Mammalian Sec16/p250 Plays a Role in Membrane Traffic from the Endoplasmic Reticulum*

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Coat protein complex II (COPII)-coated vesicles/carriers, which mediate export of proteins from the endoplasmic reticulum (ER), are formed at special ER subdomains in mammals, termed ER exit sites or transitional ER. The COPII coat consists of a small GTPase, Sar1, and two protein complexes, Sec23-Sec24 and Sec13-Sec31. Sec23-Sec24 and Sec13-Sec31 appear to constitute the inner and the outermost layers of the COPII coat, respectively. We previously isolated two mammalian proteins (p125 and p250) that bind to Sec23. p125 was found to be a mammalian-specific, phospholipase A1-like protein that participating in the organization of ER exit sites. Here we show that p250 is encoded by the KIAA0310 clone and has sequence similarity to yeast Sec16 protein. Although KIAA0310p was found to be localized at ER exit sites, subcellular fractionation revealed its predominant presence in the cytosol. Cytosolic KIAA0310p was recruited to ER membranes in a manner dependent on Sar1. Depletion of KIAA0310p mildly caused disorganization of ER exit sites and delayed protein transport from the ER, suggesting its implication in membrane traffic out of the ER. Overexpression of KIAA0310p affected ER exit sites in a manner different from that of p125. Binding experiments suggested that KIAA0310p interacts with both the inner and the outermost layer coat complexes, whereas p125 binds principally to the inner layer complex. Our results suggest that KIAA0310p, a mammalian homologue of yeast Sec16, builds up ER exit sites in cooperation with p125 and plays a role in membrane traffic from the ER.

Protein transport between intracellular membrane compartments is mediated by vesicles/tubules that bud from the donor membrane and move to and fuse with the target membrane (reviewed in Bonifacino and Glick (1) and Lee et al. (2)). Newly synthesized proteins exit the endoplasmic reticulum (ER) in COPII-coated vesicles, which are generated at ER subdomains, known as ER exit sites (ERES), in many eukaryotes (3, 4). COPII consists of heterotrimeric complexes, Sec23–Sec24, Sec13–Sec31, and a low molecular weight GTP-binding protein Sar1 (5). Sec12, an integral membrane protein of the ER (6), catalyzes nucleotide exchange on Sar1 (7, 8), leading to the exposition of its N-terminal amphipathic domain and membrane binding (9, 10). Sar1 activation results in the sequential recruitment of the Sec23–Sec24 complex and the Sec13–Sec31 complex to ER membranes. Cargo is sorted into nascent COPII vesicles via interaction with the prebudding complex components Sec24 or its homologues (11–14) and/or Sar1 (15–17). The Sec13–Sec31 complex, which can self-assemble to form minimal cages (18), occupies the outermost layer of the coat covering an inner layer of the Sec23–Sec24 complex (19). Sec23 exhibits GTPase-activating protein activity toward Sar1 (20), which results in the depolymerization of the COPII coat, thereby leading to vesicle uncoating that can take place during or after vesicle fission (21, 22).

ERES, defined cellular sites that support ER export, have been studied using mammalian cells and yeast Pichia pastoris (23–25). ERES can be generated de novo and are relatively immobile structures within the cells. COPII is dynamically exchanged at these sites supporting vesicle budding. Studies by Glick and colleagues (26, 27) have shown that a tightly ER-bound peripheral membrane protein, Sec16, is required for the organization of ERES in P. pastoris. In Saccharomyces cerevisiae, in which well organized ERES are not present, Sec16 potentiates the action of COPII components to bud transport vesicles (28, 29). A structural difference between P. pastoris and S. cerevisiae Sec16 proteins may explain why higher-order ERES exist in the former yeast but not in the latter one (27).

We previously isolated two mammalian Sec23-interacting proteins (p125 and p250) by using glutathione beads coupled to glutathione S-transferase (GST)-mouse Sec23A (30). p125, which appears to be only expressed in mammals, contains an N-terminal Pro-rich region responsible for the interaction with KIAA0310p, a mammalian homologue of yeast Sec16, builds up ER exit sites in cooperation with p125 and plays a role in membrane traffic from the ER.

