

Review

Myra E. Conway* and Christopher Lee

The redox switch that regulates molecular chaperones

DOI 10.1515/bmc-2015-0015

Received May 18, 2015; accepted July 10, 2015

Abstract: Modification of reactive cysteine residues plays an integral role in redox-regulated reactions. Oxidation of thiolate anions to sulphenic acid can result in disulphide bond formation, or overoxidation to sulphinic acid, representing reversible and irreversible endpoints of cysteine oxidation, respectively. The antioxidant systems of the cell, including the thioredoxin and glutaredoxin systems, aim to prevent these higher and irreversible oxidation states. This is important as these redox transitions have numerous roles in regulating the structure/function relationship of proteins. Proteins with redox-active switches as described for peroxiredoxin (Prx) and protein disulphide isomerase (PDI) can undergo dynamic structural rearrangement resulting in a gain of function. For Prx, transition from cysteine sulphenic acid to sulphinic acid is described as an adaptive response during increased cellular stress causing Prx to form higher molecular weight aggregates, switching its role from antioxidant to molecular chaperone. Evidence in support of PDI as a redox-regulated chaperone is also gaining impetus, where oxidation of the redox-active CXXC regions causes a structural change, exposing its hydrophobic region, facilitating polypeptide folding. In this review, we will focus on these two chaperones that are directly regulated through thiol-disulphide exchange and detail how these redox-induced switches allow for dual activity. Moreover, we will introduce a new role for a metabolic protein, the branched-chain aminotransferase, and discuss how it shares common mechanistic features with these well-documented chaperones. Together, the physiological importance of the redox regulation of these proteins under pathological conditions such as Alzheimer's disease,

Parkinson's disease, and amyotrophic lateral sclerosis will be discussed to illustrate the impact and importance of correct folding and chaperone-mediated activity.

Keywords: CXXC motifs; human branched-chain aminotransferase protein; molecular chaperones; neurodegeneration; peroxiredoxins; protein disulphide isomerase; protein folding; S-nitrosylation.

Introduction

The endoplasmic reticulum (ER) hosts a network of highly regulated mechanisms governed by molecular chaperones and folding enzymes that facilitate the translation and correct folding of proteins. The quality control systems of the ER ensures that only proteins that are correctly folded are selected to be released to the Golgi, whereas misfolded proteins are retained for further processing and ultimately degraded. These pathways can be controlled through changes in the redox environment, where the role of protein thiol-disulphide exchange in regulating and directing protein function is of particular interest (1–3). Redox-sensitive proteins can be modified by reactive oxygen or reactive nitrogen species (ROS/RNS) to higher oxidation states resulting in a loss or gain of function. Generation of excess ROS/RNS leading to these higher oxidation states is balanced by antioxidants such as glutathione and cellular reducing systems, including the thioredoxin and glutaredoxin systems (2, 3). In health, the redox potential of the cytoplasm favours an overall reduced environment, amenable to optimal cell function. However, under conditions where the generation of ROS or RNS exceeds the capacity of these reducing systems, damage to cellular macromolecules such as DNA, lipids, and proteins occur (4–6). A further consequence of increased cellular stress is a build-up of misfolded proteins that triggers an integrated protective cellular response.

The molecular chaperones are the first to respond to increased immature or misfolded proteins, and function to repair them or degrade those that cannot be rescued. This

*Corresponding author: Myra E. Conway, Department of Applied Sciences, University of the West of England, Coldharbour Lane, Bristol BS16 1QY, UK, e-mail: myra.conway@uwe.ac.uk

Christopher Lee: Department of Applied Sciences, University of the West of England, Coldharbour Lane, Bristol BS16 1QY, UK

response involves the upregulation of molecular chaperones such as the heat shock proteins (Hsps) (including the foldases and holdases) that prevent protein aggregation. This is termed the unfolded protein response, which either refold unfolded proteins or direct them for degradation through the ubiquitin proteasome or the autophagy lysosome system. This is followed by ER-associated degradation (ERAD), which specifically recognises terminally misfolded proteins. The ER stress response also includes the upregulation of protein disulphide isomerase (PDI) and glucose-regulated protein, which act as cellular protectors by alleviating the concentration of misfolded proteins. More recently, it has emerged that PDI and other enzymes such as the peroxiredoxins (Prxs) are directly regulated through changes in the redox environment and function as molecular chaperones to prevent protein aggregation. Modification of the redox-active thiols of Prx to sulphenic acid imposes structural rearrangements that trigger a switch from their enzymatic role to their role as a molecular chaperone. This nano-switch function may also explain the ability of the human branched-chain aminotransferase (hBCAT) protein to operate as a transaminase under reducing conditions but as an oxidoreductase when the environment becomes more oxidising. In this review, we will focus on these select redox-regulated chaperones including the Prxs, PDI, and the metabolic protein the hBCAT. We will conclude with an overview of their associated roles with neurodegenerative conditions such as Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS).

Redox regulation: thiol-disulphide exchange

Modifications of redox-sensitive cysteine residues

The most common types of ROS/RNS and their derivatives include the hydroxyl radical ($\cdot\text{OH}$), hydrogen peroxide (H_2O_2), the superoxide anion (O_2^-), singlet oxygen ($^1\text{O}_2$), peroxynitrite (ONOO^-), and nitric oxide (NO). At low concentrations, these species play roles as secondary messengers in the regulation of intracellular cell signalling, important for normal cell function. However, generation of excess cellular stress will damage cellular macromolecules (4–6). The most common target in proteins are redox-sensitive cysteine residues, particularly those present in CXXC motifs (7, 8). These thiol-centred motifs operate as

redox switches that facilitate the reversible regulation of proteins. Reactivity of these cysteines is dependent on their local microenvironment and their overall pK_a , where a low pK_a results in deprotonation generating a reactive thiolate anion (R-S⁻). In general, the cysteine residue closest to the N-terminus of a protein is the nucleophilic thiolate anion with the lower pK_a and is typically found at the end of α -helical structures (9). The redox potential is also dictated by the amino acids that are flanked by the reactive thiols. Oxidation of thiolate anions first generates a cysteine sulphenic acid [Figure 1A (i)], which can be identified using chemical-specific probes such as dime-done or with mass spectrometry (10). Cysteine sulphenic acids are considered highly unstable transient intermediates with a propensity to form more stable associations with other accessible cysteine residues. The potential outcomes will dictate if the reaction is reversible (e.g. disulphide bond) or irreversible (e.g. sulphinamide). The most notable is the formation of either inter- or intra-disulphide bonds [Figure 1A (ii and iii)]. Disulphide bond formation allows proteins to cycle between their active and inactive forms, creating a regulatory control switch (2, 11). The generation of mixed disulphides with glutathione, termed S-glutathionylation, also favours reversible regulation [Figure 1A (iv)]. Sulphenic acids can also react with amino groups forming sulphonamides. These oxidation products can be reversed by members of the thioredoxin or glutaredoxin systems [Figure 1A (v and vi)] or through non-enzymatic reactions with glutathione or vitamin C (2, 3, 12). If oxidation of the cysteine sulphenic acid proceeds to sulphinic or sulphonic acid or sulphinamide, the reaction is irreversible (Figure 1B). However, there is one known exception where oxidation to sulphenic acid is reversible, i.e. eukaryotic Prx. Here, the sulphenic acid form can be reduced by sulphiredoxin (Srx), offering a novel regulatory mechanism for overoxidised reactions (13, 14).

Oxidoreductase systems

For reversible regulation of intra- and intermolecular as well as mixed disulphide bonds to occur, the cell must express oxidoreductase systems. Two of the most described reducing systems in the cell are the thioredoxin and glutaredoxin systems, characterised by their redox-active CXXC motifs and typical thioredoxin structural fold (15). These systems maintain the redox state of the cell and are induced under oxidative stress. The thioredoxin system is composed of thioredoxin (Trx), thioredoxin reductase (TR), and NADPH. There are two isozymes of Trx: cytosolic Trx 1 and mitochondrial Trx 2. The redox

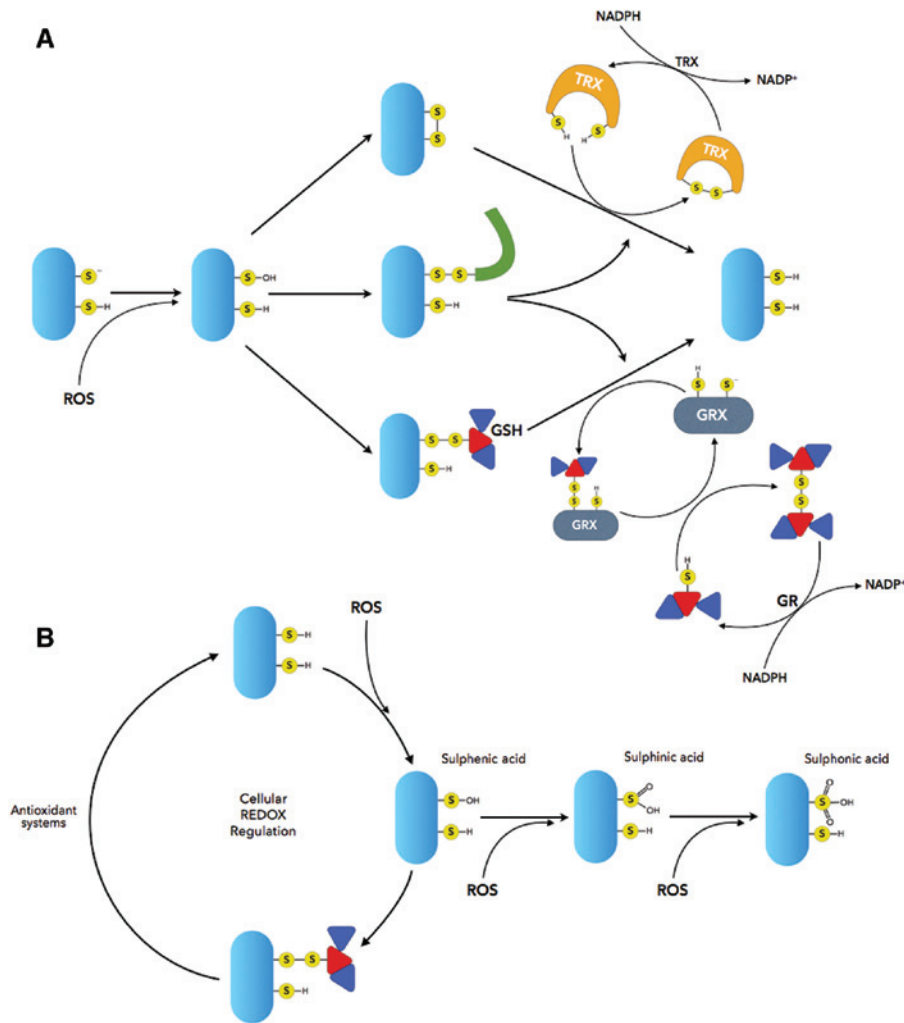


Figure 1: Redox regulation of thiol oxidation.

