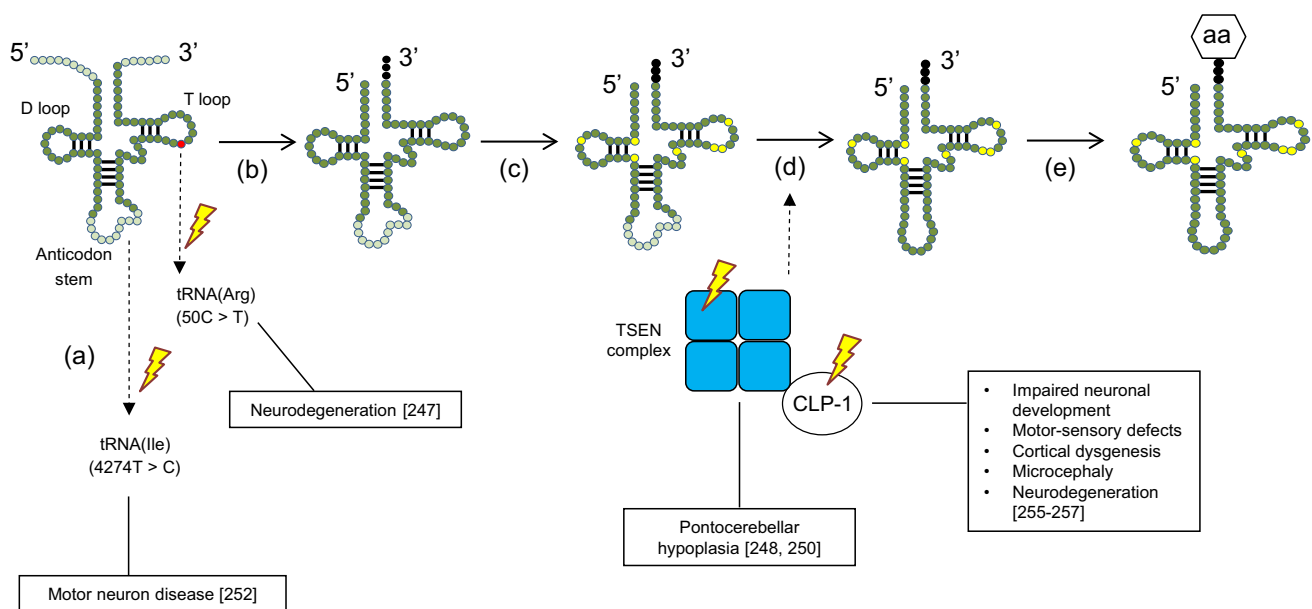


Fig. 3 Mutations in the tRNA biosynthesis pathway that lead to neurodegeneration. The point mutation (50C>T) in the T loop of one tRNA isoacceptor for arginine (Arg) provokes neurodegeneration. Another described point mutation (4274T>C) in the mitochondrial tRNA for isoleucine (Ile) has also been associated with motor neuron disease (a). Following transcription, the 5' leader sequence of the pre-tRNA is removed by RNaseP, the 3' end is processed by RNase Z and the trinucleotide CCA is added to the 3' end by a nucleotidyl

transferase (b). Different bases of the RNA transcript can undergo chemical modifications (c). The introns of the pre-tRNA are spliced out by a tRNA splicing endonuclease (TSEN). Mutations in these enzymes have been associated with pontocerebellar hypoplasia (PHC) and mutations in their co-factor CLP-1 with motor neuron loss (d). Finally, the mature tRNA is loaded with an amino acid (aa) via tRNA synthetases (e)

repertoire for protein synthesis [248, 250]. PHC6 results from a mutation in the intronic region of the mitochondrial pre-tRNA synthetase gene for arginine [251].

Finally, CLP-1 is a mammalian kinase that cooperates with the TSEN complex to remove the intronic loop of pre-tRNAs (Fig. 3c) [255]. Loss of CLP-1 results in severe impairment of spinal motor neurons in mice, ultimately leading to respiratory failure [255]. CLP-1 mutations in

affected patients have been correlated with neurodevelopment and neurological symptoms in both the central and peripheral nervous system [256, 257].

In summary, these studies demonstrate a crucial role for tRNAs in neuronal function, as either mutations in their transcript or defective post-transcriptional modifications can affect their proper processing and function, ultimately leading to neurodegeneration.

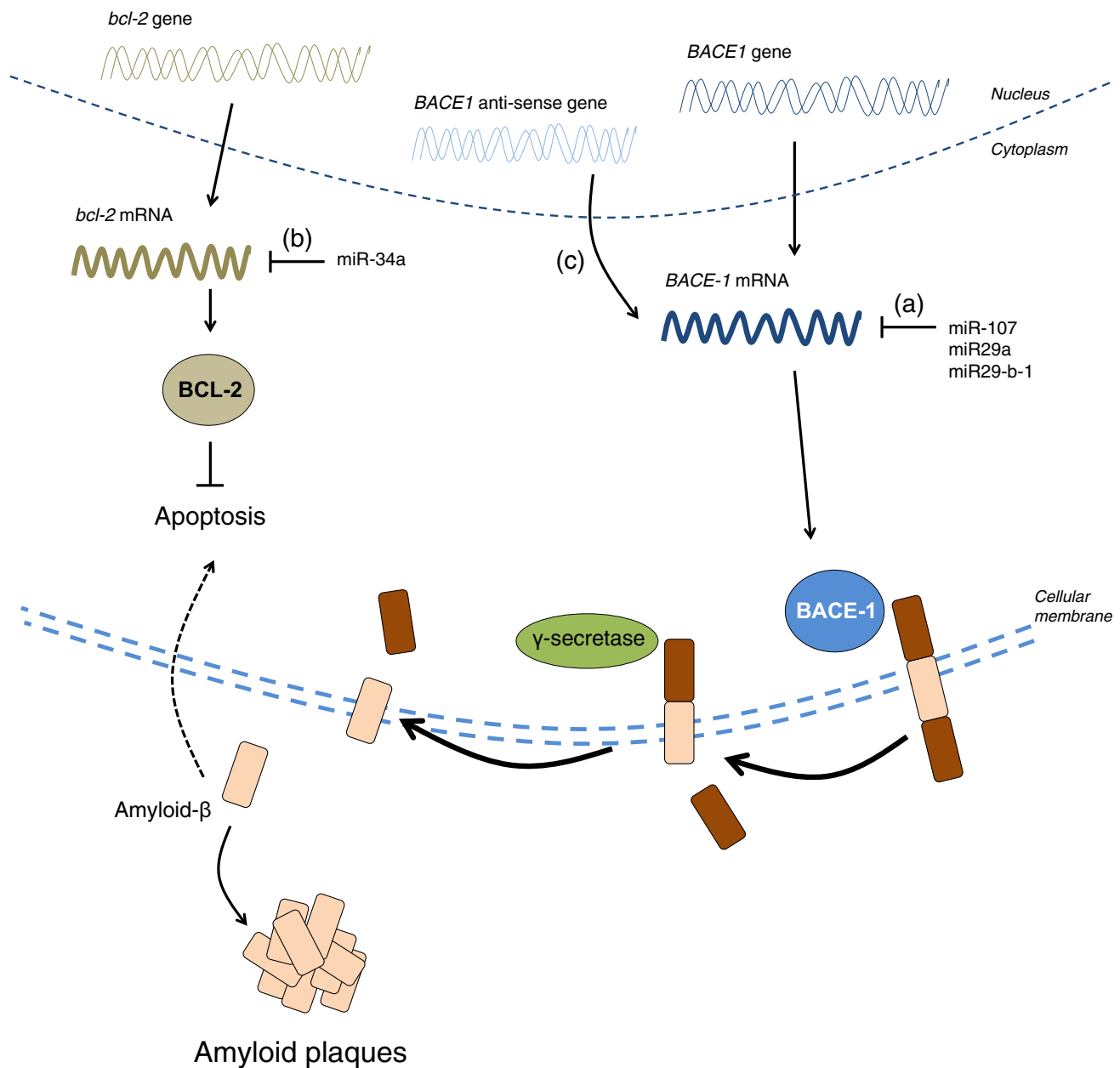


Fig. 4 Impaired BACE-1 regulation contributes to AD. miR-107, miR-29a and miR29-b-1 were shown to be decreased in the brain of AD patients while BACE-1 mRNA and protein levels were elevated (a). In an AD mouse model, elevated levels of miR-34a negatively correlate with BCL-2 protein levels, which normally prevent

apoptosis induced by amyloid-beta (b). BACE-1 anti-sense transcripts were reported to be upregulated in the brains of AD patients. BACE-1 anti-sense transcripts stabilizes BACE-1 mRNA, thereby facilitating its expression, which ultimately results in the generation of more amyloid-beta (c)

Other ncRNAs

The other non-coding RNAs shown in Table 2 have been less well studied but are nevertheless worthy of mention. Long non-coding RNAs (lncRNAs) are more than 200 nucleotides long and are mostly expressed in the nervous system (Table 2) ([223], also reviewed in [258]). Three lncRNAs have been suggested to be involved in neurodegenerative diseases. Firstly, BACE-1 anti-sense transcript is an lncRNA that competes with miR-485-5p for binding to the BACE-1 mRNA to stabilize it (Fig. 4c) [259]. In AD, the levels of BACE-1 anti-sense transcript are elevated, thereby stabilizing BACE-1 mRNA and enhancing its expression, which further promotes the generation of toxic amyloid-beta 1–42 (Fig. 4c) [259]. Secondly, in spinocerebellar ataxia type 7 (SCA7), lncSCA-7 crosstalks with miR-124 to regulate transcript levels of *atxn7* [260]. Thirdly, *Abhd11os* is an lncRNA that has been shown to be neuroprotective against mutant *huntingtin* in two mouse models for HD, although the exact mechanism of how this occurs remains to be determined [261].

