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Review

A review of ERGIC-53: Its structure, functions, regulation and relations with diseases

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Summary. ERGIC-53 is a type I transmembrane protein. It includes an N-terminal signal sequence, a carbohydrate recognition domain, which is calcium-dependent and pH-sensitive, a stalk region, a transmembrane domain, and a short cytoplasmic domain; ERGIC-53 mainly acts as a receptor of a limited number of glycoprotein and transports them from ER to ERGIC and Golgi, meanwhile it has a secondary glycoprotein quality control function. Recent research has revealed that UPR, heat shock and VIPL may regulate the expression of ERGIC-53. F5F8D, and some ER storage diseases have relationship with ERGIC-53.

Key words: ERGIC-53, MCFD2, F5F8D

Introduction

ER-Golgi intermediate compartment-53 (ERGIC-53), also called LMAN1, is a 53-kDa nonglycosylated type I single-spanning transmembrane protein that has been established as a marker protein for ER-Golgi intermediate compartment (ERGIC). As a member of the L-type animal lectin family, it is ubiquitously expressed and concentrated in ERGIC, and also locates at rough ER, cis-Golgi, and sometimes cell surface when it is overexpressed. It is a mannose-specific membrane lectin that cycles rapidly between ER, ERGIC and cis-Golgi, operating as a cargo receptor for the transport of a limited subset of glycoproteins.

ERGIC-53 exist extensively in many species, such as *Homo sapiens*, rat, mice, dog, monkey, *Xenopus laevis*, and *Saccharomyces cerevisiae* (Eiler et al., 2000). Human ERGIC-53 (hERGIC-53) is almost identical to MR60 from HL60 (Arar et al., 1995), differing only in one amino acid residue, and 89% identical to rat p58 /ERGIC-53 (Lahtinen et al., 1995) at amino acid level. Homologue of ERGIC-53 includes leguminous plant lectins, VIP36, VIPL (Neve et al., 2003), and ERGL.

hERGIC-53 gene, localized at 18q21.3-18q22, whose mRNA is 2768bp (X71661, Genebank of Medline), contains 13 exons, ranging in size from 59 bp (exon 7) to greater than 1,244 bp (exon 13). Exon 1 is composed of at least 21 bp of 5' UTR (untranslated region).

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Structure

The deduced polypeptide chain for hERGIC-53 consists of 510 amino acids (M(r) 54217), including an N-terminal signal sequence of 30 residues, a carbohydrate recognition domain (CRD) of 240 residues, a stalk region of 210 residues, a transmembrane domain (TMD) of 18 residues, and a short cytoplasmic domain of 12 residues (Fig. 1). The crystal structure of rat p58 /ERGIC-53 has shown its surface features (Fig. 2) (Velloso et al., 2002), which revealed more features of ERGIC-53.

Cytoplasmic domain

The amino acid sequence of the hERGIC-53 cytoplasmic domain is RSQQEAAAKKFF. It possesses four determinants which are required for correct targeting within the ER-ERGIC-cis-Golgi recycling pathway: (1) The double lysines in position -3 (K508) and -4 (K507) from the C-terminus act as a ER retention (pre-Golgi retention) and ER retrieval signal (Schindler et al., 1993). They interact with COPI (type I coat protein complex), and are required for efficient endocytosis of hERGIC-53 from cell surface, when

hERGIC-53 is overexpressed (2). The double phenylalanine motif in position -1 (F510) and -2 (F509) is an ER-exit determinant, and so it weakens the retrieval signal significantly, such that at steady state a substantial portion of hERGIC-53 is localized outside the ER, and overexpression results in cell surface appearance. The double phenylalanine motif also has direct interaction with COPII (type II coat protein complex). It is F509 that binds to the Sec23p component of Sec23p.Sec24p subcomplex of COPII (Kappeler et al., 1997), which mediates ER-exit of hERGIC-53 (Kappeler et al., 1997). However, evidence showed the FF ER-exit motif of hERGIC-53 can be functionally substituted by two tyrosines, leucines, isoleucines or valines. Further analysis revealed three different minimal transport motifs: a single phenylalanine or tyrosine at position -2, two leucines or isoleucines at positions -1 and -2, or a single valine at position -1 (Nufer et al., 2002). (3) Q501 is a helpful element for FF motif on ER-exit. It is not directly involved in COPII binding, but rather assists in the optimal presentation of F_{509} to the COPII machinery (Nufer et al., 2003a). (4) The C-terminal tetrapeptide KKFF is an internalization motif of hERGIC-53, and it must be at the C-terminus for efficient internalization. Two arginines or a single lysine can partially replace the two lysines in KKFF, but these mutants did not reach the wild-type internalization rate. The double phenylalanines can be changed to tyrosines in position -1 and -2, in addition to charged residues (lysines or arginines) in -3 and -4 (Itin et al., 1995).

CDR

CDR is the place where ERGIC-53 interacts with its cargo. The 3-D structure of the CRD of rat p58/ERGIC-53 confirms its structural similarity to legume lectins. The CRD domain of p58/ERGIC-53 has an overall globular shape and is composed of 15 ,-strands, a small · helix, and one turn of 310 helix. Two major twisted antiparallel ß-sheets, one seven-stranded (major) ß-sheet, and one six-stranded (minor) ß-sheet pack against each other, forming a ß-sandwich (Velloso et al., 2003) (Fig. 3).

The ß-sandwich-fold shares significant sequence similarity and many structural details with the carbohydrate binding sites of legume L-type lectins. It preferentially binds to D-mannose (Itin et al., 1996) and recognizes protein-linked high mannose-type oligosaccharides *in vivo* (Appenzeller-Herzog et al., 1999), and the binding action is calcium-dependent and pH-sensitive.

Calcium dependent

Unlike legume lectins whose CDR binds a Mn^{2+} and a Ca^{2+}, both rat p58/ERGIC-53 and hERGIC-53 bind to two Ca^{2+} respecively, while their yeast orthologs Emp46p and Emp47p bind to K^+ (Satoh et al., 2006) through their CDRs.

