FORUM REVIEW ARTICLE

Introducing Thioredoxin-Related Transmembrane Proteins: Emerging Roles of Human TMX and Clinical Implications

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Abstract

Significance: The presence of a large number of thioredoxin superfamily members suggests a complex mechanism of redox-based regulation in mammalian cells. However, whether these members are functionally redundant or play separate and distinct roles in each cellular compartment remains to be elucidated.

Recent Advances: In the mammalian endoplasmic reticulum (ER), ∼20 thioredoxin-like proteins have been identified. Most ER oxidoreductases are soluble proteins located in the luminal compartment, whereas a small family of five thioredoxin-related transmembrane proteins (TMX) also reside in the ER membrane and play crucial roles with specialized functions.

Critical Issues: In addition to the predicted function of ER protein quality control, several independent studies have suggested the diverse roles of TMX family proteins in the regulation of cellular processes, including calcium homeostasis, bioenergetics, and thiol-disulfide exchange in the extracellular space. Moreover, recent studies have provided evidence of their involvement in the pathogenesis of various diseases.

Future Directions: Extensive research is required to unravel the physiological roles of TMX family proteins. Given that membrane-associated proteins are prime targets for drug discovery in a variety of human diseases, expanding our knowledge on the mechanistic details of TMX action on the cell membrane will provide the molecular basis for developing novel diagnostic and therapeutic approaches as a potent molecular target in a clinical setting. *Antioxid. Redox Signal.* 36, 984–1000.

Keywords: disulfide, endoplasmic reticulum, oxidoreductase, redox, thioredoxin, TMX

Introduction

THIOL-DISULFIDE OXIDOREDUCTASES catalyze reduction/oxidation (redox) reactions, leading to the cleavage, formation, or isomerization of disulfide bonds between cysteine residues in substrate proteins. The formation or rearrangement of disulfide bridges is critical for the proper folding and assembly of proteins (7, 9, 41). Further, reversible thiol-disulfide exchange involves a large conformational change, and alteration of the protein redox state can positively or negatively regulate its function, thereby influencing cellular behavior (20, 37, 40, 45).

In mammalian cells, each cellular compartment contains a variety of thiol-disulfide oxidoreductases belonging to the thioredoxin superfamily (6, 31). Although the family members share a characteristic functional thioredoxin-related domain, they differ greatly in size and number of Cys-X-X-Cys (CXXC) active site motifs. The diversity among thioredoxin family members suggests a complex mechanism of redox-based regulation in mammalian cells. However, whether these members share the substrate proteins or exhibit distinctly specific roles remains elusive. Protein disulfide isomerase (PDI) belongs to the thioredoxin superfamily, and more than 20 PDI family proteins have been identified in mammalian cells (23, 64). Most PDIs are soluble proteins located in the luminal compartment of the endoplasmic reticulum (ER), whereas some are integral membrane proteins classified as members of the thioredoxin-related transmembrane protein (TMX) subfamily (27). In this review, we focus on the TMX protein family consisting of five membrane-anchored

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oxidoreductases (TMX1–5) and discuss the current understanding of their physiological functions, experimental methodology to investigate enzyme–substrate interactions, and implications in human diseases.

TMX1 (TXNDC1, PDIA11)

TMX1 is the founding member of the TMX family. Human *TMX1* was identified during a gene-trap screening for novel genes regulated by transforming growth factor-β signaling (1). This gene is located on chromosome 14 and encodes a single-pass type I integral membrane protein with 280 amino acid residues (50) (Fig. 1A). TMX1 has a cleavable N-terminal signal peptide, required for entry into the secretory pathway, yielding a mature protein with a molecular mass of ~ 30 kDa. TMX1 predominantly resides in the ER, though it lacks a classical di-lysine (KKXX)-type ER localization signal. An arginine-based motif (RQR) located near the C-terminus of TMX1 is postulated to be responsible for retrieving the protein to the ER, as shown for TMX4 (75). Topological studies have demonstrated that the catalytic thioredoxin-like domain of TMX1 is oriented toward the luminal side of the ER (54). This domain contains a unique CPAC active site motif that shuttles between the oxidized
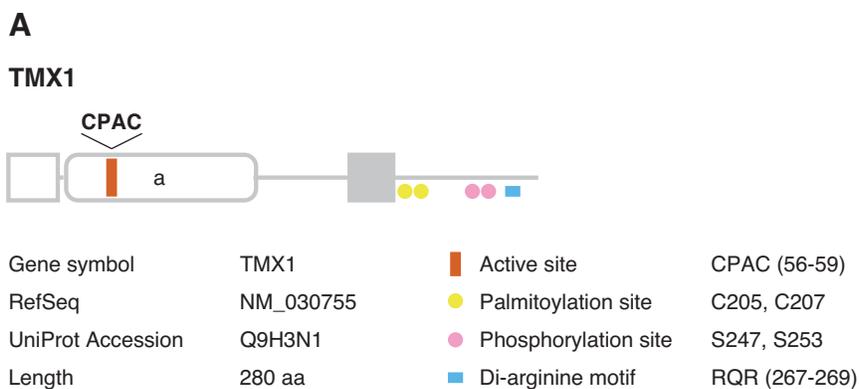

FIG. 1. Sequence features and expression profiles of human TMX1. (A) Sequence and structural characteristics of TMX1. The N-terminal signal sequence (*white rectangle*), the catalytically active thioredoxin-like domain (a-type; *rounded rectangle*), and the transmembrane domain (*grey rectangle*) are shown. The positions of the CPAC active site sequence, two palmitoylation sites, two phosphorylation sites, and the di-arginine motif are indicated. (B) Tissue distribution of *TMX1* gene. RNA expression data are obtained from HPA (https://www.proteinatlas.org) (93). Consensus NX levels for 55 tissues and 6 blood cell types are generated by combining the 3 transcriptomics datasets: RNA-seq data from HPA, RNA-seq data from GTEx project, and CAGE data from FANTOM5 project (47). CAGE, Cap Analysis of Gene Expression; GTEx, Genotype-Tissue Expression; HPA, Human Protein Atlas; NX, normalized expression; TMX, thioredoxin-related transmembrane protein. The data are available from https://www.proteinatlas.org/ENSG00000139921-TMX1/tissue.
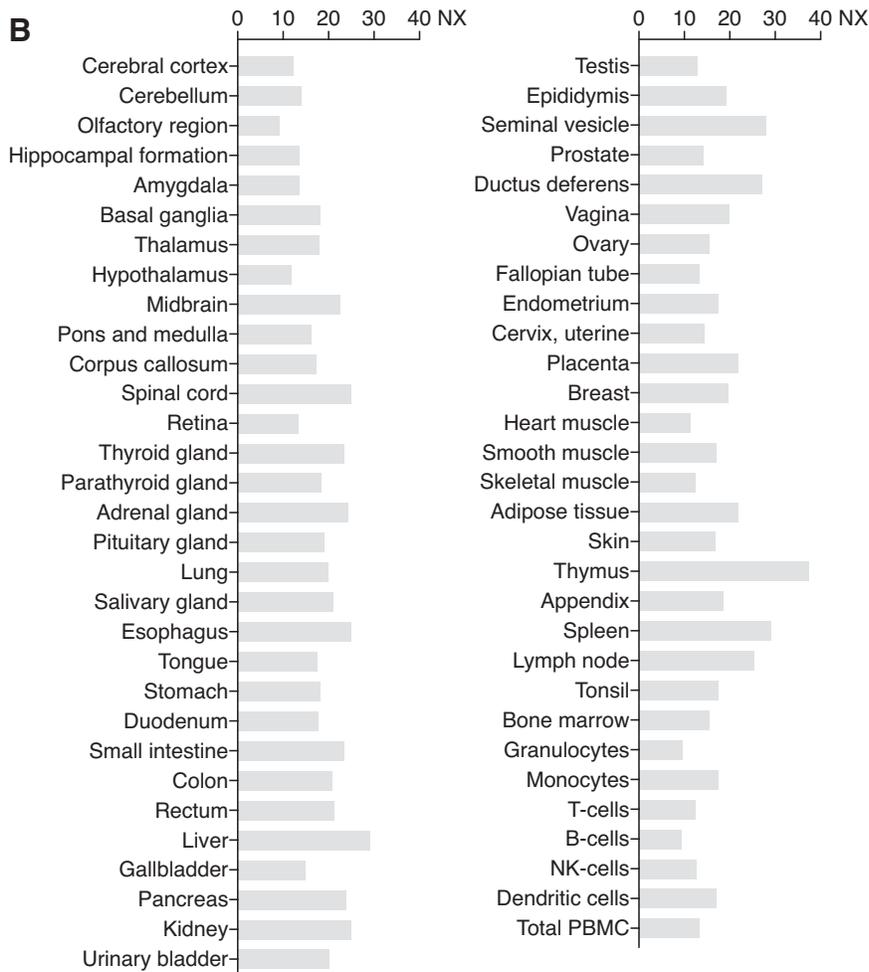
(disulfide) and reduced (dithiol) states in response to external stimuli. The short cytosolic C-terminal tail of TMX1 possesses two phosphorylation sites (Ser247 and Ser253) that have been verified by using global phosphopeptide sequencing (66). However, the biological significance of TMX1 phosphorylation remains unclear.

TMX1 messenger RNA (mRNA) expression is ubiquitous in human tissues (50) (Fig. 1B). Tmx1 knockout mice are born and develop normally (Mouse Genome Informatics, MGI [www.informatics.jax.org], ID: 1919986). However, Tmx1 deficiency causes increased sensitivity to toxic agents, such as lipopolysaccharide or thioacetamide, in a mouse model of acute liver injury associated with oxidative stress (52).

Redox properties of TMX1

Environmental and pathological perturbations to ER function can cause accumulation of malfolded or denatured proteins in the ER, thereby activating the stress response pathway termed unfolded protein response (UPR) (58, 74). TMX1 expression is not induced by UPR (53). Disruption of ER proteostasis can alter the redox properties of ER-resident oxidoreductases. The active site cysteines in TMX1 are mainly reduced in the steady state, although a small protein fraction exists in the oxidized form (53). Under conditions of ER stress elicited by protein overload, TMX1 is readily converted to the oxidized state (51). This redox transition precedes the upregulation of ER stress markers downstream of the UPR pathway. Reactive oxygen species (ROS) produced under stress conditions do not seem to directly cause TMX1 oxidation. Considering that TMX1 preferentially binds to incompletely folded membrane proteins (26, 53), ER stress-induced TMX1 oxidation may occur as a consequence of thiol-disulfide exchange with accumulated substrates. In this scenario, TMX1 may catalyze the resolution of disulfide bonds formed within aberrantly folded proteins to cope with protein overload. Supporting this, oxidized TMX1 reverted to the basal reduced state after removal of ER stressors from the culture medium (51). Thus, protein accumulation in the ER disturbs the cellular redox balance, leading to the reversible oxidation of TMX1. These findings also suggest that cells are equipped with a reductive pathway to help restore ER homeostasis during post-stress recovery. However, the cellular component responsible for maintaining TMX1 in the reduced state has yet to be identified. Reduced glutathione has been proposed to provide reducing equivalents for the reduction of disulfides formed between the active site cysteines. Alternatively, an enzyme-catalyzed pathway may regulate the TMX1 redox state, enabling continuous rounds of thiol/disulfide exchange with substrate proteins.

Interaction of TMX1 with membrane-associated proteins

Several lines of evidence suggest that TMX1 favors membrane-bound substrates. The search for endogenous substrates for TMX1 has identified a series of cysteine-containing membrane proteins (10, 53, 69). TMX1 preferentially interacts with model proteins with transmembrane domains but not with soluble variants (26, 69), confirming the specificity of TMX1 for association with membrane-bound substrates.

The selective recognition of membrane proteins by TMX1 is partially mediated through its association with calnexin, a transmembrane chaperone in the ER (12, 76). This lectin-like molecular chaperone binds to N-glycosylated proteins and assists in their folding. Despite the lack of N-glycans in TMX1, the transmembrane domain is responsible for the binding of TMX1 to calnexin (53). The catalytic luminal portion of TMX1 does not contain a peptide-binding domain, such as the b-type thioredoxin-like domain found in PDI (30, 39), suggesting that TMX1 requires calnexin as a cofactor to recruit client proteins on the ER membrane. In fact, castanospermine, which blocks the glycan-dependent interactions between glycoproteins and calnexin, suppresses the disulfide-linked TMX1–substrate interaction (53) and destabilizes the TMX1–calnexin complex (69). These results suggest that TMX1 and calnexin form a functional complex that cooperatively acts on their clients during protein folding in the ER.

