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Expression level of Sec62 modulates membrane insertion of marginally hydrophobic segments

Sung-jun Jung [1], Mekang Yun, Chewon Yim, Sujin Hong, Won-Ki Huh, Hyun Kim [*]

School of Biological Sciences and Institute of Microbiology, Seoul National University, Seoul 08826, South Korea

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ABSTRACT

In the endoplasmic reticulum (ER) membrane, transmembrane (TM) domain insertion occurs through the Sec61 channel with its auxiliary components, including Sec62. Sec62 interacts with the Sec61 channel and is located on the front side of the Sec61 lateral gate, an entry site for TM domains to the lipid bilayer. Overexpression of Sec62 led to a growth defect in yeast, and we investigated its effects on protein translocation and membrane insertion by pulse labeling of Sec62 client proteins. Our data show that the insertion efficiency of marginally hydrophobic TM segments is reduced upon Sec62 overexpression. This result suggests a potential regulatory role of Sec62 as a gatekeeper of the lateral gate, thereby modulating the insertion threshold of TM segments.

1. Introduction

Secretory and membrane proteins destined for the endomembrane system in eukaryotic cells are first targeted to the endoplasmic reticulum (ER) and translocated across or inserted into the membrane through the evolutionarily conserved Sec translocon [1]. Some proteins are translocated by the Sec61 trimer complex (Sec61 complex), which consists of Sec61(Sec61α), Sbh1(Sec61β) and Sss1(Sec61γ), while others require the Sec62/Sec63 complex in addition to the Sec61 trimer (Sec complex) [2–5].

The cryo-EM structures of the yeast Sec complex show that the pore-forming subunit Sec61 makes extensive contact with Sec63 in the cytosol, lumen and membrane [6]. On the cytosolic side of the membrane, Sec63 interacts with Sec62, and two additional subunits, Sec71 and Sec72, forming a large soluble domain of the Sec62/Sec63 complex that sits above the pore of Sec61. In the membrane, Sec63 is positioned at the back of Sec61, which is at the opposite side of the lateral gate helices where signal sequences bind and transmembrane (TM) segments exit to the membrane. When the Sec translocon structure was captured with a presecretory protein tagged with GFP, the signal peptide was found between the Sec61 lateral gate helices and the TM domains of Sec62 [7]. Thus, in the membrane, the TM domains of Sec63 and Sec62 are positioned at the back and front sides of Sec61, respectively.

Ho et al. collected and analyzed 21 proteomics datasets of protein abundance in *Saccharomyces cerevisiae* [8]. Their analysis shows that the abundance of the Sec complex subunits greatly varies depending on the method. In particular, large variations in quantitative mass spectrometry analysis data were observed, which was due to the differences in sample preparation because membrane proteins are often poorly solubilized without detergent or missed during peptide preparation by protease digestion. They found that protein abundance estimated by fluorescence measurement of GFP-tagged proteins leads to more consistent results except for low abundant proteins. Fluorescence data (both microscopy and flow cytometry) for the detection of the protein abundance of Sec61, Sec62 and Sec63 showed that the relative abundance of Sec61 compared to Sec62 and Sec63 was 3- to 11-fold higher while the relative abundance of Sec62 and Sec63 was comparable [8]. This estimation suggests that there are more Sec61 trimer complexes than Sec complexes. However, the significance of their stoichiometric balance has never been thoroughly considered.

Sec62 was poorly resolved in the first two cryo-EM structures of the Sec translocon due to its intrinsic flexibility [9,10]. When the isolated Sec complex was solubilized with increasing amounts of Triton-X 100 and resolved by blue native gel electrophoresis (BN-PAGE), subcomplexes of the Sec61 trimer, Sec62/Sec63 tetramer, and Sec63/Sec71/Sec72 trimer were found [2]. When the Sec translocon was solubilized with digitonin and separated by BN-PAGE, Sec62 was found only in the heptameric Sec complex, whereas Sec63 was found only in the Sec complex, the hexameric Sec' complex (Sec complex lacking Sec62), and the Sec63/Sec71/Sec72 trimer complex [11]. The Sec63/

* Corresponding author at: School of Biological Sciences, Building 504-421, Seoul National University, Seoul 08826, South Korea.
E-mail address: joy@snu.ac.kr (H. Kim).
[1] Current address: Department of Medical Biochemistry and Cell Biology, University of Gothenburg, 41390 Gothenburg, Sweden.

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Sec71/Sec72 complex without Sec62 was also found when microsomes were solubilized with octylglucoside/glycerol [12]. This evidence implies that the association of Sec62 in the Sec complex is labile; however, how Sec62 behaves in the native membrane remains elusive.

