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Abstract

Coat protein complex II (COPII) plays an essential role in the export of cargo molecules such as secretory proteins, membrane proteins, and lipids from the endoplasmic reticulum (ER). In yeast, the COPII machinery is critical for cell viability as most COPII knockout mutants fail to survive. In mice and fish, homozygous knockout mutants of most COPII genes are embryonic lethal, reflecting the essentiality of the COPII machinery in the early stages of vertebrate development. In humans, COPII mutations, which are often hypomorphic, cause diseases having distinct clinical features. This is interesting as the fundamental cellular defect of these diseases, that is, failure of ER export, is similar. Analyses of humans and animals carrying COPII mutations have revealed clues to why a similar ER export defect can cause such different diseases. Previous reviews have focused mainly on the deficit of secretory or membrane proteins in the final destinations because of an ER export block. In this review, we also underscore the other consequence of the ER export block, namely ER stress triggered by the accumulation of cargo proteins in the ER.

Keywords

COPII, Cell death, Endoplasmic reticulum, ER export defect, Secretion, Stress, Unfolded protein response

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Consequences of mutations in the genes of the ER export machinery COPII in vertebrates

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Key words: COPII; cell death; endoplasmic reticulum; ER export defect; secretion; stress; unfolded protein response

ABSTRACT

Coat protein complex II (COPII) plays an essential role in the export of cargo molecules such as secretory proteins, membrane proteins, and lipids from the endoplasmic reticulum (ER). In yeast, the COPII machinery is critical for cell viability as most COPII knockout mutants fail to survive. In mice and fish, homozygous knockout mutants of most COPII genes are embryonic lethal, reflecting the essentiality of the COPII machinery in the early stages of vertebrate development. In humans, COPII mutations, which are often hypomorphic, cause diseases having distinct clinical features. This is interesting as the fundamental cellular defect of these diseases, that is, failure of ER export, is similar. Analyses of humans and animals carrying COPII mutations have revealed clues to why a similar ER export defect can cause such different diseases. Previous reviews have focused mainly on the deficit of secretory or membrane proteins in the final destinations because of an ER export block. In this review, we also underscore the other consequence of the ER export block, namely ER stress triggered by the accumulation of cargo proteins in the ER.

INTRODUCTION

Protein secretion is essential for cell viability. In eukaryotes, the secretory pathway involves the endoplasmic reticulum (ER), the Golgi apparatus, and the plasma membrane. Small vesicular carriers ferry cargo molecules between two neighboring organelles. This secretory pathway is utilized not only by secretory proteins, but also by membrane proteins and lipids. The vast majority of secretory proteins, membrane proteins, and lipids are synthesized in the ER and packaged into transport vesicles coated with coat protein complex II (COPII) as they exit the ER. COPII proteins consisting of SAR1, SEC23, SEC24, SEC13, and SEC31 are recruited to the ER exit sites and generate transport vesicles (Fig. 1). SAR1, a small GTPase, is recruited to the ER exit sites by its guanine nucleotide exchange factor, SEC12, that is located in the ER exit sites (Barlowe and Schekman 1993). GTP-bound SAR1 is then inserted into the membrane through its amphipathic α-helix and in turn, recruits the SEC23/SEC24 complex (Lee et al. 2005). The SAR1-GTP/SEC23/SEC24 complex recruits the SEC13/SEC31 complex, which polymerizes to form a spherical or tubular lattice on the membrane and completes vesicle scission (Matsuoka et al. 1998; Stagg et al. 2008; Zanetti et al. 2013). SEC23 has the GTPase activating protein activity (GAP) for SAR1 and SEC24 serves as the major cargo recognition subunit (Bi et al. 2002; Miller et al. 2003). SEC31 enhances the GAP activity of SEC23, contributing to the release of SAR1 from the vesicles (Antonny et al. 2001). SEC16 is a peripheral membrane protein of the ER and regulates the SEC31 action on the GAP activity of SEC23 (Kung et al. 2011).

A disruption of the COPII genes blocks ER export, resulting in a deficit of cargo molecules in their destination as well as accumulation of them in the ER. The latter triggers the unfolded protein response (UPR) of the ER (Fig. 2). The UPR in yeast involves IRE1 which transduces ER stress signals by splicing HAC1 into an active form, whereas vertebrate UPR has

two additional signaling pathways involving PERK and ATF6 (Cox and Walter 1996; Mori et al. 1993; Wang and Kaufman 2016). In vertebrates, IRE1 transduces ER stress signals by splicing XBP1, a transcription factor (Yoshida et al. 2001). In addition, the RNase domain of IRE1 degrades multiple RNAs through a process called regulated IRE1-dependent decay (RIDD) (Gaddam et al. 2013; Hollien et al. 2009). RIDD is also observed in *Schizosaccharomyces pombe* (Kimmig et al. 2012). RIDD targets not only ER-associated mRNAs, but also pre-miRNAs (Upton et al. 2012). As for ATF6, it traffics to the Golgi upon ER stress where its N-terminal cytoplasmic domain (ATF6N) is liberated and shuttles to the nucleus to act as a transcription factor (Haze et al. 1999; Shen et al. 2002). Activated PERK phosphorylates its substrates, one of which is eIF2α, a translational regulator (Harding et al. 2000). In vertebrates, the UPR has two seemingly opposite impacts on cell fate. Initially, UPR signaling supports cell survival by alleviating ER stress, but unresolved UPR signaling triggers cell death (Ron and Walter 2007). As vertebrate UPR signaling differs significantly from yeast counterpart, it will be informative to examine the roles of the UPR in yeast and vertebrate COPII mutants. In this review, we have revisited the literature describing the effects of COPII mutations on yeast and vertebrates to summarize the principles governing the genotype-phenotype relationship and to explore the impacts of the UPR in the phenotypes.

