Spring 2020

Mutations in the COPII Vesicle Genes and the Diseases they Lead to

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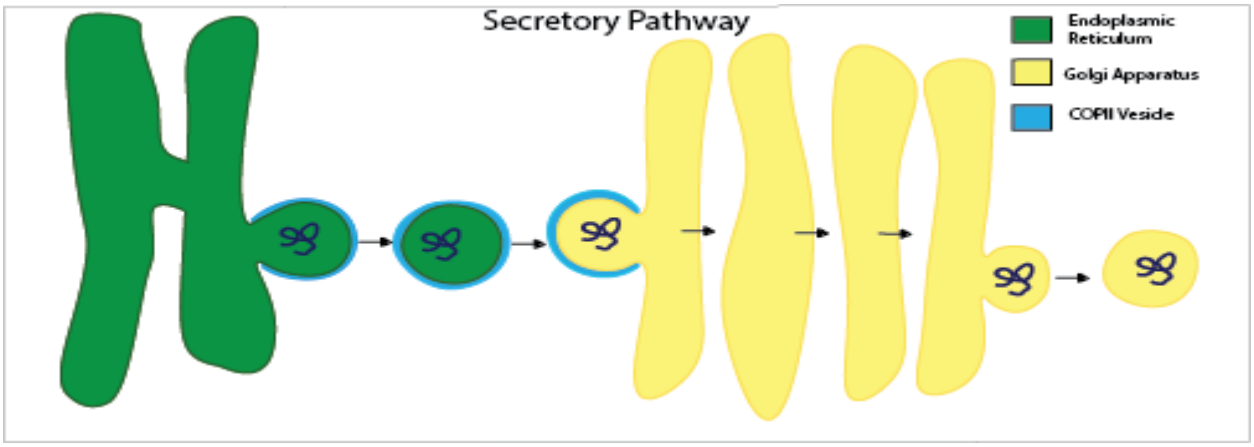
Abstract

Protein secretion is essential for cell viability. A large variety of secretory and membrane proteins are secreted through the secretory pathway. Studies have revealed how these proteins are secreted by analyzing the components of the secretory pathway. The coat protein complex II (COPII)-coated vesicles are responsible for generating vesicles at the endoplasmic reticulum. During the COPII vesicle assembly the COPII proteins package cargo molecules to nascent vesicles. COPII vesicles then fuse with ER/Golgi intermediate compartment in mammalian cells or the Golgi apparatus in yeast. The COPII proteins consist of Sar1, Sec23/Sec24 complex, and Sec13/Sec31 complex. There are paralogs of most of these proteins in vertebrates which partly explain how COPII vesicles package a large variety of proteins. Mutations in one paralog can have devastating effects on the individual, while mutations in another paralog may only cause mild health issues. By understanding how these COPII proteins function and which proteins they interact with, we can learn more about diseases caused by mutations in the genes and possibly find ways to cure or better manage these diseases.

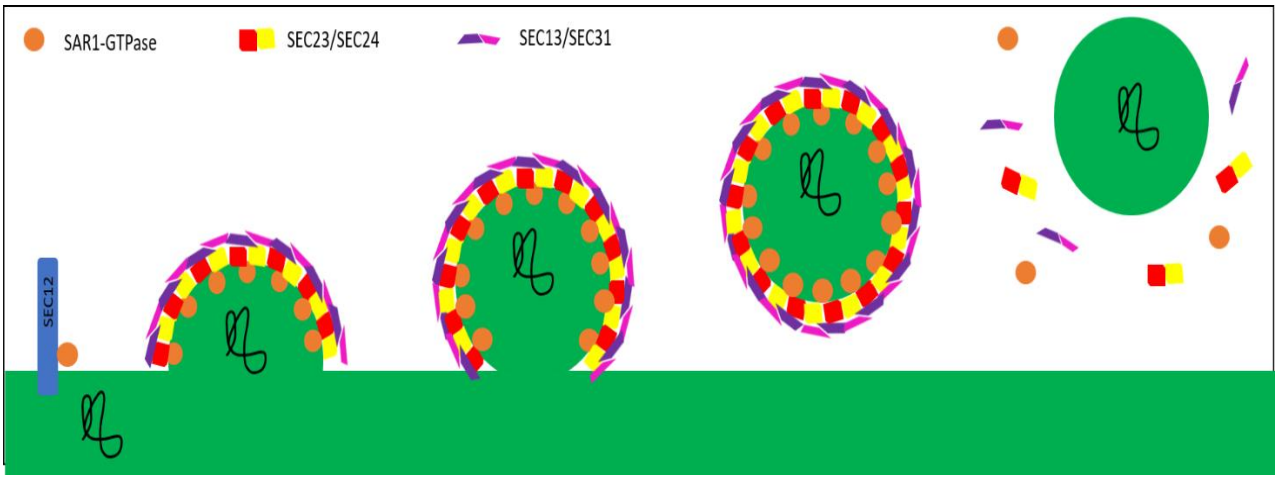
The Secretory Pathway and the Importance of COPII Vesicles