It is believed that the binding of two Ca^{2+} to the CRD of ERGIC-53 induces a localized but large conformational change in the ligand-binding site, and therefore facilitates the binding of the high-mannose glycan present on the cargo proteins. The major structural changes occurring upon Ca^{2+} binding are restricted to two loops, one between residues 161 and 172 (loop 1), and another between residues 179 and 193 (loop 2) (Figs. 4, 5). The extent of the changes to loop 1 can be illustrated by the difference in the position of residue Asn164 between the metal-bound and metal-free forms of p58/ERGIC-53. There is a shift of 7.1Å for the C^{γ} atom for the residue. The second loop (residues 179-193) undergoes a major rearrangement in order to position Asp189 between the M1 and M2 sites. The shifts observed for the C^{α} atom and for the carboxyl group of this residue are 6.2Å and 8.7Å, respectively. His186 undergoes a rearrangement with movements of 2.2Å and 6.7Å for the C^{α} and $N^{\varepsilon 2}$ atoms, respectively, moving the side-chain into the ligand-binding site. Its NÂ2 side-chain atom then corresponds to the position of a main-chain amide group that forms a hydrogen bond to mannose in the leguminous lectins. As a consequence of the conformational changes, the two loops move towards each other, narrowing the ligand-binding site: the distance between the C^{α} atoms of Asn164 and Asp189 is reduced from 17.3Å to 6Å (Velloso et al., 2003).

pH sensitive

Why does ERGIC-53 bind to its cargo in ER while releasing them at ERGIC? Appenzeller-Herzog, and Hans-Peter Hauri found that efficient binding and release is determined by a organellar pH, which is determined by the presence of active H^+ v-ATPase pumps, and the pH switch inflences calcium binding to ERGIC-53 and then indirectly affects the cargo binding activity of ERGIC-53. A conserved histidine may act as the pH-

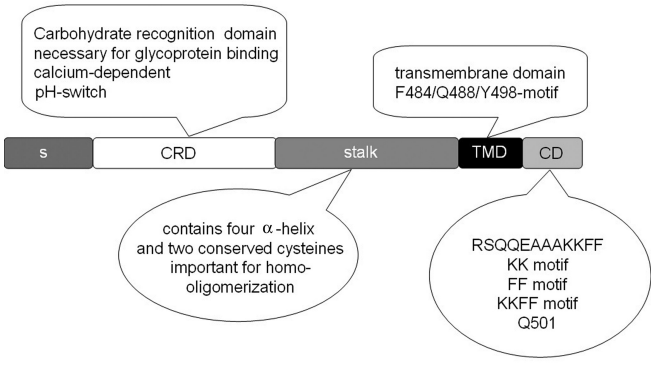

Fig. 1. hERGIC-53 contains 510 amino acids, composed of five regions-N terminal signal region, carbohydrate recognition domain, stalk region, transmembrane domain, and C-terminal cytoplasmic domain (modified from Hauri et al., 2000b).

Ca^{2+} sensor in this process. Thus, a pH-switch model is established (Fig. 6). Ca^{2+}-complexed ERGIC-53 binds its glycoprotein cargo in ER at neutral pH. While the Ca^{2+}-ERGIC-53-cargo complex arrive in ERGIC, His-178 is protonated because of lowered luminal pH, and Ca^{2+} is released. Because Ca^{2+} is required for the cargo binging activity of ERGIC-53, its loss leads to the inactivation of the mannose binding pocket, resulting in the release of its cargo. Subsequently, ERGIC-53 is recycled back to ER, where, with a deprotonated and reactivated CRD, it can start a new round of recycling (Appenzeller-Herzog et al., 2004).

Stalk

ERGIC-53 is a homo-oligomeric transmembrane protein which dimerizes immediately upon synthesis and then forms homohexamers, and only the homohexamer form is functional (Nufer et al., 2003a). The stalk region, which contains four α-helix and two conserved cysteines (C466 and C475 in hERGIC-53, and C473 and C482 in rat p58), plays an indispensable role in this process. The stalk region is absent from both VIP36 and VIPL and it has been reported that these proteins exist as monomers. It was previously believed that, in cells, ERGIC-53 hexamers and dimers exist in roughly equal amounts at steady state, and oligomers are stabilized by the two conserved cysteines of stalk region. ERGIC-53 carrying both cysteines rapidly formed dimers and hexamers, the mutants with a single replacement of either C_{466} or C_{475} only formed dimers. A construct lacking both cysteines remained monomeric (Nufer et al., 2003a). It was concluded that both cysteine residues are required for the formation of the disulfide-linked hexamers, and that only one of them is sufficient for the formation of the disulfide-linked dimers. However, in recent years, researchers found that ERGIC-53 finally exclusively exits in hexamers in cells (Neve et al., 2005) and hexamers exist in two forms (Nufer et al., 2003a; Neve at al., 2005), one as a disulfide-linked complex, and the other as a non-covalent complex made up of three disulfide-linked dimers that are likely to interact through helix-helix interaction of the stalk regions, which each contain 4 α-helix (Neve et al., 2005) or TMDs which each have an α-helix at the lipid-water interphase (Nufer et al., 2003a). In contrast to what was previously believed, neither of the membrane-proximal cysteine residues plays an essential role in the formation, or maintenance of the latter form of hexamers (Neve et al., 2005). What is more, the two cysteine residues are not essential for the intracellular distribution of ERGIC-53 (Neve et al., 2005).

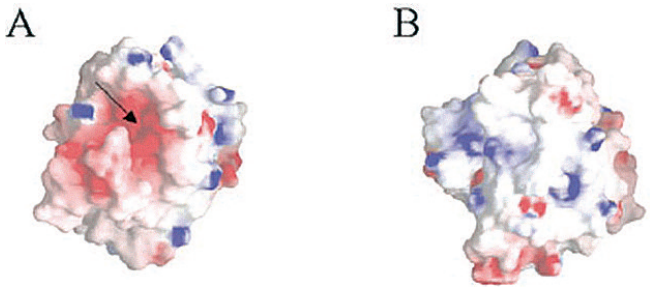

Fig. 2. Surface features of p58/ERGIC-53. A and B, electrostatic surface potential of p58. The two views are related by 180° rotation around a vertical axis in the plane of the paper and show the concave (**A**) and convex (**B**) faces of the molecule. Negative potential is denoted by red, and positive potential is denoted by blue. An arrow indicates the position of the putative ligand binding site, which is called mannose binding pocket (MBP). The maps are contoured at the 10kT level (modified from Velloso et al., 2002).