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2 The abbreviations used are: ER, endoplasmic reticulum; ERES, ER exit sites; ERGIC, ER-Golgi intermediate compartment; COPII, coat protein complex II; β-COP, β-coat protein; aa, amino acid; BFA, brefeldin A; Endo H, endoglycosidase H; GFP, green fluorescent protein; GST, glutathione S-transferase; siRNA, short interfering RNA; VSVG, vesicular stomatitis virus-encoded glycoprotein; PLASKA, phosphatidic acid-prefering phospholipase A1; MS, mass spectrometry; MALDI-QqTOF, matrix-assisted laser desorption/ionization-quadrupole time of flight.
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exhibit significant sequence homology with phospholipid-modifying proteins, especially phosphatidic acid-prefering phospholipase A₁ (PA-PLA₁) (32). p125, PA-PLA₁, and KIAA0725p constitute a family of phospholipase A₁ proteins that are localized in different cellular compartments and perhaps have different functions (30–33). Although PA-PLA₁ and KIAA0725p display phospholipase A₁ activity under certain conditions in vitro, p125 does not (32). Our recent study demonstrated that p125 is localized at ERES and contributes to their organization (34).

In the present study, we isolated p250 from mouse brain and revealed it to be a protein encoded by the KIAA0310 clone. KIAA0310p, which shows sequence similarity to yeast Sec16 protein, was found to be localized at ERES, and its depletion induced their disorganization. Pull-down experiments demonstrated that KIAA0310p differs from p125 in terms of binding to COPII coat proteins. In addition, overexpression of the two proteins differently affected the organization of ERES.

EXPERIMENTAL PROCEDURES

Antibodies and Chemicals—To produce an anti-peptide antibody against human KIAA0310p, a mixture of two peptides with an extra cysteine residue at the C-terminal or N-terminal region (MFFQGGGETENEENLC and CRLGRIGQRKHLVLN) was conjugated to keyhole limpet hemocyanin and injected into rabbits. The anti-KIAA0310p antibody was affinity-purified from the sera using antigen peptide-coupled beads. Monoclonal (M2) and polyclonal antibodies against FLAG were obtained from Sigma-Aldrich. A polyclonal antibody against GST was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Monoclonal antibodies against GM130 and Sec31A were purchased from BD Transduction Laboratories. Polyclonal antibodies against vesicular stomatitis virus-encoded glycoprotein (VSVG), β-COP, Sec23, and Sec31A were prepared in this laboratory (30, 34, 35). A monoclonal antibody against ERGIC-53 was kindly provided by Dr. H.-P. Hauri (University of Basel, Switzerland). Brefeldin A (BFA) was obtained from Sigma-Aldrich. Endoglycosidase H (Endo H) was purchased from New England Biolabs (Beverly, MA). Glutathione-Sepharose 4B beads were from Amersham Biosciences (Uppsala, Sweden).

Preparation of Mouse Brain Lysates and GST-Sec23A—Mouse brain lysates were prepared as described previously (30). GST-mouse Sec23A was expressed in Sf9 cells and isolated using glutathione beads (30).

Peptide Mass Mapping and Data Analysis—The isolated p250 was digested in-gel with trypsin and subjected to peptide mass mapping on a MALDI-QqTOF tandem MS QStar Pulsar I (Applied Biosystems, Foster City, CA). The data were analyzed against the NCBI nr protein data base by using the Mascot program.

Cell Culture and Subcellular Fractionation—Cell culture was performed as described (30). To examine KIAA0310p-depleted HeLa cells by immunofluorescence microscopy, cells were grown on poly-L-lysine-coated coverslips.

To determine the subcellular distribution of KIAA0310p, 293T cells were cultured on 10 150-mm dishes, collected, suspended in 3.3 ml of homogenization buffer (10 mM Tris-HCl, pH 7.5, containing 0.25 m sucrose and 1 mM phenylmethylsulfonyl fluoride), and homogenized. The homogenate was centrifuged at 1,000 × g for 10 min, yielding postnuclear supernatant and nuclear fractions. The postnuclear supernatant was then centrifuged at 9,000 × g for 10 min, yielding supernatant and mitochondrial fractions. The supernatant was further centrifuged at 105,000 × g for 1 h, yielding microsomal and cytosolic fractions. Mitochondrial and microsomal pellets were suspended in 300 μl each of homogenization buffer. The nuclear pellet was washed once and suspended in 0.97 ml of buffer. Protein concentrations were determined using the BCA protein assay kit (Pierce Biotechnology).

