

Critical roles of protein disulfide isomerases in balancing proteostasis in the nervous system

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Protein disulfide isomerases (PDIs) constitute a family of oxidoreductases promoting redox protein folding and quality control in the endoplasmic reticulum. PDIs catalyze disulfide bond formation, isomerization, and reduction, operating in concert with molecular chaperones to fold secretory cargoes in addition to directing misfolded proteins to be refolded or degraded. Importantly, PDIs are emerging as key components of the proteostasis network, integrating protein folding status with central surveillance mechanisms to balance proteome stability according to cellular needs. Recent advances in the field driven by the generation of new mouse models, human genetic studies, and omics methodologies, in addition to interventions using small molecules and gene therapy, have revealed the significance of PDIs to the physiology of the nervous system. PDIs are also implicated in diverse pathologies, ranging from neurodevelopmental conditions to neurodegenerative diseases and traumatic injuries. Here, we review the principles of redox protein folding in the ER with a focus on current evidence linking genetic mutations and biochemical alterations to PDIs in the etiology of neurological conditions.

Disulfide bonds are posttranslational modifications to proteins that contribute to structural stability, in addition to enabling conformational transitions involving oxidized and reduced states that confer functional versatility to proteins (1). Cysteines in disulfide bonds are the most conserved residues, with disulfides bonds driving protein evolution and positively correlating with organismal complexity (1–3). Proteins containing disulfide bonds can occur in different subcellular compartments, including the cytosol, nucleus, intermembrane space of mitochondria, and particularly the secretory route and extracellular space (1, 4).

Formation of disulfide bonds in the plasma membrane and secreted proteins of eukaryotes occurs in the endoplasmic reticulum (ER), where a complex molecular machinery exists to transfer reducing equivalents from cysteine residues of imported polypeptides to electron acceptors and promote

correct intramolecular and intermolecular pairing of cysteines (4). We refer to this process as redox protein folding (Fig. 1), which largely employs oxygen as the primary electron acceptor with generation of H₂O₂ as reactive intermediate (Fig. 1A). Thus, ER proteostasis (*i.e.*, protein homeostasis) is closely coupled to aerobic metabolism. The machinery for redox protein folding is comprised of oxidases, such as ER oxidase 1 (ERO1) and quiescin sulphydryl oxidase (QSOX), and peroxidases, such as peroxiredoxin 4 (PRDX4) and glutathione peroxidases 7 and 8 (GPX7/8), in addition to disulfide exchange proteins known as protein disulfide isomerases (PDIs) (4–8). Moreover, metabolism of vitamin K may also contribute to disulfide relay in the ER through the enzyme vitamin K epoxide reductase (VKOR) (Fig. 1A) (4).

The PDI gene family belongs to the thioredoxin (*TRX*) superfamily, with each member presenting a modular structure composed of TRX-like domains that can be either catalytically active or inactive (9). TRXs are small proteins present in prokaryotes and eukaryotes that catalyze disulfide reduction in target proteins, being reduced themselves by TRX reductases at the expense of NADPH (10). Features of the TRX structure have been widely adopted by oxidoreductases, including anti-oxidant enzymes, impacting myriad cellular functions (10). The active site motif CXXC of PDIs is widely conserved in evolution to accomplish oxidation and reduction reactions of disulfide exchange in substrates, where the nature of the amino acid residues lying between the catalytic cysteines determine the redox potential of the enzyme (7). The human proteome contains over 20 PDI family members with varied domain compositions that define their substrate specificity and cellular function (Fig. 2A) (11). Despite the significant progress in the understanding of molecular aspects of disulfide bond formation in the ER, it is still a matter of debate whether there is segregation of competing oxidative pathways or whether alternative mechanisms operate concomitantly (12).

PDIs catalyze redox protein folding in the ER by alternating between disulfide oxidized and reduced states (Fig. 1C). The PDIs in the oxidized state transfer the disulfide bond onto substrates, being converted to the reduced state (8). ERO1 is a flavoenzyme that recycles PDIs back to the oxidized state by transferring reducing equivalents to molecular oxygen with

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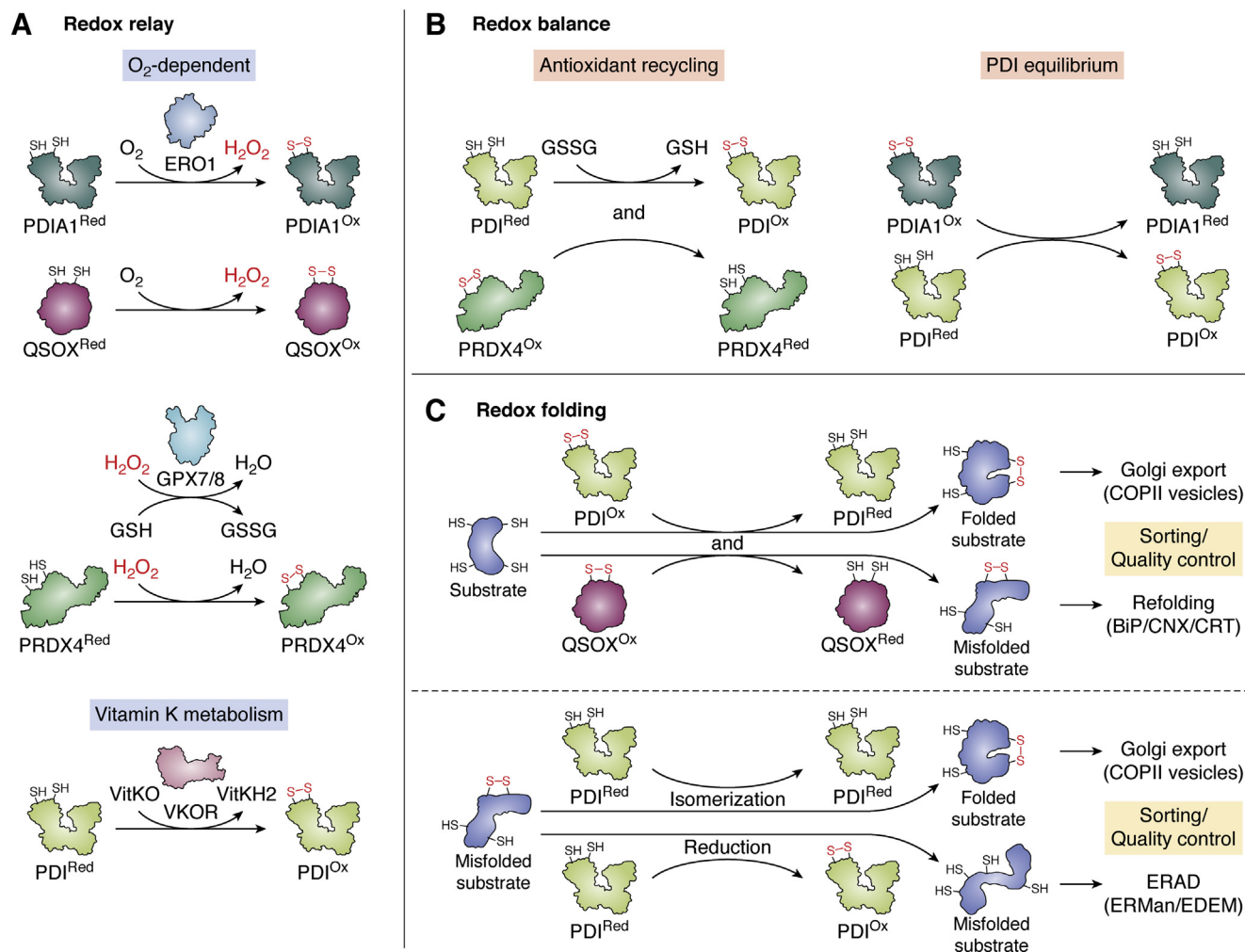


