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REVIEW ARTICLE

Let's talk about Secs: Sec61, Sec62 and Sec63 in signal transduction, oncology and personalized medicine

Maximilian Linxweiler[1], Bernhard Schick[1] and Richard Zimmermann[2]

The heterotrimeric Sec61 complex and the dimeric Sec62/Sec63 complex are located in the membrane of the human endoplasmic reticulum (ER) and play a central role in translocation of nascent and newly synthesized precursor polypeptides into the ER. This process involves targeting of the precursors to the membrane and opening of the polypeptide conducting Sec61 channel for translocation. Apart from this central role in the intracellular transport of polypeptides, several studies of the last decade uncovered additional functions of Sec proteins in intracellular signaling: Sec62 can induce ER-phagy in the process of recovery of cells from ER stress and the Sec61 channel can also act as a passive ER calcium leak channel. Furthermore, mutations, amplifications and an overexpression of the *SEC* genes were linked to various diseases including kidney and liver diseases, diabetes and human cancer. Studies of the last decade could not only elucidate the functional role of Sec proteins in the pathogenesis of these diseases, but also demonstrate a relevance of Sec62 as a prognostic and predictive biomarker in head and neck cancer, prostate and lung cancer including a basis for new therapeutic strategies. In this article, we review the current understanding of protein transport across the ER membrane as central function of Sec proteins and further focus on recent studies that gave first insights into the functional role and therapeutic relevance of Sec61, Sec62 and Sec63 in human diseases.

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PROTEIN TRANSPORT INTO AND ACROSS THE ER MEMBRANE

The transport of precursor proteins into and across the endoplasmic reticulum (ER) membrane represents a highly conserved process in eukaryotic cells and is essential for the biogenesis of many transmembrane and most secretory proteins.[1–3] Basically, this process can be divided into three major steps as follows: (i) the targeting of nascent and newly synthesized precursor polypeptides to the ER membrane; (ii) the insertion of the protein into the polypeptide conducting channel; and (iii) the lateral release of the transmembrane protein from the channel into the phospholipid bilayer or the completion of translocation into the ER lumen. As there are some mechanistic differences depending on the precursor protein being translocated during or after its synthesis at the ribosome, one can distinguish between the cotranslational[4,5] (Figure 1a) and the posttranslational transport mechanism[6,7] (Figure 1b). During co-translational transport, the ribonucleo-complex signal recognition particle (SRP)[8] binds to a hydrophobic signal sequence located at or near the N terminus of the nascent precursor polypeptide and to the ribosome.[9] Subsequently, the SRP receptor guides the ribosome nascent chain complex to the polypeptide conducting channel Sec61.[10] Following GTP hydrolysis, SRP dissociates from the ribosome and the SRP receptor[11–13] inducing a resumption of protein synthesis and the nascent polypeptide chain inserts into the Sec61 channel. Subsequently, membrane proteins diffuse laterally from the Sec61 complex into the bilayer. Alternatively, ER luminal chaperone proteins such as BiP/Grp78 can function as 'molecular ratchets' and guarantee the unidirectional transport of the nascent protein through the Sec61 channel into the ER lumen.[14–16] To facilitate an interaction between these chaperones and the precursor polypeptides in transit, J domains of ER transmembrane proteins such as Sec63 mediate their direct interaction.[17–23] As the activity of ER luminal BiP depends on ATP hydrolysis, the nucleotide-exchange factors Sil1 and GRP170 guarantee a replacement of ADP with ATP.[24] During or after the precursor protein translocation is completed, the signal sequence is cleaved off by the signal peptidase complex,[25] which is followed by folding of the translocated protein and covalent modifications such as N-glycosylation.[26]

The posttranslational transport is characterized by some crucial differences compared with the above-described co-translational transport mechanism: The precursor proteins are fully synthesized at free ribosomes because they bear a signal sequence of relatively low hydrophobicity (in yeast), or are simply too short (in mammals) to efficiently and productively interact with SRP at the ribosome, which leads to a completion of translation in the cytosol.[27,28] To maintain a protein structure compatible with translocation across the ER membrane, cytosolic Hsp40 and Hsp70 chaperones prevent extensive protein folding at this stage and keep the signal sequence free for interaction with receptors at the ER surface.[29–31] Depending on structural characteristics of the synthesized protein, for example, the chain length and the extent of folding, SRP as well as Sec62 can be required for an efficient targeting to the Sec61 translocon.[23,32–34] The subsequent steps of protein transport are comparable with the co-translational transport. Figures 1a and b give an overview of the co- and posttranslational mechanism of protein transport into and across the ER membrane.

For both the co- and posttranslational transport, the protein translocation machinery as core element is composed of the ER

