YALE JOURNAL OF BIOLOGY AND MEDICINE 69 (1996), pp. 1-8.

Rab Proteins in Gastric Parietal Cells: Evidence for the Membrane Recycling Hypothesis

Benjamin C. Calhoun and James R. Goldenring[a]

Institute for Molecular Medicine and Genetics, Departments of Medicine, Surgery and Cellular Biology and Anatomy, Medical College of Georgia and the Augusta Veterans Affairs Medical Center, Augusta, Georgia

(Received January 17, 1996; returned for revision April 22, 1996; accepted July 11, 1996).

The gastric parietal cell secretes large quantities of HCl into the lumen of the gastric gland in response to secretagogues such as histamine. In the membrane recycling hypothesis, this secretory activity requires the trafficking of the gastric H^+/K^+-ATPase to the cell surface from intracellular tubulovesicles. The Rab subclass of small GTP-binding proteins is thought to confer specificity to vesicle transport throughout the secretory pathway, and previous investigations established that Rab11 is highly expressed in gastric parietal cells. Recent discoveries in intra-Golgi transport and neuronal synaptic vesicle fusion have fortuitously converged on an evolutionarily conserved protein complex involved in vesicle docking and fusion. Recent results indicate that Rabll is involved in the apical targeting of vesicles in parietal cells and other epithelial cells throughout the gastrointestinal tract. In support of the membrane recycling hypothesis, Rab co-segregates with H^+/K^+-ATPase in parietal cells. The presence of Rab11 on tubulovesicles supports a role for this Rab protein in recycling vesicle trafficking.

INTRODUCTION

The secretion of HCl by the gastric parietal cell in response to various secretagogues is associated with profound morphological changes [1, 2]. The five to ten-fold increase in the surface area of the canaliculus at the apical pole of the parietal cell is accompanied by a reduction in the volume of intracellular tubulovesicular membranes [2]. A membrane recycling hypothesis was proposed by Forte [2] to explain the apparent recruitment of such large amounts of membrane during the stimulation of parietal cell secretion. This hypothesis attributes the expansion of the canaliculus to the fusion of tubulovesicles in the apical cytoplasm with the canaliculus. The fusion of the tubulovesicles to the canaliculus delivers the H^+/K^+-ATPase from the intracellular membranes to the cell surface, and the withdrawal of the secretagogue causes the internalization of the H^+/K^+-ATPase-containing membranes. Berglindh et al. [3] have suggested an alternative hypothesis that does not require membrane fusion. In this hypothesis, the tubulovesicular compartment is confluent with the canaliculus and exists in a supercollapsed state in the resting cell. The expansion of the canaliculus and reduction of the tubulovesicles is due to osmotic bulk flow following the secretion of HCl into the canaliculus. The only requirement for such morphological changes to take place would be the activation of the H^+/K^+-ATPase. The finding that the transition from resting to stimulated morphology can be induced by incubating parietal cells in hyperosmotic media supports the osmotic flow hypothesis [4]. The major support for the osmotic hypothesis has been mostly morphological in nature. Thus, Pettitt

[a]*To whom all correspondence should be addressed:* James R. Goldenring, M.D., Ph.D. Institute for Molecular Medicine and Genetics, CB-2803, Medical College of Georgia, 1120 Fifteenth Street Augusta, GA 30912-3175. Tel: 706-21-0682; Fax: 706-721-7915.

[b]*Abbreviations:* NEM, N-ethyl-maleimide; NSF, N-ethylmaleimide (NEM)-sensitive fusion protein (NSF); SNAP, soluble NSF attachment proteins; SNARE, SNAP receptor; VAMP, vesicle-associated membrane protein.

et al. [5] have recently reported the presence of a spiralling network of tubules in parietal cells. In agreement with the osmotic flow hypothesis, the authors suggest that these tubules represent a contiguous system of tubulovesicles in continuity with the canaliculus.