Figure 1. Redox protein folding in the ER. A, the redox relay system to remove reducing equivalents from substrates may rely on molecular oxygen or alternative substrates as electron acceptors. Flavoenzymes such as ERO1 and QSOX transfer electrons from reduced disulfides to molecular oxygen with generation of H₂O₂ as reactive intermediate. ERO1 catalytic cycle is coupled to oxidation of PDIA1, whereas QSOX acts independently. PRDX4 and GPX7/8 reduce H₂O₂ to H₂O generating oxidized PRDX4 and oxidized glutathione (GSSG), respectively. An alternative pathway includes transferring electrons from reduced disulfides to vit KO/vit K by the enzyme VKOR. B, redox balance involves reduction of GSSG and oxidized PRDX4 by PDIs to regenerate the peroxidase pathway (antioxidant recycling) and shuttle of disulfide bonds between PDIA1 and other PDIs (PDI equilibrium). C, redox folding involves insertion of disulfide bonds in client proteins by PDIs or QSOX followed by sorting and quality control steps. Folded substrates are sorted into COPII vesicles for Golgi export while misfolded substrates are directed to refolding cycles through interaction with ER chaperones BiP, CNX, CRT, among others, and disulfide isomerization. Terminally misfolded polypeptides suffer disulfide reduction and deglycosylation by ERMan and EDEM followed by degradation of the unfolded substrate by ERAD. BiP, binding-immunoglobulin protein; CNX, calnexin; COPII, coat protein II; CRT, calreticulin; EDEM, ER degradation-enhancing α -mannosidase-like protein; ER, endoplasmic reticulum; ERAD, ER-associated degradation; ERMan, ER mannosidase; ERO1, ER oxidase 1; GPX7/8, glutathione peroxidases 7 and 8; GSH, glutathione; PDI, protein disulfide isomerase; PRDX4, peroxiredoxin 4; QSOX, quiescin sulphydryl oxidase; vit K, vitamin K; vit KO, vitamin K epoxide; vit KH2, vitamin K hydroquinone; VKOR, vitamin K epoxide reductase.

formation of H₂O₂ (Fig. 1A) (5). The peroxidases expressed in the ER can couple H₂O₂ generation to disulfide bond formation (Fig. 1B). Thus, PRDX4 and GPX7/8 reduce H₂O₂ to water, resulting in disulfide oxidized PRDX4 and generation of oxidized glutathione (GSSG) (Fig. 1A) (4). PRDX4 and GSSG can transfer their disulfide bond to reduced PDIs to complete a cycle of redox relay with net formation of two disulfide bonds and reduction of molecular oxygen to water (Fig. 1B).

Despite being essential for disulfide bond formation in yeast (13, 14), genetic ablation of both ERO1 α and ERO1 β , the two ERO1 isoforms in mammals, does not lead to lethality in mice (15). ERO1 α /ERO1 β -deficient mice develop glucose intolerance due to impaired redox folding of proinsulin but show normal production of immunoglobulins (15), suggesting the

existence of alternative and efficient routes to remove reducing equivalents from cysteine residues of ER cargoes. QSOX can transfer disulfides directly to substrates without intermediacy of PDIs (Fig. 1C) (6). The reduced cysteines of QSOX are then oxidized by the enzyme-bound flavin adenine dinucleotide that finally transfer reducing equivalents to molecular oxygen generating H₂O₂ (Fig. 1A) (6). In this pathway for redox protein folding, reduced PDI is likely needed to rearrange mismatched disulfides introduced in substrates by QSOX (12).

The relative participation of ERO1 or QSOX routes in disulfide bond formation in the ER may depend on the cell type and redox status. In the ERO1 mechanism, the system should rely on a higher proportion of oxidized PDIs available to transfer the disulfide onto substrates, whereas the QSOX