Eukaryotic cells are composed of many membrane bound organelles and produce a large variety of proteins trafficking through the organelles. The trafficking pathway that is responsible for releasing the vast majority of proteins to the outside of the cell and to the cell surface is known as the secretory pathway. It is estimated that approximately one third of proteins encoded in vertebrates' genome are secreted or transported elsewhere in the cell. Proteins destined to be secreted out of the cell or incorporated to other organelles start at the endoplasmic reticulum (ER) where they are produced. Then they are packaged into COPII vesicles and are transported to the Golgi apparatus for further modification. These proteins transit through the Golgi cisternae and are routed to their destinations. COPII vesicle assembly occurs at an ER exit site, which is a special ribosome free area of the ER. (Zahoor et al., 2018) Failure in COPII vesicle formation results in accumulation of cargo molecules in the ER and deficit of the proteins to their destinations.



How COPII Vesicle Formation Works

COPII vesicle formation is triggered by SAR1, a guanosine triphosphatase (GTPase). SAR1 is activated by a guanine exchange factor protein (GEF) called SEC12 which exchanges GDP for GTP on SAR1 at the ER exit sites. When SAR1 is bound to GTP it inserts its N-terminal α-helix into the ER membrane. This leads to slight tubulation of the membrane and recruitment of the heterodimer SEC23/24. Recruited SEC23/24 molecules form the inner coat layer of the vesicle coat. Once the SEC23/24 heterodimer is in place, recruitment of the heterotetramer SEC13/31 occurs, which forms the outer layer of the vesicle coat. SEC23 acts as a GTPase activating protein (GAP) for SAR1 and SEC31 stimulates the GAP function of SEC23 further. When SAR1 hydrolyzes GTP, it leads to dissociation of the COPII coat from the membrane. To help prevent early inactivation of Sar1, a membrane bound protein called SEC16 prevents the stimulation of SEC23 GAP activity by SEC31. SEC12 also continuously loads new GTPs onto SAR1 molecules. The assembled vesicle buds off the ER membrane, SEC12 and SEC16 no longer have an effect on the coat proteins. SAR1 hydrolyzes the GTP and leads to disassembly of the coat proteins as the vesicle travels to the Golgi. (Melero et al., 2018, Vendetti et al., 2014)



It is obvious that these coat proteins have an important job when it comes to secretion of proteins. Mutations in these coat proteins have shown a variety of health issues ranging from mild health disorders to life altering ones and even lethal disorders. Here I summarize the studies on the COPII proteins that lead to better understanding their functions in vertebrates.

Mutations in SAR1B

There are two paralogs of SAR1, SAR1A and SAR1B. As of now there are no known diseases associated with mutations in the SAR1A gene.