[1]Department of Otorhinolaryngology, Head and Neck Surgery; Saarland University Medical Center, Homburg, Germany and [2]Institute of Medical Biochemistry and Molecular Biology, Saarland University, Homburg, Germany.
Correspondence: M Linxweiler (maximilian.linxweiler@uks.eu)
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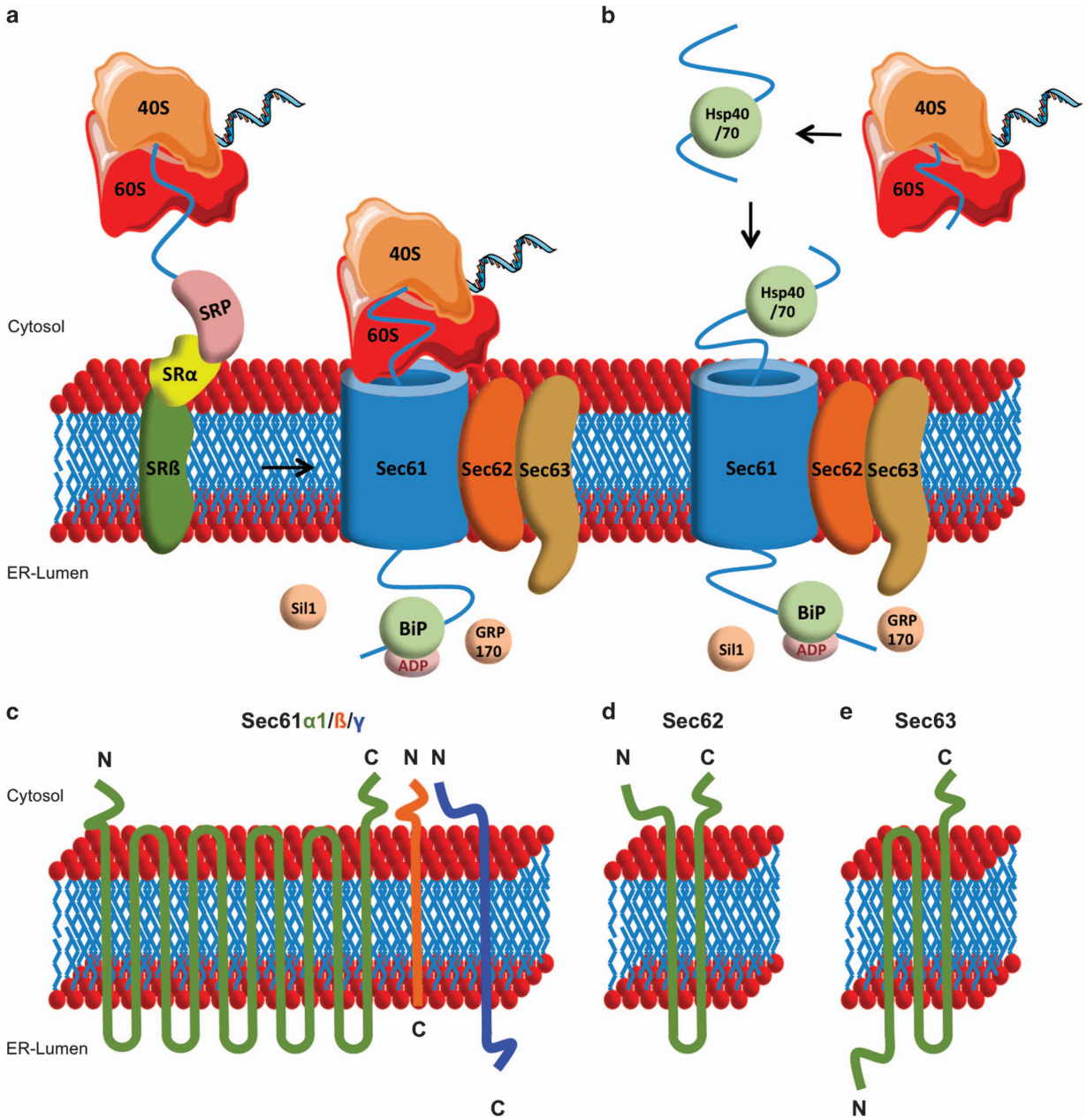

Figure 1. Protein transport across the endoplasmic reticulum membrane. Mechanism of (**a**) co-translational and (**b**) posttranslational transport of precursor proteins through the Sec61 channel. (**c**) Topological domains of Sec61α1/ß/γ, (**d**) Sec62 and (**e**) Sec63. We note that (i) Sec63 interacts with Sec62 involving a cluster of negatively charged amino-acid residues near the C terminus of Sec63 and positively charged cluster in the N-terminal domain of Sec62,[43] (ii) Sec62 interacts with the N-terminal domain of Sec61α via its C-terminal domain,[68] (iii) BiP can bind to ER luminal loop 7 of Sec61 α via its substrate-binding domain and mediated by the ATPase domain of BiP and the J-domain in the ER luminal loop of Sec63,[53] (iv) Ca^{2+}-CaM can bind to an IQ motif in the N-terminal domain of Sec61α[64] and (v) LC3 can bind to a LIR motif in the C-terminal domain of Sec62.[71] 40S, 40S ribosome subunit; 60S, 60S ribosome subunit; SR, heterodimeric SRP receptor; SRP, signal recognition particle.

transmembrane proteins Sec61, Sec62 and Sec63 ([ref. 35]) with Sec61 being composed of the three subunits Sec61α, Sec61ß and Sec61γ.[36–41] These Sec proteins form oligomers with direct interactions of Sec62 and Sec63 to each other as well as to the Sec61 channel.[42,43] However, only Sec61 and Sec62 but not Sec63 can directly interact with the ribosome.[43] The topological domains of Sec61, Sec62 and Sec63 are shown in Figures 1c–e.

Sec61 as central element of the protein translocation machinery forms a protein conducting channel with an aqueous central pore.[44] Precursor polypeptides can either completely cross the ER membrane through the Sec61 channel or exit laterally into the lipid phase of the membrane if the protein contains hydrophobic transmembrane domains.[45] This evolutionarily conserved heterotrimeric mammalian ortholog to the bacterial protein SecY was characterized in detail in its structure and function over the past years.[10,36–41,46]

After its first functional and topological description in *Saccharomyces cerevisiae* in the late 1980s,[47–49] a homolog to yeast Sec62p in mammals was identified in 1997.[35,42,50] In mammalian cells, Sec62 interacts with Sec61 as well as Sec63 and, contrary to

its yeast ortholog, harbors two conserved peptide domains at its cytosolic N terminus allowing a binding to the ribosome.[43] The detailed function of Sec62 in the protein translocation process still remains uncertain though several studies of the past decade indicated a role of Sec62 in posttranslational transport: Lakkaraju *et al.* found that posttranslationally transported precursor proteins comprising $\leqslant 100$ amino acids strongly and precursor proteins comprising 120–160 amino acids partially depend on Sec62 for efficient translocation.[33] In a study by Lang *et al.*, *SEC62* silencing led to a reduced ability for posttranslational import of small presecretory proteins without any impairment of co-translational transport or posttranslational membrane insertion of tail-anchored proteins.[23] These findings are consistent with comparable observations in yeast,[51,52] where Sec62p together with Sec61p, Sec63p, Sec71p and Sec72p forms the so-called 'posttranslational translocon'. Against this background, the question about the functional relevance of the ribosome-binding site of Sec62 remains unanswered. Eventually, further studies are needed to elucidate the detailed function of Sec62 in the protein translocation process.

