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Critical Review

The Sep15 Protein Family: Roles in Disulfide Bond Formation and Quality Control in the Endoplasmic Reticulum

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Summary

Disulfide bonds play an important role in the structure and function of membrane and secretory proteins. The formation of disulfide bonds in the endoplasmic reticulum (ER) of eukaryotic cells is catalyzed by a complex network of thiol-disulfide oxidoreductases. Whereas a number of ER-resident oxidoreductases have been identified, the function of only a few of them is firmly established. Recently, a selenocysteine-containing oxidoreductase, Sep15, has been implicated in disulfide bond assisted protein folding, and a role in quality control for this selenoprotein has been proposed. This review summarizes up-to-date information on the Sep15 family proteins and highlights new insights into their physiological function.

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Keywords Selenoprotein; disulfide bond formation; endoplasmic reticulum; protein folding; thiol-disulfide oxidoreductase.

INTRODUCTION

In eukaryotes, disulfide bonds are introduced into proteins primarily within the oxidizing environment of the endoplasmic reticulum (ER). The process of disulfide bond formation is assisted by thiol-disulfide oxidoreductases, redox-active enzymes that catalyze thiol-disulfide interchange. In addition to formation of disulfide bonds, these enzymes are also able to reduce and rearrange incorrectly formed disulfides ensuring proper folding of the substrate proteins. Active sites of many thiol-disulfide oxidoreductases contain two cysteine residues that are arranged in a characteristic C*xx*C redox motif (two cysteines separated by two other residues). The identity of the two amino acids that lie between the active-site cysteine residues determines the redox potential of the enzyme, which in turn defines its function as oxidase, reductase or isomerase (*1*). In addition, some thiol-disulfide oxidoreductases contain C*xx*C-derived motifs, in which one of the cysteines may be replaced by selenocysteine, serine, or threonine (*2*).

In mammals, PDI and ERp57 have been shown to catalyze the formation and rearrangement of disulfide bonds in secreted proteins (*1, 3*). While in the periplasmic space of *Escherichia coli* two separate pathways for oxidation and reduction/isomerization exist, thiol-disulfide oxidoreductases involved in reduction of disulfide bonds in eukaryotic cells have not been identified (*4*). Structural and biochemical analysis of members of the Sep15 family proteins revealed that these ER-resident oxidoreductases can be involved in reduction or isomerization of disulfide bonds. A model for the possible physiological roles of Sep15 in formation of disulfide bonds and quality control in the endoplasmic reticulum is discussed in this review.

THE SEP15 PROTEIN FAMILY

Selenium is an essential trace element that is incorporated into proteins in the form of selenocysteine. This rare amino acid has been shown to occur in place of one of the active-site cysteines in Sep15 and SelM, two recently reported eukaryotic thiol-disulfide oxidoreductases localized to the ER (*5, 6*). Sep15 and SelM share 31% sequence identity and form a distinct selenoprotein family. Both Sep15 and SelM are highly conserved and are found in organisms from green algae to humans. In mammals, these selenoproteins have different, but overlapping tissue expression patterns. The highest levels of Sep15 gene expression are observed in prostate, liver, kidney and testes, while SelM is expressed predominantly in brain. Moreover, Sep15 protein levels are regulated by dietary selenium, even though in some organs, for example in testes,

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its expression is less responsive to selenium availability (*7, 8*). Being a selenoprotein, SelM is also likely regulated by selenium, but details of this regulation are not known. Consistent with the ER localization, Sep15 and SelM encode an N-terminal signal peptide, which is cleaved in the mature proteins (*6, 9*). Whereas SelM is likely retained in the ER by a C-terminal H/R/K-X-DL tetrapeptide, Sep15 lacks a typical ER retention signal suggesting that it is maintained in this cellular compartment by a different retention mechanism.

