Members of the syntaxin family are key molecular components involved in diverse vesicle docking/fusion events. We report here the molecular, biochemical, and cellular characterization of a novel member (syntaxin 7) of the syntaxin family. Syntaxin 7 is structurally related to all known syntaxins. Within a 79-residue region preceding the C-terminal hydrophobic tail, syntaxin 7 is 35, 34, 34, 34, 25, and 19% identical to syntaxins 1, 2, 3, 4, 5, and 6, respectively. Northern blot analysis showed that syntaxin 7 is widely expressed. Indirect immunofluorescence microscopy revealed that syntaxin 7 is primarily associated with the early endosome. In vitro binding assays established that syntaxin 7 in membrane extracts interacts with immobilized recombinant N-ethylmaleimide-sensitive factor attachment proteins fused to glutathione S-transferase. Our results highlight the general importance of members of the syntaxin family in protein trafficking and provide new avenues for future functional and mechanistic studies of this first endosomal syntaxin as well as the endocytic pathway.

Protein transport along the exocytotic and endocytotic pathways is primarily mediated by various types of transport vesicles that bud from one membrane compartment and fuse with a specific cognate compartment (1–4). Because of the diversity of intracellular compartments and the resulting vesicles, understanding the molecular mechanisms that determine the specific docking and fusion of a given type of vesicle with the cognate acceptor compartment represents one of the central issues in cell biology.

To account for the specificity of vesicular transport, the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) hypothesis predicts that the specific docking and fusion of vesicles with the cognate compartment is mediated by the specific pairing between vesicle-associated SNAREs (v-SNAREs) with the cognate SNAREs (t-SNAREs) associated with the target membrane (2, 5–9). Most of the SNAREs are integral membrane proteins anchored to the respective membrane by their C-terminal hydrophobic tail anchors. Vesicle-associated membrane proteins (VAMPs) or synaptobrevins are v-SNAREs associated with the synaptic vesicles, while syntaxin 1 and SNAP-25 (synaptosome-associated protein of 25 kDa) are t-SNAREs associated with the presynaptic membrane. The specific pairing of VAMPs with a syntaxin-1-SNAP-25 complex plays a key role in the docking and fusion of synaptic vesicles with the presynaptic membrane (7–9).

Because of the central role of SNAREs in vesicular transport, the molecular identification, biochemical characterization, and subcellular localization of novel SNAREs are of great importance. Currently, six distinct syntaxins (syntaxin 1, 2, 3, 4, 5, and 6) have been molecularly characterized (10–14). Syntaxins 1, 2, 3, and 4 are found mainly on the cell surface and exocytotic vesicles (11–13). Syntaxin 5 is localized to the cis-Golgi apparatus and has been implicated in endoplasmic reticulum–Golgi anterograde transport (11, 15, 16). Recently, syntaxin 6, another Golgi syntaxin, has been localized to the trans-Golgi network/post-Golgi compartment (14). In this report, we describe the molecular, biochemical, and cell biological characterizations of syntaxin 7, a novel member of the syntaxin family. Antibodies against syntaxin 7 revealed that syntaxin 7 is localized to the early endosomal compartment.

**EXPERIMENTAL PROCEDURES**

*Materials—The A431 (human epidermoid carcinoma), LLC-PK1 (pig kidney), CHO (Chinese hamster ovary), NRL (normal rat liver), NIH3T3 (mouse embryonic fibroblast), CV1 (monkey kidney), Vero (African green monkey kidney), and C6 (rat glial) cell lines were obtained from American Type Culture Collection (Rockville, MD). The MDCK II (Madin-Darby canine kidney strain II) was a generous gift from Dr. K. Simons (EMBL, Germany). Synthetic oligonucleotides were either obtained from Oligos Etc. (Wilsonville, OR) or synthesized in our laboratory. The rat brain-zAP cDNA library and *Pyrococcus furiosus* DNA polymerase were obtained from Stratagene (La Jolla, CA). The rat mRNA multiple tissues Northern filter (MTF) was obtained from CLONTECH (Palo Alto, CA). [32P]dCTP, [125I]-labeled protein A, Hybond N, and Hybond C extra were obtained from Amersham Corp (Little Chalford, Buckinghamshire, United Kingdom). Oligolabeling kit and glutathione-Sepharose 4B were purchased from Pharmacia (Uppsala, Sweden). Local New Zealand White rabbits were obtained from the Sembawang Laboratory Animals Center, Singapore. Fluorescein isothiocyanate-conjugated goat anti-mouse IgG and rhodamine-conjugated goat anti-rabbit IgG were purchased from Boehringer Mannheim. Brefeldin A (BFA) was from Epicentre Technologies. Wortmannin and nocodazole were purchased from Sigma and Aldrich.