The Sec63 protein consists of three transmembrane domains with the ER luminal loop harboring a J-domain that allows an interaction with chaperones—such as BiP—to facilitate the unidirectional translocation of precursor proteins through the Sec61 translocation pore. Moreover, Lang *et al.* reported a precursor-specific role of Sec63, in cooperation with BiP, in the early phase of co-translational protein transport with proteins as pERj3, PrP and ppcecA being dependent on Sec63 for efficient initial insertion into the Sec61 channel.[23,53] However, Görlich and Rapoport showed that neither Sec63 nor BiP is required for an efficient translocation of several other precursor proteins as preprolactin and VSV G protein in an *in vitro* protein transport model.[10] Hence, the mechanism of how Sec63 acts in transport as well as its substrate specificity remains elusive. The testing of further potential substrates will be required to clarify which subset of proteins is dependent on Sec63 for efficient transport.

In addition to Sec-dependent transport of precursors with N-terminal signal peptides or internal transmembrane helices serving as signal sequences, there is a posttranslational mechanism for tail-anchored membrane proteins with C-terminal tail anchors.[3] This mechanism involves cytosolic and membrane proteins for targeting and membrane integration, the so-called transmembrane recognition complex (reviewed in Borgese and Fasana[3]).

ROLE OF SEC PROTEINS IN CELLULAR CA^{2+} HOMEOSTASIS AND AUTOPHAGY

An additional function for Sec61 apart from protein translocation was suggested by several studies, indicating that Sec61 could also serve as a Ca^{2+} channel that allows a passive efflux of Ca^{2+} ions from the ER lumen—the largest intracellular Ca^{2+} store—to the cytosol.[37,54–61] Thus, Sec61 counteracts the active import of Ca^{2+} into the ER lumen through the sarcoplasmic/ER Ca^{2+} ATPase.[62] In fact, Lang *et al.*[63] were the first who could directly measure this Ca^{2+} flow through the open Sec61 channel in planar lipid bilayer experiments and link it to the Sec61 complex at the cellular level by siRNA-mediated knockdown experiments in combination with live-cell calcium imaging.[64] As the cytosolic Ca^{2+} level crucially influences essential cellular processes as cell migration[65] and apoptosis[66]—processes that both are seriously disturbed by an uncontrolled Ca^{2+} efflux from the ER[67]—it was suggested that the passive Ca^{2+} efflux through the Sec61 channel is regulated by diversified mechanisms: as first mode of regulation, Erdmann *et al.*[64] could show that cytosolic calmodulin (CaM) can efficiently bind to the cytosolic site of Sec61 in a Ca^{2+}-dependent manner, thus limiting Ca^{2+} efflux from the ER lumen. In addition, the chaperone BiP was shown to decrease the Sec61-mediated Ca^{2+} efflux too, by binding to the loop 7 of Sec61α from the ER luminal site.[53] As a third regulatory mechanism, Linxweiler *et al.*[68] showed that *SEC62* silencing increases the Ca^{2+} efflux from the ER, suggesting that the Sec62 protein also contributes to limit the Sec61-mediated Ca^{2+} flow (Figure 2a). As a point mutation (D308A) in a putative C-terminal EF hand domain of Sec62 failed to rescue the effect of Sec62 depletion on Ca^{2+} efflux contrary to a transfection of the cells with a wild-type plasmid,[68] this regulatory effect is probably mediated by the C-terminal EF hand motif of Sec62. According to our working model, Ca^{2+} efflux leads to Ca^{2+} binding to the EF hands of Sec62 and CaM, conformational changes in these two proteins, and subsequent dissociation of Ca^{2+}-Sec62 from the N terminus of Sec61α and simultaneous binding of Ca^{2+}-CaM to an IQ motif in this N-terminal domain. Apart from that, Crowley *et al.*[69] hypothesized based on transport experiments with fluorescently labeled translocation substrates and ER luminal iodide ions for collisional quenching that the Sec61 channel is impermeable to ions during the translocation of a nascent chain with the ribosome bound to Sec61. To what extent the Sec61-mediated Ca^{2+} efflux from the ER lumen and its regulatory mechanisms contribute to the global cellular Ca^{2+} homeostasis and how this is orchestrated with the other mechanisms of intracellular calcium signaling[70] are important questions that have to be addressed by future studies.

An additional function beyond protein translocation and calcium homeostasis was recently found for the Sec62 protein as well. Fumagalli *et al.*[71] showed that Sec62 plays a crucial role in the recovery of eukaryotic cells from conditions of ER stress. The term ER stress describes conditions under which the homeostasis of protein synthesis, folding and transport at the ER is disturbed due to the perturbation of ER environment.[70] Depending on the severity of stress, the cell can either initiate compensatory mechanisms that are entirely referred to as unfolded protein response or undergo programmed cell death,[70] which involves—possibly Sec61 channel mediated—Ca^{2+} efflux from the ER. During unfolded protein response, ribosomal synthesis of the majority of proteins is blocked, the expression level of several ER luminal chaperones, such as BiP and Herp, is markedly increased to facilitate a correct folding of ER luminal polypeptides and, in the case of defective repair, misfolded proteins are degraded via a mechanism called the ER-associated protein degradation.[72,73] If the cell can finally cope with ER stress conditions, the expanded ER itself as well as the high amount of ER luminal chaperones have to be downsized to a physiological level again. Therefore, small vesicles bearing ER luminal and membrane chaperones are separated from the ER membrane, fuse with phagophores to build autophagosomes and finally are delivered to lysosomes for degradation—a process called autophagy (Figure 2b).[74] In this context, Sec62 was shown to bear a LIR motif at its C terminus functioning as a receptor for phagophore-bound LC3 on recovery from ER stress induced by cyclopiazonic acid or dithiothreitol.[71] The interaction between the LIR motifs and LC3 induces the formation of autophagosomes and their delivery to lysosomes.[74] Thus, Sec62 plays an important, Sec61- and Sec63-independent, role during the compensation of ER stress—a process the authors described as recovER-phagy.[71]

Taken together, it was shown that Sec61 and Sec62 bear important functions beyond the protein translocation process, indicating that Sec proteins can influence intracellular signaling in various manners. How these additional functions are controlled in detail and how they are linked to other cellular signaling pathways remain elusive.