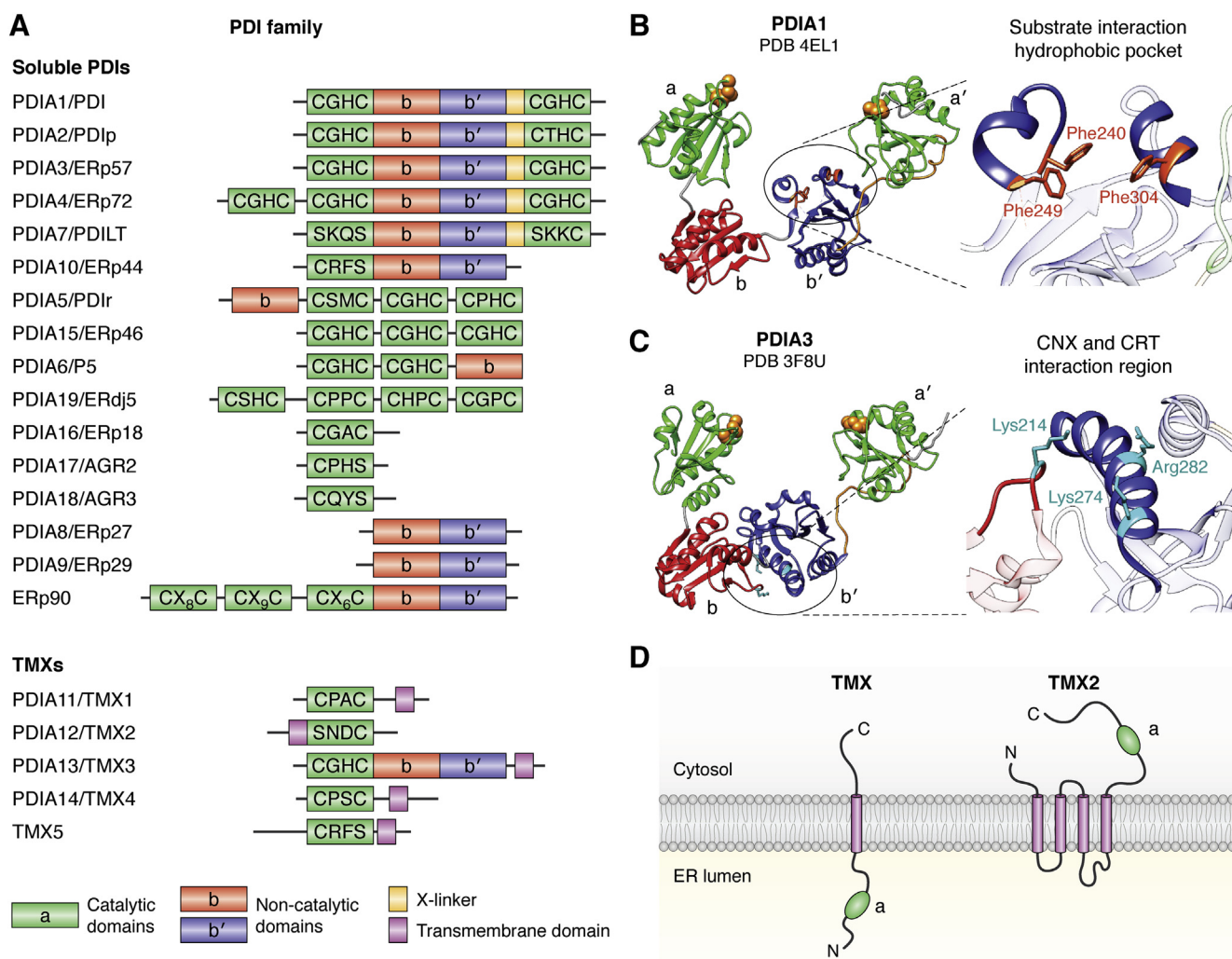


Figure 2. Structure and function of PDI family. *A*, domain structure and catalytic motif of human PDIs. *B*, PDIA1 structure with highlight of critical residues to oxidoreductase activity and chaperone function. Cysteine 53 and 56 in the a domain and 397 and 400 in the a' domain, represented in *dark yellow*. Phenylalanine 240, 249, and 304 in the b' domain are represented in *orange* and constitute the hydrophobic pocket for substrate binding. Protein data bank (PDB) code, 4EL1. *C*, PDIA3 structure with highlight of critical residues to oxidoreductase activity and protein interactions. Cysteine 57 and 60 in the a domain and 406 and 409 in the a' domain, represented in *dark yellow*. Lysine 214 and 274 and arginine 282 in the b and b' domains are represented in *light blue* and involved in PDIA3 interaction with calnexin (CNX) and calreticulin (CRT). PDB code, 3F8U. *D*, TMX1 and TMX2 topology in the ER membrane. ER, endoplasmic reticulum.

mechanism implies that PDIs should be mostly in the reduced state to catalyze isomerization reactions. The contribution of QSOX may be limited by its predominant localization in the Golgi apparatus, in addition to being secreted (4). Interestingly, QSOX has been shown to reconstitute disulfide bond formation in ERO1-deficient yeast (16), arguing for the capacity of this enzymatic system to complement redox protein folding under certain conditions. VKOR is involved in recycling vitamin K during blood coagulation (17). Despite not constituting a major pathway for redox relay in the ER (4), its contribution to redox protein folding under specific pathological states may deserve attention. Indeed, PDIA1 has been shown to boost VKOR activity (18).

The abundance and diversity of PDIs may constitute cornerstones for the relative contribution of different oxidase systems to net disulfide formation in the ER. The PDIs act in concert with ER chaperones to engage productive protein-folding cycles and exert strict quality control check to target

terminally misfolded proteins to ER-associated degradation pathway (Fig. 1C) (19), in addition to couple the folding machinery to activation of stress responses when ER proteostasis is perturbed (20). Biochemical and genetic evidence suggests that PDIs dysregulation underlies pathogenic mechanisms in several age- and injury-related neurodegenerative conditions, including protein aggregation, synaptic failure, and cell death cascades (21–24). Recent studies also uncover the relevance of PDIs to neurodevelopment, describing alterations to ER proteostasis that may contribute to neuronal malfunctioning and vulnerability in pathological conditions (25, 26). While most of the literature indicates that PDIs offer adaptive advantage over pathological insults (27, 28), there are also examples showing that PDIs may have detrimental outcomes to the nervous system (29). Thus, PDIs arise as relevant targets to be exploited for disease intervention, with reports describing beneficial effects of small molecule inhibitors (29, 30). Future translational efforts should also focus on gene therapy strategies, where

tissue- and cell-specific knockdown and overexpression of specific PDIs may provide robust therapeutic action without pleiotropic side effects. In this article we will discuss the significance of PDI family members to nervous system development and functioning and their relationship with neurodevelopmental and neurodegenerative disorders.

PDIs accomplish multiple cellular functions

The canonical function of PDIs is promoting redox protein folding and quality control by catalyzing disulfide bond formation, reduction, and isomerization in secretory pathway cargoes (31). Their domain composition and interaction with other chaperones dictate substrate specificity and possible redundancy among PDI family members. Besides their canonical function, PDIs have been implicated in myriad cellular processes in the ER and other subcellular compartments as will be discussed later. Then, their relevance to ER proteostasis balance and other molecular pathways involved in the physiology of the nervous system will be discussed considering their association to neurodevelopmental and neurodegenerative conditions, in addition to evaluating their potential therapeutic value.

PDIA1

PDIA1 (also known as PDI or P4HB) is the first discovered and most studied member of the PDI family. It is comprised of four TRX-like domains, being two catalytic at the N and C terminus termed a and a' that are separated by two non-catalytic termed b and b' (Fig. 2B). An X-linker joins the b' and a' domains. The enzyme adopts a U-shaped conformation where the b' domain of human PDIA1 provides the main binding site for clients (31). Binding of substrates to the b' domain leads to conformational changes to the a domain, resulting in the closure of the substrate-binding pocket of the b' domain that prevents interaction with other clients (31). The hydrophobic nature of this pocket confers chaperone function to PDIA1 (Fig. 2B) (31, 32). Oxidized PDIA1 alternates between open and closed conformations, while reduced PDIA1 adopts the closed conformation (31). Interestingly, the switch between oxidized and reduced states may determine selection of clients at distinct folding stages, where oxidized PDIA1 binds to more unstructured substrates while reduced PDIA1 promotes disulfide rearrangements in partially structured folding intermediates (31). This structural determinant over substrate selection may couple redox state of the enzyme to the prevailing oxidase system (*i.e.*, ERO1 or QSOX).

PDIA1 has been identified as the main partner of ERO1 for disulfide relay in the ER (Fig. 1A), while other PDIs such as PDIA3 and PDIA4 may also contribute to this process (33). According to a model of cooperative thiol-disulfide exchange between PDI family members, there is no requirement for interaction of the different PDIs with ERO1 for their turnover since disulfide transfer between several PDIs occur without kinetic constraints (Fig. 1B) (34). PDIA1 can also reduce oxidized glutathione (GSSG to GSH) and donate reducing equivalents to VKOR (4, 33), possibly occupying a high

hierarchical position for redox control and disulfide relay in the ER. Thus, disruption of PDIA1 activity could lead to a domino effect affecting redox protein folding by other PDIs under pathological conditions.

In addition to its role as oxidoreductase and chaperone, PDIA1 also accomplishes a structural function by associating with the α -subunit of microsomal triglyceride transfer protein (MTP) and the α -subunit of prolyl-4-hydroxylase (P4-H) to maintain their solubility and activity (35, 36), exerting critical roles in lipid metabolism and procollagen synthesis. Besides the ER, PDIA1 can localize to other subcellular compartments, highlighting the cytosol and the plasma membrane, where it contributes to actin cytoskeleton remodeling involving integrin signaling (37, 38). PDIA1 expression in endothelial and vascular smooth muscle cells can engage activation of NADPH oxidases, probably contributing to cytoskeleton regulation (38). In pathological conditions of the vascular system, PDIA1 localization on the extracellular face of the plasma membrane has been associated to control of vessel diameter during injury repair through organization of cytoskeleton/extracellular matrix (39), in addition to induction of thrombosis (40).

