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CHARACTERIZATION OF TWO NOVEL PROTEINS, Sec34p and Grp1p, REQUIRED FOR ER TO GOLGI TRANSPORT ALONG THE SECRETORY PATHWAY

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Secretory proteins from both prokaryotes and eukaryotes are synthesized as precursors having a conventional signal peptide. These proteins often have an N-terminal and/or a C-terminal propeptide required for protein folding/secretion etc (1). The translocation event mediated by the signal peptide across the membrane of the endoplasmic reticulum (ER) in eukaryotes is similar to the mechanism of protein translocation across the plasma membrane in prokaryotes.

In eukaryotic cells, once the proteins are translocated into the ER, transport of the proteins through the secretory pathway is mediated by membrane vesicles (2). The process is divided into three stages: i) vesicle budding from the donor compartment, ii) vesicle targeting to the acceptor membrane and iii) vesicle fusion with the acceptor membrane. Each phase is mediated by distinct sets of proteins. The formation and budding of transport vesicles involve recruitment of cytosolic coat proteins to the donor membrane. The SNAREs (soluble *N*-ethylmaleimide-sensitive fusion protein [NSF] attachment protein [SNAP] receptors) are key players in the fusion of vesicles with their acceptor membrane (3). These membrane proteins on the vesicle (v-SNAREs) and target (t-SNAREs) membranes interact with each other to form a stable complex that binds the soluble factors NSF and α-SNAP (yeast Sec18p and Sec17p, respectively). Subsequent to membrane fusion, NSF disassembles the SNARE complex and releases α-SNAP to allow the SNAREs to participate in a new round of transport (4). It is clear that the SNAREs are essential in fusing membranes (5); however, the interaction of SNAREs is not sufficient for a vesicle targeting. Recently, a growing list of proteins or protein complexes have been implicated in vesicle targeting (6).

In ER to Golgi transport in the yeast *Saccharomyces cerevisiae*, several targeting factors, Ypt1p, Uso1p, Sec35p and TRAPP complex, are involved in the tethering of vesicles to the Golgi. Uso1p, a homologue to the mammalian vesicle-docking protein p115, is a large 206 kDa cytosolic protein (7). Sec35p acts in the Uso1p-dependent docking of ER-derived vesicles to the Golgi (8). TRAPP complex , consisting of 10 subunits, resides on the *cis*-Golgi and acts upstream of the SNAREs (9). The activity of Uso1p, Sec35p and TRAPP may be regulated by the small GTP-binding protein Ypt1p.

Sec34p physically interacts with Sec35p, a protein required for ER to Golgi vesicle docking

In order to identify additional proteins that regulate the targeting/fusion activity of ER to Golgi transport vesicles with the Golgi, we performed a high copy suppressor screen with a 2-μm yeast genomic library using *sec34-2* mutant. The temperature-sensitive *sec34* mutant accumulates ER-modified precursor forms of secretory proteins and small vesicles at its restrictive temperature, implying that Sec34p is required for the targeting and/or fusion of ER to Golgi transport vesicles (10). This suppressor screen resulted in the identification of the *SEC34* structural gene, and a novel gene called *GRP1* (11). *SEC34* gene encodes a hydrophilic protein of 801 amino acids with a predicted molecular mass of 92.5 kD (actual size ~100kD on SDS-PAGE). The size of

Sec34p was confirmed by western blot analysis using an antibody directed against this protein. The disruption of *SEC34* gene results in a severe growth defect. A BLAST search of the database did not reveal any significant homologues. With a window of 28 amino acids, the amino terminus was predicted to contain a coiled-coil domain between amino acids 87-114. This domain appears to be important for function as its deletion leads to the loss of *SEC34* to suppress the *sec34* mutation. When we sequenced the two different mutations of *sec34*, *sec34-1*and *sec34-2*, by the method of gap repair, we found the *sec34-1* mutation changed a single base at position 923 (T to A), altering amino acid 308 from a leucine to an ochre stop codon. In the case of *sec34-2*, a base pair change at position 985 (C to T) altered amino acid 329 (glutamine) to an amber codon. Thus, the *sec34-1* and *sec34-2* mutants respectively encode truncated proteins of Sec34p with predicted molecular weights of ~35 kD and ~38 kD. Strains containing these truncations grew well at the permissive temperature. These findings, together with the observation that the N-terminal coiled-coil domain of Sec34p is important for function, support the hypothesis that the C-terminus of Sec34p is dispensable at the permissive temperature.