The role of thioredoxin-like ER oxidoreductases has been proposed in the reactivation of vitamin K epoxide reductase (VKOR) (10, 81, 87, 96). VKOR is an ER-resident transmembrane protein that catalyzes the conversion of vitamin K epoxide to vitamin K hydroquinone, an essential cofactor for activating blood coagulation factors (65). On catalyzing the reduction of vitamin K epoxide, VKOR is converted to the oxidized state and needs to be reverted to the reduced form through the vitamin K recycling system. TMX1 has been identified as a candidate redox partner that delivers reducing equivalents to VKOR (81). Considering that the proline residue in the CPAC motif in the TMX1 active site is required for stable interaction with VKOR, this residue may be a critical factor that determines substrate specificity and preference of TMX1 in the redox reaction. VKOR also interacts with TMX4 to a lesser extent, suggesting its preference for membrane-bound oxidoreductases. In contrast, VKOR does not form mixed disulfides with soluble ER oxidoreductases, with the exception of weak binding to ERp18 (81). No evidence has been reported on the involvement of TMX1 in thromboembolic events.

Disulfide reduction by TMX1

The active site cysteines of TMX1 are predominantly maintained in a reduced state, possibly reflecting the physiological role of TMX1 as a reductase. TMX1 exhibits reductase activity in vitro and cleaves interchain disulfide bonds in insulin (50). Moreover, it may putatively catalyze disulfide reduction; TMX1 is involved in the reductive activation of cytotoxins, such as ricin and abrin, belonging to the type 2 ribosome-inactivating protein (RIP) family (68). Type 2 RIPs are composed of two polypeptide chains linked by disulfide bonds (67). These proteins are incorporated into cells by endocytosis and translocated to the ER, where the interchain disulfide bond is cleaved to release a toxic A chain. On reduction in the ER, the catalytic A chain translocates to the cytosol and exerts cytotoxic effects by targeting eukaryotic ribosomes. In previous studies, PDI-catalyzed disulfide reduction has been implicated in the activation mechanism of these toxins (5, 85). TMX1 can efficiently reduce type 2 RIPs in the presence of reduced glutathione, and the cytotoxicity of the toxins was enhanced in TMX1-overexpressing cells. Conversely, TMX1 knockdown confers protection against type 2 RIP-induced cytotoxicity. This effect of TMX1 is specific for type 2 RIPs, and it does not increase the susceptibility of cells to other toxins with

reduction-independent activities. These results represent general roles of TMX1 in the ER-to-cytosol retro-translocation of client proteins, which may be relevant for the ER-associated degradation pathway (26, 56).

Regulation of calcium homeostasis by TMX1

The pivotal effects of ER–mitochondria interplay and crosstalk have been implicated in various cellular functions, such as calcium homeostasis, lipid metabolism, autophagy, and inflammation (18). TMX1 is enriched on the mitochondria-associated membranes (MAM), specialized subdomains of the ER. Targeting TMX1 to these mitochondria–ER contact sites requires palmitoylation of two cytosolic cysteines (Cys205 and Cys207) adjacent to the transmembrane region of TMX1 (49). Palmitoylation also serves as an MAM-targeting signal for calnexin. The recruitment of palmitoylated TMX1 to MAM allows it to interact with sarco-ER Ca^{2+} transport ATPase (SERCA) 2b. SERCA2b facilitates Ca^{2+} uptake from the cytosol to the ER, thereby regulating cellular calcium flux. Interestingly, TMX1 overexpression decreases the ER Ca^{2+} content, whereas lack of TMX1 expression leads to increased Ca^{2+} import into the ER, indicating that TMX1 is a negative regulator of SERCA2b (71). TMX1 is targeted to the MAM more efficiently under oxidizing conditions, resulting in an enhanced association with SERCA2b, whereas reducing conditions produce the opposite effects. Further, an intact CPAC active site in TMX1 is essential for binding to SERCA2b; a catalytically inactive TMX1 in which two active site cysteines are mutated fails to interact with SERCA2b. Thus, TMX1 regulates Ca^{2+} flux through its interaction with SERCA2b in a redox-dependent fashion. However, the precise mechanism of TMX1 action in the control of cellular Ca^{2+} flux remains to be elucidated.

Reduced TMX1 expression indirectly impacts ER–mitochondrial Ca^{2+} flux by activating SERCA2b. Increased ER Ca^{2+} content in *TMX1*-deficient cells impairs Ca^{2+} transfer to the mitochondria. This can be attributed to the reduced ER–mitochondria contacts observed in cells with low TMX1 expression (71). Nevertheless, it remains unclear as to how TMX1 controls the physical association between these two organelles. Although speculative, TMX1 may be linked to the ER–mitochondria tethering complex, where it could catalyze redox-dependent conformational changes in tethering molecules. Alternatively, loss of tight ER–mitochondria contacts may be a result of a compensatory response to protect cells from mitochondrial Ca^{2+} overload, which triggers apoptosis (73).

From the perspective of mitochondrial bioenergetics, TMX1 deficiency reduces mitochondrial respiration, leading to a metabolic shift toward glycolysis. This may contribute to metabolic reprogramming (the Warburg effect) that occurs in cancer cells (94).

TMX2 (TXNDC14, PDIA12)

The complementary DNA (cDNA) clone of *TMX2* was isolated from a human fetal cDNA library (55). Human *TMX2* is located on chromosome 11. This gene encodes a 296 amino acid protein with an N-terminal signal sequence (Fig. 2A). The C-terminal half of the protein contains a single thioredoxin-like domain with an atypical SNDC sequence. A topological study of TMX2 predicted it to be a multi-spanning membrane protein with both the N- and C-termini on the cytosolic side (63). Thus, in contrast to other TMX family members, the thioredoxin-like domain of TMX2 is oriented toward the cytosol. The long C-terminal tail of TMX2 possesses a di-lysine ER retention signal (KKDK) (36).

TMX2 mRNA is ubiquitously expressed in human tissues (55) (Fig. 2B). *Tmx2* deficiency in mice results in embryonic lethality before implantation (MGI ID: 1914208), implying that TMX2 is essential in the early developmental stage.

Functional properties of TMX2

Endogenous TMX2 is predominantly localized in the ER, but a significant fraction of the protein is distributed in the outer membrane of the nuclear envelope (63). In a co-immunoprecipitation assay using TMX2 as bait, several nuclear membrane-associated proteins, including importin-β, were identified as candidate TMX2-interacting partners (63). Importin-β is an adaptor protein that mediates the nuclear transport of cargo molecules *via* the nuclear localization signal (91). TMX2 itself is not targeted for nuclear import through its interaction with importin-β. The nucleocytoplasmic transport of an importin–cargo complex is controlled by the Ras-related nuclear protein (Ran) GTPase cycle. It has been suggested that TMX2 regulates the compartmentalization of GTP- and GDP-bound Ran, generating a Ran gradient across the nuclear envelope. In support of this, small interfering RNA (siRNA)-mediated knockdown of *TMX2* decreases the nuclear import of a model cargo protein expressed in cultured cells (63). Ran possesses a redox-sensitive cysteine (Cys112) (90), and the oxidized state of Ran can affect its subcellular distribution. TMX2 may control redox properties of Ran, thereby contributing to the formation of the Ran gradient that is essential for nucleocytoplasmic protein transport.

Notably, TMX2 is also targeted to MAM, where it can interact with calnexin and calcium pump SERCA2 (49, 95). It has been suggested that the interaction of TMX2 with MAM-associated proteins is crucial for regulating neuronal proliferation and migration (24, 42), which is discussed later in this article.

TMX3 (TXNDC10, PDIA13)

TMX3 was identified in a database search with a query sequence corresponding to the consensus thioredoxin-like domain (32). Human *TMX3* is localized to chromosome 18 and encodes a type-I transmembrane protein comprising 454 amino acid residues with a predicted N-terminal signal sequence (Fig. 3A). *TMX3* transcripts are found in a great variety of tissues (32) (Fig. 3B). TMX3 has two consensus sites for N-glycosylation in the luminal N-terminal portion of the protein; the presence of N-glycans was experimentally verified by sensitivity to endoglycosidase H treatment (32). TMX3 contains a classical KKXX-type consensus motif (KKKD) at the C-terminus for ER retention and, when exogenously expressed in cultured cells, TMX3 predominantly localizes to the ER. The luminal region is composed of one redox-active (a-type) and two non-catalytic (b- and b'-type) thioredoxin domains (33). The a-type domain contains a CGHC motif, a canonical active site sequence shared by most PDI family members in the ER lumen. Although the active site cysteines in TMX3 are predominantly reduced,

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TMX2

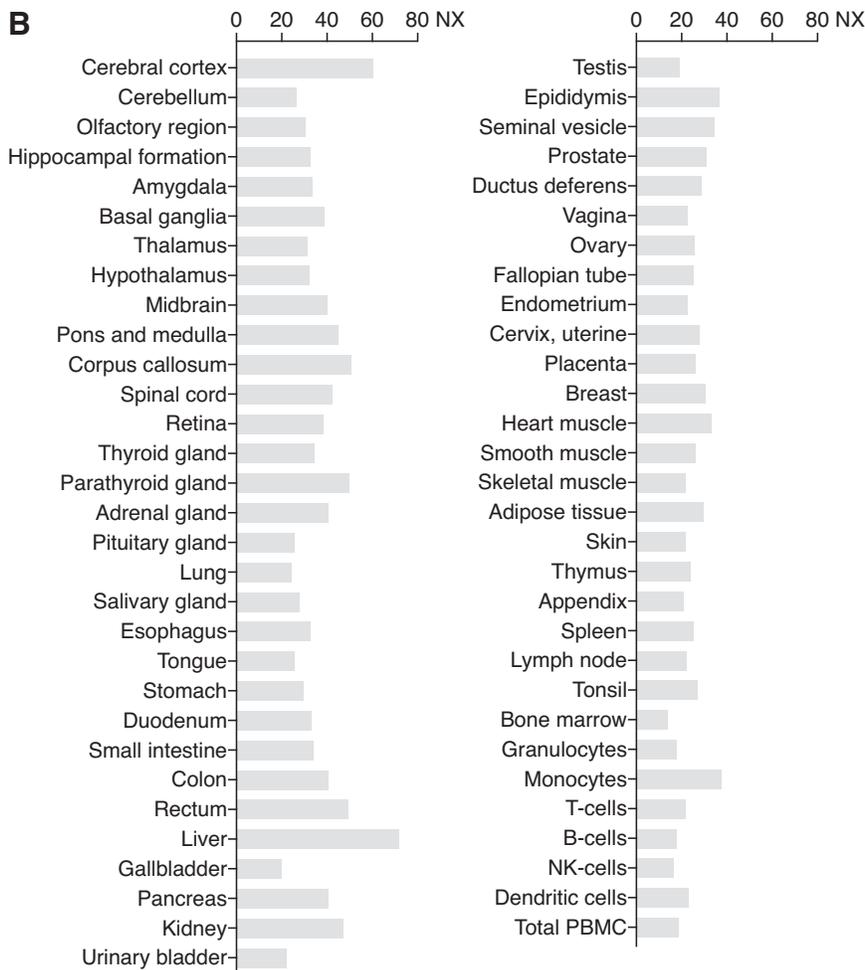|			
Gene symbol	TMX2	▌ Active site	SNDC (167-170)
RefSeq	NM_015959	▌ Di-lysine motif	KKDK (293-296)
UniProt Accession	Q9Y320		
Length	296 aa		

B

FIG. 2. **Sequence features and expression profiles of human TMX2.** (A) Sequence and structural characteristics of TMX2. The N-terminal signal sequence (*white rectangle*), the predicted transmembrane regions (*gray rectangle*), and the a-type thioredoxin-like domain (*rounded rectangle*) are shown. The positions of the SNDC active site sequence and the di-lysine motif are indicated. (B) Tissue distribution of *TMX2* gene. The RNA expression data are available from https://www.proteinatlas.org/EN SG00000213593-TMX2/tissue.

a significant amount of the oxidized protein is present in living cells (32). Considering the structural similarity with the luminal ER oxidoreductases such as PDI and its closest homolog ERp57 (31), the enzymatically inactive b-type domains of TMX3 may be involved in substrate binding and co-factor recruitment (39). The b-type domains are also suggested to mediate structural stabilization of the redox-active a-type domain for efficient catalytic activity (33).