Small nuclear RNAs (snRNAs) exist as small nuclear ribonucleoproteins (snRNPs) and are major components of the pre-mRNA splicing machinery (Table 2) [262, 263]. The survival motor neuron protein (SMN) is directly involved in the generation of snRNPs [264]. In a mouse model of spinal muscular atrophy, SMN deficiency affects the snRNA pool in a tissue-specific manner, ultimately leading to pre-mRNA splicing defects in a diverse range of genes [264]. Further evidence for the involvement of snRNAs in neurodegeneration comes from work by Jia et al., who revealed that a mutation in a U2 snRNA gene impairs alternative splicing of pre-mRNA which is directly responsible for neuron loss in the cerebellum and hippocampus of mice [265].

Neurodegeneration is clearly not exclusively caused by imbalances in protein coding genes—it can also arise from dysregulation of ncRNAs. Over the past two decades, we have begun to understand that ncRNAs are not just “transcriptional noise” and have started to define their role in the CNS and in neurodegeneration. Several reports have shown that different classes of ncRNAs influence the expression levels of the disease protein and that each class of ncRNA does so either by affecting the protein post-transcriptionally or through crosstalk with other classes of ncRNAs (miRNAs, lncRNAs). Maintaining a proper environment for protein synthesis is crucial to ensure that each mRNA molecule is effectively spliced and translated into a protein (through tRNAs and snRNAs). To establish the causal relationships between changes in ncRNAs and disease phenotypes, the targets of these ncRNAs need to be uncovered. Understanding the role of ncRNAs will provide insight into the mechanisms of neurodegenerative diseases,

which enables the identification of targets for therapeutic interventions.

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References

- Weinreb PH, Zhen W, Poon AW et al (1996) NACP, a protein implicated in Alzheimer's disease and learning, is natively unfolded. *Biochemistry* 35:13709–13715. doi:10.1021/bi961799n
- Dunker AK, Silman I, Uversky VN, Sussman JL (2008) Function and structure of inherently disordered proteins. *Curr Opin Struct Biol* 18:756–764. doi:10.1016/j.sbi.2008.10.002
- Radivojac P, Iakoucheva LM, Oldfield CJ et al (2007) Intrinsic disorder and functional proteomics. *Biophys J* 92:1439–1456. doi:10.1529/biophysj.106.094045
- Iakoucheva LM, Brown CJ, Lawson JD et al (2002) Intrinsic Disorder in Cell-signaling and Cancer-associated Proteins. *J Mol Biol* 323:573–584. doi:10.1016/S0022-2836(02)00969-5
- Uversky VN, Oldfield CJ, Dunker AK (2005) Showing your ID: intrinsic disorder as an ID for recognition, regulation and cell signaling. *J Mol Recognit* 18:343–384. doi:10.1002/jmr.747
- Dunker AK, Cortese MS, Romero P et al (2005) Flexible nets. The roles of intrinsic disorder in protein interaction networks. *FEBS J* 272:5129–5148. doi:10.1111/j.1742-4658.2005.04948.x
- Agashe VR, Guha S, Chang H-C et al (2004) Function of trigger factor and DnaK in multidomain protein folding: increase in yield at the expense of folding speed. *Cell* 117:199–209
- Frydman J, Erdjument-Bromage H, Tempst P, Hartl FU (1999) Co-translational domain folding as the structural basis for the rapid de novo folding of firefly luciferase. *Nat Struct Biol* 6:697–705. doi:10.1038/10754
- Freeman BC, Myers MP, Schumacher R, Morimoto RI (1995) Identification of a regulatory motif in Hsp70 that affects ATPase activity, substrate binding and interaction with HDJ-1. *EMBO J* 14:2281–2292
- Netzer WJ, Hartl FU (1997) Recombination of protein domains facilitated by co-translational folding in eukaryotes. *Nature* 388:343–349. doi:10.1038/41024
- Schumacher RJ, Hurst R, Sullivan WP et al (1994) ATP-dependent chaperoning activity of reticulocyte lysate. *J Biol Chem* 269:9493–9499
- Frydman J (2001) Folding of newly translated proteins in vivo: the role of molecular chaperones. *Annu Rev Biochem* 70:603–647. doi:10.1146/annurev.biochem.70.1.603
- Kim S, Schilke B, Craig EA, Horwich AL (1998) Folding in vivo of a newly translated yeast cytosolic enzyme is mediated by the SSA class of cytosolic yeast Hsp70 proteins. *Proc Natl Acad Sci* 95:12860–12865
- Crombie T, Boyle JP, Coggins JR, Brown AJ (1994) The folding of the bifunctional TRP3 protein in yeast is influenced by a

- translational pause which lies in a region of structural divergence with *Escherichia coli* indoleglycerol-phosphate synthase. *Eur J Biochem* 226:657–664
15. Hartl FU, Bracher A, Hayer-Hartl M (2011) Molecular chaperones in protein folding and proteostasis. *Nature* 475:324–332. doi:[10.1038/nature10317](https://doi.org/10.1038/nature10317)
 16. Kim YE, Hipp MS, Bracher A et al (2013) Molecular Chaperone Functions in Protein Folding and Proteostasis. *Annu Rev Biochem* 82:323–355. doi:[10.1146/annurev-biochem-060208-092442](https://doi.org/10.1146/annurev-biochem-060208-092442)
 17. Tyedmers J, Mogk A, Bukau B (2010) Cellular strategies for controlling protein aggregation. *Nature Publishing Group* 11:777–788. doi:[10.1038/nrm2993](https://doi.org/10.1038/nrm2993)
 18. Guerriero CJ, Brodsky JL (2012) The Delicate Balance Between Secreted Protein Folding and Endoplasmic Reticulum-Associated Degradation in Human Physiology. *92:537–576*. doi:[10.1152/physrev.00027.2011](https://doi.org/10.1152/physrev.00027.2011)
 19. Westerheide SD, Raynes R, Powell C et al (2012) HSF transcription factor family, heat shock response, and protein intrinsic disorder. *Curr Protein Pept Sci* 13:86–103
 20. Chen B, Retzlaff M, Roos T, Frydman J (2011) Cellular strategies of protein quality control. *Cold Spring Harb Perspect Biol* 3:a004374–a004375. doi:[10.1101/cshperspect.a004374](https://doi.org/10.1101/cshperspect.a004374)
 21. Taylor RC, Dillin A (2011) Aging as an event of proteostasis collapse. *Cold Spring Harb Perspect Biol* 3:a004440–a004441. doi:[10.1101/cshperspect.a004440](https://doi.org/10.1101/cshperspect.a004440)
 22. Morimoto RI (2008) Proteotoxic stress and inducible chaperone networks in neurodegenerative disease and aging. *Genes Dev* 22:1427–1438. doi:[10.1101/gad.1657108](https://doi.org/10.1101/gad.1657108)
 23. Åkerfelt M, Morimoto RI, Sistonen L (2010) Heat shock factors: integrators of cell stress, development and lifespan. 1–11. doi:[10.1038/nrm2938](https://doi.org/10.1038/nrm2938)
 24. Westerheide SD, Morimoto RI (2005) Heat Shock Response Modulators as Therapeutic Tools for Diseases of Protein Conformation. *J Biol Chem* 280:33097–33100. doi:[10.1074/jbc.R500010200](https://doi.org/10.1074/jbc.R500010200)
 25. Lin JH, Walter P, Yen TSB (2008) Endoplasmic Reticulum Stress in Disease Pathogenesis. *Annu Rev Pathol Mech Dis* 3:399–425. doi:[10.1146/annurev.pathmechdis.3.121806.151434](https://doi.org/10.1146/annurev.pathmechdis.3.121806.151434)
 26. Ron D, Walter P (2007) Signal integration in the endoplasmic reticulum unfolded protein response. *Nat Rev Mol Cell Biol* 8:519–529. doi:[10.1038/nrm2199](https://doi.org/10.1038/nrm2199)
 27. Lodish H, Berk A, Matsudaira P et al (2003) *Molecular Cell Biology*, 5 edn. pp 1–968
 28. Calton M, Zeng H, Urano F et al (2002) IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. *Nature* 415:92–96. doi:[10.1038/415092a](https://doi.org/10.1038/415092a)
 29. Yoshida H, Matsui T, Yamamoto A et al (2001) XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell* 107:881–891
 30. Cox JS, Walter P (1996) A novel mechanism for regulating activity of a transcription factor that controls the unfolded protein response. *Cell* 87:391–404
 31. Cox JS, Shamu CE, Walter P (1993) Transcriptional induction of genes encoding endoplasmic reticulum resident proteins requires a transmembrane protein kinase. *Cell* 73:1197–1206
 32. Mori K, Ma W, Gething MJ, Sambrook J (1993) A transmembrane protein with a cdc2+/CDC28-related kinase activity is required for signaling from the ER to the nucleus. *Cell* 74:743–756
 33. Shaffer AL, Shapiro-Shelef M, Iwakoshi NN et al (2004) XBP1, downstream of Blimp-1, expands the secretory apparatus and other organelles, and increases protein synthesis in plasma cell differentiation. *Immunity* 21:81–93. doi:[10.1016/j.immuni.2004.06.010](https://doi.org/10.1016/j.immuni.2004.06.010)
 34. Okada T, Yoshida H, Akazawa R et al (2002) Distinct roles of activating transcription factor 6 (ATF6) and double-stranded RNA-activated protein kinase-like endoplasmic reticulum kinase (PERK) in transcription during the mammalian unfolded protein response. *Biochem J* 366:585–594. doi:[10.1042/BJ20020391](https://doi.org/10.1042/BJ20020391)
 35. Travers KJ, Patil CK, Wodicka L et al (2000) Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation. *Cell* 101:249–258
 36. Ye J, Rawson RB, Komuro R et al (2000) ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. *Mol Cell* 6:1355–1364
 37. Haze K, Yoshida H, Yanagi H et al (1999) Mammalian transcription factor ATF6 is synthesized as a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress. *Mol Biol Cell* 10:3787–3799
 38. Wu J, Rutkowski DT, Dubois M et al (2007) ATF6 α optimizes long-term endoplasmic reticulum function to protect cells from chronic stress. *Dev Cell* 13:351–364. doi:[10.1016/j.devcel.2007.07.005](https://doi.org/10.1016/j.devcel.2007.07.005)
 39. Yoshida H, Okada T, Haze K et al (2000) ATF6 activated by proteolysis binds in the presence of NF-Y (CBF) directly to the cis-acting element responsible for the mammalian unfolded protein response. *Mol Cell Biol* 20:6755–6767
 40. Scheuner D, Song B, McEwen E et al (2001) Translational control is required for the unfolded protein response and in vivo glucose homeostasis. *Mol Cell* 7:1165–1176
 41. Harding HP, Novoa I, Zhang Y et al (2000) Regulated translation initiation controls stress-induced gene expression in mammalian cells. *Mol Cell* 6:1099–1108
 42. Harding HP, Zhang Y, Ron D (1999) Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. *Nature* 397:271–274. doi:[10.1038/16729](https://doi.org/10.1038/16729)
 43. Brodsky JL (2012) Cleaning up: ER-associated degradation to the rescue. *Cell* 151:1163–1167. doi:[10.1016/j.cell.2012.11.012](https://doi.org/10.1016/j.cell.2012.11.012)
 44. Araki K, Nagata K (2011) Protein folding and quality control in the ER. *Cold Spring Harb Perspect Biol* 3:a007526–a007527. doi:[10.1101/cshperspect.a007526](https://doi.org/10.1101/cshperspect.a007526)
 45. Vembar SS, Brodsky JL (2008) One step at a time: endoplasmic reticulum-associated degradation. *Nat Rev Mol Cell Biol* 9:944–957. doi:[10.1038/nrm2546](https://doi.org/10.1038/nrm2546)
 46. Määttänen P, Gehring K, Bergeron JJM, Thomas DY (2010) Protein quality control in the ER: the recognition of misfolded proteins. *Semin Cell Dev Biol* 21:500–511. doi:[10.1016/j.semcdb.2010.03.006](https://doi.org/10.1016/j.semcdb.2010.03.006)
 47. Goeckeler JL, Brodsky JL (2010) Molecular chaperones and substrate ubiquitination control the efficiency of endoplasmic reticulum-associated degradation. *Diabetes Obes Metab* 12:32–38. doi:[10.1111/j.1463-1326.2010.01273.x](https://doi.org/10.1111/j.1463-1326.2010.01273.x)
 48. Dong M, Bridges JP, Apsley K et al (2008) ERdj4 and ERdj5 are required for endoplasmic reticulum-associated protein degradation of misfolded surfactant protein C. *Mol Biol Cell* 19:2620–2630. doi:[10.1091/mbc.E07-07-0674](https://doi.org/10.1091/mbc.E07-07-0674)
 49. Nishikawa SI, Fewell SW, Kato Y et al (2001) Molecular chaperones in the yeast endoplasmic reticulum maintain the solubility of proteins for retrotranslocation and degradation. *J Cell Biol* 153:1061–1070
 50. Schmitz A, Maintz M, Kehle T, Herzog V (1995) In vivo iodination of a misfolded proinsulin reveals co-localized signals for Bip binding and for degradation in the ER. *EMBO J* 14:1091–1098
 51. Knittler MR, Dirks S, Haas IG (1995) Molecular chaperones involved in protein degradation in the endoplasmic reticulum: quantitative interaction of the heat shock cognate protein BiP with partially folded immunoglobulin light chains that are

