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Review

ER chaperones in neurodegenerative disease: Folding and beyond

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ABSTRACT

Proteins along the secretory pathway are co-translationally translocated into the lumen of the endoplasmic reticulum (ER) as unfolded polypeptide chains. Afterwards, they are usually modified with N-linked glycans, correctly folded and stabilized by disulfide bonds. ER chaperones and folding enzymes control these processes. The accumulation of unfolded proteins in the ER activates a signaling response, termed the unfolded protein response (UPR). The hallmark of this response is the coordinated transcriptional up-regulation of ER chaperones and folding enzymes. In order to discuss the importance of the proper folding of certain substrates we will address the role of ER chaperones in normal physiological conditions and examine different aspects of its contribution in neurodegenerative disease.

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Contents

1. ER chaperones and proteostasis imbalance

Protein homeostasis (proteostasis) supports several dynamic processes, including protein synthesis, folding, oligomerization and turnover, which functions to ensure the integrity of the proteome. Proteostasis imbalance is a key event in many neurodegenerative diseases (Doyle et al., 2013; Hetz and Mollereau, 2014; Valastyan and Lindquist, 2014), which are grouped together as protein misfolding disorders (PMDs). Misfolded proteins are disease-specific, tau and beta-amyloid in Alzheimer's disease (AD); α-synuclein (α-syn) in Parkinson's disease (PD); RNA binding proteins with prion-like domains in fronto temporal dementia (FTD) and amyotrophic lateral sclerosis (ALS); polyglutamine containing proteins in Huntington's disease (HD) and spinal cerebellar ataxias; and prion proteins (PrP) in Creuzfeldt-Jakob disease (Ling et al., 2013; Vidal et al., 2014; Knowles et al., 2014).

The maintenance of endoplasmic reticulum (ER) proteostasis is a highly complex process, which engages the coordination of several proteins including chaperones, foldases and co-factors. However, the disturbance in ER function due to accumulation of misfolded or unfolded proteins induce a phenomenon known as "ER stress" (Hetz, 2012). ER stress prompts an adaptive program called unfolded protein response (UPR), an integrated signaling cascade that controls the expression of target genes involved in ER

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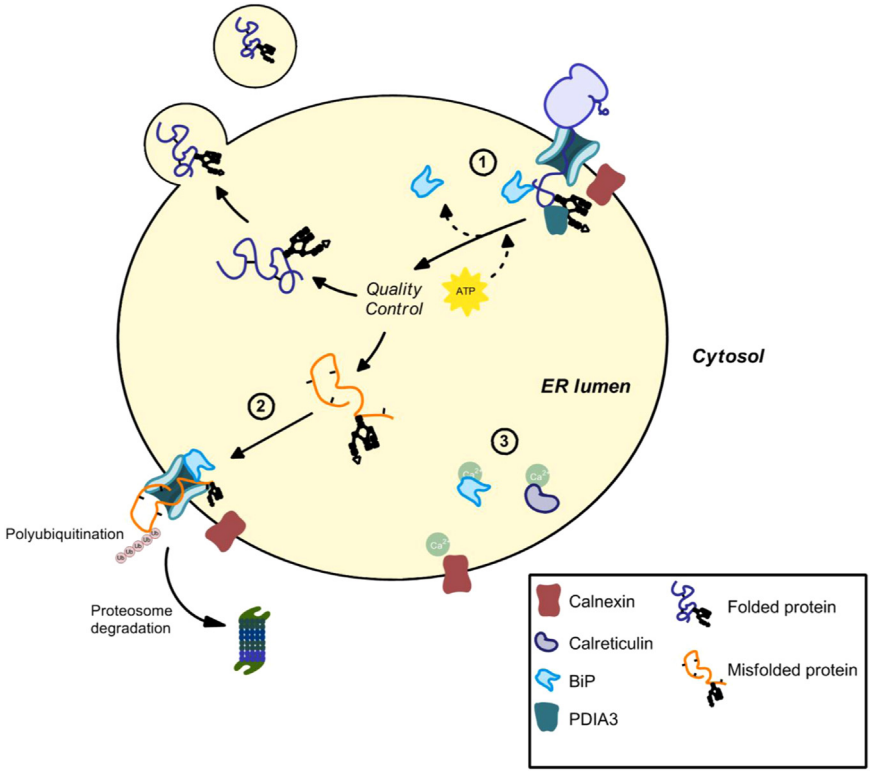

Fig. 1. Role of ER-resident chaperone functions under physiological conditions. The ER chaperones contribute to protein folding (1) facilitating co-translational translocation and helping protein folding, (2) enhancing retro-translocation and ERAD of misfolded or unfolded proteins and (3) modulating the ER luminal calcium storage.

physiology (Hetz, 2012). Initially, the UPR increases the folding capacity of the ER through an up-regulation of ER chaperones and foldases, including binding immunoglobulin protein (BiP) (also known as Grp78), Grp94, calreticulin (CRT), calnexin (CNX) and protein disulfide isomerase (PDI) family members (Schroder and Kaufman, 2005) (Fig. 1). Moreover, the removal of misfolded proteins from the ER through ER associate degradation (ERAD) is also regulated by the UPR via modulation of genes such as ER degradation-enhancing alpha-mannosidase-like 1 (EDEM1) and homocysteine-responsive endoplasmic reticulum-resident ubiquitin- like domain protein (HERP) (Vembar and Brodsky, 2008; Hetz et al., 2011) (Fig. 1). Thus, the ER is an essential component of the intracellular network able to transduce and to integrate incoming signals, to modulate and to react to its own luminal dynamics and to produce output signals in response to environmental changes (Rutkowski and Kaufman, 2004; Corbett et al., 2000).