LESSONS LEARNED FROM YEAST COPII MUTANTS

The COPII genes such as *SAR1, SEC23, SEC24, SEC13* and *SEC31* are essential for cell viability except for two Sec24 paralogs (*LST1* and *ISS1*) in yeast (Hicke and Schekman 1989; Kurihara et al. 2000; Nakano and Muramatsu 1989; Novick et al. 1980; Pryer et al. 1993; Roberg et al. 1999; Salama et al. 1997). In addition, genes closely linked to COPII vesicle assembly such as *SEC12*

and *SEC16* are essential for cell viability, emphasizing critical importance of ER export at the cellular level (Espenshade et al. 1995; Nakano et al. 1988). The two non-essential Sec24 paralogs have overlapping and distinct functional properties with Sec24. For example, deletion of *ISS1* or *LST1* exacerbates phenotypes of a hypomorphic *sec24* mutant (Miller et al. 2003) and Lst1 specifically packages the plasma membrane protein-ATPase Pma1 into COPII vesicles (Roberg et al. 1999). Thus, studies of yeast COPII mutants inform us that ER export is essential for cell viability and Sec24 paralogs show overlapping and distinct functional properties.

Yeast mutants affecting various transport steps of the secretory pathway including *temperature-sensitive* (*ts*) *sec13* and *sec23* mutants exhibit activation of IRE1 signaling (Chang et al. 2004). IRE1 signaling is necessary for cell survival as *sec13-1; ire1Δ* double mutants show partial synthetic lethality (Chang et al. 2004). Consistent with this idea, constitutive overexpression of *IRE1* or *HAC1* suppresses *ts* growth phenotypes of *sar1 E112K*, *sec12-4*, *sec13-1*, *sec16-2*, *sec23-1*, *sec24-20,* and *sec31-1* mutants (Chang et al. 2004; Higashio and Kohno 2002; Sato et al. 2002). Thus, a constitutive activation of IRE1-HAC1 signaling supports survival of yeast COPII *ts* mutants (Chang et al. 2004). However, HAC1 overexpression does not suppress the lethality of *sec13Δ* mutants (Chang et al. 2004). Clearly, the deficit of essential cargo molecules at their final destinations is the primary cause of cell death in the knockout (KO) mutants. Alternatively but not mutually exclusively, HAC1 overexpression does not suppress the other function of Sec13. As Sec13 is also a component of the nuclear pore complex (NPC) (Siniossoglou et al. 1996), the lethality of *sec13Δ* mutants may be affected by disruption in nuclear transport. Regardless, it is evident that UPR signaling is beneficial to the viability of hypomorphic COPII mutants like *ts* mutants in yeast.

PHENOTYPES OF VERTEBRATES CARRYING COPII MUTATIONS

Additional factors should be considered to understand the phenotypes of COPII gene mutations in vertebrates. For example, vertebrates have more COPII paralogs than yeast: two SAR1 (A and B), two SEC23 (A and B), four SEC24 (A-D), one SEC13, and two SEC31 (A and B) paralogs, which can provide more redundancy or distinct functions than yeast counterparts can. Maternal effects can mask mutant phenotypes at very early stages of embryo development. In addition, vertebrates have different, complex tissue organizations and go through a series of developmental processes. Consequently, spatiotemporal expression of a COPII gene as well as its paralogs likely contributes to phenotypes of a COPII mutant.

As described below, UPR activation has not been examined in all studies of COPII mutants in vertebrates. When examined, UPR activation has been commonly observed in vertebrate COPII mutants although sometimes it has been noted circumstantially. Considering the impact of the UPR in yeast COPII mutants, it is expected that the UPR is beneficial to vertebrate COPII mutants. However, it should be noted that chronic UPR activation leads to cell death in vertebrates. Thus, the UPR likely affects vertebrate COPII mutants in two opposite ways, initially beneficial, but eventually harmful as pathological COPII mutations cause a chronic UPR activation.

Functions of SAR1

SEC12 recruits SAR1 to the ER exit sites and catalyzes nucleotide exchange of SAR1. SAR1-GTP, then, inserts its amphipathic α-helix into the ER exit sites and recruits SEC23-SEC24 complex to the ER exit sites (Fig. 1).

Consequences of SAR1B mutations

Homozygous mutations of *SAR1B* cause chylomicron retention disease (CMRD) or Anderson disease (Jones et al. 2003). Affected individuals accumulate chylomicron-like particles in membrane-bound compartments of enterocytes and chylomicrons are not secreted from the basolateral surface of enterocytes. Characteristics of CMRD includes deficiency in lipophilic vitamins, low blood cholesterol levels and a selective absence of chylomicrons from the blood. CMRD with the neuromuscular disorder Marinesco-Sjogren syndrome (CMRD-MSS) is characterized by cataracts, myopathy, ataxia, skeletal abnormalities, and mild to moderate intellectual disability in addition to the typical CMRD phenotypes. Individuals with CMRD-MSS are homozygous for null-like mutations (Jones et al. 2003), suggesting that complete loss of SAR1B is related to abnormalities in eye, muscle, skeleton and brain in addition to the intestine. Analysis of human intestinal biopsies has shown that *SAR1B* is expressed about 2.5-3 times the level of *SAR1A* in the intestine of unaffected individuals and that SAR1A increases by about 50% in the intestine of CMRD patients (Georges et al. 2011). Apparently, the increased expression of *SAR1A* cannot compensate for the loss of *SAR1B*.

SAR1B knockout (KO) mice show preweaning lethality (http://www.informatics.jax.org/allele/genoview/MGI:6263418?counter=1). *Sar1b* heterozygous null mutants have decreased circulating LDL/HDL cholesterol levels (http://www.informatics.jax.org/allele/genoview/MGI:5781729?counter=2), consistent with the human phenotypes. Detailed phenotypes in other tissues of *Sar1b* KO mice are not available yet.

In zebrafish, *sar1b* is expressed broadly during development and enriched in the digestive tract organs, brain, heart and skeleton. Zebrafish *sar1b* morphants fail to clear dietary lipids (Levic et al. 2015). Dietary lipid retention in the *sar1b* morphants is rescued with the introduction of *sar1b* mRNA but not with *sar1a* mRNA, emphasizing a specific function of SAR1B in lipid clearance. SAR1B deficiency not only leads to growth failure of the liver and pancreas, but also disrupts brain patterning and skeletal morphogenesis in zebrafish (Levic et al. 2015), consistent with CMRD-MSS features. Deficits in the deposition of the extracellular matrix, intracellular accumulation of collagen, and enhanced expression of *hsp47*, a sign of UPR activation, are observed in skeletal cartilage.