- degraded in the endoplasmic reticulum. *Proc Natl Acad Sci* 92:1764–1768
52. Otero JH, Lizák B, Hendershot LM (2010) Life and death of a BiP substrate. *Semin Cell Dev Biol* 21:472–478. doi:10.1016/j.semcdb.2009.12.008
 53. Blond-Elguindi S, Cwirla SE, Dower WJ et al (1993) Affinity panning of a library of peptides displayed on bacteriophages reveals the binding specificity of BiP. *Cell* 75:717–728
 54. Petrova K, Oyadomari S, Hendershot LM, Ron D (2008) Regulated association of misfolded endoplasmic reticulum luminal proteins with P58/DNAJc3. *EMBO J* 27:2862–2872. doi:10.1038/emboj.2008.199
 55. Jin Y, Awad W, Petrova K, Hendershot LM (2008) Regulated release of ERdj3 from unfolded proteins by BiP. *EMBO J* 27:2873–2882. doi:10.1038/emboj.2008.207
 56. Weitzmann A, Volkmer J, Zimmermann R (2006) The nucleotide exchange factor activity of Grp170 may explain the non-lethal phenotype of loss of Sil1 function in man and mouse. *FEBS Lett* 580:5237–5240. doi:10.1016/j.febslet.2006.08.055
 57. Chung KT, Shen Y, Hendershot LM (2002) BAP, a Mammalian BiP-associated Protein, Is a Nucleotide Exchange Factor That Regulates the ATPase Activity of BiP. *J Biol Chem* 277:47557–47563. doi:10.1074/jbc.M208377200
 58. Liberek K, Marszałek J, Ang D et al (1991) Escherichia coli DnaJ and GrpE heat shock proteins jointly stimulate ATPase activity of DnaK. *Proc Natl Acad Sci* 88:2874–2878
 59. Jin Y, Zhuang M, Hendershot LM (2009) ERdj3, a Luminal ER DnaJ Homologue, Binds Directly to Unfolded Proteins in the Mammalian ER: identification of Critical Residues. *Biochemistry* 48:41–49. doi:10.1021/bi8015923
 60. Finley D (2009) Recognition and processing of ubiquitin-protein conjugates by the proteasome. *Annu Rev Biochem* 78:477–513. doi:10.1146/annurev.biochem.78.081507.101607
 61. Hershko A, Ciechanover A (1998) The ubiquitin system. *Annu Rev Biochem* 67:425–479. doi:10.1146/annurev.biochem.67.1.425
 62. Hochstrasser M (1996) Ubiquitin-dependent protein degradation. *Annu Rev Genet* 30:405–439. doi:10.1146/annurev.genet.30.1.405
 63. Raasi S, Orlov I, Fleming KG, Pickart CM (2004) Binding of Polyubiquitin Chains to Ubiquitin-associated (UBA) Domains of HHR23A. *J Mol Biol* 341:1367–1379. doi:10.1016/j.jmb.2004.06.057
 64. Thrower JS, Hoffman L, Rechsteiner M, Pickart CM (2000) Recognition of the polyubiquitin proteolytic signal. *EMBO J* 19:94–102. doi:10.1093/emboj/19.1.94
 65. Yao T, Cohen RE (2002) A cryptic protease couples deubiquitination and degradation by the proteasome. *Nature* 419:403–407. doi:10.1038/nature01071
 66. Verma R, Aravind L, Oania R et al (2002) Role of Rpn11 metalloprotease in deubiquitination and degradation by the 26S proteasome. *Science* 298:611–615. doi:10.1126/science.1075898
 67. Maytal-Kivity V, Reis N, Hofmann K, Glickman MH (2002) MPN + , a putative catalytic motif found in a subset of MPN domain proteins from eukaryotes and prokaryotes, is critical for Rpn11 function. *BMC Biochem* 3:28
 68. Kisselev AF, Akopian TN, Woo KM, Goldberg AL (1999) The sizes of peptides generated from protein by mammalian 26 and 20 S proteasomes. Implications for understanding the degradative mechanism and antigen presentation. *J Biol Chem* 274:3363–3371. doi:10.1074/jbc.274.6.3363
 69. Mizushima N (2012) Autophagy in Protein and Organelle Turnover. *Cold Spring Harb Symp Quant Biol* 76:397–402. doi:10.1101/sqb.2011.76.011023
 70. Mizushima N, Komatsu M (2011) Autophagy: renovation of cells and tissues. 147:728–741. doi:10.1016/j.cell.2011.10.026
 71. Yang Z, Klionsky DJ (2009) An overview of the molecular mechanism of autophagy. *Current topics in microbiology and immunology*. Springer, Berlin Heidelberg, Berlin, Heidelberg, pp 1–32
 72. Huang J, Klionsky DJ (2007) Autophagy and human disease. *cell cycle* 6:1837–1849. doi:10.4161/cc.6.15.4511
 73. Wong E, Cuervo AM (2010) Integration of clearance mechanisms: the proteasome and autophagy. *Cold Spring Harb Perspect Biol* 2:a006734–a006735. doi:10.1101/cshperspect.a006734
 74. Ylä-Anttila P, Vihinen H, Jokitalo E, Eskelinen E-L (2014) 3D tomography reveals connections between the phagophore and endoplasmic reticulum. *Autophagy* 5:1180–1185. doi:10.4161/aut.5.8.10274
 75. Hayashi-Nishino M, Fujita N, Noda T et al (2009) A subdomain of the endoplasmic reticulum forms a cradle for autophagosome formation. *Nat Cell Biol* 11:1433–1437. doi:10.1038/ncb1991
 76. Wang H, Liu J, Zong Y et al (2010) miR-106b aberrantly expressed in a double transgenic mouse model for Alzheimer's disease targets TGF- β type II receptor. *Brain Res* 1357:166–174. doi:10.1016/j.brainres.2010.08.023
 77. Ravikumar B, Moreau K, Jahreiss L et al (2010) Plasma membrane contributes to the formation of pre-autophagosomal structures. *Nat Cell Biol* 12:747–757. doi:10.1038/ncb2078
 78. Axe EL, Walker SA, Manifava M et al (2008) Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum. *J Cell Biol* 182:685–701. doi:10.1083/jcb.200803137
 79. Nazarko TY, Ozeki K, Till A et al (2014) Peroxisomal Atg37 binds Atg30 or palmitoyl-CoA to regulate phagophore formation during pexophagy. *J Cell Biol* 204:541–557. doi:10.1083/jcb.201307050
 80. Motley AM, Nuttall JM, Hettema EH (2012) Pex3-anchored Atg36 tags peroxisomes for degradation in *Saccharomyces cerevisiae*. *EMBO J* 31:2852–2868. doi:10.1038/emboj.2012.151
 81. Nazarko VY, Nazarko TY, Farre J-C et al (2011) Atg35, a micropexophagy-specific protein that regulates micropexophagic apparatus formation in *Pichia pastoris*. *Autophagy* 7:375–385. doi:10.4161/aut.7.4.14369
 82. Suzuki K, Kondo C, Morimoto M, Ohsumi Y (2010) Selective transport of alpha-mannosidase by autophagic pathways: identification of a novel receptor, Atg34p. *J Biol Chem* 285:30019–30025. doi:10.1074/jbc.M110.143511
 83. Kanki T, Wang K, Baba M et al (2009) A genomic screen for yeast mutants defective in selective mitochondria autophagy. *Mol Biol Cell* 20:4730–4738. doi:10.1091/mbc.E09-03-0225
 84. Kanki T, Wang K, Cao Y et al (2009) Atg32 is a mitochondrial protein that confers selectivity during mitophagy. *Dev Cell* 17:98–109. doi:10.1016/j.devcel.2009.06.014
 85. Farre J-C, Manjithaya R, Mathewson RD, Subramani S (2008) PpAtg30 tags peroxisomes for turnover by selective autophagy. *Dev Cell* 14:365–376. doi:10.1016/j.devcel.2007.12.011
 86. Stasyk OV, Stasyk OG, Mathewson RD et al (2006) Atg28, a novel coiled-coil protein involved in autophagic degradation of peroxisomes in the methylotrophic yeast *Pichia pastoris*. *Autophagy* 2:30–38. doi:10.4161/aut.2226
 87. Kabeya Y, Kawamata T, Suzuki K, Ohsumi Y (2007) Cis1/Atg31 is required for autophagosome formation in *Saccharomyces cerevisiae*. *Biochemical and Biophysical Research Communications* 356:405–410. doi:10.1016/j.bbrc.2007.02.150