Upon overexpression of Sec62, we observed defective growth in *Saccharomyces cerevisiae* and investigated its phenotypes. Localization of overexpressed Sec62 was assessed by fluorescence microscopy, and translocation and membrane insertion of Sec62-dependent proteins were determined by pulse-labeling and N-linked glycosylation assays. Our data show that the threshold hydrophobicity for membrane insertion increased when Sec62 was overexpressed, suggesting that overexpressed Sec62 impedes membrane insertion of marginally hydrophobic TM segments.

2. Materials and methods

2.1. Strains and growth conditions

The JRY4 strain (W303-1α, *sec62Δ::HIS3*, pRS416 *1 kb + SEC62*) [15] was transformed with pRS415 or pRS425GPD plasmids encoding *SEC62-FLAG* or pRS315 vector encoding *SEC63-IDGR-SEC62-FLAG* under the endogenous promoter of *SEC63* and subjected to 5′ fluoroorotic acid (FOA) selection to remove *SEC62* in the URA-borne pRS416 vector (plasmid shuffling). The JRY4 strain was also used for plasmid shuffling of *sec62*Δ strains having *SEC62-yEGFP-FLAG* under the endogenous *SEC62* promoter or glyceraldehyde-3-phosphate dehydrogenase (*GPD*) promoter.

Yeast strains with chromosomally mRFP-tagged Sec61 were generated by a PCR-based gene modification method as previously described [32]. The mRFP module was amplified by PCR from plasmid *pFA6a-mRFP-KlURA3* (provided by Prof. Won-Ki Huh). Proper chromosomal integration of the mRFP-KlURA3 fragment was verified by colony PCR.

2.2. Plasmid construction

All plasmids were constructed by homologous recombination as previously described [16,17] or with a Gibson assembly kit (NEB, USA). Truncation and introduction of the FLAG epitope were performed by site-directed mutagenesis (Toyobo, Japan). Genes of interest were amplified from the genomic DNA of W303-1α by PCR using the oligonucleotide synthesized. For the *SEC63-SEC62* fusion, *1 kb + SEC63* was cloned into the pRS315-3×HA vector by homologous recombination. The plasmid was linearized by digestion of the introduced *Sma*I site downstream of *SEC63-3×HA*. The amplified *SEC62-FLAG* gene was introduced into the *Sma*I-linearized vector to generate pRS315 *1 kb + SEC63-3×HA-SEC62-FLAG*. The 3×HA epitope was mutated to IDGR residues, generating the final *SEC63-SEC62* fusion construct used in this study. All sequences were confirmed by DNA sequencing.

2.3. Fluorescence microscopy

Fluorescence microscopy was performed on a Nikon Eclipse E1 microscope with a Plan Fluor 100×/1.30 NA oil immersion objective. Image analysis was performed using NIS Elements imaging software (Nikon).

2.4. Pulse labeling and Western blotting

Cells with the plasmids encoding the indicated proteins were incubated at 30 °C. Subsequently, 3.2 OD_{600} unit cells were harvested when the OD_{600} reached 0.4–1.0. Half of the cells were solubilized with 1× SDS–PAGE sample buffer (50 mM Tris-HCl, pH 7.5, 5 % SDS, 5 % glycerol, 50 mM EDTA, pH 7.4, 50 mM DTT, 1× protease inhibitor cocktail, 1 mM PMSF) for Western blotting with HA, FLAG and Pgk1 antibodies. The remaining cells were starved in 1 ml of -Met medium for 15 min at 30 °C. Cells were resuspended in 150 μl of the same medium, and 2 μl ^{35}S[MET] was added. After 5 or 10 min of incubation at 30 °C, labeling stop solution (20 mM Tris-HCl, pH 7.5, and 20 mM sodium azide) was added and the cells were collected. Cells were lysed with lysis buffer (20 mM Tris-HCl, pH 7.5, 1 % SDS, 1 mM DTT, 1 mM PMSF, and protease inhibitor mixture) and vortexed for 4 min. After incubation at 65 °C for 10 min, lysates were transferred to IP buffer (15 mM Tris-HCl, pH 7.5, 0.1 % SDS, 1 % Triton X-100, and 150 mM NaCl) containing 2 μl HA antibody and protein G-agarose and rotated overnight at 4 °C. Beads were washed twice with IP buffer, ConA (500 mM NaCl, 20 mM Tris-HCl, pH 7.5, and 1 % Triton X-100) and Buffer C (50 mM NaCl and 10 mM Tris-HCl, pH 7.5) and added to the 1× SDS–PAGE sample buffer. After 5 min of incubation at 95 °C, endoglycosidase H was treated with the indicated samples. Proteins were analyzed by SDS–PAGE and autoradiography.

