Exo84p Is an Exocyst Protein Essential for Secretion*

Wei Guo‡, Althea Grant, and Peter Novick§
From the Department of Cell Biology, Yale University School of Medicine, New Haven, Connecticut 06520-8002

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The exocyst is a multiprotein complex that plays an important role in secretory vesicle targeting and docking at the plasma membrane. Here we report the identification and characterization of a new component of the exocyst, Exo84p, in the yeast Saccharomyces cerevisiae. Yeast cells depleted of Exo84p cannot survive. These cells are defective in invertase secretion and accumulate vesicles similar to those in the late sec mutants. Exo84p co-immunoprecipitates with the exocyst components, and a portion of the Exo84p co-sediments with the exocyst complex in velocity gradients. The assembly of Exo84p into the exocyst complex requires two other subunits, Sec5p and Sec10p. Exo84p interacts with both Sec5p and Sec10p in a two-hybrid assay. Overexpression of Exo84p selectively suppresses the temperature sensitivity of a sec5 mutant. Exo84p specifically localizes to the bud tip or mother/daughter connection, sites of polarized secretion in the yeast S. cerevisiae. Exo84p is mislocalized in a sec5 mutant. These studies suggest that Exo84p is an essential protein that plays an important role in polarized secretion.

Spatial regulation of secretion is fundamental to a wide range of biological processes such as epithelial cell polarity establishment and neuronal growth cone formation. The budding yeast Saccharomyces cerevisiae provides a particularly useful system to study the spatial regulation of secretion. S. cerevisiae cells reproduce by budding, a process that requires a sophisticated system for polarized delivery and docking of vesicles containing proteins and lipids for localized plasma membrane expansion. A set of SEC genes was isolated from yeast that are required for secretion (1). 10 of these genes (SEC1, -2, -3, -4, -5, -6, -8, -9, -10, and -15) are required at the post-Golgi stage of the secretory pathway. Sec4p and Sec2p, in concert with the yeast cytoskeleton, are thought to be important for polarized delivery of secretory vesicles to the plasma membrane (2). Sec3, -5, -6, -8, -10, and -15p and Exo70p interact with each other and form a multisubunit complex termed the exocyst (3, 4). Components of the complex are localized to the emerging bud tip and mother/daughter connection, regions of active exocytosis (3, 5, 21, 22, 25). The localization of Sec3p is independent of the secretory pathway and actin cytoskeleton, suggesting that it may provide a spatial landmark for vesicle docking at the plasma membrane (5). Another component of the exocyst, Sec15p, can associate with secretory vesicles and interact with GTP-bound Sec4p, thus providing a molecular connection between the vesicles and the specialized exocytic sites on the plasma membrane (6). The exocyst appears to play a key role in vesicle docking and may act to couple the Rab/GTPase to the membrane fusion machinery (6, 23).

A complex homologous to the exocyst is present in mammalian cells. In Madin-Darby canine kidney cells, the exocyst proteins are localized to sites of new plasma membrane addition. Antibodies directed against Sec6p block basolateral secretion (7). In cultured hippocampal neurons, the complex is found in the tip of growing neurites, filopodia, and growth cones, regions of active membrane addition during synaptogenesis and neuronal maturation (8). These studies support the hypothesis that the exocyst plays an important role in vesicle targeting and docking.

The rat brain exocyst complex contains eight subunits (9). Among the eight proteins, seven are believed to be homologues of Sec3p, Sec5p, Sec6p, Sec8p, Sec10p, Sec15p, and Exo70p (9–13). Proteins identified in the immunopurified exocyst complex from yeast lysates. The other mammalian protein, rat Exo84p (rExo84p) (9), does not have a counterpart present in the originally purified seven-subunit exocyst complex (3). In this study, we report the identification of Exo84p from the budding yeast S. cerevisiae. Further characterization of this protein suggests that it is an essential component of the exocytic machinery that plays an important role in polarized secretion.