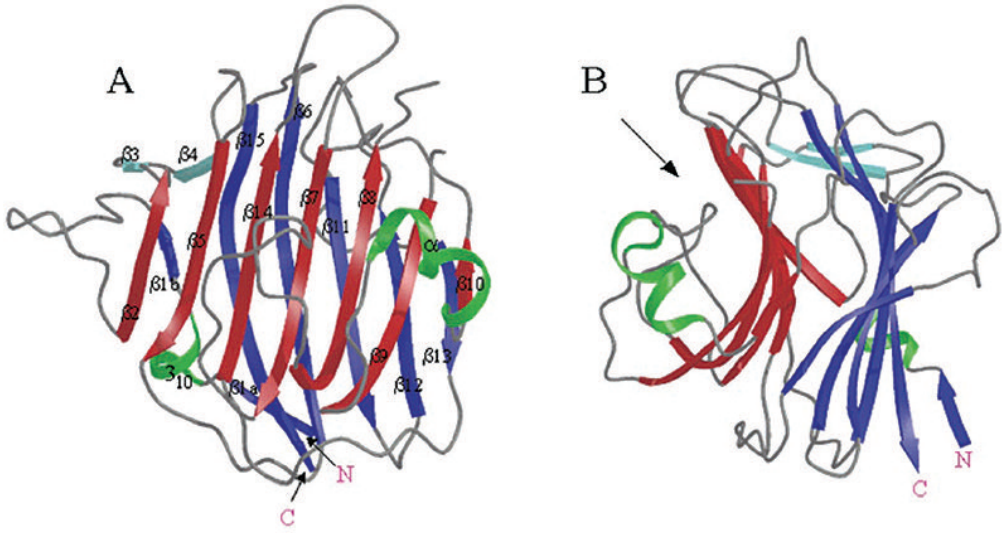

Fig. 3. Overall structure of CDR of p58/ERGIC-53. Ribbon diagram of p58/ERGIC-53 monomer shown (**A**) perpendicular to the -sheets and (**B**) rotated 90° around a vertical axis. Positions of the N and C termini are indicated. ß-sheets are shown in red and dark blue, respectively. Loops and helices are shown in gray and green, respectively. The arrow in **B** indicates the position of the ligand binding site (modified from Velloso et al., 2002).

TMD

The length of TMD affects ER-exit efficiency. Evidence showed the optimal length is 21 amino acids. The wild type is 18 amino acids long and it is suboptimal for efficient ER-exit of hERGIC-53, but has some helpful effects. Besides the length, a three residue motif $F_{484}/Q_{488}/Y_{498}$ is important. Polar residues (Q_{488} and T_{489}) is a notable feature of the hERGIC-53 TMD, which is unusual for single-spanning. TMDs, while aromatic residues are very common. Evidence showed $F_{484}/Q_{488}/Y_{498}$ positioned on the same side of the α helix, which is formed at the lipid-water interphase, influencing the transport efficiency of ERGIC-53. One possible reason is that the $F_{484}/Q_{488}/Y_{498}$ motif contributes to the hexamerization of ERGIC-53 (Nufer et al., 2003a). What is more, $F_{484}/Q_{488}/Y_{498}$ (Eilerset et al., 2000; Adamian and Liang, 2001) help to stabilize the hexamer through helix-helix interaction of TMD.

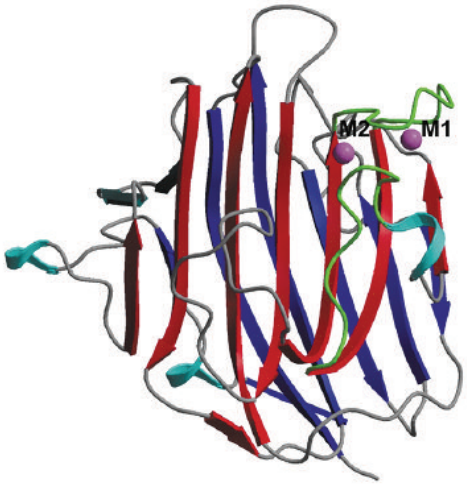

Fig. 4. Overall structure of the CRD of p58/ERGIC-53 showing the location of the two bound calcium ions (pink spheres). Two loops changing conformation upon metal ion binding are shown in green (Velloso et al., 2003).

Function

Cargo receptor and recycling protein

ERGIC-53 is a recycling protein in the early secretory pathway. It mainly operates as a cargo receptor and selectively transports its cargo from ER to ERGIC. It is the hexameric form of ERGIC-53 that binds glycoprotein cargo, and it only recognizes a limited mumber of folded, high-mannose type glycoproteins in the ER after they have been released from the quality control machinery for glycoprotein folding and subjected to glucose trimming. Its cargo is a specific subset of glycoproteins, including blood coagulation factors V and VIII (Nichols et al., 1998; Zhang et al., 2003), two lysosomal enzymes glycoproteins cathepsin Z (Appenzeller-Herzog et al., 1999) and cathepsin C (Vollenweiderr et al., 1998; Nyfeler et al., 2006), procathepsin Z (Appenzeller-Herzog et al., 2005), N-glycosylated human nicastrin (Morais et al., 2006), and alpha 1-antitrypsin (Nyfeler et al., 2008a). Besides, ERGIC-53 is also involved in the traffic of IgM (Mattioli et al., 2006), fibroblast growth factor receptor 3 (Lievens et al., 2008) and Sulfatase modifying factor 1 (Fraldi et al., 2008). Multiple coagulation factor deficiency 2 (MCFD2) is dispensable for the transport of the lysosomal enzymes, and N-glycosylated human nicastrin, but required for the transport of factors V and VIII (Nyfeler et al., 2006; Kawasaki et al., 2008).