Functions of SEC23

SEC23 forms a heterodimer with SEC24 (Bi et al. 2002). SEC23 has a GAP activity for SAR1. SAR1-GDP is released from the membrane. Thus, SEC23 is critical for the recycling of SAR1. There are two paralogs of SEC23 in vertebrates.

Consequences of SEC23A mutations

SEC23A missense mutations cause cranio-lenticulo-sutural dysplasia (CLSD) in humans (Boyadjiev et al. 2006; Boyadjiev et al. 2011). CLSD is characterized by late-closing fontanels, sutural cataracts, facial dysmorphism, and skeletal defects. As *SEC23B* is not expressed in calvarial osteoblasts (Fromme et al. 2007), ER export relies on functional SEC23A. This is partly why *SEC23A* mutations disrupt craniofacial formation.

Mouse phenotypes caused by *Sec23* mutations are quite different from human phenotypes caused by *SEC23* mutations. *Sec23a* KO mutants carrying a gene trap allele (*Sec23a*$^{gt/gt}$) begin to have a neural tube opening at midbrain with an exencephaly phenotype at E11.5 and the majority of the mutants die between E11.5 and E12.5 (Zhu et al. 2015). Apoptosis occurs in the mutant brains. This reopening of the neural tube differs from neural tube defects observed in *Sec24b* KO mice (see the SEC24B section for more detail). In the *Sec23a* KO embryos, reopening of the neural tube is likely due to secretion defects of collagens, which compromises the tensile strength of the skin tissue (Zhu et al. 2015). In addition, the majority of yolk sacs of the mutant embryos are broken. In yolk sac and amnion, UPR genes such as *Atf4*, *Chop*, and *Trb3* (a target of CHOP/ATF4) are drastically overexpressed, suggesting preferential activation of the PERK pathway (Zhu et al. 2015). The examination of thin sections of the head of the mutants has revealed normal secretion of fibronectins, but defective secretion of type I collagens (Zhu et al. 2015), suggesting a specific role of SEC23A in collagen secretion. The neural and yolk sac phenotypes of the mutant mice are consistent with expression profiles of *Sec23a* and *Sec23b* in mice: although both *Sec23a* and *Sec23b* are widely expressed in mouse tissues, *Sec23a* expression is high in the brain and the yolk sac and *Sec23b* expression is low at least in the brain (Tao et al. 2012). The differences in expression profiles of *Sec23a* and *Sec23b* between mouse and human likely contribute to the phenotypic differences of corresponding mutants.

The zebrafish *crusher* mutant carries a nonsense homozygous L402X mutation in the *sec23a* gene. The *crusher* mutants are viable up to 9 days post fertilization (dpf) (Lang et al. 2006). Similarly, *sec23a* morphants fail to survive beyond 9 dpf (Boyadjiev et al. 2006). *sec23a* is expressed in cartilages of the head and the pectoral fin (Boyadjiev et al. 2006). The *crusher* mutants and *sec23a* morphants show a short body length and malformations of the cranial

skeleton and pectoral fins (Boyadjiev et al. 2006; Lang et al. 2006). Chondrocytes of the *crusher* mutants accumulate type II collagens in the ER and expression levels of *hsp47* are much higher in *crusher* than in wildtype, indicating a strong UPR activation (Lang et al. 2006). It should be noted, though, that overexpression of hsp47 may represent activation of a specific branch of the UPR rather than a global UPR.

Consequences of SEC23B mutations

SEC23B mutations cause congenital dyserythropoietic anemia type II (CDAII) (Schwarz et al. 2009). CDAII is characterized by ineffective erythropoiesis and by the presence of bi- and multinucleated erythroblasts in bone marrow, with nuclei of equal size and DNA content, suggesting a cytokinesis disturbance (Queisser et al. 1971). Consistent with the impaired glycosylation of erythrocyte membrane proteins in CDAII (Anselstetter et al. 1977), band 3 (anion exchange protein 1) is hypoglycosylated in patients with *SEC23B* mutations (Bianchi et al. 2009). Transcription in the region around *SEC23A* is repressed in human erythroid cells whereas the equivalent region around mouse *Sec23a* is more transcriptionally permissive (Ulirsch et al. 2014), partly explaining why humans with *SEC23B* mutations show the erythroid specific phenotype, but not *Sec23b* KO mutants (Schwarz et al. 2009). CDAII-linked *SEC23B* mutations involve either two hypomorphic alleles or one hypomorphic allele and a null allele (Bianchi et al. 2009; Schwarz et al. 2009). A homozygous *SEC23B* null mutation likely causes embryonic lethality.

Sec23b KO mice die shortly after birth with degeneration of secretory tissues such as the pancreas and salivary glands, reflecting an abundant expression of *Sec23b* and low expression of

Sec23a in these tissues (Tao et al. 2012). In *Sec23b* KO mice, ER stress-induced apoptosis/degeneration of professional secretory tissues is enhanced after cells start to synthesize a large amount of secretory proteins (Tao et al. 2012), suggesting that excessive accumulation of secretory proteins is the trigger of tissue degeneration. The proapoptotic pathway of the UPR plays a central role in degeneration of the professional secretory tissues, as *Chop* and *Trb3* are markedly upregulated. SEC23B deficiency does not lead to collagen secretion defects (Zhu et al. 2015). The *Sec23b* KO mice also fail to suckle, which is suggestive of other abnormalities. Importantly, SEC23A can functionally replace SEC23B as mice expressing *Sec23a* on the *Sec23b* locus ($Sec23b^{b\text{-}a}$) are indistinguishable from wild type (Khoriaty et al. 2018). It has not been shown, however, if $Sec23a^{a\text{-}b}$ mice are also indistinguishable from wild type.