Plasmid Construction, Transfection, and Pull-down Assay—The KIAA0310 clone was obtained from the Kazusa DNA Research Institute (Kazusa, Japan). The obtained clone lacked nucleotides corresponding to the N-terminal 550 amino acids (aa) and corresponding to aa 2,265–2,310. (The full-length cDNA for KIAA0310p, now available from the Kazusa DNA Research Institute, encodes a protein of 2,357 aa.) The C-terminal deletion may be generated by alternative splicing. We constructed a full-length cDNA for KIAA0310p by joining the 5′-end of the cDNA prepared by PCR to the obtained clone. The full-length cDNA for KIAA0310p and a partial cDNA encoding aa 374–2,357 (KIAA0310pΔN), both of which lack aa 2,265–2,310, were inserted into pFLAG-CMV-6c and -6b, respectively, to give rise to the pFLAG-KIAA0310p and pFLAG-KIAA0310pΔN. The mammalian expression plasmids for GST-mouse Sec23A, GST-human Sec24C, FLAG-Sar1(H79G), and FLAG-p125 were constructed previously (30, 34, 35). Plasmids for GST-human Sec31A and GST-human Sec13 were prepared in this study using the pEBG vector.

For the expression of proteins, HeLa or 293T cells plated on 35-mm dishes were transfected with 1–2 μg of expression plasmids using the Lipofectamine PLUS reagent (Invitrogen) according to the manufacturer’s instructions. The pull-down assay was conducted as described (30).

Immunofluorescence Microscopy—Immunofluorescence microscopy was performed as described (36).

Short Interfering RNA (siRNA)-mediated Protein Knockdown—The RNA duplexes used for targeting KIAA0310p (oligonucleotide 1, 5′-ACCCGCCAUCGUAUGAAUU-3′; oligonucleotide 3, 5′-CCAGGUGUUAAGUACUCAUA-3′); and lamin A/C, 5′-AACUGAGGUCCAGAAGAACAUC-3′) were purchased from Japan Bioservice, Inc. Transfection of HeLa cells was performed using Oligofectamine (Invitrogen). The final concentration of an RNA duplex was 200 nM. At 48 h after transfection, the cells were processed for immunoblotting and immunofluorescence.

Membrane Binding Assay—Recombinant Sar1 proteins were purified as described previously (37). Microsomal membranes and cytosol were prepared as described previously (38), except that HeLa cells were used instead of rat liver. Membrane binding of KIAA0310p was analyzed by using microsomal membranes (20–40 μg) supplemented with recombinant Sar1 proteins and cytosol, as described for the analysis of Sec23 binding (39, 40).

Protein Transport Assay—The expression plasmid for VSVG fused with green fluorescent protein (GFP) was kindly donated.
by Dr. J. Lippincott-Schwartz (National Institutes of Health, Bethesda, MD). HeLa cells were grown on 35-mm dishes, transfected with duplex RNAs (200 nM), and incubated at 37 °C for 48 h. The cells were then transfected with 1 µg of the plasmid for VSVG-GFP and incubated at 40 °C for 24 h. Cycloheximide was added to a final concentration of 100 µg/ml, and then the cells were shifted to 32 °C to allow transport. At the indicated times, the cells were fixed and processed for immunofluorescence analysis. Alternatively, the cells were solubilized with 0.5% SDS and 1% 2-mercaptoethanol (0.1 ml/35-mm dish) and heated at 100 °C for 10 min. A portion of the lysate was digested with Endo H according to the manufacturer’s protocol and then subjected to SDS-PAGE on 5% gels and analyzed by immunoblotting with a polyclonal anti-VSVG antibody, and GST proteins (10% gels) were used to detect GST proteins.