In normal conditions, after synthesis, proteins must be folded to accomplish their biological function. Membrane or secretion proteins are incorporated to the ER where they acquire their proper folded state facilitated by chaperones. In ER stress conditions, chaperones can avoid the aggregation of unfolded polypeptide chains (Hartl et al., 2011; Kim et al., 2013), cooperate with proteases to facilitate protein degradation (Alexopoulos et al., 2012; Li and Lucius, 2013) and disaggregate protein aggregates (Hodson et al., 2012; Winkler et al., 2012). Increasing evidence implicates chaperone proteins defects in the etiology of PMDs concurring aggregative processes. Recently, several studies demonstrated that chaperone proteins have a neuroprotective role in different mouse models of neurodegeneration (Carman et al., 2013; Witt, 2013; Wyatt et al., 2012). Upregulation of ER chaperones, such as BiP, Grp94, CRT, CNX, and some members of the PDI family, has been observed in early stages of activation of the UPR (Schroder and Kaufman, 2005), during neurodegenerative diseases. Moreover, these ER chaperones are involved in Ca^{2+} homeostasis (Higo et al., 2005; Li and Camacho, 2004) and ERAD, ameliorating protein misfolding within the ER (Gillece et al., 1999; Molinari et al., 2002; Tsai et al., 2001). PDI family members perform crucial roles regulating ER stress by maintaining native protein conformation and facilitating protein degradation (Ellgaard and Ruddock, 2005). This family play key functions as ER foldases catalyzing disulfide reduction (breakage), formation (oxidation) and isomerization thereby promoting native protein folding (Feige and Hendershot, 2011) of proteins that traffick through the secretory pathway (Hatahet and Ruddock, 2009) (Fig. 1). Although several PDI family members have been related to neurodegeneration, their specific contribution to pathological conditions is only beginning to be understood. On the other hand, CNX and CRT are two critical ER localized lectins, involved in folding and quality control of newly synthesized peptides (Hebert and Molinari, 2007). These proteins interact with the monoglycosylation found on some nascent polypeptide chains. Both proteins, together with PDIA3, also called Grp58 or Erp57 (here after referred as PDIA3), make up the major chaperone complex in the CNX/CRT cycle, which prevent protein aggregation by ERAD (Tannous et al., 2015; Leitman et al., 2014).

In this review, we overview recent findings showing important and divergent roles of ER chaperones in neurodegeneration, suggesting their contribution in protein folding and quality control, and also in other processes such as apoptosis, synaptic connectivity, cellular adhesion, protein secretion, protein aggregation, and ERAD.

2. PDI family members in neurodegeneration

PDIs are a protein family that has important functions in the folding of different proteins synthetized through the secretory pathway. This family is conformed by 21 known members classified by sequence and structural homology (Appenzeller-Herzog

and Ellgaard, 2008). PDIs are important targets of the UPR pathway as they act as foldases and molecular chaperones that help to decrease the load of unfolded/misfolded proteins inside the ER (Feige and Hendershot, 2011). The last studies in the field have been related to the role of ER resident chaperones and foldases in PMDs, particularly focused in PDIA1 and PDIA3 (reviewed in (Andreu et al., 2012)). In this article we describe the implication of ER foldadeses and chaperons in PMDs.

PDI, also called PDIA1, is the most studied PDI family member and the first to be identified with a role in the catalysis of disulfide bond formation (Givol et al., 1964). PDIA1 is subunit of different protein complexes (Kozlov et al., 2010), recognized as a versatile protein in a variety of cellular processes in different locations (Laurindo and Pescatore, 2012).

The PDIA3 foldase, is mainly located in the ER lumen, although it has also been found in the nucleus and cytoplasm (Coppari et al., 2002). Its principal function in the folding of glycoproteins is mediated by a cooperative interaction with the CNX/CRT cycle (Turano et al., 2002), catalyzing the formation, isomerization and reduction of disulfide bonds in the glycoproteins that enter this cycle (Ellgaard and Frickel, 2003). The specific role of PDIA3 in this process is linked to the isomerization of non-native disulfide bridges from specific glycoprotein substrates; thereby increasing its folding (Jessop et al., 2007).

As mentioned above, both PDIA1 and PDIA3 expression may be altered during the neurodegenerative process. In cellular models and tissue from PD patients, PDIA1 has been found transcriptionally upregulated and it is present in Lewy bodies (Conn et al., 2004). In addition, PDIA1 was described in neurofibrillary tangles in brain tissue derived from AD patients. Curiously, in both diseases, PDIA1 is S-nitrosylated; and this modification inhibits its enzymatic activity enhancing protein aggregation (Uehara et al., 2006). In normal conditions, in cerebrospinal fluid, it is known that bulk of beta-amyloids are bound to ER chaperones like PDIA3 and CRT, suggesting that these may be carrier proteins which prevent aggregation (Erickson et al., 2005). Furthermore, progranulin (PGRN), a glycoprotein deficient in FTD, has been describe to have a role in cell survival and cell growth, neuronal development and regulation of neurite outgrowth by the cleaved form of PGRN (granulins) (Van Damme et al., 2008). Interestingly, suppression of PDIA3 gene decreases PGRN secretion, that can be associated with the evidence that PDIA3 is essential for the proper folding of many membrane and secreted proteins and their transport through the ER-Golgi pathway (Rutkevich et al., 2010). Moreover, PGRN has been described as a substrate for PDIA3 and PDIA1, and strongly interacts with the chaperons BiP, CRT, PDIA3 and Grp94 (Almeida et al., 2011) (Fig. 2). In the case of prion diseases, it has been found that both, PDIA1 and PDIA3, are upregulated in patients and experimental models (Yoo et al., 2002; Hetz et al., 2003, 2005; Rane et al., 2008; Wang et al., 2012). Furthermore, the overexpression of those foldases induced neuroprotective effects (Hetz et al., 2005; Wang et al., 2012). We have recently characterized the role of PDIA3 as a modulator of mature and total levels of Prion protein (PrP) associated with the human disease, where also we could demonstrate the physical interaction between PDIA3 and PrP (Torres et al., 2015).

In ALS, chaperone levels also showed alteration. In cellular and animal models of the disease, PDIA1 is up regulated (Atkin et al., 2006; Massignan et al., 2007; Hetz et al., 2009) and S-nitrosylated, as in PD and AD, decreasing the enzymatic activity and folding capacity (Walker et al., 2010). Moreover, PDI co-localizes with neuronal cytoplasmic inclusions in tissue derived from fALS and sALS patients (familial and sporadic ALS, respectively) (Honjo et al., 2011), and in a TDP-43 mouse model (Walker et al., 2013). Recently, a genetic screening identified some rare point mutations present in PDIA1 and PDIA3 genes that were selectively present in