MUTATION AND OVEREXPRESSION OF *SEC* GENES IN HUMAN DISEASES

Over the past years, mutations, amplification and overexpression of *SEC61*, *SEC62* and *SEC63* have been linked to numerous human

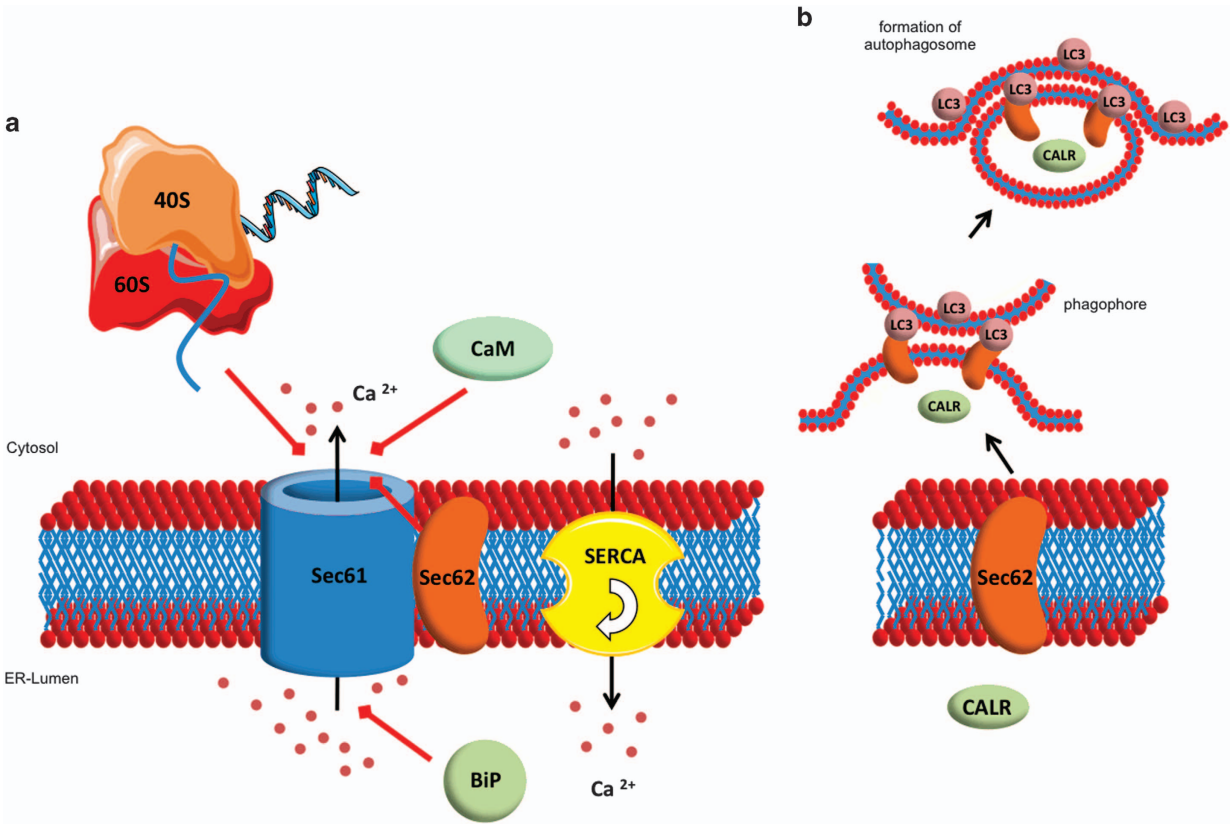

Figure 2. Regulation of Ca^{2+} homeostasis at the endoplasmic reticulum membrane and Sec62-mediated autophagy. (**a**) Regulation of Ca2+ efflux through the Sec61 channel. (**b**) Sec62-mediated autophagy. The red arrows in **a** indicate inhibitory effects on the passive Ca^{2+} efflux through the Sec61 channel. 40S, 40S ribosome subunit; 60S, 60S ribosome subunit; CaM, calmodulin; CALR, calreticulin; LC3, 1A/1B-light chain 3; SERCA, sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase.

diseases (Figure 3). In 2004, Sec63 was the first human Sec protein being linked to a human disease, as Davila *et al.*[75] showed that autosomal-dominant polycystic liver disease can be caused either by mutations in the *SEC63* gene or mutations in the protein kinase C substrate 80K-H gene (*PRKCSH*). For *SEC63*, two frameshift mutations, two nonsense mutations and two mutations predicted to disrupt splice donor–acceptor sites were described in a collective of 63 individuals, all leading to a loss of gene function. Further studies confirmed these results and strengthened the role of *SEC63* as a driver gene in the pathogenesis of autosomal-dominant polycystic liver disease by the disruption of co-translational transport of proteins, such as polycystins I and II, into the ER.[76–79]

Comparably, loss-of-function mutations have also been described for the *SEC61A1* gene and could be linked to autosomal-dominant tubulo-interstitial kidney disease in humans (ADTKD)[80] as well as diabetes and hepatosteatosis in mice.[81] Bolar *et al.* investigated renal tissue samples from two families with ADTKD and identified two different missense variants of *SEC61A1* (c.553A < G (p.Thr185Ala) and c.200T < G (p.Val67Gly)), whereas none of the otherwise frequently mutated genes *UMOD*, *MUC1* and *REN* were altered. The defective Sec61α1 variants were delocalized to the Golgi apparatus and, when induced in zebrafish embryos, led to convolution defects of the pronephric tubules consistent with the histological findings in ADTKD patients.