FIGURE 1. p250 is a protein encoded by the KIAA0310 clone. 293T cells were transfected with 1 µg of the plasmid for GST or GST-Sec23A (A) or transfected with pFLAG-KIAA0310pΔN plus the plasmid for GST or GST-Sec23A (1 µg each) (B). At 24 h after transfection, the cells were lysed, and the GST proteins were pulled down with glutathione-Sepharose 4B. The precipitates were subjected to SDS-PAGE on 5% gels and analyzed by immunoblotting with an anti-KIAA0310p (A, top panel) or anti-FLAG (B, top panel). Expression of endogenous KIAA0310p (A, middle panel), FLAG-KIAA0310pΔN (B, middle panel), and GST proteins (A and B, bottom panels) was monitored (5% of input). 10% gels were used to detect GST proteins.

RESULTS

Identification of p250—Sec23-interacting proteins were purified from lysates of mouse brain using GST-mouse Sec23A as described previously (30). p250 was separated by SDS-PAGE and subjected to MALDI-TOF MS analysis after tryptic digestion. The sequences of more than 10 peptide fragments derived from p250 were found in a protein encoded by the mouse KIAA0310 clone (41). To confirm that p250 is KIAA0310p, we raised a polyclonal antibody against a mixture of two peptides derived from the sequence of human KIAA0310p and examined whether endogenous KIAA0310p is pulled down with GST-Sec23A expressed in 293T cells. As shown in Fig. 1A, the human p250 pulled down with GST-Sec23A was recognized by the anti-KIAA0310p. The interaction between Sec23A and KIAA0310p was also examined by expressing the two proteins tagged with GST and FLAG, respectively, in 293T cells (Fig. 1B). In this experiment, we used a KIAA0310 construct lacking the N-terminal 373 aa (FLAG-KIAA0310pΔN) because the full-length construct was poorly expressed. As shown in Fig. 1B, FLAG-KIAA0310pΔN was pulled down with GST-Sec23A.

The results of these experiments unequivocally indicated that p250 is KIAA0310p.

The predicted coding region of KIAA0310p is 2,357 aa long, which is rich in Pro (13.2%), Gly (7.8%), Ser (11.2%), Ala (9.5%), and Leu (8.4%) and includes three Pro-rich regions (aa 3–276, aa 1,113–1,181, and aa 1,931–2,263) and one Tyr-rich region (aa 1,159–1,300). A FASTA search revealed that KIAA0310p shows 19.8% amino acid identity with S. cerevisiae Sec16 protein.

*KIAA0310p Is Localized at ERES—*We next analyzed the localization of KIAA0310p in HeLa cells by immunofluorescence microscopy. As shown in Fig. 2A, immunostaining for KIAA0310p showed a punctuated pattern with some concentration at the perinuclear region, typical for proteins located at ERES. Indeed, KIAA0310p was almost completely colocalized with the COPII component Sec31A (Fig. 2A, top row) and substantially with an ER-Golgi intermediate compartment (ERGIC) marker, ERGIC-53 (middle row). On the other hand, the KIAA0310p-positive puncta, except at the perinuclear region, were negative for the Golgi marker GM130 (bottom row). To confirm the localization of KIAA0310p in ERES, cells were incubated with BFA to disrupt the Golgi apparatus (42) or transfected with a GTP-restricted form of Sar1 (Sar1(H79G)) to stabilize and nucleate ERES (43). Although BFA treatment induces Golgi disassembly, peripheral ERES are not significantly affected (34, 44). As shown in Fig. 2B, BFA treatment induced dispersion of the COPI component β-COP (second row) but did not significantly affect the localization of KIAA0310p (top row) or Sec31A (top and second rows). When the GTP-restricted form of Sar1 was expressed, KIAA0310p (Fig. 2B, third row), like Sec31A (bottom row), accumulated at the perinuclear region, perhaps reflecting the consequence of inhibition of uncoating of the COPII coat.