Functional properties of TMX3

The role for TMX3 as a foldase acting on membrane proteins has been demonstrated; TMX3 has been identified as a key cofactor for functional expression of insect nicotinic acetylcholine receptors (AChRs) (35). Co-expression of TMX3 can facilitate proper folding and assembly of the receptor subunits in the *Xenopus* oocyte system, resulting in the robust expression of functional AChRs. AChRs belong to the cysteine-loop ligand-gated ion channel superfamily that is characterized by a highly conserved cysteine loop motif consisting of two disulfide bond-forming cysteines (25, 61). Thus, TMX3 may catalyze the formation of disulfide bonds that are critical for the maturation of heteromeric AChRs.

TMX4 (TXNDC13, PDIA14)

Human *TMX4* was identified in a database search for proteins with thioredoxin-like catalytic domains (75, 89).
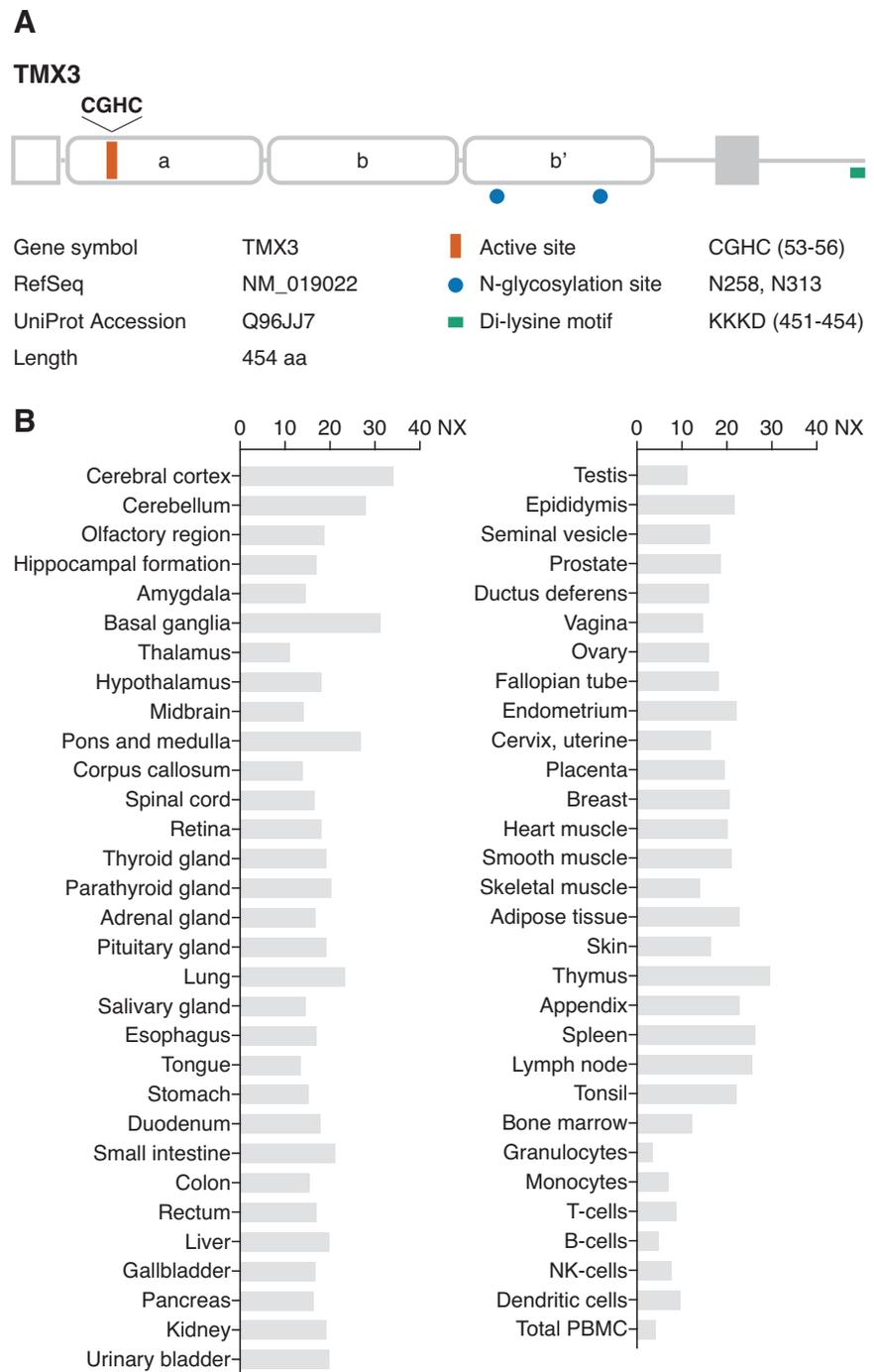
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TMX3

FIG. 3. Sequence features and expression profiles of human TMX3. (**A**) Sequence and structural characteristics of TMX3. The catalytically active thioredoxin-like domain (a-type) and the two noncatalytic b-type domains (b and b′) are represented by *rounded rectangles*. The N-terminal signal sequence (*white rectangle*) and the transmembrane domain (*gray rectangle*) are shown. The positions of the CGHC active site sequence, two consensus sequences for N-glycosylation, and the di-lysine motif are indicated. (**B**) Tissue distribution of *TMX3* gene. The RNA expression data are available from https://www.proteinatlas.org/ENSG00000166479-TMX3/tissue.

The gene is located on chromosome 20 and encodes a 349 amino acid protein with a cleavable signal sequence at the N-terminus and a single membrane-spanning region (Fig. 4A). *TMX4* mRNA is ubiquitously expressed in human tissues (75, 89) (Fig. 4B). The membrane topology of TMX4 was established by determining sensitivity to proteinase treatment, revealing it to be a type I transmembrane protein with an N-terminal luminal region containing a thioredoxin-like catalytic domain (75, 89). TMX4 has a consensus N-glycosylation site in the luminal region, and the protein is sensitive to endoglycosidase H. TMX4 contains two phosphorylation sites (Sr251 and Ser259) within the C-terminal cytosolic tail (66). Exogenously expressed TMX4 is localized to the ER membrane, despite lacking a classical di-lysine motif that is required for ER localization. TMX4 possesses an RQR sequence that is located close to its C-terminus, which is conserved among TMX4 orthologs in different species. This di-arginine motif may be involved in the ER targeting of TMX4 (75).

Functional properties of TMX4

The thioredoxin-like domain of TMX4 has a non-canonical CPSC active site sequence and shows the highest similarity with TMX1 among the five family members (75). The presence of a proline residue at the second position of

A

TMX4

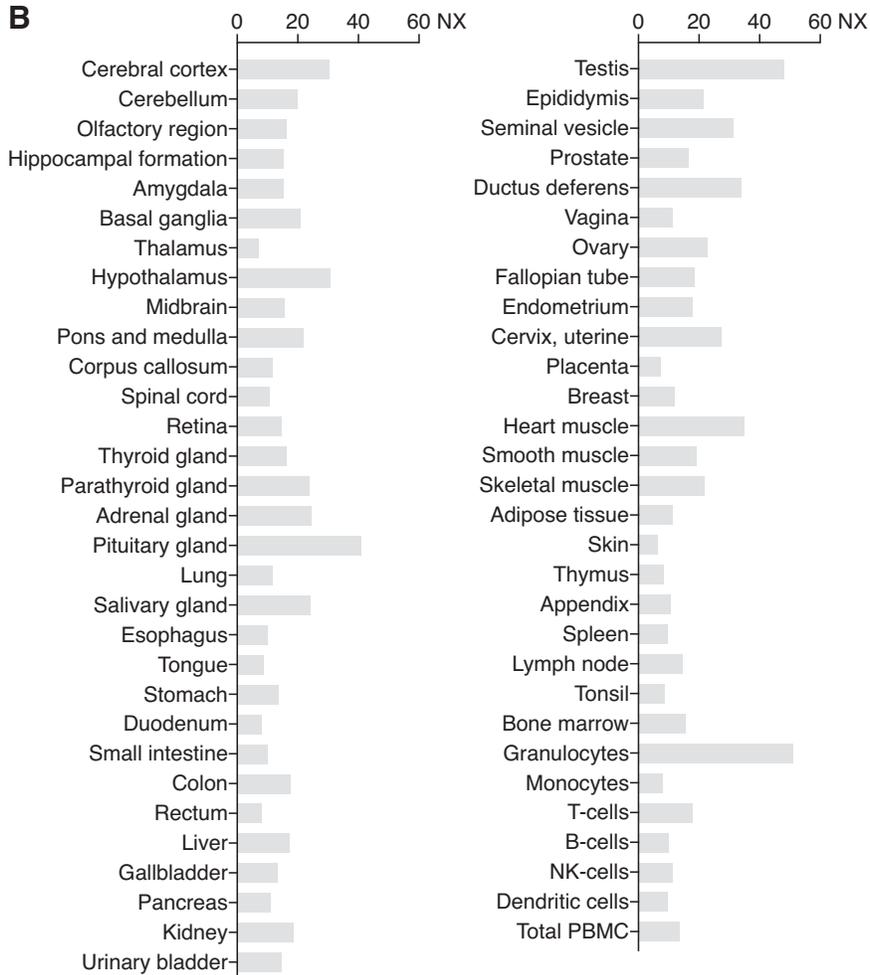|				
Gene symbol	TMX4	▮ Active site	CPSC (64-67)	
RefSeq	NM_021156	● N-glycosylation site	N46	
UniProt Accession	Q9H1E5	● Phosphorylation site	S251, S259	
Length	349 aa	■ Di-arginine motif	RQR (338-340)	

B

FIG. 4. Sequence features and expression profiles of human TMX4. (A) Sequence and structural characteristics of TMX4. The N-terminal signal sequence (*white rectangle*), the catalytically active thioredoxin-like domain (a-type; *rounded rectangle*), and the transmembrane domain (*gray rectangle*) are shown. The positions of the CPSC active site sequence, the N-glycosylation site, two phosphorylation sites, and the di-arginine motif are indicated. (B) Tissue distribution of *TMX4* gene. The RNA expression data are available from https://www.proteinatlas.org/ENSG00000125827-TMX4/tissue.

the CXXC active site motif is a unique feature shared by TMX1 and TMX4. Both TMX1 and TMX4 form a mixed-disulfide intermediate with VKOR (81). The fact that VKOR exhibits a preference for these membrane-anchored thioredoxin family members suggests that the thiol-mediated ER quality control mechanisms are differentially regulated between luminal soluble proteins and membrane-associated molecules.

Phylogenetic analysis shows that TMX4 is a close paralog of TMX1 (Fig. 5A). However, the sequence similarities between TMX1 and TMX4 are confined to their thioredoxin-like domains, and TMX4 has some structural features that are different from TMX1, such as N-glycosylation and a longer cytoplasmic tail. In the steady state, TMX1 exists mainly in the reduced form (53), whereas TMX4 is primarily oxidized (75, 89). These properties suggest a distinct role for TMX4 in redox regulation on cell membranes. Moreover, molecular dynamics simulations in a comparative *in silico* analysis revealed that sequence-dependent structural and dynamical features of TMX4 are distinguishable from TMX1 and other ER oxidoreductases (87). Focusing on the CXXC active site motif, the folding state and the geometry (*i.e.*, a distance between the cysteine residues and a dihedral angle) of the motif are predicted to be noticeably different among the

A

	Active site	TRX domain
PDI (a)	CGHC	25-131
PDI (a')	CGHC	368-472
TMX3	CGHC	26-128
TMX2	SNDC	137-142
TRX	CGPC	2-103
TMX1	CPAC	30-129
TMX4	CPSC	38-137
TMX5	CRFS	191-293
ERp44	CRFS	30-138