Another mutation of the *SEC61A1* gene (Y344H) was found to cause excessive ER stress and, as a consequence, inducing apoptosis of pancreatic ß-cells in C57BL/6 mice, which finally led to diabetes and hepatosteatosis. Thereby, transgenic ß-cell-specific expression of normal *SEC61A1* could rescue diabetes and ß-cell loss in mutant mice proving a critical role of Sec61α1 in the ß-cell response to glucose. One study introduced the mutant *SEC61A1* variant together with *SEC61A1* targeting siRNA into human cells and observed that the mutant Sec61 channel could not substitute for the wild-type channel with respect to BiP-dependent protein transport into the ER and cellular calcium homeostasis.[53] Therefore, this study suggested that the mutated *SEC61A1* gene causes apoptosis of professional secretory cells such as ß-cells because of disturbed calcium homeostasis. However, no study has investigated so far the impact of this point mutation in human patients suffering from diabetes so that a direct link to human disease is still missing.

Apart from the mentioned kidney, liver and metabolic diseases, mutations and especially an amplification and overexpression of *SEC* genes were found to be frequent molecular characteristics of various human tumor diseases (Table 1). For *SEC63*, frameshift mutations caused by microsatellite instability were found in 37.5% of microsatellite-unstable gastric cancers,[82] 48.8% of colorectal cancers,[82] 56% of small-bowel cancer associated with hereditary non-polyposis colorectal cancer[83] and in one case of hepatocellular carcinoma associated with Lynch syndrome.[84] However, functional analyses to further uncover the role of Sec63 in human carcinogenesis were solely conducted by Casper *et al.*,[84] who showed that a low hepatic expression of *SEC63* correlated with a decreased apoptosis rate and an increased proliferative activity of hepatocytes in BXD mice. Altogether, these studies indicate a potential role of *SEC63* as a tumor suppressor gene in the carcinogenesis of gastric cancer, colorectal cancer and hepatocellular cancer (HCC) without, however, providing a link of disrupted Sec63 function to tumor cell biology in these entities.

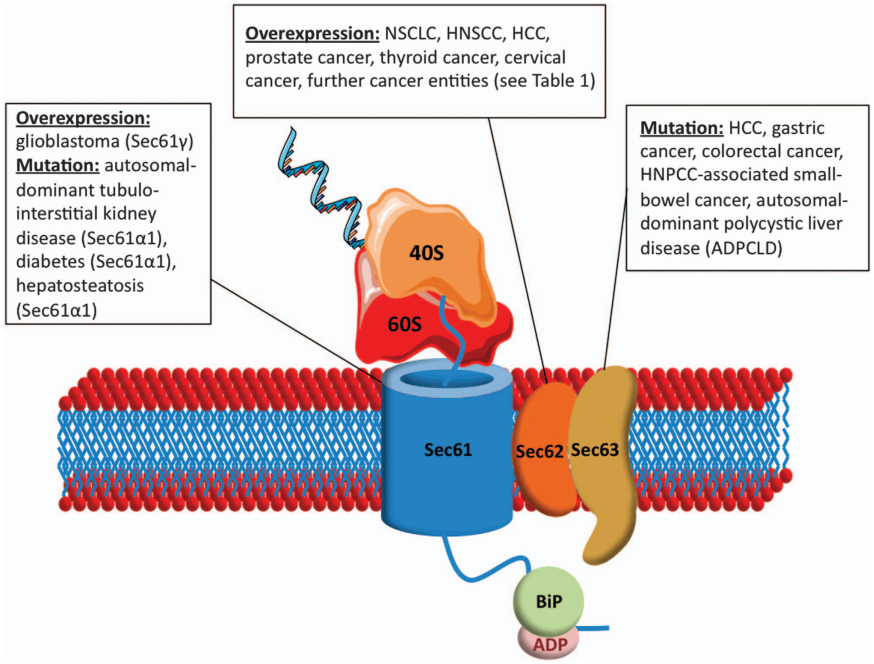

Figure 3. Overexpression and mutation of *SEC61*, *SEC62* and *SEC63* in human diseases. 40S, 40S ribosome subunit; 60S, 60S ribosome subunit; HCC, hepatocellular carcinoma; HNPCC, hereditary non-polyposis colorectal cancer; HNSCC, head and neck squamous cell carcinoma; NSCLC, non-small-cell lung cancer.

For *SEC61*, only one study reported a potential relevance in human cancer so far.[85] In this study, copy-number changes and the messenger RNA (mRNA) expression of the Sec61γ-coding gene (*SEC61G*) were investigated in 43 human glioblastoma samples using quantitative PCR. Thereby, high copy-number gains (>4-fold) were found in 47% and an overexpression compared with healthy brain tissue in 77% of cases. Neither the genes coding for Sec61α (*SEC61A1*) nor for Sec61ß (*SEC61B*) showed comparably high expression levels with, however, a tendency to an elevated expression of both genes in glioblastoma samples compared with healthy brain tissue. When silencing the *SEC61G* gene in the human glioblastoma cell line H80, Lu *et al.* could observe reduced cell viability with an increased rate of apoptosis. Induction of ER stress by treating H80 and HeLa cells with tunicamycin—an inhibitor of N-linked glycosylation—led to an increase in *SEC61G* expression, indicating a potential role of Sec61γ in ER stress response. As several other studies reported a general ER expansion under conditions of ER stress,[86,87] it is uncertain if Sec61γ has further functions during the compensation of ER stress apart from expanding ER capacity.

The strongest evidence for a causative role in cancer development and tumor cell biology exists for *SEC62* with the first association with human cancer having been reported in 2006: Jung *et al.*[88] investigated copy-number changes in 22 prostate cancer samples and found copy-number gains of the *SEC62* gene in 50% of cases as well as increased *SEC62*-mRNA levels in all analyzed samples. Following the promising results of this study, Greiner *et al.*[89] investigated *SEC62* expression at the protein level in 2071 tissue samples from 55 different tumor entities in an immunohistochemical multitissue tumor microarray. Thereby, 72% of all tumors showed detectable expression levels of *SEC62* with the highest percentage of an increased expression compared with healthy tissue from the same origin in lung cancer (93–97%, depending on the subtype) and thyroid cancer (87–100%, depending on the subtype). These results for lung and thyroid cancer were confirmed in a study by Linxweiler *et al.*,[90] including 70 non-small-cell lung cancer cases and 10 thyroid cancer cases. Increased Sec62 protein and *SEC62*-mRNA levels compared with healthy lung tissue from the same patients were detected in 80% and 60.9% of cases, respectively. For thyroid cancer, increased Sec62 protein levels were observed in 40% and increased *SEC62*-mRNA levels in 60% of cases. A study focusing on *SEC62* expression in dysplastic cervical lesions[91] found *SEC62* amplifications in 23% and increased Sec62 protein levels in 100% of cervical cancer cases with a gradually increasing *SEC62* expression depending on the severity of dysplasia. Wemmert *et al.*[92] investigated the expression of *SEC62* in 35 cases of head and neck squamous cell carcinomas using immunohistochemistry and found a strong staining intensity in 34% and a moderate staining intensity in 23% of cases. Another study focused on the expression of *SEC62* in peripheral blood mononuclear cells from 80 HCC patients and 30 healthy individuals. Hereby, Sec62-mRNA and protein content were significantly higher in the blood samples from HCC patients compared with healthy controls.[93]