MATERIALS AND METHODS

Yeast Strains and Media—Yeast cells were grown in VP medium containing 1% Bacto-yeast extract, 2% Bacto-peptone (Difco) with 2% glucose (YPD), 2% galactose (YPG), or 2% raffinose plus 0.5% galactose (YPGR).

Construction of GAL-EXO84—A fragment containing nucleotides 1–560 of EXO84 was amplified by PCR using oligonucleotides containing BglII and HindIII restriction sites. The fragment was cloned into pBluescript II SK(+) and then into pBlueScript II SK(+) containing the GAL1 promoter and the LEU2 gene as a selectable marker. The sequence of the construct was verified by the Keck Foundation DNA Sequencing Laboratory at Yale University. The resulting plasmid was digested with BamHI and introduced into NY1523 diploid cells. Transformants were sporulated, and haploid cells (NY2135) harboring the GAL1-EXO84 as the sole copy of EXO84 were selected by tetrad dissection. For depletion of Exo84p, NY2135 cells were first grown in YPGR overnight at 25 °C. The cells were harvested and resuspended in YPD at 0.02 A(600) units/ml for further growth.

Invertase Secretion Assay—Wild-type cells (NY2136) and GAL-EXO84 cells (NY2135) were grown for 16 h in YPG at 25 °C. The cells were then transferred to YPD, and growth continued for an additional 16, 20, and 24 h, respectively. 2.0 A(600) units of cells were collected at each time point. For each sample, half was immediately pelleted, resuspended in 1 ml of ice-cold 10 mM NaOH, and stored on ice. The other half was incubated in YP plus 0.1% glucose for 2 h at 25 °C for invertase induction. Measurement of internal and external invertase activity was performed on all samples as described previously (14).

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‡ Supported in part by an NIH postdoctoral fellowship.
§ To whom correspondence should be addressed: Dept. of Cell Biology, Yale University School of Medicine, P.O. Box 208002, New Haven, CT 06520-8002. Tel.: 203-785-5871; Fax: 203-785-7226; E-mail: peter.novick@yale.edu.

1 The abbreviations used are: rExo84p, rat Exo84p; PCR, polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis; GFP, green fluorescent protein.
Bam

Tates were collected and washed in buffer A. The immunoprecipitates were incubation with protein A-Sepharose for 45 min and then incubated lysed using glass beads in buffer A. The lysates were first cleared by rotor (Beckman) at 50,000 rpm for 4 h at 4 °C. Fractions were collected lished from the top of the gradients, and proteins in each fraction were sepa-

rated. The resulting plasmid was digested at a unique Bgl site, a 3 HI site, and a 3 Xho HI site, and a 3 Xho HI site, and generated by PCR with oligonucleotides containing Clal (forward primer) and XhoI (reverse primer) restriction sites. The fragment was subcloned into a vector modified from pRS306 with triple HA sequences and ACTI1 tag, were used for fusion PCR as described previously (18). The yeast cells were grown to early log phase and lysed using glass beads in buffer A containing 20 NcoI and BamHI sites into pASt2 vector and expressed as a glutathione S-transferase fusion protein. This recombinant protein was purified using glutathione-Sepharose and injected into rabbits for antibody production. The resulting antibody (YU165) was used at a 1:2,000 dilution for Western blot, and at a 1:300 dilution for immunoprecipitation.

HA Tagging of Exo84p—A DNA fragment containing nucleotides 1687—2263 of EXO84 was generated by PCR with oligonucleotides containing Clal (forward primer) and XhoI (reverse primer) restriction sites. The fragment was subcloned into a vector modified from pRS306 with triple HA sequences and ACTI1 tag, were used for fusion PCR as described previously (18). The yeast cells were grown to early log phase and lysed using glass beads in buffer A containing 20 NcoI and BamHI sites into pASt2 vector and expressed as a glutathione S-transferase fusion protein. This recombinant protein was purified using glutathione-Sepharose and injected into rabbits for antibody production. The resulting antibody (YU165) was used at a 1:2,000 dilution for Western blot, and at a 1:300 dilution for immunoprecipitation.