Mutations in SAR1B causes Anderson's disease (AD), also known as chylomicron retention disease. AD is characterized with symptoms of chronic diarrhea, retarded growth and distended abdomen in infants. Endoscopies also show a white frosting on the mucosal surface of the small intestine. A study was done on a few children with AD, who at a very young age had the symptoms and signs described above. They were all found to have a mutation in the SAR1B gene. Biopsies were taken from the small intestine of the AD patients and were compared to

healthy individuals. They found that in patients with AD the enterocytes had accumulated a large amount of fatty acids and membrane bound lipoprotein sized structures compared to those without AD. Blood analysis was also done with blood draws after 12 hours of fasting, then 90 minutes and 180 minutes after a lipid loaded meal. Fasting lipid levels in AD patients were slightly lower than normal compared to healthy subjects, but after eating a lipid loaded meal the lipid levels did not significantly change in the AD patients, where the healthy subjects had increased triglycerides and chylomicrons. Those with AD can be treated with vitamin supplementation and a low-fat diet. Some are able to eat a normal diet and tolerate the amount of fats just fine. (Georges et al., 2011)

A study with Sar1b transgenic mice compared the effects of Sar1b overexpression on mice. They found the transgenic mice to have higher bodyweights than wildtype, as well as increased glucose and insulin levels causing insulin resistance. The transgenic mice also had hepatic and plasma lipid abnormalities and chylomicron hyperproduction, which suggests that intestinal cells have increased lipid trafficking with overexpression of Sar1b. (Marcil et al., 2014)

In another study, a depletion of SAR1B in Caco-2/15 cells resulted in lower amounts of apolipoprotein B-48, apolipoprotein A-I and very low-density lipoprotein (VLDL) in the cells. The amounts of these lipoproteins were also lower in the media compared to the control cell line. The amounts of chylomicrons were also measured and found to have lowered amounts in the media of the SAR1B-depleted cells. (Theophile Sane et al., 2017)

Mutations in SEC23A

SEC23 is one of two proteins that form the inner coat of COPII vesicles. There are two paralogs for this protein, SEC23A and SEC23B. Homozygous mutations in the *SEC23A* gene cause cranio-lenticulo-sutural dysplasia (CLSD). CLSD is characterized by late closing fontanels, sutural cataracts, facial dysmorphisms and skeletal defects. Studies show that mutations in SEC23A leads to significant decrease in collagen secretion, which could partly explain the skeletal features of CLSD. One study analyzed the ER of cells with a heterozygous or homozygous mutation of the *SEC23A* gene. The heterozygous mutant showed a moderate dilation of the ER and the homozygous cells showed a severe dilation of the ER due to accumulation of cargo molecules compared to the wildtype. (Boyadjiev et al., 2006)

When Sec23a is mutated in zebrafish, they show similar characteristics as CLSD in humans. Mutant fish have truncated body length, various cranial defects, dorsal curvature of the body and kinking of the pectoral fins. (Boyadjiev et al., 2006, Lang et al., 2006) Chondrocytes from the Sec23a mutant zebrafish showed enlarged ER similar to that shown in cultured cell with Sec23a mutations. In zebrafish, knocking out both Sec23a and Sec23b showed similar results to knocking out Sec23a alone, but with a moderately smaller cranium. This result suggests that both genes play a role in craniofacial cartilage development, and loss of one gene is not compensated for by the other paralog. (Lang et al., 2006)

Mutations in SEC23B

Mutations in the SEC23B gene cause congenital dyserythropoietic anemia type II (CDAII). It is an autosomal recessive disease and is characterized by mild to moderate anemia due to ineffective erythropoiesis, an increase in multinucleated erythroblasts in bone marrow, jaundice and splenomegaly. Red blood cells of affected individuals have a double membrane appearance, a sign of ineffective erythropoiesis. There have been more than 60 different mutations identified in the SEC23B gene that cause CDAII. (Khoriaty et al., 2014). A study was conducted recently with mice having mutations in SEC23B, but unfortunately, they were unable to observe phenotypes similar to those seen in humans with comparable mutations. Mice born with a homozygous *Sec23b* null mutation died within 24 hours of birth. (Khoriaty et al., 2014).