On the whole, all studies addressing the expression of *SEC62* in human cancer so far consistently reported an increased *SEC62* expression level for the majority of investigated cases both in the tumor tissue[88–92,94] and in peripheral blood mononuclear cells,[93] suggesting that *SEC62* plays a crucial role in the pathogenesis of various tumor entities and bears a potential oncogenic function. This hypothesis is further substantiated by the fact that the *SEC62*-encoding chromosomal region 3q26 is amplified in numerous human cancer entities including cervical cancer,[95,96] non-small-cell lung cancer,[97] esophageal cancer,[98] ovarian cancer,[99] and head and neck cancer.[100,101] Hagerstrand *et al.*[102] screened 3131 tumor samples from 26 different tumor entities for somatic copy-number alterations and found the *SEC62*-encoding region 3q26 to be amplified in 22% of cases. Indeed, a following systematic interrogation of 3q26 by gain- and loss-of-function studies identified *SEC62/TLOC1* as a 'tumor-driver gene' encoded in this region. The same group investigated the effect of short hairpin RNA-mediated *SEC62* knockout on the proliferation of 16 different human cell lines and found that cell lines harboring an 3q26 amplification rely on *SEC62* for normal proliferative activity.

Table 1. Sec proteins in human cancer

Protein	*Study*	*Tumor entity*	*No. of patients*	*Animal model*	*Cell lines*	*Findings*
Sec61γ	Lu *et al.*[85]	Glioblastoma	*N* = 59	/	H80, HeLa	*SEC61G* is amplified and overexpressed in glioblastomas; *SEC61G* silencing suppresses cell growth and induces apoptosis; ER stress induces *SEC61G* expression
Sec62	Jung *et al.*[88]	Prostate cancer	*N* = 22	/	PC3, U145, DU145MN1	*SEC62* copy-number gains in 50% of all prostate cancer samples+increased Sec62 protein level; however, copy-number gain in patients with lower risk of and longer time to progression
	Greiner *et al.*[89]	55 different tumor entities	*N* = 2071	/	DU145, PC3, LNCaP	*SEC62* silencing sensitizes DU145, PC3 and LNCaP cells to thapsigargin treatment; correlation of thapsigargin sensitivity with *SEC62* expression in DU145, PC3 and LNCaP cells; 72% of all tumors show *SEC62* expression; *SEC62* overexpression in tumor tissue compared with healthy tissue of the same organ in lung cancer (93–97%) and thyroid cancer (87–100%); *SEC62* silencing reduces the migration of all tested cell lines
	Greiner *et al.*[94]	Prostate cancer	*N* = 32	/	A549, H1299, HT1080, TX3868, PC3	*SEC62* overexpression in the majority of prostate cancer cases correlating with the Gleason score
	Linxweiler *et al.*[90]	NSCLC thyroid cancer	*N* = 70 *N* = 10	/ /	A549, BC01, BHT101, ML1, HEK293	*SEC62* overexpression in tumor tissue compared with healthy lung tissue; high expression levels correlate lymph node metastases and poor tumor differentiation; *SEC62* silencing inhibits the migration of NSCLC cells; increased Sec62 protein (40%) and mRNA (60%) levels in thyroid cancer compared with tumor-free tissue; *SEC62* silencing inhibits the migration of BC01, BHT101 and ML1 cells, and sensitizes the cells to thapsigargin-induced ER stress; *SEC62* overexpression stimulates the migration of HEK293 cells
	Weng *et al.*[93]	HCC	*N* = 110	/	/	High *SEC62* expression in PBMCs correlates with reduced recurrent-free survival; Sec62 as an independent predictor of HCC recurrence
	Linxweiler *et al.*[68]	NSCLC	*N* = 70	/	PC3, HeLa, A549, BC01, BHT101, ML1, HEK293	High *SEC62* expression correlates with a poorer OS; effect of *SEC62* silencing on tumor cell migration and ER stress tolerance can be mimicked by CaM anatgonists; *SEC62* overexpression in HEK293 cells increases ER stress tolerance
	Hagerstrand *et al.*[102]	26 different tumor entities	*N* = 3131	C.Cg/AnNTac-Foxn1^{nunu} mice	T47D, HCC1937, H3255, HCC95, H1819, H26, TE6, RPMI8226, Fu-Ov-O1, COLO320, MCF7, MDA-MB-231, ZR75-1, HMEC, HCC364, DLD1, HMLE	3q26 amplification: 22% of tumor samples (43.7% in ovarian cancer, 31.7% in breast cancer, 31.2% in non-small-cell lung cancer). 3q26-encoded *SEC62* is required for the proliferation of celll lines with 3q26 amplification; *SEC62* overexpression in HMLE cells induces subcutaneous tumor growth in C.Cg/AnNTac-Foxn1^{nunu} mice; *SEC62* expression level correlates wtih 3q26 amplification; *SEC62* as a tumor-driver gene of the 3q26 region
	Wemmert *et al.*[92]	HNSCC	*N* = 35	/	/	High Sec62 protein level is associated with poorer OS and PFS
	Linxweiler *et al.*[91]	Cervical cancer	*N* = 107	/	HeLa, MCF7	Stepwise increase in *SEC62* expression depending on the severity of dysplasia with the highest expression in invasive cervical cancer; *SEC62* silencing inhibits and *SEC62* overexpression stimulates the migration of cervical cancer cells
Sec63	Mori *et al.*[82]	Gastric cancer CRC	*N* = 16 *N* = 43	/ /	/ /	Frameshift mutations of the *SEC63* gene due to microsatellite instability in 37.5% of gastric cancers and 48.8% of colorectal cancers
	Schulmann *et al.*[83]	HNPCC-associated SBC	*N* = 17	/	/	Frameshift mutations of the *SEC63* gene due to microsatellite instaibility in 56% of HNPCC-associated small-bowel cancers
	Casper *et al.*[84]	HCC	*N* = 1	BXD mice	/	Microsatellite instability in the *SEC63* gene in the tested HCC case; correlation of low hepatic *SEC63* expression with decreased apoptosis and increased proliferation rate in the mouse model