Velocity Gradient Fractionation of Exo84p—Yeast cells were grown to early log phase and lysed using glass beads in buffer A containing 20 mM Hepes, pH 6.8, 100 mM NaCl, 1 mM dithiothreitol, 1 mM EDTA, 0.5% Tween 20, and protease inhibitor mixture. 150 μl of the lysates were loaded on 10–30% glycerol gradients and centrifuged in an SW50.1 rotor (Beckman) at 50,000 rpm for 5 h at 4 °C. Fractions were collected from the top of the gradients, and proteins in each fraction were separated by 10% SDS-PAGE. The sedimentation of EXO84 was monitored by Western blot analysis using anti-Exo84p antibody.

Immunoprecipitation—Yeast cells were grown to early log phase and lysed using glass beads in buffer A. The lysates were first cleared by incubation with 5 μg/ml of protein A-Sepharose for 45 min and then incubated with immunoprecipitation antibodies at 1,300 dilution for 4 h at 4 °C. Protein A-Sepharose was added to the reaction, and immunoprecipitates were collected and washed in buffer A. The immunoprecipitates were separated by 10% SDS-PAGE and analyzed by Western blot.

Suppression of Late sec Mutants by 2 EXO84—The 2 μm gene was subcloned into the 2 μm vector pRS426 with HIS as the selection marker, and expressed in the sec7-1 mutant strain. The growth of the transformants at 25, 30, 34, and 37 °C was examined.

GFP Tagging and Localization of Exo84p—A fragment containing the last 612 base pairs of 

EXO84 was fused to the GFP gene of A. victoria using PCR. Three primers, a 5′ outer primer that introduced a BamHI site, a 3′ outer primer that introduced a NotI site, and a 3′ primer, were used for fusion PCR as described previously (18). The construct was verified by sequencing. The amplified fragment was cloned into yeast integrating vector containing UR3 as the selectable marker. The resulting plasmid was digested at a unique BamHI site within EXO84 and used for transformation, replacing the C terminus of the genomic copy of EXO84 with the corresponding C-terminal fragment fused to GFP. The result is a yeast strain with EXO84-GFP as the sole full-length EXO84 allele within the genome. Because EXO84 is essential, the growth of yeast cells at 23, 30, and 37 °C confirmed that the EXO84-GFP allele was able to complement wild-type EXO84 function in cells.

To localize Exo84p, cells from an early log phase culture grown in dropout medium lacking uracil were transferred to 37 °C for 1 h. Cells were then pelleted in ice-cold phosphate-buffered saline and resuspended in 1 ml of buffer. After 1 h at 4 °C, cells were fixed for 10 min in 1% formaldehyde in methanol at −20 °C, pelleted, and washed with acetone at −20 °C. Cells were subsequently rehydrated, and washed three times with phosphate-buffered saline at 4 °C. Cells were then immediately examined by fluorescence microscopy.

Fluorescence Microscopy—Fluorescence microscopy was done with a Zeiss Axioshot2 microscope fitted with a 100× oil immersion objective (1.3 N.A.) and standard filter sets. The fluorescence image was recorded with a Quantix HCCD camera (Photometrics Ltd., Tucson, AZ), digi-

zed, and stored using IPLab Imaging Software (Scanalytics Inc., Fair-

fax, Virginia).

RESULTS

Sequence Analysis—The yeast S. cerevisiae data base was searched using the amino acid sequence of rExo84p. The open reading frame that shares the highest sequence homology is YBR102C. BESTFIT analysis (Genetics Computer Group) indi-

cates that YBR102C amino acids 148–719 and rExo84p amino acids 4–532 share 23.2% identity and 35.4% similarity (gap weight = 3, and length weight = 3) (Fig. 1). This similarity, although not strikingly high, is nonetheless comparable with those of other yeast-mammalian exocyst component sequence comparisons (10–13). The quality score of the BESTFIT was compared with the scores obtained by randomization of the YBR102C sequence. The score (Z value) is about 13 S.D. values above the average score of the randomized sequences (Z = (quality score of the alignment – mean quality score of 30 alignments)/S.D.). Therefore, the homology between rExo84p and YBR102C is highly significant and is not due to an overall similarity of their amino acid compositions.