dDNA Cloning and Sequencing—A human EST clone (accession no. T811994) that encodes an open reading frame that is homologous to known syntaxins was revealed by using the BLAST program.

The cDNA insert of this EST clone was used to screen a rat brain-zAP cDNA library as described previously (19). Among 10 positive clones, one clone (no. 9) with an insert size of 2.0 kilobase pairs was sequenced completely.
Northern Blot Analysis—The rat cDNA was used as a probe on a rat multiple tissues blot of poly(A) mRNA (CLONTech) using the procedure as described previously (19).

Expression of Recombinant Proteins in Bacteria—For expression of the putative syntaxin, recombinant protein was produced in M15(pREP4). Recombinant protein was purified as described previously (47). Expression and purification of GST recombinant proteins were performed as described elsewhere (19).

Preparation and Purification of Polyclonal Antibodies—HisX6-tagged proteins (300 μg) emulsified in complete Freund’s adjuvant were injected subcutaneously into two local New Zealand rabbits. Booster injections containing a similar amount of antigen emulsified in incomplete Freund’s adjuvant were administered every 2 weeks. Rabbits were bled 10 days after the second or third booster injections prior to affinity purification. Serum was diluted twofold with PBS before incubating with the antigen (identical to the one used for injection) coupled to cyanogen bromide-activated Sepharose (3 mg/ml Sepharose beads) for 2 h at room temperature. The beads were washed extensively, and specific antibodies were eluted (19).

Immunofluorescence Microscopy—Immunofluorescence microscopy was performed as described previously (17–19). Briefly, cells grown on coverslips were washed twice with PBSCM (PBS containing 1 mM CaCl2 and 1 mM MgCl2) and then fixed with 3% paraformaldehyde in PBSCM for 30 min at room temperature. The fixed cells were sequentially washed twice with PBSCM, PBSCMN (PBSCM containing 50 mM NH4Cl) and PBSCM. The cells were then permeabilized with PBSCMS (PBSCM containing 0.2% saponin) for 20 min and then incubated with rabbit antibodies (5–10 μg/ml) in fluorescence dilution buffer (PBSCM with 5% normal goat serum, 5% fetal bovine serum and 2% bovine serum albumin pH 7.6) for 1 h at room temperature. After washing three times with PBSCMS, cells were incubated with rhodamine-conjugated goat anti-rabbit IgG antibody for 1 h at room temperature. Cells were then washed five times with PBSCMS, mounted in a drop of Vectashield (Vector Labs), observed using the axiophot microscope (Carl Zeiss), and photographed with Kodak Tri-X 400 film. For the treatment of cells with BFA, noclodazole, or wortmannin, A431 cells grown on coverslips were incubated with OKT9 monoclonal antibody against human transferrin receptor for 30 min, washed once with Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum, and then treated with BFA (10 μg/ml), noclodazole (10 μg/ml), or wortmannin (1 μM). After incubating for 1 h at 37 °C, cells were washed twice with PBSCM and then fixed in 3% paraformaldehyde. Fixed cells were then permeabilized and incubated with antibodies against syntaxin 7 (polyclonal) for 1 h at room temperature. After washing three times with PBSCMS, cells were incubated with rhodamine-conjugated goat anti-rabbit IgG antibody and fluorescein isothiocyanate-conjugated goat anti-mouse antibodies (10 μg/ml) for 1 h at room temperature. Coverslips were then mounted as described above after washing extensively with PBSCMS.

In Vitro Translation—In vitro translation was performed using the RNA polymerase-coupled TNT system of Promega (Madison, WI) and processed as described previously (19).

