BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **224,** 126–133 (1996)
ARTICLE NO. 0995

The Putative Membrane Anchor Protein for Yeast Sec7p Recruitment

Joseph R. Wolf,[1] Robert S. Lasher, and Alex Franzusoff[2]

Department of Cellular and Structural Biology, CU Cancer Center, University of Colorado Health Sciences Center, Box B-111, 4200 East Ninth Avenue, Denver, Colorado 80262

Received May 22, 1996

Proteins required for yeast secretory pathway function have been identified by genetic selection and characterization of the temperature-sensitive secretory (*sec*) mutants. The use of genetic and biochemical approaches has expanded the catalog of components of the secretory pathway, yet many proteins, especially membrane and lumenal proteins, remain to be identified. Sec7p, one of the original *SEC* gene products to be described, is required at multiple stages of the yeast secretory pathway in the coating of transport vesicles. A chemical cross-linking approach was used to identify proteins associated with Sec7p protein complexes from yeast cell lysates. A 90 kDa integral membrane protein (p90) was isolated whose interactions with Sec7p were reproduced in the absence of chemical cross-linking. Further biochemical analysis indicated that p90 may act as the anchor protein for Sec7p membrane recruitment in transport vesicle assembly.

Studies of *S. cerevisiae* strains exhibiting temperature-sensitive defects in cell growth and secretion have facilitated the characterization of proteins essential to yeast secretory pathway function (for reviews, see 1-4). Vesicle-mediated traffic between compartments, and the molecules that participate in the assembly of vesicles include coat proteins that are recruited from the cytosol onto the membrane surface (5). For vesicle budding from the yeast ER, two different coat protein assemblies have been identified, *i.e.* the soluble COPI and COPII coat proteins (6). In the case of COPII coats, the membrane protein Sec12p binds the Sar1 GTPase, thereby recruiting the Sec23p/Sec24p and Sec13p/Sec31p complexes from the cytosol (7). The mechanisms for incorporating other cytosolic and integral membrane proteins, such as Sec16p, Sec22p, Bos1p and Emp24p, into COPII coated vesicles are not understood (8-10). For COPI coated vesicles, the cytosolic Arf1 GTPase promotes the binding of COPI coat proteins, yet the membrane anchor protein has not been identified (for reviews, see 7, 11). The assembly of vesicle coats is presumably not only essential for membrane bilayer deformation, but also for the packaging of lumenal and membrane cargo destined for other organelles (11). Information for targeting the vesicles and recruiting other molecules to facilitate membrane fusion must also be packaged with the budding vesicle. The identification of components required in vesicle formation, especially the membrane and lumenal proteins, is currently incomplete.

One vesicle coat protein, Sec7 protein (Sec7p), acts at multiple stages of the yeast secretory pathway from the ER and through the Golgi apparatus (12-15). Sec7p is a 230 kDa phosphoprotein found in cytosolic protein complexes and in membrane-associated protein networks (15-17). To participate in vesicle coat formation, the cytosolic Sec7p-associated protein complexes are proposed to bind to the surface of ER and Golgi membranes (5, 14, 17, 18). After budding, vesicles uncoat for membrane fusion with the target organelles, and Sec7p is recycled to the cytosolic protein pool in an ARF-dependent manner (19, 20).

[1] Current address: Peace College, 15 E. Peace Street, Raleigh, NC 27604.
[2] Corresponding author: Fax: (303) 270-4729; E-mail: Alex.Franzusoff@UCHSC.EDU.

0006-291X/96 $18.00

Given these observations, Sec7p must interact with proteins on membrane surfaces to be recruited onto transport vesicles. Both genetic and biochemical approaches are being used to identify Sec7p-associated proteins. Chemical crosslinking approaches have been successful in previous studies for demonstrating protein-protein interactions of components involved in secretory pathway function (e.g. 21, 22). Here we describe the use of chemical crosslinking to identify proteins associated with Sec7p in whole yeast cell lysates.