PDIA3

PDIA3 (also known as GRP58 or ERp57) is a close paralog of PDIA1, containing the same domain composition conforming a U-shape structure and the active site motif CXXC (Fig. 2C). Unlike PDIA1, however, PDIA3 is not a major reducing partner of ERO1 and lacks the hydrophobic pocket in the b' domain to chaperone substrates (31). Instead, PDIA3 appears to have evolved a specialized function catalyzing redox folding of glycosylated substrates recruited by the lectin-like chaperones calnexin (CNX) and calreticulin (CRT) (Fig. 2C) (41, 42). After polypeptides are imported into the ER, they can be N-glycosylated by the transfer of a preassembled carbohydrate consisting of two units of GlcNAc, nine of mannose (Man), and three of glucose (Glc) to the asparagine residue of the consensus sequence Asn-X-Ser/Thr, where X is any amino acid but proline (43). The cleavage of glucose residues dictates interactions of polypeptides with ER chaperones, functioning as a molecular signal for protein folding and quality control. The two outermost glucose residues are quickly cleaved by α -glucosidase I and II (43). Monoglucosylated polypeptides are bound by CNX or CRT and presented to PDIA3 for isomerization of disulfide bonds. The substrates can undergo several binding and release events until the innermost glucose of the carbohydrate moiety is cleaved by α -glucosidase II (43). Deglucosylated polypeptides interact with UDP-glucose glycoprotein glucosyltransferase (UGGT1), a multifunctional protein that can recognize folding defects in proteins by binding to hydrophobic stretches and transfer a glucose residue to the carbohydrate moiety promoting further interaction of substrates with CNX and CRT, a mechanism termed the CNX cycle (43). Correctly folded substrates passing quality control by UGGT1 are exported to the Golgi apparatus while terminally misfolded proteins are directed to degradation (19).

The interaction with CNX and CRT extends protein folding to allow sufficient time for correct disulfide rearrangements by PDIA3 (43).

Glycosylated substrates showed to depend on PDIA3 for redox folding include proteins participating in cell adhesion and extracellular matrix formation that are important for nervous system development and functioning, such as integrins and laminins (41, 44, 45). In addition to protein folding, PDIA3 has been found to regulate sarcoplasmic/endoplasmic reticulum calcium ATPase 2b (SERCA2b) calcium pump, which promotes translocation of calcium from cytosol into the ER lumen coupled to ATP hydrolysis, through a redox switch that is sensitive to luminal calcium concentration (46). The interaction of PDIA3 with SERCA2b under high calcium concentrations is mediated by CRT and keeps the channel in an oxidized and closed state. The release of PDIA3 upon calcium depletion allows SERCA2b disulfide reduction and calcium influx into the ER (46).

Moreover, PDIA3 has been found to accomplish an important scaffold function during peptide loading in the major histocompatibility complex class I (MHC-I) (47). PDIA3 forms a covalent heterodimer with tapasin through a disulfide bond involving cysteine-57 of PDIA3, the first cysteine residue in the a domain active site motif, and cysteine-95 of tapasin (48, 49). This heterodimer assists the transfer of peptides carried by transporter associated with antigen processing I and II (TAPI and II) to MHC-I in the ER membrane (50–52). Remarkably, this PDIA3 function is independent of its oxidoreductase activity (53).

PDIA3 can also distribute to other subcellular locations including the plasma membrane, cytosol, and the nucleus, having alternative functions beyond disulfide bond formation. PDIA3 has been identified as plasma membrane receptor for 1,25-dihydroxyvitamin D₃ (1,25(OH)₂-vitamin D₃, the active form of vitamin D), being termed 1,25D₃-membrane-associated, rapid response steroid binding (MARRS) receptor (54). PDIA3 can mediate fast response to 1,25(OH)₂-vitamin D₃, promoting phosphate and calcium uptake in the intestine (55). Moreover, PDIA3 has been detected in association with signal transducer and activator of transcription 3 (STAT3) (56, 57), a protein resident on the plasma membrane that translocates to the nucleus upon phosphorylation downstream of receptor activation to act as a transcription factor controlling multiple cellular pathways (58). In the cytosol and plasma membrane, PDIA3 is proposed to sequester inactive and activated STAT3 (56). On the other hand, PDIA3 has been shown to form a complex with STAT3 binding DNA in the nucleus, possibly sustaining its transcriptional activity (57).

PDIA4

PDIA4 (also known as ERp72) is a close paralog of PDIA3 with 40% identity that contains five TRX-like domains (59), being three catalytic termed a^o, a, and a' containing the active site motif CXXC and two noncatalytic, b and b'. Like PDIA1 and PDIA3, PDIA4 has a U-shaped domain architecture, but its function remains poorly understood (60). The hydrophobic

patches that confer chaperone function to PDIA1 are not conserved in the b and b' domains of PDIA4 (60). Despite being reported to compensate for PDIA3 deficiency in cell culture (61), PDIA4 does not appear to bind the lectin-like chaperones CNX and CRT (59).

TMX

Most PDI family members are soluble in the ER lumen (8). TRX-related transmembrane proteins (TMX) compose a PDI subfamily anchored to the ER membrane (Fig. 2, A and D) (62). TMX1 is the most studied member of the family, catalyzing redox folding of membrane-tethered clients in association with CNX (62). TMX1 active site motif has CPAC sequence, where the proline residue in position 2 favors the reductase activity because it destabilizes the disulfide state (62). Besides redox folding, TMX1 has been shown to contribute to ER-associated degradation by reducing misfolded substrates (63) and calcium flux by regulating SERCA2b activity (64). TMX4, possessing CPSC sequence in the catalytic site, is a close member to TMX1 whose function remains to be further investigated (62). TMX3 has one TRX-like catalytic a domain with CGHC active site motif followed by two noncatalytic b and b' domains involved in substrate recognition and stabilization of the catalytic domain (65), thus being closely related to PDIA1. TMX5 is a poorly understood member possessing an unconventional CRFS active site, where the absence of the C-terminal cysteine residue restricts its catalytic activity as isomerase.

The TMX members just described are characterized by a single-pass transmembrane region and N-terminal catalytic domain in the ER lumen (62). TMX2, on the other hand, is a multipass transmembrane protein with N- and C-terminal in the cytosolic side (Fig. 2D) (62). The SNDC active site faces the cytosol in the C-terminal region of the protein and its function remains unknown. TMX2 appears to be involved in nucleocytoplasmic transport and mitochondria-ER contact sites (25, 62), with genetic ablation being embryonic lethal in mice.

PDIs control the unfolded protein response

Accumulation of misfolded and aggregated proteins in the ER lumen leads to a condition termed ER stress marked by activation of a signal transduction pathway known as unfolded protein response (UPR) (66). The mammalian UPR operates through three stress sensors and transducers localized in the ER membrane, named inositol-requiring enzyme 1 (IRE1), PKR-like ER kinase (PERK), and activating transcription factor 6 (ATF6) (67). These proteins are activated upon ER stress, triggering signaling cascades that reduce translation and reprogram gene expression to restore ER proteostasis or eliminate irreversibly damaged cells (67). The ER-sensing mechanism has been shown to be coupled to the ER chaperone BiP through repressive interactions (68) and by the direct binding of misfolded proteins to the luminal domain of UPR sensors (in the case of PERK and IRE1) (69). The ER chaperone and collagen carrier Hsp47 also contributes to the UPR activation by modulating the binding of BiP to IRE1 (70). Several PDIs have been shown to regulate the activation of the

three UPR sensors (20), coupling the redox folding machinery to signal transduction.