Far greater evidence supports the membrane recycling hypothesis. A number of investigations suggest separate canalicular and tubulovesicular domains in parietal cells. Thus, beneath the secretory canaliculus lies an F-actin-containing domain, which also contains the F-actin-associated protein, ezrin [6, 7]. This F-actin domain appears to separate the secretory canaliculus from the H^+/K^+-ATPase-containing tubulovesicles [8]. This distinction is especially well seen in cultured parietal cells, where the secretory canaliculus exists primarily as an intracellular vesicle [8]. Stimulation of the parietal cells with histamine results in the co-localization of H^+/K^+-ATPase immunostaining with that for F-actin lining the canaliculus [8]. Extensive biochemical data have also accumulated indicating that tubulovesicles and the canaliculus are separate entities. Forte and colleagues [9, 10] have demonstrated that following stimulation H^+/K^+-ATPase activity redistributes from light parietal cell membranes enriched for tubulovesicles into heavier membrane fractions (so-called stimulus-associated or "SA" vesicles). We have observed a similar redistribution of H^+/K^+-ATPase immunoreactivity following stimulation of isolated rabbit parietal cells [11]. All of these results have provided both biochemical and morphological support for the membrane recycling hypothesis, but neither the membrane recycling hypothesis nor the osmotic expansion hypothesis has been directly proven.

RAB PROTEINS IN PARIETAL CELLS

An important prediction of the membrane recycling hypothesis is that tubulovesicles should possess the appropriate markers of vesicle trafficking to an apically directed compartment. The family of Rab small GTP-binding proteins has been implicated in many aspects of vesicular trafficking along both endocytotic and exocytotic pathways [12]. Thus, particular Rab proteins have been associated with discrete intracellular vesicle populations [13]. Importantly, to date, no Rab proteins have been observed in association with plasma membrane compartments. Indeed, in the case of vesicular secretion from zymogen-secreting cells [14], synaptosomes [15] and adrenal chromaffin cells [16], Rab3 isoforms appear to dissociate from the vesicle during the process of exocytosis. We have, therefore, sought to determine whether tubulovesicles of parietal cells contain Rab proteins. Our original investigations indicated that a Rab2-immunoreactive protein was associated with tubulovesicles and translocated to heavier membrane fractions in concert with H^+/K^+-ATPase during stimulation [11, 17]. However, [α-^{32}P]-GTP overlays of enriched tubulovesicle preparations also demonstrated that at least two other small GTP-binding proteins were associated with tubulovesicles [11]. Therefore, we set out to identitify parietal cell Rab proteins by using a 3′-RACE protocol to clone small GTP-binding proteins based on the conserved WDTAGQE sequence in the Rab GTP-binding site. These studies led to the isolation of a number of clones for small GTP-binding proteins including Rab1, Rab10, Rab11, Rab14, Ran and a novel protein, Rab25 [18].

Greater than half of the 3′-RACE clones sequenced coded for Rab11. We therefore focused our efforts on Rab11 in parietal cells. We compared the distribution of Rab11 with that of the H^+/K^+-ATPase. Figure 1 compares the H^+/K^+-ATPase immunoreactivity with that for Rab11 in vesicle fractions prepared from gastric glands by differential centrifugation and sucrose density gradient sedimentation. Rab11 immunoreactivity co-segregated with H^+/K^+-ATPase. These studies have now been verified with both polyclonal and specific monoclonal antibodies against Rab11 [19, 20]. While our initial studies with a polyclonal antiserum indicated that Rab11 was enriched in parietal cells [19], we have now performed higher resolution studies with a specific monoclonal antibody raised against recombinant rabbit Rab11 [20]. Figure 2 compares the staining for H^+/K^+-ATPase with