MATERIALS AND METHODS

Strains and growth media. GPY59 yeast (23) were grown in YPD medium (1% yeast extract, 2% peptone, 2% glucose) or in YNB + glucose medium (0.67% yeast nitrogen base + amino acid supplements). Growth was monitored at 600 nm, where 1 OD_{600} unit is equivalent to 10^7 cells.

Chemical crosslinking of yeast spheroplast lysates. GPY59 yeast were grown in YNB at 30°C to 0.7 OD_{600}/ml. The cells (5 OD_{600}) were washed with 0.1 M Tris, pH 9.4 then converted to spheroplasts with 150 U oxalyticase (Enzogenetics, Corvallis, OR) in 1% YNB, 0.7 M Sorbitol, 0.1% glucose, 5 mM $MgCl_2$, 1% raffinose, pH 6.8 for 30 min at 30°C. Spheroplasts were washed in labeling medium (YNB, 0.7 M Sorbitol, 2% glucose), resuspended to 5 OD_{600}/ml in the same medium, then metabolically radiolabeled with Trans^{35}S label (ICN Biomedicals) for 30 min at 30°C. After labeling, spheroplasts were washed then resuspended in 200 μl crosslinking buffer (0.1 M $NaPO_4$, 1.2 M Sorbitol, 5 mM EDTA, 0.1 M PMSF, proteinase inhibitor cocktail (PIC: 0.25 μg/ml pepstatin A, 0.25 μg/ml leupeptin, 0.25 μg/ml chymostatin, 0.25 μg/ml aprotinin, 0.025 μg/ml antipain). 800 μl H_2O containing 1 mM PMSF and PIC was added to lyse the spheroplasts, then 200 μg/ml dithiobis(succinimidyl-propionate) (Pierce Chemicals) was added for 30 min at 25°C. The reaction was quenched with 0.1 M NH_4Ac. Laemmli sample buffer without β-mercaptoethanol was then added and the samples were heated. The crosslinked lysate was incubated with #2849 antibody to Sec7p (Sec7-Ab, (17)) and protein A Sepharose (Pharmacia) overnight at 4°C for immunoprecipitations, as described previously (24). The immunoprecipitates were washed, solubilized with Laemmli sample buffer without reducing agent, then re-immunoprecipitated with Sec7-Ab. The second immunoprecipitates were heated with sample buffer containing 50 mM dithiothreitol. In other experiments, samples immunoprecipitated under native, non-denaturing conditions were incubated at 4°C with Sec7-Ab and protein A Sepharose in buffer 88 plus 1 mg/ml BSA, 1 mM PMSF and PIC before washing and solubilization in denaturing Laemmli sample buffer.

Monoclonal antibody to p90. The 38 kDa polypeptide antigen for the injection of mice was purified by electroelution from SDS gel slices, then concentrated and emulsified in Hunter's TiterMax (CytRx Corporation). The emulsion was then injected subcutaneously into Balb/c mice. The mice were boosted twice with the same antigen. Four days before sacrifice, mice were injected intraperitoneally with the emulsion. Spleen cells from the immunized mice were fused to P3X63Ag8.653 myeloma cells 25 using polyethylene glycol 4000 and hybridomas were selected in HAT medium. Hybridoma culture supernatants were screened for immunoreactivity to gel purified 38 kDa antigen and the antigen in crude yeast lysates by immunoblotting. Conditioned culture medium from expanded immunoreactive clone 8-4 was centrifuged to remove particulates, brought to 0.05 M Tris pH 7, 3 mM NaN_3 and stored at 4°C.