*Sar1-dependent Recruitment of KIAA0310p to ER Membranes—*In addition to the peripheral and perinuclear dot-like localization, KIAA0310p was shown to be present in the cytosol by immunofluorescence microscopy (data not shown). To confirm this observation, we performed subcellular fractionation. Homogenates of 293T cells were fractionated by differential centrifugation, and each fraction was analyzed by immunoblotting with antibodies against KIAA0310p and Sec23. As shown in Fig. 3A, KIAA0310p was predominantly present in the cytosol. On the other hand, Sec23 was found to be equally distributed in the cytosolic and microsomal fractions. The distribution of KIAA0310p between cytosolic and microsomal fractions was similar when the subcellular fractionation was performed in the absence (Fig. 3A) or presence (not shown) of EDTA.

The finding that KIAA0310p is predominantly cytosolic prompted us to investigate whether the recruitment or association of KIAA0310p to ER membranes is dependent on Sar1, as observed for COPII coat components (5, 11, 45). To this end, microsomal membranes were incubated with cytosol in the presence of the GTP- or GDP-restricted form of Sar1, and then the membranes were recovered by centrifugation. As shown in Fig. 3B, the amount of KIAA0310p associated with microsomal membranes increased as the
amount of the active Sar1 increased. On the other hand, no enhancement in the binding of KIAA0310p was observed by the addition of the inactive Sar1. These results suggest that the binding of KIAA0310p to ER membranes is regulated by the GTP/GDP cycle of Sar1, as observed for COPII coat components (5, 11, 45).

Depletion of KIAA0310p Mildly Disturbs the Organization of ERES and Induces Delay in Protein Transport—To explore the possible role of KIAA0310p, we examined the effect of depletion of KIAA0310p on the morphology of ERES and the Golgi apparatus. siRNA for KIAA0310p (oligonucleotide 1 (Fig. 4, Oligo 1)) was transfected into HeLa cells, and at 48 h after transfection, the cells were analyzed by immunoblotting (Fig. 4A) and immunofluorescence microscopy (Fig. 4B). Oligonucleotide 1 effectively reduced the expression of KIAA0310p without affecting the expression of Sec31A and p125 (Fig. 4A). The number of Sec31A-positive dots was somewhat decreased in KIAA0310p-depleted cells when compared with that in control (lamin A/C-depleted) cells (Fig. 4B, top row). In addition, the perinuclear accumulation of Sec31A was lost, and Sec31A-positive dots were distributed in less polarized perinuclear region. Redistribution was also observed for ERGIC-53 (third row), and the Golgi apparatus as marked by GM130 was partially disassembled (fifth row). Similar results were obtained when another siRNA (oligonucleotide 3) was used to knock down KIAA0310p (data not shown). These data may suggest that KIAA0310p participates in the organization of ERES and the ER-Golgi interface.

We previously showed that depletion of p125 affects the organization of ERES but does not significantly inhibit the transport of VSVG-GFP, a well characterized temperature-sensitive biosynthetic transport reporter (46), from the ER to the Golgi apparatus (34). Next, we investigated whether depletion of KIAA0310p interferes with VSVG-GFP transport (Fig. 5). For this purpose, HeLa cells were first transfected with oligonucleotide 1 (Oligo 1) and subsequently transfected with a plasmid encoding VSVG-GFP. The transfected cells were incubated at 40 °C to accumulate VSVG-GFP in the ER. Upon a shift...
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FIGURE 4. Depletion of KIAA0310p perturbs the structural organization of the early secretory pathway. A, HeLa cells were transfected with siRNA for lamin A/C or KIAA0310p (oligonucleotide 1 (Oligo 1)). At 48 h after transfection, cell lysates were prepared, and proteins (25 μg of each) were analyzed by immunoblotting with the antibodies against KIAA0310p, p125, and Sec31A. Mock, mock-transfected. B, cells depleted of lamin A/C or KIAA0310p were double-stained with antibodies against KIAA0310p and Sec31A (top two rows), ERGIC-53 (middle two rows), or GM130 (bottom two rows). Bar, 10 μm.

to the permissive temperature, VSVG-GFP was capable of exiting the ER and was mobilized through the Golgi apparatus to the plasma membrane in KIAA0310p-depleted cells, as observed in the control lamin A/C-depleted cells (Fig. 5A). The time course of VSVG-GFP transport in KIAA0310p-depleted cells (upper row) appeared not to be substantially different from that in the control (lower row). When oligonucleotide 3 was used to suppress KIAA0310p expression, a slight delay in VSVG-GFP transport from the ER was observed (data not shown). To detect subtle changes in transport, we analyzed the kinetics of acquisition of Endo H resistance, which is a hallmark shown). To detect subtle changes in transport, we analyzed the time course of VSVG-GFP transport in KIAA0310p-depleted cells.