In zebrafish, *sec23b* morphants show similar phenotypes to the *crusher* mutants (Lang et al. 2006). However, CRISPR/Cas9-mediated zebrafish *sec23b* null (*Δexon5*) mutants do not survive beyond 3 weeks of age albeit no gross abnormalities except that they are smaller (Khoriaty et al. 2018). *sec23b* morphants have previously been reported to exhibit an increase of circulating bi-nucleated erythrocytes (Schwarz et al. 2009). However, such erythroid phenotype is not observed in the $sec23b^{\Delta exon5}$ mutant. As zebrafish communities have raised concerns for the issue that morpholino-induced phenotypes can be different from those of the corresponding stable mutants despite careful application of the guidelines for morpholino use (Stainier et al. 2017), the $sec23b^{\Delta exon5}$ mutant likely reflects the functions of Sec23b in zebrafish.

Functions of SEC24

SEC24 forms a heterodimer with SEC23. It is mainly responsible for cargo capture and has multiple cargo binding pockets. Four paralogs of SEC24 partially overlapping and distinct cargo binding activities.

Consequences of SEC24A mutations

Sec24a KO mice are grossly indistinguishable from wild type and develop normally to adulthood, but they exhibit reduced cholesterol levels in the plasma because PCSK9, a soluble negative regulator of LDLR, depends on SEC24A for efficient exit from the ER (Chen et al. 2013). Disruption of one copy of *Sec24b* in *Sec24a* KO mice reduces plasma cholesterol levels further, suggesting that there is a partial redundancy in function between SEC24A and SEC24B in the regulation of plasma cholesterol levels.

Consequences of SEC24B mutations

Four rare missense heterozygous *SEC24B* mutations have been identified in human cases with neural tube defects (Yang et al. 2013). However, those mutations have been found in volunteers of large-scale sequence projects (Lek et al. 2016). Perhaps, a genetic defect in another gene is involved in the neural tube defects (see mouse studies below). The neural tube defects are classified into three groups: a failure of caudal tube closure leads to spina bifida, a failure of rostral neural tube closure leads to anencephaly, and a closure failure of the neural tube along the entire anterior–posterior axis from the midbrain–hindbrain boundary to the most caudal end leads to craniorachischisis (Greene and Copp 2014; Merte et al. 2010). Homozygous *Sec24b* KO embryos such as *Sec24b*^{Y613}, *Sec24b*^{Y578}, and *Sec24b*^{S135X} exhibit craniorachischisis and are

embryonic lethal near E18.5 (Merte et al. 2010). This phenotype is caused by ER exit failure of VANGL2 which relies on SEC24B for ER export (Merte et al. 2010). VANGL2 is a core component of planar cell polarity that regulates convergent extension immediately preceding neural tube closure (Bailly et al. 2018; Montcouquiol et al. 2003). Zebrafish *sec24b* morphants show marked shortening of the anterior-posterior axis, a defect of convergent extension in zebrafish (Yang et al. 2013). The mouse *Sec24b* mutations described above result in SEC24B truncation. The heterozygous $Sec24b^{Y613}$ mutation alone does not cause a neural tube defect and a heterozygous $Vangl2^{LP}$ mutation causes spina bifida with low penetrance (~5%). Interestingly, mice heterozygous for both $Sec24b^{Y613}$ and $Vangl2^{LP}$ have a strong propensity (~68%) to develop spina bifida (Merte et al. 2010). Probably, the human SEC24B cases with neural tube defects involve another heterozygous mutation(s) in *VANGL2* or a related gene.

Consequences of SEC24C mutations

SEC24C is expressed ubiquitously in humans (Tang et al. 1999). *Sec24c* KO mice are embryonic lethal near E7.5 and SEC24C seems to be required in the embryonic ectoderm just prior to gastrulation (Adams et al. 2014). SEC24C is dispensable in hepatocytes, pancreatic cells, smooth muscle cells, and intestinal epithelial cells in mice (Adams et al. 2014). The embryonic lethality at E7.5 of *Sec24c* KO mice can be rescued by replacing *Sec24c* with *Sec24d* at the *Sec24c* locus ($Sec24c^{c\text{-}d}$) (Wang et al. 2018). However, the $Sec24c^{c\text{-}d}$ mice fail to suckle and die shortly after birth. Thus, there is only a partial functional overlap between SEC24C and SEC24D. Conditional KO (cKO) of *Sec24c* in neural progenitors causes perinatal mortality and microcephaly due to UPR activation and apoptotic cell death of postmitotic neurons (Wang et al. 2018). Expression of

UPR genes such as *Atf4*, *Chop*, *Gadd34*, *Herp*, *Bip*, and spliced *Xbp1* is upregulated in the cortices of the mutant mice.

Zebrafish *sec24c* morphants are shorter than wild types, but their head skeleton and cartilages appear normal (Sarmah et al. 2010).

Consequences of SEC24D mutations

Compound heterozygous *SEC24D* mutations cause a syndromic form of osteogenesis imperfecta with craniofacial malformations (Garbes et al. 2015). The craniofacial phenotypes of the affected individuals are reminiscent of those of CLSD patients. *SEC24D* is expressed broadly in a wide range of tissues both in humans and in mice (Krupp et al. 2012; Tang et al. 1999). *Sec24d* KO mice carrying a gene trap allele ($Sec24d^{gt1/gt1}$) are embryonic lethal and no null embryos are recovered even at the blastocyst stage (Baines et al. 2013). Another line of *Sec24d* KO mutant ($Sec24d^{gt2/gt2}$) survives to mid-embryogenesis (E11.5) due to a low level of residual normal splicing around the gene trap (Baines et al. 2013).

Zebrafish *bulldog* mutations are located in the *sec24d* gene (Sarmah et al. 2010). The *bulldog* mutations result in either a premature stop codon or a frameshift and a premature stop codon. *sec24d* is expressed throughout the entire embryo, but is prominently found in the craniofacial cartilage in zebrafish. Secretion of cartilage matrix proteins is blocked in *sec24d* mutant fish, resulting in cartilage malformation and severe craniofacial/skeletal abnormalities (Ohisa et al. 2010; Sarmah et al. 2010). Craniofacial development in double *sec23c*/*sec24d*-deficient animals is stopped earlier than in *bulldog* mutants, implying that *sec24c* has a partially overlapping function with *sec24d* during early chondrogenesis stages. *sec24d* inactivation also

leads to stimulation of collagen biosynthesis and triggers ER stress in chondrocytes as levels of BiP, Sil1 (co-chaperone for BiP) are increased by about 5-fold and Hsp47 is elevated significantly (Sarmah et al. 2010).