Sequence analysis of YBR102C did not identify any potential transmembrane sequences, although several short stretches of hydrophobic residues are present in the sequence. Using the Msaeterpro program (window size = 28), coiled-coil regions were predicted in YBR102C (amino acids 226–278, amino acids 542–584). A coiled-coil stretch is also predicted in rExo84p (amino acids 282–291). In addition, a pleckstrin homology domain sequence (amino acids 173–273) is predicted in rExo84p using Profile Scan. However, no pleckstrin homology domain sequence is predicted in YBR102C.

Exo84p Associates with the Exocyst Complex—Since
YBR102C shares some degree of similarity to rExo84p, which is a component of the rat brain exocyst complex, we have examined if YBR102C protein associates with the yeast exocyst complex. Yeast cells expressing YBR102C tagged with a triple HA epitope were used in immunoprecipitation experiments using anti-HA antibody. An untagged strain was used as a negative control. As shown in Fig. 2, an anti-HA polyclonal antibody (HA1.1, Babco) immunoprecipitated HA-tagged YBR102C (left panel); Sec8p, a known exocyst protein, co-immunoprecipitated with YBR102C (right panel). As a control, this antibody did not precipitate Sec8p from the untagged strain lysate (right panel), indicating specificity of the immunoprecipitation. This result, combined with the sequence analysis (Fig. 1), indicates that YBR102C indeed encodes the yeast Exo84p.

The exocyst complex sediments at 19.5S in a velocity gradient. We examined if Exo84p co-sediments with the exocyst complex. Yeast lysates were prepared and loaded onto 10–30% glycerol gradients. The lysates were centrifuged at 50,000 rpm for 5 h at 4 °C. 15 fractions were obtained for Western blot analysis. As shown in Fig. 3, a portion of Exo84p was found to co-migrate with Sec8p; however, the majority of Exo84p was found to sediment more slowly. This result suggests that not all of Exo84p associates with the exocyst complex in lysates. It is possible that Exo84p has a major free pool in the cells in addition to its presence in the exocyst complex. Alternatively, it is possible that the association of Exo84p with the exocyst is weak, and it may dissociate from the complex during the lysate preparation and subsequent centrifugation.

**Exo84p Is Essential for Cell Viability**—To determine whether EXO84 is required for cell viability, one chromosomal copy of EXO84 in a diploid strain was engineered under the control of the GAL1 promoter with LEU2 as a selectable marker. The transformants were sporulated, and tetrads were dissected. In each tetrad, only two spores were viable on YPD plates, although all spores were viable on YPGal (Fig. 4A). This suggests that the lack of EXO84 expression in glucose medium leads to the loss of cell viability. We conclude that EXO84 is an essential gene in yeast S. cerevisiae.

The growth properties of wild-type and GAL-EXO84 strains were also examined in liquid medium. The cells were first grown in YPRG for 16 h and then switched to YPD to deplete cells of Exo84p. The rates of growth of both strains were monitored by A600 measurement. We found that the growth of the GAL-EXO84 strain slowed and then stopped after 20 h of shift, while the wild-type yeast strains continued to grow at a logarithmic rate (Fig. 4B). Western blot analysis of lysates made from the GAL-EXO84 strain during growth in YPD was carried out using a polyclonal antibody against Exo84p (YU165). Exo84p was found to be undetectable after 16 h of growth in YPD medium (Fig. 4C).
Fig. 5. Invertase accumulation in wild-type (black bars) and GAL-EXO84 cells (shaded bars) after 16-, 20-, and 24-h shift to YPD medium. Cells were grown for 16 h in YPRG at 25 °C and then transferred to YPD, and growth continued for an additional 16, 20, and 24 h, respectively. 2.0Accepted for publication February 18, 2002.  