Despite not having an animal model mimicking the human phenotypes, there have been multiple human studies of CDAII. One study compared *SEC23B* transcript levels and SEC23B protein content from erythroblast cells in CDAII patients and healthy participants. It was found that CDAII patients had a 40-60% decrease in mRNA for SEC23B compared to the healthy patients and a decrease in protein production in CDAII patients corresponded to the decrease in mRNA. (Punzo et al., 2011) No patients were found completely lacking the SEC23B protein which suggests that absence of SEC23B gene expression leads to lethality. Since there are a wide variety of SEC23B mutations linked to CDAII, a study was done to analyze if a type of SEC23B mutations correlates with severity of the disease. 42 people with CDAII participated and their hemoglobin levels were checked and their *SEC23B* genes were sequenced. Patients with addition of one missense mutation and one nonsense mutation had the most severe form of anemia and patients with two missense mutations had milder conditions. (Iolascon et al., 2010).

Mutations in SEC24A

SEC24 forms a heterodimeric complex with SEC23. There are four SEC24 paralogs in vertebrates, SEC24A, SEC24B, SEC24C and SEC24D. Deficiency of SEC24A leads to normal healthy mice, other than much lower blood cholesterol levels. In the study homozygous or heterozygous mice for a *Sec24a* null mutation were created and compared to wildtype mice. Western blots showed absence of SEC24A in the homozygous mutant and significantly less protein in the heterozygous mutant than wild type. Amounts of SEC24C and SEC24D were the same in all mice but SEC24B was increased significantly in the homozygous mutants and only slightly increased in the heterozygous mutants, suggesting that SEC24B was increased to compensate for loss of SEC24A. Grossly, there were no differences between wildtype and the mutant mice, and they all developed into healthy adults. However, when protein levels in the blood plasma were compared, there was a 30-70% decrease in apolipoprotein A-I (APO-A1) in the homozygous mutants compared to wildtype. There was also a 40% reduction in total cholesterol and high density lipoprotein (HDL) and a 60% decrease in low density lipoprotein (LDL) in the blood plasma compared to wildtype. To identify the mechanism underlining this observation, they measured levels of proteins known to interact with cholesterol in the blood and found that secretion of PCSK9 was reduced by 55%. PCSK9 is a negative regulator of LDL receptors (LDLR). The amount of PCSK9 mRNA was the same in mutant and wildtype mice. Further analyses of mouse liver cells showed that the amount of PCSK9 was the same between mutant and wildtype mice, but in the mutant mice PCSK9 was accumulated in the ER.

Introduction GFP-tagged SEC24A in T cells lead to a reduction of intracellular levels of PCSK9. Similar observations were made with SEC24B, albeit to a lesser extent but not with SEC24C and SEC24D. (Chen et al., 2013) This shows that SEC24A is necessary for PCSK9 secretion. With a reduced secretion of PCSK9, plasma cholesterol levels become lower. However, SEC24A null mice are still healthy. It is unclear if SEC24A mutations affect blood cholesterol levels or other conditions in humans.

Mutations in SEC24B

Studies on neural tube defects have shown a possible link of these defects to SEC24B. One study looked at possible causes of a condition called craniorachischisis, which is a lethal defect of neural tube closure from hindbrain to the tailbone area resulting is exposure of the spinal cord. A forward genetic screen was done looking for recessive mutations that can affect neural development in mice. Mouse line 811 was distinguished because homozygous mutants in the line developed craniorachischisis. When breeding within this mouse line was done, about 12% of the embryos had fully open neural tubes. Sequencing on the embryos with open neural tubes identified a causal mutation in *Sec24b*. The *Sec24b* mutant embryos were shorter and wider than wildtype. They also displayed omphalocele, which is a congenital birth defect where the organs develop outside the abdomen, in 45% of the mutant embryos compared to 1% of the control embryos. Eyelid fusion was also defective in the mutants with 99% occurrence compared to 2% in the control group. Neural tube defects are often associated with aberrations in the planar cell polarity (PCP) signaling pathway. It turned out that VANGL2, a protein in the PCP pathway, was not found in the plasma membrane in the *Sec24b* mutant cells. To assess if SEC24B was responsible for packaging VANGL2 into COPII vesicles, an invitro budding reaction was done where they measured how much VANGL2 was packaged into COPII vesicles with each SEC24 paralog. There was a significant increase of VANGL2 in the vesicles when SEC24B was present but not when other SEC24 paralogs were included, indicating that SEC24B is responsible for packaging VANGL2 into vesicles. (Merte et al., 2010).