The cargo leaves the ER by a receptor-mediated

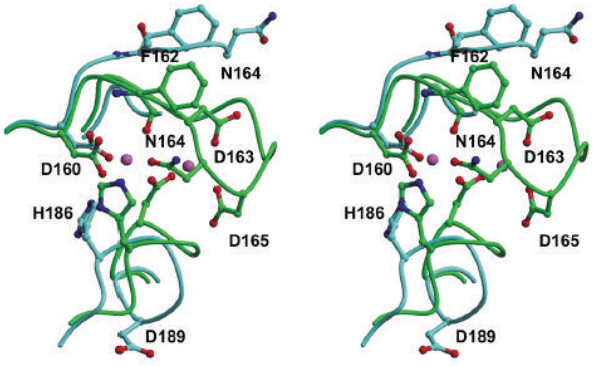

Fig. 5. Conformational changes in p58/ERGIC-53 as a result of calcium binding. Stereo view of the region close to the ligand-binding site, showing residues that undergo significant conformational changes in ball-and-stick representation. The two calcium ions are shown as pink spheres. The metal free p58/ERGIC-53 structure is colored in light blue, and the metal-bound structure is colored in green (Velloso et al.,2003).

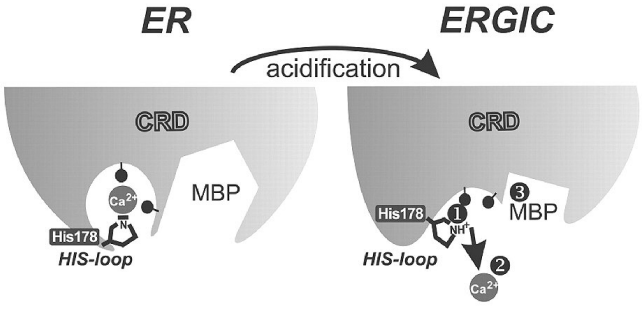

Fig. 6. Model for cargo release by pH-induced loss of Ca^{2+}. Upon arrival in the ERGIC, His-178 in the HIS-loop of ERGIC-53 is protonated because of lowered luminal pH, leading to the loss of one (or more) Ca^{2+} . Loss of Ca^{2+} inactivates the mannose binding pocket, and triggers cargo release (Appenzeller-Herzog et al., 2004). MBP, mannose binding pocket; HIS-loop: histidine ion sensor loop, the α-helical loop comprising His-178.

cargo capture mechanism, but the signals required for the cargo-receptor interaction are largely unknown until 2005. In 2005, Christian Appenzeller-Herzog and his colleagues discovered a novel conformation-based targeting motif that is composed of a high-mannose type oligosaccharide intimately associated with a surface-exposed peptide ß-hairpin loop, which contributes to efficient binging of cargo to active lectin domain of ERGIC-53. The conformation-based N-glycan/ß-hairpin loop motif (Fig. 7) (Appenzeller-Herzog et al., 2005) is only present in fully folded procathepsin Z and its recognition by ERGIC-53 reflects a quality control mechanism that acts complementary to the primary folding machinery in the ER, similar oligosaccharide/ß-hairpin loop structure is present in cathepsin C, suggesting the general nature of this ER-exit signal.

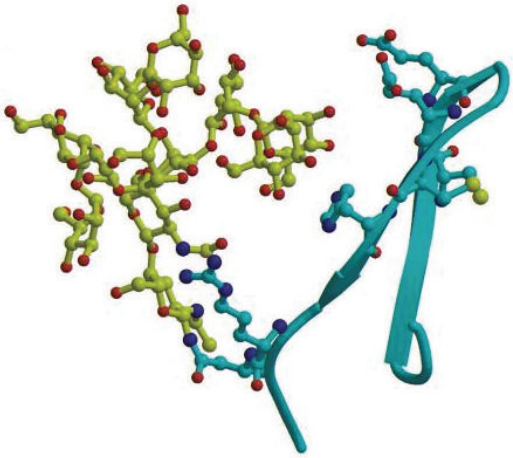

Fig. 7. 3-D model of the peptide ß-hairpin loop (cyan) and the high-mannose N-linked glycan (yellow) (Appenzeller-Herzog et al., 2005).

The transport process includes anterograde and retrograde traffic, following the steps beblow: (Fig. 8).

(1) Translocation of newly synthesized ERGIC-53 monomers into the lumen of the rough ER and anchored in the ER membrane by their single transmembrane domain.

(2) Formation of disulfide-linked or non-covalent homo-oligomers that bind to N-glycans of correctly folded cargo glycoproteins at suitable pH and Ca^{2+} concentration.

(3) The cargo-receptor complex is actively recruited by the cytosolic COPII machinery (through phenylalanine-dependent interaction of COPII with the cytosolic tail of ERGIC-53) and enters the vesicles formed by COPII.

(4) COP II-vesicle budding and vesicular transport to the ERGIC.

(5) Release of the cargo in the ERGIC at lower pH, which would in turn result in the dissociation of calcium from ERGIC-53 and concomitant loss of its lectin activity. And the cargo will be transported to Golgi and then to plasma membrane through Golgi network.

(6) Recruitment of free ERGIC-53 to COPI vesicle-budding sites by direct interaction of the cytosolic di-lysine signal with COPI. Most ERGIC-53 returns to the