Moreover, *SEC62* overexpression increased anchorage-independent growth in human mammary epithelial cells and induced subcutaneous tumor growth of otherwise non-tumor-forming murine embryo fibroblasts (NIH3T3) in C.Cg/AnNTac-Foxn1^{nunu} mice[102] again pointing to a potential oncogenic function of *SEC62*.

To identify further roles of Sec62 in tumor cell biology apart from the known function in protein translocation across the ER membrane, several studies investigated the effect of *SEC62* silencing and *SEC62* overexpression on neoplastic and non-neoplastic human cell lines. Consistently, a *SEC62* knockdown markedly reduced the migration and invasion potential of prostate cancer cells[90] as well as the migration of NSCLC cells,[90] thyroid cancer cells[90] and cervical cancer cells,[91] whereas *SEC62* overexpression stimulated the migration of cervical cancer cells[91] and even human embryonic kidney cells.[90] The latter is particularly telling since it provided a direct link between Sec62 and cell migration. Of note, neither *SEC62* silencing nor *SEC62* overexpression markedly affected cell proliferation in these studies.[89–91] However, other studies reported an impairment of cell proliferation in Sec62-depleted cell lines harboring a 3q26 amplification[102] as well as in Sec62-depleted HeLa cells.[32] Though all of these studies indicate a crucial role of *SEC62* in cancer cell migration and invasion—molecular processes that are essential for tumor metastasis—it is not yet clear how this function of Sec62 is mediated on the molecular level. As Sec62 is involved in the protein translocation process at the ER, it is conceivable that a distinct subset of migration-relevant precursor proteins rely on Sec62 for efficient transport. However, no according substrates have been identified so far. Linxweiler *et al.*[91] investigated a potential role of *SEC62* in the induction of epithelial–mesenchymal transition, a highly conserved molecular process that is essential for metastasis formation, but found no influence of Sec62 on the expression of epithelial–mesenchymal transition markers as vimentin and E-cadherin. Apart from that, the inhibitory effect of high *SEC62* expression levels on the Sec61-mediated Ca^{2+} efflux from the ER lumen[68] represents a possible connection between Sec62 and cell migration. Though the exact molecular mechanisms linking Sec62 and the Ca^{2+} efflux as well as Ca^{2+} and cellular migration remain elusive.

In addition to an increased potential for migration and invasion, *SEC62* overexpressing cancer cells were found to exhibit a higher tolerance to cellular stress, such as thapsigargin (TG)-induced ER stress,[89,90] another hallmark of cancer cells. Again, increased calcium stress tolerance could be induced by *SEC62* overexpression in human embryonic kidney cells[90] and was reduced by *SEC62* knockdown from cancer cells, thereby providing a direct link between *SEC62* overexpression and tolerance to cellular calcium stress (see below).

Taken together, functional analyses investigating the role of Sec62 in tumor cell biology have shown that tumor cells could profit from an increased *SEC62* expression level in terms of an increased capability to migrate and invade the surrounding tissue, which is essential for the formation of metastases. In addition, the recently observed function of Sec62 in the recovery from ER stress[71] represents a further potentially beneficial effect of high *SEC62* expression levels for tumor cells and maybe linked to the role of Sec62 in stress tolerance.

SEC PROTEINS AS MOLECULAR BIOMARKERS AND THERAPEUTIC TARGETS IN HUMAN CANCER

To explore the clinical relevance of Sec62 as a potential biomarker especially in tumor diseases, the *SEC62* expression level of the respective tumor tissue as well as peripheral blood mononuclear cells was correlated with the patients' clinical data, including TNM stage and survival in several of the before-mentioned studies. Hereby, Greiner *et al.*[94] found a correlation between the Sec62 protein levels in prostate cancer tissue with the patients' Gleason score—a histopathological grading system with high scores indicating a poor prognosis—indicating a worse outcome for patients with higher Sec62 levels. Comparably, the Sec62 protein levels in NSCLC as well as head and neck squamous cell carcinomas tissue samples significantly correlated with a shorter overall survival.[68,92] For NSCLC patients, an additional correlation of high Sec62 levels with a dedifferentiation of the tumors and the occurrence of lymph node metastases was found,[90] again pointing to a potential function of Sec62 in tumor metastasis. In HCC patients, a high expression of *SEC62* in peripheral blood mononuclear cells correlated with a reduced recurrence-free survival characterizing Sec62 as an independent predictor of HCC recurrence.[93] In contrast, prostate cancer patients with *SEC62* copy-number gains showed a lower risk of and a longer time to progression compared with patients without *SEC62* copy-number gains in the study by Jung *et al.*[88] Considering the comparably low number of patients in this study ($n = 22$) and the consistent findings of the other studies, Sec62 seems to be an independent biomarker for the patients' prognosis in several human cancers. To confirm this potential role of Sec62 as a prognostic marker and to establish a valid basis for clinical applications, further tumor entities enclosing larger patient cohorts have to be investigated in future studies.