3. Results

3.1. Growth phenotypes of Sec62-overexpressing cells

Growth of the *sec62* deletion (*sec62*Δ) strains carrying a plasmid encoding the *SEC62* gene under its own promoter (*SEC62*) or under a strong glyceraldehyde-3-phosphate dehydrogenase gene (GPD) promoter (*SEC62*↑) was assessed at three different temperatures (25 °C, 30 °C and 37 °C). Overexpression of Sec62 caused a growth defect at all three temperatures (Fig. 1A). To check whether the growth defect is specific to the *sec62*Δ strain or due to the backbone plasmid, a growth assay was performed with the W303-1α strain and empty plasmids as controls (EV or EV↑) [13]. The W303-1α strain transformed with empty plasmids or that carrying the *SEC62* gene under its own promoter grew normally at all tested temperatures (Fig. 1B). However, cells carrying the *SEC62* overexpression vector showed reduced growth at 25 °C and 30 °C and a severe growth defect at 37 °C, thus confirming that overexpression of Sec62 caused a growth defect (Fig. 1B).

Since Sec62 interacts with Sec63, we sought to determine whether the enhanced association between Sec62 and Sec63 causes a growth defect. To test this idea, the C-terminus of *SEC63* was fused with the N-terminus of *SEC62* to mimic the enhanced association between Sec62 and Sec63. Both ends are naturally oriented to the cytosol so that the fusion should not alter their membrane topology. The fusion construct was transformed into the *sec62Δ* strain and confirmed to functionally replace *SEC62* by plasmid shuffling, indicating that Sec62 can function while being attached to Sec63. Both *sec62*Δ and W303-1α strains carrying the *SEC63-SEC62* fusion plasmid grew normally at the test temperatures, suggesting that the enhanced association between Sec62 and Sec63 is unlikely to cause a growth defect. (Fig. 1A and B).

3.2. Subcellular localization of overexpressed Sec62

To determine whether overexpressed Sec62 aggregates in the cytosol and causes cytotoxicity, the subcellular localization of Sec62 was examined using fluorescence microscopy (Fig. 2). Sec62 was tagged with yeast enhanced GFP (yEGFP) [14]. In the *sec62Δ* strain, *SEC61* was chromosomally tagged with red fluorescent protein (mRFP) as an ER localization marker. Sec62-yEGFP expressed under its own promoter colocalized with Sec61-mRFP. Sec62 overexpression was not detected in some cells, because the 2-μ overexpression vector could mis-segregate, leaving some cells without the vector. Overexpressed Sec62-yEGFP in the cells, however, showed intense fluorescence around the nuclear envelope, which colocalized with Sec61-mRFP, and puncta were not observed in the cytoplasm. These data show that overexpressed Sec62 was correctly localized to the ER membrane, thereby excluding the possibility of cytosolic aggregation.

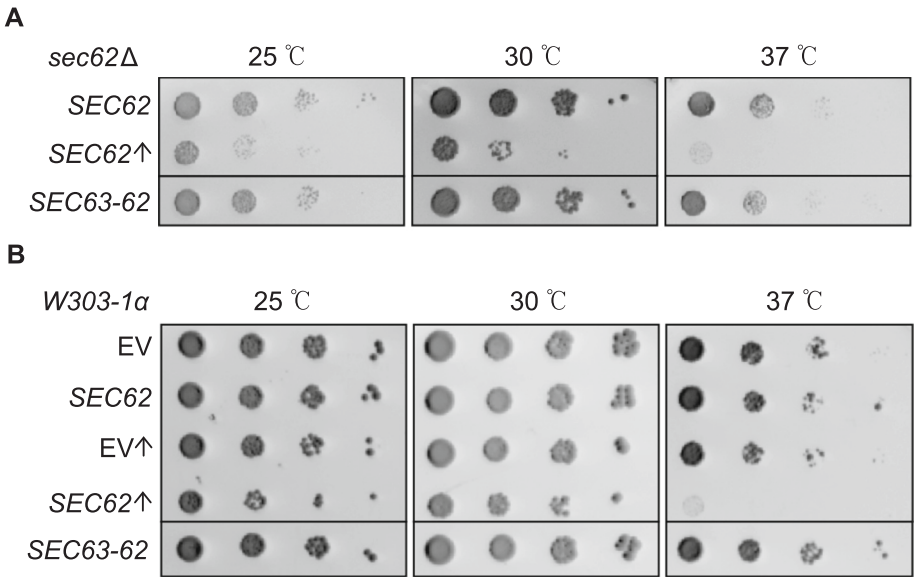

Fig. 1. Growth phenotypes of the cells overexpressing Sec62. (A) Growth assay of *sec62*Δ strains expressing *SEC62* under the endogenous or *GPD* promoter (↑) and *SEC63-SEC62* under the endogenous promoter of *SEC63*. (B) Growth assay of W303-1α strains expressing *SEC62* under the endogenous or *GPD* promoter (↑) and *SEC63-SEC62* under the endogenous promoter of *SEC63*. EV (empty vector) indicates pRS415, and EV↑ indicates pRS425GPD.