Preparation of Golgi-enriched Membranes—Preparation and subfractionation of membranes were performed as described previously (20). Briefly, livers from overnight-fasted Harlan Sprague-Dawley rats were rinsed once in ice-cold PBS, once in homogenization buffer (25 mM Hepes, pH 7.3, 5 mM MgCl2, 1 mM phenylmethylsulfonyl fluoride) containing 0.25 mM sucrose, and then weighed. All subsequent fractionation steps were carried out at 4 °C. Rinsed livers were homogenized in 3 volumes (g/ml) of the homogenization buffer containing 0.25 mM sucrose with a Teflon pestle appended to a homogenizer (6–10 strokes). Homogenate was centrifuged at 10,000 × g for 10 min to remove unbroken cells, nuclei, and mitochondrial. The supernatants were then recentlyrifuged at 100,000 × g in a Beckman Ty45TI rotor for 1 h. The supernatant, which consisted mainly of cytosol, was collected, and the total membrane pellets were resuspended in an equal volume of homogenization buffer containing 0.25 mM sucrose using a Dounce homogenizer. The membrane suspension was then adjusted to a final concentration of 1.25 mM sucrose by adding a sufficient volume of 2.0 mM sucrose homogenization buffer and then overlaying with step gradients of 10 ml 1 M sucrose, 10 ml 1.0 M sucrose, and 5.0 ml 0.5 M sucrose in homogenization buffer. The samples were then centrifuged at 28,000 rpm for 4 h in a Beckman SW 28 rotor, and three distinct fractions were obtained, the Golgi-enriched fraction (G1) at the 0.5 M/1.0 M sucrose interphase, the endosomes/Golgi-enriched fraction (G2) at the 1.0 M/2.1 M sucrose interphase, and the microsome pellet. The G1 and G2 fractions were collected and diluted to a final concentration of 0.25% sucrose by adding a sufficient volume of 25 mM Hepes, pH 7.3, 1 mM phenylmethylsulfonyl fluoride, and pelleted by a 100,000 × g centrifugation step as above. The membrane pellet was then resuspended in a minimal volume of homogenization buffer containing 0.25 mM sucrose. The microsome pellet was resuspended in minimal volume of homogenization buffer (containing 0.25 mM sucrose) with a Dounce homogenizer.

In Vitro Binding Assays—Syntaxin 7-enriched membranes (3 mg) were resuspended at 12,000 × g at 15 min at 4 °C, and the pellet was extracted in 500 μl of incubation buffer (100 mM KCl, 20 mM Hepes, pH 7.3, 2 mM EDTA, 2 mM dithiothreitol, 0.2 mM ATP) containing 1% Triton X-100 prior to incubating at 4 °C for 1 h with agitation. The extracted membranes were diluted with 500 μl of incubation buffer minus Triton X-100 and then centrifuged at 100,000 × g at 4 °C for 1 h. The extracted proteins in the supernatant were used for the binding study. GST-α-SNAP beads (2–5 μg) were washed twice with incubation buffer containing 0.5% Triton X-100 (1 ml each) and then incubated with the different amounts of membrane extract (10, 20, 40, 80, 160, 200, and 300 μg) in a total volume of 100 μl at 4 °C for 3 h with agitation. GST-α-SNAP beads were then washed twice with incubation buffer with 0.5% Triton X-100, once with incubation buffer with 0.1% Triton X-100, and twice with incubation buffer minus Triton X-100 prior to the addition of 10 μl of 3 × SDS sample buffer, separation on SDS-PAGE, and immunoblotting analysis.

RESULTS AND DISCUSSION

Syntaxin 7 Is a New Member of the Syntaxin Protein Family—Using the amino acid sequence of syntaxin 1b to search the sequence data bases by the BLAST program resulted in the identification of a novel EST sequence (accession no. T81994) that could potentially encode a protein fragment bearing amino acid sequence similarities with known syntaxins. The cDNA insert of this EST clone was used to screen a rat brain cDNA library, and several clones of different sizes were identified. One full-length clone was completely sequenced, and the nucleotide and the deduced amino acid sequences are shown in Fig. 1A. Since the derived 261-amino acid sequence is clearly related to all known mammalian syntaxins, we have designated this protein as syntaxin 7 (10, 11, 14). The region of syntaxin 7 that is most related to known syntaxins lies between residues 157 and 255, just preceding the C-terminal hydrophobic membrane anchor. This region is 35, 34, 34, 34, 25, and 19% identical to syntaxin 1, 2, 3, 4, 5, and 6, respectively (Fig. 1B). Furthermore, this region is predicted to form coiled-coil domains (21, 22), a feature of other syntaxins.