88. Kawamata T, Kamada Y, Suzuki K et al (2005) Characterization of a novel autophagy-specific gene, ATG29. *Biochem Biophys Res Commun* 338:1884–1889. doi:10.1016/j.bbrc.2005.10.163
89. Klionsky DJ, Cregg JM, Dunn WA et al (2003) A unified nomenclature for yeast autophagy-related genes. *Dev Cell* 5:539–545
90. Yang Z, Klionsky DJ (2010) Eaten alive: a history of macroautophagy. *Nat Cell Biol* 12:814–822. doi:10.1038/ncb0910-814
91. Xie Z, Klionsky DJ (2007) Autophagosome formation: core machinery and adaptations. *Nat Cell Biol* 9:1102–1109. doi:10.1038/ncb1007-1102
92. Mijaljica D, Prescott M, Devenish RJ (2014) Microautophagy in mammalian cells: revisiting a 40-year-old conundrum. *Autophagy* 7:673–682. doi:10.4161/aut.7.7.14733
93. Koga H, Martinez-Vicente M, Macian F et al (2011) A photo-convertible fluorescent reporter to track chaperone-mediated autophagy. *Nat Commun* 2:386. doi:10.1038/ncomms1393
94. Cuervo AM, Dice JF (1996) A receptor for the selective uptake and degradation of proteins by lysosomes. *Science* 273:501–503
95. Chiang HL, Terlecky SR, Plant CP, Dice JF (1989) A role for a 70-kilodalton heat shock protein in lysosomal degradation of intracellular proteins. *Science* 246:382–385
96. Chiang HL, Dice JF (1988) Peptide sequences that target proteins for enhanced degradation during serum withdrawal. *J Biol Chem* 263:6797–6805
97. Agarraberes FA, Terlecky SR, Dice JF (1997) An intralysosomal hsp70 is required for a selective pathway of lysosomal protein degradation. *J Cell Biol* 137:825–834
98. Salvador N, Aguado C, Horst M, Knecht E (2000) Import of a cytosolic protein into lysosomes by chaperone-mediated autophagy depends on its folding state. *J Biol Chem* 275:27447–27456. doi:10.1074/jbc.M001394200
99. Hara T, Nakamura K, Matsui M et al (2006) Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. *Nature* 441:885–889. doi:10.1038/nature04724
100. Komatsu M, Waguri S, Chiba T et al (2006) Loss of autophagy in the central nervous system causes neurodegeneration in mice. *Nature* 441:880–884. doi:10.1038/nature04723
101. Ding Q, Dimayuga E, Martin S et al (2004) Characterization of chronic low-level proteasome inhibition on neural homeostasis. *J Neurochem* 86:489–497. doi:10.1046/j.1471-4159.2003.01885.x
102. Pandey UB, Nie Z, Batlevi Y et al (2007) HDAC6 rescues neurodegeneration and provides an essential link between autophagy and the UPS. *Nature* 447:859–863. doi:10.1038/nature05853
103. Massey AC, Kaushik S, Sovak G et al (2006) Consequences of the selective blockage of chaperone-mediated autophagy. *Proc Natl Acad Sci* 103:5805–5810. doi:10.1073/pnas.0507436103
104. Escusa-Toret S, Vonk WIM, Frydman J (2013) Spatial sequestration of misfolded proteins by a dynamic chaperone pathway enhances cellular fitness during stress. *Nat Cell Biol* 15:1231–1243. doi:10.1038/ncb2838
105. Malinowska L, Kroschwald S, Munder MC et al (2012) Molecular chaperones and stress-inducible protein-sorting factors coordinate the spatiotemporal distribution of protein aggregates. *Mol Biol Cell* 23:3041–3056. doi:10.1091/mbc.E12-03-0194
106. Liu X-D, Ko S, Xu Y et al (2012) Transient aggregation of ubiquitinated proteins is a cytosolic unfolded protein response to inflammation and endoplasmic reticulum stress. *J Biol Chem* 287:19687–19698. doi:10.1074/jbc.M112.350934
107. Specht S, Miller SBM, Mogk A, Bukau B (2011) Hsp42 is required for sequestration of protein aggregates into deposition sites in *Saccharomyces cerevisiae*. *J Cell Biol* 195:617–629. doi:10.1083/jcb.201106037
108. Kaganovich D, Kopito R, Frydman J (2008) Misfolded proteins partition between two distinct quality control compartments. *Nature* 454:1088–1095. doi:10.1038/nature07195
109. Szeto J, Kaniuk NA, Canadien V et al (2006) ALIS are stress-induced protein storage compartments for substrates of the proteasome and autophagy. *Autophagy* 2:189–199
110. Sontag EM, Vonk WI, Frydman J (2014) Sorting out the trash: the spatial nature of eukaryotic protein quality control. *Curr Opin Cell Biol* 26:139–146. doi:10.1016/j.ceb.2013.12.006
111. Bagola K, Sommer T (2008) Protein quality control: on IPODs and other JUNQ. *Curr Biol* 18:R1019–R1021. doi:10.1016/j.cub.2008.09.036
112. Ogrodnik M, Salmonowicz H, Brown R et al (2014) Dynamic JUNQ inclusion bodies are asymmetrically inherited in mammalian cell lines through the asymmetric partitioning of vimentin. *Proc Natl Acad Sci* 111:8049–8054. doi:10.1073/pnas.1324035111
113. Weisberg SJ, Lyakhovetsky R, Werdiger A-C et al (2012) Compartmentalization of superoxide dismutase 1 (SOD1G93A) aggregates determines their toxicity. *Proc Natl Acad Sci USA* 109:15811–15816. doi:10.1073/pnas.1205829109
114. Johnston JA, Ward CL, Kopito RR (1998) Aggresomes: a cellular response to misfolded proteins. *J Cell Biol* 143:1883–1898
115. Johnston JA, Illing ME, Kopito RR (2002) Cytoplasmic dynein/dynactin mediates the assembly of aggresomes. *Cell Motil Cytoskeleton* 53:26–38. doi:10.1002/cm.10057
116. Canadien V, Tan T, Zilber R et al (2005) Cutting edge: microbial products elicit formation of dendritic cell aggresome-like induced structures in macrophages. *J Immunol* 174:2471–2475. doi:10.4049/jimmunol.174.5.2471
117. Nyström T (2010) Spatial protein quality control and the evolution of lineage-specific ageing. *Philos Trans Royal Soc B Biol Sci* 366:71–75. doi:10.1098/rstb.2010.0282
118. Sabate R, de Groot NS, Ventura S (2010) Protein folding and aggregation in bacteria. *Cell Mol Life Sci* 67:2695–2715. doi:10.1007/s00018-010-0344-4
119. Coelho M, Dereli A, Haese A et al (2013) Fission Yeast Does Not Age under Favorable Conditions, but Does So after Stress. *Curr Biol* 23:1844–1852. doi:10.1016/j.cub.2013.07.084
120. Liu B, Larsson L, Caballero A et al (2010) The Polarosome Is Required for Segregation and Retrograde Transport of Protein Aggregates. *Cell* 140:257–267. doi:10.1016/j.cell.2009.12.031
121. Rokney A, Shagan M, Kessel M et al (2009) E. coli Transports Aggregated Proteins to the Poles by a Specific and Energy-Dependent Process. *J Mol Biol* 392:589–601. doi:10.1016/j.jmb.2009.07.009
122. Erjavec N, Cvijovic M, Klipp E, Nyström T (2008) Selective benefits of damage partitioning in unicellular systems and its effects on aging. *Proc Natl Acad Sci USA* 105:18764–18769. doi:10.1073/pnas.0804550105
123. Lindner AB, Madden R, Demarez A et al (2008) Asymmetric segregation of protein aggregates is associated with cellular aging and rejuvenation. *Proc Natl Acad Sci USA* 105:3076–3081. doi:10.1073/pnas.0708931105
124. Spokoini R, Moldavski O, Nahmias Y et al (2012) Confinement to organelle-associated inclusion structures mediates asymmetric inheritance of aggregated protein in budding yeast. *Cell Rep* 2:738–747. doi:10.1016/j.celrep.2012.08.024
125. Knowles TPJ, Vendruscolo M, Dobson CM (2014) The amyloid state and its association with protein misfolding diseases. *Nature Publishing Group* 15:384–396. doi:10.1038/nrm3810
126. Soto C (2003) Unfolding the role of protein misfolding in neurodegenerative diseases. *Nat Rev Neurosci* 4:49–60. doi:10.1038/nrn1007