B

FIG. 5. Phylogenetic relationship of the human TMX family. (A) Phylogenetic tree of selected thioredoxin superfamily members. An aligned set of thioredoxin domain sequences was subjected to the phylogenetic analysis. The active site motifs and the positions of the catalytic thioredoxin domain based on the Pfam database (57) are shown. Multiple sequence alignment was performed by MEGA X (43, 86) using the ClustalW algorithm, and the phylogenetic tree was constructed by the neighbor-joining method. **(B)** Graphical representation of amino acid conservation in the catalytic thioredoxin-like domains. With the same set of aligned sequences used in **(A)**, the sequence logo (79) was generated by using WebLogo 3 (http://weblogo.threeplusone.com) (15). Amino acids are colored according to their chemical properties: polar (*green*), neutral (*purple*), basic (*blue*), acidic (*red*), and hydrophobic (*black*). The positions of the active site motif (*shaded*) and the conserved proline residue (*asterisk*) corresponding to cis-Pro151 of *Escherichia coli* DsbA are shown. TRX, thioredoxin.
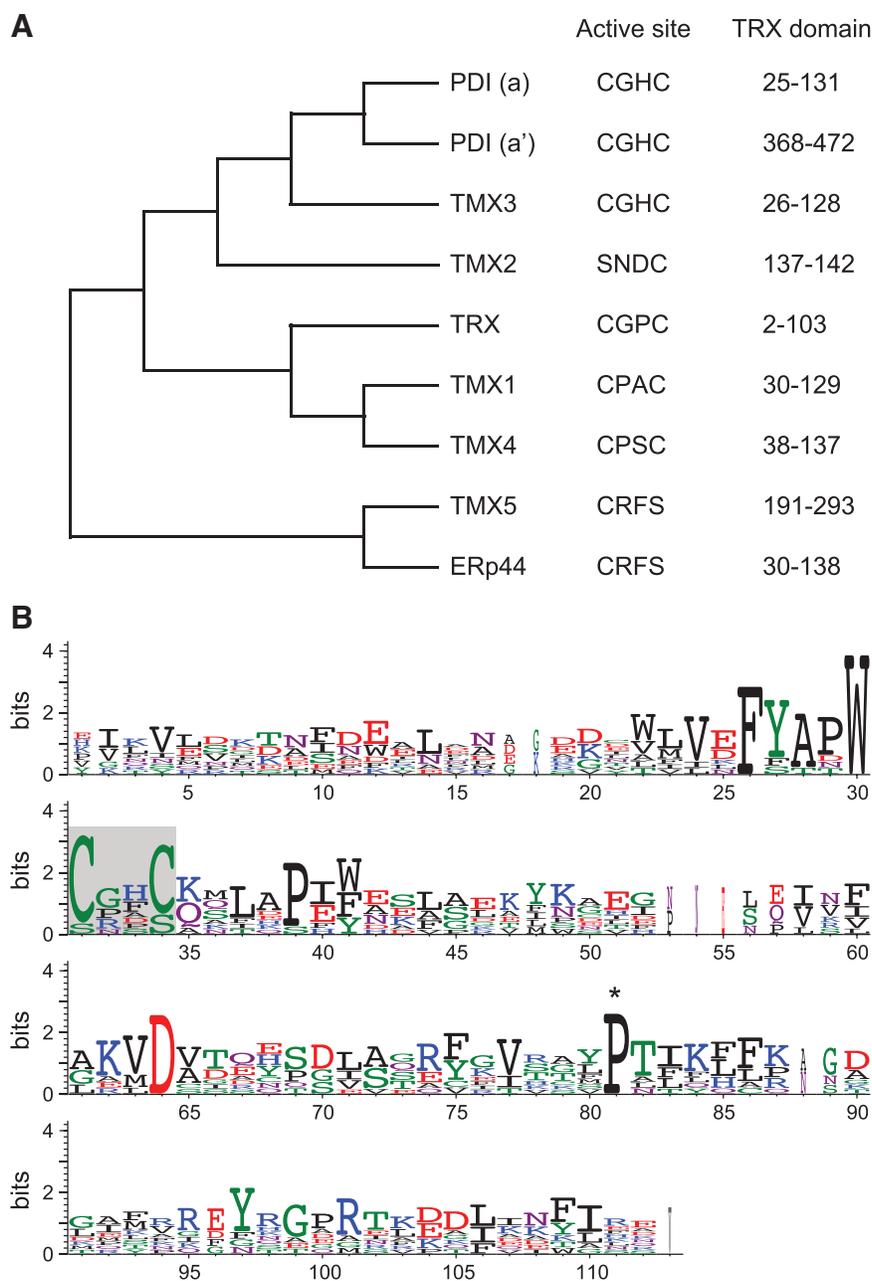
oxidoreductases of the thioredoxin family. The CPAC motif of TMX1 constitutes a part of the well-folded α-helix, with the thiol groups in a restrained cis-configuration. In TMX4, the CPSC active site is located on a coiled linker connecting the β-strand and the α-helix. The coiled structure allows a highly divergent configuration of the TMX4 CPSC motif, and the thiol groups can adopt varying orientations from the synperiplanar to antiperiplanar conformation. These conformational differences may influence their specificity in target recognition during the thiol-disulfide reactions.

Recently, TMX4 was shown to localize on the inner membrane of the nuclear envelope in mesenchyme-derived cells (13). Although there are no reports on the physiological function of TMX4 on the nuclear membrane, this suggests that TMX4 may participate in redox-based regulation of the nuclear envelope structure.

TMX5 (TXNDC15)

Human *TMX5* (*TXNDC15*) was cloned during a large-scale screening for novel secreted and transmembrane proteins (14). The gene is localized to chromosome 5 and encodes a putative type I transmembrane protein comprising 360 amino acids with an N-terminal signal peptide (Fig. 6A). *TMX5* transcripts are widely expressed in human tissues (Fig. 6B). Motif analysis indicates that the N-terminal region contains a single thioredoxin-like domain with a non-canonical CRFS active site motif and four predicted N-glycosylation sites. The protein lacks ER retention motifs, and its subcellular localization has not been precisely determined. According to the Human Pr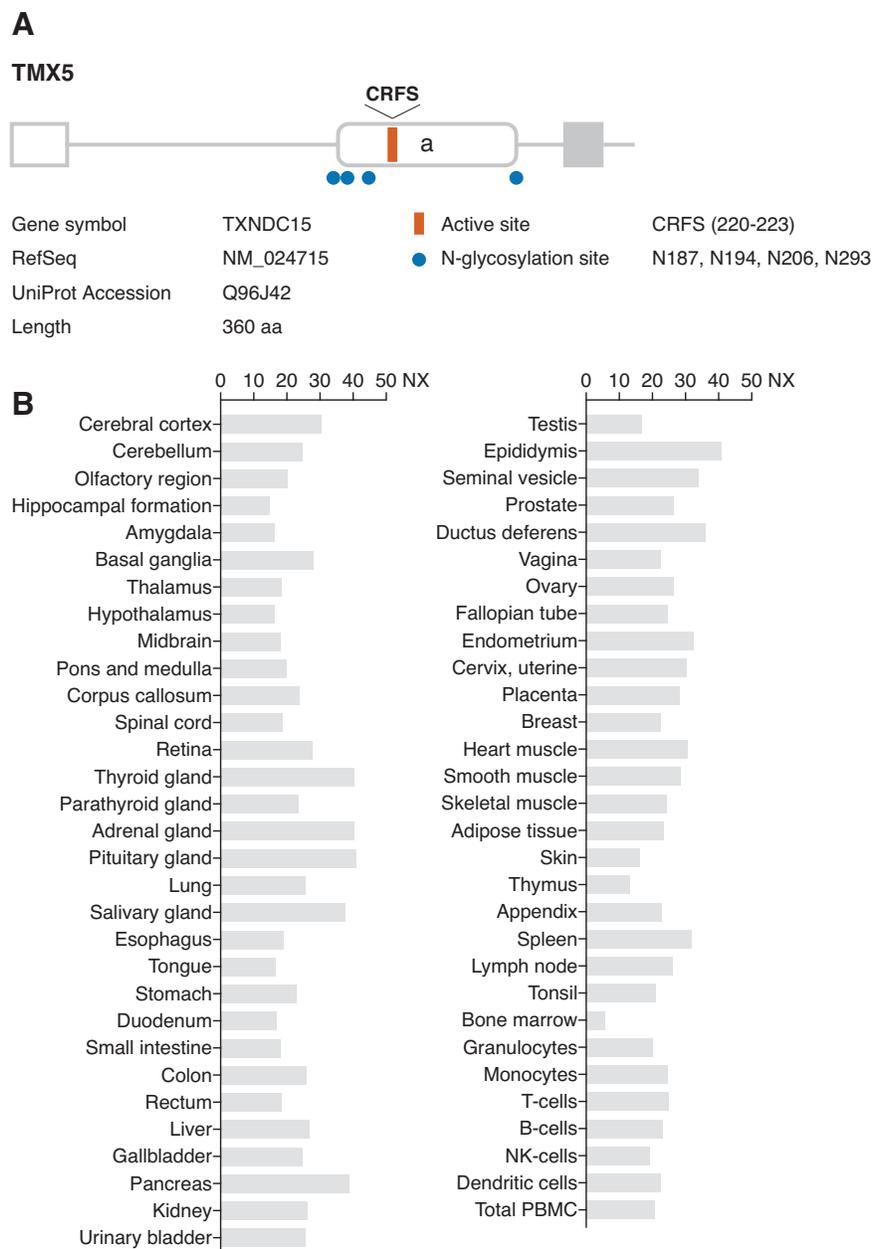otein Atlas (92), TMX5 was detected in the Golgi apparatus (https://www.proteinatlas.org/ENSG00000 113621-TXNDC15/cell).

A

TMX5

Gene symbol	TXNDC15	▮ Active site	CRFS (220-223)
RefSeq	NM_024715	● N-glycosylation site	N187, N194, N206, N293
UniProt Accession	Q96J42		
Length	360 aa		

B

FIG. 6. **Sequence features and expression profiles of human TMX5/TXNDC15. (A)** Sequence and structural characteristics of TMX5. The N-terminal signal sequence (*white rectangle*), the a-type thioredoxin-like domain (*rounded rectangle*), and the transmembrane domain (*gray rectangle*) are shown. The positions of the CRFS active site sequence and the four predicted N-glycosylation sites are indicated. **(B)** Tissue distribution of *TXNDC15* gene. The RNA expression data are available from https://www.proteinatlas.org/ENSG00000113621-TXNDC15/tissue.

Functional properties of TMX5

Our understanding of TMX5 is very limited, and its physiological function has not been well characterized thus far. The thioredoxin-like domain of TMX5 is closer in structure to that of ERp44 (3), a soluble oxidoreductase of the PDI family in the ER lumen (Fig. 5A). ERp44 has been shown to form intermolecular disulfide bonds with client proteins, and the long-lived nature of this interaction allows for efficient retention of its substrates within the ER (2). The absence of the C-terminal active site cysteine in TMX5 suggests its ability to form stabilized intermolecular disulfide bonds with the client proteins. TMX5 may function in a manner similar to ERp44, regulating thiol-mediated protein quality control.

Identification of TMX Substrates

To determine whether these redox proteins share overlapping biological activities or whether they have separate

and distinct functions, it is necessary to identify binding partners and substrate proteins for each oxidoreductase. The identification of physiological substrates of the thiol-disulfide isomerases has been challenging, which is in part due to the instability of the enzyme–substrate interaction during the thiol-disulfide exchange reaction. In this section, we describe the application of mechanism-based approaches to identify target proteins that engage in redox reactions with thiol-disulfide oxidoreductases. These methods should be applicable to a wide range of studies analyzing thiol-disulfide exchange reactions occurring inside or outside the cells.

Thiol-disulfide exchange reactions

The activity of thiol-disulfide oxidoreductases depends on a pair of cysteine residues in a conserved CXXC active site motif that shuttles between the oxidized (disulfide) and reduced (dithiol) states during the catalytic cycle (Fig. 7). The
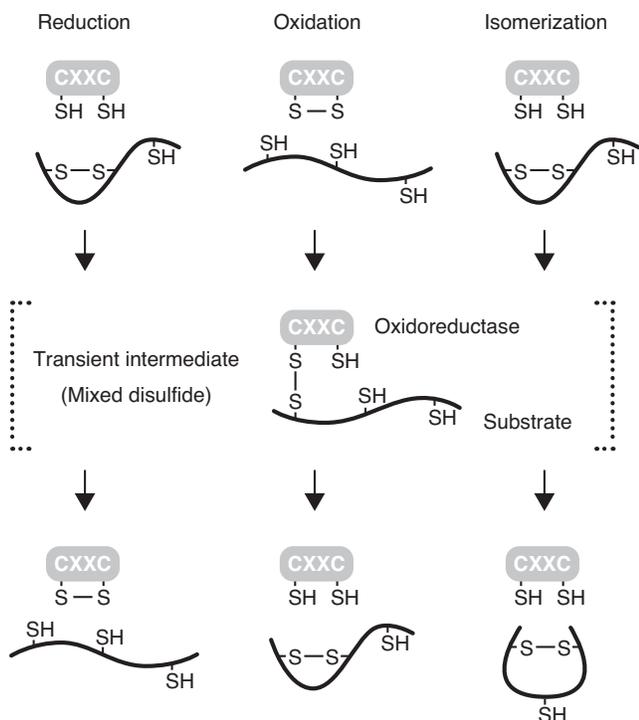
FIG. 7. Thiol-disulfide exchange reactions. The thioredoxin family proteins have the potential to catalyze reduction (disulfide bond cleavage), oxidation (disulfide bond formation), and isomerization (disulfide bond rearrangement). During the catalytic reactions, a transient disulfide-linked intermediate is formed between an oxidoreductase and its substrate.

reduced form of the enzyme transfers reducing equivalents to disulfides in a substrate protein and catalyzes their reduction. Thereafter, a reduced substrate protein is released, and the oxidoreductase is converted to its oxidized form. When the active site cysteine residues are in the oxidized form, the oxidoreductase can receive electrons from a substrate and introduce a disulfide bond in the substrate protein. In this oxidative pathway, the reaction breaks the disulfide residue in the enzyme active site motif. Sequential reduction and oxidation result in the isomerization or rearrangement of a disulfide bond in a substrate protein.