Though Sec61 and Sec63 have been linked to human diseases as well, a potential function of these proteins as diagnostic or prognostic biomarkers seems unlikely, as all diseases showing *SEC61A1* and *SEC63* mutations can also be caused by other genetic alterations.[80,81,103] In the context of human cancer, the percentage of gastric cancer,[82] small-bowel cancer[83] and colorectal cancer cases[82] showing frameshift mutations of *SEC63* as well as the percentage of glioblastoma cases showing a *SEC61G* amplification and overexpression[85] is too low to allow diagnostic conclusions. A potential correlation of *SEC63* and *SEC61G* expression levels with the patients' survival has not been investigated so far.

Regarding the relevance of Sec62 as a prognostic biomarker in different cancer entities and the stimulation of tumor cell migration and invasion by high *SEC62* expression levels in cell culture experiments, one can hypothesize that a suppression of *SEC62* gene function could possibly influence tumor metastasis *in vivo* too. Unfortunately, there are many known synthetic (eeyarestatin I and cotransin) and natural inhibitors (exotoxin A, mycolactone and apratoxin A) of the Sec61 channel (Figure 4),[104–109] but no direct inhibitors of Sec62 and Sec63. However, it is possible to antagonize the function of Sec62 in cellular Ca^{2+} homeostasis by either inhibiting the sarcoplasmic/ER Ca^{2+} ATPase and thereby decreasing the active Ca^{2+} import from the cytosol into the ER lumen, or antagonizing the CaM-mediated Sec61 closure with CaM antagonists and thereby increasing the passive Ca^{2+} efflux from the ER lumen through the Sec61 channel (Figure 4). Linxweiler *et al.*[68] performed *in vitro* experiments to investigate a possible phenocopy of *SEC62* gene silencing in human tumor cells by the treatment with CaM antagonists. Indeed, the CaM antagonists trifluoperazine (TFP) and ophiobolin A induced a dose-dependent inhibition of tumor cell migration, an additional inhibition of tumor cell proliferation at higher concentrations and sensitized the cells to TG)-induced ER stress—the same effects that were reported for Sec62-depleted tumor cells.[89–91] Hence, a treatment with CaM antagonists represents a potential mechanism how to achieve a functional *SEC62* knockdown in a living organism and thereby inhibiting the migratory and proliferative potential of tumor cells. As TFP was used for the treatment of patients with psychiatric disorders over many years[110] and one retrospective study observed a beneficial side effect of TFP treatment on the clinical course of tumor diseases,[111] TFP appears to be a promising new substance for a targeted therapy approach, especially in tumors with high *SEC62* expression levels. In addition, the observation that low Sec62 levels sensitize tumor cells to TG-

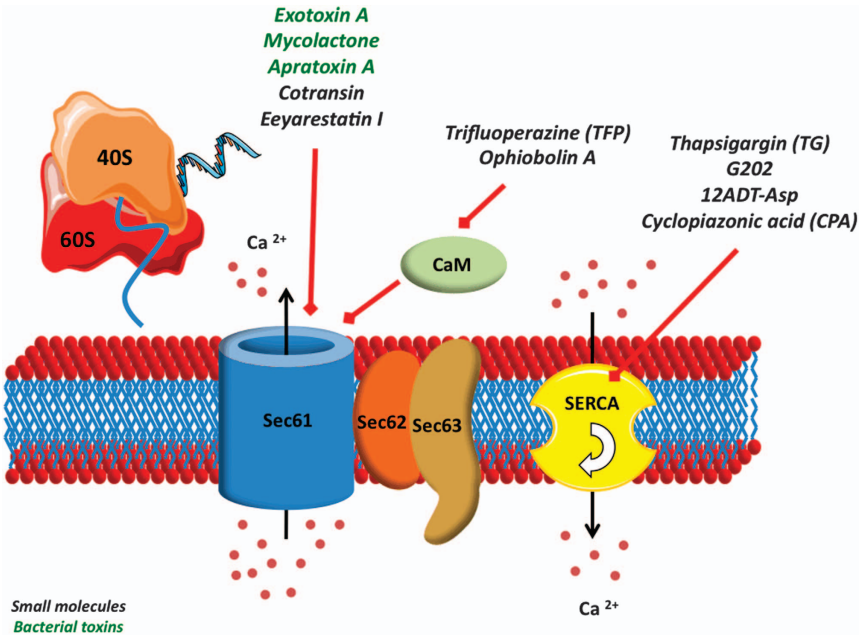

Figure 4. Protein translocation complex as a target of bacterial toxins and small molecule therapeutics. Small molecules are written in italic and black letters; bacterial toxins in italic and green letters. The red arrows indicate inhibitory effects directed against the respective target structure. 40S, 40S ribosome subunit; 60S, 60S ribosome subunit; CaM, calmodulin; SERCA, sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase.

induced ER stress[68,89] is of potential clinical relevance. Over the past years, several *in vitro* and *in vivo* studies investigated the applicability of TG and TG prodrugs (G202 and 12ADT-Asp) for the treatment of cancer patients with promising results.[112–114] In addition, a first clinical phase I trial including 44 patients with locally advanced or metastatic solid tumors was finished in august 2013 ('dose-escalation phase I study of G202 in patients with advanced solid tumors', clinical trials gov. identifier NCT01056029) and reported an acceptable tolerability and favorable pharmacokinetic profile for G202, also called mipsagargin.[115] For this treatment approach, Sec62 could serve as a predictive biomarker, as it was shown that high *SEC62* expression levels attenuate the therapeutic efficacy of sarcoplasmic/ER Ca^{2+} ATPase inhibitors.[68,89] With regard to the molecular background, a combined treatment of cancer cells with a sarcoplasmic/ER Ca^{2+} ATPase inhibitor and a CaM antagonist could overcome Sec62-mediated resistance and show synergistic therapeutic effects[68]—a new therapy approach that has to be evaluated in future studies.

Taken together, studies of the past decades have not only further illuminated the detailed mechanism of protein translocation into and across the ER membrane but gave also first insights into the role of proteins Sec61, Sec62 and Sec63 in human diseases including cancer, diabetes, liver and kidney diseases. Further investigations of the pathophysiological relevance of Sec proteins have the potential to provide a better understanding of disease emergence and progression, to enable a better prognostication and therapy planning in various cancer entities and to uncover new therapeutic targets.

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COMPETING INTERESTS

The authors declare no conflict of interest.

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