A study was done on 163 stillborn and miscarried fetuses with neural tube defects and compared them to healthy infants born. Sequencing of the *SEC24B* gene in the defective fetuses and healthy counterparts found four single amino acid substitutions in fetuses and none in any of the healthy infants. All four had heterozygous mutations (Phe227Ser, Phe682Leu, Arg1248Gln, Ala1251Gly). (Yang et al., 2013) SEC24B may have an effect on neural tube formation defects in humans.

Mutations in SEC24C

Sec24c knockout mice are embryonic lethal at E7.5. To understand how mutations in SEC24C can affect mammals, mouse studies have been done where mice with a floxed *Sec24c* allele were crossed bred with Nes-Cre-transgenic mice. These offspring were then cross bred and gave birth to offspring containing homozygous *Sec24c* conditional knockout (cKO). The homozygous *Sec24c* cKO mutants had microcephaly and died shortly after birth. Their brains weighed approximately 50% less than those of wildtype, and staining showed smaller size of cortex in the mutants. An immunoblot assay confirmed loss of *Sec24c* expression in the brains of the mutants,

but no change for *Sec24a, Sec24b,* or *Sec24d.* There were markedly less neurons in the cortical layers of the mutants than controls, which was correlated with an increase of cleaved caspase-3 in the cortex and thalamus. These results suggest that the microcephaly is caused by apoptotic neuronal death. At 16.5 days of gestation, neuron numbers between the mutants and control embryos were not altered, suggestion neurogenesis was normal. To evaluate the effect of *Sec24c* mutations postnatally, they were able to delete *Sec24c* in forebrain excitatory neurons by crossing floxed *Sec24c* mice with Camk2a-Cre-trangenic mice. By 12 months of age, the mutant mice showed a significant decrease in brain weight, but not body weight and showed greater spontaneous locomotor activity compared to the control mice. Ventricles became larger and cortexes became thinner in the mutant mice, than those in control mice. The 2 month old mice showed no differences in anatomical structures. They then tested if SEC24D could compensate for the loss of SEC24C by creating knock-in mice with *Sec24d* in place of *Sec24c*. The embryonic lethality at E7.5, which was associated with the germline disruption of *Sec24c* expression, was rescued in the knock-in mice. The brain development between the knock-in mice and control at 16.5 days gestation were similar in size and neuron number. The brains of the knock-in mice showed no increase in cleaved caspase-3 staining compared with that in littermate controls. However, the knock-in mice died shortly after birth, and their body weights were noticeably reduced compared to control mice. (Wang et al., 2018) This shows that SEC24C and SEC24D are important for controlling apoptosis in neurons and SEC24C and SEC24D have non-redundant function. There are no human studies as of now.

Mutations in SEC24D

Mutations in SEC24D have been shown to decrease collagen secretion, causing osteogenesis imperfecta (OI) in humans. Chondrocytes of zebrafish *bulldog* mutants fail to secrete type 2 collagen and the mutant embryos have reduced head size, shorter body length, and kinked pectoral fins compared to wildtype embryos. The causal mutations were located in the sec24d gene. To confirm that sec24d mutations are responsible for the *bulldog* phenotypes, antisense morpholino oligonucleotides (MO) targeting the sec24d 5'UTR were designed. The MO injected embryos developed similar characteristics as *bulldog* mutants. When assessing distribution of collagen2α1 at 59 and 62 hours post fertilization (hpf) and 3, 4, and 5 days post fertilization (dpf), they found that the collagen primarily localized in the extracellular space and small juxtanuclear compartments in the wildtype embryos. In the *bulldog* mutants, the collagen was not detected in the extracellular space and accumulated in chondrocytes. Sec24c is broadly expressed throughout the head and jaw region during the first 48 hpf and was present for the following two days. When sec24c expression was knocked down using the morpholino strategy the resulting embryos were shorter than wildtype but had normal skeletal development of the head suggesting sec24c is not involved in cranial development.