During the thiol-disulfide exchange reactions, a transient disulfide-linked intermediate (mixed disulfide) is formed between an oxidoreductase and its substrate (60). Because such transient mixed disulfides tend to be unstable, the identification of substrates for oxidoreductases has proven to be technically difficult. Several experimental approaches have been developed to stabilize reaction intermediates (4, 17, 21, 22, 28, 83), and these strategies can facilitate the identification of specific substrates of thioredoxin superfamily members (Fig. 8A).

Trapping substrates using a single-cysteine mutant

Considering the instability of the intermediates, experimental approaches have been used to trap substrates by mutating the C-terminal cysteine in the CXXC active sites of thiol-disulfide oxidoreductases. Thiol-disulfide oxidoreductase variants (trapping mutants), in which the C-terminal
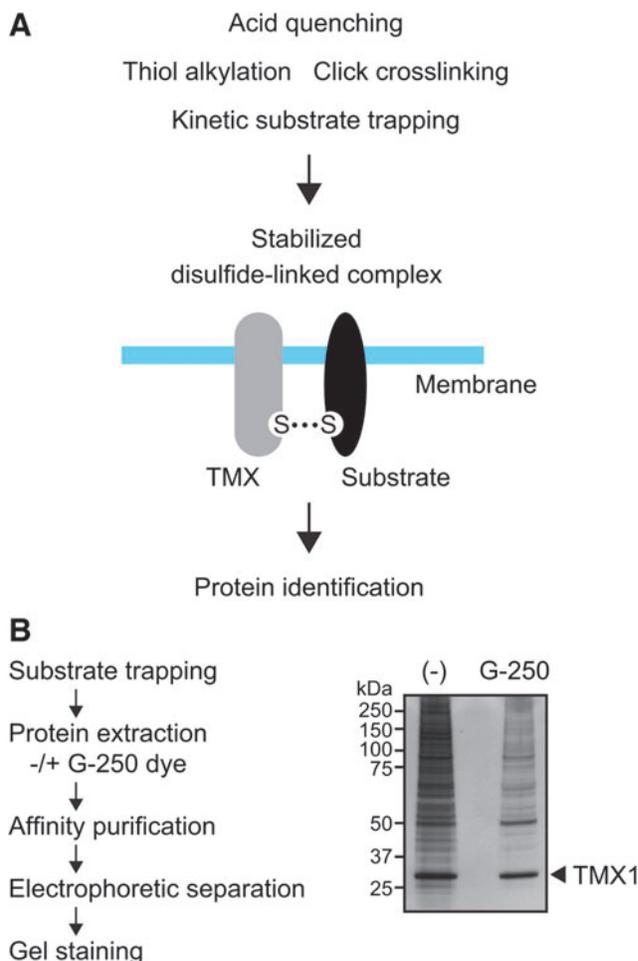

FIG. 8. Strategies for identifying physiological substrates for TMX. (A) To minimize the possibility of post-lysis redox reactions, the samples are typically exposed to highly acidic conditions before protein extraction. A membrane-permeable alkylating agent is also used to block free thiols, quenching the cellular redox status. Several substrate trapping techniques have been developed. Based on the kinetics of the thiol-disulfide exchange, selected conserved amino acid residues in the catalytic thioredoxin domain are mutated, leading to the stabilization of the disulfide-linked enzyme–substrate complexes. **(B)** The effect of coomassie brilliant blue G-250 in reducing non-specific protein binding to affinity resins. Cells expressing TMX1 fused to a poly-histidine tag were lysed, and the proteins were affinity-purified. The addition of G-250 dye in the extraction buffer significantly reduces the co-purification of contaminants, which can facilitate the isolation of specific substrates of TMX1.

cysteine in the active site is replaced by Ala (CXXA) or Ser (CXXS), have been utilized to trap and identify substrate proteins (21). As the N-terminal cysteine is intact, the mutant retains the ability to attack the target disulfide in the substrate, leading to the formation of a mixed disulfide conjugate. Substitution of the C-terminal cysteine in the active site, however, retards resolution of the mixed-disulfide complex, trapping the substrate as a conjugate with the enzyme. The stabilized enzyme–substrate complex enables efficient recovery and isolation of the substrate protein for downstream analysis. Exogenous expression of oxidoreductase-

trapping mutants causes accumulation of enzyme–substrate complexes linked by an intermolecular disulfide bond, leading to the successful identification of potential targets of various thiol-disulfide oxidoreductases, including TMX1 and TMX4 (53, 81, 89).

As an alternative approach, an oxidoreductase-trapping mutant may be prepared as a recombinant protein produced by bacteria or mammalian cells and administered to living cells to isolate the substrate on the cell surface. For example, a human thioredoxin-trapping mutant, in which the resolving cysteine at position 35 was replaced with serine, was allowed to react with the cell surface of lymphocytes, leading to the identification of CD30, a member of the tumor necrosis factor receptor superfamily, as the principal target of thioredoxin (82). Thioredoxin-mediated thiol-disulfide exchange causes a conformational change in the extracellular domain of CD30 and interferes with ligand–receptor binding. The recombinant trapping mutant was also used to capture substrates in tissue lysates and biological fluids. This method would be applicable to the catalytic domains of TMXs expressed as soluble proteins without the transmembrane regions. Identification of extracellular substrates for TMX family proteins will provide insights for elucidating the mechanism of action of TMXs on the cell surface.

Alternative approaches to stabilize mixed-disulfides

A drawback of the approach employing CXXA or CXXS trapping variants is that the method can only be used to identify substrates undergoing reduction or isomerization. To capture a substrate for oxidase, both cysteines in the active site must be intact and form disulfide bonds. Therefore, another strategy for substrate identification is required. To overcome the limitation of substrate trapping methods using CXXA or CXXS oxidoreductase mutants, researchers have generated several thiol-disulfide oxidoreductase trapping variants by substituting conserved amino acid residues other than cystine in the active site motif.

Conversion of the cis-proline 151 residue of DsbA, an *Escherichia coli* oxidase in the periplasm, results in the accumulation of disulfide-linked DsbA–substrate complexes (38). The crystal structure of the DsbA-substrate complex revealed that proline 151 is positioned close to the CXXC active site, suggesting its importance for substrate recognition by DsbA. Substitution of proline 151 with threonine causes a defect in the release of substrate from the enzyme. Thus, P151T substitution may affect the positioning of the mixed disulfide formed between DsbA and its substrate, leading to retarded resolution of the reaction intermediates. The corresponding proline residue is also conserved among mammalian thioredoxin-like proteins, including TMX family members (Fig. 5B). The TMX1 mutant harboring an analogous P101T substitution is capable of capturing disulfide-linked complexes (53). Thus, cis-proline mutants are useful for stabilizing mixed disulfides formed during thiol-disulfide exchange, leading to the identification of the substrates of TMX1. Based on these observations, it is suggested that the mutation of the corresponding proline residues may be useful in mammalian systems for the detection of mixed disulfide intermediates.

Another approach employing trapping variants involves changing the intervening residues between the cysteines in the CXXC motif. Screening for PDI variants that exhibit slower reaction kinetics against substrates revealed that substitution of the intervening histidine in the CGHC active site to either proline (CGPC) or arginine (CGRC) prolongs the enzyme–substrate interaction (88). The mechanism of action of these histidine trapping mutants has been attributed to a slower transition of the enzyme between the oxidized and reduced forms during the thiol-disulfide exchange catalytic cycle. It should be noted that the histidine-substituted trapping mutant retains both the active site cysteine residues and is enzymatically redox active. The histidine mutant of PDI with the reduced active site can trap substrates with disulfide bonds targeted for reduction by PDI. On the other hand, when the active site exists in the oxidized state, the histidine-trapping mutant is able to capture the substrate undergoing PDI-catalyzed oxidation. Thus, PDI histidine mutants allow the identification of protein substrates in both reductive and oxidative pathways. In fact, recombinant PDI variants with histidine substitution could capture candidate substrate proteins undergoing either reduction or oxidation by PDI during platelet activation. Considering the presence of CGHC active site motif in TMX3, this method would be beneficial to identify its physiological substrates.

Analysis of TMX–substrate complexes

In general, stabilized enzyme–substrate conjugates are purified by using specific antibodies or affinity chromatography resins, followed by mass spectrometry-based analysis. Considering that high background is a major problem in proteomic analysis, especially for integral membrane proteins with hydrophobic stretches (such as TMXs), it is necessary to minimize non-specific binding of contaminants to affinity matrices. In our approach to identify molecules interacting with TMX1, we included anionic Coomassie Brilliant Blue G-250 dye in the cell lysis buffer and successfully decreased the amount of co-purified contaminants (Fig. 8B). Coomassie G-250 dye has been used for blue-native polyacrylamide gel electrophoresis (97). It binds nonspecifically to proteins and confers a negative charge. The dye potentially masks hydrophobic sites, and negatively charged protein surfaces repel each other. Thus, the membrane proteins lose their hydrophobic properties on binding to the dye, and the G-250-treated molecules are expected to be less prone to aggregation (78). This approach is promising and would be useful for isolating specific interacting partners and physiological substrates for membrane-bound TMX family proteins.

Clinical Implications of TMX

A growing number of studies have provided evidence that dysregulation of TMX function is associated with the pathogenesis of a wide range of human diseases (Table 1).

Developmental disorders

Pathogenic variants of *TMX2* have been identified in patients with neuronal migration disorders, such as microcephaly, lissencephaly, and polymicrogyria (Online Mendelian Inheritance in Man [OMIM; https://omim.org] Phenotype MIM number: 618730), using whole-exome sequencing (24, 95). These variants result in reduced *TMX2* expression. RNA-seq analysis revealed dysregulated cellular pathways,

TABLE 1. HUMAN DISEASES ASSOCIATED WITH DYSREGULATION OF THIOREDOXIN-RELATED
TRANSMEMBRANE PROTEIN FUNCTION

Gene	Location	MIM	Disease	Expression/activity	References
TMX1	14q22.1	610527	Melanoma	Downregulated	(71)
			Melanoma	Upregulated	(100)
TMX2	11q12.1	616715	Microcephaly, polymicrogyria	Downregulated (mutated)	(95)
			Microlissencephaly	Downregulated (mutated)	(24)
TMX3	18q22.1	616102	Microphthalmia, anophthalmia	Downregulated (mutated)	(11)
			Coronary artery disease	Unknown (mutated)	(46)
			Melanoma	Upregulated	(100)
TMX4	20p12.3	616766	Not reported	—	—
TXNDC15 (TMX5)	5q31.1	617778	Meckel–Gruber syndrome	Downregulated (mutated)	(84)
			Meckel–Gruber syndrome	Unknown (mutated)	(70)
			Meckel–Gruber syndrome	Downregulated (mutated)	(72)

MIM, entry number in the Mendelian Inheritance in Man database.

including post-translational modification (*i.e.*, disulfide bond formation and N-glycosylation), synaptic function, and calcium binding, in *TMX2*-mutated cells from affected individuals. TMX2 is localized on the MAM, where it interacts with calnexin and other protein-folding factors (49). In addition, TMX2 binds to regulators of calcium homeostasis located at the ER–mitochondria interface. Indeed, skin fibroblasts harboring *TMX2* variants showed decreased mitochondrial respiratory capacity compared with healthy control cells (95). Significant upregulation of glycolytic activity was observed in the fibroblasts with *TMX2* variants, which may reflect a compensatory mechanism against the mitochondrial bioenergetic defects associated with reduced TMX2 activity. Collectively, these data imply an essential role of TMX2 in the maintenance of proper protein homeostasis, and the disruption of cellular redox adaptation mechanisms may result in developmental abnormalities in the human brain.