Phosphorylation of PDIA1 under ER stress has been shown to induce a conformational change in the protein switching its function from foldase to holdase, which acts on activated IRE1, limiting its signaling output to adaptive responses (71). Moreover, PDIA6 has been found to mediate the decay of IRE1 signaling by forming mixed disulfides with the sensor (72). PDIA6 has also been proven to attenuate the PERK pathway, with its deficiency leading to constitutively active UPR and lethality during *C. elegans* development (72). Another study, however, has demonstrated that PDIA6 activates IRE1 signaling upon calcium depletion in the ER (73). The functional outcome of UPR regulation by PDIA6 may be dependent on the tissue and deserves to be investigated in the context of neurological disorders. Several PDI family members can physically associate with ATF6 (20). PDIA5 has been identified as an inducer of ATF6 activity in cancer cells by promoting rearrangement of disulfide bonds in the sensor and its transport to Golgi apparatus in coat protein complex II (COPII) vesicles (74). PDIA16, also known as ERp18, has also been shown to catalyze disulfide exchange in ATF6, which permits optimal processing of the sensor in the Golgi apparatus and downstream transcriptional response (75).

Pathobiology of the nervous system

Sustaining the quality and stability of the neuronal proteome is essential to drive higher functions of the nervous system. Most of the pathways monitoring protein misfolding in the cytosol, the ER, and other subcellular compartments have been involved with the regulation of neurophysiology, and their alterations contribute to pathology (76). PDIs have been implicated in different pathological conditions of the nervous system, either marked by ER stress and chronic activation of the UPR or involving abnormal morphogenesis of brain structures and malfunctioning of neural circuits. The next sections describe the evidence linking PDI dysregulation with pathogenic mechanisms of neurological disorders and highlight possible therapeutic opportunities.

Neurodevelopmental disorders

Neurodevelopmental disorders encompass a wide range of neurological conditions such as intellectual disability (ID), autism spectrum disorders, schizophrenia, epilepsy, among others (77). The etiology of neurological problems is varied and complex, ranging from environmental factors such as alcohol abuse and viral infections to genetic alterations (77). Affected subjects can present gross morphological abnormalities in the nervous system including microcephaly, cortical malformations such as lissencephaly (*i.e.*, absence of normal folds in the cerebral cortex), as well as more subtle changes to structure of neural circuits and synapses (77–81).

Mutations in *TMX2* coding gene, *TMX2*, cause microcephaly accompanied by lissencephaly, cortical polymicrogyria, and other cell migration defects (25, 82). Even though the pathogenic mechanism associated to *TMX2* mutants remains

unknown, it may reflect disturbed redox regulation at mitochondria–ER contact sites resulting in impaired energy production (25). We have recently described a recessive mutation in *PDIA3*, encoding PDIA3, causing severe ID with no evident brain malformation (26). The ID-linked C57Y mutation affects the active site motif of the α domain by substituting the first cysteine for an aromatic residue, which is predicted to be pathogenic by blunting enzymatic activity and destabilizing protein structure (Fig. 3) (26). Expression of *PDIA3*^{C57Y} altered memory consolidation, neuronal connectivity, and synaptic plasticity. At the molecular level, mutant PDIA3 presented loss of enzymatic activity and propensity to form aggregates that abnormally interact with ER chaperones. These effects were associated to altered biogenesis and signaling of integrins (26), key adhesion molecules supporting synaptic activity (44, 83). In this model, mutant PDIA3 limits the production of integrins, which signal to the cytosol to enhance neurite outgrowth and connectivity possibly involving changes in actin cytoskeleton dynamics (Fig. 3) (26). Despite the association between cognitive dysfunction and altered ER proteostasis, neurodevelopmental impairment may be caused, at least in part, by defects in MHC-I pathway in the nervous system since *PDIA3*^{C57Y} should no longer be capable of binding tapasin and promoting peptide loading (48, 50). MHC-I has been discovered to participate in nervous system development and synaptic plasticity (84, 85), but whether antigen presentation contributes to such function remains to be further explored.

Neurodegenerative diseases

The involvement of PDIs in neurodegenerative diseases has been supported by several lines of evidence, ranging from their detection in histopathological and biochemical studies of patient postmortem samples to identification of genetic variants as disease risk factors. PDIs are generally viewed as protective factors against neurodegeneration due to their role in redox folding and quality control of proteins counteracting ER stress, which contributes to neuronal dysfunction and demise in several neurological disorders (21, 28). However, there is also evidence of detrimental action of PDIs in neurodegeneration (23, 29). In this section, we discuss the role of PDIs on different disease contexts.

Prion-related disorders

Several fatal and transmissible neurodegenerative diseases in humans and other mammals marked by spongiform encephalopathy and astrogliosis are caused by an infectious form of the Prion protein (PrP), which are collectively called prion-related disorders (PrDs) and include Creutzfeldt–Jakob disease, kuru, scrapie, among others (86). The normal, cellular PrP (PrP^C) is expressed in the nervous system, where it participates in myelination of peripheral nerves and possibly synaptic function (87). PrP^C is converted in the infectious form, referred to as scrapie PrP (PrP^{Sc}), through a conformational transition into a state of high β -sheet content (88). PrP^{Sc} can self-propagate by acting as a template inducing PrP^C conversion into PrP^{Sc}, leading to propagation of toxic