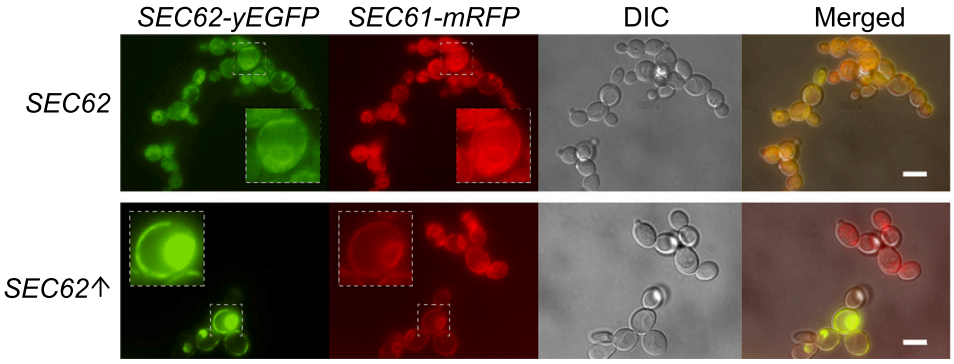

Fig. 2. Subcellular localization of overexpressed Sec62. Fluorescence microscopy of the *sec62*Δ, *SEC61-mRFP* strain expressing *SEC62-yEGFP* under endogenous or *GPD* promoter conditions (↑). A larger view is shown in insets. Sec61-mRFP was used as a localization marker for the ER. Cells were grown to the logarithmic phase in SC-leucine medium and visualized under a fluorescence microscope. Scale bars, 5 μm.

3.3. Translocation and membrane insertion of selective proteins in Sec62-overexpressing cells

3.3.1. CPY and Dap2

To determine whether translocation of Sec62-dependent precursors was defective in the Sec62 overexpression strain, we first assessed translocation of a Sec62-dependent client, carboxypeptidase Y (CPY), along with a Sec62-independent client, dipeptidyl aminopeptidase (Dap2). Both proteins have multiple N-linked glycosylation sites that are glycosylated upon ER translocation. Regardless of the Sec62 expression levels, both proteins were efficiently glycosylated, indicating that they were properly targeted and translocated to the ER lumen (Fig. 3).

3.3.2. Lep-H1(5L), a single-pass membrane protein

Next, we tested the translocation of other Sec62-dependent model membrane proteins that we previously characterized [15,16]. Sec62 mediates membrane insertion of marginally hydrophobic TM segments, the membrane insertion ratio of which is partial. Depending on whether it is a single-pass or double-pass membrane protein, we observed slightly different threshold hydrophobicities for membrane insertion (50 % membrane insertion). For a single-pass membrane protein to be inserted, higher hydrophobicity is needed, whereas for the second TM of the

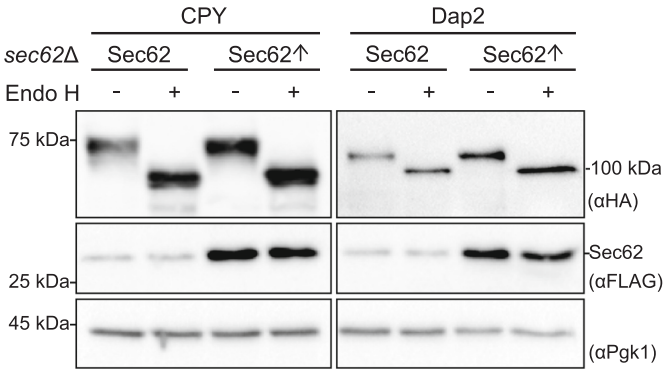

Fig. 3. Targeting and translocation of Sec62-dependent and -independent clients in Sec62-overexpressing cells. CPY and Dap2 that are tagged with three copies of HA epitopes in the C-terminus were expressed in *sec62*Δ strains expressing *SEC62* under endogenous or *GPD* promoter conditions (↑). Proteins were analyzed by SDS–PAGE and Western blotting using αHA (CPY and Dap2), αFLAG (Sec62) and αPgk1 antibodies.

double-pass membrane protein to be inserted, less hydrophobicity is required, possibly due to the effect of the accompanying TM segment [17,18].

Lep-H1(5 L) is an *E. coli* leader peptidase (Lep) whose N-terminal two TM segments are replaced with an engineered hydrophobic stretch containing 5 leucines and 14 alanines [17]. Lep-H1(5L) possesses three N-linked glycosylation sites, one in the N-terminus and two in the C-terminus (Fig. 4A). It generates two different glycosylated forms depending on the orientation of the TM segment: the singly glycosylated N_{lumen}-$C_{cytosol}$ form and the doubly glycosylated $N_{cytosol}$-C_{lumen} form. If not targeted, an unglycosylated species appears; thus, protein targeting and membrane insertion with different topologies can be distinguished with this model protein (Fig. 4A).