Exo84p Depletion Blocks Invertase Secretion—We have examined the effect of Exo84p depletion on the secretion of invertase in yeast cells. At 16, 20, and 24 h after the shift of GAL-EXO84 cells to YPD, the secretion of invertase was partially blocked in GAL-EXO84 cells. In contrast, invertase secretion is normal in wild-type cells grown under the same conditions (Fig. 5). The severity of the secretory block correlates with the time of the shift to YPD medium. The extent of the block is comparable with that seen upon depletion of Gdi1p (Sec19p) using similar experimental conditions (19).

Exo84p Depletion Results in Secretory Vesicle Accumulation—We have examined the effect of Exo84p depletion on cell morphology using thin section electron microscopy. The GAL-EXO84 strain and wild-type control strains were grown first in YPRG and then shifted to YPD for 20 h, at which time the cells were depleted of Exo84p. As shown in Fig. 6, a large number of vesicles were accumulated as a result of Exo84p depletion. These vesicles are similar in size ($81 \pm 4$ nm, $n = 30$) and shape to those accumulated in the late sec mutants shifted to nonpermissive temperature, therefore representing post-Golgi secretory vesicles. In addition, a number of toroidal, membrane-bound structures were seen. The appearance of these structures, termed Berkeley bodies, has been attributed to a build up of transported material in the Golgi apparatus. In contrast, few secretory vesicles and no Berkeley bodies were seen in wild-type cells grown in YPD. This result, in combination with the invertase assay, indicates that Exo84p, like the other components of the exocyst, plays an important role at a late stage of the secretory pathway.

EXO84 Is a High Copy Suppressor of the sec5–24 Temperature-sensitive Mutant—Dosage suppression can be used to explore the possible functional relationship between proteins (20). The phenotypes of some sec mutations can be suppressed by overexpression of other SEC genes, suggesting that their gene products function on the same pathway. We have examined the effect of introducing a high copy number (2μ circle-based) EXO84 plasmid on the growth of the late sec mutants at various temperatures. Among the mutants examined (sec1–1, sec2–41, sec3–2, sec4–8, sec5–24, sec6–4, sec8–9, sec9–4, sec10–2, and sec15–1), only sec5–24 cells were rescued at 34 °C and, to a lesser extent, at 37 °C (Table I). No effects were found in the other mutants examined. Sec5p is a component of the exocyst complex, and the genetic interaction identified here is consistent with the finding that Exo84p is also a component of the exocyst complex. Furthermore, it suggests that Exo84p probably has a close relationship with Sec5p within the exocyst complex.

Molecular Interactions of Exo84p in the Exocyst Complex—To identify the binding partner(s) of Exo84p using the two-hybrid system, EXO84 was subcloned into the pAS1-CYH2 vector (“bait”). The construct was used to test the pairwise interactions of Exo84p with a panel of late Sec proteins using the yeast two-hybrid system. The results were quantitated using a β-galactosidase assay. As shown in Table II, Exo84p interacts with Sec5p and Sec10p. Previous studies indicated that Sec5p interacts with Sec10p (6). The interactions found between Exo84p and Sec5p and Sec10p could be the result of either a direct protein-protein interaction or a “bridging” effect of the proteins. The possible interaction of Exo84p with Sec5p is consistent with the finding that Exo84p is also a component of the exocyst complex. The presence of Sec10p in the exocyst suggests that the possible interaction of Exo84p with Sec5p is consistent with the finding that Exo84p is also a component of the exocyst complex.
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**Table I**