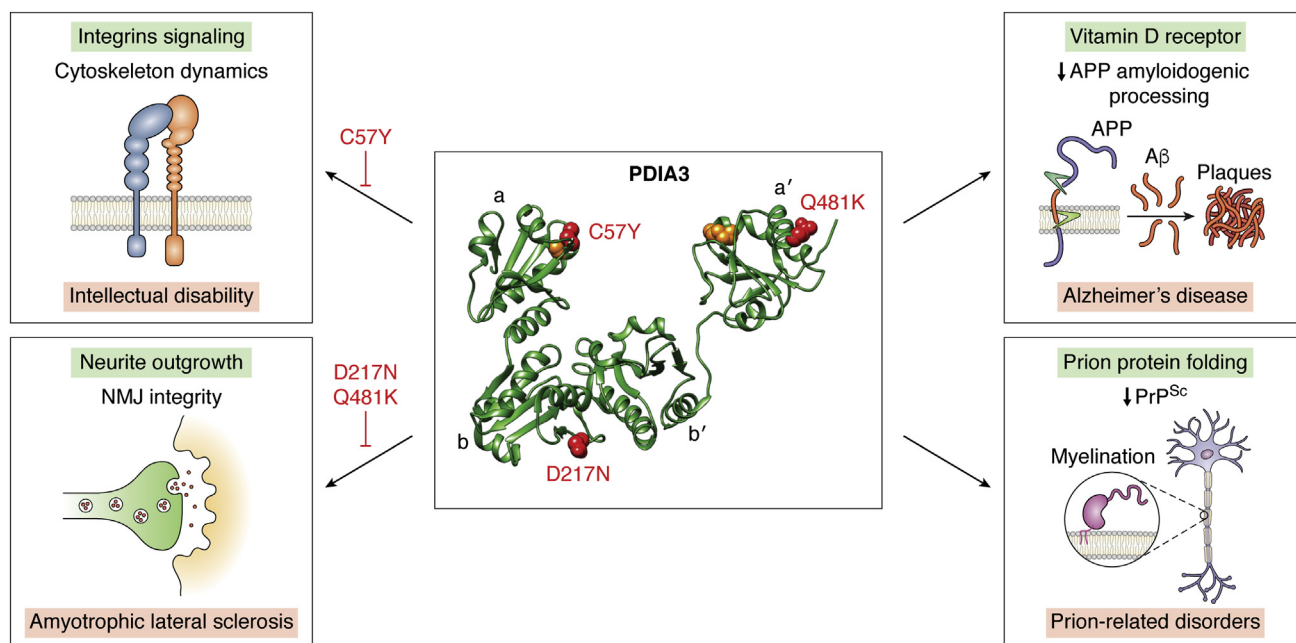


Figure 3. Pathogenic variants of PDIA3 and their impact on neurophysiology. Central panel, PDIA3 structure with highlight of residues substituted in neurological disorders (pathogenic variants indicated next to the residues). Cysteine 60 in the a domain and 406 and 409 in the a' domain, represented in dark yellow. Cysteine 57, aspartate 217, and glutamine 481 represented in red. Protein data bank code, 3F8U. Side panels, PDIA3 functional output in the nervous system and their association to neurological disorders. The C57Y variant associated to intellectual disability impair integrins signaling and downstream events related to cytoskeleton dynamics. The variants D217N and Q481K linked to ALS disturb neuritogenesis, which is likely related to compromised neuromuscular junction (NMJ) integrity in the disease. PDIA3 may positively impact the outcome of Alzheimer's disease by suppressing amyloidogenic processing of amyloid precursor protein (APP). The folding of Prion protein (PrP) is enhanced by PDIA3, which may decrease formation of the scrapie form of PrP (PrP^{Sc}) causing Prion-related disorders and improve axonal myelination.

proteinaceous species throughout the nervous system and causing neurodegeneration (86–88).

Despite lack of evidence for activation of the UPR in histopathological studies in humans (89, 90), the upregulation of PDIA1 and PDIA3, in addition to ER chaperones glucose-related protein 94 (Grp94) and BiP, has been reported in postmortem tissue of PrDs patients (91, 92). Of note, we have found that PDIA3 contributes to PrP^C folding and quality control (Fig. 3) (93). Furthermore, PDIA3 has been shown to be upregulated in multiple animal and cellular models of PrDs (91, 94–98), temporally correlating with accumulation of PrP^{Sc} in mice (95). In the brain of infected hamsters, upregulation of PDIA1, PDIA3, and BiP has been reported (98), with a possible role of S-nitrosylated PDIA1 in induction of apoptotic cell death (Fig. 4). Importantly, overexpression of PDIA3 has been shown to protect against prion replication and toxicity in cellular and mouse models (95, 99), which could be related to accelerated folding of PrP^C limiting conformational transitions that otherwise would facilitate its conversion into PrP^{Sc}. It would also be interesting determining whether PDIA3 could target PrP^{Sc} to degradation or blunt its replication activity.

Alzheimer's disease

Affecting one quarter of the elderly population aged over 80 years, Alzheimer's disease (AD) is the most common neurodegenerative disease marked by progressive loss of memory and dementia (100, 101). AD pathology is

characterized by accumulation of intracellular neurofibrillary tangles (NFTs) composed of the hyperphosphorylated Tau protein and extracellular plaques of amyloid- β peptide triggering synaptic loss and neuroinflammation (102–104).

S-nitrosylation of PDIA1 and PDIA6 has been shown in AD postmortem samples (105, 106), indicating possible dysfunction of redox protein folding and UPR regulation in the disease etiology. Surprisingly, inhibition of PDIA1 has been found to protect against toxicity of amyloid- β peptide by blunting a cell death pathway mediated by the protein localized in ER-membrane contact sites (Fig. 4) (29). Whether PDIA1 modified by S-nitrosylation or other oxidative modification, acquires toxic properties in the nervous system warrants further investigation. Histopathological analysis of AD brains identified NFTs positive for PDIA1 and PDIA6 (106, 107), linking ER proteostasis to a molecular hallmark of the disease. It would be important to determine whether the same neurons exhibiting PDIA1- and PDIA6-positive NFT also present active UPR sensors such as phosphorylated IRE1 and phosphorylated PERK, previously documented in postmortem tissue of AD patients (89, 108). Moreover, the levels of PDIA1 and PDIA6 have been found to be reduced in oligodendrocytes in an AD mouse model before typical pathological alterations, in addition to postmortem samples of AD patients (109). Another histopathological study in postmortem tissue, however, has reported staining of PDIA1 exclusively in neurons with no differences between AD patients and controls (110). While total levels of PDIA1 are primary indicators of their dysregulation in disease, posttranslational modifications and

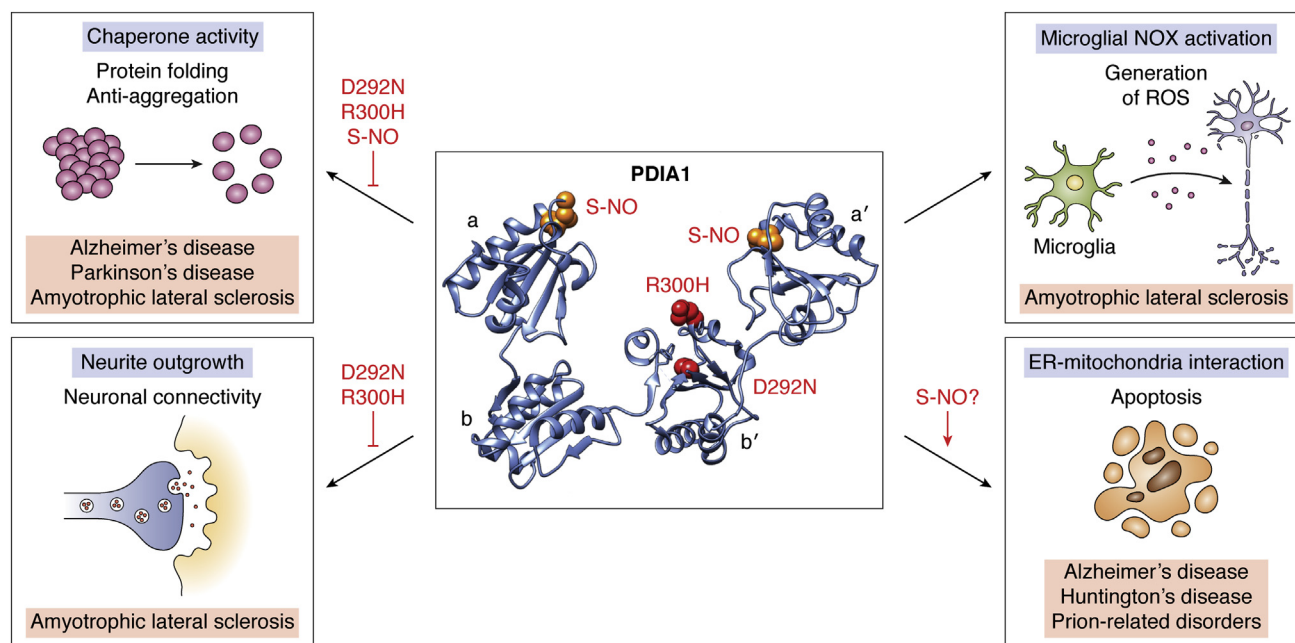


Figure 4. Posttranslational modifications and pathogenic variants disrupting PDIA1 function in the nervous system. *Central panel*, PDIA1 structure with highlight of posttranslational modifications and residues substituted in neurological disorders (pathogenic variants indicated next to the residues). Cysteine 53 and 56 in the a domain and 397 and 400 in the a' domain, represented in *dark yellow* are prone to suffer S-nitrosylation (S-NO). Aspartate 292 and arginine 300 represented in *red*. Protein data bank code, 4EL1. *Side panels*, PDIA1 function in the nervous system and their association to neurological disorders. The variants D292N and R300H linked to ALS, in addition to S-NO modification of cysteines reported in Alzheimer's disease (AD), Parkinson's disease, and ALS, can disturb enzymatic activity leading to protein aggregation. The ALS-linked variants can also impair neuritogenesis, affecting neuronal connectivity. PDIA1 can participate in activation of NADPH oxidases (NOX) and production of reactive oxygen species (ROS) in microglia that can damage motoneurons in ALS. Moreover, PDIA1 accumulates in endoplasmic reticulum (ER)–mitochondria contact sites contributing to apoptosis in AD and Huntington's disease. This mechanism may be mediated by S-NO modification and also contribute to Prion-related disorders.