(A) (i) Oxidation of a thiolate anion generates a cysteine sulphenic acid. (ii) Thiols that are structurally within 3 Å, often in a -CXXC- motif, can form intramolecular disulphide bonds or (iii) intermolecular disulphide bonds with other protein thiols (iv). The generation of mixed disulphides for example with glutathione, termed S-glutathionylation, favours reversible regulation. These oxidation products can be reversed by members of the thioredoxin (Trx) or glutaredoxin (Grx) systems. (v) Reduction of protein disulphide bonds is initiated through the nucleophilic attack of the reactive cysteine of Trx, resulting in the formation of a mixed disulphide bond, subsequently releasing the reduced protein. Reduced Trx is regenerated through redox cycling with thioredoxin reductase (TrxR) and NADPH. (vi) The Grx system utilises glutathione (GSH) and operates in a series of thiol exchange reactions producing GSSG that is subsequently reduced by an NADPH-dependent GSH reductase. (B) Sequential oxidation of cysteine sulphenic acid results in the formation of sulphinic or sulphonyl acid.

potential for human Trx is -230 mV and is believed to remain 90% reduced in healthy cells (16). Reduction of protein disulphide bonds is initiated through the nucleophilic attack of the reactive cysteine of Trx, which has a low pK_a , resulting in formation of a mixed disulphide bond (17) [Figure 1 (vi)]. The reduced protein is subsequently released by the interaction of the N-terminal cysteine of the -CGPC- motif of Trx forming an intra-disulphide bond. Reduced Trx is then regenerated through redox cycling with TrxR and the reducing equivalent NADPH. The glutaredoxin system has a similar composition including

NADPH, glutathione reductase (GR), glutathione (GSH), and glutaredoxin (Grx). Glutaredoxin has a dithiol and monothiol form characterised by a -C-P-Y-C- and -C-G-F-S- motif, respectively (15). Dithiol Grx is a general thiol-disulphide oxidoreductase, which utilise GSH and operate in a series of thiol exchange reactions producing GSSG, that is subsequently reduced by an NADPH-dependent GSH reductase [(i) (GR) $R-S_2 + Grx-(SH)_2 \rightarrow R-(SH)_2 + Grx-S_2$; (ii) $Grx-S_2 + 2GSH \rightarrow Grx-(SH)_2 + GSSG$] (15). The monothiol Grx system preferentially reduces proteins that are S-glutathionylated, but can also reduce mixed disulphides

[R-S-SG+Grx-(SH)₂→R-SH+Grx-S-SG]. Here, the mixed disulphide substrate is reduced by Grx forming Grx-S-SG [Figure 1 (vi)]. Grx-S-SG is subsequently reduced with GSH, which becomes oxidised, producing GSSG in the reaction [Grx-S-SG+GSH→Grx-(SH)₂+GSSG]. Levels of GSH are maintained through the NADPH-dependent GR (Figure 1). The true importance of these systems is as gatekeepers of cellular redox status, preventing higher and typically irreversible modifications. Throughout this review, we will focus on how these redox regulatory systems play more intricate roles, where enzymes share dual roles and switch between functions depending on the redox state.

Molecular chaperones

Molecular chaperones can be categorised into two main groups based on their dependence on ATP: the foldases (e.g. Hsp60) are ATP dependent and the holdases (e.g. HspB1–10) are independent of ATP (18). The Hsps are ubiquitously expressed in all cells and in all subcellular compartments. They function through hydrophobic interactions with unfolded/misfolded proteins, forming intermediates that prevent protein aggregation (19, 20). Molecular chaperones also rearrange proteins within more complex protein structures such as oligomers, translocate proteins in and out of organelles, and assist in the turnover of proteins tagged for degradation (21). Although their expression is characteristically linked with induction by stress such as heat-shock or hypoxia, they also play a role in protein stabilisation under non-stress conditions.

Heat shock proteins: ATP-dependent foldases

The foldases represent the more characterised families of Hsp, including Hsp60, Hsp70, Hsp90, and Hsp100. Here, the binding and hydrolysis of ATP facilitates a change in substrate affinity between the chaperone and the unfolded protein, preventing unfavourable molecular interactions such as aggregation, promoting correct folding. The substrate may need to undergo several sequential rounds of binding and release to attain the ultimate correctly folded state. The *foldases*, such as the Hsp70 family, have an ATPase domain (N-terminal) and a C-terminal substrate-binding domain that undergo an orchestrated conformational change to facilitate their functional role, which also depends on their interaction with other chaperones such as Hsp40 (also known as the J protein) (Figure 2A) (22). This is initiated through ATP binding, which opens the helical

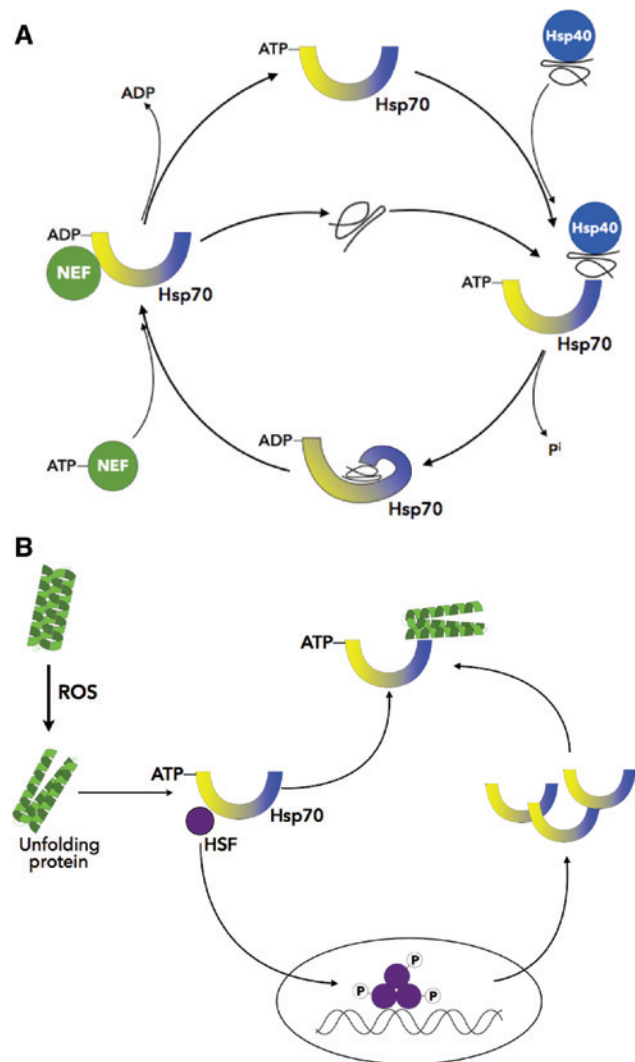


Figure 2: Chaperone activity and regulation of HSP70.

(A) Substrate is directed to HSP70 by HSP40 when ATP is bound. On ATP hydrolysis, the helical lid closes, stabilizing the substrate-Hsp70 complex. NEFs regulate substrate binding and release by supporting the exchange of ADP for ATP. Peptides may undergo several rounds of refolding, before their correct folded state is achieved, preventing aggregation. (B) An increase in ROS triggers the binding of unfolded proteins to HSP70, which releases the HSFs. These proteins are directed to the nucleus where they are hyperphosphorylated and form trimers. Binding of HSF1 to HSE results in the increased transcription of molecular chaperones.

lid, exposing the substrate (unfolded protein) binding site. Together with Hsp40, the substrate is recruited, but only when ATP hydrolysis occurs and the helical lid closes is the substrate-Hsp70 complex stabilised. Peptides may undergo several rounds of refolding, where binding and release is rapid but controlled, until their correct folded state is achieved, preventing aggregation. In addition to Hsp40, co-factors such as the nucleotide exchange factors

(NEFs) regulate binding and release, where NEFs reinitiate the Hsp70 cycle by supporting ADP release and rebinding of ATP (23). Hsp70 also interacts with Hsp100 in disaggregating large aggregates (24). During oxidative stress, cellular levels of ATP rapidly decrease (24–26), which will impact the function of the foldases, leading to misfolding and aggregation of proteins. This is where the holdases are particularly important as they can bind to the hydrophobic regions of misfolded proteins, preventing aggregation without the need for ATP.

Heat-shock proteins: ATP-independent holdases

The holdases are small Hsp (sHsp, approximately 10–40 kDa), composed of 10 members (HspB1–10) that regulate proteome stability in an ATP-independent manner and expressed under stress (18, 27–29). Although the sHsps have widespread heterogeneity, they share common properties, including structural motifs with a conserved α -crystallin domain that permits dimerisation and the ability to form oligomers (30). A conserved triad motif (Ile-Xaa-Ile/Val), in most but not all sHsp, is linked to the oligomerisation of these sHsps, which change in size and organisation dependent on their substrates or stress indicator (31). Their major role is to prevent aggregation of partially misfolded proteins and function as a ‘docking’ station that sequesters the non-native proteins prior to refolding by other chaperones such as Hsp70 or Hsp100. This ability to form oligomers is also shared by the Prxs, discussed later in this review. The holdases also interact with the cytoskeletal system, which provides scaffolds to prevent aggregation of misfolded proteins (32).