FIGURE 5. VSVG-GFP transport is partly impaired in KIAA0310p-depleted cells. A, HeLa cells were successively transfected with siRNA for lamin A/C or KIAA0310p (oligonucleotide 1 (Oligo 1)) and with the plasmid for VSVG-GFP as described under "Experimental Procedures." To allow VSVG-GFP transport, the cells were shifted to 32 °C. At the indicated times, the cells were fixed and processed for immunofluorescence analysis (A) or lysed and subjected to Endo H treatment (B). Bar, 10 μm. R and S denote Endo H-resistant and -sensitive forms, respectively. C, the ratio (percentage) of the amount of Endo H-resistant form to that of the total amount (Endo H-resistant + Endo H-sensitive form) is plotted. White bar, lamin A/C-depleted cells; black bar, KIAA0310p-depleted cells.

whether KIAA0310p and p125 have different roles in the biogenesis of ERES, we compared the effects of overexpression of KIAA0310p and p125 on the distribution of ERES and ER-Golgi interface proteins. When expressed at a low level, full-length KIAA0310p was found to be targeted to ERES (data not shown). When overexpressed, on the other hand, full-length FLAG-KIAA0310p exhibited diffuse cytosolic staining with dot-like structures and caused a marked decrease in the perinuclear and peripheral staining for Sec31A (Fig. 6A, top row) and p125 (bottom row), suggesting the disruption of ERES. Concomitant with this change, the ERGIC (ERGIC-53) was redistributed to the ER (second row), and a Golgi protein (GM130) was considerably dispersed (third row), perhaps due to an inhibition of membrane traffic from the ER. In contrast, overexpression of FLAG-p125 at a level similar to that of FLAG-KIAA0310p induced aggregation of Sec31A at the perinuclear region (Fig. 6B, top row), as reported previously (30). In addition, ERGIC-53 (second row) and GM130 (third row) were also observed in p125-positive aggregates, suggesting the coalescence of ERES and the ER-Golgi interface. Interestingly, KIAA0310p, unlike Sec31A, was not accumulated at p125-positive aggregates (bottom row), suggesting that KIAA0310p dissociates from the coalesced structures. These different effects of overexpression of KIAA0310p and p125 on marker proteins may reflect different roles of KIAA0310p and p125 in the organization and function of ERES.

Interactions of COPII Components with KIAA0310p and p125—To further compare the properties of KIAA0310p and p125, we examined their interactions with COPII components. FLAG-KIAA0310pΔN was expressed in 293T cells with GST fusion proteins of Sec23A, Sec24C, Sec13, and Sec31A, and
pull-down experiments were performed. As shown in Fig. 7A, FLAG-KIAA0310pΔN was effectively pulled down with GST-Sec23A and GST-Sec13 but was less effectively pulled down with GST-Sec24C and GST-Sec31A. As Sec23 and Sec13 form different subcomplexes (5, 47), it is unlikely that the observed binding between GST-Sec13 and FLAG-KIAA0310pΔN is mediated by endogenous Sec23. As shown in Fig. 7B, p125 was also found to interact with GST-Sec24C as well as GST-Sec23A but found to interact much less or not at all with GST-Sec31A or GST-Sec13, suggesting that p125 interacts primarily with the inner layer coat complex. No interaction was observed between KIAA0310p and p125 (data not shown).

**DISCUSSION**

In the present study, we identified and characterized p250, a Sec23-interacting protein that we previously isolated by affinity precipitation (30). MALDI-TOF MS analysis revealed that p250 is a protein encoded by the KIAA0310 clone. KIAA0310p was found to be a cytosolic protein and recruited to membranes by Sar1. Overexpression and depletion of KIAA0310p resulted in the disorganization of ERES and the Golgi apparatus, suggesting that KIAA0310p is involved in the structural organization of the early secretory pathway.