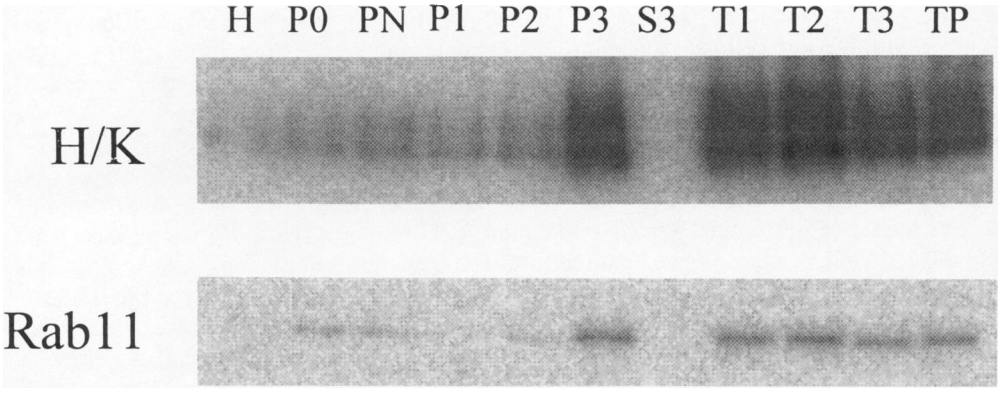

Figure 1. Distribution of Rab11 and H+/K+-ATPase immunoreactivity in gastric membrane subfractions and enriched preparations of tubulovesicles. Membrane protein subfractions from rabbit gastric mucosa were prepared by differential centrifugation, and enriched tubulovesicles membranes were prepared by discontinuous sucrose gradient: H, homogenate; P0, 40g microsomes; PN, post-nuclear supernate; P1, 4000g membranes; P2, 15,000g membranes; P3, 100,000g membranes; S3, 100,000g supernate; T1, 20 percent sucrose buoyant vesicles; T2, 27 percent sucrose buoyant vesicles, T3, 33 percent sucrose buoyant vesicles and TP, vesicles pelleting through 33 percent sucrose. Western blots of 50 ug of protein from each fraction were probed for Rab11 while blots of 5 ug of protein each were probed for H^+/K^+-ATPase. Rab11 immunoreactivity co-segregated with H^+/K^+-ATPase immunoreactivity into enriched tubulovesicle fractions [20].

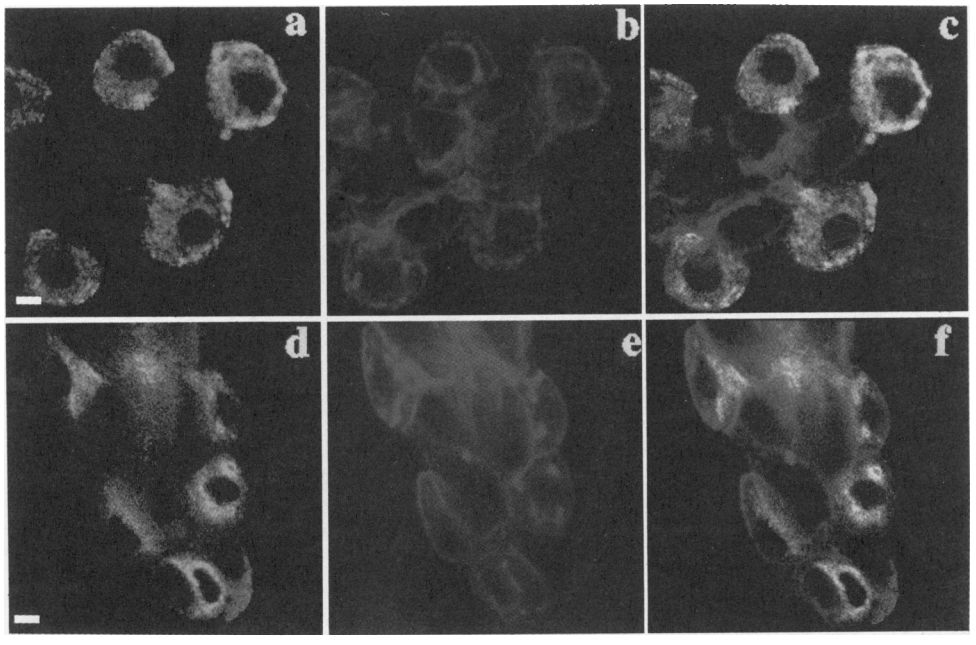

Figure 2. Distribution of H^+/K^+-ATPase and Rab11 in isolated rabbit gastric glands. Isolated gastric glands fixed in four percent paraformaldehyde were stained with either monoclonal anti-H^+/K^+-ATPase (a) (a gift of Dr. Adam Smolka) plus bodipy phallacidin (b) or monoclonal anti-Rab11 (d) and bodipy phallacidin (e). Anaglyph superimpositions are shown in c and f. Bar = 20 μm.

that for Rab11 in isolated rabbit gastric glands. In both cases, glands were double-labelled with Bodipy-phallacidin to demarcate the location of the intracellular canaliculus. The confocal immunofluorescence optical sections show the similar pattern of labelling for H^+/K^+-ATPase and Rab11 as punctate intracellular staining lying deep to or in close proximity with the intracellular canaliculus. These data support the notion that Rab11 is present on tubulovesicles.