Indirect redox regulation of molecular chaperones

The chaperones are tightly regulated at both the transcriptional and translational levels. Although not directly redox regulated at the protein level, the genes of these chaperones are controlled through transcription factors called heat shock factors (HSFs), which are induced through oxidative stress (Figure 2B) (33). In mammals, there are four HSFs, with HSF1 predominantly expressed. In response to increased cellular stress, HSF1 binds to a consensus heat shock element (HSE) within the promoter region of HSF1, resulting in increased gene expression of HSP. Under normal physiological conditions, HSF1 is inactive, does not bind to DNA, but is reported as bound to

chaperones such as HSP70 or HSP90. An increase in cellular ROS or heat shock causes the chaperone complex to bind the unfolded proteins, releasing HSF1. In a series of reactions that involves hyperphosphorylation, trimerisation, and redistribution to the nucleus, HSF1 binds to the HSE upregulating these chaperones (Figure 2B) (34). The rate-limiting step to this reaction is the binding of chaperones such as Hsp70, which renders the HSF inactive, attenuating the response (33). There are, however, exceptional examples where chaperones are directly regulated through changes in their redox environment, including Prx, PDI, and hBCAT.

Redox-regulated molecular chaperones

Peroxiredoxin

Peroxiredoxin antioxidant activity

Peroxiredoxins are primarily classed as antioxidants that reduce hydrogen peroxide, peroxyxynitrite, and alkyl hydroperoxides (35). The rate of Prx-mediated detoxification of hydrogen peroxide is lower ($1 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$) relative to other antioxidants such as catalase ($1 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$), and Prx appears to be highly sensitive to overoxidation leading to inactivation (27). Overoxidation to higher oxidation states has, however, assigned a novel role for Prx in chaperone-mediated activity, but the physiological importance still remains to be fully characterised. There are six isoforms of Prxs (PrxI–VI), which show cell and tissue specificity (36). Based on the role of cysteinyl residues involved in catalysis, the Prx are classified into two categories: the 1-Cys and 2-Cys Prx – the latter is further subdivided into ‘typical’ and ‘atypical’ 2-Cys Prxs (37). Common to all Prx is a conserved N-terminal active-site cysteine (termed the peroxidatic cysteine, Cp), where the substrate (e.g. hydrogen peroxide) is reduced through a sulphenic acid intermediate (35–37). It is the second step, disulphide bond formation, which differentiates between categories. Important to this review is the ‘typical’ 2-Cys Prx (T2-CP), where the N- and C-terminal redox-active cysteine residues are conserved (Figure 3). The N-terminal residue is typically at position 51 and the C-terminal residue at position 171. T2-CP operates as a homodimer with a catalytic active site in each subunit. In T2-CP, once Cys51 is oxidised, the resolving cysteine at position 171, located on the other subunit, reduces the N-terminal oxidised thiol, in a top to tail arrangement (38,

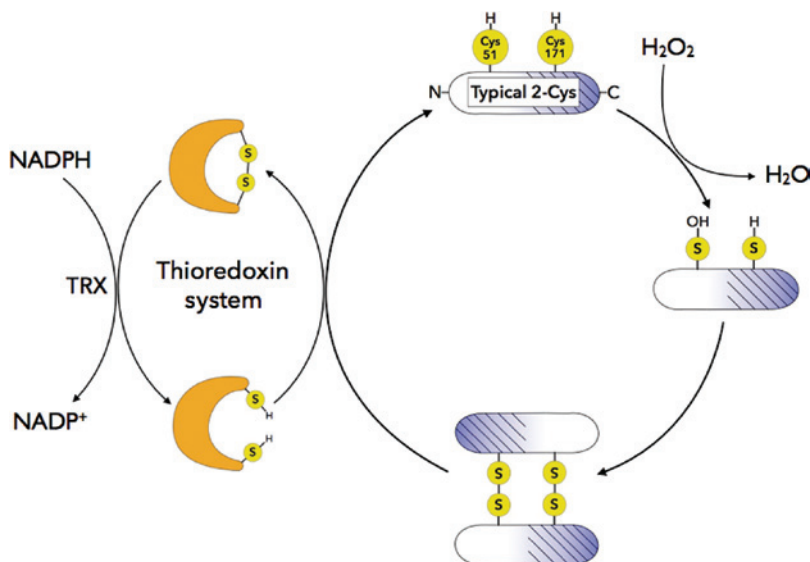


Figure 3: Antioxidant activity of typical 2-Cys peroxiredoxin.

Reduction of hydrogen peroxide occurs through a sulphenic acid intermediate forming an intermolecular disulphide bond with the resolving cysteine of the -CXXC- motif on the other subunit. The disulphide bond is then reduced by the Trx system, using NADPH as a cofactor, recycling the protein.

39). The two molecules are bound together by *inter*-subunit disulphide bond formation, which is then reduced by oxidoreductases, such as the Trx system, recycling the protein (40) (Figure 3). For ‘atypical’ Prx (e.g. PrxV), although two cysteines are also required for catalysis, and the endpoint, resulting in the recycling of thiols by Trx, is the same as described for T2-CP, the resolving cysteine is located in the same subunit as the N-terminal cysteine, resulting in the formation of an *intra*-molecular disulphide bond (37). In the case of 1-Cys Prx (e.g. Prx VI), only the N-terminal cysteine is required for catalysis and substrate release is thought to be regenerated through vitamin C or low molecular weight (LMW) thiols (27).

Peroxide-induced conformational change for Prx, insight into dual role

As with all proteins, conformational change and structural dynamics strongly influence catalysis and function. When reduced, T2-CP is fully folded and the reactive thiols are substantially distant (≥ 13 Å apart), not to mention the fact that they are on opposite subunits, with the resolving cysteine partially buried (38). Thus, for catalysis, partial protein unfolding of the active site to expose the reactive thiol is required, and it remains in this form until the thiols are recycled by Trx. The rate at which unfolding occurs is delayed by a C-terminal extension, which can result in the overoxidation of the N-terminal cysteine, in particular

under oxidative stress, which will impact its antioxidant activity (see below). However, as the sulphenic acid form of Prx could be reversed by Srx, it provided the scope for this reaction to have dual mechanistic roles (41, 39, 42). A key factor to emerge was the ability of Prx to adopt several structural conformations, including oligomerisation, dependent on the redox environment.

Its role as a redox chaperone was first described in yeast where two cytosolic isoforms of Prx were shown to alternate their function between an antioxidant and a molecular chaperone, dependent on the redox environment (43). Under normal physiological conditions, where low levels of ROS are generated, Prx forms LMW structures such as dimer, tetramer, and oligomer conformations. Under these conditions, peroxidase activity predominates (Figure 4). However, when cellular stress increases, mediated through heat shock or increased ROS, higher molecular weight (HMW) structures accumulate (Figure 4). Notably, as described above, the reactive N-terminal cysteine becomes overoxidised to sulphinic acid, triggering the chaperone activity of yeast Prx. Moreover, the C-terminal contains a -GGLG- loop and an additional α -helix with a conserved YF domain that cover the active site when T2-CP is reduced. This additional C-terminal extension confers structural stability to the reactive cysteine and appears to slow transition to disulphide bond formation, rendering the Cp-sulphenic acid susceptible to further oxidation to sulphinic acid formation (44). *In vitro* studies have indicated that these structural transitions of

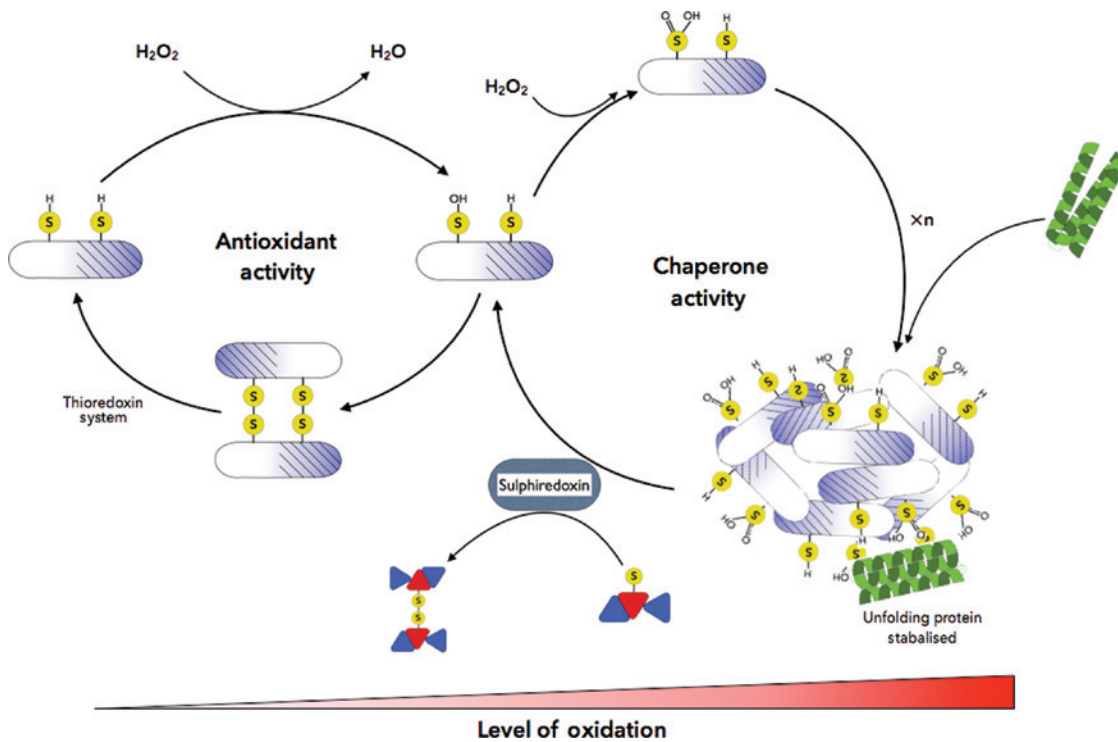


Figure 4: The redox nano-switch of 'typical' 2-Cys peroxiredoxin.

The antioxidant activity of T2 CP is considered to predominate when levels of ROS are low. However, an increase in cellular stress creates HMW structures, where T2 CP switches its role from antioxidant to chaperone, stabilizing unfolded proteins. This switch is believed to be regulated through overoxidation to sulphinic acid, which can be reversed by sulphiredoxin, regenerating reduced T2 CP.