More recently, a new member of Sep15 protein family has been identified (*10*). This selenoprotein is found only in fish and was designated as Fep15 (for fish Sep15-like protein). Fep15 is also targeted to the ER by its N-terminal signal peptide and has a C-terminal ER retention signal (RDEL). Although Sep15, SelM and Fep15 share regions of significant sequence identity, they have several unique features that may specify differences in their substrate specificity and/or physiological functions. Multiple sequence alignment of the Sep15 family members revealed that SelM and Fep15 have an elongated C-terminus, whereas Sep15 possesses a distinct cysteine-rich N-terminal domain (Fig. 1). In contrast to SelM, in which the active-site cysteine and selenocysteine are organized into highly conserved C*xx*U motif (U is selenocysteine), Sep15 possesses an unusual motif, in which cysteine and selenocysteine are separated by only one amino acid (C*x*U). Moreover, Fep15 encodes a single selenocysteine residue (U) and has valine in place of the conserved cysteine. Remarkably, Fep15 does not have any conserved cysteines, and in some of the Fep15 sequences cysteines are not present at all.

THIOL-DISULFIDE OXIDOREDUCTASE FUNCTION

Thiol-disulfide oxidoreductases often contain one or more thioredoxin-like domains with a characteristic redox-active motif. The structures of Sep15 from *Drosophila melanogaster*, which naturally encodes a cysteine residue in place of selenocysteine, and the U48C mutant of SelM from *Mus musculus* have been solved by nuclear magnetic resonance