Western blots. Gels were transferred to nitrocellulose overnight at 4°C, 0.2A, in transfer buffer (20mM Tris, 150mM glycine, 20% methanol). Non-specific antibody binding was blocked by either PBN (4% BSA, PBS, 3mM NaN_3) for monoclonal antibodies, or BB (2.5% non-fat dry milk, 50mM Tris pH 7.5, 150mM NaCl, 0.1% NP40, 3mM NaN_3) for polyclonal antibodies. Monoclonal antibody to p90 was used as hybridoma culture supernatant. Polyclonal antibody to Sec7p (#2898) was used at a 1:500 dilution (17). Polyclonal antibody to Sec61p (#8320) was kindly provided by Randy Schekman and used at 1:500 dilution (21). To detect the monoclonal antibodies, at 1:1000 dilution of rabbit anti-mouse IgG (Zymed) was used, followed by ^{125}I-protein A (ICN).

Membrane extractions and $CHCl_3$/MeOH precipitations. Carbonate pH 11.5, NaCl, and Urea/EGTA extractions were performed by diluting lysate into 0.1M Carbonate pH 11.5, 1M NaCl, or Urea/EGTA (3.3M Urea, 2mM EGTA, 50mM Tris pH 8.8, 0.7M sorbitol, 0.15M NaCl, 2mM DTT) and incubating 30 min, 4°C. The samples were subjected to ultracentrifugation at 100,000 × g 4°C for 1 hour. Supernatant and pellet fractions were collected and precipitated with $CHCl_3$/MeOH. TX-114 extractions were performed as described (26). Aqueous and detergent phases were pooled and precipitated with $CHCl_3$/MeOH. $CHCl_3$/MeOH precipitations were performed as described (27).

RESULTS

A chemical crosslinking approach was utilized to identify proteins that interact with Sec7p for vesicular transport through the yeast secretory pathway. The membrane-impermeant, cleavable crosslinking reagent, dithiobis succinimidypropionate (DSP), forms covalent bonds between primary amine groups in close proximity (28). Lysates prepared by osmotic lysis of radiola-

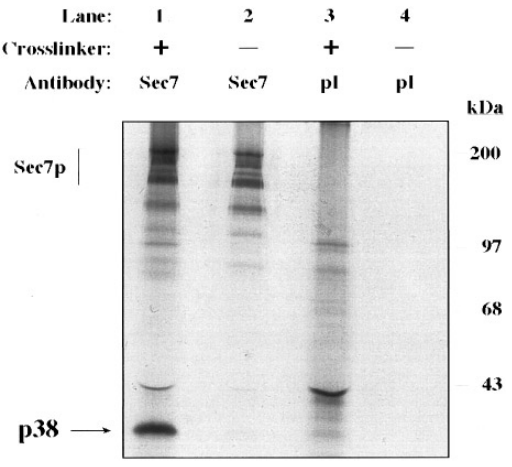

FIG. 1. A 38 kDa polypeptide is crosslinked to seC7p by DSP. Lysates made from radiolabeled GPY59 spheroplasts were treated with DSP. The reaction was quenched and samples were denatured under non-reducing conditions, then Sec7p and its crosslinked proteins were immunoprecipitated with Sec7-Ab. The immunoprecipitates were resolved on SDS-gels under reducing conditions. DSP-treated lysates and immunoprecipitated with Sec7-Ab (Lane 1) or with pre-immune antisera (lane 4); mock treated lysate immunoprecipitated with Sec7-Ab (lane 2); or with pre-immune antibody (lane 3).

belled yeast spheroplasts were incubated with DSP. Sec7p and its crosslinked proteins were immunoprecipitated with Sec7p-specific antibodies (Sec7-Ab) under non-reducing conditions. The immunoprecipitates were dissociated under reducing conditions to break the crosslinks, and the radiolabeled proteins were resolved on SDS-gels. Some bands precipitated by Sec7-Ab in the absence of DSP were Sec7p fragments due to its protease sensitivity in yeast lysates (Fig. 1, lane 2). However, an abundant 38 kDa polypeptide and a minor species at ~100 kDa were precipitated together with Sec7p, but only in the presence of DSP (Fig. 1, *cf.* lane 1-3).