To examine the effects of overexpression of Sec62 on targeting and membrane insertion, Lep-H1(5L) was pulse labeled for 5 min with Met-^{35}S in yeast cells expressing different levels of Sec62 and the Sec63-Sec62 fusion protein, immunoprecipitated and analyzed by autoradiography. Overexpression of Sec62 and expression of the Sec63-Sec62 fusion protein at the expected size on SDS-gels were confirmed by Western blotting (Fig. 4A).

The relative amounts of unglycosylated forms (0 g) in the cells overexpressing Sec62 or expressing Sec63-62 fusion were subtly increased, indicating that the targeting and membrane insertion of Lep-H1(5 L) is affected by the expression levels of Sec62 or the association status of Sec62 with Sec63 (Fig. 4A). Our previous studies have shown that Sec62 selectively mediates membrane insertion of Lep-H1(5L) in

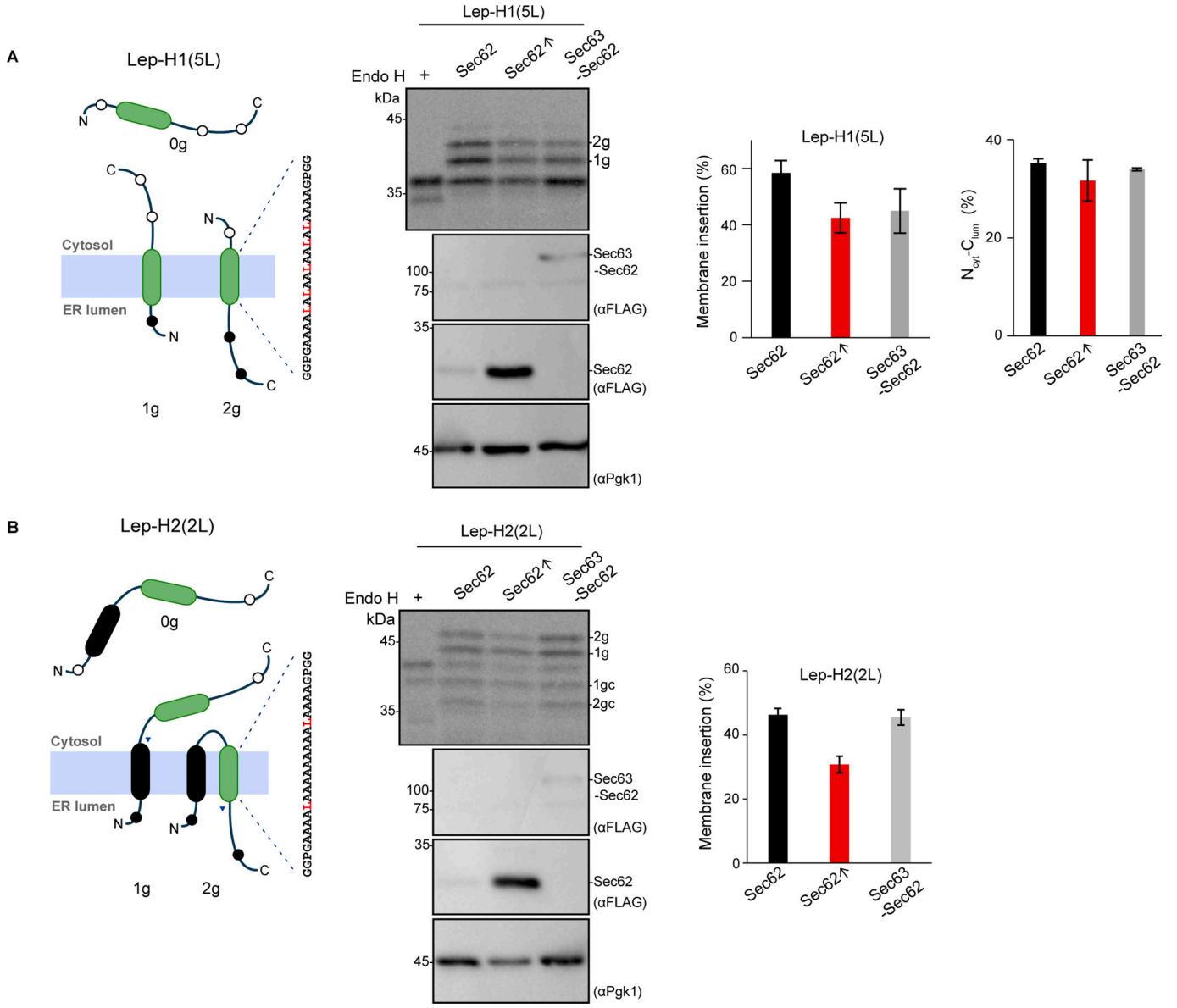

Fig. 4. Membrane insertion of marginally hydrophobic membrane proteins. (A) Schematics of Lep-H1 (5L). A hydrophobic segment is indicated in green, and the sequence is shown. Open and closed circles indicate nonglycosylated and glycosylated N-sites, respectively. Lep-H1(5L) in the *sec62*Δ strains expressing *SEC62* under the endogenous or *GPD* promoter (↑) or *SEC63-SEC62* under the endogenous promoter of *SEC63* was analyzed by pulse labeling and Western blotting. The membrane insertion efficiency (%) was calculated as [(1 g + 2 g) × 100 / Total]. The N_{cyt}-C_{lum} (%) was calculated as [2 g × 100/(1 g + 2 g)]. (B) Schematics of Lep-H2(2L). The test hydrophobic segments are indicated in green, and the sequence is shown. Cleavage points are indicated in blue arrow. Lep-H2(2L) was pulse-labeled and analyzed as described in (A). Singly and doubly glycosylated forms are indicated as 1 g and 2 g, respectively. Singly and doubly glycosylated, cleaved forms are indicated as 1gc and 2gc, respectively. The membrane insertion efficiency (%) was calculated as [(2 g + 2gc) × 100 / (Total − 0 g)]. Sec63-Sec62, Sec62 were detected by Western blotting using αFLAG antibodies that recognized the C-terminal FLAG tag in Sec62. Pgk1 was used as a loading control and detected by αPgk1 antibodies. Average values of at least three independent experiments are shown with standard error of the mean (SEM).