Very recently, Stephens and colleagues (48) reported the identification of KIAA0310p as mammalian Sec16. They used a central conserved region between *S. cerevisiae* and *P. pastoris* to search the human genome data base and found sequence homology between KIAA0310p and yeast Sec16 protein. Furthermore, Bhattacharyya and Glick (49) also reported similar findings. Their results are almost consistent with ours, except for the localization of KIAA0310p. Although Stephens and colleagues (48) showed that KIAA0310p is exclusively associated with microsomal membranes isolated in low ionic strength buffers, our results demonstrated that KIAA0310p is dominantly cytosolic and that Sar1 activation stabilizes the interaction of KIAA0310p with membranes, thus regulating its function.

Although KIAA0310p appears to be a mammalian homologue of yeast Sec16, the amino acid sequence similarity
between the two proteins is relatively low (only 19.8% identity). This is in contrast to Sec23, a partner of Sec16/KIAA0310p. Mammalian Sec23A and B show high sequence similarity with their yeast orthologue (about 49% amino acid identity). In addition, yeast Sec16 and KIAA0310p are different in terms of protein-protein and protein-membrane interactions. Sec16 is tightly associated with ER membranes, whereas KIAA0310p is predominantly cytosolic. Although Sec16 interacts directly with Sec23, Sec24, and Sec31 (50, 51), KIAA0310p appears to interact strongly with Sec13, in addition to Sec23. Despite these differences, the results by us and others (48, 49) suggest that KIAA0310p participates in the organization of ERES, as does P. pastoris Sec16 (26, 27).

We now know that there are at least two factors, KIAA0310p and p125, which may be responsible for the organization of ERES in mammals. p115/Usol plays a role for the organization of ERES in Drosophila (52). Although organized ERES are not detected in S. cerevisiae, Usol as well as Rab GTPase Ypt1 and the Sec34 and Sec35 tethers are required to support differential protein sorting into subclass of COPII vesicles (53). ERES assembly may be similarly required to regulate selective cargo incorporation into COPII vesicles. However, no evidence for a role of p115/USol in ERES organization in mammals has been reported so far.

Why are multiple factors, p125 (phospholipase A₂-like protein) and KIAA0310p (Sec16), required for the organization of ERES in mammalian cells, whereas one factor (Sec16p) is enough for P. pastoris? One possible explanation is that KIAA0310p and p125 have different functions and differently contribute to the organization of ERES. When overexpressed at moderate levels, p125 induces the coalescence of ERES and ER-Golgi interface at the perinuclear region and displaces KIAA0310p from the coalesced ERES structures. This may imply that p125 plays a role in anchoring COPII components to ER membranes. As p125 contains phospholipase A₂-like domain (30) and interacts with Sec23A and Sec24C (this study), it is tempting to speculate that p125 may serve as a linker between phospholipid membranes and the inner layer of the coat. A recent study demonstrated the role of phosphatidylinositol 4-phosphate in regulating the nucleation of COPII at ERES (54). p125 was also found to bind to phosphatidylinositol phosphate. In contrast to the effect of p125 overexpression, overexpression of full-length KIAA0310p caused displacement of Sec31A and Sec24C from ERES (Ref. 48 and this study). Perhaps expressed KIAA0310p cannot efficiently accumulate at ERES due to its low affinity for membranes but may effectively capture COPII components, leading to displacement of COPII components from ERES. The different roles of p125 and KIAA0310p may be reflected by their different binding properties for COPII components and membranes. KIAA0310p may support COPII coat assembly by linking proteins in the inner and outermost layers.

Finally, we would like to point out the presence of a putative homologue of KIAA0310p. A BLAST homology search revealed the presence of a putative KIAA0310p homologue of 1,061 aa (KIAA1928p). This protein, regucalcin gene promoter region-related protein (RCRP), was originally characterized as a transcription factor that regulates the expression of regucalcin (55, 56). Our preliminary data showed that RCRP is capable of associating with several COPII components. Future work will disclose how this protein contributes to the organization of ERES.

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