To initiate characterization of the 38 kDa polypeptide, microsequencing of the whole polypeptide or smaller peptides derived from it proved unsuccessful. The chemical modifications resulting from crosslinking of primary amines were apparently not compatible with microsequencing. However, a mouse monoclonal antibody generated against the protein purified from SDS-gels recognized the 38 kDa polypeptide precipitated with Sec7-Ab in the crosslinking experiments (Fig. 2A), but not from whole yeast lysates in the absence of crosslinking (Fig. 2B). Instead, the monoclonal antibody recognized a 90 kDa polypeptide (p90) in whole yeast lysates (Fig. 2B). These results indicated that the crosslinked 38 kDa polypeptide was a fragment of p90, protected from further degradation by its association with Sec7p.

To test whether p90 was associated with Sec7p in the absence of crosslinking, native immunoprecipitations with Sec7-Ab from whole yeast lysates were performed using pre-immune antisera (Fig. 3, lanes 1, 2), Sec7-Ab (lane 3) or p90-MAb (lane 4). These results demonstrated that the p90 polypeptide (and not a 38 kDa species) co-immunoprecipitated with Sec7p under native conditions. Similar experiments but with lysates prepared under denaturing conditions failed to reveal p90 co-immunoprecipitation with Sec7p (data not shown). The converse experiment using p90 monoclonal antibodies for the immunoprecipitations could not be performed as the antisera did not precipitate p90 under either native or denatured conditions. However, these results supported the hypothesis that p90 is normally found in association with Sec7p, and that the 38 kDa fragment from crosslinking with Sec7p was derived from the p90 molecule by proteolysis in the cell extracts during the experiment.

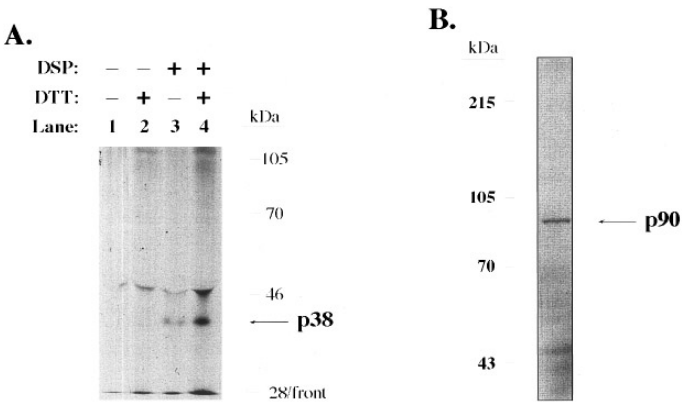

FIG. 2. Monoclonal antibody raised against the 38 kDa crosslinked polypeptide recognizes a 90 kDa protein from whole cell lysates. A. Yeast spheroplast lysates were treated with or without DSP, as in Figure 1. After immunoprecipitation with Sec7-Ab, the samples were denatured in urea-SDS sample buffer, in the absence or presence of DTT for reducing the crosslinks before resolution on SDS gels. Immunoblotting was performed with monoclonal antibodies raised against gel-purified 38 kDa polypeptide. Note the presence of the p38 polypeptide that co-immunoprecipitated with Sec7-Ab (at arrow), but only from lysates treated with DSP (lanes 3 and 4). The species at ~46 kDa is due to compression from the heavy chain IgG antibodies used in the immunoprecipitation, and does not represent precipitated protein. B. Proteins from whole yeast lysates rapidly denatured with urea-SDS gel buffer under reducing conditions were resolved on gels and the resultant blot was probed with monoclonal antibodies generated to the gel-purified 38 kDa Sec7p-crosslinked polypeptide. Only a 90 kDa polypeptide is recognized from rapidly denatured yeast lysates.