subcellular localization may be more informative of their actual participation in pathogenic mechanisms.

The activation of PDIA3 in the plasma membrane, where it functions as 1,25(OH)₂-vitamin D₃ receptor, with a plant-derived steroidal sapogenin has been shown to rescue memory deficits in AD mice, an effect accompanied by reduction of NFT, amyloid plaques, and degenerating axons and presynaptic terminals (111). Accordingly, PDIA3 has been shown to regulate expression of several components involved in processing amyloid precursor protein (APP) to amyloid-β peptide (Fig. 3) (112). Of note, STAT3 inhibition has also been found to reduce amyloid plaques (113). It would be interesting to determine whether PDIA3 activation on the plasma membrane can decrease APP processing by sequestering STAT3 in AD. Finally, PDIA3 and CRT have been detected in complex with soluble amyloid-β peptide in cerebrospinal fluid of normal subjects (114), suggested to function as carrier protein that prevent plaques deposition.

Whether conditions disrupting PDI function and redox protein folding in the ER could favor amyloidogenic APP processing, amyloid plaques deposition, impairment of ER–mitochondria communication in a feed-forward cycle of neurodegenerative cascades warrants clarification. In the case that posttranslational modifications inactivating PDIs such as S-nitrosylation act as molecular switches that trigger neuronal demise, overexpression approaches to boost PDI activity may instead worsen disease progression. On the other hand, the translation potential of broad spectrum PDI inhibitors need to

be critically assessed, as safe drugs should target pools of PDIs with particular posttranslational modifications in the subcellular compartments where they exert detrimental effects.

Parkinson's disease

Clinically characterized by resting tremors, rigidity, bradykinesia, and difficulty with walking, Parkinson's disease (PD) is the second most common neurodegenerative disease (115). PD is caused by degeneration of dopaminergic neurons in the *substantia nigra pars compacta* leading to reduced levels of the neurotransmitter dopamine in the striatum and loss of movement control (115). While genetic causes of PD have been mapped and cellular pathways dissected (116), most cases are idiopathic and may occur due to environmental factors (117). The accumulation of proteinaceous inclusions containing the protein α-synuclein inside dopaminergic neurons, so-called Lewy bodies, is a prominent feature of PD (118).

As for AD, S-nitrosylation of PDIA1 has also been detected in postmortem samples of PD patients (Fig. 4) (105). While upregulation of PDIA1 has been described in spinal cord of a transgenic mouse model expressing PD-linked mutant α-synuclein (119), induction of PDIA3 has been reported in a drug-based PD model (120). PDIA3 forms aggresome-like structures in dopaminergic neurons in a neurotoxin-based mouse model through an oxidative pathway and has been identified as a target for covalent modification by dopamine metabolites (121, 122). Because of PDIA3 aggregation and posttranslational modifications, redox protein folding in the ER might become

compromised in PD. Another study has showed decreased levels of PDIA3 and CRT in the midbrain of a toxicological model of PD (123). However, overexpression of PDIA3 using transgenic mice has not afforded any protection in animals exposed to a PD-inducing neurotoxin (124). Failure of the overexpression strategy may reflect susceptibility of PDIA3 to inactivating modifications or deficiency of chaperones of the CNX cycle, a possibility worth to be investigated in future preclinical studies.

Also known as Lou Gehrig's disease, amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease affecting motoneurons that lead to muscle denervation, paralysis, and death by respiratory failure (125). Whereas most cases have no familial history being termed sporadic ALS (sALS), about 10% are hereditary and called familial ALS (126). Genetic studies in familial ALS cases have led to discovery of many mutations causing the disease, the most common being hexanucleotide repeat expansions in *C9ORF72* and missense mutations in *SOD1*, encoding superoxide dismutase 1 (SOD1), *TARDBP*, encoding TAR-DNA binding protein-43 (TDP-43), and *FUS*, encoding fused in sarcoma (FUS) (126). The accumulation of misfolded and aggregated proteins linked to genetic mutations is a disease hallmark (127). Misfolding and aggregation of WT form of TDP-43 and SOD1 have also been implicated in sALS pathology (128–132).

Several unbiased studies in patient postmortem tissue and experimental models of ALS have identified ER stress as an early and transversal pathogenic mechanism in the disease (133–136). We and others have also shown increased levels of PDIA4 in spinal cord of sALS patients (132, 137). Proteomic analysis has identified upregulation of PDIA1 in the spinal cord of presymptomatic ALS mice (138). S-nitrosylation of PDIA1 has been detected in ALS mice and postmortem tissue of sALS patients (139), being linked to mutant SOD1 aggregation and neuronal death (140). Of note, motoneurons show much lower PDIA1 expression compared to neighbor cells (135), which may be linked to their selective vulnerability in ALS. Genetic studies have found intronic mutations in *P4HB*, encoding PDIA1, associated with decreased survival of two different populations of ALS patients (141, 142).

We have previously identified missense variants of *P4HB* and *PDIA3* as risk factors for ALS (143). These variants include D292N and R300H substitutions in the b' domain of PDIA1 and D217N and Q481K mapping to the b and catalytic a' domains of PDIA3, respectively (Figs. 3 and 4). Expression of WT PDIA1 and PDIA3 enhances neurite outgrowth in cell culture (Figs. 3 and 4), while the ALS-linked variants impair this process and cause motor dysfunction in zebrafish (144). Overexpression of PDIA1 and PDIA3 has been shown to counteract mutant SOD1 and TDP-43 aggregation in cell culture (139, 145), an activity lost by ALS-linked mutants (Fig. 4) (146). On the other hand, PDIA1 induced in microglia of ALS mice has been reported to activate NADPH oxidase (147), fueling production of deleterious reactive oxygen species (ROS) that can damage motoneurons (Fig. 4). Increased levels of PDIA3 have been reported in sALS tissue (137, 148). Relevantly, we have shown that conditional KO of *Pdia3* in the

nervous system disturbs maturation of neuromuscular junctions in mice (Fig. 3), possibly due to impaired biogenesis of synaptic proteins (144). Although we observed some degree of lethality in PDIA3-deficient animals and the occurrence of motor problems even under partial loss of function, this model did not develop paralysis, suggesting that PDIA3 alterations operate as a risk factor to develop ALS and may contribute to early disease stages involving muscle denervation. To understand PDIA3 significance to disease pathogenesis, we have generated ALS mice overexpressing the WT protein together with mutant SOD1 by employing a double transgenic approach (149). We have reported that PDIA3 overexpression improves motor performance and electrophysiological activity of affected muscle at early disease stages, an effect correlated with delayed muscle denervation and increased levels of proteins involved in axonal maintenance and synaptic function (149). However, animal survival and markers of disease end stage were not modified by PDIA3 overexpression, again suggesting that it modulates early events of ALS pathogenesis.