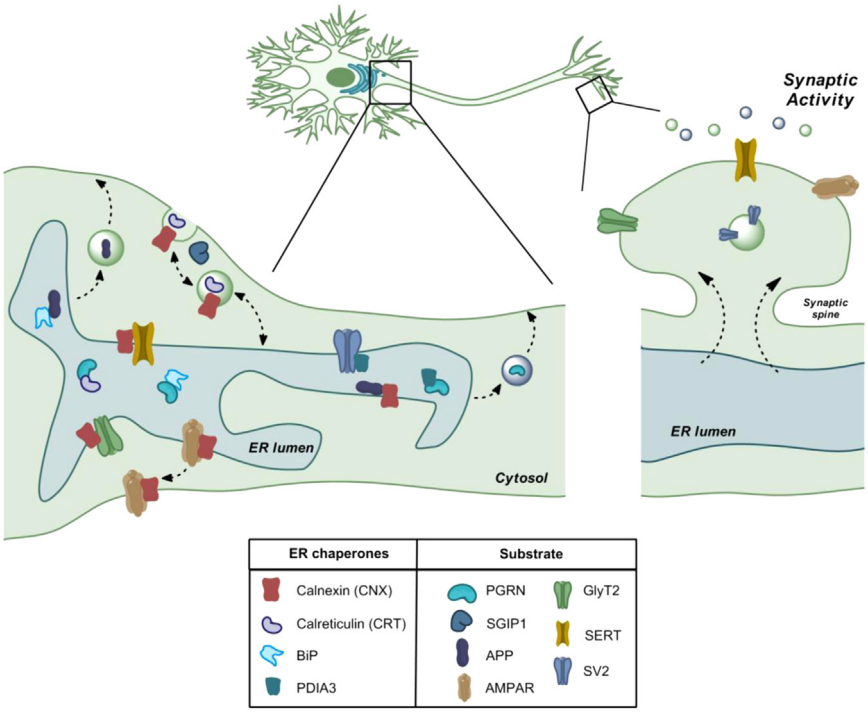

Fig. 2. Role of folding process on neuronal functions. Proper folding of neurotransmitters transporters such as SERT and GlyT2 is assisted by ER chaperones: CNX, CRT and BiP. Vesicle trafficking and mobility is enhanced by SV2, whose tertiary structure is facilitated by PDIA3. BiP, CNX, CRT and PDIA3 also have a role in folding of secretion of proteins, for example Aβ peptide or granulins products of the cleavage of APP and PGRN respectively. Outside the ER, CNX is located in the cell membrane co-localizing with AMPA receptors in a NMDA activation-dependent manner suggesting a role in synaptic plasticity. A role in the recycling of synaptic membrane proteins is proposed for CNX as an interaction with the regulator of endocytosis (SGIP1) has been described. Moreover, both CNX and CRT can be found in endo- and exocytosis vesicles supporting a role in autophagy.

ALS cases (Gonzalez-Perez et al., 2015). We have recently analyzed the effect of these PDI variants in cellular and animal models and their impact in neurodegeneration (Woehlbier et al., 2016). In this report, through different *in vitro* and *in vivo* approaches, we described a novel role for PDIA1 and PDIA3 in neurite outgrowth process and motoneuron functions (Woehlbier et al., 2016). Targeting PDIA3 *in vivo,* through the use of a conditional knockout mouse, mimicked early stages of ALS phenotypes, associated to abnormalities in morphology and function of neuromuscular junctions. Interestingly and related to these results, we detected a reduction in the levels of synaptic vesicle 2 (SV2) protein. Further, by biochemical analyses we might observe that these mutations directly affect the enzymatic functions of PDIA1 and the physical interaction with CNX and CRT by PDIA3 (Woehlbier et al., 2016). Therefore, we speculate that PDIA3 is a key component in the folding of certain substrates that could be linked to neural connections (Fig. 2). In this context, we could demonstrate the beneficial effect of PDIA3 expression in the context of axonal regeneration in peripheral nervous system (Castillo et al., 2015). However, no effects in dopaminergic neuron loss was observed *in vivo* through the challenge of the neurotoxin 6-OHDA in a transgenic mouse that overexpress PDIA3, probably due to inactivation of ectopic PDIA3 by redox imbalance associated to 6-OHDA toxin (Castillo et al., 2015). On the other hand, new evidence support that PDIA3 does not significantly have any effect in cell fate under ER stress conditions (Torres et al., 2015; Woehlbier et al., 2016). In agreement with these results, PDIA3 deficiency *in vivo* did not show signs of ER stress (Woehlbier et al., 2016).

Besides, PDIA3 has been associated with antigen presentation, specifically, in the assembly of peptide-loading complex (PLC). CRT and PDIA3 are included in the PLC, which is necessary for the formation of the MHC-I and for the loading of antigenic peptides inside the ER (Appenzeller-Herzog and Ellgaard, 2008; Cresswell et al., 2005). PDIA3 and CRT, play a role in PLC stability through the formation of stable disulfide bonds, allowing generating the proper structure of the complex (Peaper et al., 2005). Otherwise, PDIA3 is related to the reduction of the α2 HC subunit disulfide bond (Antoniou et al., 2002; Kienast et al., 2007) and it has been suggested that PDIA1 fulfills the opposite role as the oxidant of the HC of MHC-I (Park et al., 2006). Also, it is worth to mention that, chaperones BiP and CNX associate with HC after its translation, being these proteins crucial in the first steps of MHC-I formation (Hulpke and Tampe, 2013). The role of ER chaperones in MHC-I assembly acquires great significance in neurodegeneration since it was recently reported that astrocytes derived from ALS patients and SOD1 mutant mice reduce the expression of MHC-I molecules in motoneurons, making these cells more sensitive to astrocyte-induced cell death (Song et al., 2016). This interesting observation is complemented with the neuroprotective effect of MHC-I in ALS mouse model, where MHC-I expression via AAV injection leads an increase in survival and motor performance, protecting against astrocyte toxicity. Furthermore, this effect has also been observed in motoneurons derived from human when HLA-F, a single MHC-I molecule, is expressed (Song et al., 2016). Thus, the impact of PDIA3 and potentially of PDIA1 is an attractive topic to be deeply studied not only in the context of neurodegeneration and protein misfolding, but also in neuronal physiology.

3. Calnexin and calreticulin: more than folding

Mammalian CNX is a ~570 residue type I membrane protein of the ER whereas CRT is a ~400 amino acid soluble protein that resides primarily within the ER lumen. Both proteins bind Ca^{2+} and ATP, although no ATPase activity has been detected yet. CNX and its soluble orthologous CRT, are lectins with specificity for a monoglycosylated oligosaccharide. This sugar structure is the product of ER glycosidases that recognize the N-glycosylation present at asparagine residues in proteins that are going to be secreted (reviewed in (Williams, 2006)). CNX and CRT form the CNX/CRT cycle or system, responsible for retaining misfolded substrates at the ER, preventing protein aggregation and premature degradation, and promoting disulfide bond formation through interactions with another ER chaperone, ERp57 (reviewed in (Anelli and Sitia, 2008)). This process can be co-translational and is usually coupled with transport from the ER to the Golgi complex. CNX and CRT are able to bind the same glycoprotein but trough different domains acting in different stages of the folding and protein maturation process (Ellgaard and Frickel, 2003).

Several brain diseases are characterized by the presence of mis- or unfolded proteins including superoxide dismutase 1 (SOD1), Tar DNA binding protein 43KDa (TDP-43), fused in sarcoma (FUS), Huntingtin (Htt), α-syn, PrP and others, and the possible role of ER chaperons in the folding process involving those proteins have been previously reviewed (for examples, see in (Andreu et al., 2012)). Here, we will to discuss the role of ER chaperones in quality control components in synaptic functions, in physiological and pathological conditions. We will consider how folding processes can directly or indirectly affect, through its interaction with receptors, transporters and other proteins, the neuronal functions.