The distribution of p90 in whole yeast cell lysates was examined by several approaches. Subcellular fractionation showed that the p90 polypeptide was recovered in the 100,000 × g pellet fraction, and Sec7p was equally distributed in both pellet and supernatant fractions from whole yeast lysates ((17) and Fig. 4A, lanes 1-3). The p90 sedimentation characteristics were further evaluated by treatment with either 0.1 M carbonate pH 11.5, or with 1 M NaCl (Fig. 4A). The carbonate/high pH treatment ruptures membranes into sheets, releasing luminal contents, and both treatments promote the release of peripheral membrane proteins into the supernatant, without effect on integral membrane proteins (29). Neither treatment affected p90 or Sec7p sedimentation with the pellet fractions (Fig. 4A, and (17)). To release the sedimentable Sec7p into the supernatant, treatment of the sample with urea plus EGTA was previously

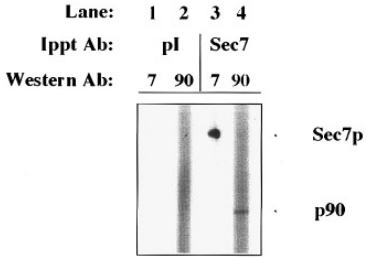

FIG. 3. The 90 kDa protein associates with seC7p in yeast lysates, even in the absence of crosslinking. Yeast lysates were immunoprecipitated with Sec7-Ab under native, non-denaturing conditions. Blots of samples immunoprecipitated with pre-immune antibody (Lane 1, 2) or Sec7-Ab (lane 3, 4) were probed with Sec7-Ab (labeled as 7, lanes 1 and 3) or p90-MAb (labeled as **90,** lanes 2 and 4). The heavy chain IgG protein (50 kDa) in all lanes also indicated equal loading.

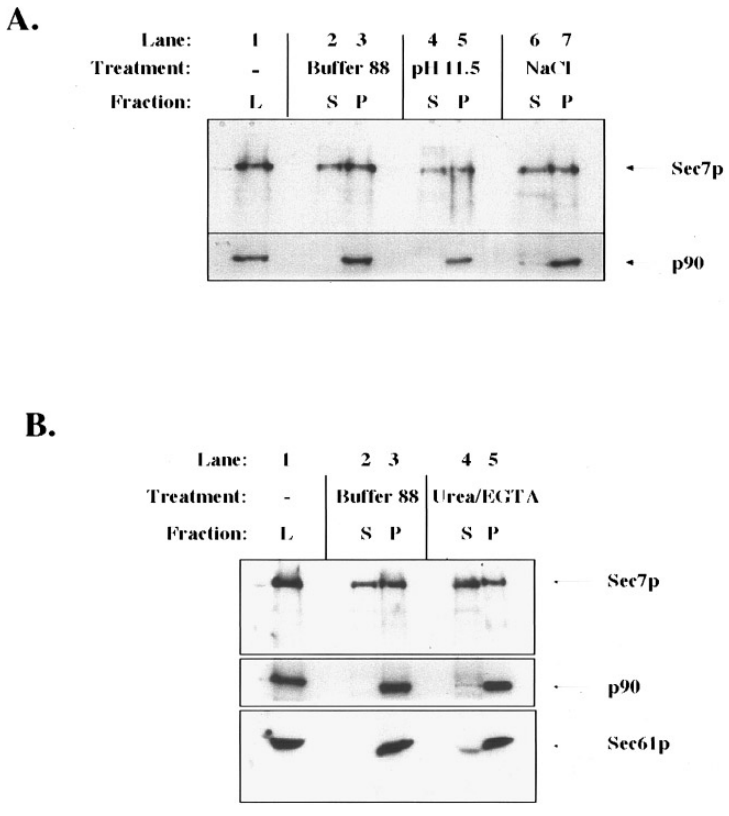

FIG. 4. P90 is an integral membrane protein. A. Yeast lysates were incubated with buffer 88, 0.1 M Na_2CO_3, pH 11.5 or 1 M NaCl, then separated into supernatant (S) and pellet (P) fractions by centrifugation at 100K × g. Immunoblotting were performed with Sec7-Ab (top) or p90-MAb (bottom). B. Yeast lysate (L) was incubated with buffer 88 or 3.3M urea/10mM EGTA buffer then separated into supernatant (S) and pellet (P) fractions by centrifugation at 100K × g for immunoblotting as above, with Sec7-Ab (top), p90-MAb (middle), or antibody to the integral membrane protein Sec61p (bottom).