Prx prevent protein aggregation of unfolded proteins generated in response to heat shock (45). Thus, overoxidation of its reactive thiol to a sulphinic acid creates a nanoswitch regulating the proteins' chaperone activity but only when extreme ROS occurs. This type of regulation could prevent T2-CP interfering with the chaperone *foldases* under mild oxidative stress when ATP is available, preventing little crossover and promoting efficient folding. It has also been proposed as an adaptive response to increased ROS, where more potent antioxidants, such as catalase, take over this role in the cell (45, 46).

Protein disulphide isomerase

PDI: thiol disulphide exchange

PDIs (EC 5.3.4.1), part of the thioredoxin superfamily, primarily reside in the ER and operate in the maturation, transport, and folding of secretory proteins (47). These enzymes catalyse the oxidation, reduction, and isomerisation of disulphide bonds, whereas molecular chaperones mediate correct protein folding and stabilise structures, yet do not

increase the rate of protein folding like the isomerases (18, 21). These oxidoreductase proteins also operate as chaperones in polypeptide refolding, involving redox-dependent and -independent reactions (27). Like Trx and Grx, the PDI family have characteristic thioredoxin-like fold structures with most including one to three redox-active CXXC motifs (48). In human PDI (hPDI) and yeast, there are four thioredoxin-like domains (**a**, **a'**, **b**, and **b'**). Structurally, these are arranged in a 'U-shaped' assembly where the **a/a'** domains face each other, each containing a solvent-exposed catalytic redox-active -CGHC- motif (49), where the thiols can cycle between an intramolecular disulphide bond or a reduced state and are responsible for the thiol-disulphide isomerase activity of PDI. As an oxidoreductase, oxidised PDI transfers its electrons to reduced substrates, forming a mixed disulphide. Reoxidation of the PDI **a** domain occurs through the nucleophilic attack of the reactive thiols of the structurally opposing **a'** domain. This reaction is catalysed by Ero1, which is responsible for the transfer of oxidising equivalents to PDI, where electrons are ultimately channelled to molecular oxygen (reviewed in Ref. 19). PDI, in its reduced form, targets its substrate disulphide through a nucleophilic attack and the resulting mixed disulphide is resolved with the free thiol group, oxidising PDI. As an

isomerase, the mechanistic details are not entirely elucidated; however, a sequence of thiol/disulphide exchange reactions occur that rearrange the pattern of disulphide bonds, generating the correctly folded protein. Substrate specificity is considered to be governed by the highly hydrophobic **b** domains, which are flanked by the **a** and **a'** domains. The **a'** and **b'** domains are connected through a 19-amino-acid inter-domain link, called the x-linker, which is predicted to promote flexibility, influencing substrate binding (50). Another key feature is the highly acidic C-terminus, which is involved in calcium binding and contains the ER-retrieval motif KDEL.

PDI: redox-independent chaperone activity

The binding of polypeptides (10–15 amino acids in length) to PDI has more often been associated with the hydrophobic **b** domains, independent of redox regulation (51). However, for full-length proteins, a larger proportion of PDI is required (52). *In vitro* experiments demonstrated that PDI had chaperone-mediated activity with glyceraldehyde dehydrogenase and rhodanase, respectively, proteins without disulphide bonds (53, 54). Chaperone activity in addition to isomerase activity is required for maximal refolding and reactivation of reduced/denatured acidic phospholipase A2 (55). Physiologically, a chaperone-mediated role for PDI has also been assigned in the ERAD response, and in its ability to enhance tissue factor coagulant activity on microvesicles (56, 57). Binding of ERE-containing DNA to the oestrogen receptor was recently reported to be enhanced by PDI, independent of its isomerase activity, as evidenced by the ability of the -CGHC- site-directed mutants to also promote binding (58). Also, PDILT, a PDI homolog, without a redox-active -CGHC-, was shown to display chaperone activity, when found to interact with somatostatin (59). In these examples, the activity of the thioredoxin domains is not required and the chaperone activity is independent of the redox environment.

PDI: redox-dependent chaperone activity

The first evidence in support of a redox-regulated chaperone mechanism originated from work with cholera toxin (60, 61). Cholera toxin consists of A and B subunits, where only a fragment of the A subunit, A1, is active and is released into the cytosol. For this to occur, the toxin must first make its way to the ER, where it is initially nicked by proteases to undergo unfolding, a step required to release the A1 segment. Here, the hydrophobic segments of the

A1 chain are exposed and present as a substrate for PDI, which must be reduced for binding to occur and on oxidation is released, unfolding the protein (60). The authors of this study emphasised that the redox cycling was not from the cysteines in the substrates but most certainly from PDI. Building on these studies, the authors showed that the latter oxidation reaction is catalysed by Ero1, releasing the A1 chain. Release of the A1 chain to a protein at the luminal side of the ER completes the chaperone role of PDI (61). This mechanism of action was compared with the ATP-driven chaperone activity of Hsp70, where a conformational change, causing an opening and closing of the chaperone-binding site, regulates folding. However, in this instance, the ATP/ADP regulatory switch is replaced by the reactive thiols. Similarly, in bacteria, Hsp33, which protects bacteria against oxidative stress, has also been identified as a redox-dependent chaperone (27). PDI was also shown to associate with NAD(P)H oxidase, a prominent source of ROS in the vasculature (62). In neutrophils, NAD(P)H oxidase activity was regulated through redox-chaperone PDI activity. Here, using colocalisation and pull-down assays, PDI associated with distinct subunits of the oxidase complex. The authors proposed that both redox and non-redox interactions with p47^{phos} contribute to a role for PDI as a 'NADPH oxidase complex organiser protein', indicating a novel role for PDI in oxidase regulation (63).

Although the concept of PDI as a redox-regulated chaperone was challenged at the time, largely because this redox-regulated chaperone function could not be repeated for other proteins (64), the elucidation of the 3D structure of PDI in its reduced and oxidised form now provides strong evidence in support of this mechanism (49, 63, 62). Initial studies by Tsai et al. in 2001 showed that PDI adopted a more compact structure in the reduced form. In the reduced and oxidised forms, a conformational shift induced by oxidation of the -CGHC- motif of the **a'** subunit created a change in the rotation of the C-terminal of the x-linker, exposing a greater surface area of the hydrophobic area of the **b'** domain (Figure 5) (49). When oxidised, the distance between the two active -CGHC- regions increases by 12.7 Å. This more open structure promotes the binding of unfolded peptides, where high binding affinity is gained. Now PDI can oxidise the substrate, where PDI itself becomes reduced and the structural conformation becomes more compact. Once correctly folded, the substrate is released. Reported by several groups, homodimerisation of the **bb'** domain, regulated by the x-linker region, is suggested to block substrate access, regulating PDI activity (65–67). Molecular dynamic simulation of three-dimensional structures suggested that in solution, hPDI may adopt more rigid structures facilitated

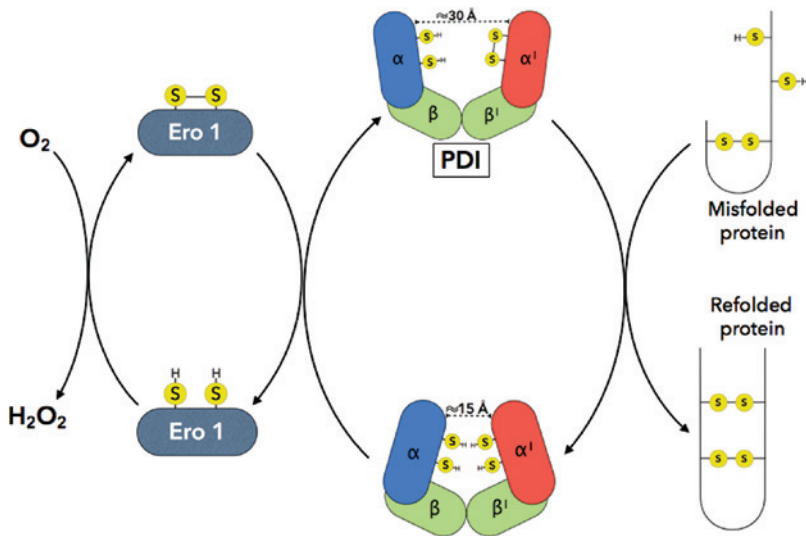


Figure 5: Protein disulphide isomerase as a redox-chaperone.

Oxidation of PDI increases the distance between the two active sites, exposing a larger surface area of the hydrophobic region, promoting the binding of unfolded proteins. Oxidation of the substrate, introducing disulphide bonds, results in the reduction of PDI, where the structure now adopts a more compact conformation. Once correctly folded, the substrate is released.

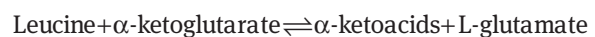
by inter-domain salt bridges, encouraging inter-domain disulphide bond formation, where a role for domain **a** was also supported (68). Clearly, these proteins are more than just an oxidoreductase and also operate as chaperones in polypeptide refolding, where evidence in support of redox-dependent and -independent reactions exists, indicating that changes in the redox environment linked to changes in the structural dynamics may direct substrate specificity under normal and pathophysiological conditions.

Metabolic redox chaperones: the hBCAT proteins

Transamination and redox regulation

The hBCAT proteins are PLP-dependent aminotransferases, where transamination consists of the coupled half-reaction in which the PLP cofactor transfers between its PLP and pyridoxamine (PMP) form via a Ping-Pong Bi-Bi mechanism (69, 70). These proteins have a mitochondrial (m) and cytosolic (c) isoform with tissue-specific locations, with hBCATm, the most ubiquitous isoform, thought to be responsible for the majority of transamination outside the central nervous system with the highest levels of hBCATm recorded in the pancreas, kidney, stomach, and brain (70). hBCATc, is largely associated with neuronal cells and is predominantly found in the soma and proximal dendrites of

glutamatergic and GABAergic neuronal cells, supporting its role in contributing to both the storage and metabolic pools of the neurotransmitter glutamate and as the precursor for GABA production in GABAergic neurons (71–73). Although hBCATm is ubiquitously expressed throughout the body, there is extensive immuno-positive staining for hBCATm specific to brain vasculature, indicating a new role for hBCATm in brain glutamate regulation (71, 74). BCAT catalyse the transfer of the α -amino group from the hydrophobic branched-chain amino acids (BCAAs) leucine, isoleucine, and valine, to α -ketoglutarate releasing their respective keto acids, ketoisocaproate, keto methyl valerate, and ketoisovaline and glutamate, regenerating the enzyme (75, 76). The committed step in BCAA oxidation is catalysed by the second enzyme in the catabolic pathway, the branched-chain α -keto acid dehydrogenase complex (BCKDC). The enzyme complex catalyses multistep reactions that lead to the irreversible oxidative decarboxylation of the branched-chain keto acids giving rise to branched-chain acyl-CoAs. The BCKDC complex is organised around a 24-meric core scaffold consisting of dihydrolipoamide acyltransferase (E2) subunits, where multiple copies of the branched-chain keto acid dehydrogenase (E1) and dihydrolipoamide dehydrogenase (E3) are non-covalently attached.