SEC24D mutations can cause OI in humans. This condition is characterized by reduced bone mass, increased bone fragility, bone deformity, and growth deficiency. Up to 90% of patients with OI have mutations in the *COL1A1* and *COL1A2* genes which encode for two pro-alpha chains on type 1 collagen. Skin fibroblasts taken from a patient having SEC24D mutations

showed procollagen accumulation in the ER and dilated ER tubules. (Garbes et al., 2015) Based on this data SEC24D appears to be responsible for the export of procollagen from the ER.

Mutations in SEC13

Sec13 mutations cause a variety of developmental problems in zebrafish. *Sec13*^{sq198} mutant zebrafish carries a new splicing acceptor in the sec13 gene. At 2 dpf the mutants and wildtype were indistinguishable. At 3 and 4 dpf the expansion of the liver and exocrine pancreas was arrested in the mutants. The intestine was also less expanded at the intestinal bulb region in mutants. Staining of the embryos at 4 and 5 dpf revealed malformed skeleton cartilage. Development of the liver, pancreas, intestine and skeletal cartilage were all impaired in the mutants but not differentiation of these tissues. When the amount of phosphorylated histone 3 (pH3) was measured to see if the cells were arresting the cell cycle it was lowered 2.8 fold in intestines of the mutant. In the pancreas it was lowered 4.7 fold in mutants, but there was no significant difference in the liver. Apoptotic activity in the liver and intestine of mutant embryos was significantly higher than wildtype. Thus, cell cycle arrest and elevated apoptosis lead to the hypoplastic digestive organs. Transmission electron microscopy revealed that chondrocytes were deformed and disorganized in the mutant embryos. The cytoplasm of the chondrocytes also had accumulated dilated vacuole like structures. Antibodies against Col2a showed that Col2a was significantly retained in the mutant chondrocytes, while it was secreted in the wildtype chondrocytes. (Niu et al., 2012)

Another zebrafish study suppressed Sec13 and had similar phenotypes as mentioned in the previous study, but they also noticed hypomorphic eyes in the zebrafish. Scanning electron microscopy at 5dpf revealed the retina was greatly reduced in most of the Sec13 morphants but the lens size was similar to wildtype. (Schmidt et al., 2013)

Mutations in SEC31A

A recent study showed that mutations in SEC31A can cause a neurological syndrome. Two siblings having a homozygous SEC31A mutation presented with intrauterine growth retardation, developmental delay, spastic quadriplegia, pseudobulbar palsy, epilepsy, neurosensory deafness and optic nerve atrophy. Unfortunately, they both died by the age of 4. Homozygous mapping of the siblings, their parents and five other healthy family members was done. It revealed the siblings both had a homozygous two-nucleotide insertion mutation in exon 22 of SEC31A that caused a truncation of the protein. This mutation also triggers a nonsense mediated decay, indicating that this mutation is a null mutation.

Drosophila sec31a knockout mutants are lethal early on. No larvae or pupae formed with homozygous mutations. They also tried knock down studies with the *Drosophila* but had similar early lethality. Knock down of *sec31a* in the *Drosophila* eye resulted in severe rough eye phenotype with disorganized and fused ommatids. *Sec31a* knockdown in the *Drosophila* neurons also proved to be lethal early on, but most made it to the larvae stage before they died. Neuroblastoma cells where *sec31a* is deleted by CRISPR/Cas9 failed to expand and generate viable clones. (Harperin et al., 2019)

Mutations in SEC31B

In a zebrafish study, *sec31a* and *sec31b* morphants showed phenotypes similar to *sec13* morphants with small livers, intestine and pancreas. For eye development *sec31b* morphants had a more profound eye phenotype than *sec31a morphants*, and the double morphant showed even worse eye phenotype in addition to a curved body. Morphants of *sec23a*, *sec23b* and *sec24c* did not show defects in retinal lamination. This showed that COPII dysfunction is not the primary cause for disruption of retinal lamination. (Niu et al., 2014)