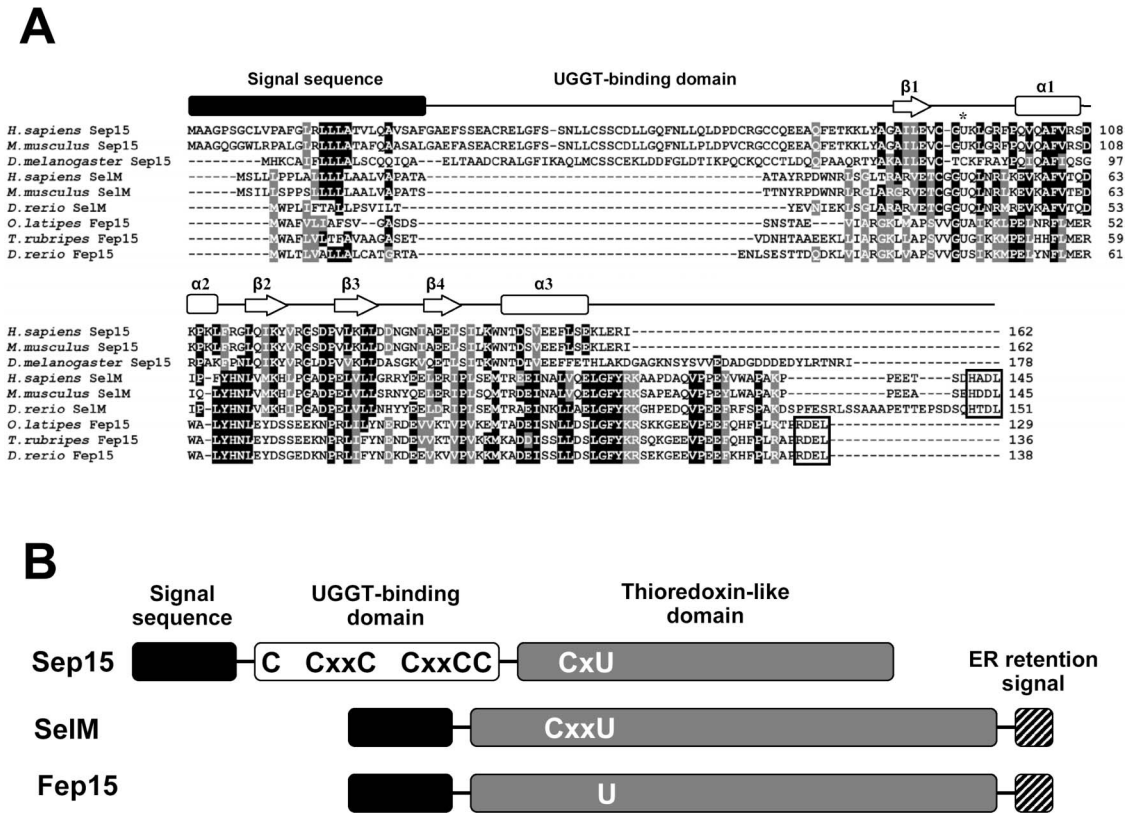

Figure 1. Alignment of the Sep15 family members. (A) Multiple sequence alignment of Sep15, SelM and Fep15 proteins. Identical residues are shaded in black and similar residues in gray. The genetically encoded selenocysteine (U) residues and the corresponding cysteine residue found in the *D. melanogaster* Sep15 are marked with an asterisk. Predicted signal sequences and secondary structure elements are shown at the top. ER retention signals of SelM and Fep15 are highlighted with solid-line boxes. (B) Schematic representation of domain arrangement of Sep15, SelM and Fep15. The Sep15 family proteins share an N-terminal signal sequence (colored black) and a common thioredoxin-like domain (colored gray). The cysteine-rich N-terminal extension of Sep15 has been labeled as the UGGT-binding domain.

(NMR) (*7*). The structural studies revealed that both Sep15 and SelM have a two layer α/β-fold with a central β-sheet surrounded by α-helices, typical of thioredoxin-like proteins (Fig. 2A). Similar to other oxidoreductases with thioredoxin-like domains, the active-site redox motifs of Sep15 and SelM are located in a loop between the C-terminus of strand β1 and the N-terminus of helix α1. Moreover, under oxidizing conditions, cysteines in the redox-active motifs of both proteins formed intramolecular disulfide bonds suggesting that these residues are able to form reversible disulfides during the catalytic cycle of oxidation and reduction (Fig. 2B).

Although Sep15 and SelM are structurally similar to the thioredoxin superfamily, they form a distinct evolutionary family. In contrast to thioredoxin, the active-site cysteine residues in both proteins are surface accessible, as found in an a-domain of protein disulfide isomerase. In addition, the consensus sequences of the active-site motifs in Sep15 (CGU) and SelM (CGGU) are different from those of thioredoxins (CGPC), protein disulfide isomerases (CGHC) and disulfide oxidases (CPHC) (*1, 11*). Many structurally defined members of the thioredoxin superfamily have a conserved *cis*-proline residue placed near the N-terminus of strand β3 (*11*). Although Sep15 and SelM do have prolines located at similar positions, they are in the *trans*-conformation. Moreover, a charge pair that is involved in the proton transfer reaction in thioredoxin (*1, 12*) and protein disulfide isomerase (*1, 13*) is not found in Sep15 and SelM.

The presence of thioredoxin-fold together with the location of the redox motifs implied a thiol-disulfide oxidoreductase function for Sep15 and SelM. Whether oxidoreductase is involved in the formation, reduction or isomerization of disulfides, may be predicted from its equilibrium redox potential. The redox potential of fruit fly Sep15 [-225 mV (*7*)] lies between the redox potentials of protein disulfide isomerase [-175 mV (*14*)] and disulfide reductase thioredoxin [-270 mV (*14, 15*)] suggesting that Sep15 may plausibly catalyze the isomerization or reduction of disulfide bonds (*1*).