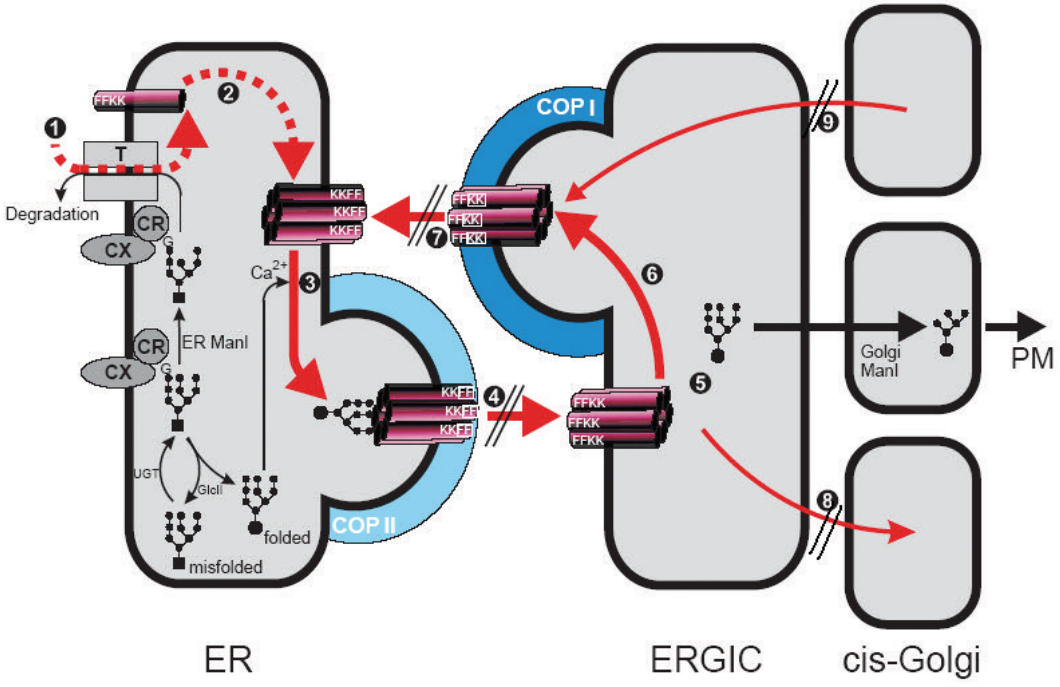

Fig. 8. Cargo receptor function and recycling of hERGIC-53. Anterograde traffic includes 1-8, retrograde traffic includes 6, 7, and 9 (Hauri et al., 2000b).

ER largely bypassing the Golgi.
(7) COPI-vesicle budding and retrograde transport to the ER.
(8) Escape of a subpopulation of ERGIC-53 to the cis-Golgi. Recycling of ERGIC-53 through the cis-Golgi is only a minor pathway.
(9) Retrieval of this subpopulation to the ERGIC by COPI vesicles.
In the process, two important details must be mentioned:
Firstly, four points are critical for efficient ER-exit:
(1) a double phenylalanine motif at the C-terminus: it mediates the interaction between ERGIC-53 and COPII, assisted by glutamine 501 (Nufer et al., 2003a) to increase ER-exit efficiency.
(2) stabilized hexamerization status of ERGIC-53.
(3) length of transmembrane domain: 21 amino acids is optimal, while 18 amino acids is suboptimal (Nufer et al., 2003a).
(4) folded glycoprotein cargo with ERGIC-53-specific conformation.

Secondly, factors or reagents that block ERGIC-53 recycling:
The recycling of ERGIC-53 can be blocked either in the ER, ERGIC or Golgi by various factors (Table 1).

Secondary quality control

Recent studies have shown ERGIC-53 appears to represent a secondary quality control mechanism that acts complementary to the ER folding machinery and ensures that preferentially folded cargo will be withdrawn from the folding environment of the ER.

Then, where will the incompletely folded glycoprotein cargo go, when they are rejected by ERGIC-53? They bind to calnexin (CX) and/or calreticulin (CR) after removal of the two outermost glucose residues. Subsequently, they are trimmed by glucosidase II (Glc II) and, if incompletely folded, reglucosylated by UDP glucose: glycoprotein glucosyltransferase (UGT). After folding is completed, the glycoprotein cargo molecule can bind to ERGIC-53. After a prolonged time, ERα1, 2-mannosidase (ER Man I) acts on the middle branch of the glycan, removing one mannose residue. Mono-glucosylated Man8 is a targeting signal for calnexin- dependent retranslocation of the misfolded protein to the cytosol, and subsequent degradation, possibly due to attenuated deglucosylation by Glc II (Liu et al., 1999; Hauri et al., 2000b) (Fig. 8). ER-associated degradation (ERAD) will be significantly delayed when cells are treated with specific inhibitors of ER α-mannosidase (kifunensine or deoxymanano-jirimycin) that block the formation of Man8GlcNAc2 isomer b (Man8b) (Cabral et al., 2001). However, recent studies have demonstrated that the carbohydrate determinant triggering ERAD may not be restricted to Man8b, as previously thought. EDEM1 (Olivari et al., 2006), EDEM2 (Olivari et al., 2005), and EDEM3 (Hirao et al., 2006) are all active mannosidases that produce Man5, Man6 and Man7 species, and such oligosaccharides attached to misfolded glycoproteins target them for ERAD (Frenkel et al., 2003; Hosokawa et al., 2003).

Regulation

UPR and ERGIC-53

Newly synthesized proteins undergo quality control

Table 1. Factors blocking ERGIC-53 recycling in the early secretory pathway and respective mechanisms.

Blocking of ER-exit	Blocking of ERGIC-exit	Blocking of cis- Golgi exit
Phosphatase inhibitor. okadaic acid (inhibiting the association of COPI components and ERGIC-53) (Pryde et al., 1998)	15°C (antrograde and retrograde) (interruptting the balance between ERGIC-associated kinesin and dynein) (Lippincott et al., 1990; Saraste and Svensson, 1991;Klumperman et al., 1998)	PLA2 antagonist (ONO-RS-082) (inhibiting Ca^{2+}-independent PLA2) (Figueiredo et al., 2000)
Deoxyglucose/azide (lowering of the ATP levels) (Appenzeller-Herzog et al., 1999)	AlF_4- (antrograde and retrograde) (inhibiting trimeric GTPases) (Kappeler et al., 1997; Klumperman et al.,1998; Andersson et al.,1999; Hauri et al., 2000b)	
A GTP-restricted dominant negative mutant of the small GTPase Sar1 (inhibiting COPI-associated vesicle budding) (Shima et al., 1998; Andersson et al., 1999)	Bafilomycin A1 (Baf A), (retrograde, but not antrograde) (inhibiting the H^+-ATPase of the pre-Golgi structures) (Palokangas et al., 1999)	
Osmotically induced cell-volume change (inability of COPI) (Lee and Linstedt, 1999)	3-cyclohexane-bis(methylamine) (CBM), (mechanism not clear yet) (Hauri et al., 2000b)	
During mitosis (depletion of COPI component Sec13p) (Farmaki et al., 1999)	Nordihydroguararetic acid (NDGA) (retrograde) (mechanism not clear yet) (Fujiwara et al., 1998; Hauri et al., 2000b)	
Nordihydroguaiaretic acid (NDGA) (inducing Golgi disassembly) (Fujiwara et al., 1998)	Brefeldin A (BFA) (antrograde, not retrograde) (inhibiting budding and formation of COPI vesicles) (Fujiwara et al., 1998; Lippincott et al., 1990; Saraste and Svensson, 1991)	
10°C (mechanism not clear yet) (Hauri et al., 2000b)	Proton ionophpre monensin (mechanism not clear yet) (Hauri et al., 2000b)	

in the ER. The process involves the monitoring of the state of protein folding and oligomerization. Accumulation of unfolded proteins in the ER triggers the unfolded protein response (UPR), also known as ER stress response. The accumulation of unfolded proteins in the ER triggers a signaling response known as unfolded protein response (UPR). UPR increases the folding capacity that is overwhelmed by stress conditions that can result from perturbation in calcium homeostasis or redox status, altered glycosylation, glucose/nutrient deprivation, expression of misfolded proteins, or elevated protein synthesis.