127. Andersson V, Hanzén S, Liu B et al (2013) Enhancing protein disaggregation restores proteasome activity in aged cells. *Aging* (Albany NY) 5:802–812
128. Demontis F, Perrimon N (2010) FOXO/4E-BP signaling in drosophila muscles regulates organism-wide proteostasis during aging. *Cell* 143:813–825. doi:10.1016/j.cell.2010.10.007
129. Ben-Zvi A, Miller EA, Morimoto RI (2009) Collapse of proteostasis represents an early molecular event in *Caenorhabditis elegans* aging. *Proc Natl Acad Sci* 106:14914–14919. doi:10.1073/pnas.0902882106
130. Vernace VA, Arnaud L, Schmidt-Glenewinkel T, Figueiredo-Pereira ME (2007) Aging perturbs 26S proteasome assembly in *Drosophila melanogaster*. *FASEB J* 21:2672–2682. doi:10.1096/fj.06-6751com
131. (2005) Altered proteasome structure, function, and oxidation in aged muscle. 19:644–646. doi: 10.1096/fj.04-2578fje
132. Cuervo AM, Dice JF (2000) Age-related Decline in Chaperone-mediated Autophagy. *J Biol Chem* 275:31505–31513. doi:10.1074/jbc.M002102200
133. Vittorini S, Paradiso C, Donati A et al (1999) The age-related accumulation of protein carbonyl in rat liver correlates with the age-related decline in liver proteolytic activities. *J Gerontol Series A Bio Sci Med Sci* 54:B318–B323
134. Gupta R, Kasturi P, Bracher A et al (2011) Firefly luciferase mutants as sensors of proteome stress. *Nat Methods* 8:879–884. doi:10.1038/nmeth.1697
135. Olzscha H, Schermann SM, Woerner AC et al (2011) Amyloid-like aggregates sequester numerous metastable proteins with essential cellular functions. *Cell* 144:67–78. doi:10.1016/j.cell.2010.11.050
136. Gidalevitz T, Ben-Zvi A, Ho KH et al (2006) Progressive disruption of cellular protein folding in models of polyglutamine diseases. *Science* 311:1471–1474. doi:10.1126/science.1124514
137. Bence NF (2001) Impairment of the Ubiquitin-Proteasome System by Protein Aggregation. *Science* 292:1552–1555. doi:10.1126/science.292.5521.1552
138. Walther DM, Kasturi P, Zheng M et al (2015) Widespread Proteome Remodeling and Aggregation in Aging *C. elegans*. *Cell* 161:919–932. doi:10.1016/j.cell.2015.03.032
139. Reis-Rodrigues P, Czerwieniec G, Peters TW et al (2012) Proteomic analysis of age-dependent changes in protein solubility identifies genes that modulate lifespan. *Aging Cell* 11:120–127. doi:10.1111/j.1474-9726.2011.00765.x
140. David DC, Ollikainen N, Trinidad JC et al (2010) Widespread protein aggregation as an inherent part of aging in *C. elegans*. *PLoS Biol* 8:e1000450. doi:10.1371/journal.pbio.1000450
141. Bigio EH, Wu JY, Deng H-X et al (2013) Inclusions in frontotemporal lobar degeneration with TDP-43 proteinopathy (FTLD-TDP) and amyotrophic lateral sclerosis (ALS), but not FTLD with FUS proteinopathy (FTLD-FUS), have properties of amyloid. *Acta Neuropathol* 125:463–465. doi:10.1007/s00401-013-1089-6
142. Lashley T, Rohrer JD, Bandopadhyay R et al (2011) A comparative clinical, pathological, biochemical and genetic study of fused in sarcoma proteinopathies. *Brain* 134:2548–2564. doi:10.1093/brain/awr160
143. Shelkovich TA (2013) Modelling FUSopathies: focus on protein aggregation. *Biochem Soc Trans* 41:1613–1617. doi:10.1042/BST20130212
144. Haass C, Selkoe DJ (2007) Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid β -peptide. *Nat Rev Mol Cell Biol* 8:101–112. doi:10.1038/nrm2101
145. Eichner T, Radford SE (2011) A Diversity of Assembly Mechanisms of a Generic Amyloid Fold. 43:8–18. doi:10.1016/j.molcel.2011.05.012
146. Chiti F, Dobson CM (2006) Protein misfolding, functional amyloid, and human disease. *Annu Rev Biochem* 75:333–366. doi:10.1146/annurev.biochem.75.101304.123901
147. De Felice FG (2004) Formation of amyloid aggregates from human lysozyme and its disease-associated variants using hydrostatic pressure. *FASEB J*. doi:10.1096/fj.03-1072fje
148. Schmittschmitt JP, Scholtz JM (2003) The role of protein stability, solubility, and net charge in amyloid fibril formation. *Protein Sci* 12:2374–2378. doi:10.1110/ps.03152903
149. Fändrich M, Fletcher MA, Dobson CM (2001) Amyloid fibrils from muscle myoglobin. *Nature* 410:165–166. doi:10.1038/35065514
150. Ferrão-Gonzales AD, Souto SO, Silva JL, Foguel D (2000) The preaggregated state of an amyloidogenic protein: hydrostatic pressure converts native transthyretin into the amyloidogenic state. *Proc Natl Acad Sci* 97:6445–6450
151. McParland VJ, Kad NM, Kalverda AP et al (2000) Partially Unfolded States of β 2-Microglobulin and Amyloid Formation in Vitro. *Biochemistry* 39:8735–8746. doi:10.1021/bi000276j
152. Chiti F, Webster P, Taddei N et al (1999) Designing conditions for in vitro formation of amyloid protofilaments and fibrils. *Proc Natl Acad Sci* 96:3590–3594
153. Guijarro JI, Sunde M, Jones JA et al (1998) Amyloid fibril formation by an SH3 domain. *Proc Natl Acad Sci* 95:4224–4228
154. Litvinovich SV, Brew SA, Aota S et al (1998) Formation of amyloid-like fibrils by self-association of a partially unfolded fibronectin type III module. *J Mol Biol* 280:245–258. doi:10.1006/jmbi.1998.1863
155. Maurer-Stroh S, Debulpaep M, Kummerer N et al (2010) Exploring the sequence determinants of amyloid structure using position-specific scoring matrices. *Nat Methods* 7:237–242. doi:10.1038/nmeth.1432
156. Fernandez-Escamilla A-M, Rousseau F, Schymkowitz J, Serano L (2004) Prediction of sequence-dependent and mutational effects on the aggregation of peptides and proteins. *Nat Biotechnol* 22:1302–1306. doi:10.1038/nbt1012
157. Walsh DM, Klyubin I, Fadeeva JV et al (2002) Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. *Nature* 416:535–539. doi:10.1038/416535a
158. Lambert MP, Barlow AK, Chromy BA et al (1998) Diffusible, nonfibrillar ligands derived from A β 1–42 are potent central nervous system neurotoxins. *Proc Natl Acad Sci* 95:6448–6453
159. Kuo YM, Emmerling MR, Vigo-Pelfrey C et al (1996) Water-soluble A β (N-40, N-42) oligomers in normal and Alzheimer disease brains. *J Biol Chem* 271:4077–4081
160. Walsh DM, Hartley DM, Kusumoto Y et al (1999) Amyloid beta-protein fibrillogenesis. Structure and biological activity of protofibrillar intermediates. *J Biol Chem* 274:25945–25952. doi:10.1074/jbc.274.36.25945
161. Walsh DM, Lomakin A, Benedek GB et al (1997) Amyloid beta-protein fibrillogenesis. Detection of a protofibrillar intermediate. *J Biol Chem* 272:22364–22372
162. Miller J, Arrasate M, Brooks E et al (2011) Identifying polyglutamine protein species in situ that best predict neurodegeneration. *Nat Chem Biol* 7:925–934. doi:10.1038/nchembio.694
163. Bolognesi B, Kumita JR, Barros TP et al (2010) ANS Binding Reveals Common Features of Cytotoxic Amyloid Species. 5:735–740. doi:10.1021/cb1001203
164. Arrasate M, Mitra S, Schweitzer ES et al (2004) Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. *Nature* 431:805–810. doi:10.1038/nature02998
165. Kaye R, Head E, Thompson JL et al (2003) Common structure of soluble amyloid oligomers implies common mechanism of