Gene	**Human Diseases**	**Results from Studies**
SAR1A	Unknown	
SAR1B	Chylomicron retention disease	In humans with mutations, large amounts of fatty acid accumulation in enterocytes and lower cholesterol levels. Over expression in mice showed elevated lipid levels, and chylomicron production
SEC23A	Cranio-lenticulo-sutural dysplasia	Zebrafish showed similar characteristics as CLSD patients and dilated ERs.
SEC23B	Congenital dyserythropoietic anemia type II	In humans, mild to moderate anemia, multinucleated erythroblasts in bone marrow, jaundice and splenomegaly. Never completely lacking the protein. Mice died within 24 hours of birth.
SEC24A	Unknown	Lower blood cholesterol levels in mice and decreased secretion of PCSK9.
SEC24B	Possible neural Tube defects	In mice, neural tube defects present consistent with craniorachischisis and decreased VANGL2 secretion. Fetus study showed 4 cases of miscarried fetuses with mutations in SEC24B and neural tube defects.
SEC24C	Unknown	Mouse studies showed microcephaly and decreased number of neurons with signs of increased apoptosis of neurons.
SEC24D	Osteogenesis imperfecta	Zebrafish studies showed phenotypes consistent OI. Human study showed procollagen accumulation in the ER of skin fibroblasts.
SEC13	Unknown	In zebrafish, impaired development of the intestines, liver, pancreas, skeletal cartilage and retinas.
SEC31A	Neurological syndromes	Human studies showed neurological syndrome with intrauterine growth retardation, developmental delay, spastic quadriplegia, pseudobulbar palsy, epilepsy, neurosensory deafness and optic nerve atrophy. Both subjects died by age of 4. Drosophila studies showed to be lethal early on.
SEC31B	Unknown	In zebrafish, impaired development of the intestines, liver, pancreas, and skeletal cartilage similar to Sec13 mutants.

Possible Explanation for Different Diseases caused by COPII Mutations

Mutations of the COPII genes seem to cause vastly different diseases and conditions despite that the COPII proteins all work at the same cellular process. There are many possible explanations for why this is the case. For SAR1B it has a weaker affinity to the SEC13/31 heterotetramer than SAR1A. This may allow the vesicle to be more flexible for larger cargo like chylomicrons and collagen to be loaded into COPII vesicles. Absence of functional SAR1B likely leads to failure of packaging larger cargo into COPII vesicles. A similar concept maybe applicable to mutations in SEC23A, which leads to decreased collagen export. Perhaps SEC23A has poor recruitment of the SEC13/31 heteroteramer, leading to larger vesicles, but if mutated the vesicles remain small. (Venditti et al., 2014). COPII paralogs may be responsible for loading specific cargo into the COPII vesicles for secretion. For example, the four SEC24 paralogs are classified into two groups (SEC24A/SEC24B and SEC24C/SEC24D) based on their amino acid sequence homology. Within each group there is 50% identities between the pairs but only 20% identities between the two groups. SEC24A is responsible for packaging PCSK9 and SEC24B is responsible for packaging VANGL2 into COPII vesicles. (Unlu et al., 2014). Mouse mutants of Sec24a or Sec24b showed phenotypes associated with deficiency of PCSK9 and VANGL2, respective.

Tissue specific expression is another possible explanation for why certain diseases occur with COPII mutations. SEC23A mutations in humans leads to CLSD and zebrafish have similar phenotypes. These were due to decreased collagen secretion and accumulation in the ER of fibroblasts. However, in mice SEC23A mutations are lethal in embryos and they developed neural tube openings at the midbrain. This indicates that SEC23A may be expressed in different tissues across species, causing different phenotypic defects. (Zhu et al., 2015). Differences in the expression for each COPII gene may explain why mutations effect certain organ systems or tissues.