Syntaxin 7 Is Widely Expressed—To understand whether syntaxin 7 is involved in a general transport event or its function is restricted to certain tissues, Northern blot analysis was performed to examine the level of mRNA in various rat tissues (Fig. 2). Two different species of mRNA were detected for syntaxin 7. The larger transcript of about 2.4 kilobase pairs exists at high levels in all the tissues examined. Despite the fact that the mRNA for syntaxin 7 is significantly longer than that of the cloned cDNA, the derived amino acid sequence is of full-length because the initiation Met codon is flanked in frame with stop
codons at the 5' end, and the nucleotide sequence around the initiation codons is in agreement with the Kozak sequence (23). Consistent with this, the in vitro translated product of this clone has a size similar to the endogenous protein identified by syntaxin 7-specific antibodies (see below).

Syntaxin 7 Is a 42-kDa Protein—The predicted size of syntaxin 7 is approximately 30 kDa. However, as shown in Fig. 3 (lane 1), the size of the in vitro translation product of syntaxin 7 is approximately 42 kDa when analyzed by SDS-PAGE. Since the difference in size between the predicted and the translation product of syntaxin 7 is significant, it is important to determine the size of the endogenous syntaxin 7 in mammalian cells. The predicted cytoplasmic domain (residues 1–250) was expressed as a HisX6 fusion protein (HisX6-syntaxin 7) and used to raise polyclonal antibodies against syntaxin 7. Immunoblot analysis was used to detect syntaxin 7 in the total membrane preparation from MDCK II cells. As shown in Fig. 3 (lane 2), syntaxin 7-specific antibodies detect a 42-kDa polypeptide (similar to the in vitro translated syntaxin 7) in the MDCK II total membrane extract. Detection of this polypeptide by syntaxin 7 antibodies was blocked by preincubation of antibodies with HisX6-syntaxin 7 (Fig. 3, lane 4) but not by a mixture of GST-syntaxin 3 and GST-syntaxin 4 (Fig. 3, lane 3) recombinant proteins, demonstrating the specificity of the antibodies.

Syntaxin 7 Is an Integral Membrane Protein Enriched in the Asialoglycoprotein Receptor-enriched Membrane Fraction—The preparation and fractionation of rat liver membranes were performed as described previously (20). Fig. 4A shows that the affinity-purified antibodies detect the syntaxin 7 polypeptide in the lane containing the G1 (membrane fraction at the 0.5 M/1.0 M sucrose gradient interphase) and even more in G2 (membrane fraction at the 1.0 M/1.1 M sucrose interface) membrane fractions. A small amount of syntaxin 7 could also be detected in the total membrane fraction but not in the microsome membrane fraction. Syntaxin 7 is therefore enriched in the G1 and G2 membrane fractions. The G1 and G2 membrane fractions were also enriched in the asialoglycoprotein receptor subunit R1 (46 kDa) that is localized to the early endosomal compart-
A GST fusion protein (the entire polypeptide of the E. coli was expressed from the pGEX-KG vector in S protein fused to the C terminus of the glutathione nonidet P-40. Syntaxin 7 was not solubilized in PBS, 2M KCl, 2.5 M urea, and 0.15 M sodium bicarbonate, pH 11.0, 2M KCl, 1% Triton X-100, and 1% sodium bicarbonate, pH 11.0, 2M KCl, 1% Triton X-100, and 1% sodium bicarbonate, pH 11.0, but effectively membrane fractions were extracted with PBS, 2.5M urea, 0.15M sodium bicarbonate, pH 11.0, 1% Triton X-100, and 1% Nonidet P-40, Syntaxin 7 was not solubilized in PBS, 2M KCl, 2.5 M urea, and 0.15 M sodium bicarbonate, pH 11.0, but effectively by 1% Triton X-100 and 1% Nonidet P-40 (Fig. 5), confirming that syntaxin 7 is indeed an integral membrane protein.