the $N_{cytosol}$-C_{lumen} orientation [15,16]. To determine whether this was the case in Sec62-overexpressing cells, the relative ratio of the two topology forms was compared (singly vs. doubly glycosylated forms). However, differences were not observed in the ratio of the two forms in the cells overexpressing Sec62 or in the cells expressing endogenous levels of Sec62. These data suggest that the targeting and membrane insertion of a marginally hydrophobic single-pass membrane protein is mildly reduced when Sec62 was overexpressed.

3.3.3. *Lep-H2(2 L), a double-pass membrane protein*

Next, we tested Lep-H2(2L), a potential double-pass membrane protein (Fig. 4B) [17]. The first TM domain mediates targeting to and anchoring of the protein in the ER membrane. The following engineered hydrophobic segment is membrane inserted if it is hydrophobic and noninserted if the segment is less hydrophobic. Lep-H2(2L) has two N-linked glycosylation sites, one in the N-terminus and the other in the C-terminus. Upon the insertion of the first TM domain, the N-terminal glycosylation site is modified, indicative of proper targeting to the ER. If the following hydrophobic segment is inserted, the C-terminal glycosylation site is used, and the protein becomes doubly glycosylated. It has been confirmed in earlier studies that both singly and doubly glycosylated products generate each cleaved product [15–17]. The ratio of singly versus doubly glycosylated proteins thus represents the insertion efficiency of the second hydrophobic segment.

For the hydrophobic segment with 2 leucines and 17 alanines, previous studies have shown that it results in mixed pools of singly and doubly glycosylated forms, indicating partial membrane insertion [17]. Glycosylation of Lep-H2(2L) in the cells expressing endogenous, overexpression levels of Sec62, or the Sec63-Sec62 fusion was assessed by pulse labeling as above. Unglycosylated proteins were not detected, indicating efficient protein targeting to the ER (Fig. 4B). The relative amount of the doubly glycosylated form was reduced in Sec62-overexpressing cells compared to that in cells expressing endogenous levels of Sec62 or the Sec63-Sec62 fusion, suggesting that membrane insertion of the hydrophobic segment or translocation of the C-terminus is reduced in Sec62-overexpressing cells (Fig. 4B).