- pathogenesis. *Science* 300:486–489. doi:[10.1126/science.1079469](https://doi.org/10.1126/science.1079469)
166. De Genst E, Messer A, Dobson CM (2014) Antibodies and protein misfolding: from structural research tools to therapeutic strategies. *BBA - Proteins and Proteomics* 1844:1907–1919. doi:[10.1016/j.bbapap.2014.08.016](https://doi.org/10.1016/j.bbapap.2014.08.016)
 167. Benilova I, Karran E, De Strooper B (2012) The toxic A β oligomer and Alzheimer's disease: an emperor in need of clothes. *Nat Neurosci* 15:349–357. doi:[10.1038/nm.3028](https://doi.org/10.1038/nm.3028)
 168. Lashuel HA, Lansbury PT (2006) Are amyloid diseases caused by protein aggregates that mimic bacterial pore-forming toxins? *Q Rev Biophys* 39:167–201. doi:[10.1017/S0033583506004422](https://doi.org/10.1017/S0033583506004422)
 169. Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT (2011) Neuropathological alterations in Alzheimer disease. *Cold Spring Harb Perspect Med* 1:a006189. doi:[10.1101/cshperspect.a006189](https://doi.org/10.1101/cshperspect.a006189)
 170. van Ham TJ, Breitling R, Swertz MA, Nollen EAA (2009) Neurodegenerative diseases: lessons from genome-wide screens in small model organisms. *EMBO Mol Med* 1:360–370. doi:[10.1002/emmm.200900051](https://doi.org/10.1002/emmm.200900051)
 171. Miller-Fleming L, Giorgini F, Outeiro TF (2008) Yeast as a model for studying human neurodegenerative disorders. *Biotechnol J* 3:325–338. doi:[10.1002/biot.200700217](https://doi.org/10.1002/biot.200700217)
 172. Spillantini MG, Schmidt ML, Lee VM et al (1997) Alpha-synuclein in Lewy bodies. *Nature* 388:839–840. doi:[10.1038/42166](https://doi.org/10.1038/42166)
 173. Kim WS, Kågedal K, Halliday GM (2014) Alpha-synuclein biology in Lewy body diseases. *Alzheimers Res Ther* 6:73. doi:[10.1186/s13195-014-0073-2](https://doi.org/10.1186/s13195-014-0073-2)
 174. Outeiro TF, Lindquist S (2003) Yeast cells provide insight into alpha-synuclein biology and pathobiology. *Science* 302:1772–1775. doi:[10.1126/science.1090439](https://doi.org/10.1126/science.1090439)
 175. Winderickx J, Delay C, De Vos A et al (2008) Protein folding diseases and neurodegeneration: lessons learned from yeast. *Biochim Biophys Acta* 1783:1381–1395. doi:[10.1016/j.bbamcr.2008.01.020](https://doi.org/10.1016/j.bbamcr.2008.01.020)
 176. Cooper AA, Gitler AD, Cashikar A et al (2006) Alpha-synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson's models. *Science* 313:324–328. doi:[10.1126/science.1129462](https://doi.org/10.1126/science.1129462)
 177. Gitler AD, Chesi A, Geddie ML et al (2009) Alpha-Synuclein is part of a diverse and highly conserved interaction network that includes PARK9 and manganese toxicity. *Nat Genet* 41:308–315. doi:[10.1038/ng.300](https://doi.org/10.1038/ng.300)
 178. Chesi A, Kilaru A, Fang X et al (2012) The role of the Parkinson's disease gene PARK9 in essential cellular pathways and the manganese homeostasis network in yeast. *PLoS One* 7:e34178. doi:[10.1371/journal.pone.0034178](https://doi.org/10.1371/journal.pone.0034178)
 179. Kuwahara T, Koyama A, Koyama S et al (2008) A systematic RNAi screen reveals involvement of endocytic pathway in neuronal dysfunction in alpha-synuclein transgenic *C. elegans*. *Hum Mol Genet* 17:2997–3009. doi:[10.1093/hmg/ddn198](https://doi.org/10.1093/hmg/ddn198)
 180. van Ham TJ, Thijssen KL, Breitling R et al (2008) *C. elegans* model identifies genetic modifiers of alpha-synuclein inclusion formation during aging. *PLoS Genet* 4:e1000027. doi:[10.1371/journal.pgen.1000027](https://doi.org/10.1371/journal.pgen.1000027)
 181. Hamamichi S, Rivas RN, Knight AL et al (2008) Hypothesis-based RNAi screening identifies neuroprotective genes in a Parkinson's disease model. *Proc Natl Acad Sci USA* 105:728–733. doi:[10.1073/pnas.0711018105](https://doi.org/10.1073/pnas.0711018105)
 182. van der Goot AT, Zhu W, Vazquez-Manrique RP et al (2012) Delaying aging and the aging-associated decline in protein homeostasis by inhibition of tryptophan degradation. *Proc Natl Acad Sci USA* 109:14912–14917. doi:[10.1073/pnas.1203083109](https://doi.org/10.1073/pnas.1203083109)
 183. Komatsu M, Wang QJ, Holstein GR et al (2007) Essential role for autophagy protein Atg7 in the maintenance of axonal homeostasis and the prevention of axonal degeneration. *Proc Natl Acad Sci* 104:14489–14494. doi:[10.1073/pnas.0701311104](https://doi.org/10.1073/pnas.0701311104)
 184. Ballard C, Gauthier S, Corbett A et al (2011) Alzheimer's disease. *Lancet* 377:1019–1031. doi:[10.1016/S0140-6736\(10\)61349-9](https://doi.org/10.1016/S0140-6736(10)61349-9)
 185. Takashima A (2009) Amyloid-beta, tau, and dementia. *J Alzheimers Dis* 17:729–736. doi:[10.3233/JAD-2009-1090](https://doi.org/10.3233/JAD-2009-1090)
 186. Bettens K, Sleegers K, Van Broeckhoven C (2013) Genetic insights in Alzheimer's disease. *Lancet Neurol* 12:92–104. doi:[10.1016/S1474-4422\(12\)70259-4](https://doi.org/10.1016/S1474-4422(12)70259-4)
 187. Flachsbart F, Caliebe A, Kleindorp R et al (2009) Association of FOXO3A variation with human longevity confirmed in German centenarians. *Proc Natl Acad Sci USA* 106:2700–2705. doi:[10.1073/pnas.0809594106](https://doi.org/10.1073/pnas.0809594106)
 188. Suh Y, Atzmon G, Cho M-O et al (2008) Functionally significant insulin-like growth factor I receptor mutations in centenarians. *Proc Natl Acad Sci* 105:3438–3442. doi:[10.1073/pnas.0705467105](https://doi.org/10.1073/pnas.0705467105)
 189. Willcox BJ, Donlon TA, He Q et al (2008) FOXO3A genotype is strongly associated with human longevity. *Proc Natl Acad Sci USA* 105:13987–13992. doi:[10.1073/pnas.0801030105](https://doi.org/10.1073/pnas.0801030105)
 190. Blüher M, Kahn BB, Kahn CR (2003) Extended longevity in mice lacking the insulin receptor in adipose tissue. *Science* 299:572–574. doi:[10.1126/science.1078223](https://doi.org/10.1126/science.1078223)
 191. Holzenberger M, Dupont J, Ducos B et al (2003) IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. *Nature* 421:182–187. doi:[10.1038/nature01298](https://doi.org/10.1038/nature01298)
 192. Tatar M (2001) A mutant drosophila insulin receptor homolog that extends life-span and impairs neuroendocrine function. *Science* 292:107–110. doi:[10.1126/science.1057987](https://doi.org/10.1126/science.1057987)
 193. Kenyon C, Chang J, Gensch E et al (1993) A *C. elegans* mutant that lives twice as long as wild type. *Nature* 366:461–464. doi:[10.1038/366461a0](https://doi.org/10.1038/366461a0)
 194. Link CD (1995) Expression of human beta-amyloid peptide in transgenic *Caenorhabditis elegans*. *Proc Natl Acad Sci* 92:9368–9372
 195. Cohen E, Bieschke J, Perciavalle RM et al (2006) Opposing activities protect against age-onset proteotoxicity. *Science* 313:1604–1610. doi:[10.1126/science.1124646](https://doi.org/10.1126/science.1124646)
 196. Hsu A-L, Murphy CT, Kenyon C (2003) Regulation of aging and age-related disease by DAF-16 and heat-shock factor. *Science* 300:1142–1145. doi:[10.1126/science.1083701](https://doi.org/10.1126/science.1083701)
 197. Cohen E, Paulsson JF, Blinder P et al (2009) Reduced IGF-1 signaling delays age-associated proteotoxicity in mice. *Cell* 139:1157–1169. doi:[10.1016/j.cell.2009.11.014](https://doi.org/10.1016/j.cell.2009.11.014)
 198. Treusch S, Hamamichi S, Goodman JL et al (2011) Functional links between A β toxicity, endocytic trafficking, and Alzheimer's disease risk factors in yeast. *Science* 334:1241–1245. doi:[10.1126/science.1213210](https://doi.org/10.1126/science.1213210)
 199. Shulman JM, Chipendo P, Chibnik LB et al (2011) Functional screening of Alzheimer pathology genome-wide association signals in *Drosophila*. *Am J Hum Genet* 88:232–238. doi:[10.1016/j.ajhg.2011.01.006](https://doi.org/10.1016/j.ajhg.2011.01.006)
 200. Morley JF, Brignull HR, Weyers JJ, Morimoto RI (2002) The threshold for polyglutamine-expansion protein aggregation and cellular toxicity is dynamic and influenced by aging in *Caenorhabditis elegans*. *Proc Natl Acad Sci* 99:10417–10422. doi:[10.1073/pnas.152161099](https://doi.org/10.1073/pnas.152161099)
 201. Silva MC, Fox S, Beam M et al (2011) A genetic screening strategy identifies novel regulators of the proteostasis network. *PLoS Genet* 7:e1002438. doi:[10.1371/journal.pgen.1002438](https://doi.org/10.1371/journal.pgen.1002438)
 202. Nollen EAA, Garcia SM, van Haaften G et al (2004) Genome-wide RNA interference screen identifies previously undescribed