**Syntaxin 7 Interacts with α-SNAP**—An in vitro binding assay was performed to determine whether syntaxin 7 interacts with the general docking/fusion component, α-SNAP. α-SNAP was expressed from the pGEX-KG vector in E. coli bacteria as a GST fusion protein (the entire polypeptide of the α-SNAP protein fused to the C terminus of the glutathione S-transferase protein) and bound to glutathione-agarose beads. Beads containing 3 μg of GST-α-SNAP fusion protein were incubated with increasing amounts of detergent-soluble membrane extracts of the G2 fraction (10, 20, 40, 80, 160, 200, and 300 μg). In another set of experiments, GST proteins bound to beads were used as binding controls. Syntaxin 7 bound to the beads was detected by immunoblotting. As shown in Fig. 6, syntaxin 7 binds to α-SNAP in a dose-dependent manner. Under similar conditions, syntaxin 7 did not bind GST-coupled beads (data not shown). These results suggest that syntaxin 7 in the G2 membrane extract interacts with α-SNAP, and syntaxin 7 thus could be a SNARE. It has been shown previously that the binding of synaptobrevin to syntaxin is essential for efficient α-SNAP binding, either by forming a composite receptor surface for α-SNAP or inducing a conformational change in syntaxin that results in high affinity binding (27). Therefore, the interaction of syntaxin 7 with α-SNAP is most likely mediated by a syntaxin 7-containing SNARE complex.

**Syntaxin 7 Is Associated with Vesicular Structures in Several Cell Types**—In an attempt to study the function of syntaxin 7, affinity-purified antibodies were used to detect the endogenous syntaxin 7 by indirect immunofluorescent microscopy. As shown in Fig. 7, perinuclear and punctate structures labeling for syntaxin 7 were identified in eight different cell types (CV1, Vero, MDCK II, NIH3T3, CHO, LLC-PK1, C6, and NRL cells) derived from six different species. This specific labeling was abolished by preincubation of the antibodies with HisX6-syntaxin 7 recombinant protein (data not shown).

**Syntaxin 7 Is Associated with the Endocytic Compartment Marked by Cell Surface Internalized Transferrin Receptor**—The enrichment of syntaxin 7 in the asialoglycoprotein receptor-enriched membrane fractions, in conjunction with the perinuclear and punctate labeling of syntaxin 7 in all the cell types, suggests that syntaxin 7 may be localized to the endo-
cistic compartments. To determine the exact subcellular localization of syntaxin 7, colocalization studies were performed using surface internalized monoclonal antibody against the transferrin receptor as a marker for the early endosomes in A431 cells. As shown in Fig. 8, the labeling of syntaxin 7 colocalized well with that of internalized transferrin receptor, particularly in the perinuclear vesicular structures (Fig. 8, A and B), suggesting that syntaxin 7 is associated with the early endosome.

It has been previously reported that the early endosomes fuse with the trans-Golgi network after treatment of cells with BFA (28, 29). In addition, during the course of the BFA treatment, tubular structures were formed that concentrated to the microtubule organizing center (MTOC). When A431 cells were treated with BFA for 60 min and then processed for indirect immunofluorescence microscopy, syntaxin 7 was found to colocalize with internalized transferrin receptor in a tubular network that was concentrated in the perinuclear region (MTOC) (Fig. 8, C and D). Prolonged treatment with BFA (120 min) reduced the visibility of the tubular network but increased the compactness of the MTOC staining (Fig. 8, E and F). The BFA-induced redistribution of internalized transferrin receptor to the tubular network and subsequently to compact structures around the MTOC has been well documented (30–32). The similar BFA response of syntaxin 7 and transferrin receptor further suggests that syntaxin 7 is associated with the early endosome.