RAB11 AS A MARKER OF H^+/K^+-ATPASE SORTING

Recently, Forte and colleagues have noted that treatment of rabbits for five days with omeprazole, the irreversible inhibitor of the H^+/K^+-ATPase, led to the redistribution of H^+/K^+-ATPase into leakier vesicle populations [21]. When 100,000 Xg membranes from the gastric mucosa of untreated animals are separated on discontinuous sucrose gradients of 20, 27 and 33 percent sucrose, the majority of H^+/K^+-ATPase is observed in the less-dense vesicles layering above the 20 and 27 percent interfaces. These "less dense" vesicles are characterized as higher resistance vesicles with greater acid pumping ability. In vesicles derived from the mucosa of omeprazole-treated rabbits, there is a redistribution of H^+/K^+-ATPase into the denser vesicles at 33 percent sucrose interface (Figure 3). This redistribution of H^+/K^+-ATPase is thought to reflect either the recycling of inactive proton pump or the creation of new tubulovesicles to replace inactivated enzyme. We found that omeprazole treatment elicited a redistribution of Rab11 immunoreactivity similar to that observed for H^+/K^+-ATPase (Figure 3). These data indicate that Rab11 may be a marker for the targeting or recycling of H^+/K^+-ATPase-containing tubulovesicles.

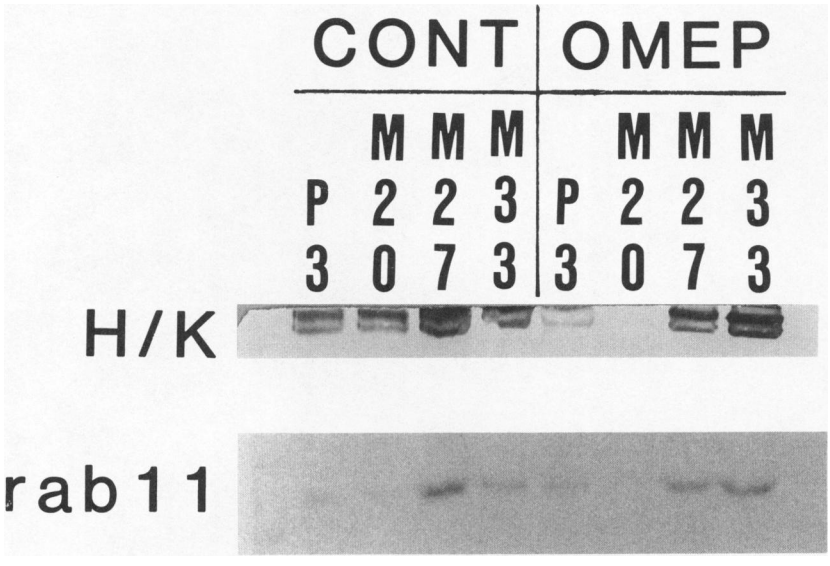

Figure 3. Redistribution of H^+/K^+-ATPase and Rab11 following treatment with omeprazole. Vesicles were isolated from the fundic mucosa of either control rabbits or those treated with omeprazole for five days (P3, 100,000g vesicles; M20, 20 percent sucrose buoyant vesicles; M27, 27 percent sucrose buoyant vesicles and M33, 33 percent sucrose buoyant vesicles). In omeprazole-treated animals, both H^+/K^+-ATPase and Rab11 redistributed to more dense vesicle fractions layering above the 33 percent sucrose gradient layer.

RAB11 AS A GENERAL APICAL VESICLE TARGETING PROTEIN

Preparation of specific Rab11 monoclonal antibodies has allowed us to compare the localization of Rab11 in a number of epithelial cell populations [20]. Throughout the gastrointestinal tract, Rab11 immunoreactivity was observed in an apical vesicular staining pattern. A pattern of subapical punctate staining was observed in the surface mucous cells of the gastric fundus and antrum, jejunal and ileal enterocytes, as well as in colonic surface cells. No significant staining was observed in goblet cells. In the liver, hepatocyes were stained in a vesicular pattern lying just deep to the bile canaliculus. In the pancreas, a fine apical vesicular staining was observed in pancreatic acinar cells that was not accounted for by staining of zymogen granules. In the kidney, fine subapical punctate staining was observed in collecting duct epithelia. Similarly, apical vesicular staining was also observed in the cells of the prostate gland. Finally, supranuclear staining was observed in the deep layers of the squamous epithelia of skin and esophagus. All of these patterns indicate that Rab11 may be a general apical vesicular sorting signal. With the exception of the parietal cell tubulovesicles, the exact nature of these apically located vesicles remains obscure.