- regulators of polyglutamine aggregation. *Proc Natl Acad Sci* 101:6403–6408. doi:[10.1073/pnas.0307697101](https://doi.org/10.1073/pnas.0307697101)
203. van Ham TJ, Holmberg MA, van der Goot AT et al (2010) Identification of MOAG-4/SERF as a regulator of age-related proteotoxicity. *Cell* 142:601–612. doi:[10.1016/j.cell.2010.07.020](https://doi.org/10.1016/j.cell.2010.07.020)
204. Falsone SF, Meyer NH, Schrank E et al (2012) SERF protein is a direct modifier of amyloid fiber assembly. *Cell Rep* 2:358–371. doi:[10.1016/j.celrep.2012.06.012](https://doi.org/10.1016/j.celrep.2012.06.012)
205. Lejeune F-X, Mesrob L, Parmentier F et al (2012) Large-scale functional RNAi screen in *C. elegans* identifies genes that regulate the dysfunction of mutant polyglutamine neurons. *BMC Genom* 13:91. doi:[10.1186/1471-2164-13-91](https://doi.org/10.1186/1471-2164-13-91)
206. Behrends C, Langer CA, Boteva R et al (2006) Chaperonin TRiC promotes the assembly of polyQ expansion proteins into nontoxic oligomers. *Mol Cell* 23:887–897. doi:[10.1016/j.molcel.2006.08.017](https://doi.org/10.1016/j.molcel.2006.08.017)
207. Tam S, Geller R, Spiess C, Frydman J (2006) The chaperonin TRiC controls polyglutamine aggregation and toxicity through subunit-specific interactions. *Nat Cell Biol* 8:1155–1162. doi:[10.1038/ncb1477](https://doi.org/10.1038/ncb1477)
208. Giorgini F, Guidetti P, Nguyen Q et al (2005) A genomic screen in yeast implicates kynurenine 3-monooxygenase as a therapeutic target for Huntington disease. *Nat Genet* 37:526–531. doi:[10.1038/ng1542](https://doi.org/10.1038/ng1542)
209. Campesan S, Green EW, Breda C et al (2011) The kynurenine pathway modulates neurodegeneration in a *Drosophila* model of Huntington's disease. *Curr Biol* 21:961–966. doi:[10.1016/j.cub.2011.04.028](https://doi.org/10.1016/j.cub.2011.04.028)
210. Zwilling D, Huang S-Y, Sathyaikumar KV et al (2011) Kynurenine 3-monooxygenase inhibition in blood ameliorates neurodegeneration. *Cell* 145:863–874. doi:[10.1016/j.cell.2011.05.020](https://doi.org/10.1016/j.cell.2011.05.020)
211. Lander ES, Linton LM, Birren B et al (2001) Initial sequencing and analysis of the human genome. *Nature* 409:860–921. doi:[10.1038/35057062](https://doi.org/10.1038/35057062)
212. Clamp M, Fry B, Kamal M et al (2007) Distinguishing protein-coding and noncoding genes in the human genome. *Proc Natl Acad Sci USA* 104:19428–19433. doi:[10.1073/pnas.0709013104](https://doi.org/10.1073/pnas.0709013104)
213. Mouse Genome Sequencing Consortium, Waterston RH, Lindblad-Toh K et al (2002) Initial sequencing and comparative analysis of the mouse genome. *Nature* 420:520–562. doi:[10.1038/nature01262](https://doi.org/10.1038/nature01262)
214. ENCODE Project Consortium (2012) An integrated encyclopedia of DNA elements in the human genome. *Nature* 489:57–74. doi:[10.1038/nature11247](https://doi.org/10.1038/nature11247)
215. Kapranov P, Cheng J, Dike S et al (2007) RNA Maps Reveal New RNA Classes and a Possible Function for Pervasive Transcription. *Science* 316:1484–1488. doi:[10.1126/science.1138341](https://doi.org/10.1126/science.1138341)
216. Carninci P, Kasukawa T, Katayama S et al (2005) The transcriptional landscape of the mammalian genome. *Science* 309:1559–1563. doi:[10.1126/science.1112014](https://doi.org/10.1126/science.1112014)
217. Cech TR, Steitz JA (2014) The Noncoding RNA Revolution—Trashing Old Rules to Forge New Ones. *Cell* 157:77–94. doi:[10.1016/j.cell.2014.03.008](https://doi.org/10.1016/j.cell.2014.03.008)
218. Kapranov P, Laurent GS, Raz T et al (2010) The majority of total nuclear-encoded non-ribosomal RNA in a human cell is “dark matter” un-annotated RNA. *BMC Biol* 8:149. doi:[10.1186/1741-7007-8-149](https://doi.org/10.1186/1741-7007-8-149)
219. Donnelly CJ, Zhang P-W, Pham JT et al (2013) RNA toxicity from the ALS/FTD C9ORF72 expansion is mitigated by antisense intervention. *Neuron* 80:415–428. doi:[10.1016/j.neuron.2013.10.015](https://doi.org/10.1016/j.neuron.2013.10.015)
220. Miller JW, Urbinati CR, Teng-Umuay P et al (2000) Recruitment of human muscleblind proteins to (CUG)(n) expansions associated with myotonic dystrophy. *EMBO J* 19:4439–4448. doi:[10.1093/emboj/19.17.4439](https://doi.org/10.1093/emboj/19.17.4439)
221. Krol J, Loedige I, Filipowicz W (2010) The widespread regulation of microRNA biogenesis, function and decay. *Nat Rev Genet* 11:597–610. doi:[10.1038/nrg2843](https://doi.org/10.1038/nrg2843)
222. Bak M, Silahtaroglu A, Moller M et al (2008) MicroRNA expression in the adult mouse central nervous system. *RNA* 14:432–444. doi:[10.1261/rna.783108](https://doi.org/10.1261/rna.783108)
223. Mercer TR, Dinger ME, Sunkin SM et al (2008) Specific expression of long noncoding RNAs in the mouse brain. *Proc Natl Acad Sci USA* 105:716–721. doi:[10.1073/pnas.0706729105](https://doi.org/10.1073/pnas.0706729105)
224. Kapsimali M, Kloosterman WP, de Bruijn E et al (2007) MicroRNAs show a wide diversity of expression profiles in the developing and mature central nervous system. *Genome Biol* 8:R173. doi:[10.1186/gb-2007-8-8-r173](https://doi.org/10.1186/gb-2007-8-8-r173)
225. Visvanathan J, Lee S, Lee B et al (2007) The microRNA miR-124 antagonizes the anti-neural REST/SCP1 pathway during embryonic CNS development. *Genes Dev* 21:744–749. doi:[10.1101/gad.1519107](https://doi.org/10.1101/gad.1519107)
226. Smirnova L, Gräfe A, Seiler A et al (2005) Regulation of miRNA expression during neural cell specification. *Eur J Neurosci* 21:1469–1477. doi:[10.1111/j.1460-9568.2005.03978.x](https://doi.org/10.1111/j.1460-9568.2005.03978.x)
227. Miska EA, Alvarez-Saavedra E, Townsend M et al (2004) Microarray analysis of microRNA expression in the developing mammalian brain. *Genome Biol* 5:R68–R69. doi:[10.1186/gb-2004-5-9-r68](https://doi.org/10.1186/gb-2004-5-9-r68)
228. Sempere LF, Freemantle S, Pitha-Rowe I et al (2004) Expression profiling of mammalian microRNAs uncovers a subset of brain-expressed microRNAs with possible roles in murine and human neuronal differentiation. *Genome Biol* 5:R13–R14. doi:[10.1186/gb-2004-5-3-r13](https://doi.org/10.1186/gb-2004-5-3-r13)
229. Krichevsky AM, King KS, Donahue CP et al (2003) A microRNA array reveals extensive regulation of microRNAs during brain development. *RNA* 9:1274–1281. doi:[10.1261/rna.5980303](https://doi.org/10.1261/rna.5980303)
230. Rege SD, Geetha T, Pondugula SR et al (2013) Noncoding RNAs in Neurodegenerative Diseases. *International Scholarly Research Notices* 2013:1–5. doi:[10.1155/2013/375852](https://doi.org/10.1155/2013/375852)
231. Johnson R, Noble W, Tartaglia GG, Buckley NJ (2012) Neurodegeneration as an RNA disorder. *Prog Neurobiol* 99:293–315. doi:[10.1016/j.pneurobio.2012.09.006](https://doi.org/10.1016/j.pneurobio.2012.09.006)
232. Salta E, De Strooper B (2012) Non-coding RNAs with essential roles in neurodegenerative disorders. *Lancet Neurol* 11:189–200. doi:[10.1016/S1474-4422\(11\)70286-1](https://doi.org/10.1016/S1474-4422(11)70286-1)
233. Smith P, Hashimi AI, Girard J et al (2011) In vivo regulation of amyloid precursor protein neuronal splicing by microRNAs. *J Neurochem* 116:240–247. doi:[10.1111/j.1471-4159.2010.07097.x](https://doi.org/10.1111/j.1471-4159.2010.07097.x)
234. Wang X, Liu P, Zhu H et al (2009) miR-34a, a microRNA up-regulated in a double transgenic mouse model of Alzheimer's disease, inhibits bcl2 translation. *Brain Res Bull* 80:268–273. doi:[10.1016/j.brainresbull.2009.08.006](https://doi.org/10.1016/j.brainresbull.2009.08.006)
235. Hébert SS, Horré K, Nicolai L et al (2008) Loss of microRNA cluster miR-29a/b-1 in sporadic Alzheimer's disease correlates with increased BACE1/beta-secretase expression. *Proc Natl Acad Sci USA* 105:6415–6420. doi:[10.1073/pnas.0710263105](https://doi.org/10.1073/pnas.0710263105)
236. Wang W-X, Rajeev BW, Stromberg AJ et al (2008) The expression of microRNA miR-107 decreases early in Alzheimer's disease and may accelerate disease progression through regulation of beta-site amyloid precursor protein-cleaving enzyme 1. *J Neurosci* 28:1213–1223. doi:[10.1523/JNEUROSCI.5065-07.2008](https://doi.org/10.1523/JNEUROSCI.5065-07.2008)