The serotonin transporter (SERT) was the first member of the neurotransmitter transporter family whose folding has been showed, in insect cells, to be assisted by the molecular chaperones CNX, CRT, and BiP (Tate et al., 1999). Tate et al. determined that the expression of SERT was enhanced by the co-expression of CNX and to a lesser degree on co-expression of CRT and BiP. Moreover, a physical interaction between SERT and CNX or CRT, independent from the presence of the *N*-glycan, was demonstrated (Tate et al., 1999). Similarly, it has been showed that the foldase *NinaA*, a membrane-bound isomerase from *Drosophila*, can increase the cell surface expression of the dopamine transporter (Lenhard and Reilander, 1997), a homologue of SERT (Torres et al., 2003). In agreement with this, the expression of the glycine transporter 2 (GlyT2) was also sensitive to the expression of the CNX (Arribas-Gonzalez et al., 2013) where, again, no correlation between glycan removal and CNX binding was observed (Arribas-Gonzalez et al., 2013). These results suggest that CNX may contribute to the maturation of immature molecules increasing the levels of transporter at the cell surface and thus transport activity. Since modulation of extracellular monoamine concentrations are directly involved in the maintenance of presynaptic homeostasis, delivery of functional transporters at the membrane could directly impacting onto neuronal activity (Fig. 2). Similar results were obtained for CNX, as both a molecular chaperone required for rhodopsin maturation and a regulator of Ca^{2+}. Mutations in *Drosophila* CNX lead to retinal degeneration enhanced by light, suggesting its possible function as a Ca^{2+} buffer contribute for photoreceptor cell survival (Rosenbaum et al., 2006). These results reflect the possible impact in signaling by ER chaperons, which could relays on their folding capacity to deliver functional molecules to the cell surface. Growing data suggest ER components may localize away from neural body where *in situ* protein synthesis could be occurring (Willis et al., 2005), although those results were seen in an *in vitro* model, it could assign a role for *in vivo* axonal regeneration (Fig. 2).

Regarding PMDs, in a pharmacological Parkinson's disease (PD) model has been described alteration of chaperones levels. Intrastriatal 6-hydroxydopamine (6-OHDA) lesions resulted in decreased levels of CRT and PDIA3 in the midbrain. The reduction of the components of CNX/CRT glycoprotein quality-control system may play a role in neuronal injury in PD and other neurodegenerative disorders associated with dysfunction of the ubiquitin-proteasome system. Besides, proteasome inhibitor treatments

reduce protein levels of CRT and PDIA3 but not of CNX. In addition, knockdown of CRT increases the vulnerability of neuronal cell line against 6-OHDA toxicity (Kuang et al., 2014). In a fALS model (mutant SOD1 (mSOD1) transgenic mouse), results in the same direction have been found. Pettmann's group have reported decreased levels of CRT, but no CNX, in vulnerable motoneurons before the appearance of disease symptoms. Moreover, the correlation between low CRT levels and high ER stress sensitivity of SOD1 mutant motoneurons was proposed to be contributing to the neurodegenerative process (Bernard-Marissal et al., 2012). In a genetic approach, the same group demonstrated that targeting CRT gene in an mSOD1 model provokes early muscle denervation. CRT deficiency was associated to exacerbated levels of both UPR and mTOR signaling pathway (Bernard-Marissal et al., 2015). Together, those results showed that the maintenance of regular levels of CRT or CNX, is important to neuron survival in different CNS diseases.

CNX and CRT are resident ER proteins, however, a recently discovery has reported non-canonical function for these proteins, particularly associated to the secretory pathway. Despite its ubiquitous expression, the absence of CNX has different effects depending on the organism. CNX deficiency is lethal in *Schizosaccharomyces pombe* (Parlati et al., 1995) but not in *Saccharomyces cerevisiae* (Parlati et al., 1995), *Dictyostelium* (Fajardo et al., 2004; Muller-Taubenberger et al., 2001), or *Caenorhabditis elegans* (Lee et al., 2006; Xu et al., 2001). In mice, early studies reported that deletion of the *calnexin* gene (Canx) results in early postnatal death (Denzel et al., 2002). Opposite, recent studies demonstrate that *Canx* deficiency in the mouse did not result in early postnatal death, probably due to differences in the genetic background of the animals and/or the difference between gene inactivation (Kraus et al., 2010). In both studies, the consequences of glycoprotein maturation caused a dramatic loss of large to medium myelinated nerve fibers, thereby decreasing the size of the sciatic nerve, implying that CNX plays a tissue-specific role in mammalian physiology. Besides, calnexin-deficient spinal cord had a thinner, wavy, and decompacted myelin without affecting the number of motoneurons (Kraus et al., 2010). The demyelinating phenotype described unveiled the emerging importance of CNX and ER-associated pathways as contributors to severe neurological disorders.

On the other hand, an enhanced clathrin-dependent endocytosis has been observed in CNX-deficient mice which may contribute to the neurological phenotype of this model (Li et al., 2011). In this context, surprisingly, several reports have showed that proteins usually located on the ER (such as CNX and CRT), are present in phagosome preparations suggesting a role in the phagocytic process (Garin et al., 2001). CNX was present in phagocytic cups and early autophagosomes confirming that ER is recruited very early during phagosome formation. The possible role of this protein in the delivery of substrates to the phagosomes could potentially be exploited as an alternative route to degradation for improperly folded proteins along the quality control pathway (Gagnon et al., 2002). Curiously, a decline in the rate of phagocytosis was observed in double mutants lacking CNX and CRT, whereas only mild changes occurred in single mutants (Muller-Taubenberger et al., 2001). Moreover, an interaction between the cytoplasmic tail of CNX and endophilin interacting protein 1 (SGIP1) has been described (Trevaskis et al., 2005). SGIP1, a neuronal specific regulator of endocytosis, binds the important endocytic protein, endophilin, suggesting that CNX may participate in the recycling of synaptic membrane proteins between endosomes and the plasma membrane. Indeed, transferrin uptake is increased due to enhanced endocytosis in calnexin-deficient cerebellar granule cells (Li et al., 2011) (Fig. 2).

CNX was found in both non-synaptic and synaptic membrane fractions of hippocampal neurons, co-localizing with the AMPA subunit GluA2 (Itakura et al., 2013). Curiously, this localization is regulated in an NMDA receptor-dependent manner suggesting that CNX participates in the incorporation of synaptic proteins to the neuronal plasma membrane (Itakura et al., 2013). In hippocampus, the number of AMPA receptors increases in the synaptic membrane after NMDA receptor activation (Lu et al., 2001). This is believed to be an important mechanism underlying synaptic plasticity, including long-term potentiation. The amount of cell surface CNX increased markedly after NMDA receptor activation, suggesting that the incorporation of CNX into the plasma membrane is NMDA receptor-dependent (Itakura et al., 2013) (Fig. 2). However, further studies are necessary to elucidate the functional implications of cell surface of this chaperone in synaptic plasticity.