(Scheme 1)

Structurally, the BCAT proteins operate as homodimers with each monomer existing as a large and small

domain (77, 78). Although they share the same substrate specificity, they have subtle catalytic differences and are differentially regulated through changes in the redox environment, through their peroxide-sensitive redox switch, which is ~ 10 Å from the active site (9, 11, 79–81). Through biochemical and X-ray crystallography investigations, it was determined that reduction and oxidation of the -CXXC- motif represented the active and inactive forms of the proteins respectively. Using peptide mapping, the reactive thiols were identified as -Cys315-XX-Cys318- for hBCATm and -Cys335-XX-Cys338- for hBCATc (9, 11). In each isoform, the N-terminal cysteine residue is the most reactive, sensing changes in the redox environment, whereas the C-terminal residue permits reversible regulation through the formation of a disulphide bond via a sulphenic acid intermediate (11, 82). ROS-mediated oxidation resulted in the complete reversible inactivation of hBCATm, whereas air oxidation alone resulted in a loss of 40–45% functional activity for hBCATc, with no further loss on treatment with hydrogen peroxide (11, 83). X-ray crystallography studies and kinetic analysis demonstrated that the predominant effect of oxidation was on the second half-reaction rather than the first half-reaction, where disruption of the CXXC centre results in altered substrate orientation and an unprotonated PMP amino group, thus rendering the enzyme catalytically inactive (83). The reactive cysteines of both isoforms are also targets for the S-nitrosylating agent, S-nitroso glutathione (GSNO), where a transition between S-nitrosation and S-glutathionylation was reported dependent on the level of RNS exposure (81). The glutaredoxin/glutathione system reversed this inactive form, supporting a role for hBCAT in cellular redox control.

Redox-dependent substrate channelling

A redox-chaperone function of hBCAT has been shown to be important for the formation of a metabolon, a supra-molecular complex, where metabolic enzymes associate, to facilitate substrate channelling (84). Here, the physical association of hBCATm to the E1 subunit of the BCKDC was required for the channelling of the BCKA product to the active site of E1. In this instance, mutation of the redox-active thiols impaired the association of hBCATm with the BCKDC, supporting a role for redox signalling in the regulation of this metabolon. As described for PDI, the CXXC motif is located at the opening of the active site, where the reduced state and the net dipole charge are important for complex formation between hBCATm and the E1 complex. Oxidation or mutation of the reactive thiols increases the

net dipole charge, destabilizing the substrate and the PLP cofactor, interfering with the metabolon. Several other metabolic proteins, isolated from neuronal IMR32 cells, were also shown to have peroxide-mediated redox associations with hBCAT (80). These proteins have either known reactive cysteine residues or CXXC motifs with phosphorylation sites and/or are directly involved or controlled by G-protein cell signalling, known to be modulated by peroxide.

The role of hBCAT in protein folding and redox-chaperone activity with PDI

More recently, we showed a novel functional role for hBCAT in redox protein folding (85). The hBCAT proteins were shown to catalyse the refolding of reduced and denatured RNA. As the redox environment became more oxidising, the oxidase activity of hBCATm continued to increase relative to hBCATc. Although both hBCAT proteins had thiol isomerase activity, it was 50% less than their oxidase activity. Site-directed mutagenesis of the reactive thiols of hBCAT showed that during catalysis, the N-terminal cysteine is responsible for the first step in forming an intermediate with RNase and although the single C-terminal cysteine mutants could form this initial step, substrate release was impossible as illustrated by the inability of either mutant to catalyse refolding (85). Notably, when these proteins were treated with GSNO, HMW structures were reported and the rate of refolding increased, a feature reported for Prxs when overoxidised. Thus, under reduced conditions, the role of hBCAT favours transamination and metabolon formation with BCKDC, whereas oxidation changes the structural conformation opening the active site to novel substrates to promote protein folding and chaperone activity, predicted to be supported by its three hydrophobic pockets in the active site. Further evidence in support of the hBCAT proteins, in particular hBCATm, as a redox chaperone was corroborated with findings to show that hBCATm colocalised with PDI, in both human brain and cell models, indicating that these two proteins were interacting. Using confocal and electron microscopy, our studies established that the interaction of hBCAT with PDI was redox regulated (85). Although hBCAT did not appear to impact the overall PDI activity, it did increase the rate of refolding. We hypothesised that hBCATm may be a novel chaperone for PDI operating through a thiol disulphide exchange mechanism or independently as an oxidoreductase regulated through the redox environment (Figure 6). The physiological role of this interaction has not yet been elucidated, but new data have isolated a protein-folding

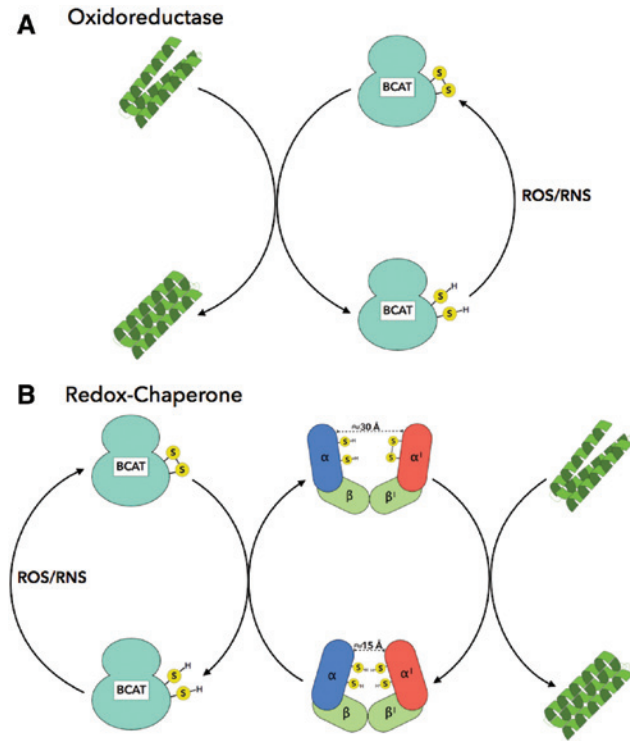


Figure 6: Redox activity of the hBCAT.

(A) As an oxidoreductase, hBCAT can catalyse the introduction of disulphide bonds, refolding proteins. This reaction is redox regulated. (B) As a chaperone, it is hypothesised that through a thiol disulphide exchange mechanism, hBCATm can oxidize PDI, becoming reduced and promoting the refolding of substrate proteins. Of note is the increase in rate of refolding when hBCATm is incubated with PDI during refolding of reduced denatured RNase.

metabolon, offering exciting new roles for these metabolic proteins as redox chaperones (Lee et al., unpublished observations).

Molecular chaperones and their role in neurodegenerative conditions

The cell is equipped to combat an increase in cellular stress; however, these ‘quality control systems’ seem to become overwhelmed, and under sustained cellular stress cell function is compromised. One consequence of increased ROS/RNS is the build-up of unfolded proteins creating aggregates. Accumulation of these aggregates contributes to a cascade of events that ultimately results in cell death, implicated in the manifestation of numerous disease conditions, including cancer, cardiovascular disease, and neurodegenerative conditions (86, 87, 41,

88–90). Triggered by increased ROS/RNS, the holdases and foldases respond and try to evoke their chaperone activity. However, for reasons not entirely understood, they cannot refold these ‘disease’ proteins and are often found associated with these aggregates, indicating an imbalance between the production and activity of these Hsp (91, 92). In neuronal cell models, however, overexpression of Hsp70 reduces aggregation, in particular with respect to A β and τ , aggregates particular to AD, indicating their role as neuroprotectors; however, it remains unclear why this does translate to the human brain (93, 94). A neuroprotective role has also been assigned for Prx, in particular with respect to resisting neurotoxicity by A β (95–97). In human brain, isoforms of Prx were found to be differentially expressed in AD, Pick’s disease, and Down syndrome, indicating that these antioxidants show regional brain regulation, in response to increased cellular stress, dependent on the type of neurodegenerative condition identified (54). Other conditions where aggregated proteins accumulate such as PD, Creutzfeldt-Jakob disease, and ALS also show increased Prx expression (98–100). Given the role of Prx as an antioxidant and as a redox chaperone, this increased expression should reduce the accumulation of misfolded proteins in neurodegenerative conditions. However, in reality, aggregates still form; therefore, the proposed neuroprotective role is insufficient or the activity is compromised through another pathway yet to be discovered. Clearly, further studies are required to manipulate these pathways and offer insight into why these fine-tuned quality control systems lose their neuroprotective capacity.

A role for PDI in protein misfolding has emerged as a key link to understanding how aggregates accumulate in the brain, and play a role in the pathogenesis of neurodegenerative conditions including AD, PD, Huntington’s disease, and ALS (19, 20, 101–106). PDI has been shown to accumulate with neurofibrillary tangles and the dystrophic neurites of senile plaques in AD brain, and with FUS-positive ubiquitinated inclusions in human ALS patient spinal cords (107, 108). Not only was there an increase in PDI expression in AD, PD, and ALS brain, the amount of S-nitrosylated PDI was also substantial (107, 109–115). S-nitrosylation of PDI, through the redox-active thiols of the α and α' domains, inhibits enzyme activity. Cell models of AD pathology show that PDI inactivated through NO triggers an accumulation of poly-ubiquitinated proteins, an increase in ER stress, and induction of apoptosis (92, 99). Similar reports using ALS and PD models show that overexpression prevents aggregation, whereas inhibition of PDI results in the accumulation of misfolded proteins (107, 116–119). Further understanding of these

pathways will determine if inhibitors of S-nitrosylated PDI are therapeutically beneficial or if increased expression of PDI will generate unfavourable metabolic reactions, attenuating its potential role as a target therapeutic.