In yeast, the UPR affects several hundred genes that encode ER chaperones and proteins operating at later stages of secretion. In mammalian cells, the UPR appears to be more limited to chaperones of the ER and genes assumed to be important after cell recovery from ER stress which are not important for secretion. Induced UPR increase the mRNA level of ERGIC-53 and VIP36, and their de novo synthesis of ERGIC-53 and VIP36 protein is in parallel to its mRNA synthesis relatively. That is, UPR upregulates the transcription of ERGIC-53 and VIP36. As to the mechanism, Beat Nyfeler speculated that the upregulatin of ERGIC-53 by UPR might be accounted for by the ATF6 (activating transcriptionfactor 6) pathway of UPR (Nyfeler et al., 2003), and that ERSEs (ER stress response elements) in the promoter region of ERGIC-53 might be the UPR targeting gene. Under normal conditions, ATF6 is retained in the ER by association with the ER chaperone BiP. Upon ER stress, ATF6 is transported to the Golgi, where proteases cleave ATF6 to release its cytoplasmically exposed transcription factor domain. The transcription factor domain is translocated to the nucleus and activates UPR-responsive genes by directly binding to ERSEs in their promoter region. However, the ERSE was not found in hERGIC-53 until 2007. Identified ERSEs include ERSEI, ERSEII and UPRE (unfolded protein response element). ERSE-I contains a CCAAT site at its 5'-end for the constitutive transcription factor NFY/CBF, a 9 bp spacer containing the CGG triplet which is the TFII-I binding site (Yoshida et al., 1998; Roy and Lee, 1999), and a CCACG motif at the 3'-end that is required for ATF6α recruitment. ERSE-II (5'-ATTGGNCCAC(G/A)-3') retains a reversed NFY/CBF binding site at its 5'-end and a flanking ATF6α site. The UPRE contains the ATF6α binding site on its complementary strand and is also the preferred binding site for the transcription factor XBP1 (X-box-binding protein 1). But the putative promoter region of hERGIC-53 (500 bp) does not contain these special sequences. It contains two CCAAT motifs and five GC boxes (Fig. 9) (Spatuzza et al., 2004), which may bind to NFY/CBF and Sp1 proteins relatively. In 2007, Maurizio Renna (Renna et al., 2007) found a novel structure of ERSE in ERGIC-53 . The newly identified ERSE contains a 5'-end CCAAT sequence that constitutively binds NFY/CBF and, 9 nucleotides away, a 3'-end region (5'-CCCTGTTGGCCATC-3') that is equally important for ER stress mediated induction of

Fig. 9. Putative promoter region of hERGIC-53. It contains a ERGIC-53 ERSE, three parts of which bind to NFY/CBF, YY1, and undefined factors relatively, and three GC boxes which bind to SP1 proteins (modified from Spatuzza et al., 2004).

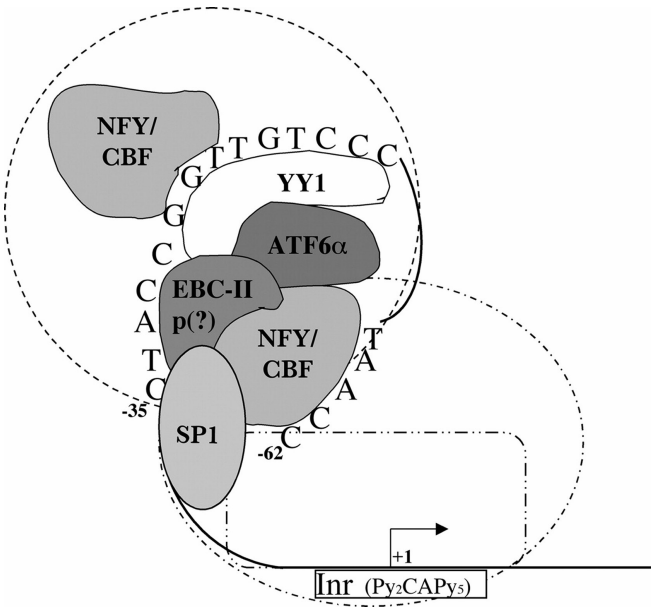

Fig. 10. Proposed model for the ERGIC-53 promoter transactivation in response to ER stress. In response to ER stress, ATF6α is rapidly activated and combined with the constitutively expressed YY1, which is part of a multiprotein complex able to bind to the ERGIC-53 ERSE and to ensure promoter activation. NFY/CBF and Sp1 are needed as co-activators for ATF6α. Other components (EBC-II p (?)) of the transcriptional complex could act as bridging factors between the ERSE and basal transcription machinery (depicted by dashed shapes). Inr is the initiator sequence required for the transcription of TATA-less promoters (Renna et al., 2007).

the gene. The 5'-CCCTGTTGG-3'part is the binding site of YY1, which is a co-activitor of the ER stress response in mammalian cells and interacts with ATF6α through the b-Zip domain of ATF6α and the zinc finger domain of YY1. The 3'-end part CCATC is the binding site for accessory and as yet unidentified regulatory proteins (Fig. 9). Maurizio Renna also proposed a molecular model for the transcriptional regulation of ERGIC-53. Fig. 10 illustrates how the transcription factors could converge to form the transcriptional complex required for UPR-mediated control of ERGIC-53. In response to ER stress, ATF6α is rapidly activated and associates with YY1. The ATF6α-YY1 complex could bind the ERGIC-53 promoter to the CCCTGTTGG part of the ERSE. The nuclear proteins that form the EBC-I (ERSE binding complexI) in association with ATF6α-YY1 could act as coactivators for NFY/CBF present in the EBC-II. In this context, Sp1 proteins could contribute to the formation of the complex by recognizing the GC box in the regulatory region (Fig. 10).