In an effort to identify genes whose products may interact with Sec34p, we employed a second genetic screen. This screen relied on the ability of *SEC34* to suppress the growth defect of known mutants whose defective products may stably or transiently interact with Sec34p. When the ability of *SEC34* to suppress all secretory mutants that block membrane traffic between the ER and Golgi complex was tested, we observed that *SEC34* only suppressed *sec35-1* and not mutants blocked at other stages of the pathway. This suppression was strong and specific, suggesting a possible physical interaction between Sec34p and Sec35p. To further define the genetic interactions between *SEC34* and *SEC35*, we crossed *sec34-1* to *sec35-1* and performed tetrad analysis. *sec34-1sec35-1* double mutations displayed a synthetic growth defect. To determine if Sec34p behaves like Sec35p, differential centrifugation studies were performed. Sec34p was largely found in the soluble fraction along with Sec35p, while the integral membrane protein Bos1p was in the insoluble fraction. The nature of the association of a little of Sec34p/Sec35p with membranes was assessed by the ability of different reagents, 1% Triton X-100, 1 M NaCl and 0.1 M Na_2CO_3, pH 11, to extract these proteins. Most of the Bos1p was solubilized by 1% Triton X-100, as was some of the Sec34p and Sec35p. Other reagents such as NaCl partially released Sec34p and Sec35p from the pellet, while Na_2CO_3 efficiently released it. Neither NaCl or Na_2CO_3 released Bos1p from membranes. Thus, Sec34p and Sec35p co-fractionate with each other in differential fractionation experiments and behave like peripheral membrane proteins. These data suggest that Sec34p and Sec35p may be members of the same complex. Sec35p is a protein required for ER to Golgi vesicle docking (8). To test the hypothesis that Sec34p physically interacts with Sec35p, immunoprecipitation experiment was performed using a strain with Sec34p-myc. Sec35p co-precipitated with Sec34p only under non-denaturing conditions, but not under denatured condition with 1% SDS, indicating that Sec34p physically interact with Sec35p. The size of the Sec34p/Sec35p complex was estimated by gel filtration chromatography of cell extracts on a Superose 6 column. This analysis revealed that Sec34p always co-fractionates with Sec35p at an estimated molecular weight of 480kD. To identify other putative members of this complex, we prepared a radiolabeled lysate from a strain containing Sec35p-myc and precipitated the Sec35p-associated proteins with anti-c-myc. In addition to Sec35p-myc, five polypeptides (p91, p73, p68, p51, and Sec34p) were precipitated. The interaction between Sec34p and Sec35p seems to be important for function because the complex was disassembled in the *sec34-1* mutant. These data indicate that Sec34p acts in conjunction with Sec35p to mediate a common step in vesicular traffic.

Recently, we have identified and characterized a new subunit called Sgf1p of the Sec34p/Sec35p complex. The pulse-chase study of carboxypeptidase Y and electron microscopy (EM) study using *sgf1* or *sec35* mutation show accumulation of Golgi form/Golgi structure as

well as ER form/ER structure, indicating that Sec34p/Sec35p complex may act at multiple stages of the secretory pathway (12). Genetic studies support this hypothesis. For example, the overexpressed *SEC34* was found to inhibit the growth of the *sec9-4* mutant, which is defective in post-Golgi secretion (11). *SNC2*, a post-Golgi vesicle SNARE, has been reported to suppress *sec35-1* (8). Grd20p (another name of Sec34p) was also recently found to be required for protein sorting in the *trans*-Golgi network/endosomal system (13). Taken together, these data suggest that the Sec34p/Sec35p complex may tether different classes of vesicles to their target membranes (Figure 1).

Grp1p facilitates membrane traffic indirectly by maintaining Golgi function

The *GRP1* gene was isolated as a suppressor of the *sec34-2* mutant (11). *GRP1* encodes a highly hydrophilic protein of 484 amino acids with a predicted molecular mass of 56 kD (apparent size is ~ 80 kD on SDS-PAGE). It has no predicted signal peptide and no significant hydrophobic stretch of amino acids that may serve as a transmembrane domain. Interestingly, approximately 57% of the protein is predicted to form a coiled-coil structure. Grp1p is related to the mammalian Golgi protein golgin-160 (160kD), which is one of two autoantigens that cross-reacts with the sera of patients with autoimmune diseases (14). Like Grp1p, a large portion of the protein (amino acids 708-1124) is predicted to form a coiled-coil structure. Golgin-160 resides on the Golgi and is part of a matrix that cannot be extracted from membranes with the detergent Triton X-100 (14). Grp1p localizes to the Golgi complex and co-localizes with the *cis*-Golgi markers Anp1p or Och1p by sucrose velocity gradient or immunofluorescence study (15). Grp1p pellets with membrane and is insoluble in Triton X-100 like golgin-160, indicating that it may be a matrix protein (15). Although *GRP1* is not essential for growth, the *grp1_* mutation displays synthetic lethal interactions with several mutations that result in ER-accumulation and block the late stages of ER to Golgi transport, but not those that fail to bud vesicles from the ER (11). Inspite of these synthetic lethal interactions, pulse chase analysis using Grp1p depleted cells did not reveal a significant delay in the ER to Golgi transit of the vacuolar protease carboxypeptidase Y (11). These findings suggest that Grp1p facilitates membrane traffic indirectly by maintaining Golgi structure and function as a Golgi matrix protein (15).

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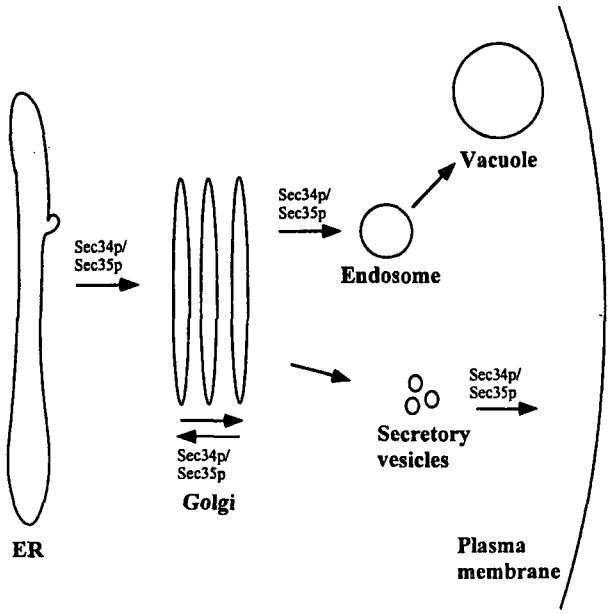

Figure 1. Sec34p/Sec35p complex may function at multiple stages of the secretory pathway