The hBCAT proteins have also been implicated in the pathogenesis of AD, predicted to be linked more with its metabolic function rather than its role in redox regulation (120). In brief, a regional increase in hBCATc expression in the hippocampal region of AD patients was reported, whereas an increase of 160% was reported for hBCATm in the frontal and temporal cortex compared to matched control brains. Increased hBCATm expression correlated with Braak stage, suggesting that the expression of this enzyme is directly related to the severity of the disease (120). Here, a neuroprotective role was assigned to the hBCAT proteins, as neuronal cells were morphologically intact, with no evidence of apoptotic features. Increased expression of hBCAT would, however, be expected to increase the glutamate pool, and sustained release may destroy surrounding cells. Thus, their intended role in neuroprotection may switch to neurotoxic. Alternatively, we can speculate that similar to that described for the Prxs, the redox switch of hBCAT may signal a change in function from its metabolic role to a role as an oxidoreductase or redox chaperone when the redox environment becomes more oxidizing.

Conclusion

One of the most intriguing aspects of molecular chaperone activity is the extensive structural remodelling that is required to correctly fold and translocate proteins. The recent advances in our understanding of how these structural realignments change in response to the redox environment have made it clearer that the function of molecular chaperones can be redox-dependent and redox-independent. This may allow the cell to operate its stress response more efficiently, optimizing its chances for survival. However, evidently, this is not enough. Despite these elaborate transitions between LMW and HMW structures to prevent protein misfolding, protein aggregation and accumulation persists and clinically presents as inclusions or plaques. A major challenge of this work is translating these *in vitro* studies to understand the complexities of protein folding, in particular in the human brain. Future studies need to focus on the intricacies of these structural changes and aim to elucidate the molecular components of the crosstalk between redox signalling and molecular chaperone activity. Lack

of robust cell/animal models, together with the complexities of brain metabolism, explains the barriers that challenge the translation to our *in vivo* understanding of protein misfolding. However, what is clear is that in the human brain, these molecular chaperones and folding enzymes are upregulated, found to be associated with aggregates, and in the case of PDI, show evidence of S-nitrosylation, that impacts refolding capacity. Clearly, the need exists to continue to understand how these mechanisms are regulated, as they present with new avenues for therapeutics to target protein aggregation, a major clinical need for the treatment of neurodegenerative conditions.

References

1. Droge W. Redox regulation in anabolic and catabolic processes. *Curr Opin Clin Nutr* 2006; 9: 190–5.
2. Holmgren A, Johansson C, Berndt C, Lonn ME, Hudemann C, Lillig CH. Thiol redox control via thioredoxin and glutaredoxin systems. *Biochem Soc Trans* 2005; 33: 1375–7.
3. Lu J, Holmgren A. The thioredoxin antioxidant system. *Free Radical Biol Med* 2014; 66: 75–87.
4. Lipinski B. Hydroxyl radical and its scavengers in health and disease. *Oxid Med Cell Longev* 2011; 2011: 809696.
5. Ghezzi P. Protein glutathionylation in health and disease. *Biochim Biophys Acta* 2013; 1830: 3165–72.
6. Alfadda AA, Sallam RM. Reactive oxygen species in health and disease. *J Biomed Biotechnol* 2012; 2012: 936486.
7. Groitl B, Jakob U. Thiol-based redox switches. *Biochim Biophys Acta* 2014; 1844: 1335–43.
8. Poole LB. The basics of thiols and cysteines in redox biology and chemistry. *Free Radic Biol Med* 2015; 80: 148–57.
9. Conway ME, Yennawar N, Wallin R, Poole LB, Hutson SM. Identification of a peroxide-sensitive redox switch at the CXXC motif in the human mitochondrial branched chain aminotransferase. *Biochemistry* 2002; 41: 9070–8.
10. Furdui CM, Poole LB. Chemical approaches to detect and analyze protein sulfenic acids. *Mass Spectrom Rev* 2014; 33: 126–46.
11. Conway ME, Poole LB, Hutson SM. Roles for cysteine residues in the regulatory CXXC motif of human mitochondrial branched chain aminotransferase enzyme. *Biochemistry* 2004; 43: 7356–64.
12. Ginter E, Simko V, Panakova V. Antioxidants in health and disease. *Bratisl Lek Listy* 2014; 115: 603–6.
13. Woo HA, Jeong W, Chang TS, Park KJ, Park SJ, Yang JS, Rhee SG. Reduction of cysteine sulfenic acid by sulfiredoxin is specific to 2-cys peroxiredoxins. *J Biol Chem* 2005; 280: 3125–8.
14. Jeong W, Bae SH, Toledano MB, Rhee SG. Role of sulfiredoxin as a regulator of peroxiredoxin function and regulation of its expression. *Free Radic Biol Med* 2012; 53: 447–56.
15. Berndt C, Lillig CH, Holmgren A. Thioredoxins and glutaredoxins as facilitators of protein folding. *Biochim Biophys Acta* 2008; 1783: 641–50.

16. Coles SJ, Hancock JT, Conway ME. Differential redox potential between the human cytosolic and mitochondrial branched-chain aminotransferase. *Acta Biochim Biophys Sin* 2012; 44: 172–6.
17. Kallis GB, Holmgren A. Differential reactivity of the functional sulfhydryl groups of cysteine-32 and cysteine-35 present in the reduced form of thioredoxin from *Escherichia coli*. *J Biol Chem* 1980; 255: 10261–5.
18. Niforou K, Cheimonidou C, Trougakos IP. Molecular chaperones and proteostasis regulation during redox imbalance. *Redox Biol* 2014; 2: 323–32.
19. Conway ME, Harris M. S-nitrosylation of the thioredoxin-like domains of protein disulfide isomerase and its role in neurodegenerative conditions. *Front Chem* 2015; 3: 27.
20. Blair LJ, Baker JD, Sabbagh JJ, Dickey CA. The emerging role of peptidyl-prolyl isomerase chaperones in tau oligomerization, amyloid processing, and Alzheimer's disease. *J Neurochem* 2015; 133: 1–13.
21. Gething MJ, Sambrook J. Protein folding in the cell. *Nature* 1992; 355: 33–45.
22. Saibil H. Chaperone machines for protein folding, unfolding and disaggregation. *Nat Rev Mol Cell Biol* 2013; 14: 630–42.
23. Goloubinoff P, De Los Rios P. The mechanism of Hsp70 chaperones: (entropic) pulling the models together. *Trends Biochem Sci* 2007; 32: 372–80.
24. Shorter J. The mammalian disaggregase machinery: Hsp110 synergizes with Hsp70 and Hsp40 to catalyze protein disaggregation and reactivation in a cell-free system. *PLoS One* 2011; 6: e26319.
25. Tiwari BS, Belenghi B, Levine A. Oxidative stress increased respiration and generation of reactive oxygen species, resulting in ATP depletion, opening of mitochondrial permeability transition, and programmed cell death. *Plant Physiol* 2002; 128: 1271–81.
26. Leist M, Single B, Naumann H, Fava E, Simon B, Kuhnle S, Nicotera P. Nitric oxide inhibits execution of apoptosis at two distinct ATP-dependent steps upstream and downstream of mitochondrial cytochrome c release. *Biochem Biophys Res Commun* 1999; 258: 215–21.
27. Kumsta C, Jakob U. Redox-regulated chaperones. *Biochemistry* 2009; 48: 4666–76.
28. Garrido C, Paul C, Seigneuric R, Kampinga HH. The small heat shock proteins family: the long forgotten chaperones. *Int J Biochem Cell B* 2012; 44: 1588–92.
29. Kampinga HH, Garrido C. HSPBs: small proteins with big implications in human disease. *Int J Biochem Cell B* 2012; 44: 1706–10.
30. De Jong WW, Leunissen JA, Voorter CE. Evolution of the α -crystallin/small heat-shock protein family. *Mol Biol Evol* 1993; 10: 103–26.
31. Fuchs M, Poirier DJ, Seguin SJ, Lambert H, Carra S, Charette SJ, Landry J. Identification of the key structural motifs involved in HspB8/HspB6-Bag3 interaction. *Biochem J* 2010; 425: 245–55.
32. Pivovarova AV, Chebotareva NA, Kremneva EV, Lappalainen P, Levitsky DI. Effects of actin-binding proteins on the thermal stability of monomeric actin. *Biochemistry* 2013; 52: 152–60.
33. Batista-Nascimento L, Neef DW, Liu PC, Rodrigues-Pousada C, Thiele DJ. Deciphering human heat shock transcription factor 1 regulation via post-translational modification in yeast. *PLoS One* 2011; 6: e15976.
34. Herbolme G, Kloster-Landsberg M, Folco EG, Col E, Usson Y, Vourc'h C, Delon A, Souchier C. Dynamics of the full length and mutated heat shock factor 1 in human cells. *PLoS One* 2013; 8: e67566.
35. Rhee SG, Woo HA, Kil IS, Bae SH. Peroxiredoxin functions as a peroxidase and a regulator and sensor of local peroxides. *J Biol Chem* 2012; 287: 4403–10.
36. Hall A, Nelson K, Poole LB, Karplus PA. Structure-based insights into the catalytic power and conformational dexterity of peroxiredoxins. *Antioxid Redox Signal* 2011; 15: 795–815.
37. Hall A, Karplus PA, Poole LB. Typical 2-Cys peroxiredoxins – structures, mechanisms and functions. *FEBS J* 2009; 276: 2469–77.
38. Wood ZA, Poole LB, Hantgan RR, Karplus PA. Dimers to doughnuts: redox-sensitive oligomerization of 2-cysteine peroxiredoxins. *Biochemistry* 2002; 41: 5493–504.
39. Woo HA, Kang SW, Kim HK, Yang KS, Chae HZ, Rhee SG. Reversible oxidation of the active site cysteine of peroxiredoxins to cysteine sulfinic acid. Immunoblot detection with antibodies specific for the hyperoxidized cysteine-containing sequence. *J Biol Chem* 2003; 278: 47361–4.
40. Knoop B, Loumaye E, Van Der Eecken V. Evolution of the peroxiredoxins. *Subcell Biochem* 2007; 44: 27–40.
41. Gil-Mohapel J, Brocardo PS, Christie BR. The role of oxidative stress in Huntington's disease: are antioxidants good therapeutic candidates? *Curr Drug Targets* 2014; 15: 454–68.
42. Woo HA, Chae HZ, Hwang SC, Yang KS, Kang SW, Kim K, Rhee SG. Reversing the inactivation of peroxiredoxins caused by cysteine sulfinic acid formation. *Science* 2003; 300: 653–6.
43. Jang HH, Lee KO, Chi YH, Jung BG, Park SK, Park JH, Lee JR, Lee SS, Moon JC, Yun JW, Choi YO, Kim WY, Kang JS, Cheong GW, Yun DJ, Rhee SG, Cho MJ, Lee SY. Two enzymes in one; two yeast peroxiredoxins display oxidative stress-dependent switching from a peroxidase to a molecular chaperone function. *Cell* 2004; 117: 625–35.
44. Moon JC, Hah YS, Kim WY, Jung BG, Jang HH, Lee JR, Kim SY, Lee YM, Jeon MG, Kim CW, Cho MJ, Lee SY. Oxidative stress-dependent structural and functional switching of a human 2-Cys peroxiredoxin isotype II that enhances HeLa cell resistance to H₂O₂-induced cell death. *J Biol Chem* 2005; 280: 28775–84.
45. Hofmann B, Hecht HJ, Flohe L. Peroxiredoxins. *Biol Chem* 2002; 383: 347–64.
46. Wood ZA, Schroder E, Robin Harris J, Poole LB. Structure, mechanism and regulation of peroxiredoxins. *Trends Biochem Sci* 2003; 28: 32–40.
47. Wilkinson B, Gilbert HF. Protein disulfide isomerase. *Biochim Biophys Acta* 2004; 1699: 35–44.
48. Appenzeller-Herzog C, Ellgaard L. The human PDI family: versatility packed into a single fold. *Biochim Biophys Acta* 2008; 1783: 535–48.
49. Wang C, Li W, Ren J, Fang J, Ke H, Gong W, Feng W, Wang C. Structural insights into the redox-regulated dynamic conformations of human protein disulfide isomerase. *Antioxid Redox Signal* 2013; 19: 36–45.
50. Wang C, Chen S, Wang X, Wang L, Wallis AK, Freedman RB, Wang CC. Plasticity of human protein disulfide isomerase: evidence for mobility around the X-linker region and its functional significance. *J Biol Chem* 2010; 285: 26788–97.
51. Klappa P, Ruddock LW, Darby NJ, Freedman RB. The b' domain provides the principal peptide-binding site of protein disulfide isomerase but all domains contribute to binding of misfolded proteins. *EMBO J* 1998; 17: 927–35.