Nocodazole and Wortmannin Treatment Caused Redistribution of Syntaxin 7—It has been proposed previously that the perinuclear structures marked by the transferrin receptor represent recycling endosomes (described by Mellman and colleagues (33) as recycling vesicles) and these structures disperse upon treatment with the microtubule depolymerizing agent, nocodazole (33–36). Therefore, we examined the effect of nocodazole on the perinuclear vesicular structures (Fig. 9, A and B) which contain syntaxin 7 and the cell surface internalized transferrin receptor. As shown in Fig. 9 (panels C and D), nocodazole caused both syntaxin 7 and the transferrin receptor to disperse into smaller punctate structures throughout the cytosol. The colocalization between syntaxin 7 and transferrin receptor is perfect in these structures and thus show that syntaxin 7 is mostly enriched in the recycling endosomes. The treatment of cells with wortmannin, an inhibitor of phosphatidylinositol 3-kinases, has been proposed previously to cause recycling endosomes to redistribute to swollen vacuole-like structures (37). Wortmannin has been known to inhibit several
Fig. 9. Syntaxin 7 is redistributed by nuncadole and wortmannin treatment. A431 cells were incubated with monoclonal antibody against transferrin receptor for 30 min at 37°C and then either untreated (A and B) or treated with either nuncadole (C and D) or wortmannin (E and F) for 60 min. Cells were processed for indirect immunofluorescence microscopy to double-label syntaxin 7 (A, C, and E) and the internalized transferrin receptor (B, D, and F). Bar, 10 μm.

membrane-trafficking pathways including recycling from the late endosomal compartment to the trans-Golgi network (37), transport from the late endosomal compartment to the lysosome (37), the transport of activated platelet-derived growth factor receptor to the degradative compartment of the endocytic some (37), the transport of activated platelet-derived growth fac-

Conclusions—SNAREs are key molecules mediating docking and fusion of intracellular vesicular transport, and therefore identification of novel SNAREs is essential for the molecular dissection of the respective transport event. In this report, we have molecularly identified syntaxin 7, a novel protein and the first member of the syntaxin family that is localized predomin-