Growth properties of late sec mutants transformed with 2μ vector or plasmid containing EXO84

|        | 2μ vector control | 2μ EXO84 |
|--------|-------------------|----------|
|        | 25 °C | 30 °C | 34 °C | 37 °C | 25 °C | 30 °C | 34 °C | 37 °C |
| sec1–1 | +     | +     | +     | +     | +     | +     | +     | +     |
| sec2–41 | +     | +     | +     | +     | +     | +     | +     | +     |
| sec5–2 | +     | +     | +     | +     | +     | +     | +     | +     |
| sec6–4 | +     | +     | +     | +     | +     | +     | +     | +     |
| sec8–9 | +     | +     | +     | +     | +     | +     | +     | +     |
| sec9–4 | +     | +     | +     | +     | +     | +     | +     | +     |
| sec10–2 | +    | +     | +     | +     | +     | +     | +     | +     |
| sec15–1 | +    | +     | +     | +     | +     | +     | +     | +     |

**Table II**

Exo84 two-hybrid interactions

| pACT II constructs | Activitya |
|--------------------|-----------|
| Sec3p              | 0.21 ± 0.05 |
| Sec5p              | 49.01 ± 0.91 |
| Sec6p              | 0.34 ± 0.03 |
| Sec8p              | 0.21 ± 0.10 |
| Sec10p             | 8.11 ± 0.12 |
| Sec15p             | 1.43 ± 0.39 |
| Sec70p             | 0.25 ± 0.05 |
| Sec84p             | 0.31 ± 0.05 |
| Sec1p              | 0.12 ± 0.07 |
| Sec2p              | 0.12 ± 0.07 |
| Sec4p              | 0.18 ± 0.03 |

a β-Galactosidase activities (units) are presented as mean ± S.E. obtained from three independent transformations.

with the result that Exo84p overexpression rescues sec5–24 temperature sensitivity.

Efficient Association of Exo84p with the Exocyst Complex Requires Sec5p and Sec10p—Previous studies have characterized the molecular interactions of all of the exocyst components except for Exo84p (6). The two-hybrid assay mentioned above suggests that Exo84p associates with Sec10p and/or Sec5p. To gain further insight into the molecular organization of the exocyst, particularly concerning the role of Sec10p and Sec5p in the association of Exo84p to the exocyst, we have carried out immunoprecipitation experiments in various exocyst mutant strains.

Anti-Exo84p antibody was used in the immunoprecipitation reactions, and Western blot analysis was performed to detect the ability of Sec8p, a peripheral component of the exocyst complex, to co-immunoprecipitate with Exo84p from various mutant lysates (Fig. 7). Compared with wild-type cells, the amounts of Sec8p precipitated from sec5–24 and sec10–2 mutant strains were greatly reduced (Fig. 7A, upper panel). As controls, the amounts of Sec8p co-precipitated with Exo84p in all of the other exocyst mutants were not affected (Table III). This result indicates that the association of Exo84p with the exocyst complex requires Sec5p and Sec10p, and mutations in these two proteins lead to disruption of the link between Exo84p and Sec8p (Fig. 7B). In all of the mutants, the association of Exo84p with Sec10p was unaffected (Fig. 7A and Table III), suggesting a possible direct interaction between Exo84p and Sec10p. Especially, the association of Exo84p with Sec10p was not disrupted in the sec5 mutant, suggesting that the connection between Sec10p and Exo84p is probably not through Sec5p. We have also examined the association of Sec5p with Exo84p. Due to the lack of anti-Sec5p antibody, we used the strains containing HA-tagged Sec5p. As shown in Fig. 7A (lower panel), the level of co-precipitation of Sec5p-HA was reduced in the sec10–2 mutant strain. Therefore, it is possible that Exo84p is linked to Sec5p through Sec10p. Alternatively, it is possible that Exo84p directly binds both Sec5p and Sec10p; however, the loss of Sec10p reduces the overall association of Exo84p with the exocyst complex. These observations are consistent with the two-hybrid assay results (Table II).