VESICLE FUSION MECHANISMS: IMPLICATIONS FOR PARIETAL CELL SECRETION.

The coordinated movement of small GTP-binding proteins with the gastric H^+/K^+-ATPase in response to secretagogues suggests that the regulation of vesicle trafficking is critical to HCl secretion by the parietal cell [17, 19]. While the scope of membrane fusion in parietal cells is particularly impressive, the mechanisms that underlie many aspects of vesicular fusion are well conserved in cells. Thus, recent advances in the studies of intra-Golgi transport, neurotransmitter release and temperature-sensitive yeast secretory mutants have surprisingly converged on a common group of proteins involved in vesicle docking and fusion. The first of these proteins to be purified was the N-ethylmaleimide $(NEM)^b$-sensitive fusion protein (NSF) [22], which restored transport in isolated Golgi membranes treated with the cysteine alkylating agent NEM [23]. The membrane association of NSF is mediated in part by a group of soluble NSF attachment proteins [α–, β–, γ–SNAPs) [24, 25].

Experiments using brain detergent extracts facilitated the discovery of integral membrane protein receptors for SNAPs or SNAP receptors (SNAREs) [26]. An immobilized complex of SNAPs and NSF stabilized with a non-hydrolyzable analog of ATP [27] was used to affinity purify three previously discovered neuronal membrane proteins and identify them as SNAREs. Syntaxin [28] and vesicle-associated membrane protein (VAMP)/synaptobrevin [29] are integral membrane proteins of the plasma membrane and the synaptic vesicle, respectively. SNAP-25 (synaptosomal associated protein of 25kDa) is palmitoylated on several cysteines and behaves as an integral membrane protein of the plasma membrane [30]. The discovery that cleavage of each of these proteins by a specific clostridial neurotoxin abolishes neurotransmission [31-34] validated the functional significance of the identification of syntaxin, SNAP-25 and VAMP as SNAREs. Synaptobrevin, syntaxin, and SNAP-25 bind to each other in an SDS resistant 7S complex in detergent extracts of brain membranes [35, 36]. NSF and α-SNAP bind to this core complex to form the 20S fusion particle [35]. Rothman and colleagues have proposed that the vesicle membrane proteins (v-SNAREs) and target membrane proteins (t-SNAREs) selectively bind to each other to facilitate the docking of the vesicle at the appropriate site on the target membrane [35]. The identification of SNARE homologs essential for various steps in the secretory pathway in yeast indicates that the components of the SNAP-SNARE complex are evolutionarily conserved and involved in many stages of the secretory pathway [37-39].

Syntaxin and synaptobrevin are now known to be representatives of larger protein families, and some members of these families are expressed in tissues outside of the nervous system [40-42]. SNAP-25 has also been found in tissues outside the nervous system such as the adrenal medulla [43]. The entire SNARE complex has been isolated from adrenal chromaffin cells [43]. VAMPs are resident proteins of the GluT-4-containing vesicles of adipocytes [44], and water channel vesicles in the principal cells of the renal medullary collecting duct [45]. Northern analysis of a variety of non-neural tissues demonstrated syntaxins in liver, muscle, spleen and heart [40]. The presence of isoforms of these proteins in a wide variety of nonneural tissues suggests a role for them in many nonneural secretory events.

The preponderance of morphological and biochemical data support the membrane recycling hypothesis of gastric HCl secretion. Furthermore, the mechanism of a wide variety of regulated secretory processes including zymogen granule release, neurotransmitter release and the reversible movement of glucose transporters and water channels appears to require a membrane fusion event. The identification of elements of the SNAP-SNARE fusion complex in all of these systems suggests that a tightly controlled vesicle docking and fusion reaction is involved in all of these secretory processes. We have recently found VAMP-2 immunoreactivity in immunoisolated gastric tubulovesicles [46], and thus, VAMP-2 may be the parietal cell v-SNARE

The presence of a VAMP-2 immunoreactive protein in the gastric H^+/K^+-ATPase-containing vesicles indicates that components of the evolutionarily conserved SNARE complex may be involved in HCl secretion by the gastric parietal cell. Rigorous testing of this hypothesis is needed. If VAMP-2 is the putative parietal cell, v-SNARE, the parietal cell t-SNARE, remains to be identified. Perhaps the study of the parietal cell will lead to the discovery of variations on the SNARE complex theme specific to the regulated trafficking of an ATPase.

