Thioredoxin-Related Transmembrane Proteins

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The endoplasmic reticulum (ER) is site of synthesis and maturation of membrane and secretory proteins in eukaryotic cells. The ER contains more than 20 members of the Protein Disulfide Isomerase (PDI) family. These enzymes regulate formation, isomerization and disassembly of covalent bonds between cysteine residues. As such, PDIs ensure protein folding, which is required to attain functional and transport-competent structure, and protein unfolding, which facilitates dislocation of defective gene products across the ER membrane for ER-associated degradation (ERAD). The PDI family includes over a dozen of soluble members and few membrane-bound ones. Among these latter, there are five PDIs grouped in the thioredoxin-related transmembrane (TMX) protein family.

endoplasmic reticulum protein folding ERAD PDI TMXs

1. Introduction

About one third of the proteome in eukaryotic cells is made of membrane and secretory proteins [1]. Their production and maturation occurs within the ER with help and under surveillance of resident chaperones and folding enzymes, such as the members of the PDI family [2]. PDIs assist protein folding by catalyzing the formation of the native set of intra- and inter-molecular disulfide bonds (oxidation); they can also correct structural errors by disassembling non-native disulfides to promote their conversion into the native set (isomerization) [3][4]; they can facilitate the translocation across the ER membrane of terminally misfolded polypeptides by dissolving intra- and inter-molecular disulfide bonds (reduction), in a step that precedes their degradation by cytosolic 26S-proteasomes [5][6]. In addition to these activities, PDIs can also act as regulators of the luminal calcium homeostasis [7] and participate to multimeric structures such as the prolyl 4-hydroxylase [8] or the oligosaccharyltransferase complexes [9].

More than 20 PDI family members have been identified, so far [10]. The reasons for such a high number is not fully understood. However, their tissue distribution, membrane topology, and organization of the active site hint at client-specificity and high functional versatility [11]. Most PDI family members are soluble in the ER lumen, with few membrane-anchored exceptions [4]. The TMX protein family comprises five membrane-tethered PDIs (TMX1, TMX2, TMX3, TMX4 and TMX5) [12][13][14][15][16] (Figure 1 and Table 1). These proteins are all characterized by an N-terminal signal sequence for ER targeting and one catalytically active thioredoxin (TRX)-like domain (known as type-a TRX-like domain), containing the active site. TMX1, the best characterized member of the TMX family, preferentially interacts with membrane-bound folding-competent and folding-defective clients [17][18]. In contrast, the other members of the family have been poorly studied, if at all.