Possible Treatment Options

All of these mutations discussed are rare and are difficult to treat because of the lack of knowledge on some of these mutations, and difficultly to do clinical trials on such rare conditions. However, some possible treatment options are available. One option is to introduce the correct gene into the genome. By using viral vectors, liposomes, microspheres or plasmid DNA, a correct gene can be incorporated into the genome so that it produces the correct proteins. However, there are still many issues that have to be sorted out before this becomes a realistic option. Lifetime is an issue because many of the tissues regenerate cells quickly so it would only last for a short period of time, unless the stem cells of tissues are targeted. Transfection efficacy is another issue because a low efficiency only has a limited effect on target tissues. Getting the DNA into the cell and into the nucleus is difficult, especially if the gene is large. Another issue with this approach is safety, especially with viral vectors. There is a chance these altered viruses can revert back to their normal functions and become damaging to the body. They also may incorporate their own genes into the cell genome, which could lead to other unwanted mutations. Lastly, high cost is still an issue with this technique. (Rang et al., 2016) The idea of being able to fix mutated genes is appealing, but it has many concerns that need to be addressed.

Another treatment option could be the use of CRISPR/Cas9 system. This would work best for mutations that are tissue specific such as mutations in SEC23B, which causes congenital dyserythropoietic anemia type II. By replacing the mutant gene with the wildtype gene in bone marrow stem cells taken from the patient, and then by placing the edited cells back in the patient, this approach would allow the cells to produce the correct proteins. Similar trials have been completed in mice with success. (Dever et al., 2016). A similar approach could be taken with stem cells of the GI tract for patients with SAR1B mutations. The diseases involving multiple organ systems and tissues types would be difficult to adopt the CRISPR system to cure the diseases. There are still many issues with this gene editing. It is possible that an off-target gene can be edited, leading to unwanted mutations. In addition, ethical issues with using the CRISPR/Cas9 system have not been resolved in humans.

Intracellular protein delivery systems would be another option. By using nanocarrier systems composed of silica, gold nanoparticles, or polymers, proteins can be delivered intracellularly without being degraded. This way those affected by mutations in the COPII coat proteins can have functional forms of the proteins for normal protein secretion functions. Many of these nanocarriers are relatively non-toxic also and can deliver large proteins. However, this technology is still undergoing testing. There are also issues with how effective it is with targeting the proteins to the cells. Proteins have different lifespans, so if this becomes a possible treatment option, there will need to be ongoing treatment throughout the patient's life to keep proteins levels adequate for function. (Lee et al., 2019).

Some treatment options have been completed for people with chylomicron retention disease. By eating a low fat diet supplemented with lipid soluble vitamins, children resume normal growth and the gastrointestinal symptoms improve. There has also been success in controlling symptoms in patients taking lipid soluble vitamins and exogenous pancreatic enzyme supplementation while eating a normal diet. (Georges et al., 2011)

Some of the mutations discussed are lethal. To prevent children being born with these mutations, parents may seek genetic testing and counseling to evaluate the likelihood of having children born with lethal mutations or a disease. However, these mutations are rare, and it is unlikely they will be tested for these mutations unless they have specific reasons, such as family history of lethal conditions in infancy. Perhaps in vitro fertilization and accompanying selective testing of the embryos beforehand for any mutations should be considered for families of high risk.

Conclusion

Normal formation of the COPII vesicle coat is important for the health of the individual. Human and animal studies have shown solid evidence of what happens when certain COPII coat proteins are mutated. We can see that each paralog leads to a vastly different disease, despite working in the same cellular process. However, we still have incomplete understanding about these proteins and how they affect human health when they become defective. We don't know if mutations of SAR1A, SEC24A, SEC24B, SEC24C, SEC13, and SEC31B cause a disease in humans. It is possible that these mutations are lethal in humans so no one is born with it, or it could be that these mutations cause such mild symptoms, if any at all, that no disease is detected. For example,

according to mouse studies, SEC24A mutations caused lower blood cholesterol, and if this is the case there would be no noticeable health issues in humans.

The other COPII genes are linked to human diseases, but it is still unclear how mutations of the COPII genes cause such distinctive diseases. To treat or even cure the COPII diseases we need to understand more about physiological functions of COPII proteins and develop disease-specific and safer treatment approaches.

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