Array-based comparative genomic hybridization analysis in patients with microphthalmia, a developmental defect of the eye, identified a deletion in chromosome 18, and *TMX3* was included in the deleted region (99). Moreover, two missense mutations in *TMX3* have been identified in patients with microphthalmia or anophthalmia (OMIM Phenotype MIM number: 300345) (11). These nucleotide changes result in substitution of amino acids (R39Q and D108N) in the catalytic thioredoxin-like domain of TMX3, possibly disrupting protein function. In a zebrafish model, morpholino-mediated gene knockdown of a *TMX3* ortholog resulted in a small eye phenotype and abnormal retinal formation (11). In these knockdown larvae, co-injection of human *TMX3* mRNA significantly reduced the frequency of ocular abnormalities; in contrast, co-injecting an mRNA encoding *TMX3* mutant (R39Q) did not rescue the small eye phenotype, supporting the impaired functionality of the patient-derived mutant. Thus, despite its broad tissue distribution, deletion or haploinsufficiency of *TMX3* causes more severe developmental defects in the eye than in other organs. Further studies are warranted to clarify the precise mechanism of TMX3 action in vertebrate eye development. The identification of physiological substrates of TMX3 in the developing eye would be of particular interest, as it may help further our understanding of the pathogenesis of human microphthalmia.

Genomic analysis of a large ciliopathy cohort identified loss-of-function mutations in *TMX5* (84). *TMX5* was found to be independently mutated in families with features of

Meckel–Gruber syndrome (MKS), a lethal autosomal recessive ciliopathy caused by defective primary cilium formation. Lack of functional TMX5 causes mislocalization of the TMEM67 ciliary receptor to the transition zone, resulting in the perturbation of ciliary signaling and aberrant ciliogenesis. Several clinical studies have also reported pathogenic variants of *TMX5* in fetuses with MKS (70, 72). In addition, an experimental approach based on genome-wide CRISPR-mediated gene disruption identified *TMX5* as a candidate ciliopathy-associated gene (8). *TMX5*-knockout cells harbored a defect in Hedgehog signaling, which is indispensable for the normal development of embryo, and exhibited abnormal morphology, supporting its causative role in MKS. These reports suggest that *TMX5* is a novel MKS-associated gene. However, the molecular basis for TMX5 action in ciliary signaling pathways remains poorly understood.

Neurodegenerative diseases

Mutations in the C9ORF72 gene are the most common causes of amyotrophic lateral sclerosis (ALS). The pathogenic mutation is a hexanucleotide-repeat expansion that results in the massive production of toxic dipeptide-repeat (DPR) proteins (16). DPR proteins are prone to aggregation and contribute to neurodegeneration. In CRISPR–Cas9 genome-wide genetic deletion screens, *TMX2* was identified as a potent genetic modifier of DRP toxicity (42). *TMX2* knockout suppressed DPR-induced toxicity in primary neurons. The DPR proteins induce ER stress that is believed to be involved in DPR toxicity and ALS pathogenesis. It has been suggested that *TMX2* deficiency confers protection against DRP toxicity by modulating the ER stress response induced by DRP proteins. In view of disease prevention mechanisms, suppression of TMX2 would be beneficial for protecting neurons from DRP-induced cytotoxicity and may serve as a therapeutic target for ALS pathogenesis.

Accumulation of N-terminal fragments of mutant huntingtin protein (mHTT) has been implicated in the progression of Huntington's disease (HD) (77). It is suggested that the mHTT fragments containing expanded polyglutamine repeats are oxidized to form stable aggregates, thereby exerting their toxicity (19). In an experimental model of HD, TMX3 decreased the levels of exogenously expressed mHTT (48). Since mHTT accumulates in the cytosol, it is improbable that TMX3 directly regulates the redox properties of mHTT.

The authors of that study also showed that overexpression of cytosolic thioredoxin attenuates the toxic effects of mHTT in the HD models (48). These results indicate that these oxidoreductases act to reduce the burden of protein aggregation by controlling protein redox states, thereby preventing mHTT-induced cell death. Although the implications of thiol-mediated protective mechanisms against neurodegenerative diseases can be postulated, the precise roles of oxidoreductases in the control of disease states have not yet been explored.

Cardiovascular diseases

The ER oxidoreductases, such as PDI, ERp57, and ERp5, are translocated to the cell surface where they regulate platelet function extracellularly (80). A recent study revealed that TMX1 is localized on the surface of human platelets and that its expression is increased on platelet activation (101). Moreover, the inhibition of cell surface TMX1 resulted in increased platelet aggregation. *Tmx1*-deficiency in mice potentiates platelet function with elevated platelet incorporation into the thrombus and shortened tail bleeding times, supporting the *in vitro* studies. These results indicate that TMX1 is a negative regulator of platelet aggregation and thrombosis. It has been proposed that TMX1 oxidizes integrin $\alpha IIb\beta 3$, maintaining the integrin in an inactive or quiescent state and inhibiting its signal. Consistently, platelets from *Tmx1*-deficient mice showed increased thiols in the $\beta 3$ subunit of $\alpha IIb\beta 3$.

Whole-exome sequencing identified a novel pathogenic indel mutation in *TMX3* in a Chinese cohort with a family history of coronary artery disease (46). A cell surface biotinylation assay revealed the presence of TMX3 on the platelet surface (34). Although speculative at present, TMX3 may act on certain extracellular substrates to control platelet function in a manner similar to TMX1 (98). Given that platelet aggregation and thrombosis are the major causes of coronary artery disease, it may be possible that TMX3 is trafficked from the ER to the platelet surface, where it regulates thrombus formation.

Cancer

The role of TMX1 in tumorigenesis remains controversial. Decreased TMX1 expression has been reported in metastatic melanoma (71). Low TMX1 expression in cancer cell lines decreases Ca^{2+} transport to the mitochondria, dampens mitochondrial respiration, and produces higher levels of ROS. Despite the growth suppressive phenotype of *TMX1*-knockdown cells *in vitro*, reduced TMX1 expression led to increased tumor growth in a mouse xenograft model, suggesting tumor suppressive properties of TMX1. These opposing effects of TMX1 on cell proliferation should be further explored. Further, lower expression levels of TMX1 were also reported in aggressive breast cancer cells (44) and asbestos-exposed lung cells (62).

Conversely, elevated expression of TMX1, as well as TMX3, has been reported in melanoma cells and patient-derived samples (100). Moreover, TMX1 expression levels positively correlate with the disease stage of aggressive melanoma, and patients with increased TMX1 expression have a lower survival expectancy. From a mechanistic point of view, TMX1 regulates nuclear factor of activated T cells 1 (NFAT1) signaling that is implicated in melanoma growth (59). TMX1 downregulation suppresses the nuclear translocation of NFAT1 in an ROS-dependent manner, thereby inhibiting the proliferation and invasive potential of melanoma cells. These results suggest that the TMX1–NFAT1 axis promotes cancer progression, and elevated TMX1 expression is indicative of cancer aggressiveness and poor survival outcome.

TP53-regulated inhibitor of apoptosis 1 (TRIAP1), an oncogenic protein highly expressed in lung adenocarcinoma, has been shown to mediate the upregulation of some antioxidative proteins, including TMX1 and TMX2 (29). The expression levels of TMX1 and TMX2 were inversely correlated with the overall survival of the patients. Further, their expression was enhanced in lung cancer cell lines on exposure to radiation. The results may imply that the pro-oncogenic property of TRIAP1 involves upregulation of the antioxidative capacity of cancer cells, thereby contributing to tumor resistance against radiation-induced oxidative damage.

Conclusion

Since the discovery of TMX1 in 2001, four additional members have been added to the human TMX family. They share the common property of being anchored to cellular membranes. The collective body of evidence indicates their preference for interacting with membrane-associated molecules. Most TMX members are localized mainly to the ER membrane, suggesting their primary roles in assisting the folding and assembly of membrane proteins in the secretory pathway. Further, recent studies have revealed that they have diverse functions outside of the ER. However, the precise function of individual TMX family members in human physiology and the underlying mechanism of action remain to be elucidated. The cellular membrane serves as a platform for signal transduction and cell–cell interaction, and membrane proteins are prime targets for drug discovery in several human diseases. Expanding our knowledge of the physiological roles of these membrane-bound oxidoreductases of the TMX family and determining the mechanistic details of their action will be of the utmost importance for developing diagnostics, potent molecular targets, and therapeutic approaches for the treatment of human disorders taken together with the elucidation of functional roles against other PDI family proteins in the cells, although extensive research is still required to obtain a complete picture.

References

1. Akiyama N, Matsuo Y, Sai H, Noda M, and Kizaka-Kondoh S. Identification of a series of transforming growth factor beta-responsive genes by retrovirus-mediated gene trap screening. *Mol Cell Biol* 20: 3266–3273, 2000.

2. Anelli T, Alessio M, Bachi A, Bergamelli L, Bertoli G, Camerini S, Mezghrani A, Ruffato E, Simmen T, and Sitia R. Thiol-mediated protein retention in the endoplasmic reticulum: the role of ERp44. *EMBO J* 22: 5015–5022, 2003.

3. Anelli T, Alessio M, Mezghrani A, Simmen T, Talamo F, Bachi A, and Sitia R. ERp44, a novel endoplasmic reticulum folding assistant of the thioredoxin family. *EMBO J* 21: 835–844, 2002.

4. Araki K, Ushioda R, Kusano H, Tanaka R, Hatta T, Fukui K, Nagata K, and Natsume T. A crosslinker-based identification of redox relay targets. *Anal Biochem* 520: 22–26, 2017.

5. Bellisola G, Fracasso G, Ippoliti R, Menestrina G, Rosen A, Solda S, Udali S, Tomazzolli R, Tridente G, and Colombatti M. Reductive activation of ricin and ricin A-chain immunotoxins by protein disulfide isomerase and thioredoxin reductase. *Biochem Pharmacol* 67: 1721–1731, 2004.

6. Berndt C, Lillig CH, and Holmgren A. Thioredoxins and glutaredoxins as facilitators of protein folding. *Biochim Biophys Acta* 1783: 641–650, 2008.

7. Braakman I and Bulleid NJ. Protein folding and modification in the mammalian endoplasmic reticulum. *Annu Rev Biochem* 80: 71–99, 2011.

8. Breslow DK, Hoogendoorn S, Kopp AR, Morgens DW, Vu BK, Kennedy MC, Han K, Li A, Hess GT, Bassik MC, Chen JK, and Nachury MV. A CRISPR-based screen for Hedgehog signaling provides insights into ciliary function and ciliopathies. *Nat Genet* 50: 460–471, 2018.

9. Bulleid NJ. Disulfide bond formation in the mammalian endoplasmic reticulum. *Cold Spring Harb Perspect Biol* 4: a013219, 2012.

10. Cao Z, van Lith M, Mitchell LJ, Pringle MA, Inaba K, and Bulleid NJ. The membrane topology of vitamin K epoxide reductase is conserved between human isoforms and the bacterial enzyme. *Biochem J* 473: 851–858, 2016.

11. Chao R, Nevin L, Agarwal P, Riemer J, Bai X, Delaney A, Akana M, JimenezLopez N, Bardakjian T, Schneider A, Chassaing N, Schorderet DF, FitzPatrick D, Kwok PY, Ellgaard L, Gould DB, Zhang Y, Malicki J, Baier H, and Slavotinek A. A male with unilateral microphthalmia reveals a role for TMX3 in eye development. *PLoS One* 5: e10565, 2010.

12. Chen W, Helenius J, Braakman I, and Helenius A. Cotranslational folding and calnexin binding during glycoprotein synthesis. *Proc Natl Acad Sci U S A* 92: 6229–6233, 1995.

13. Cheng LC, Baboo S, Lindsay C, Brusman L, Martinez-Bartolomé S, Tapia O, Zhang X, Yates JR, 3rd, and Gerace L. Identification of new transmembrane proteins concentrated at the nuclear envelope using organellar proteomics of mesenchymal cells. *Nucleus* 10: 126–143, 2019.

14. Clark HF, Gurney AL, Abaya E, Baker K, Baldwin D, Brush J, Chen J, Chow B, Chui C, Crowley C, Currell B, Deuel B, Dowd P, Eaton D, Foster J, Grimaldi C, Gu Q, Hass PE, Heldens S, Huang A, Kim HS, Klimowski L, Jin Y, Johnson S, Lee J, Lewis L, Liao D, Mark M, Robbie E, Sanchez C, Schoenfeld J, Seshagiri S, Simmons L, Singh J, Smith V, Stinson J, Vagts A, Vandlen R, Watanabe C, Wieand D, Woods K, Xie MH, Yansura D, Yi S, Yu G, Yuan J, Zhang M, Zhang Z, Goddard A, Wood WI, Godowski P, and Gray A. The secreted protein discovery initiative (SPDI), a large-scale effort to identify novel human secreted and transmembrane proteins: a bioinformatics assessment. *Genome Res* 13: 2265–2270, 2003.