Finally, like CRT, CNX is predominantly located in the ER, but it has also been identified at the cell surface of a number of cells (Okazaki et al., 2000). Direct fusion of the ER with the plasma membrane would explain how ER proteins, including CNX and CRT, are present in the plasma membrane (Okazaki et al., 2000; Johnson et al., 2001). CNX is continuously delivered to the cell surface and then internalized for lysosomal degradation suggesting that there is continuous exocytosis and endocytosis of this protein, and the CNX levels on the plasma membrane results from the balance of these events. Indeed, proteasome inhibitor treatments, reduced protein levels of CRT and PDIA3 but not of CNX. These treatments increased protein levels of CRT in culture media; an effect blocked by brefeldin A, indicating that these proteins are substrates of the secretion route. This phenomenon is not reported for PDIA3 (Kuang et al., 2014). Particularly the glycoprotein-binding domain of CNX is necessary for this function. These findings suggest that the surface expression of CNX depends on the association with glycoproteins and that may play a certain role as a chaperone on the plasma membrane as well (Okazaki et al., 2000).

4. The folding sensor: BiP

The glucose-regulated protein 78-kDa (GRP78), also known as immunoglobulin heavy chain binding protein (BiP), is an essential regulator of ER homeostasis. This protein participates in several regulatory processes within the ER: translocation, protein folding, ERAD and control of the activation of the ER stress sensors initiating the ER stress response. The initial model suggests that under normal conditions, the ER chaperone BiP binds to the luminal domain of IRE1, maintaining the protein in an inactive state as a monomer (Kimata et al., 2003; Bertolotti et al., 2000). Conversely, in cells undergoing ER stress, BiP is released and binds to unfolded proteins. This event allows IRE1 multimerization and autophosphorylation, activating the RNase domain through a putative conformational change.

However, besides just folding, ER chaperones are implicated in ER-Golgi transit of proteins directly involved in neurodegenerative diseases. This is the case of the amyloid precursor protein (APP) that has been showed to physically interact with BiP. Overexpression of BiP decreases the level of both mature APP and the secretion of the Aβ peptide (Vattemi et al., 2004; Yang et al., 1998) (Fig. 2). As previously mentioned, several studies directly involves ER chaperons with pathological misfolded proteins: accumulation of BiP in senile plaques, the up-regulation of ER chaperones in the brains of AD patients and the co-localization of ER chaperones with Aβ have been reported (Yoo et al., 2001; Hoozemans et al., 2005; Kakimura et al., 2002). Moreover, various ER chaperones, including BiP and CNX, suppress the generation of Aβ *in vitro* probably due to inhibition of the secretase-dependent proteolytic processing of APP through direct interaction between ER chaperones and APP, resulting in the inhibition of APP maturation.

Furthermore, ER chaperones are up-regulated not only in cultured neuronal cells overproducing mutant forms of APP or treated with synthetic Aβ42, but also in the cortex and hippocampus of transgenic mice expressing mutant APP (Hoshino et al., 2007). Levels and localization of BiP is also altered in different models of Parkinson's disease (PD). For example, in a rabbit model of PD it has been demonstrated that BiP translocate from the ER to the nucleus and cytosol. They used a PD model based in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Ghribi et al., 2003), which is a pro-toxin converted by monoamine oxidase B to the active toxin, 1-methyl-4-phenylpyridinium (MPP+). MPTP/MPP+ is widely used as a pharmacological model of PD in non-human primates and rodents, in which it induces a selective loss of dopaminergic neurons in the substantia nigra. Similarly, in a neuroblastoma cell line, treatment with MPP^+ leads to a down-regulation of BiP mRNA (Holtz and O'Malley, 2003), while treatment of the same cells with 6-OHDA has the opposite effect (Holtz and O'Malley, 2003; Chen et al., 2004). Moreover, in a HD model, treatment with ER stressors, decreased BiP-GFP mobility and accelerated UPR activation suggesting that mutant Htt protein (mHtt) indirectly affects the flux of misfolded peptides out of the ER for degradation as previously demonstrated (Lu et al., 2001; Okazaki et al., 2000). In addition, mHtt-expressing cells exhibited decreased misfolded protein flux as a result of ERAD dysfunction. Thus, mHtt expression impairs misfolded secretory protein turnover, decreases the ER stress threshold, and increases cell vulnerability to insults (Lajoie and Snapp, 2011). Curiously, data from human patients showed that BiP is upregulated in post-mortem samples of HD brains (Carnemolla et al., 2009).

Finally, according to the demyelinating results obtained for *CNX* deficiency (Kraus et al., 2010), mice lacking functional *BiP* gene expression in oligodendrocytes during development, showed tremors, ataxia and hind-limb paralysis due to oligodendrocytes loss and corresponding severe myelin abnormalities. Furthermore, mice in which the *BiP* gene was specifically inactivated in developing Schwann cells displayed tremor that progressed to hind-limb paralysis demonstrating that BiP is critical for myelinating cell survival and contributes to the protective response of oligodendrocytes against inflammatory demyelination (Hussien et al., 2015).

5. Concluding remarks

ER chaperons and foldases actively participate in give, nascent proteins at the ER, the proper conformation. Nevertheless, its role in maintaining cell protein homeostasis in physiological and pathological conditions its now being explored. Accumulating evidence have uncover an essential participation of those ER proteins in processes further its classical folding role. Targeting CNX/CRT or folding components have revealed a physiological function in neuronal function and connectivity, including endocytosis, autophagy, phagocytosis and keeping synaptic proteins in neuronal surface and can directly interfere in delivery of synaptic receptors or transporters, with drastic consequences neuronal functions. Moreover, genetic modulation of CNX/CRT members, modify vulnerability to neurodegenerative conditions.

In the last years, converging evidence is also pointing a direct role of those ER chaperones in neurodegenerative diseases, in particular PMDs. Co-localization or direct interaction of chaperons with brain pathogenic proteins or inclusions, changing levels associated to CNS affecting diseases has reinforced the concept of its functional role in PMDs. Studies PMDs associated mutations in ER chaperons have also contributed into revealed the physiological function in synaptic connectivity.

Altogether, this evidence places the ER network, including foldases and chaperons, in a new key role in the synaptic function. These results open new opportunities for therapeutic intervention for PMDs, as using ER chaperons as therapeutic targets is now starting to be explored. Thus, in the next few years, new findings will support the role of ER chaperons in different process beyond the folding function already described.