Functions of SEC31

SEC31 forms a heterotetramer with SEC13 (Fath et al. 2007). SEC13-SEC31 hetertetramers can form different cage structures amenable to vesicles and tubules (Fath et al. 2007; Stagg et al. 2008; Zanetti et al. 2013). SEC13-SEC31 heterotetramers are recruited to the ER exit sites through interaction between SEC23 and SEC31. SEC31 enhances the SEC23-GAP activity further, facilitating SAR1 recycling (Antonny et al. 2001). There are two paralogs of SEC31 in vertebrates.

Consequences of SEC31 mutations

SEC31A is expressed broadly and enriched in the ovary in humans (Halperin et al. 2019; Krupp et al. 2012). *SEC31B* is also expressed broadly, but expressed in high amounts in kidneys and low amounts in livers (Krupp et al. 2012). Overall, *SEC31A* is expressed much more than *SEC31B* (Krupp et al. 2012). A homozygous *SEC31A* truncation mutation causes a neurological syndrome (Halperin et al. 2019). This mutation triggers nonsense-mediated decay, resulting in a null mutation. Affected individuals die by the age of four and present with intrauterine growth retardation, neural and neuromuscular abnormalities, and bilateral nuclear cataracts. They also exhibit dysmorphic craniofacial features and skeletal deformities, but do not display lipid malabsorption, neural tube defects, and dyserythropoiesis. It is yet to be determined if SEC31A

is functionally redundant with or distinct from SEC31B because the SEC31A phenotypes may be affected by the insufficiency of SEC31B in the affected tissues.

Zebrafish *sec31a* morphants do not reproduce the human phenotypes as the *sec31a* morphants display small eyes, hypoplastic liver, exocrine pancreas, intestinal tube, and malformed skeletal cartilage (Niu et al. 2012). Gross phenotypes of *sec31b* morphants are very similar to those of *sec31a* morphants although the skeletal phenotype has not been documented for the *sec31b* morphants (Niu et al. 2014). Sec31b plays a more important role than Sec31a in eye development as *sec31b* morphants show a more profound eye phenotype than *sec31a* morphants (Niu et al. 2014). Type II collagens accumulate in the ER of chondrocytes in the *sec31a* morphants.

Functions of SEC13

SEC13 is a subunit of the SEC13-SEC31 heterotetramer as well as a subunit of the NPC (Fath et al. 2007; Siniossoglou et al. 1996). There is no paralog of SEC13 both in yeast and in vertebrates.

Consequences of SEC13 mutations

Sec13 KO mice are not viable and these mice have not been analyzed in detail (Moreira et al. 2015). *Sec13* hypomorphic mice ($SEC13^{H/-}$) which express low levels of *Sec13* are viable and do not present with any gross defects (Moreira et al. 2015). However, they show aberrant expression of immunological factors in macrophages and T cells.

In zebrafish, *sec13* is expressed in the head and trunk and enriched in developing digestive organs (Niu et al. 2012). Zebrafish *sec13* morphants are viable at least until 5 dpf (Townley et al. 2008). They exhibit defects in craniofacial development and malformation of the pectoral fins, reminiscent of the *crusher* phenotypes and *sec31a* morphant phenotypes. Zebrafish *sec13*^{sq198} mutants have a new splicing acceptor site in intron 7 of the *sec13* gene, resulting in a C-terminal truncation of Sec13 and loss of affinity for Sec31 (Niu et al. 2012). This mutant exhibits hypoplastic digestive organs (the liver, exocrine pancreas, and intestine), small eyes with disrupted retinal lamination, and malformed skeletal cartilage. The retinal phenotype of this mutant is due to loss of the NPC function of the truncated Sec13 (Niu et al. 2014). Type II collagen is retained in the ER of chondrocytes and laminin is retained in the ER of intestinal epithelial cells (Niu et al. 2012). Consistent with these ER phenotypes, expression of UPR genes such as *bip*, *chop, grp94*, *atf6*, *ire1a*, and *perk* is enhanced and protein levels of Chop and p-eIF2α are increased, which leads to cell-cycle arrest and apoptosis, arresting the growth of the digestive organs and skeletal cartilage (Niu et al. 2012).

CLUES TO SPECIFIC PHENOTYPES OF COPII MUTANTS

Studies of COPII mutants have provided us with critical information about physiological functions of individual COPII components. Important clues that contribute to specific phenotypes of COPII mutants have emerged.

A requirement of a specific paralog for ER export of a certain cargo molecule(s)

As SEC24s are mainly responsible for cargo recognition, a mutation of a *SEC24* paralog likely affects ER export of specific cargo molecules. Indeed, an ER export defect of PCSK9 and VANGL2 can account for the phenotypes of *Sec24a* KO mice and *Sec24b* KO mice, respectively (Chen et al. 2013; Merte et al. 2010). SEC24C is critical for early embryo development and brain formation in mice, but cargo molecules responsible for the phenotypes remain to be identified (Adams et al. 2014). SEC24D plays a role in ER export of procollagens as SEC24D mutations cause skeletal defects in different vertebrates where its expression profile would differ (Garbes et al. 2015; Ohisa et al. 2010; Sarmah et al. 2010). Collagen secretion is defective in *Sec24d* KO embryos (unpublished data, C.L. Lu and J. Kim).