15. Crooks GE, Hon G, Chandonia JM, and Brenner SE. WebLogo: a sequence logo generator. *Genome Res* 14: 1188–1190, 2004.

16. DeJesus-Hernandez M, Mackenzie IR, Boeve BF, Boxer AL, Baker M, Rutherford NJ, Nicholson AM, Finch NA, Flynn H, Adamson J, Kouri N, Wojtas A, Sengdy P, Hsiung GY, Karydas A, Seeley WW, Josephs KA, Coppola G, Geschwind DH, Wszolek ZK, Feldman H, Knopman DS, Petersen RC, Miller BL, Dickson DW, Boylan KB, Graff-Radford NR, and Rademakers R. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron* 72: 245–256, 2011.

17. Eriksson O, Stopa J, and Furie B. Identification of PDI substrates by mechanism-based kinetic trapping. *Methods Mol Biol* 1967: 165–182, 2019.

18. Fan Y and Simmen T. Mechanistic connections between endoplasmic reticulum (ER) redox control and mitochondrial metabolism. *Cells* 8: 1071, 2019.

19. Fox JH, Connor T, Stiles M, Kama J, Lu Z, Dorsey K, Lieberman G, Sapp E, Cherny RA, Banks M, Volitakis I, DiFiglia M, Berezovska O, Bush AI, and Hersch SM. Cysteine oxidation within N-terminal mutant huntingtin promotes oligomerization and delays clearance of soluble protein. *J Biol Chem* 286: 18320–18330, 2011.

20. Fra A, Yoboue ED, and Sitia R. Cysteines as redox molecular switches and targets of disease. *Front Mol Neurosci* 10: 167, 2017.

21. Fujimoto T, Inaba K, and Kadokura H. Methods to identify the substrates of thiol-disulfide oxidoreductases. *Protein Sci* 28: 30–40, 2019.

22. Fujimoto T, Nakamura O, Saito M, Tsuru A, Matsumoto M, Kohno K, Inaba K, and Kadokura H. Identification of the physiological substrates of PDIp, a pancreas-specific protein-disulfide isomerase family member. *J Biol Chem* 293: 18421–18433, 2018.

23. Galligan JJ and Petersen DR. The human protein disulfide isomerase gene family. *Hum Genomics* 6: 6, 2012.

24. Ghosh SG, Wang L, Breuss MW, Green JD, Stanley V, Yang X, Ross D, Traynor BJ, Alhashem AM, Azam M, Selim L, Bastaki L, Elbastawisy HI, Temtamy S, Zaki M, and Gleeson JG. Recurrent homozygous damaging mutation in TMX2, encoding a protein disulfide isomerase, in four families with microlissencephaly. *J Med Genet* 57: 274–282, 2020.

25. Green WN and Wanamaker CP. The role of the cystine loop in acetylcholine receptor assembly. *J Biol Chem* 272: 20945–20953, 1997.

26. Guerra C, Brambilla Pisoni G, Soldà T, and Molinari M. The reductase TMX1 contributes to ERAD by preferentially acting on membrane-associated folding-defective polypeptides. *Biochem Biophys Res Commun* 503: 938–943, 2018.

27. Guerra C and Molinari M. Thioredoxin-related transmembrane proteins: TMX1 and little brothers TMX2, TMX3, TMX4 and TMX5. *Cells* 9: 2000, 2020.

28. Hansen RE and Winther JR. An introduction to methods for analyzing thiols and disulfides: reactions, reagents, and practical considerations. *Anal Biochem* 394: 147–158, 2009.

29. Hao CC, Luo JN, Xu CY, Zhao XY, Zhong ZB, Hu XN, Jin XM, and Ge X. TRIAP1 knockdown sensitizes non-small cell lung cancer to ionizing radiation by disrupting redox homeostasis. *Thorac Cancer* 11: 1015–1025, 2020.

30. Hatahet F and Ruddock LW. Substrate recognition by the protein disulfide isomerases. *FEBS J* 274: 5223–5234, 2007.

31. Hatahet F and Ruddock LW. Protein disulfide isomerase: a critical evaluation of its function in disulfide bond formation. *Antioxid Redox Signal* 11: 2807–2850, 2009.

32. Haugstetter J, Blicher T, and Ellgaard L. Identification and characterization of a novel thioredoxin-related transmembrane protein of the endoplasmic reticulum. *J Biol Chem* 280: 8371–8380, 2005.

33. Haugstetter J, Maurer MA, Blicher T, Pagac M, Wider G, and Ellgaard L. Structure-function analysis of the endoplasmic reticulum oxidoreductase TMX3 reveals interdomain stabilization of the N-terminal redox-active domain. *J Biol Chem* 282: 33859–33867, 2007.

34. Holbrook LM, Watkins NA, Simmonds AD, Jones CI, Ouwehand WH, and Gibbins JM. Platelets release novel thiol isomerase enzymes which are recruited to the cell surface following activation. *Br J Haematol* 148: 627–637, 2010.

35. Ihara M, Furutani S, Shigetou S, Shimada S, Niki K, Komori Y, Kamiya M, Koizumi W, Magara L, Hikida M, Noguchi A, Okuhara D, Yoshinari Y, Kondo S, Tanimoto H, Niwa R, Sattelle DB, and Matsuda K. Cofactor-enabled functional expression of fruit fly, honeybee, and bumblebee nicotinic receptors reveals picomolar neonicotinoid actions. *Proc Natl Acad Sci U S A* 117: 16283–16291, 2020.

36. Jackson MR, Nilsson T, and Peterson PA. Identification of a consensus motif for retention of transmembrane proteins in the endoplasmic reticulum. *EMBO J* 9: 3153–3162, 1990.

37. Jordan PA and Gibbins JM. Extracellular disulfide exchange and the regulation of cellular function. *Antioxid Redox Signal* 8: 312–324, 2006.

38. Kadokura H, Tian H, Zander T, Bardwell JC, and Beckwith J. Snapshots of DsbA in action: detection of proteins in the process of oxidative folding. *Science* 303: 534–537, 2004.

39. Klappa P, Ruddock LW, Darby NJ, and 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* 17: 927–935, 1998.

40. Kondo N, Nakamura H, Masutani H, and Yodoi J. Redox regulation of human thioredoxin network. *Antioxid Redox Signal* 8: 1881–1890, 2006.

41. Kosuri P, Alegre-Cebollada J, Feng J, Kaplan A, Ingles-Prieto A, Badilla CL, Stockwell BR, Sanchez-Ruiz JM, Holmgren A, and Fernandez JM. Protein folding drives disulfide formation. *Cell* 151: 794–806, 2012.

42. Kramer NJ, Haney MS, Morgens DW, Jovičić A, Couthouis J, Li A, Ousey J, Ma R, Bieri G, Tsui CK, Shi Y, Hertz NT, Tessier-Lavigne M, Ichida JK, Bassik MC, and Gitler AD. CRISPR-Cas9 screens in human cells and primary neurons identify modifiers of C9ORF72 dipeptide-repeat-protein toxicity. *Nat Genet* 50: 603–612, 2018.

43. Kumar S, Stecher G, Li M, Knyaz C, and Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Mol Biol Evol* 35: 1547–1549, 2018.

44. Lagadec C, Dekmezian C, Bauche L, and Pajonk F. Oxygen levels do not determine radiation survival of breast cancer stem cells. *PLoS One* 7: e34545, 2012.

45. Leichert LI and Dick TP. Incidence and physiological relevance of protein thiol switches. *Biol Chem* 396: 389–399, 2015.

46. Li M, Wen Y, Wen H, Gui C, Huang F, and Zeng Z. Discovery of PPP2R3A and TMX3 pathogenic variants in a Zhuang family with coronary artery disease using whole-exome sequencing. *Int J Clin Exp Pathol* 11: 3678–3684, 2018.

47. Lizio M, Harshbarger J, Shimoji H, Severin J, Kasukawa T, Sahin S, Abugessaisa I, Fukuda S, Hori F, Ishikawa-Kato S, Mungall CJ, Arner E, Baillie JK, Bertin N, Bono H, de Hoon M, Diehl AD, Dimont E, Freeman TC, Fujieda K, Hide W, Kaliyaperumal R, Katayama T, Lassmann T, Meehan TF, Nishikata K, Ono H, Rehli M, Sandelin A, Schultes EA, t Hoen PA, Tatum Z, Thompson M, Toyoda T, Wright DW, Daub CO, Itoh M, Carninci P, Hayashizaki Y, Forrest AR, and Kawaji H; FANTOM Consortium. Gateways to the FANTOM5 promoter level mammalian expression atlas. *Genome Biol* 16: 22, 2015.

48. Lu Z, Barrows L, and Fox J. Thiol-disulfide oxidoreductases TRX1 and TMX3 decrease neuronal atrophy in a lentiviral mouse model of Huntington's disease. *PLoS Curr* 7, ecurrents.hd.b966ec2eca8e2d89d2bb4d020be4351e, 2015.

49. Lynes EM, Bui M, Yap MC, Benson MD, Schneider B, Ellgaard L, Berthiaume LG, and Simmen T. Palmitoylated TMX and calnexin target to the mitochondria-associated membrane. *EMBO J* 31: 457–470, 2012.

50. Matsuo Y, Akiyama N, Nakamura H, Yodoi J, Noda M, and Kizaka-Kondoh S. Identification of a novel thioredoxin-related transmembrane protein. *J Biol Chem* 276: 10032–10038, 2001.

51. Matsuo Y and Hirota K. Transmembrane thioredoxin-related protein TMX1 is reversibly oxidized in response to protein accumulation in the endoplasmic reticulum. *FEBS Open Bio* 7: 1768–1777, 2017.

52. Matsuo Y, Irie K, Kiyonari H, Okuyama H, Nakamura H, Son A, Lopez-Ramos DA, Tian H, Oka S, Okawa K, Kizaka-Kondoh S, Masutani H, and Yodoi J. The protective role of the transmembrane thioredoxin-related protein TMX in inflammatory liver injury. *Antioxid Redox Signal* 18: 1263–1272, 2013.

53. Matsuo Y, Masutani H, Son A, Kizaka-Kondoh S, and Yodoi J. Physical and functional interaction of transmembrane thioredoxin-related protein with major histocompatibility complex class I heavy chain: redox-based protein quality control and its potential relevance to immune responses. *Mol Biol Cell* 20: 4552–4562, 2009.

54. Matsuo Y, Nishinaka Y, Suzuki S, Kojima M, Kizaka-Kondoh S, Kondo N, Son A, Sakakura-Nishiyama J, Yamaguchi Y, Masutani H, Ishii Y, and Yodoi J. TMX, a human transmembrane oxidoreductase of the thioredoxin family: the possible role in disulfide-linked protein folding in the endoplasmic reticulum. *Arch Biochem Biophys* 423: 81–87, 2004.

55. Meng X, Zhang C, Chen J, Peng S, Cao Y, Ying K, Xie Y, and Mao Y. Cloning and identification of a novel cDNA coding thioredoxin-related transmembrane protein 2. *Biochem Genet* 41: 99–106, 2003.

56. Meusser B, Hirsch C, Jarosch E, and Sommer T. ERAD: the long road to destruction. *Nat Cell Biol* 7: 766–772, 2005.

57. Mistry J, Chuguransky S, Williams L, Qureshi M, Salazar GA, Sonnhammer ELL, Tosatto SCE, Paladin L, Raj S, Richardson LJ, Finn RD, and Bateman A. Pfam: the protein families database in 2021. *Nucleic Acids Res* 49: D412–D419, 2021.

58. Mori K. Tripartite management of unfolded proteins in the endoplasmic reticulum. *Cell* 101: 451–454, 2000.

59. Muller MR and Rao A. NFAT, immunity and cancer: a transcription factor comes of age. *Nat Rev Immunol* 10: 645–656, 2010.

60. Nagy P. Kinetics and mechanisms of thiol-disulfide exchange covering direct substitution and thiol oxidation-mediated pathways. *Antioxid Redox Signal* 18: 1623–1641, 2013.

61. Nemecz A, Prevost MS, Menny A, and Corringer PJ. Emerging molecular mechanisms of signal transduction in pentameric ligand-gated ion channels. *Neuron* 90: 452–470, 2016.

62. Nymark P, Lindholm PM, Korpela MV, Lahti L, Ruosaari S, Kaski S, Hollmen J, Anttila S, Kinnula VL, and Knuutila S. Gene expression profiles in asbestos-exposed epithelial and mesothelial lung cell lines. *BMC Genomics* 8: 62, 2007.