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References

Alexopoulos, J.A., Guarne, A., Ortega, J., 2012. ClpP: a structurally dynamic protease regulated by AAA+ proteins. J. Struct. Biol. 179 (2), 202–210.
Almeida, S., Zhou, L., Gao, F.B., 2011. Progranulin, a glycoprotein deficient in frontotemporal dementia, is a novel substrate of several protein disulfide isomerase family proteins. PLoS One 6 (10), e26454.
Andreu, C.I., et al., 2012. Protein disulfide isomerases in neurodegeneration: from disease mechanisms to biomedical applications. FEBS Lett. 586 (18), 2826–2834.
Anelli, T., Sitia, R., 2008. Protein quality control in the early secretory pathway. EMBO J. 27 (2), 315–327.
Antoniou, A.N., et al., 2002. The oxidoreductase ERp57 efficiently reduces partially folded in preference to fully folded MHC class I molecules. EMBO J. 21 (11), 2655–2663.
Appenzeller-Herzog, C., Ellgaard, L., 2008. The human PDI family: versatility packed into a single fold. Biochim. Biophys. Acta 1783 (4), 535–548.
Arribas-Gonzalez, E., et al., 2013. Calnexin-assisted biogenesis of the neuronal glycine transporter 2 (GlyT2). PLoS One 8 (5), e63230.
Atkin, J.D., et al., 2006. Induction of the unfolded protein response in familial amyotrophic lateral sclerosis and association of protein-disulfide isomerase with superoxide dismutase 1. J. Biol. Chem. 281 (40), 30152–30165.
Bernard-Marissal, N., et al., 2012. Reduced calreticulin levels link endoplasmic reticulum stress and Fas-triggered cell death in motoneurons vulnerable to ALS. J. Neurosci. 32 (14), 4901–4912.
Bernard-Marissal, N., et al., 2015. Calreticulin levels determine onset of early muscle denervation by fast motoneurons of ALS model mice. Neurobiol. Dis. 73, 130–136.
Bertolotti, A., et al., 2000. Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. Nat. Cell Biol. 2 (6), 326–332.
Carman, A., et al., 2013. Chaperone-dependent neurodegeneration: a molecular perspective on therapeutic intervention. J. Alzheimers Dis. Park. 2013 (Suppl. 10).
Carnemolla, A., et al., 2009. Rrs1 is involved in endoplasmic reticulum stress response in Huntington disease. J. Biol. Chem. 284 (27), 18167–18173.
Castillo, V., et al., 2015. Functional role of the disulfide isomerase ERp57 in axonal regeneration. PLoS One 10 (9), e0136620.
Chen, G., et al., 2004. Glycogen synthase kinase 3beta (GSK3beta) mediates 6-hydroxydopamine-induced neuronal death. FASEB J. 18 (10), 1162–1164.
Conn, K.J., et al., 2004. Identification of the protein disulfide isomerase family member PDIp in experimental Parkinson's disease and Lewy body pathology. Brain Res. 1022 (1–2), 164–172.
Coppari, S., et al., 2002. Nuclear localization and DNA interaction of protein disulfide isomerase ERp57 in mammalian cells. J. Cell Biochem 85 (2), 325–333.
Corbett, E.F., et al., 2000. The conformation of calreticulin is influenced by the endoplasmic reticulum luminal environment. J. Biol. Chem. 275 (35), 27177–27185.
Cresswell, P., et al., 2005. Mechanisms of MHC class I-restricted antigen processing and cross-presentation. Immunol. Rev. 207, 145–157.
Denzel, A., et al., 2002. Early postnatal death and motor disorders in mice congenitally deficient in calnexin expression. Mol. Cell. Biol. 22 (21), 7398–7404.
Doyle, S.M., Genest, O., Wickner, S., 2013. Protein rescue from aggregates by powerful molecular chaperone machines. Nat. Rev. Mol. Cell Biol. 14 (10), 617–629.
Ellgaard, L., Frickel, E.M., 2003. Calnexin, calreticulin, and ERp57: teammates in glycoprotein folding. Cell Biochem. Biophys. 39 (3), 223–247.
Ellgaard, L., Ruddock, L.W., 2005. The human protein disulphide isomerase family: substrate interactions and functional properties. EMBO Rep. 6 (1), 28–32.
Erickson, R.R., et al., 2005. In cerebrospinal fluid ER chaperones ERp57 and calreticulin bind beta-amyloid. Biochem. Biophys. Res. Commun. 332 (1), 50–57.
Fajardo, M., et al., 2004. Calnexin, calreticulin and cytoskeleton-associated proteins modulate uptake and growth of Legionella pneumophila in Dictyostelium discoideum. Microbiology 150 (Pt 9), 2825–2835.
Feige, M.J., Hendershot, L.M., 2011. Disulfide bonds in ER protein folding and homeostasis. Curr. Opin. Cell Biol. 23 (2), 167–175.