Other COPII components appear to have a specific role in secretion of certain cargo molecules. For example, dietary lipid retention in the *sar1b* morphants is rescued with the introduction of *sar1b* mRNA but not *sar1a* mRNA (Levic et al. 2015). SEC23A appears to have a unique role in collagen secretion as disruption of *Sec23a* leads to a secretion block of collagen but not fibronectin and SEC23B deficiency does not lead to collagen secretion defects in mice (Zhu et al. 2015). The secretion defect of collagen in *Sec23a* KO mice doesn't appear to arise from lack of SEC23B in the affected tissues as *Sec23b* is normally expressed in the tissues (Zhu et al. 2015). It is possible, however, that the observed amounts of SEC23B in the tissues are not sufficient to support collagen secretion. The zebrafish *sec23a crusher* mutant also displays collagen secretion defects (Sarmah et al. 2010). A recent report suggests that SEC23A and SEC23B are interchangeable as $Sec23b^{b\text{-}a}$ mice are indistinguishable from wild type mice (Khoriaty et al. 2018). However, whether SEC23B can replace SEC23A remains to be tested. If SEC23A has a unique role in collagen secretion, secretion of collagen will be defective in $Sec23a^{a\text{-}b}$ mice.

Collagens and chylomicrons are either too long or too big to be packaged into standard COPII vesicles of 60 – 80 nm in diameter. Interestingly, while ER export of procollagens involves SAR1B, SEC23A, SEC24D, SEC13, and SEC31A, that of chylomicrons requires SAR1B. CMRD phenotypes have not been observed in patients with *SEC23A* mutations, *SEC24D* mutations, and *SEC31A* mutations, suggesting that other COPII paralogs can support ER export of chylomicrons. Phenotypic assessments of human patients and animal models indicate that TANGO1, cTAGE5, SLY, SYT18, CUL3-KLHL12, SEDL, BBF2H7 are also required for ER export of procollagens or chylomicrons (Saito and Katada 2015). Mechanisms for differential utilization of COPII proteins and related proteins by procollagens and chylomicrons remain elusive at present.

Abundant paralog in a tissue

SEC23A is much more abundant than SEC23B in human calvarial osteoblasts (Fromme et al. 2007). In the diseased calvarial osteoblast, both forms of SEC23 are likely missing because *SEC23A* has a defective mutation and *SEC23B* is not expressed sufficiently, which partly explains why *SEC23A* mutations affect skull formation in humans (Boyadjiev et al. 2006; Boyadjiev et al. 2011). Similarly, SEC23B is far more abundant than SEC23A in erythroid cells (Ulirsch et al. 2014), which accounts for the erythroid phenotype of the patients with *SEC23B* mutations (Schwarz et al. 2009). Phenotypes caused by mutations of an abundant paralog can differ in different species as expression profiles of the paralogs vary significantly in different species. Indeed a genetic disruption of *Sec23a* or *Sec23b* in mice does not produce respective human phenotypes (Tao et al. 2012; Zhu et al. 2015).

Differential sensitivity of tissues to an ER export block

SEC13 disruption should cause a general ER export defect in every tissue, as there is no SEC13 paralog. Indeed secretion of collagen (a large cargo molecule) as well as laminin (a small cargo molecule) is blocked in zebrafish *sec13*^{sq198} mutants (Niu et al. 2012). However, digestive organs and skeletal cartilage are the tissues with the most severe impairments in the mutants. It is possible that the *sec13*^{sq198} mutation (a new splicing acceptor site in intron 7 of the *sec13* gene) is hypomorphic in nature and complete ablation of *sec13* would affect all the tissue similarly. Regardless, it is clear that some tissues are more sensitive than other tissues to a similar ER export block. It remains to be determined why a similar ER export block spares some tissues. Perhaps Sec13^{sq198}, a truncated Sec13, can support a basal ER export activity in tissues where secretion demand is low but not in tissues where the demand is high. The tissues with high secretion demand would trigger rampant UPR and ensuing cell death. In support of this idea, *Sec23b* deletion triggers cell death after cells start to synthesize large amounts of secretory proteins in mice (Tao et al. 2012). Alternatively, normal Sec13 proteins are synthesized more in certain tissues with the help of different splicing components.

UPR MODULATION IN COPII MUTANTS

Genetic manipulation in the UPR and cell death

In yeast, *HAC1* overexpression suppresses growth phenotypes of *ts* mutants in the COPII genes, but not *sec13*Δ mutant. Considering the dual function of Sec13 in the assembly of both COPII vesicles and the NPC, it is possible that *HAC1* overexpression does not suppress the NPC defect

of *sec13*Δ mutants. Thus, it should be tested whether *HAC1* overexpression suppresses the growth phenotype in other COPII deletion mutants such as *sar1*, *sec23*, *sec24*, and *sec31* that have no role in the NPC.

An important question is whether UPR modulation affects the phenotypes of vertebrate COPII mutants. One challenge to this approach is that overexpression of a UPR gene can be harmful by itself. In fact, overexpression of *Atf4* induces early onset of hyperlipidemia and hepatic steatosis in zebrafish (Yeh et al. 2017). Skeletal muscle-specific overexpression of a recombinant *Perk* induces skeletal muscle atrophy in transgenic mice (Miyake et al. 2017). In cultured cells, overexpression of active *ATF6* induces apoptosis of myoblasts (Morishima et al. 2011) and overexpression of *IRE1* leads to apoptosis of transfected cells (Wang et al. 1998). It remains to be determined if different tissues react differently to overexpression of UPR sensor genes or the genes linked to the UPR.

Cell death pathways may be considered to alleviate UPR-induced cell death. UPR activation and apoptotic cell death are observed in postmitotic neurons of *Sec24c* cKO mice where expression of UPR genes such as *Atf4*, *Chop*, *Gadd34*, *Herp*, *Bip*, and spliced *Xbp1* is upregulated (Wang et al. 2018). However, deletion of *Chop* in the *Sec24c* cKO mice does not delay cell death (Wang et al. 2018), indicating that CHOP activation is not solely responsible for cell death in the tissue. Perhaps multiple UPR pathways are involved in the cell death of the mutant. It is also possible that lack of cargo molecules in their destinations is the primary cause of cell death in the tissue. Thus, it remains to be determined which pathway is responsible for cell death in this and other COPII mutants.