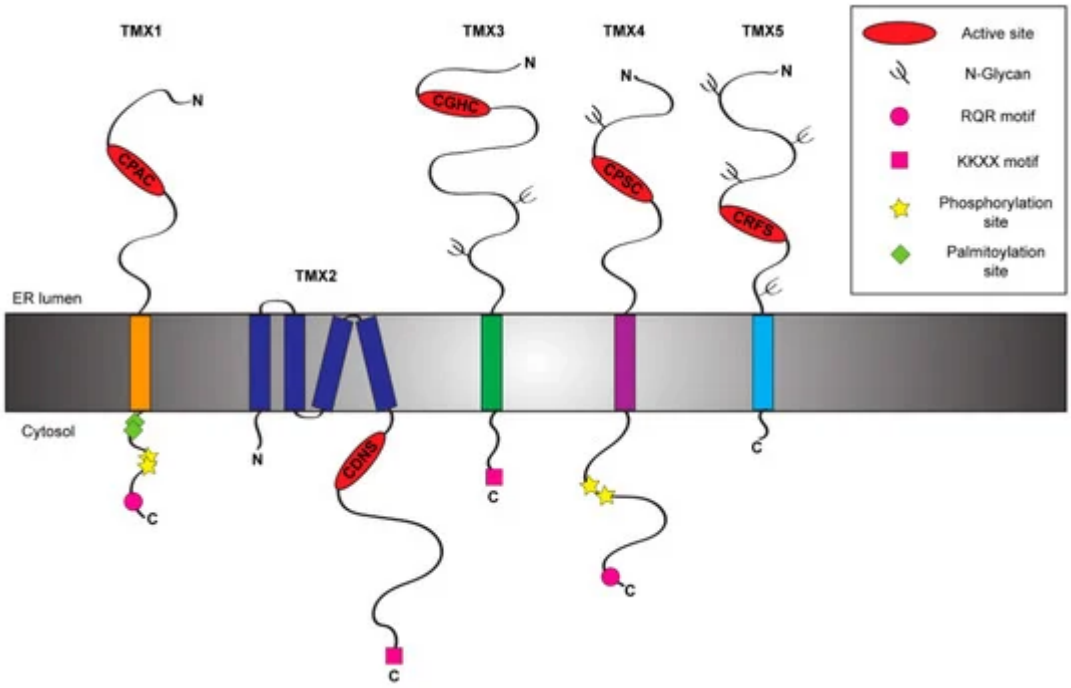

Figure 1. Schematic representation of the TMX protein family members. The figure shows the topology and the main structural and functional features of the five members of the TMX family [12][13][14][15][16].

Table 1. List of the TMX family members. The table displays the main features of the five TMXs including their active site sequences and biological functions. a, active type-a TRX-like domain; b, inactive type-b TRX-like domain; R, reductase activity; O, oxidase activity.

Protein	TRX-like domains	Active site	Activities	Biological functions
TMX1	a	CPAC	R	Protein folding and ERAD Ca^{2+} flux regulation
TMX2	a	SNDC	?	Nuclear protein import Ca^{2+} flux regulation
TMX3	abb'	CGHC	O	?
TMX4	a	CPSC	R	Protein folding
TMX5	a	CRFS	?	?

2. TMX1: A Topology-Specific ER-Resident Reductase

TMX1 (other name TXNDC1) is the best-known member of the TMX family. It has been identified in 2001 by Matsuo and colleagues [16] among the genes up-regulated by TGF-β [19]. TMX1 is a single-pass type I protein of 280 residues with a large luminal N-terminal region harboring a TRX-like domain and a short cytosolic tail [16] (Figure 1). TMX1 displays a di-arginine motif that ensures its retention within the ER [16][20][21] (Figure 1). The cytosolic tail of TMX1 also contains both palmitoylation [22] and phosphorylation sites [23] (Figure 1). These modifications affect the sub-ER localization of TMX1 and may determine the spectrum of clients [21]. TMX1 is ubiquitously expressed in human tissues, with the highest levels in kidney, lungs, placenta and liver [16]. Unlike other members of the PDI family, TMX1 does not contain an ER stress-responsive element (ERSE) within its promoter region [24] and indeed it is not up-regulated upon ER stress [25]. Deletion of the TMX1 gene is innocuous at the cellular level. This suggests the activation of compensatory mechanisms in cultured cells, where other members of the PDI family may play a role [26]. At the organism level, however, the absence of TMX1 has consequences, as it increases susceptibility to liver damage induced by lipopolysaccharides [26]. From a functional point of view, TMX1 displays a non-canonical CPAC active site in its type-a TRX-like domain [16] (Figure 1 and Table 1). The proline in position 2 suggests a role as reductase [27], since it destabilizes the disulfide state and favors the di-thiol reduced form of the active site [28]. Consistently, TMX1 is predominantly reduced in vivo [25], and in vitro it reduces insulin disulfides [16]. Of additional support to the putative function of TMX1 as an ER reductase, it has been demonstrated that TMX1 overexpression enhances the cytotoxicity of the toxins ricin and abrin, two type-2 ribosome-inactivating proteins requiring a step of reduction in the ER before the dislocation of the catalytic subunits to the cytosol [29]. TMX1 has been also found to interact with vitamin K epoxide reductase (VKOR), an enzyme involved in the process of blood coagulation working with membrane-tethered TRX-like proteins, which serve as redox partners [30].

From the functional point of view, TMX1 represents the first example of topology-specific redox catalyst involved in both protein folding and ERAD pathways [17][18]. Indeed, TMX1 recruits membrane-bound N-glycosylated clients through a cooperative interaction with the ER lectin CNX catalyzing their folding [17]. Moreover, TMX1 reductase activity is required for the clearance of membrane-tethered faulty gene products from the ER [18].

In addition to its role as topology-selective reductase in the folding and degradation pathways [17][18], palmitoylated TMX1 localizes at ER-mitochondria contact sites, aka MAM (mitochondria-associated membranes), to regulate Ca^{2+} flux between ER and mitochondria [31].