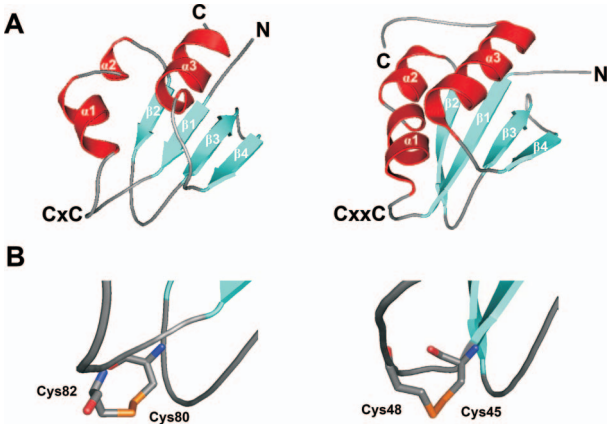

Figure 2. Solution NMR structures of the redox-active domain of *D. melanogaster* Sep15 and U48C mutant of *M. musculus* SelM. (A) Ribbon representation of the Sep15 (left panel, PDB accession code 2A4H) and SelM (right panel, PDB accession code 2A2P) structures. α-Helices (α1–α3) are shown in red, β-strands (β1–β4) in blue, and loops in gray. The locations of the redox-active motifs for Sep15 (C*x*C) and SelM (C*xx*C) are indicated. Residues 62–70 and 150–178 of Sep15 as well as residues 25–34 and 121–145 of SelM are not shown because these regions are flexible. (B) Close view of the Sep15 (left) and SelM (right) active sites. The amino acid side chains of the two active-site cysteine residues are shown as stick models. This figure was prepared with PyMol (*30*).

ROLE IN QUALITY CONTROL

Recent studies identified UDP-glucose:glycoprotein glucosyltransferase (UGGT) as a binding partner of Sep15. UGGT is a large ER chaperone that regulates the calnexin (CNX) cycle (*9*). The CNX cycle is an ER-based quality control pathway that specifically assists in the folding of N-linked glycoproteins (*16*). UGGT is also a participant in this quality control pathway and functions as the folding sensor that recognizes partially unfolded or improperly folded glycoproteins (Fig. 3). The regulation involves the UGGT-catalyzed transfer of glucose residue from UDP-glucose to the glycan core (*17*). This reaction creates a signal that keeps the substrate in the ER and promotes binding of membrane-bound CNX and its lumenal homologue, calreticulin (CRT), to the glycan (*18–21*). In turn, this triggers the binding of a lumenal protein disulfide isomerase, ERp57, to CNX and CRT, and accelerates folding by catalyzing disulfide bond exchange (*22–26*).

The role of Sep15 in this process is not known. However, the tight association of Sep15 and UGGT, in 1:1 ratio and with apparent K_d of 20 nM, suggests that Sep15 may either modulate the enzymatic activity of UGGT or be involved in assessing disulfide bonding or thiol/disulfide state of the UGGT substrates (*27*).

In addition to binding UGGT and ER location, the role of Sep15 in quality control in the ER is supported by the observation that expression of Sep15 is elevated by treatments that activate the unfolded protein response (UPR) (Labunskyy and Gladyshev, unpublished data). UPR is a signaling pathway activated in response to accumulation of unfolded proteins in the ER (*28*). This pathway leads to a block in synthesis of cytosolic proteins and enhanced expression of proteins that facilitate protein folding. In essence, UPR helps cells remove incorrectly folded proteins in the ER of stressed cells.

It is possible that, in contrast to general protein disulfide isomerases characterized by broad substrate specificity, Sep15 targets a restricted group of proteins that are substrates of UGGT. This situation may be similar to that of protein