52. Freedman RB, Klappa P, Ruddock LW. Model peptide substrates and ligands in analysis of action of mammalian protein disulfide-isomerase. *Methods Enzymol* 2002; 348: 342–54.
53. Cai H, Wang CC, Tsou CL. Chaperone-like activity of protein disulfide isomerase in the refolding of a protein with no disulfide bonds. *J Biol Chem* 1994; 269: 24550–2.
54. Song JL, Wang CC. Chaperone-like activity of protein disulfide-isomerase in the refolding of rhodanese. *Eur J Biochem* 1995; 231: 312–6.
55. Yao Y, Zhou Y, Wand C. Both the isomerases and chaperone activities of protein disulfide isomerase are required for the reactivation of reduced and denatured acidic phospholipase A2. *EMBO J* 1997; 16: 651–8.
56. Molinari M, Galli C, Piccaluga V, Pieren M, Paganetti P. Sequential assistance of molecular chaperones and transient formation of covalent complexes during protein degradation from the ER. *J Cell Biol* 2002; 158: 247–57.
57. Versteeg HH, Ruf W. Tissue factor coagulant function is enhanced by protein-disulfide isomerase independent of oxidoreductase activity. *J Biol Chem* 2007; 282: 25416–24.
58. Schultz-Norton J, McDonald W, Yates J, Narduli A. Protein disulfide isomerase serves as a molecular chaperone to maintain estrogen receptor alpha structure and function. *Mol Endocrinol* 2006; 20: 1982–95.
59. Van Lith M, Karala AR, Bown D, Gatehouse JA, Ruddock LW, Saunders PT, Benham AM. A developmentally regulated chaperone complex for the endoplasmic reticulum of male haploid germ cells. *Mol Biol Cell* 2007; 18: 2795–804.
60. Tsai B, Rodighiero C, Lencer WI, Rapoport TA. Protein disulfide isomerase acts as a redox-dependent chaperone to unfold cholera toxin. *Cell* 2001; 104: 937–48.
61. Tsai B, Rapoport TA. Unfolded cholera toxin is transferred to the ER membrane and released from protein disulfide isomerase upon oxidation by Ero1. *J Cell Biol* 2002; 159: 207–16.
62. Janiszewski M, Lopes LR, Carmo AO, Pedro MA, Brandes RP, Santos CX, Laurindo FR. Regulation of NAD(P)H oxidase by associated protein disulfide isomerase in vascular smooth muscle cells. *J Biol Chem* 2005; 280: 40813–9.
63. De APaM, Verissimo-Filho S, Guimaraes LL, Silva AC, Takiuti JT, Santos CX, Janiszewski M, Laurindo FR, Lopes LR. Protein disulfide isomerase redox-dependent association with p47(phox): evidence for an organizer role in leukocyte NADPH oxidase activation. *J Leukoc Biol* 2011; 90: 799–810.
64. Lumb RA, Bulleid NJ. Is protein disulfide isomerase a redox-dependent molecular chaperone? *EMBO J* 2002; 21: 6763–70.
65. Wallis AK, Sidhu A, Byrne LJ, Howard MJ, Ruddock LW, Williamson RA, Freedman RB. The ligand-binding b' domain of human protein disulphide-isomerase mediates homodimerization. *Protein Sci* 2009; 18: 2569–77.
66. Byrne LJ, Sidhu A, Wallis AK, Ruddock LW, Freedman RB, Howard MJ, Williamson RA. Mapping of the ligand-binding site on the b' domain of human PDI: interaction with peptide ligands and the x-linker region. *Biochem J* 2009; 423: 209–17.
67. Bastos-Aristizabal S, Kozlov G, Gehring K. Structural insight into the dimerization of human protein disulfide isomerase. *Protein Sci* 2014; 23: 618–26.
68. Yang S, Wang X, Cui L, Ding X, Niu L, Yang F, Wang C, Wang CC, Lou J. Compact conformations of human protein disulfide isomerase. *PLoS One* 2014; 9: e103472.
69. Conway ME, Hustson S. The cytosolic and mitochondrial branched chain amino transferase. In: Rajendram R, Preedy V, Vinood P, editors. *Branched chain amino acids in clinical nutrition*. New York: Springer, 2012: 25–40.
70. Suryawan A, Hawes JW, Harris RA, Shimomura Y, Jenkins AE, Hutson SM. A molecular model of human branched-chain amino acid metabolism. *Am J Clin Nutr* 1998; 68: 72–81.
71. Hull J, Hindy ME, Kehoe PG, Chalmers K, Love S, Conway ME. Distribution of the branched chain aminotransferase proteins in the human brain and their role in glutamate regulation. *J Neurochem* 2012; 123: 997–1009.
72. Sweatt AJ, Wood M, Suryawan A, Wallin R, Willingham MC, Hutson SM. Branched-chain amino acid catabolism: unique segregation of pathway enzymes in organ systems and peripheral nerves. *Am J Physiol Endocrinol Metab* 2004; 286: E64–76.
73. Garcia-Espinosa MA, Wallin R, Hutson SM, Sweatt AJ. Widespread neuronal expression of branched-chain aminotransferase in the CNS: implications for leucine/glutamate metabolism and for signaling by amino acids. *J Neurochem* 2007; 100: 1458–68.
74. Karpeisky MY, Ivanov VI. A molecular mechanism for enzymatic transamination. *Nature* 1966; 210: 493–6.
75. Ichihara A, Koyama E. Transaminase of branched chain amino acids: I. Branched chain amino acids- α -ketoglutarate transaminase. *J Biochem* 1966; 59: 160–9.
76. Conway E, Hutson SM. The cytosolic and mitochondrial branched chain aminotransferase. In: Rajendram R, Preedy V, Petel V, editors. *Branched chain amino acids in clinical nutrition*. New York: Springer, 2015. 25–40.
77. Yennawar NH, Conway ME, Yennawar HP, Farber GK, Hutson SM. Crystal structures of human mitochondrial branched chain aminotransferase reaction intermediates: ketimine and pyridoxamine phosphate forms. *Biochemistry* 2002; 41: 11592–601.
78. Goto M, Miyahara I, Hirotsu K, Conway M, Yennawar N, Islam MM, Hutson SM. Structural determinants for branched-chain aminotransferase isozyme-specific inhibition by the anticonvulsant drug gabapentin. *J Biol Chem* 2005; 280: 37246–56.
79. Conway ME, Yennawar N, Wallin R, Poole LB, Hutson SM. Human mitochondrial branched chain aminotransferase: structural basis for substrate specificity and role of redox active cysteines. *Biochim Biophys Acta* 2003; 1647: 61–5.
80. Conway ME, Coles SJ, Islam MM, Hutson SM. Regulatory control of human cytosolic branched-chain aminotransferase by oxidation and S-glutathionylation and its interactions with redox sensitive neuronal proteins. *Biochemistry* 2008; 47: 5465–79.
81. Coles SJ, Easton P, Sharrod H, Hutson SM, Hancock J, Patel VB, Conway ME. S-nitrosoglutathione inactivation of the mitochondrial and cytosolic BCAT proteins: S-nitrosation and S-thiolation. *Biochemistry* 2009; 48: 645–56.
82. Hutson SM, Poole LB, Coles SJ, Conway ME. Redox regulation and trapping sulphenic acid in the peroxide sensitive human mitochondrial branched chain aminotransferase. *Method Mol Biol* 2009; 476: 135–48.
83. Yennawar NH, Islam MM, Conway M, Wallin R, Hutson SM. Human mitochondrial branched chain aminotransferase isozyme: structural role of the CXXC center in catalysis. *J Biol Chem* 2006; 281: 39660–71.
84. Islam MM, Nautiyal M, Wynn RM, Mobley JA, Chuang DT, Hutson SM. Branched-chain amino acid metabolism: interaction of glutamate dehydrogenase with the mitochondrial branched-chain aminotransferase (BCATm). *J Biol Chem* 2010; 285: 265–76.