Gagnon, E., et al., 2002. Endoplasmic reticulum-mediated phagocytosis is a mechanism of entry into macrophages. Cell 110 (1), 119–131.
Garin, J., et al., 2001. The phagosome proteome: insight into phagosome functions. J. Cell Biol. 152 (1), 165–180.
Ghribi, O., et al., 2003. MPP+ induces the endoplasmic reticulum stress response in rabbit brain involving activation of the ATF-6 and NF-kappaB signaling pathways. J. Neuropathol. Exp. Neurol. 62 (11), 1144–1153.
Gillece, P., et al., 1999. Export of a cysteine-free misfolded secretory protein from the endoplasmic reticulum for degradation requires interaction with protein disulfide isomerase. J. Cell Biol. 147 (7), 1443–1456.
Givol, D., Goldberger, R.F., Anfinsen, C.B., 1964. Oxidation and disulfide interchange in the reactivation of reduced ribonuclease. J. Biol. Chem. 239, PC3114–PC3116.
Gonzalez-Perez, P., et al., 2015. Identification of rare protein disulfide isomerase gene variants in amyotrophic lateral sclerosis patients. Gene 566 (2), 158–165.
Hartl, F.U., Bracher, A., Hayer-Hartl, M., 2011. Molecular chaperones in protein folding and proteostasis. Nature 475 (7356), 324–332.
Hatahet, F., Ruddock, L.W., 2009. Protein disulfide isomerase: a critical evaluation of its function in disulfide bond formation. Antioxid. Redox Signal. 11 (11), 2807–2850.
Hebert, D.N., Molinari, M., 2007. In and out of the ER: protein folding, quality control, degradation, and related human diseases. Physiol. Rev. 87 (4), 1377–1408.
Hetz, C., et al., 2003. Caspase-12 and endoplasmic reticulum stress mediate neurotoxicity of pathological prion protein. EMBO J. 22 (20), 5435–5445.
Hetz, C., et al., 2005. The disulfide isomerase Grp58 is a protective factor against prion neurotoxicity. J. Neurosci. 25 (11), 2793–2802.
Hetz, C., et al., 2009. XBP-1 deficiency in the nervous system protects against amyotrophic lateral sclerosis by increasing autophagy. Genes Dev. 23 (19), 2294–2306.
Hetz, C., et al., 2011. The unfolded protein response: integrating stress signals through the stress sensor IRE1α. Physiol. Rev. 91 (4), 1219–1243.
Hetz, C., 2012. The unfolded protein response: controlling cell fate decisions under ER stress and beyond. Nat. Rev. Mol. Cell Biol. 13, 1–14.
Hetz, C., Mollereau, B., 2014. Disturbance of endoplasmic reticulum proteostasis in neurodegenerative diseases. Nat. Rev. Neurosci. 15 (4), 233–249.
Higo, T., et al., 2005. Subtype-specific and ER lumenal environment-dependent regulation of inositol 1,4,5-trisphosphate receptor type 1 by ERp44. Cell 120 (1), 85–98.
Hodson, S., Marshall, J.J., Burston, S.G., 2012. Mapping the road to recovery: the ClpB/Hsp104 molecular chaperone. J. Struct. Biol. 179 (2), 161–171.
Holtz, W.A., O'Malley, K.L., 2003. Parkinsonian mimetics induce aspects of unfolded protein response in death of dopaminergic neurons. J. Biol. Chem. 278 (21), 19367–19377.
Honjo, Y., et al., 2011. Protein disulfide isomerase-immunopositive inclusions in patients with amyotrophic lateral sclerosis. Amyotroph. Lateral Scler. 12 (6), 444–450.
Hoozemans, J.J., et al., 2005. The unfolded protein response is activated in Alzheimer's disease. Acta Neuropathol. 110 (2), 165–172.
Hoshino, T., et al., 2007. Endoplasmic reticulum chaperones inhibit the production of amyloid-beta peptides. Biochem. J. 402 (3), 581–589.
Hulpke, S., Tampe, R., 2013. The MHC I loading complex: a multitasking machinery in adaptive immunity. Trends Biochem. Sci. 38 (8), 412–420.
Hussien, Y., et al., 2015. ER chaperone BiP/GRP78 is required for myelinating cell survival and provides protection during experimental autoimmune encephalomyelitis. J. Neurosci. 35 (48), 15921–15933.
Itakura, M., et al., 2013. NMDA receptor-dependent recruitment of calnexin to the neuronal plasma membrane. Neurosci. Lett. 550, 173–178.
Jessop, C.E., et al., 2007. ERp57 is essential for efficient folding of glycoproteins sharing common structural domains. EMBO J. 26 (1), 28–40.
Johnson, S., et al., 2001. The ins and outs of calreticulin: from the ER lumen to the extracellular space. Trends Cell Biol. 11 (3), 122–129.
Kakimura, J., et al., 2002. Possible involvement of ER chaperone Grp78 on reduced formation of amyloid-beta deposits. Ann. N.Y. Acad. Sci. 977, 327–332.
Kienast, A., et al., 2007. Redox regulation of peptide receptivity of major histocompatibility complex class I molecules by ERp57 and tapasin. Nat. Immunol. 8 (8), 864–872.
Kim, Y.E., et al., 2013. Molecular chaperone functions in protein folding and proteostasis. Annu. Rev. Biochem. 82, 323–355.
Kimata, Y., et al., 2003. Genetic evidence for a role of BiP/Kar2 that regulates Ire1 in response to accumulation of unfolded proteins. Mol. Biol. Cell 14 (6), 2559–2569.
Knowles, T.P., Vendruscolo, M., Dobson, C.M., 2014. The amyloid state and its association with protein misfolding diseases. Nat. Rev. Mol. Cell Biol. 15 (6), 384–396.
Kozlov, G., et al., 2010. A structural overview of the PDI family of proteins. FEBS J. 277 (19), 3924–3936.
Kraus, A., et al., 2010. Calnexin deficiency leads to dysmyelination. J. Biol. Chem. 285 (24), 18928–18938.
Kuang, X.L., et al., 2014. Reductions of the components of the calreticulin/calnexin quality-control system by proteasome inhibitors and their relevance in a rodent model of Parkinson's disease. J. Neurosci. Res. 92 (10), 1319–1329.
Lajoie, P., Snapp, E.L., 2011. Changes in BiP availability reveal hypersensitivity to acute endoplasmic reticulum stress in cells expressing mutant huntingtin. J. Cell Sci. 124 (Pt 19), 3332–3343.
Laurindo, F.R., Pescatore, L.A., Fernandes Dde, C., 2012. Protein disulfide isomerase in redox cell signaling and homeostasis. Free Radic. Biol. Med. 52 (9), 1954–1969.
Lee, W., et al., 2006. Alternative chaperone machinery may compensate for calreticulin/calnexin deficiency in Caenorhabditis elegans. Proteomics 6 (4), 1329–1339.
Leitman, J., et al., 2014. Herp coordinates compartmentalization and recruitment of HRD1 and misfolded proteins for ERAD. Mol. Biol. Cell 25 (7), 1050–1060.
Lenhard, T., Reilander, H., 1997. Engineering the folding pathway of insect cells: generation of a stably transformed insect cell line showing improved folding of a recombinant membrane protein. Biochem. Biophys. Res. Commun. 238 (3), 823–830.
Li, H.D., Liu, W.X., Michalak, M., 2011. Enhanced clathrin-dependent endocytosis in the absence of calnexin. PLoS One 6 (7), e21678.
Li, T., Lucius, A.L., 2013. Examination of the polypeptide substrate specificity for *Escherichia coli* ClpA. Biochemistry 52 (29), 4941–4954.
Li, Y., Camacho, P., 2004. Ca2+-dependent redox modulation of SERCA 2b by ERp57. J. Cell Biol. 164 (1), 35–46.
Ling, S.C., Polymenidou, M., Cleveland, D.W., 2013. Converging mechanisms in ALS and FTD: disrupted RNA and protein homeostasis. Neuron 79 (3), 416–438.
Lu, W., et al., 2001. Activation of synaptic NMDA receptors induces membrane insertion of new AMPA receptors and LTP in cultured hippocampal neurons. Neuron 29 (1), 243–254.
Massignan, T., et al., 2007. Proteomic analysis of spinal cord of presymptomatic amyotrophic lateral sclerosis G93A SOD1 mouse. Biochem. Biophys. Res. Commun. 353 (3), 719–725.
Molinari, M., et al., 2002. Sequential assistance of molecular chaperones and transient formation of covalent complexes during protein degradation from the ER. J. Cell Biol. 158 (2), 247–257.
Muller-Taubenberger, A., et al., 2001. Calreticulin and calnexin in the endoplasmic reticulum are important for phagocytosis. EMBO J. 20 (23), 6772–6782.
Okazaki, Y., et al., 2000. Cell surface expression of calnexin, a molecular chaperone in the endoplasmic reticulum. J. Biol. Chem. 275 (46), 35751–35758.
Park, B., et al., 2006. Redox regulation facilitates optimal peptide selection by MHC class I during antigen processing. Cell 127 (2), 369–382.
Parlati, F., et al., 1995. The calnexin homologue cnx1+ in Schizosaccharomyces pombe, is an essential gene which can be complemented by its soluble ER domain. EMBO J. 14 (13), 3064–3072.
Peaper, D.R., Wearsch, P.A., Cresswell, P., 2005. Tapasin and ERp57 form a stable disulfide-linked dimer within the MHC class I peptide-loading complex. EMBO J. 24 (20), 3613–3623.
Rane, N.S., et al., 2008. Reduced translocation of nascent prion protein during ER stress contributes to neurodegeneration. Dev. Cell 15 (3), 359–370.
Rosenbaum, E.E., Hardie, R.C., Colley, N.J., 2006. Calnexin is essential for rhodopsin maturation, Ca^{2+} regulation, and photoreceptor cell survival. Neuron 49 (2), 229–241.
Rutkevich, L.A., et al., 2010. Functional relationship between protein disulfide isomerase family members during the oxidative folding of human secretory proteins. Mol. Biol. Cell 21 (18), 3093–3105.
Rutkowski, D.T., Kaufman, R.J., 2004. A trip to the ER: coping with stress. Trends Cell Biol. 14 (1), 20–28.
Schroder, M., Kaufman, R.J., 2005. The mammalian unfolded protein response. Annu. Rev. Biochem. 74, 739–789.
Schroder, M., Kaufman, R.J., 2005. ER stress and the unfolded protein response. Mutat. Res. 569 (1–2), 29–63.
Song, S., et al., 2016. Major histocompatibility complex class I molecules protect motor neurons from astrocyte-induced toxicity in amyotrophic lateral sclerosis. Nat. Med.
Tannous, A., et al., 2015. N-linked sugar-regulated protein folding and quality control in the ER. Semin. Cell Dev. Biol. 41, 79–89.
Tate, C.G., Whiteley, E., Betenbaugh, M.J., 1999. Molecular chaperones stimulate the functional expression of the cocaine-sensitive serotonin transporter. J. Biol. Chem. 274 (25), 17551–17558.
Torres, G.E., Gainetdinov, R.R., Caron, M.G., 2003. Plasma membrane monoamine transporters: structure, regulation and function. Nat. Rev. Neurosci. 4 (1), 13–25.
Torres, M., et al., 2015. The protein-disulfide isomerase ERp57 regulates the steady-state levels of the prion protein. J. Biol. Chem. 290 (39), 23631–23645.
Trevaskis, J., et al., 2005. Src homology 3-domain growth factor receptor-bound 2-like (endophilin) interacting protein 1, a novel neuronal protein that regulates energy balance. Endocrinology 146 (9), 3757–3764.
Tsai, B., et al., 2001. Protein disulfide isomerase acts as a redox-dependent chaperone to unfold cholera toxin. Cell 104 (6), 937–948.
Turano, C., et al., 2002. Proteins of the PDI family: unpredicted non-ER locations and functions. J. Cell Physiol. 193 (2), 154–163.
Uehara, T., et al., 2006. S-nitrosylated protein-disulphide isomerase links protein misfolding to neurodegeneration. Nature 441 (7092), 513–517.
Valastyan, J.S., Lindquist, S., 2014. Mechanisms of protein-folding diseases at a glance. Dis. Model Mech. 7 (1), 9–14.
Van Damme, P., et al., 2008. Progranulin functions as a neurotrophic factor to regulate neurite outgrowth and enhance neuronal survival. J. Cell Biol. 181 (1), 37–41.
Vattemi, G., et al., 2004. Endoplasmic reticulum stress and unfolded protein response in inclusion body myositis muscle. Am. J. Pathol. 164 (1), 1–7.
Vembar, S.S., Brodsky, J.L., 2008. One step at a time: endoplasmic reticulum-associated degradation. Nat. Rev. Mol. Cell Biol. 9 (12), 944–957.
Vidal, R.L., et al., 2014. Targeting autophagy in neurodegenerative diseases. Trends Pharmacol. Sci. 35 (11), 583–591.