A Novel Syntaxin of the Early Endosome

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REFERENCES

1. Pryer, N. K., Wuestehube, J. J., and Schekman, R. (1992) Annu. Rev. Biochem. 61, 471–516
2. Rothman, J. E. (1994) Nature 372, 55–63
3. Rothman, J. E., and Wieland, F. T. (1996) Science 272, 227–234
4. Schekman, R., and Orci, L. (1996) Science 271, 1526–1532
5. Ferro-Novick, S., and John, R. (1994) Nature 370, 191–193
6. Rothman, J. E., and Warren, G. (1994) Curr. Biol. 4, 220–233
7. Scheller, R. H. (1995) Neuron 14, 893–897
8. Solhner, T., Whiteheart, S. W., Brunner, M., Erdjument-Bromage, H., Geromanos, S., Tempe, P., and Rothman, J. E. (1995) Nature 372, 318–324
9. Sudhof, T. C. (1995) Nature 373, 645–653
10. Bennett, M. K., Calakos, N., and Scheller, R. H. (1992) Science 257, 255–259
11. Bennett, M. K., Garcia-Arraras, J. E., Effernki, L. A., Peterson, K., Fleming, A. M., Haraka, C. D., and Scheller, R. H. (1993) Cell 74, 863–873
12. Low, S. H., Chapin, S. J., Weimbs, T., Konmues, L. G., Bennett, M. K., and Mostov, K. E. (1996) Mol. Biol. Cell 7, 2007–2018
13. Galasso, H. Y., Ghai, M., Makkus, P. N., Sheu, L., Bouquillon, A., Bennett, M. K., and Trimble, W. S. (1996) Mol. Biol. Cell 7, 2019–2027
14. Bock, J. B., Lin, R. C., and Scheller, R. H. (1996) J. Biol. Chem. 271, 17961–17965
15. Banfield, D. K., Lewis, M. J., Rabouille, C., Warren, G., and Pelham, H. R. B. (1994) J. Cell Biol. 127, 357–371
16. Bascher, C., Mattesone, J., and Balch, W. E. (1994) J. Biol. Chem. 269, 29093–29096
17. Wong, S. H., Low, S. H., and Hong, W. (1992) J. Cell Biol. 117, 245–258
18. Wong, S. H., and Hong, W. (1993) J. Biol. Chem. 268, 22853–22862
19. Lowe, S. L., Wong, S. H., and Hong, W. (1996) J. Cell Sci. 109, 209–220
20. Subramaniam, V. N. n, m, and Devau, R. B. Wong S. H., Lim, G. B., Chew, M., and Hong W. (1991) J. Biol. Chem. 267, 12016–12021
21. Lupas, A. (1996) Methods Enzymol. 266, 513–524
22. Lupas, A., Van Dyke, M., and Stock, J. (1991) Science 252, 1162–1164
23. Koza, M. (1986) Cell 44, 283–292
24. Spies, M. (1990) Biochemistry 29, 10018–10022
25. Graebe, L., Fatza, A., Drickamer, K., and Rodriguez-Boulan, E. (1990) J. Biol. Chem. 265, 1216–1224
26. Lodish, H. F. (1991) Trends Biochem. Sci. 16, 374–377
27. McMahon, H. T., and Sudhof, T. C. (1995) J. Biol. Chem. 270, 22123–22127
28. Woof, S. A., Park, J. E., and Brown, W. J. (1991) Cell 67, 591–600
29. Lippincott-Schwartz, J., Yuan, L. C., Tipper, C., Amherdt, M., Orci, L., and Klumper, R. D. (1991) Cell 67, 601–617
30. Klassen, R. D., Klassen, R. D., and Lippincott-Schwartz, J. (1999) J. Cell Biol. 126, 1017–1020
31. Wood, S. A., and Brown, W. J. (1992) J. Cell Biol. 119, 273–285
32. Whitney, J. A., Gomez, M., Sheff, D., Kreis, T. E., and Mellman, I. (1995) Cell 83, 703–713
33. Doro, E., Slujis, P. V. D., Galili, T., and Mellman, I. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 9505–9506
34. Tomiz, T. and Hollisheim, M. (1991) J. Cell Biol. 115, 655–653
35. Sakai, T., Yamashina, S., and Ohnishi, S. (1991) J. Biochem. (Tokyo) 109, 828–833
36. Mcgraw, T. E., Dunn, K. W., and Maxfield, F. R. (1993) J. Cell. Physiol. 155, 579–594
37. Reaves, B., Bright, N. A., Mulliek, B. M., and Luzio, J. P. (1996) J. Cell Sci. 109, 749–762
38. Joly, M., Kaulauskas, A., Fay, F. S., and Corvera, S. (1994) Science 263, 684–687
39. Li, G., D’Souza-Schorey, C., Barbieri, M. A., Roberts, R. L., Klippel, A., Hazeki, O., Ui, M., and Ebina, Y. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 9207–9211
40. Joly, M., Kaulauskas, A., Fay, F. S., and Corvera, S. (1994) Science 263, 684–687
41. Li, G., D’Souza-Schorey, C., Barbieri, M. A., Roberts, R. L., Klippel, A., Hazeki, O., Ui, M., and Ebina, Y. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 9207–9211
42. Jones, A. T., and Clague, M. J. (1995) Biochim. J. 311, 51–34
43. Clague, M., Thorpe, C., and Jones, A. T. (1995) FEBS Lett. 367, 272–274
44. Kasai, F., Taka, K., Tokuda, M., Hayashi, H., Komuro, S., Ishii, K., Okada, T., Hazeki, O., Ui, M., and Ebina, Y. (1993) Biochem. Biophys. Res. Commun. 195, 762–768
45. Hansen, S. H., Adess, A., and Casanueva, J. E. (1995) J. Biol. Chem. 270, 33824–33832
46. Shepherd, P. R., Soos, M. A., and Sidle, K. (1995) Biochem. Biophys. Res. Commun. 211, 535–539
47. Mayor, S., Presley, J. F., and Maxfield, F. R. (1993) J. Cell Biol. 121, 1257–1269
48. Bock, J. B., and Scheller, R. H. (1997) Nature 387, 133–135
49. Subramaniam, V. N., Peter, F., Philip, R., Wong S. H., and Hong W. (1996) Science 273, 1161–1163