shown to be necessary (Fig. 4B, and (17)). This chaotropic treatment does not disrupt membrane integrity, as soluble lumenal proteins were not released by this extraction procedure (17). However, the urea extraction did not displace p90 from the pellet fraction (Fig. 4B). These results suggested that either p90 is more tightly bound to membranes than Sec7p, or that p90 is an integral membrane protein.

Another technique commonly used to examine the distribution of proteins in membranes is extraction with the non-ionic detergent Triton X-114 (TX-114). The cloud point for TX-114 at 30°C causes separation of the detergent and aqueous phases upon centrifugation at 1000 × g. Integral membrane and other highly hydrophobic proteins partition into the detergent phase (26). The aqueous and detergent phase fractions from yeast lysates solubilized with TX-114 were examined. Most of p90 and a known integral membrane protein of the ER, Sec61p, partitioned mostly into the detergent phase (Fig. 5A, and (30)). In contrast, neither Sec7p (Fig. 5A), nor carboxypeptidase Y (a soluble vacuolar enzyme, data not shown), partitioned into the detergent phase. These results support the hypothesis that p90 is an integral membrane protein.

The organization of p90 into higher order complexes was revealed by selective membrane

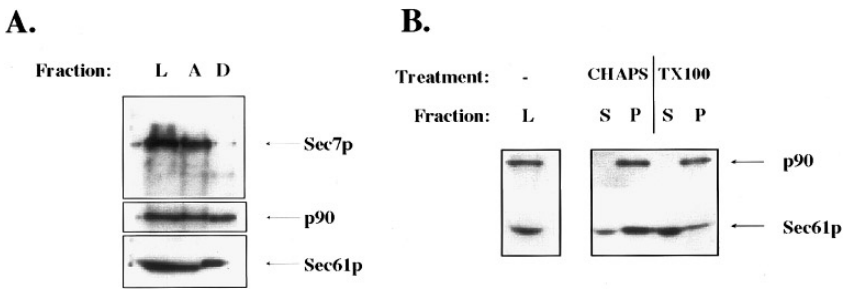

FIG. 5. Selective detergent solubilization of membrane proteins. A. Yeast lysates (L) were incubated in TX-114 then separated into aqueous (A) and detergent (D) phases as described in Methods. Immunoblots were performed with Sec7-Ab (top), p90-MAb (middle), and antibody to the integral membrane protein Sec61p (bottom). B. Yeast lysate (L) membranes were sedimented then treated with 40 mM CHAPS or 1% TX-100 before separation into supernatant (S) and pellet (P) fractions by centrifugation at 100K × g. Immunoblotting was performed with p90-MAb (top) or antibody to Sec61p (bottom).

solubilization. The zwitterionic detergent CHAPS exhibits a moderate critical micellar concentration (cmc, 4-8 mM), yet only very few (2-4) detergent molecules participate in micelle formation (31). By comparison, the non-ionic detergent Triton X-100 (TX-100) exhibits a low cmc (0.24 mM), yet pure detergent micelles are formed with ~140 molecules. Hence, protein-protein and protein-lipid interactions are more likely to be dispersed in TX-100, whereas CHAPS is less likely to disrupt these interactions.