As for other neurodegenerative diseases, posttranslational modifications in PDIs during disease progression may limit their activity, impairing redox protein folding and biogenesis of essential proteins for synaptic function. The beneficial effects of PDIA3 overexpression at early, but not late, pathological stages may reflect the increase burden of ROS leading to enzyme inactivation over the disease course, a hypothesis that needs to be tested for further development of proof-of-principle therapeutics. In addition, the balance between levels of PDIA3, CNX, and CRT should be critically evaluated when designing prototypical interventions tackling neurodegeneration.

Huntington's disease

Huntington's disease (HD) is an inherited autosomal dominant neurodegenerative disease characterized by motor, affective, and cognitive impairment resulting from loss of striatal and cortical neurons (150). HD is caused by CAG repeat expansion in the gene coding for huntingtin (151), resulting in a long polyglutamine tail in the N-terminal that leads to protein aggregation (150). Increased levels of PDIA3 have been found in cellular and animal models of HD, in addition to postmortem tissue of HD patients (30). As in AD cellular models, PDI inhibitors have also been shown to protect against mutant huntingtin toxicity (29, 30), possibly through inhibition of apoptotic cell death triggered by PDIA1 at ER-mitochondria contact sites (Fig. 4) (29).

Trauma and ischemia/reperfusion injury

Stroke, traumatic brain injury, and spinal cord injury constitute major disabling health problems that exert important socioeconomic burden worldwide (152–154). ER stress emerges as a significant cellular alteration influencing the pathological outcome of these conditions (155–157). Deprivation of O₂ during ischemia is expected to halt redox protein folding in the ER, transiently leading to accumulation of abnormally disulfide-crosslinked protein complexes.

In this context, PDIs are suggested as relevant molecular targets in different conditions that could be exploited therapeutically.

PDIA1 has been shown to be upregulated upon transient forebrain ischemia in rats and gerbils (158, 159), and its overexpression reduced cellular death in the hippocampus of ischemic rats (158). Nonetheless, inhibition by cyclooxygenase-2 metabolites may limit PDIA1 action during ischemic damage and offer a rationale for design of novel therapies (160). It will be interesting to investigate whether neuroprotection afforded by PDIA1 in ischemia also involves controlled remodeling of brain vasculature by organization of actin cytoskeleton/extracellular matrix as reported for artery injury (39). PDIA1 has also been identified among major proteins induced in a rat model of spinal cord injury by quantitative proteomics (161). Transient S-nitrosylation of PDIA1 has been reported 1 day after sciatic nerve injury (162), possibly associated with impaired redox relay in the ER.

In ischemia/reperfusion injury to the spinal cord in rabbits, levels of PDIA3 have been reported to fluctuate at earlier time points after reperfusion with later increase and higher accumulation in interneurons compared to motoneurons, which correlated to differential neuronal vulnerability in the model (163). Delivery of PDIA3 fused to the TAT carrier peptide has been shown to afford neuroprotection against oxidative and ER stress caused by ischemia/reperfusion in cell culture, rabbit spinal cord and gerbil brain (164, 165). In a model of optic nerve crush, PDIA3 has been found induced after stimulation of α 2-adrenergic receptors and correlate with sustained axonal growth (166), in line with our studies in ALS showing increased levels of protein related to axonal maintenance upon PDIA3 overexpression (149). We have observed that PDIA3 overexpression enhances axonal regeneration in sciatic nerve crush model associated with increased macrophages recruitment and myelin removal (124). It remains to be determined whether increased macrophages recruitment resulting from PDIA3 overexpression is related to enhanced antigen presentation by the MHC-I. Finally, PDIA4 has been reported to be induced upon transient forebrain ischemia in rats (167).

Concluding remarks

The ER contributes to the intricate architecture and dynamic function of the nervous system by supporting neuronal morphogenesis and synaptic activity. The extreme polarization and branching of neurons support formation of numerous neuron–neuron and neuron–glia contacts that build neural circuits and networks (168). The accurate positioning of neurons and growth of neuronal processes, in addition to synaptic remodeling evoked by fast neurotransmission, is predicted to require multiple PDI clients, contributing to maintain healthy neuronal proteostasis. Thus, PDI function is crucial for proper development and activity of the nervous system. Disturbance to PDI molecular networks early in life may lead to improper formation and malfunctioning of the nervous system that predispose an individual to suffer

neurological deficits and neurodegeneration triggered by traumatic events and aging.

Different PDIs have been associated to disease states of the nervous system, among them PDIA1 and PDIA3 arising as the most common dysregulated enzymes. Accumulating evidence suggests that PDIs may counteract pathological cascades by (i) sustaining production of an important fraction of the neuronal proteome (*e.g.*, those proteins involved in synapsis, connectivity, and morphogenesis), (ii) reducing abnormal protein misfolding and aggregation. On the other hand, PDIs may engage ROS production and suffer oxidative modifications themselves such as S-nitrosylation that may switch their neuroprotective role into deleterious outcome, such as apoptosis induction. In this regard, PDIA1 may constitute a primary target for oxidative inactivation that may perturb redox relay and the entire redox protein folding network in the ER leading to severe consequences to neuronal function. Several outstanding questions that warrant further investigation are listed in [Box 1](#), where we highlight efforts to define the

Box 1. Outstanding questions

Overall, PDIs are proposed as crucial components for nervous system development and adaptation to pathological insults over the organism lifespan. Key points to be addressed hereafter include the following:

- Are different PDI family members folding different subsets of clients in the nervous system?
- What is the relative contribution of PDIs to ER proteostasis in neurons and glia?
- Where are different PDIs localized in neurons?
- Is the expression of certain PDIs restricted to specific neuronal subtypes?
- Do PDIs regulate the UPR in the nervous system?
- What are the posttranslational modifications or protein interactions regulating PDI activity?
- Do PDIs determine neuronal vulnerability in neurodegenerative diseases?

specific clients of PDIs and how they relate to neurophysiology and disease. Whether PDIs are involved in the widespread activation of the UPR under physiological and pathological conditions also remains unknown. PDIs are emerging as a central node of the proteostasis network, which may even impact cellular processes beyond secretory pathway function. Gene therapy and pharmacological strategies to modulate PDI activity may have important translational value. For instance, PDIA1 confers survival advantages to cancer cells (169, 170), with inhibitors been shown to reduce cancer progression (170, 171). The challenge in the field is to generate small molecules selectively targeting different PDI family members to avoid broad side effects. Future translational efforts will offer proof of concept for the therapeutic value of manipulating the redox folding machinery in the ER to treat neurological disorders.

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Abbreviations—The abbreviations used are: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; ER, endoplasmic reticulum; HD, Huntington's disease; ID, intellectual disability; MHC-I, major histocompatibility complex class I; NFT, neurofibrillary tangle; PD, Parkinson's disease; PrD, prion-related disorder; ROS, reactive oxygen species; sALS, sporadic ALS; UPR, unfolded protein response.

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