3. TMX2 and its Cytosolic Active Site

Among TMX family members, TMX2 (alternative name TXNDC14) undoubtedly is the most mysterious. It is a non-glycosylated protein of 296 amino acids, which has been identified in 2003 upon cloning from a fetal cDNA library [32]. The topology of TMX2 has been only recently characterized. Initially, it has been described as a type I membrane protein [32]. A recent study clarified its topology showing that TMX2 is a multi-spanning protein

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displaying both the N- and the C-terminal regions within the cytosol [12] (Figure 1). As such, the peculiar SNDC catalytic site of TMX2 is oriented towards the cytosol [12], in contrast with the other members of the TMX family (Figure 1). The long C-terminal tail of TMX2 contains a canonical ER retention signal (-KKDK) [32] (Figure 1). TMX2 is localized in different ER sub-compartments, such as in the nuclear outer membrane [12], or at the MAM [22]. TMX2 expression is ubiquitous with the highest levels in brain, heart, liver, kidney and pancreas [32]. Moreover, TMX2 is up-regulated upon oxidative stress, but not upon hypoxia, heat shock, or ER stress, consistently with the lack of an ERSE motif within its promoter region [12]. TMX2 gene deletion in mice is embryonic lethal, implying a crucial role of this protein at early stages of development [33]. So far, no information is available on the physiologic function of TMX2 (Table 1) with the exception of a possible participation of TMX2 in the importin-β:Ran complex that controls nuclear targeting of select cargo proteins [12][34]. The localization of TMX2 in the outer nuclear membrane, which is contiguous to the ER membrane, is crucial for the binding of the importin-β:Ran complex and the maintenance of the nucleocytoplasmic Ran protein gradient, whereas mutations in the TMX2 active site only partially impair them [12]. TMX2 can also localize at the MAM [22], where it forms a functional complex with CNX and the Ca^{2+} pump SERCA2 to modulate the calcium flux [33] in a function that mimics the one reported for TMX1 [35] and that could be exerted in a tissue-specific manner. Consistently with its high expression in brain tissue, missense mutations of TMX2 have recently been associated with brain developmental abnormalities [33] and microlissencephaly [36], a rare congenital brain disorder [37]. This phenotype could result from a loss of the protective role of TMX2 from ER stresses [38], which represents an important concurrent cause of neuronal death [39].

4. TMX3, a Classic PDI

TMX3 is a single-pass type I glycoprotein of 454 amino acids (Figure 1), which has been identified in 2005 among the uncharacterized proteins containing the consensus sequence for a TRX-like domain [13]. This PDI family member, also known as TXNDC10, displays within its large N-terminal region two N-glycosylation sites (Figure 1) and three TRX-like domains, one catalytically active type-a domain followed by two inactive type-b (b and b′) domains [40]. Additionally, its C-terminal region contains a classical KKKD retention sequence [13] (Figure 1). TMX3 transcripts have been found in a great variety of tissues with the highest levels in heart and skeletal muscle [13]. Consistent with other TMX family members, TMX3 does not contain an ERSE motif and it is not upregulated upon ER stress [13]. The type-a TRX-like domain of TMX3 is characterized by a canonical CGHC sequence [13] (Figure 1 and Table 1), which corresponds to the catalytic sequence of PDI [41]. TMX3 acts as an oxidase in vitro [13] and its b′ domain is possibly involved in substrate recruitment [40]. The precise role of TMX3 in cells has not been established, yet. Preliminary studies show a protective function of TMX3 against neuronal atrophy in mice models for Huntington's disease [42], a progressive brain disorder caused by an inherited CAG trinucleotide repeat expansion in the huntingtin (HTT) gene [43]. The molecular basis of this protective effect is unclear, also because HTT is a cytosolic protein and a direct interaction with the functional portion of TMX3 can be ruled out. Since it has been shown that the expression of mutated HTT triggers ER stress, an hypothesis is that TMX3 protects cells against neuronal atrophy mitigating the stress situation [44]. Both deletion and missense mutations in TMX3 gene

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have been linked to coronary artery disease [44] and Leber's congenital amaurosis [45], a disease associated with retarded growth of the eye [46].