63. Oguro A and Imaoka S. Thioredoxin-related transmembrane protein 2 (TMX2) regulates the Ran protein gradient and importin-β-dependent nuclear cargo transport. *Sci Rep* 9: 15296, 2019.

64. Okumura M, Kadokura H, and Inaba K. Structures and functions of protein disulfide isomerase family members involved in proteostasis in the endoplasmic reticulum. *Free Radic Biol Med* 83: 314–322, 2015.

65. Oldenburg J, Bevans CG, Muller CR, and Watzka M. Vitamin K epoxide reductase complex subunit 1 (VKORC1): the key protein of the vitamin K cycle. *Antioxid Redox Signal* 8: 347–353, 2006.

66. Olsen JV, Blagoev B, Gnad F, Macek B, Kumar C, Mortensen P, and Mann M. Global, in vivo, and site-specific phosphorylation dynamics in signaling networks. *Cell* 127: 635–648, 2006.

67. Olsnes S. The history of ricin, abrin and related toxins. *Toxicon* 44: 361–370, 2004.

68. Pasetto M, Barison E, Castagna M, Della Cristina P, Anselmi C, and Colombatti M. Reductive activation of type 2 ribosome-inactivating proteins is promoted by transmembrane thioredoxin-related protein. *J Biol Chem* 287: 7367–7373, 2012.

69. Pisoni GB, Ruddock LW, Bulleid N, and Molinari M. Division of labor among oxidoreductases: TMX1 preferentially acts on transmembrane polypeptides. *Mol Biol Cell* 26: 3390–3400, 2015.

70. Radhakrishnan P, Nayak SS, Shukla A, Lindstrand A, and Girisha KM. Meckel syndrome: clinical and mutation profile in six fetuses. *Clin Genet* 96: 560–565, 2019.

71. Raturi A, Gutiérrez T, Ortiz-Sandoval C, Ruangkittisakul A, Herrera-Cruz MS, Rockley JP, Gesson K, Ourdev D, Lou PH, Lucchinetti E, Tahbaz N, Zaugg M, Baksh S, Ballanyi K, and Simmen T. TMX1 determines cancer cell metabolism as a thiol-based modulator of ER-mitochondria Ca^{2+} flux. *J Cell Biol* 214: 433–444, 2016.

72. Ridnoi K, Sois M, Vaidla E, Pajusalu S, Kelder L, Reimand T, and Ounap K. A prenatally diagnosed case of Meckel-Gruber syndrome with novel compound heterozygous pathogenic variants in the TXNDC15 gene. *Mol Genet Genomic Med* 7: e614, 2019.

73. Rizzuto R, De Stefani D, Raffaello A, and Mammucari C. Mitochondria as sensors and regulators of calcium signalling. *Nat Rev Mol Cell Biol* 13: 566–578, 2012.

74. Ron D and Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat Rev Mol Cell Biol* 8: 519–529, 2007.

75. Roth D, Lynes E, Riemer J, Hansen HG, Althaus N, Simmen T, and Ellgaard L. A di-arginine motif contributes to the ER localization of the type I transmembrane ER oxidoreductase TMX4. *Biochem J* 425: 195–205, 2009.

76. Ruddock LW and Molinari M. N-glycan processing in ER quality control. *J Cell Sci* 119: 4373–4380, 2006.

77. Sanchez I, Mahlke C, and Yuan J. Pivotal role of oligomerization in expanded polyglutamine neurodegenerative disorders. *Nature* 421: 373–379, 2003.

78. Schagger H. Blue-native gels to isolate protein complexes from mitochondria. *Methods Cell Biol* 65: 231–244, 2001.

79. Schneider TD and Stephens RM. Sequence logos: a new way to display consensus sequences. *Nucleic Acids Res* 18: 6097–6100, 1990.

80. Schulman S, Bendapudi P, Sharda A, Chen V, Bellido-Martin L, Jasuja R, Furie BC, Flaumenhaft R, and Furie B. Extracellular thiol isomerases and their role in thrombus formation. *Antioxid Redox Signal* 24: 1–15, 2016.

81. Schulman S, Wang B, Li W, and Rapoport TA. Vitamin K epoxide reductase prefers ER membrane-anchored thioredoxin-like redox partners. *Proc Natl Acad Sci U S A* 107: 15027–15032, 2010.

82. Schwertassek U, Balmer Y, Gutscher M, Weingarten L, Preuss M, Engelhard J, Winkler M, and Dick TP. Selective redox regulation of cytokine receptor signaling by extracellular thioredoxin-1. *EMBO J* 26: 3086–3097, 2007.

83. Schwertassek U, Weingarten L, and Dick TP. Identification of redox-active cell-surface proteins by mechanism-based kinetic trapping. *Sci STKE* 2007: pl8, 2007.

84. Shaheen R, Szymanska K, Basu B, Patel N, Ewida N, Faqeih E, Al Hashem A, Derar N, Alsharif H, Aldahmesh MA, Alazami AM, Hashem M, Ibrahim N, Abdulwahab FM, Sonbul R, Alkuraya H, Alnemer M, Al Tala S, Al-Husain M, Morsy H, Seidahmed MZ, Meriki N, Al-Owain M, AlShahwan S, Tabarki B, Salih MA, Faquih T, El-Kalioby M, Ueffing M, Boldt K, Logan CV, Parry DA, Al Tassan N, Monies D, Megarbane A, Abouelhoda M, Halees A, Johnson CA, and Alkuraya FS. Characterizing the morbid genome of ciliopathies. *Genome Biol* 17: 242, 2016.

85. Spooner RA, Watson PD, Marsden CJ, Smith DC, Moore KA, Cook JP, Lord JM, and Roberts LM. Protein disulphide-isomerase reduces ricin to its A and B chains in the endoplasmic reticulum. *Biochem J* 383: 285–293, 2004.

86. Stecher G, Tamura K, and Kumar S. Molecular Evolutionary Genetics Analysis (MEGA) for macOS. *Mol Biol Evol* 37: 1237–1239, 2020.

87. Stolyarchuk M, Ledoux J, Maignant E, Trouvé A, and Tchertanov L. Identification of the primary factors determining the specificity of human VKORC1 recognition by thioredoxin-fold proteins. *Int J Mol Sci* 22: 802, 2021.

88. Stopa JD, Baker KM, Grover SP, Flaumenhaft R, and Furie B. Kinetic-based trapping by intervening sequence

variants of the active sites of protein-disulfide isomerase identifies platelet protein substrates. *J Biol Chem* 292: 9063–9074, 2017.

89. Sugiura Y, Araki K, Iemura S, Natsume T, Hoseki J, and Nagata K. Novel thioredoxin-related transmembrane protein TMX4 has reductase activity. *J Biol Chem* 285: 7135–7142, 2010.

90. Tao GZ, Zhou Q, Strnad P, Salemi MR, Lee YM, and Omary MB. Human Ran cysteine 112 oxidation by pervanadate regulates its binding to keratins. *J Biol Chem* 280: 12162–12167, 2005.

91. Terry LJ, Shows EB, and Wente SR. Crossing the nuclear envelope: hierarchical regulation of nucleocytoplasmic transport. *Science* 318: 1412–1416, 2007.

92. Thul PJ, Akesson L, Wiking M, Mahdessian D, Geladaki A, Ait Blal H, Alm T, Asplund A, Bjork L, Breckels LM, Backstrom A, Danielsson F, Fagerberg L, Fall J, Gatto L, Gnann C, Hober S, Hjelmare M, Johansson F, Lee S, Lindskog C, Mulder J, Mulvey CM, Nilsson P, Oksvold P, Rockberg J, Schutten R, Schwenk JM, Sivertsson A, Sjostedt E, Skogs M, Stadler C, Sullivan DP, Tegel H, Winsnes C, Zhang C, Zwahlen M, Mardinoglu A, Ponten F, von Feilitzen K, Lilley KS, Uhlen M, and Lundberg E. A subcellular map of the human proteome. *Science* 356: eaal3321, 2017.

93. Uhlen M, Fagerberg L, Hallstrom BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson A, Kampf C, Sjostedt E, Asplund A, Olsson I, Edlund K, Lundberg E, Navani S, Szigyarto CA, Odeberg J, Djureinovic D, Takanen JO, Hober S, Alm T, Edqvist PH, Berling H, Tegel H, Mulder J, Rockberg J, Nilsson P, Schwenk JM, Hamsten M, von Feilitzen K, Forsberg M, Persson L, Johansson F, Zwahlen M, von Heijne G, Nielsen J, and Ponten F. Tissue-based map of the human proteome. *Science* 347: 1260419, 2015.

94. Vander Heiden MG and DeBerardinis RJ. Understanding the intersections between metabolism and cancer biology. *Cell* 168: 657–669, 2017.

95. Vandervore LV, Schot R, Milanese C, Smits DJ, Kasteleijn E, Fry AE, Pilz DT, Brock S, Börklü-Yücel E, Post M, Bahi-Buisson N, Sánchez-Soler MJ, van Slegtenhorst M, Keren B, Afenjar A, Coury SA, Tan WH, Oegema R, de Vries LS, Fawcett KA, Nikkels PGJ, Bertoli-Avella A, Al Hashem A, Alwabel AA, Tlili-Graiess K, Efthymiou S, Zafar F, Rana N, Bibi F, Houlden H, Maroofian R, Person RE, Crunk A, Savatt JM, Turner L, Doosti M, Karimiani EG, Saadi NW, Akhondian J, Lequin MH, Kayserili H, van der Spek PJ, Jansen AC, Kros JM, Verdijk RM, Milošević NJ, Fornerod M, Mastroberardino PG, and Mancini GMS. TMX2 is a crucial regulator of cellular redox state, and its dysfunction causes severe brain developmental abnormalities. *Am J Hum Genet* 105: 1126–1147, 2019.

96. Wajih N, Hutson SM, and Wallin R. Disulfide-dependent protein folding is linked to operation of the vitamin K cycle in the endoplasmic reticulum. A protein disulfide isomerase-VKORC1 redox enzyme complex appears to be responsible for vitamin K1 2,3-epoxide reduction. *J Biol Chem* 282: 2626–2635, 2007.

97. Wittig I, Braun HP, and Schagger H. Blue native PAGE. *Nat Protoc* 1: 418–428, 2006.

98. Wu Y and Essex DW. Vascular thiol isomerases in thrombosis: the yin and yang. *J Thromb Haemost* 18: 2790–2800, 2020.

99. Zayed H, Chao R, Moshrefi A, Lopezjimenez N, Delaney A, Chen J, Shaw GM, and Slavotinek AM. A maternally inherited chromosome 18q22.1 deletion in a male with late-presenting diaphragmatic hernia and microphthalmia-evaluation of DSEL as a candidate gene for the diaphragmatic defect. *Am J Med Genet A* 152a: 916–923, 2010.

100. Zhang X, Gibhardt CS, Will T, Stanisz H, Körbel C, Mitkovski M, Stejerean I, Cappello S, Pacheu-Grau D, Dudek J, Tahbaz N, Mina L, Simmen T, Laschke MW, Menger MD, Schön MP, Helms V, Niemeyer BA, Rehling P, Vultur A, and Bogeski I. Redox signals at the ER-mitochondria interface control melanoma progression. *EMBO J* 38: e100871, 2019.

101. Zhao Z, Wu Y, Zhou J, Chen F, Yang A, and Essex DW. The transmembrane protein disulfide isomerase TMX1 negatively regulates platelet responses. *Blood* 133: 246–251, 2019.

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Abbreviations Used

AChR = acetylcholine receptor
ALS = amyotrophic lateral sclerosis
CAGE = Cap Analysis of Gene Expression
cDNA = complementary DNA
CXXC = Cys-X-X-Cys
DPR = dipeptide-repeat
ER = endoplasmic reticulum
GTEx = Genotype-Tissue Expression
HD = Huntington's disease
HPA = Human Protein Atlas
MAM = mitochondria-associated membranes
MGI = Mouse Genome Informatics
mHTT = mutant huntingtin protein
MKS = Meckel–Gruber syndrome
mRNA = messenger RNA
NFAT1 = nuclear factor of activated T cells 1
NX = normalized expression
OMIM = Online Mendelian Inheritance in Man
PDI = protein disulfide isomerase
Ran = Ras-related nuclear protein
RIP = ribosome-inactivating protein
ROS = reactive oxygen species
SERCA = sarco-ER Ca^{2+} transport ATPase
TMX = thioredoxin-related transmembrane protein
TRIAP1 = TP53-regulated inhibitor of apoptosis 1
TRX = thioredoxin
UPR = unfolded protein response
VKOR = vitamin K epoxide reductase