85. El Hindy M, Hezwani M, Corry D, Hull J, El Amraoui F, Harris M, Lee C, Forshaw T, Wilson A, Mansbridge A, Hassler M, Patel VB, Kehoe PG, Love S, Conway ME. The branched-chain aminotransferase proteins: novel redox chaperones for protein disulfide isomerase – implications in Alzheimer's disease. *Antioxid Redox Signal* 2014; 20: 2497–513.
86. Zhong H, Yin H. Role of lipid peroxidation derived 4-hydroxynonenal (4-HNE) in cancer: focusing on mitochondria. *Redox Biol* 2015; 4: 193–9.
87. Lloret A, Fuchsberger T, Giraldo E, Vina J. Molecular mechanisms linking amyloid β toxicity and Tau hyperphosphorylation in Alzheimer's disease. *Free Radic Biol Med* 2015; 83: 186–91.
88. Borza LR. A review on the cause-effect relationship between oxidative stress and toxic proteins in the pathogenesis of neurodegenerative diseases. *Rev Med Chir Soc Med Nat Iasi* 2014; 118: 19–27.
89. El Eter E, Al Masri A, Habib S, Al Zamil H, Al Hersi A, Al Hussein F, Al Omran M. Novel links among peroxiredoxins, endothelial dysfunction, and severity of atherosclerosis in type 2 diabetic patients with peripheral atherosclerotic disease. *Cell Stress Chaperon* 2014; 19: 173–81.
90. Fischer R, Maier O. Interrelation of oxidative stress and inflammation in neurodegenerative disease: role of TNF. *Oxid Med Cell Longev* 2015; 2015: 610813.
91. Ranford JC, Henderson B. Chaperonins in disease: mechanisms, models, and treatments. *Mol Pathol* 2002; 55: 209–13.
92. Bakthisaran R, Tangirala R, Rao CM. Small heat shock proteins: role in cellular functions and pathology. *Biochim Biophys Acta* 2015; 1854: 291–319.
93. Magrane J, Smith RC, Walsh K, Querfurth HW. Heat shock protein 70 participates in the neuroprotective response to intracellularly expressed β -amyloid in neurons. *J Neurosci* 2004; 24: 1700–6.
94. Wu Y, Cao Z, Klein WL, Luo Y. Heat shock treatment reduces β amyloid toxicity in vivo by diminishing oligomers. *Neurobiol Aging* 2010; 31: 1055–8.
95. Cumming RC, Dargusch R, Fischer WH, Schubert D. Increase in expression levels and resistance to sulfhydryl oxidation of peroxiredoxin isoforms in amyloid β -resistant nerve cells. *J Biol Chem* 2007; 282: 30523–34.
96. Cimini A, Gentile R, Angelucci F, Benedetti E, Pitari G, Giordano A, Ippoliti R. Neuroprotective effects of Prxl over-expression in an in vitro human Alzheimer's disease model. *J Cell Biochem* 2013; 114: 708–15.
97. Davey GP, Bolanos JP. Peroxiredoxin 5 links mitochondrial redox signalling with calcium dynamics: impact on Parkinson's disease. *J Neurochem* 2013; 125: 332–3.
98. Krapfenbauer K, Yoo BC, Fountoulakis M, Mitrova E, Lubec G. Expression patterns of antioxidant proteins in brains of patients with sporadic Creutzfeldt-Jakob disease. *Electrophoresis* 2002; 23: 2541–7.
99. Basso M, Giraud S, Corpillo D, Bergamasco B, Lopiano L, Fasano M. Proteome analysis of human substantia nigra in Parkinson's disease. *Proteomics* 2004; 4: 3943–52.
100. Kato S, Kato M, Abe Y, Matsumura T, Nishino T, Aoki M, Itoyama Y, Asayama K, Awaya A, Hirano A, Ohama E. Redox system expression in the motor neurons in amyotrophic lateral sclerosis (ALS): immunohistochemical studies on sporadic ALS, superoxide dismutase 1 (SOD1)-mutated familial ALS, and SOD1-mutated ALS animal models. *Acta Neuropathol* 2005; 110: 101–12.
101. Kim HT, Russell RL, Raina AK, Harris PL, Siedlak SL, Zhu X, Petersen RB, Shimohama S, Smith MA, Perry G. Protein disulfide isomerase in Alzheimer disease. *Antioxid Redox Signal* 2000; 2: 485–9.
102. Muchowski PJ. Protein misfolding, amyloid formation, and neurodegeneration: a critical role for molecular chaperones? *Neuron* 2002; 35: 9–12.
103. Tuite MF, Melki R. Protein misfolding and aggregation in ageing and disease: molecular processes and therapeutic perspectives. *Prion* 2007; 1: 116–20.
104. Honjo Y, Horibe T, Torisawa A, Ito H, Nakanishi A, Mori H, Komiya T, Takahashi R, Kawakami K. Protein disulfide isomerase P5-immunopositive inclusions in patients with Alzheimer's disease. *J Alzheimers Dis* 2014; 38: 601–9.
105. Jin J, Li GJ, Davis J, Zhu D, Wang Y, Pan C, Zhang J. Identification of novel proteins associated with both alpha-synuclein and DJ-1. *Mol Cell Proteomics* 2007; 6: 845–59.
106. Honjo Y, Kaneko S, Ito H, Horibe T, Nagashima M, Nakamura M, Fujita K, Takahashi R, Kusaka H, Kawakami K. Protein disulfide isomerase-immunopositive inclusions in patients with amyotrophic lateral sclerosis. *Amyotroph Lateral Scler* 2011; 12: 444–50.
107. Honjo Y, Ito H, Horibe T, Shimada H, Nakanishi A, Mori H, Takahashi R, Kawakami K. Derlin-1-immunopositive inclusions in patients with Alzheimer's disease. *Neuroreport* 2012; 23: 611–5.
108. Farg MA, Soo KY, Walker AK, Pham H, Orian J, Horne MK, Warraich ST, Williams KL, Blair IP, Atkin JD. Mutant FUS induces endoplasmic reticulum stress in amyotrophic lateral sclerosis and interacts with protein disulfide-isomerase. *Neurobiol Aging* 2012; 33: 2855–68.
109. Uehara T, Nakamura T, Yao D, Shi ZQ, Gu Z, Ma Y, Masliah E, Nomura Y, Lipton SA. S-nitrosylated protein-disulphide isomerase links protein misfolding to neurodegeneration. *Nature* 2006; 441: 513–7.
110. Uehara T. Accumulation of misfolded protein through nitrosative stress linked to neurodegenerative disorders. *Antioxid Redox Signal* 2007; 9: 597–601.
111. Calabrese V, Sultana R, Scapagnini G, Guagliano E, Sapienza M, Bella R, Kanski J, Pennisi G, Mancuso C, Stella AM, Butterfield DA. Nitrosative stress, cellular stress response, and thiol homeostasis in patients with Alzheimer's disease. *Antioxid Redox Signal* 2006; 8: 1975–86.
112. Andreu CI, Woehlbier U, Torres M, Hetz C. Protein disulfide isomerases in neurodegeneration: from disease mechanisms to biomedical applications. *FEBS Lett* 2012; 586: 2826–34.
113. Hoffstrom BG, Kaplan A, Letso R, Schmid RS, Turmel GJ, Lo DC, Stockwell BR. Inhibitors of protein disulfide isomerase suppress apoptosis induced by misfolded proteins. *Nat Chem Biol* 2010; 6: 900–6.
114. Atkin JD, Farg MA, Turner BJ, Tomas D, Lysaght JA, Nunan J, Rembach A, Nagley P, Beart PM, Cheema SS, Horne MK. Induction of the unfolded protein response in familial amyotrophic lateral sclerosis and association of protein-disulfide isomerase with superoxide dismutase 1. *J Biol Chem* 2006; 281: 30152–65.
115. Massignan T, Casoni F, Basso M, Stefanazzi P, Biasini E, Tortarolo M, Salmona M, Gianazza E, Bendotti C, Bonetto V. Proteomic analysis of spinal cord of presymptomatic amyotrophic lateral sclerosis G93A SOD1 mouse. *Biochem Biophys Res Commun* 2007; 353: 719–25.

116. Nakamura T, Lipton SA. Cell death: protein misfolding and neurodegenerative diseases. *Apoptosis* 2009; 14: 455–68.
117. Xu B, Jin CH, Deng Y, Liu W, Yang TY, Feng S, Xu ZF. Alpha-synuclein oligomerization in manganese-induced nerve cell injury in brain slices: a role of NO-mediated S-nitrosylation of protein disulfide isomerase. *Mol Neurobiol* 2014; 50: 1098–110.
118. Walker AK, Farg MA, Bye CR, Mclean CA, Horne MK, Atkin JD. Protein disulphide isomerase protects against protein aggregation and is S-nitrosylated in amyotrophic lateral sclerosis. *Brain* 2010; 133: 105–16.
119. Jeon GS, Nakamura T, Lee JS, Choi WJ, Ahn SW, Lee KW, Sung JJ, Lipton SA. Potential effect of S-nitrosylated protein disulfide isomerase on mutant SOD1 aggregation and neuronal cell death in amyotrophic lateral sclerosis. *Mol Neurobiol* 2014; 49: 796–807.
120. Hull J, Patel V, El Hindy M, Lee C, Odeleye E, Hezwani M, Love S, Kehoe P, Chalmers K, Conway M. Regional increase in the expression of the BCAT proteins in Alzheimer's disease brain: implications in glutamate toxicity. *J Alzheimers Dis* 2015; 45: 891–905.