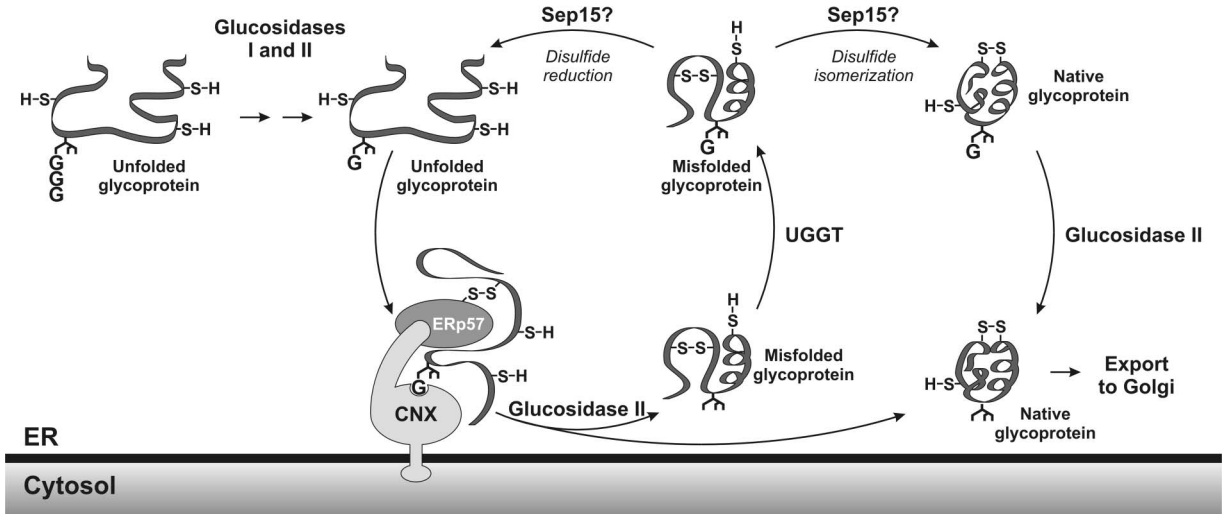

Figure 3. A model for disulfide bond formation and quality control in the ER. After removal of the two outermost glucose residues (G) from the N-linked oligosaccharide by glucosidases I and II, a newly synthesized glycoprotein binds to CNX or CRT (for simplicity only CNX is shown). ERp57, a lumenal protein disulfide isomerase associated with CNX (and CRT), catalyzes the formation of disulfide bonds in the unfolded glycoprotein substrate. When the remaining glucose residue is cleaved by glucosidase II, the glycoprotein dissociates. If the protein is properly folded, it is transported to Golgi. However, misfolded glycoprotein is reglucosylated by UGGT allowing it to reenter the CNX/CRT cycle. Sep15 is potentially involved in either rearrangement of disulfide bonds (isomerase function) or reduction of incorrectly formed disulfide bonds (reductase function) in misfolded glycoproteins bound to UGGT.

disulfide isomerase ERp57, which was reported to function as a disulfide isomerase exclusively for partially folded glycoproteins that are bound to the chaperones CNX and CRT (*22, 25, 29*). If so, UGGT, by recognizing partially folded glycoproteins, may serve as a primary binding site for Sep15 substrates, which may be analogous to the role of non-catalytic b′-domain of human protein disulfide isomerase (Fig. 3). Consistent with this possibility is the observation that Sep15 is kept in the ER by association with UGGT rather than by a C-terminal retention signal. For this purpose, Sep15 has an N-terminal UGGT-binding domain, which is missing in SelM and Fep15. The binding partners of these latter proteins are not known.

PERSPECTIVES

Selenoproteins have not been previously shown to participate in the quality control networks involved in assessing structural fidelity of proteins, or generally in the ER function. Although emerging evidence suggests that members of the Sep15 protein family may participate in the reduction or rearrangement of incorrectly formed disulfide bonds in misfolded glycoproteins, further studies are required in order to clarify these potential roles.

Despite the significant recent progress in characterizing members of the Sep15 family, the physiological function and catalytic mechanism of Sep15 remain poorly understood. The major obstacle is our lack of knowledge about its specificity and identity of endogenous donors/acceptors of reducing equivalents. Identification of potential target proteins may aid in the development of assays with endogenous substrates.

Whereas Sep15, SelM and Fep15 share regions of significant sequence identity, it will be useful to determine whether the different enzymes have overlapping or distinct substrate specificities and functions. Finally, in order to address the role of Sep15 family proteins in the quality control of protein folding, it would be necessary to test whether inactivation of Sep15 and/or SelM would lead to accumulation of unfolded proteins in the ER.

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