Heat shock response and ERGIC-53

Heat shock is a conserved response resulting from environmental stress during hyperthermia, exposure to heavy metals, amino acid analogues, several other agents, etc. Evidence showed that heat shock response increases the ERGIC-53 protein level, but not its mRNA level. That is, at variance with UPR, which affects transcription of ERGIC-53, heat shock only has a positive influence on its translation. In addition, the half-life of the protein does not change during heat shock. Heat shock also affects the recycling pathway of ERGIC-53 without any effect on ER exit sites, and morphological change of ER, ERGIC, and Golgi. The protein rapidly redistributes in a more peripheral area of the cell during heat shock, in a vesicular compartment that has a lighter sedimentation density on sucrose gradient in comparison to the compartment that contains the majority of ERGIC-53 at 37°C. Analysis of the 5'-untranslated region (UTR) of ERGIC-53 revealed its strikingly complementary to 18S rRNA (Fig. 11). In addition, RNA structure prediction analysis (Zuker, 2003) showed that the 5'-UTR of ERGIC-53 might be able to form a stable RNA/RNA hybrid with 18 S rRNA. Since complementarity with the 18 S rRNA is an essential requirement for the preferential translation of viral and cellular genes during down-regulation of general translation, we suggest that ERGIC-53 could be preferentially translated by a ribosomal shunting mechanism in response to Heat shock (Spatuzza et al., 2004).

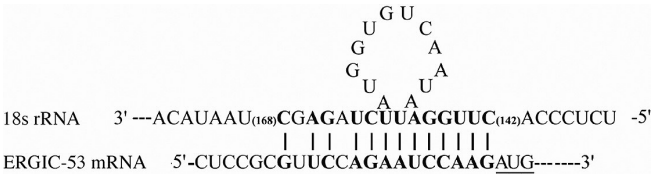

Fig. 11. 5'UTR (21nt) of hERGIC-53 is complementary to 18S rRNA (Spatuzza et al., 2004)

VIPL and ERGIC-53

VIPL is a N-glycosylated type I membrane glycoprotein and a non-cycling resident protein of the ER. It is a homologue of ERGIC-53 and VIP36, 35.2% and 57.8% identity respectively. Over expression of VIPL leads to a striking redistribution of ERGIC-53 to the ER, without detectable morphology change of the early secretory pathway or the cycling of VIP36 and other recycling proteins in the secretory pathway. Researches suggest that VIPL may function as a regulator of ERGIC-53. However, in vitro, interaction between ERGIC-53 and VIPL by co-immuno-precipitation or by chemical cross-linking is

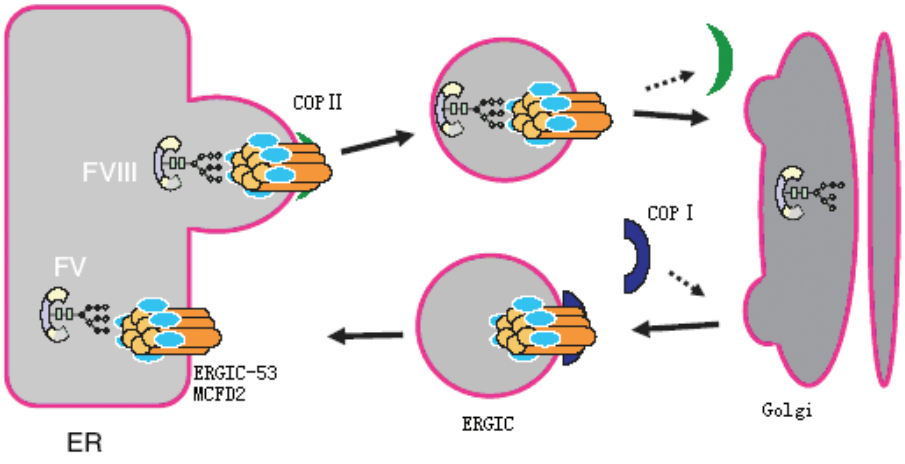

Fig. 12. Model of receptor-mediate ER to Golgi transport of FV and FVIII. Correctly folded FV/FVIII molecules are envisioned to be recruited to the COPII-coated vesicles budding from the ER by binding to the ERGIC-53-MCFD2 complex. Release of FV/FVIII from ERGIC-53-MCFD2 occurs in the ERGIC. The ERGIC-53-MCFD2 complex is recycled back to the ER in COPI-coated retrograde vesicles as FV and FVIII are transported to the Golgi (Zhang and Ginsburg, 2004).

undetectable. Whether VIPL regulate ERGIC-53 directly or indirectly, and how, remains uncertain now (Neve et al., 2003; Nufer et al., 2003b).

Diseases

F5F8D

Combined deficiency of factors V (FV) and factor VIII (FVIII) (F5F8D) first described in 1954 by Oeri et al. (1954) is an autosomal recessive bleeding disorder associated with plasma levels of coagulation factors V and VIII approximately 5% to 30% of normal (Nichols et al., 1999). To our knowledge, at least 140 patients in 81 families have been diagnosed with F5F8D, with over half of the families from the Mediterranean region. However, it is likely that F5F8D is under-diagnosed or misdiagnosed as mild hemophilia or parahemophilia. This disorder appears to be particularly prevalent among Middle Eastern Jews and non-Jewish Iranians, estimated at 1:100 000 (Seligsohn et al., 1982).

Significiant progress has been made these years on the molecular mechanism of F5F8D. The disorder is mostly caused by mutation of two genes: ERGIC-53 and MCFD2. MCFD2 is a soluble EF-hand-containing protein, whose folding is calcium-dependent (Guy et al., 2008). It is retained in the ER through its direct calcium-dependent (Zhang et al., 2003) interaction with ERGIC-53, ERGIC-53 and MCDF2 form a 1:1 complex that functions as a cargo receptor, ferrying FV and FVIII from the ER to the Golgi (Fig. 12). MCFD2 could function at the point of either cargo loading or cargo

Table 2. Mutations found in ERGIC-53 or MCFD2 genes.