Finally, while many of the SNAP-SNARE proteins may be shared among vesicle fusion events, some level of specificity must be needed within cells that utilize membrane docking and fusion at multiple intracellular sites. Since Rab proteins appear to be confined to defined vesicle populations within particular cells [13], it is tempting to suggest that Rab proteins may provide an element of specificity. Furthermore, different cells likely utilize different Rab proteins to regulate the processing of exocytotic vesicles. Thus, while the association of Rab3 family members with synaptic vesicles, zymogen granules and adrenal chromaffin granules is well established, we have been unable to demonstrate the presence of any Rab3 family members in parietal cells (Tang and Goldenring, unpublished observations). In the gastric parietal cell, it appears likely that Rab11 regulates the vesicular trafficking of the H^+/K^+-ATPase. Given the apical localization of Rab11 in a number of epithelial cells, it is likely that Rab11 also regulates apical vesicle fusion in other epithelial cells. Future research will be required to elucidate the relationship of Rab11 with parietal cell SNARE complexes.

ACKNOWLEDGEMENTS: This work was supported by grants to JRG from awards from NIH NIDDKD (DK48370 and DK43405) and a Veterans Administration Merit Award. We are grateful to Drs. James Crothers and John Forte for the gift of tubulovesicles prepared from omeprazole-treated rabbits.

REFERENCES

1. Black, J.A., Forte, T.M., and Forte, J.G. Structure of oxyntic cell membranes during conditions of rest and secretion of HCl as revealed by freeze-fracture. Anat. Rec. 196:163-172, 1980.
2. Forte, T.M., Machen, T.E., and Forte, J.G. Ultrastructural changes in oxyntic cells associated with secretory function: a membrane recycling hypothesis. Gastroenterol. 73:941-955, 1977.
3. Berglindh, T., Helander, H.F., and Obrink, K.J. Effects of secretogogues on oxygen consumption, aminopyrine accumulation and morphology in isolated gastric glands. Acta Physiol. Scand. 97:401-414, 1976.