237. Lukiw WJ (2007) Micro-RNA speciation in fetal, adult and Alzheimer's disease hippocampus. *NeuroReport* 18:297–300. doi:[10.1097/WNR.0b013e3280148e8b](https://doi.org/10.1097/WNR.0b013e3280148e8b)
238. Miñones-Moyano E, Porta S, Escaramís G et al (2011) Micro-RNA profiling of Parkinson's disease brains identifies early downregulation of miR-34b/c which modulate mitochondrial function. *Hum Mol Genet* 20:3067–3078. doi:[10.1093/hmg/ddr210](https://doi.org/10.1093/hmg/ddr210)
239. Kabaria S, Choi DC, Chaudhuri AD et al (2015) Inhibition of miR-34b and miR-34c enhances α -synuclein expression in Parkinson's disease. *FEBS Lett* 589:319–325. doi:[10.1016/j.febslet.2014.12.014](https://doi.org/10.1016/j.febslet.2014.12.014)
240. Lee S-T, Chu K, Im W-S et al (2011) Altered microRNA regulation in Huntington's disease models. *Exp Neurol* 227:172–179. doi:[10.1016/j.expneurol.2010.10.012](https://doi.org/10.1016/j.expneurol.2010.10.012)
241. Marti E, Pantano L, Banez-Coronel M et al (2010) A myriad of miRNA variants in control and Huntington's disease brain regions detected by massively parallel sequencing. *Nucleic Acids Res* 38:7219–7235. doi:[10.1093/nar/gkq575](https://doi.org/10.1093/nar/gkq575)
242. Johnson R, Zuccato C, Belyaev ND et al (2008) A microRNA-based gene dysregulation pathway in Huntington's disease. *Neurobiology of Disease* 29:438–445. doi:[10.1016/j.nbd.2007.11.001](https://doi.org/10.1016/j.nbd.2007.11.001)
243. Lee Y, Samaco RC, Gatchel JR et al (2008) miR-19, miR-101 and miR-130 co-regulate ATXN1 levels to potentially modulate SCA1 pathogenesis. *Nat Neurosci* 11:1137–1139. doi:[10.1038/nn.2183](https://doi.org/10.1038/nn.2183)
244. Packer AN, Xing Y, Harper SQ et al (2008) The Bifunctional microRNA miR-9/miR-9* Regulates REST and CoREST and Is Downregulated in Huntington's Disease. *J Neurosci* 28:14341–14346. doi:[10.1523/JNEUROSCI.2390-08.2008](https://doi.org/10.1523/JNEUROSCI.2390-08.2008)
245. Novoa EM, Ribas de Pouplana L (2012) Speeding with control: codon usage, tRNAs, and ribosomes. *Trends Genet* 28:574–581. doi:[10.1016/j.tig.2012.07.006](https://doi.org/10.1016/j.tig.2012.07.006)
246. Goodenbour JM, Pan T (2006) Diversity of tRNA genes in eukaryotes. *Nucleic Acids Res* 34:6137–6146. doi:[10.1093/nar/gkl725](https://doi.org/10.1093/nar/gkl725)
247. Ishimura R, Nagy G, Dotu I et al (2014) RNA function. Ribosome stalling induced by mutation of a CNS-specific tRNA causes neurodegeneration. *Science* 345:455–459. doi:[10.1126/science.1249749](https://doi.org/10.1126/science.1249749)
248. Kasher PR, Namavar Y, van Tijn P et al (2011) Impairment of the tRNA-splicing endonuclease subunit 54 (*tsen54*) gene causes neurological abnormalities and larval death in zebrafish models of pontocerebellar hypoplasia. *Hum Mol Genet* 20:1574–1584. doi:[10.1093/hmg/ddr034](https://doi.org/10.1093/hmg/ddr034)
249. Namavar Y, Barth PG, Kasher PR et al (2011) Clinical, neuroradiological and genetic findings in pontocerebellar hypoplasia. *Brain* 134:143–156. doi:[10.1093/brain/awq287](https://doi.org/10.1093/brain/awq287)
250. Budde BS, Namavar Y, Barth PG et al (2008) tRNA splicing endonuclease mutations cause pontocerebellar hypoplasia. *Nat Genet* 40:1113–1118. doi:[10.1038/ng.204](https://doi.org/10.1038/ng.204)
251. Edvardson S, Shaag A, Kolesnikova O et al (2007) Deleterious mutation in the mitochondrial arginyl-transfer RNA synthetase gene is associated with pontocerebellar hypoplasia. *Am J Hum Genet* 81:857–862. doi:[10.1086/521227](https://doi.org/10.1086/521227)
252. Borthwick GM, Taylor RW, Walls TJ et al (2006) Motor neuron disease in a patient with a mitochondrial tRNA^{Ala} mutation. *Ann Neurol* 59:570–574. doi:[10.1002/ana.20758](https://doi.org/10.1002/ana.20758)
253. Rudnik-Schöneborn S, Barth PG, Zerres K (2014) Pontocerebellar hypoplasia. *Am J Med Genet C Semin Med Genet* 166C:173–183. doi:[10.1002/ajmg.c.31403](https://doi.org/10.1002/ajmg.c.31403)
254. Trotta CR, Paushkin SV, Patel M et al (2006) Cleavage of pre-tRNAs by the splicing endonuclease requires a composite active site. *Nature* 441:375–377. doi:[10.1038/nature04741](https://doi.org/10.1038/nature04741)
255. Hanada T, Weitzer S, Mair B et al (2013) CLP1 links tRNA metabolism to progressive motor-neuron loss. *Nature*. doi:[10.1038/nature11923](https://doi.org/10.1038/nature11923)
256. Karaca E, Weitzer S, Pehlivan D et al (2014) Human CLP1 mutations Alter tRNA biogenesis, affecting Both peripheral and central nervous system function. *Cell* 157:636–650. doi:[10.1016/j.cell.2014.02.058](https://doi.org/10.1016/j.cell.2014.02.058)
257. Schaffer AE, Eggens VRC, Caglayan AO et al (2014) CLP1 Founder Mutation Links tRNA Splicing and Maturation to Cerebellar Development and Neurodegeneration. *Cell* 157:651–663. doi:[10.1016/j.cell.2014.03.049](https://doi.org/10.1016/j.cell.2014.03.049)
258. Qureshi IA, Mattick JS, Mehler MF (2010) Long non-coding RNAs in nervous system function and disease. *Brain Res* 1338:20–35. doi:[10.1016/j.brainres.2010.03.110](https://doi.org/10.1016/j.brainres.2010.03.110)
259. Faghihi MA, Zhang M, Huang J et al (2010) Evidence for natural antisense transcript-mediated inhibition of microRNA function. *Genome Biol* 11:R56. doi:[10.1186/gb-2010-11-5-r56](https://doi.org/10.1186/gb-2010-11-5-r56)
260. Tan JY, Vance KW, Varela MA et al (2014) Cross-talking noncoding RNAs contribute to cell-specific neurodegeneration in SCA7. *Nat Struct Mol Biol* 21:955–961. doi:[10.1038/nsmb.2902](https://doi.org/10.1038/nsmb.2902)
261. Francelle L, Galvan L, Gaillard M-C et al (2015) The striatal long noncoding RNA *Abhd11os* is neuroprotective against an N-terminal fragment of mutant huntingtin in vivo. *Neurobiol Aging* 36(1601):e7–e16. doi:[10.1016/j.neurobiolaging.2014.11.014](https://doi.org/10.1016/j.neurobiolaging.2014.11.014)
262. Guthrie C, Patterson B (1988) Spliceosomal snRNAs. *Annu Rev Genet* 22:387–419. doi:[10.1146/annurev.ge.22.120188.002131](https://doi.org/10.1146/annurev.ge.22.120188.002131)
263. Maniatis T, Reed R (1987) The role of small nuclear ribonucleoprotein particles in pre-mRNA splicing. *Nature* 325:673–678. doi:[10.1038/325673a0](https://doi.org/10.1038/325673a0)
264. Zhang Z, Lotti F, Dittmar K et al (2008) SMN deficiency causes tissue-specific perturbations in the repertoire of snRNAs and widespread defects in splicing. *Cell* 133:585–600. doi:[10.1016/j.cell.2008.03.031](https://doi.org/10.1016/j.cell.2008.03.031)
265. Jia Y, Mu JC, Ackerman SL (2012) Mutation of a U2 snRNA gene causes global disruption of alternative splicing and neurodegeneration. *Cell* 148:296–308. doi:[10.1016/j.cell.2011.11.057](https://doi.org/10.1016/j.cell.2011.11.057)