3.3.4. *SP-Lep variants*

To distinguish whether membrane insertion or C-terminal translocation is defective in Sec62-overexpressing cells and to determine the membrane insertion efficiencies of TM segments in detail, we used another set of proteins containing controlled hydrophobicity. These Lep variants carry a cleavable signal peptide of yeast Suc2 protein (invertase) that targets and initiates translocation of the Lep protein containing the engineered leucine/alanine hydrophobic segment (SP-Lep) (Fig. 5A) [19]. The hydrophobic segment is flanked by two N-linked glycosylation sites. If targeting is defective, an unglycosylated product results. If the hydrophobic segment is insufficient to be membrane inserted, it is translocated to the lumen, and a doubly glycosylated form is generated. If the hydrophobic segment is membrane inserted, it is oriented as N_{lumen}-$C_{cytosol}$ topology, and a singly glycosylated form is generated. While we could not distinguish whether membrane insertion

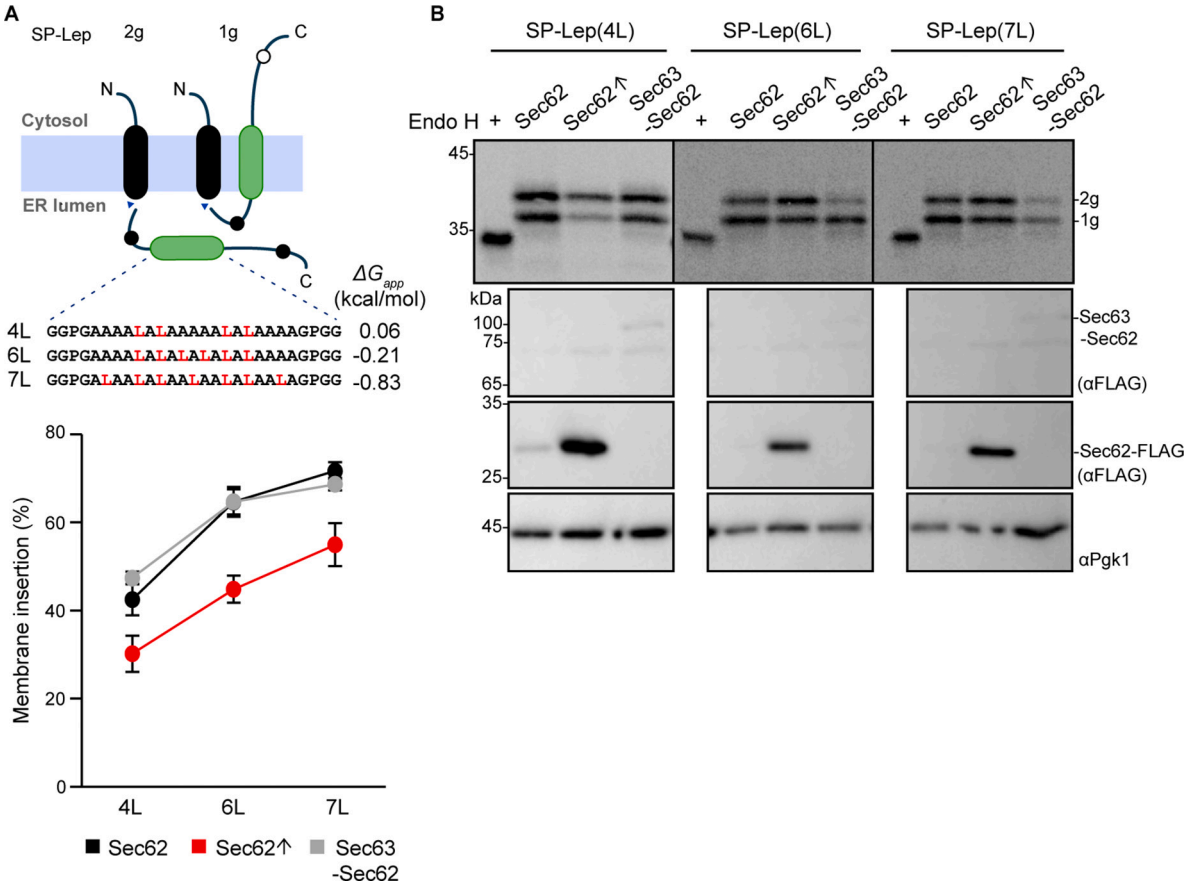

Fig. 5. Membrane insertion efficiency of hydrophobic segments in Sec62-overexpressing cells. (A) Schematics of SP-Lep. A signal sequence of Suc2 is shown in black, and the hydrophobic segment is shown in green. Signal sequence cleavage site is indicated in blue arrow. Filled and unfilled circles indicate used and unused N-glycosylation sites, respectively. The sequences of test hydrophobic segments are shown with the measured hydrophobicity in ΔG_{app} (kcal/mol) in [19]. (B) SP-Lep was pulse-labeled and analyzed as shown in Fig. 4A and B. Singly and doubly glycosylated forms are indicated as 1 g and 2 g, respectively. (C) The membrane insertion efficiency (%) was calculated as [1 g × 100 / (1 g + 2 g)]. Average values of at least three independent experiments are shown with standard error of the mean (SEM).

or C-terminal translocation was impaired in Sec62-overexpressing cells with Lep-H2(2L) model protein, SP-Lep variants could be used to distinguish these two possibilities. Furthermore, the ratio between singly and doubly glycosylated forms indicates the membrane insertion efficiency of the potential TM segment.