4. Dibona, D.R., Ito, S., Berglindh, T., and Sachs, G. Cellular site of gastric acid secretion. Proc. Natl. Acad. Sci. USA 76:6689-6693, 1979.
5. Pettitt, J.M., Humphris, D.C., Barrett, S.P., Toh, B., van Driel, I.R., and Gleeson, P.A. Fast freeze-fixation/freeze-substitution reveals the secretory membranes of the gastric parietal cell as a network of helically coiled tubule: a new model for parietal cell transformation. J. Cell Sci. 108:127-1141, 1995.
6. Hanzel, D., Urushidani, T., Usinger, W.R., Smolka, A., and Forte, J.G. Immunological localization of an 80-kDa phosphoprotein to the apical membrane of gastric parietal cells. Am. J. Physiol. 256:G1082-G1089, 1989.
7. Yao, X., Chaponnier, C., Gabbiani, G., and Forte, J.G. Polarized distribution of actin isoforms in gastric parietal cells. Mol. Biol. Cell 6:541-557, 1995.
8. Soroka, C.J., Chew, C.S., Modlin, I.M., Hanzel, D., Smolka, A., and Goldenring, J.R. Characterization of membrane and cytoskeletal compartments in cultured parietal cells using immunofluorescence and confocal microscopy. Eur. J. Cell Biol. 60:76-87, 1993.
9. Hirst, B.H. and Forte, J.G. Redistribution and characterization of H^+/K^+-ATPase membranes from resting and stimulated parietal cells. Biochem. J. 231:641-649, 1985.
10. Urushidani, T. and Forte, J.G. Stimulation-associated redistribution of H^+/K^+-ATPase activity in isolated gastric glands. Am. J. Physiol. 252:G458-G465, 1987.
11. Basson, M.D., Goldenring, J.R., Tang, L.H., Lewis, J.J., Padfield, P., Jamieson, J.D., and Modlin, I.M. Redistribution of 23 kDa tubulovesicle-associated GTP-binding proteins during parietal cell stimulation. Biochem. J. 279:43-48, 1991.
12. Nuoffer, C. and Balch, W.E. GTPases: Multifunctional molecular switches regulating vesicular traffic. Ann. Rev. Biochem. 63:949-990, 1994.
13. Chavrier, P., Parton, R.G., Hauri, H.P., Simons, K., and Zerial, M. Localization of low molecular weight GTP-binding proteins to exocytic and endocytic compartments. Cell 62:317-329, 1990.
14. Jena, B.P., Gumkowski, F.D., Konieczko, E.M., von Mollard, G.F., Jahn, R., and Jamieson, J.D. Redistribution of a rab3-like GTP-binding protein from secretory granules to the Golgi complex in pancreatic acinar cells during regulated exocytosis. J. Cell Biol. 124:43-53, 1994.
15. Fischer von Mollard, G., Sudhof, T.C., and Jahn, R. A small GTP-binding protein dissociates from synaptic vesicles during exocytosis. Nature 349:79-80, 1991.
16. Darchen, F., Zahraoui, A., Hammel, F., Monteils, M.P., Tavitian, A., and Scherman, D. Association of GTP-binding protein Rab3A with bovine adrenal chromaffin granules. Proc. Natl. Acad. Sci. USA 87:5692-5696, 1990.
17. Tang, L.H., Stoch, S.A., Modlin, I.M., and Goldenring, J.R. Identification of Rab2 as a tubulovesicle-membrane associated protein in rabbit gastric parietal cells. Biochem. J. 285:715-719, 1992.
18. Goldenring, J.R., Shen, K.R., Vaughan, H.D., and Modlin, I.M. Identification of a small GTP-binding protein, Rab25, expressed in the gastrointestinal mucosa, kidney and lung. J. Biol. Chem. 268:18419-18422, 1993.
19. Goldenring, J.R., Soroka, C.J., Shen, K.R., Tang, L.H., Rodriguez, W., Vaughan, H.D., Stoch, S.A., and Modlin, I.M. Enrichment of Rab11, a small GTP-binding protein, in gastric parietal cells. Am. J. Physiol. 267:G187-G194, 1994.
20. Goldenring, J.R., Smith, J., Vaughan, H.D., Cameron, P., Hawkins, W., and Navarre, J. Rab11 is an apically located small GTP-binding protein in epithelial tissues. Am. J. Physiol. 270:G515-G525, 1996.
21. Karam, S.M. and Forte, J.G. Inhibiting gastric H^+/K^+-ATPase activity by omeprazole promotes degeneration and production of parietal cells. Am. J. Physiol. 266:G745-G758, 1994.
22. Block, M.R., Glick, B.S., Wilcox, C.A., Wieland, F.T., and Rothman, J.E. Purification of an N-ethylmaleimide-sensitive protein catalyzing vesicular transport. Proc. Natl. Acad. Sci. USA 85:7852-7856, 1988.
23. Malhotra, V., Orci, L., Glick, B.S., Block, M.R., and Rothman, J.E. Role of an N-ethylmaleimide-sensitive transport component in promoting fusion of transport vesicles with cisternae of the Golgi stack. Cell 54:221-227, 1988.
24. Clary, D.O., Griff, I.C., and Rothman, J.E. SNAPs: a family of NSF attachment proteins involved in intracellular membrane fusion in animals and yeast. Cell 61:709-721, 1990.
25. Weidman, P.J., Melancon, P., Block, M.R., and Rothman, J.E. Binding of an N-ethylmaleimide-sensitive fusion protein to Golgi membranes requires both a soluble protein(s) and an integral membrane receptor. J. Cell Biol. 108:1589-1596, 1989.