Gene	Mutation	Country of origin	Reference
ERGIC-53	C604>T (exon 5)	Japan	Nichols et al., 1999
ERGIC-53	Del C422 (exon 3)	Japan	Nichols et al., 1999
ERGIC-53	T2>C (exon 1)	Italy	Nichols et al., 1999
ERGIC-53	Ins G85-89 (exon 1)	Venezuela (Iranian Jewish)	Nichols et al., 1999
ERGIC-53	Del A720-A735 (exon 6)	Venezuela	Nichols et al., 1999
ERGIC-53	A904>T (exon 8)	France	Nichols et al., 1999
ERGIC-53	Del A1519-1524 (exon13)	US	Nichols et al., 1999
ERGIC-53	Del TC1109-112 (exon 9)	US	Nichols et al., 1999
ERGIC-53	Ins A912-913 (cd305)	Iran	Neerman et al., 1999
ERGIC-53	822G>A (IVS7 DS-1)	Iran	Neerman et al., 1999
ERGIC-53	Ins G89-90 (cd30)	Iran	Neerman et al., 1999
ERGIC-53	IVS9+2T>G	Iran	Neerman et al., 1999
ERGIC-53	Del 1214-1218AAATG(cd406)	Iran	Neerman et al., 1999
ERGIC-53	Del G23 (cd8)	Iran	Neerman et al., 1999
ERGIC-53	R202X(CGA>TGA)	Iran	Neerman et al., 1999
ERGIC-53	K302X(AAA>TAA)	Pakistan	Neerman et al., 1999
ERGIC-53	R456X(CGA>TGA)	Pakistan; China?	Neerman et al., 1999
ERGIC-53	M1T(ATG>ACG)	Italy	Neerman et al., 1999
ERGIC-53	K302X(AAA>TAA)	Pakistan	Neerman et al., 1999
ERGIC-53	IVS5+1G>T	Italy	Neerman et al., 1999
ERGIC-53	Ins T1208-1209 (cd 403)	Italy	Neerman et al., 1999
ERGIC-53	Del G31 (cd 11)	Algeria	Neerman et al., 1999
ERGIC-53	G340>T (exon 2)	Indian	Mohanty et al., 2005
ERGIC-53	Ins G86-89 (exon 1)	Jew; Iran; Italy	Neerman et al., 1999; Nichols et al., 1999; Nichols et al., 1998; Segal et al., 2004
ERGIC-53	IVS9+2 T>C	Djerba Jews	Nichols et al., 1998; Segal et al., 2004
ERGIC-53	C604>T (exon5)	Japan; Iran	Nichols et al., 1999; Neerman et al., 1999; Dansako et al., 2001
ERGIC-53	Del T780	Austria	Zhang et al., 2006
ERGIC-53	Del A839-841	Poland	Zhang et al., 2006
ERGIC-53	E321X	Iraq	Zhang et al., 2006
ERGIC-53	C475R	Italian	Zhang et al., 2006
ERGIC-53	G823>C	Thai	Sirachainan et al., 2005
ERGIC-53	C1366>T	Thai	Sirachainan et al., 2005
MCFD2	149+5G>A (exon2)	Yugolavia; Italion; Indian; US; Swiss; Bangladeshi	Zhang et al., 2003
MCFD2	309+1G>A	Iran; Turkey	Zhang et al., 2003
MCFD2	Del C103	Italy	Zhang et al., 2003
MCFD2	Del T249	Turkish	Zhang et al., 2003
MCFD2	Del 263-270TTGATGGC	South Africa	Zhang et al., 2003
MCFD2	C387>G	Venezuela	Zhang et al., 2003
MCFD2	T407>C(I136T)	Yugolavia; Venezuelar	Zhang et al., 2003
MCFD2	-6-1G>C (exon2)	Iran	Zhang et al., 2006
MCFD2	c.431C>G; p.Ser144X (ΔSLQ) (exon 4)	South America	Nyfeler et al., 2008b

unloading. Evidence has shown interactions of both ERGIC-53 and MCFD2 with factor VIII, have the B domain of factor VIII as the most likely site of interaction. Interestingly, both interactions are calcium dependent, but FVIII glycosylation state independent (Zhang et al., 2005).

Mutations in ERGIC-53 or MCFD2 genes account for most cases of F5F8D (Table 2), and probably more genes are related to this disorder (Zhang et al., 2003), because in some F5F8D cases, we cannot find any mutation in ERGIC-53 or MCF2 genes.

ER storage diseases-Russell bodies

Mutant Ig-μ chains and other Ig isotypes that lack the first constant domain accumulate as detergent-insoluble aggregates in ER, called Russell bodies (RB). The presence of similar structures hallmarks many ER storage diseases. Recent research showed that RB form when the production of μΔCH1 (mutant Ig-μ lacking CH1) exceeds the degradation capacity, and condensation of μΔCH1 occurs in different sub-cellular locations, depending on the interacting molecules present in the host cell: if Ig light chains are co-expressed, μΔCH1-light chain oligomers accumulate in large ribosome-coated structures (rough Russell bodies); if μΔCH1 interacts with hERGIC-53, μΔCH1-hERGIC-53 oligomers aggregate in smooth tubular vesicles (smooth Russell bodies) in ER. ERGIC-53 binds μΔCH1 in a calcium-dependent and N-glycan of μΔCH1-dependent way via its CDR, which is consistent with the principle of hERGIC-53 interacting with its substrates. However, ERGIC-53 and μΔCH1 remain in dynamic contact: once the insoluble complexes are formed, they do not require the continuous interaction with ERGIC-53; while ERGIC-53 can re-localize in sRB upon readdition of calcium (Mattioli et al., 2006).

Conclusion

ERGIC-53 is a significant transmembrane proteion. Besides transporting some specific glycoproteins, it also has a quality control function. Great progress on its structure, function, regulation, and relations with diseases has been made these years. However, there remains some questions to be further investigated. As the crystal structure of human ERGIC-53 is unidentified, does it have any differrances with p58 in 3-Dimension structure? What is its specific regulation mechanism of the expression of ERGIC-53? Is there any important proteins or other factors upstream or downstream in its regulation chain? Does it have any relationships with other diseases?

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