Walker, A.K., et al., 2010. Protein disulphide isomerase protects against protein aggregation and is S-nitrosylated in amyotrophic lateral sclerosis. Brain 133 (Pt 1), 105–116.
Walker, A.K., et al., 2013. ALS-associated TDP-43 induces endoplasmic reticulum stress, which drives cytoplasmic TDP-43 accumulation and stress granule formation. PLoS One 8 (11), e81170.
Wang, S.B., et al., 2012. Protein disulfide isomerase regulates endoplasmic reticulum stress and the apoptotic process during prion infection and PrP mutant-induced cytotoxicity. PLoS One 7 (6), e38221.
Williams, D.B., 2006. Beyond lectins: the calnexin/calreticulin chaperone system of the endoplasmic reticulum. J. Cell Sci. 119 (Pt 4), 615–623.
Willis, D., et al., 2005. Differential transport and local translation of cytoskeletal, injury-response, and neurodegeneration protein mRNAs in axons. J. Neurosci. 25 (4), 778–791.
Winkler, J., et al., 2012. Chaperone networks in protein disaggregation and prion propagation. J. Struct. Biol. 179 (2), 152–160.
Witt, S.N., 2013. Molecular chaperones, alpha-synuclein, and neurodegeneration. Mol. Neurobiol. 47 (2), 552–560.
Woehlbier, U., et al., 2016. ALS-linked protein disulfide isomerase variants cause motor dysfunction. EMBO J.
Wyatt, A.R., et al., 2012. Roles of extracellular chaperones in amyloidosis. J. Mol. Biol. 421 (4–5), 499–516.
Xu, K., Tavernarakis, N., Driscoll, M., 2001. Necrotic cell death in *C. elegans* requires the function of calreticulin and regulators of Ca^{2+} release from the endoplasmic reticulum. Neuron 31 (6), 957–971.
Yang, Y., Turner, R.S., Gaut, J.R., 1998. The chaperone BiP/GRP78 binds to amyloid precursor protein and decreases Abeta40 and Abeta42 secretion. J. Biol. Chem. 273 (40), 25552–25555.
Yoo, B.C., et al., 2001. Deranged expression of molecular chaperones in brains of patients with Alzheimer's disease. Biochem. Biophys. Res. Commun. 280 (1), 249–258.
Yoo, B.C., et al., 2002. Overexpressed protein disulfide isomerase in brains of patients with sporadic Creutzfeldt-Jakob disease. Neurosci. Lett. 334 (3), 196–200.