The effects of detergent extraction on p90 and Sec61p were evaluated. Using CHAPS, both p90 and Sec61p were found in the pellet fraction (Fig. 5B, lane 3). However, TX-100 treatment released most of Sec61p into the supernatant, while p90 remained in the detergent-insoluble pellet (lane 5). These results were indicative of interactions with other proteins. Even though Sec61p is a membrane protein required for protein translocation, earlier studies demonstrated interactions with a soluble luminal protein known as Kar2p/BiP in the ER (32, 33). The differential solubilization of Sec61p and p90 by TX-100 treatment indicate that the Sec61p interactions with other proteins must have been disrupted, while p90 complexes were unaffected. We had previously shown that Sec7p was organized into complexes on membranes yet these interactions were unaffected by treatment with a variety of detergents, including CHAPS and TX-100 (17). Hence, the TX100 results suggest that p90 is in a protein complex, at least partly with Sec7p, causing both molecules to behave anomalously in detergent extraction procedures.

DISCUSSION

Because Sec7p is organized into cytosolic and membrane protein complexes, we set out to biochemically isolate the Sec7p-associated proteins. Treating yeast lysates with the chemical crosslinker DSP, a major 38 kDa polypeptide is found associated with Sec7p. However, monoclonal antibodies raised against the 38 kDa species recognize a 90 kDa protein (p90) from whole yeast lysates. The 38 kDa protein was shown by several criteria to represent a fragment of p90, where p90 is the polypeptide that interacts with Sec7p even in the absence of crosslinker. The results from different extraction procedures show that p90 is an integral membrane protein, and is complexed with other proteins. The interactions between p90 and Sec7p apparently cause both molecules to behave anomalously to different treatments, implying that p90 serves as an anchor protein for Sec7p membrane binding.

To our knowledge, a p90 protein that functions in the yeast secretory pathway has not been

previously described, suggesting that we have identified a novel component. Its association with Sec7p implies that p90 is also involved in secretory pathway function, but this remains to be shown. Experiments using p90 monoclonal antibodies to inhibit yeast cell-free assays of ER to Golgi protein transport have not been successful, most likely because the p90-MAb fail to immunoprecipitate p90 under either native or denaturing conditions. With the completion of the yeast genome sequencing project, the improved ability to identify the yeast gene encoding p90 will support further biochemical efforts to characterize its role in vesicular traffic.

The cytosolic Sec7p-associated protein complex apparently cycles on and off the membrane in an ARF GTPase dependent manner (20). Since ARFs have been implicated in the recruitment and disassembly of coat proteins with membrane components, the association of Sec7p with an integral membrane protein for regulating vesicular traffic is predicted (13, 15, 18, 20). The manner in which p90 was identified by its association with Sec7p leads us to speculate that p90 is the putative membrane anchor for Sec7p binding. The abundance of polypeptide cross-linked to Sec7p (Fig. 1) is consistent with potentially stoichiometric interactions between the two molecules. Furthermore, p90 fractionation into the CHAPS-insoluble pellet from whole cell lysates supports the hypothesis that the p90 is tethered into higher order protein structures. An alternative explanation is that p90 was non-specifically trapped into a network aggregate upon detergent treatment. The likelihood of this possibility is diminished by efficient CHAPS solubilization from the ER membranes of Sec61p, a member of a protein complex involved in protein translocation. Further work will elucidate the distribution, topology and role of p90 in vesicular traffic.

ACKNOWLEDGMENTS

We gratefully acknowledge gifts of antibodies from Drs. Randy Schekman (Sec61p) and Dieter Gallwitz (Ypt1p). We thank Dr. Kathryn Howell for helpful comments and criticisms during the course of this work; Michael Nicks for his technical assistance and Stephen Deitz for help in preparing the figures; the CU Cancer Center for support of Dr. J. Wolf during the course of this work and a grant to A.F. from the American Cancer Society (CD-498).