Modulation of the UPR with small molecules

Alternatively, small molecules that modulate the UPR may alleviate phenotypes of COPII mutants. A recent study of a prion disease has provided insight into this possibility. Prion protein (PrP) is a cell surface glycoprotein synthesized with an N-terminal signal sequence and a C-terminal transmembrane domain that is cleaved off after translocation to the ER (Rudd et al. 2002). Subsequently, PrP is anchored to the membrane with glycosylphosphatidylinositol. Expression of a misfolded form of PrP (PrP^{Sc}) disrupts ER Ca^{2+} homeostasis and causes ER stress (Hetz et al. 2003). Strikingly, GSK2606414, a PERK inhibitor, ameliorates brain atrophy and prevents neurodegeneration in a prion disease model mouse line at the cost of pancreatic toxicity (Moreno et al. 2013). Integrated stress response inhibitor (ISRIB) targets downstream of eIF2α phosphorylation (Sidrauski et al. 2013; Sidrauski et al. 2015). Although this molecule has a poor water solubility, it prevents neurodegeneration without pancreatic toxicity in a mouse model of prion disease (Halliday et al. 2015). In addition, a UPR intervention has been successfully used in a myopathy model. Mutations in the type 1 ryanodine receptor (*RYR1*) gene disrupt ER Ca^{2+} homeostasis and cause muscle weakness and atrophy (Wilmshurst et al. 2010). FDA-approved 4-phenylbutyrate (4PBA) that ameliorates the UPR improves muscle functions in a RYR1 myopathy model mouse line (Lee et al. 2017). 4PBA can bind to a cargo binding pocket (the B site) of SEC24s, which somehow enhances export of ER resident proteins and ER-trapped misfolded proteins from the ER (Ma et al. 2017). Thus, in principle, a modulation of the UPR and/or ER export with a small molecule can provide viable strategies for treatment of COPII-related diseases.

CONCLUSION

The COPII mutations described above trigger rampant UPR because of excessive accumulation of cargo molecules in the ER. It has been well established that persistent and severe ER stress eventually induces cell death in vertebrates (Walter and Ron 2011). Indeed particular tissues are degenerated or fail to grow in COPII mutants. As the root cause of the COPII-related diseases is the ER export defects, small molecules that can improve such defects likely ameliorate ER stress as well. However, when such molecules are not available or only partially effective that cell death continues to occur, UPR interventions that can maintain beneficial effects of the UPR and curb harmful effects of the UPR would be useful to treat the diseases. If cell death in the affected tissues were delayed, cells would manage to secrete secretory molecules albeit to a low level. Cell death may be modulated by lowering the amounts of cargo molecules, considering the observation that tissue degeneration coincides with the onset of synthesis of a large amount of secretory proteins. In fact, a low-fat diet that lessens the ER load of lipids in enterocytes is a recommended treatment for CMRD (Peretti et al. 2010). This treatment regimen also includes fat-soluble vitamin supplements and large amounts of vitamin E, which are normally supplied through chylomicrons in healthy individuals. Interventions targeting the UPR likely benefit patients carrying hypomorphic mutations. As a proof of concept, UPR interventions restore viability of yeast hypomorphic COPII mutants (Chang et al. 2004; Higashio and Kohno 2002; Sato et al. 2002). As genome-editing tools are improving, correcting COPII mutations via genetic means may be possible in the future. In the meantime, small molecules that can improve ER export and/or modulate ER stress should be explored for interventions of COPII-related diseases.

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FIGURE LEGENDS

Figure 1. COPII vesicle assembly. GDP-bound SAR1 (D) is converted to GTP-bound SAR1 (T) by SEC12 at the ER exit sites. GTP-SAR1 recruits the SEC23-SEC24 complex and then the SEC13-SEC31 complex in a sequential manner to the ER exit sites. Lateral polymerization of SEC13-SEC31 complexes helps fission of COPII vesicles. COPII vesicles generated with purified COPII proteins, GTP and microsomes lack SAR1 (Barlowe et al. 1994). COPII vesicles shed coats upon interaction with CK1δ kinase in Golgi membranes (Lord et al. 2011).

Figure 2. The UPR of the ER. UPR signaling alleviates ER stress by enhancing protein-folding capacity and decreasing protein load in the ER. (A) The UPR in yeast. ER stress triggers IRE1 polymerization and trans-autophosphorylation, which activates its RNase domain. The activated RNase domain, then, catalyzes splicing of HAC1 (unspliced form to spliced form). The activated RNase domain also degrades multiple RNAs through a process called regulated IRE1-dependent decay (RIDD). (B) The UPR in vertebrates. Vertebrate IRE1 behaves similarly to yeast IRE1 except that the activated RNase domain catalyzes splicing of XBP1. Phosphorylated IRE1 can influence activities of MAP kinases and c-Jun N-terminal kinase (JNK) via scaffolding protein TRAF2. When ER stress becomes chronic and excessive (red arrow), TRAF2 activates caspase-12, which leads to apoptotic cell death (Yoneda et al. 2001). ER stress induces PERK oligomerization and trans-autophosphorylation, which leads to activation of its kinase domain. Active PERK phosphorylates eIF2α, resulting in inhibition of protein translation. This process decreases protein load in the ER and increases the selective translation of ATF4. When cells are stressed chronically and excessively (red arrow), ATF4 induces expression of CHOP (Fawcett et

al. 1999), which causes CHOP-dependent cell death (Zinszner et al. 1998). Activated PERK also phosphorylates a transcription factor for antioxidant genes NRF2, leading to stabilization of NRF2. Upon ER stress, ATF6 is packaged into COPII vesicles and transported to the Golgi where the N-terminal cytosolic domain (ATF6N) is liberated by proteolytic processing.