Localization of Exo84p in Yeast Cells—A common characteristic of the exocyst proteins is that they are concentrated at the bud tip and mother/daughter neck, regions of active exocytosis in the budding yeast. We have examined the localization of Exo84p in various exocyst mutants (sec3–2, sec4–8, sec5–24, sec6–4, sec8–9, sec10–2, and sec15–1). The strains were first grown at 25 °C and then shifted to 37 °C for 1 h. The localization patterns of Exo84-GFP in the cells before (Fig. 8B) and after temperature shift (Fig. 8C) were examined. We found that the localization of Exo84p in sec5–24 mutant was altered after the temperature shift (Fig. 8C). Instead of being concentrated at sites of active secretion, Exo84p seemed to be randomly distributed in a punctuate pattern throughout the cell (Fig. 8C). The localization of Exo84p was not affected in other exocyst mutant strains examined.

**DISCUSSION**

We report here the identification and characterization of a component of the exocyst, Exo84p, in the yeast *S. cerevisiae*. Exo84p is an essential protein. Yeast cells depleted of Exo84p are defective in invertase secretion and accumulate vesicles similar to those in the late sec mutants. Exo84p co-immunoprecipitates with the exocyst components, and a portion of Exo84p co-sediments with the exocyst complex in velocity gradients. Overexpression of Exo84 selectively suppresses the temperature sensitivity of a sec5 mutant. The association of Exo84p to the exocyst complex is dependent on Sec5p and Sec10p. Furthermore, Exo84 specifically localizes to the bud tip or mother/daughter connection, regions of polarized secre-
In the yeast *S. cerevisiae*, these studies suggest that Exo84p plays an important role in polarized secretion. Based upon the molecular characterization of Exo84p summarized above, it is reasonable to classify this protein as a component of the exocyst complex. In fact, the mammalian Exo84p co-purified with, and had a 1:1 stoichiometry with other exocyst members (9). Exo84p was not detected in the immunopurified yeast complex (3, 4), probably because its association with the complex is not stable and it was lost during the purification. Velocity gradient fractionation demonstrates that the majority of Exo84p is in a free pool instead of the fully assembled complex. This free pool may be due to the dissociation of Exo84p from the complex during the experimental procedure. This low affinity of Exo84p to the complex may explain the absence of Exo84p in the immunopurified complex (4). Alternatively, it is possible that the “free pool” is indeed present in vivo and has some biological significance.

Within the exocyst complex, Exo84p seems to be most proximal to Sec5p and Sec10p. Genetically, overexpression of Exo84p suppressed sec5 temperature sensitivity. Using the yeast two-hybrid assay, positive interactions were found between Exo84p and Sec5p and Sec10p (Table II). Furthermore, incorporation of Exo84p into the exocyst required functional Sec5p and Sec10p (Fig. 7). It is possible that Exo84p directly interacts with Sec10p. When Sec10p is defective, Exo84p loses its connection with the other exocyst components. Since Sec10p itself needs Sec5p for its association with the other members (4, 6), this proposal is consistent with the observation that Exo84p

**TABLE III**

| Subunit | Wild type | sec3–2 | sec5–24 | sec6–4 | sec10–2 | sec15–1 |
|---------|-----------|--------|--------|--------|--------|--------|
| Exo84p  | +         | +      | +      | +      | +      | +      |
| Sec5p   | +         | +      | –      | +      | –      | –      |
| Sec10p  | +         | +      | +      | +      | –      | –      |
| Sec5p(HA)| +        | +      | +      | ND*    | +      | –      |

* ND: Not determined.
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Exo84p antibody indicates that the profile of Exo84p in sec5 mutant cells is equivalent to that of wild-type cells (data not shown). We speculate that Sec5p is crucial for retaining Exo84p in those regions, considering the biochemical and genetic relationship of Exo84p with Sec5p mentioned above as well as the central role of Sec5p in the molecular organization of the exocyst complex (3, 6).

In summary, we have identified and characterized yeast Exo84p, a protein essential for exocytosis in \textit{S. cerevisiae}. Future studies will be focused on identification of proteins that may interact with Exo84p. These studies should help us understand the molecular mechanisms that regulate post-Golgi vesicle docking and fusion in eukaryotic cells.

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