5. TMX4, the Paralogue of TMX1

TMX4 (alternative name TXNDC13) is a single-pass type I glycoprotein (Figure 1) of 349 amino acids that has been identified in 2010 during a database search for TRX-like domain containing proteins [14]. Phylogenetic analysis showed that TMX4 represents the paralogue of TMX1 [21], with whom it shares high similarity within the N-terminal luminal regions, despite the presence of an N-glycosylation site [14][21] and a di-arginine ROR retention motif within the C-terminal domain [21]. Both proteins display two phosphorylation sites within the cytosolic domain [23] (Figure 1), which could modulate sub-ER localization upon recruitment of select binding partners [21]. TMX4 expression is ubiquitous with the highest levels in heart tissue [14]. Consistently with the lack of an ERSE motif within its promoter region, TMX4 is not up-regulated during ER stresses [14]. TMX4 has one luminal type-a TRX-like domain, which contains a non-canonical CPSC active site [14] (Figure 1 and Table 1). The proline in position 2 hints at a possible role in protein folding [37]. Consistently, TMX4 efficiently reduces insulin disulfides [14]. Additionally, TMX4 also has the ability to pair cysteines [14], even though its activity is weaker compared to the one established by VKOR and TMX1 [30]. From the functional point of view, no evidence has been reported on substrate preference and possible roles of TMX4 in cells, even though different hypothesis have been formulated. The structural similarities with TMX1 strongly hint at a role of TMX4 as ER reductase possibly acting in both protein folding and degradation pathways.Finally, a recent study showed that TMX4 also distributes at the inner membrane of the nuclear envelope (NE) with the N-terminal portion facing the NE lumen [47]. Since many protein complexes resident at the NE (including the LINC/Torsin A complex) are modulated by redox cycles, a role for TMX4 in the regulation of the NE structure can be envisioned [47].

6. TMX5, a Natural Trapping Mutant Member of the TMX Family

TMX5 (TXNDC15) is another poorly characterized member of the TMX family. It is a predicted single-span type I protein of 360 amino acids (Figure 1). It has been identified in a large scale protein screening in 2003 [48]. Structurally, TMX5 has a large N-terminal luminal domain and a very short C-terminal cytoplasmic tail of 18 amino acids which lacks basic residues, characteristic of tail-anchored proteins [48]. TMX5 could potentially leave the ER and traffic through the secretory pathway. Ubiquitously expressed TMX5 is a putative N-glycosylated protein disulfide isomerase, and mutations in TXNDC15 have been found to cause a rare form of lissencephaly [49]. TMX5 possesses four cysteine residues and one type-a TRX-like domain within its N-terminal luminal portion. The core of its TRX-like domain is represented by a non-canonical CRFS active site. The absence of the C-terminal cysteine residue within its active site defines TMX5 as a natural trapping mutant protein [49]. Indeed, the N-terminal cysteine of the TMX5 active site can nucleophilically attack free thiol group in protein substrates, but the interaction can be resolved only by the intervention of an external cysteine provided by another PDI or by the protein substrate itself. As such, the mixed disulfide between TMX5 and client proteins results stabilized and it could be long-living.

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To date, no evidences has been reported on the physiological role of TMX5 gene. However, recently mutations in TMX5 have been associated with the development of the Meckel-Gruber syndrome (MKS), a rare perinatally lethal autosomal recessive disease caused by defective ciliogenesis [50]. Deletions and missense mutations result in the generation of truncated forms of TMX5 that do not localize within primary cilium or periciliary regions as the wild type [51][52][53]. Thus, the mis-localization or the premature degradation of TMX5 might correlate with the onset of such ciliopathies. Indeed, it has been found that patients' derived mutated fibroblasts as well as cells subjected to siRNA knockdown have a reduced number of ciliated cells, abnormal ciliary morphology and an aberrant localization to the transition zone of TMEM67 [52][54], a crucial regulator of cilia function [54].

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7. Conclusion

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We recapitulate the current knowledge about the features and roles of the members of the TMX family. These are five membrane-tethered PDIs, which are characterized by an ER signal sequence and a type-a TRX-like domain. Despite their similarities, the TMXs also show some structural differences, which could hint at a certain degree of functional specialization among the different members of the TMX family. Future dissection of their individual roles are needed to enlarge our knowledge about PDIs functions, and to allow the comparison between the members of the same TMX family and between membrane-tethered and soluble PDIs.

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