26. Sollner, T., Whiteheart, S.W., Brunner, M., Erdjument-Bromage, H., Geromanos, S., Tempst, P., and Rothman, J.E. SNAP receptors implicated in vesicle targeting and fusion. Nature 362:318-324, 1993.
27. Wilson, D.W., Whiteheart, S.W., Wiedmann, M., Brunner, M., and Rothman, J.E. A multisubunit particle implicated in membrane fusion. J. Cell Biol. 117:531-538, 1992.
28. Bennett, M.K., Calakos, N., and Scheller, R.H. Syntaxin: a synaptic protein implicated in docking of synaptic vesicles at presynaptic active zones. Science 257:255-259, 1992.
29. Trimble, W.S., Cowan, D.M., and Scheller, R.H. VAMP-1: a synaptic vesicle-associated integral membrane protein. Proc. Natl. Acad. Sci. USA 85:4538-4542, 1988.
30. Oyler, G.A., Higgins, G.A., Hart, R.A., Battenberg, E., Billingsley, M., Bloom, F.E., and Wilson, M.C. The identification of a novel synaptosomal-associated protein, SNAP-25, differentially expressed by neuronal subpopulations. J. Cell Biol. 109:3039-3052, 1989.
31. Blasi, J., Binz, T., Yamasaki, S., Link, E., Niemann, H., and Jahn, R. Inhibition of neurotransmitter release by clostridial neurotoxins correlates with specific proteolysis of synaptosomal proteins. J. Physiol. (Paris) 88:235-241, 1994.
32. Blasi, J., Chapman, E.R., Link, E., Binz, T., Yamasaki, S., De Camilli, P., Sudhof, T.C., Niemann, H., and Jahn, R. Botulinum neurotoxin A selectively cleaves the synaptic protein SNAP-25. Nature 365:160-163, 1993.
33. Link, E., Edelmann, L., Chou, J.H., Binz, T., Yamasaki, S., Eisel, U., Baumert, M., Sudhof, T.C., Niemann, H., and Jahn, R. Tetanus toxin action: inhibition of neurotransmitter release linked to synaptobrevin proteolysis. Biochem. Biophys. Res. Comm. 189:1017-1023, 1992.
34. Schiavo, G., Shone, C.C., Bennett, M.K., Scheller, R.H., and Montecucco, C. Botulinum neurotoxin type C cleaves a single Lys-Ala bond within the carboxyl-terminal region of syntaxins. J. Biol. Chem. 270:10566-10570, 1995.
35. Sollner, T., Bennett, M.K., Whiteheart, S.W., Scheller, R.H., and Rothman, J.E. A protein assembly-disassembly pathway in vitro that may correspond to sequential steps of synaptic vesicle docking, activation, and fusion. Cell 75:409-418, 1993.
36. Hayashi, T., McMahon, H., Yamasaki, S., Binz, T., Hata, Y., Sudhof, T.C., and Niemann, H. Synaptic vesicle membrane fusion complex: action of clostridial neurotoxins on assembly. EMBO J. 13:5051-5061, 1994.
37. Bennett, M.K. and Scheller, R.H. The molecular machinery for secretion is conserved from yeast to neurons. Proc. Natl. Acad. Sci. USA 90:2559-2563, 1993.
38. Ferro-Novick, S. and Jahn, R. Vesicle fusion from yeast to man. Nature 370:191-193, 1994.
39. Rothman, J.E. Mechanisms of intracellular protein transport. Nature 372:55-63, 1994.
40. Bennett, M.K., Garcia-Arraras, J.E., Elferink, L.A., Peterson, K., Fleming, A.M., Hazuka, C.D., and Scheller, R.H. The syntaxin family of vesicular transport receptors. Cell 74:863-873, 1993.
41. McMahon, H.T. and Sudhof, T.C. Synaptic core complex of synaptobrevin, syntaxin, and SNAP25 forms high affinity alpha-SNAP binding site. J. Biol. Chem. 270:2213-2217, 1995.
42. Gaisano, H.Y., Sheu, L., Foskett, J.K., and Trimble, W.S. Tetanus toxin light chain cleaves a vesicle-associated membrane protein (VAMP) isoform 2 in rat pancreatic zymogen granules and inhibits enzyme secretion. J. Biol. Chem. 269:17062-17066, 1994.
43. Roth, D. and Burgoyne, R.D. SNAP-25 is present in a SNARE complex in adrenal chromaffin cells. FEBS Lett. 351:207-210, 1994.
44. Cain, C.C., Trimble, W.S., and Lienhard, G.E. Members of the VAMP family of synaptic vesicle proteins are components of glucose-transporter-containing vesicles of rat adipocytes. J. Biol. Chem. 267:11681-11684, 1992.
45. Gorelick, F.S., Cohn, J.A., Freedman, S.D., Delahunt, N.G., Gershoni, J.M., and Jamieson, J.D. Calmodulin-stimulated protein kinase activity from rat pancreas. J. Cell Biol. 97:1294-1298, 1983.
46. Calhoun, B.C. and Goldenring, J.R. Co-isolation of Rab11, VAMP-2 and H^+/K^+-ATPase, but not syntaxin, on gastric parietal cell tubulovesicles. Mol. Biol. Cell 6:186a, 1995.