Figure 1

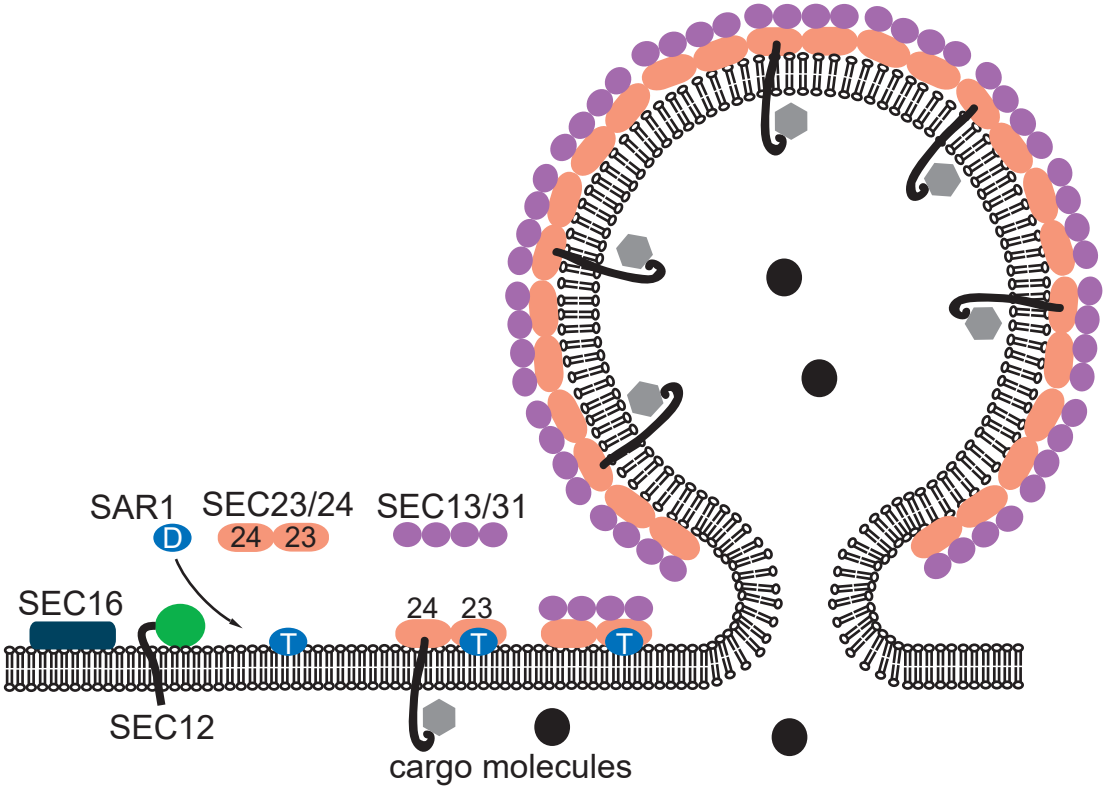
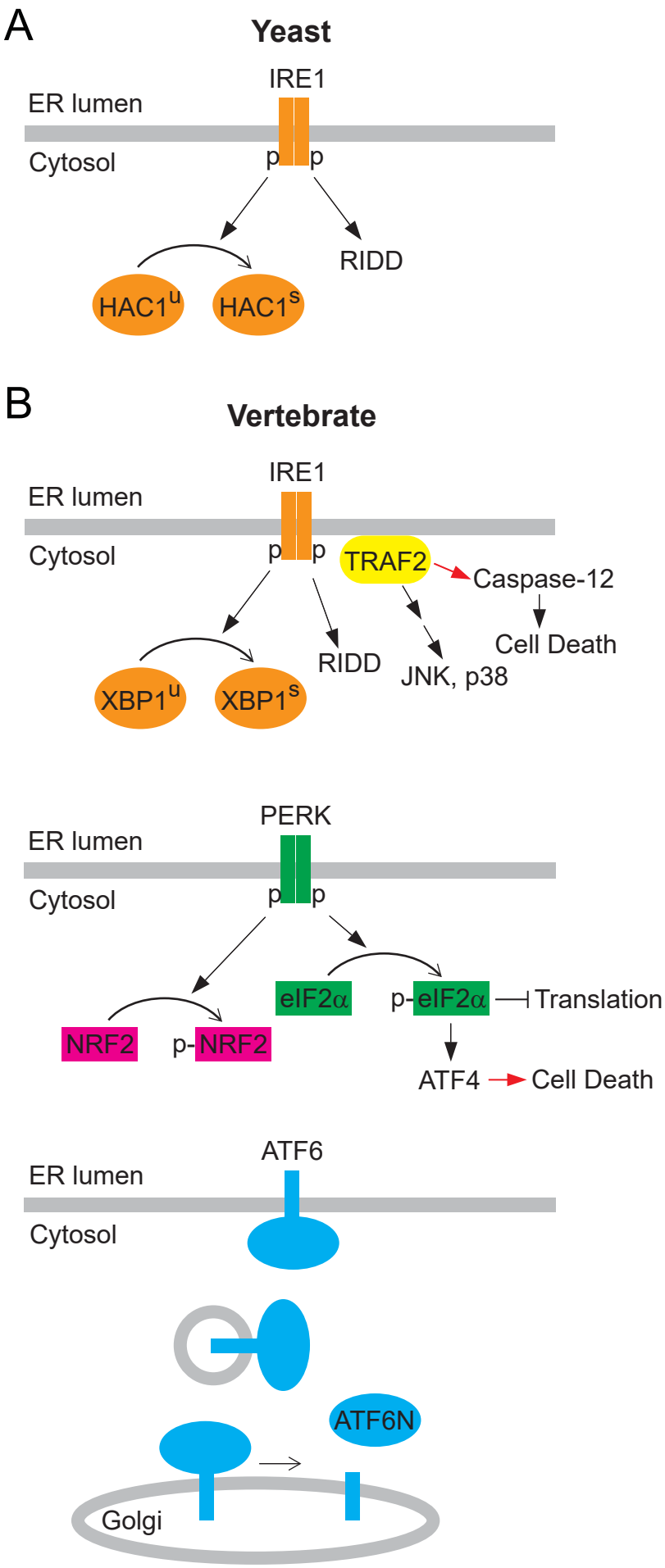

SAR1
SEC23/24
SEC13/31
D
24
23
SEC16
T
24
23
T
T
SEC12
cargo molecules

Figure 2


A
Yeast
IRE1
ER lumen
Cytosol
p
p
RIDD
HAC1u
HAC1s
B
Vertebrate
IRE1
ER lumen
Cytosol
p
p
TRAF2
Caspase-12
Cell Death
RIDD
JNK, p38
XBP1u
XBP1s
PERK
ER lumen
Cytosol
p
p
eIF2α
p-eIF2α
Translation
NRF2
p-NRF2
ATF4
Cell Death
ATF6
ER lumen
Cytosol
ATF6N
Golgi