REFERENCES

1. Barinaga, M. (1993) *Science* **260,** 487–489.
2. Franzusoff, A. (1992) *Seminars Cell Biol.* **3,** 309–324.
3. Pryer, N. K., Wuestehube, L. J., and Schekman, R. (1992) *Ann. Rev. Biochem.* **61,** 471–516.
4. Ferro-Novick, S. and Jahn, R. (1994) *Nature* **370,** 191–193.
5. Schekman, R. and Orci, L. (1996) *Science* **271,** 1526–1533.
6. Bednarek, S. Y., Ravazzola, M., Hosobuchi, M., Amherdt, M., Perrelet, A., Schekman, R. and Orci, L. (1995) *Cell* **83,** 1183–1196.
7. Salama, N. R. and Schekman, R. W. (1995) *Curr. Opin. Cell Biol.* **7,** 536–543.
8. Schimmöller, F., Singer-Krüger, B., Schröder, S., Krüger, U., Barlowe, C. and Riezman, H. (1995) *EMBO J.* **14,** 1329–1339.
9. Espenshade, P., Gimeno, R. E., Holzmacher, E., Teung, P. and Kaiser, C. A. (1995) *J. Cell Biol.* **131,** 311–324.
10. Lian, J. P. and Ferro-Novick, S. (1993) *Cell* **73,** 735–745.
11. Mallabiabarrena, A. and Malhotra, V. (1995) *Cell* **83,** 667–669.
12. Novick, P., Ferro, S. and Schekman, R. (1981) *Cell* **25,** 461–469.
13. Franzusoff, A. and Schekman, R. (1989) *EMBO J.* **8,** 2695–2702.
14. Wolf, J., Nicks, M., Deitz, S., Steiner, E., van Tuinen, E. and Franzusoff, A. (1996) Submitted.
15. Lupashin, V. V., Hamamoto, S. and Schekman, R. W. (1996) *J. Cell Biol.* **132,** 277–289.
16. Achstetter, T. A., Franzusoff, A., Field, C. and Schekman, R. (1988) *J. Biol. Chem.* **263,** 11711–11717.
17. Franzusoff, A., Redding, K., Crosby, J., Fuller, R. S. and Schekman, R. (1991) *J. Cell Biol.* **112,** 27–37.
18. Franzusoff, A., Lauzé, E. and Howell, K. E. (1992) *Nature* **355,** 173–175.
19. Melançon, P., Franzusoff, A. and Howell, K. E. (1991) *Trends Cell Biol.* **1,** 165–171.
20. Deitz, S. B., Wu, C., Silve, S., Howell, K. E., Melançon, P., Kahn, R. and Franzusoff, A. (1996) *Mol. Cell Biol.*, in press.
21. Deshaies, R. J., Sanders, S. L., Feldheim, D. A. and Schekman, R. (1991) *Nature* **349,** 806–808.

22. Stack, J. H., Herman, P. K., Schu, P. V. and Emr, S. D. (1993) *EMBO J.* **12,** 2195–2204.
23. Baker, D., Hicke, L., Rexach, M., Schleyer, M. and Schekman, R. (1988) *Cell* **54,** 335–344.
24. Franzusoff, A., Rothblatt, J. and Schekman, R. (1991) *Meth. Enzymol.* **194,** 662–675.
25. Kearney, J. F., Radbruch, A., Liesegang, B. and Rajewsky, K. (1979) *J. Immunol.* **123,** 1548–1550.
26. Bordier, C. (1981) *J. Biol. Chem.* **256,** 1604–1607.
27. Wessel, D. and Flugge, U. I. (1984) *Anal. Biochem.* **138,** 141–143.
28. Lomant, A. J. and Fairbanks, G. (1976) *J. Mol. Biol.* **104,** 243–261.
29. Fujiki, Y., Hubbard, A. L., Fowler, S. and Lazarow, P. B. (1982) *J. Cell Biol.* **93,** 97–102.
30. Deshaies, R. J. and Schekman, R. (1987) *J. Cell Biol.* **105,** 633–645.
31. Helenius, A. and Simons, K. (1975) *BBA* **415,** 27–79.
32. Brodsky, J. L. and Schekman, R. (1993) *J. Cell Biol.* **123,** 1355–1363.
33. Sanders, S. L., Whitfield, K. M., Vogel, J. P., Rose, M. D. and Schekman, R. W. (1992) *Cell* **69,** 353–365.