Translocation and membrane insertion of SP-Lep variants carrying the 4 L/15A(4 L), 6 L/13A(6 L) or 7 L/12A(7 L) segment in cells expressing different levels of Sec62 and the Sec63-Sec62 fusion were assessed by pulse labeling. The relative amount of the doubly glycosylated form compared to the singly glycosylated form was increased in Sec62-overexpressing cells, indicating that the C-terminal translocation of SP-Lep variants occurred efficiently, whereas membrane insertion efficiency was decreased (Fig. 5B).

The threshold membrane insertion (50 % membrane insertion) of SP-Lep variants was ~5 L/14A for the cells expressing endogenous levels of Sec62 or the Sec63-Sec62 fusion but changed to ~7 L/12A for the cells overexpressing Sec62 (Fig. 5C). The measured apparent free energy (ΔG_{app}) of the 5 L/14A segment with GGPG and GPGG flanking residues in the wild-type strain was −0.23 kcal/mol, whereas that of the 7 L/12A segment with the same flanking residues was −0.83 kcal/mol in [19]. These results suggest that the TM segment needs to be more hydrophobic to be inserted into the membrane when Sec62 is overexpressed.

4. Discussion

Here, we present the unique observation we made with yeast cells overexpressing Sec62. In the Sec62 overexpression strain, targeting of test proteins occurred efficiently, while membrane insertion of marginally hydrophobic TM segments was impaired regardless of their membrane orientations. These phenotypes are different from Sec62 mutants that result from impaired interaction with Sec63 [15]. In those Sec62 mutants, translocation of CPY was impaired and the C-terminal translocation and membrane insertion with the $N_{cytosol}$-C_{lumen} orientation of Lep-H1(5L) and Lep-H2(2L) were selectively defective [15,20].

The membrane insertion efficiencies of SP-Lep variants in Sec62-overexpressing cells were assessed, and we found that the threshold hydrophobicity for membrane insertion increased, meaning that TM segments need to be more hydrophobic to be inserted into Sec62-overexpressing cells.

The observed function of Sec62 in the insertion of TM segments in this study is similar to the function of Mgr2, a subunit of the TIM23 complex that mediates membrane insertion and translocation of nuclear encoded proteins in the mitochondrial inner membrane [21]. Overexpression of Mgr2 reduced the membrane insertion efficiencies of marginally hydrophobic TM segments into the mitochondrial inner membrane, which function as gatekeepers for the sorting of TM domains to the mitochondrial inner membrane [22,23].

The cryo-EM structures of the Sec translocon show that the TM domains of Sec62 are located in the front side of the Sec61 lateral gate through which the TM segments of membrane proteins are released to the lipid bilayer [6,7]. Our data indicate that overexpressed Sec62 may accidentally act as a gatekeeper of the translocon for membrane insertion of marginally hydrophobic TM segments in the ER membrane.

How does Sec62 sense marginally hydrophobic TM segments? These segments are borderline hydrophobic, sampling between the aqueous and nonpolar environments at the Sec61 lateral gate. Thus, they are likely to linger at the lateral gate relatively longer than less or more hydrophobic segments, both of which must be rapidly translocated across or integrated into the membrane, respectively. We envision that this prolonged presence of the marginally hydrophobic segment at the lateral gate may be a signal for the Sec62 recruitment to the site. When Sec62 is abundant, association rate of Sec62 with Sec61 lateral gate would increase and the prolonged presence of Sec62 TMs at the lateral gate may transiently act as a shield, preventing/delaying efficient insertion of the marginally hydrophobic TM segment into the membrane.

Overexpression of Sec62 is a biomarker in numerous cancers [24–26]. Subunits of many protein complexes are coordinated in expression so that they form a functional complex [27]. Excess or orphaned proteins then undergo quality control and degradation due to stoichiometry imbalance [28]. Our data and that from a previous membrane proteomics study in yeast indicate that overexpressed Sec62 is not readily degraded even though stoichiometry with other subunits of the Sec complex is imbalanced [29], suggesting that overexpressed Sec62 is stable without associating with other subunits of the Sec complex. Thus, stably overexpressed Sec62 may exert unexpected misregulation of cellular processes in the degradation of selective ER proteins (recovER-phagy) [30], Ca^{2+} homeostasis [31] in human cells, and insertion of membrane proteins in yeast cells. Although our observation in the yeast cells is yet to be validated in the human cells, the similar effects may also contribute to the etiology of various human cancers.

CRediT authorship contribution statement

SJ, MY, and HK conceived the project and designed the experiments. SJ, MY, CY, and SH performed the experiments and analyzed the data. SJ and HK wrote the manuscript. SJ, MY, CY, SH, W—K H, and HK reviewed the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

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