Pergamon

0028-3908(95)00113-1

Neuropharmacology, Vol. 34, No. 11, pp. 1351–1360, 1995

0028-3908/95 $29.00 + 0.00

Review

v- and t-SNAREs in Neuronal Exocytosis: A Need for Additional Components to Define Sites of Release

T. GALLI, E. P. GARCIA, O. MUNDIGL, T. J. CHILCOTE and P. DE CAMILLI*

Department of Cell Biology and Howard Hughes Medical Institute, Yale University School of Medicine, Boyer Center For Molecular Medicine, 295 Congress Avenue, New Haven, CT 06510, U.S.A.

(Accepted 12 July 1995)

Summary—Synaptic vesicle recycling is a specialized form of membrane recycling which takes place in all cells between early endosomes and the plasmalemma. Synaptic vesicles exocytosis is highly regulated and occurs only at presynaptic active zones. In contrast, exocytosis of endosome-derived vesicles of the housekeeping recycling pathway takes place constitutively and throughout the cell surface. Since v- and t-SNAREs play a key role in membrane interactions leading to fusion, unique v- and t-SNAREs may be implicated in synaptic vesicle exocytosis. It was found, however, that the same v-SNAREs of the synaptobrevin family are found both on synaptic vesicles and on endosome-derived vesicles which undergo constitutive fusion. Likewise, t-SNAREs which act as plasmalemmal receptors for synaptic vesicles are not restricted to synaptic active zones. Thus, v- and t-SNAREs interactions may define which organelles can fuse with the plasmalemma, but require additional components to define properties of the exocytotic reaction which are specific for distinct classes of secretory organelles.

Keywords—Exocytosis, v-SNAREs, t-SNAREs.

The function of eukaryotic cells involves a continuous interaction among membranes which delimit intracellular subcompartments. In order to mantain the distinct characteristics of the compartments, docking and fusion of membranes occurs with a high specificity which is controlled at a minimum of three major levels. First, membranes destined to fuse are endowed with the property of recognizing each other, so that only these interactions are permitted. Second, the efficiency of fusion between cognate membranes undergoes regulation to account for plasticity of cellular functions. Third, the site where a given membrane can fuse with another may be topologically restricted. A good example of these three levels of control apply to the case of synaptic vesicle exocytosis. Synaptic vesicles, like other secretory vesicles, fuse with the plasmalemma, but not with other intracellular membrane compartments. Their rate of exocytosis increases by several orders of magnitude in response to a stimulus and is therefore highly regulated. Their fusion takes place selectively at the presynaptic plasmalemma and is therefore highly controlled in space.

A large body of information accumulated over the last few years from a variety of experimental systems has provided a first insight into some of the mechanisms which mediate and regulate membrane interactions leading to fusion (Rothman, 1994; Bennett and Scheller, 1993; Südhof *et al.*, 1993). This information has led to the formulation of the SNARE hypothesis (Söllner *et al.*, 1993). This hypothesis predicts that membrane docking and fusion requires the presence on the partner membranes of cognate proteins, referred to as SNAREs which recognize each other in a lock and key fashion (Rothman, 1994). Formation of the SNARE complex may then progress to the fusion reaction in the presence of appropriate additional factors, although the molecular mechanism leading to fusion remains to be elucidated (Burgoyne and Morgan, 1995). The SNARE hypothesis is supported by the convergence of a large number of studies which preceded and followed its formulation, including genetic studies in yeast, studies of membrane fusion in cell free systems and studies on the mechanism of action of clostridial neurotoxins [for review see

*To whom correspondence should be addressed.

Söllner and Rothman (1994), Ferro-Novick and Jahn (1994) and Schiavo *et al.* (1994)]. The precise level of specificity in membrane interactions which is accounted for by v- and t-SNAREs, however, is still unclear.

v- and t-SNARE appear to provide a general recognition system which defines sets of membranes which can interact with each other to accomplish vectorial transport from one compartment to another in the secretory and endocytic pathways. More specifically, they have been implicated in fusion reactions at the boundaries between the four major compartments of these pathways: the ER, the Golgi complex, the lysosomal system and the plasmalemma. Vesicles generated from one of these compartments carry a v-SNARE which interacts with a cognate t-SNARE localized on the acceptor membrane. v- and t-SNAREs implicated in each of these classes of fusion have been identified in yeast (Bennett and Scheller, 1993; Ferro-Novick and Jahn, 1994). Related cognate v- and t-SNAREs have been found on some of the corresponding membrane compartments in mammalian cells. The best characterized mammalian SNAREs are the ones which participate in exocytosis. These are the v-SNAREs of the synaptobrevin family (synaptobrevin 1, synaptobrevin 2 and cellubrevin) and the plasmamembrane t-SNAREs: SNAP-25 and the syntaxins. SNAP-25 and syntaxin 1 are neuronal t-SNAREs (Ferro-Novick and Jahn, 1994). Several other syntaxins are expressed outside the nervous system (Bennett *et al.*, 1993), while non-neuronal isoforms of SNAP-25 remain to be identified. Mammalian SNAREs which participate at other stations of the secretory and endocytotic pathways have also been described (Cain *et al.*, 1992; Jo *et al.*, 1995) [including a Golgi specific member of the syntaxin family (Bennett *et al.*, 1993)] and the list of known mammalian SNAREs is rapidly growing. The elucidation of the localization of v- and t-SNARE is important for a precise understanding of their role in defining the specificity of membrane interactions. The following sections review evidence indicating that the localization of v- and t-SNARE which participate in exocytosis at the synapse may account for their selective involvement in exocytosis, but not for additional levels of temporal and spatial specificity.

SYNAPTOBREVIN 1, 2 AND CELLUBREVIN HAVE DIFFERENT TISSUE DISTRIBUTION BUT SIMILAR SUBCELLULAR LOCALIZATIONS

Mammalian cells contain a multiplicity of vesicular carriers destined to fuse with the plasmalemma (De Camilli, 1993). These include Golgi complex-derived organelles and organelles generated at the cell periphery by membrane recycling from the plasmalemma. Examples of the first class of organelles are vesicles of the constitutive pathway, and the secretory granules containing peptide hormones in endocrine cells and neurons. Examples of the second class of organelles are endosome-derived vesicles which recycle plasmalemma receptors, synaptic vesicles, and the vesicles which contain glucose transporters in a variety of insulin-sensitive cells. The fate of each of these organelles is to fuse with the plasmalemma, but each of them fuse with distinct spatial and temporal specificity. For example, in neurons, synaptic vesicles fuse only at the presynaptic plasmalemma, while peptide-containing secretory granules (referred to as large dense-core vesicles) undergo exocytosis primarily in distal axonal segments but not selectively at active zones (De Camilli and Jahn, 1990). Vesicular carriers of the constitutive secretory pathway and of the constitutively recycling pathway have been less thoroughly characterized in neurons but do not appear to fuse at special sites of the cell surface. Furthermore, exocytosis of these various organelles is differentially regulated. Only exocytosis of synaptic vesicles and large dense-core vesicles is triggered by action potentials, and large dense-core vesicle exocytosis is preferentially triggered by trains of action potentials (De Camilli and Jahn, 1990).

An attractive possibility is that distinct vesicular carriers use different v-SNAREs and that each v-SNARE participates in defining the properties of the exocytotic process. Synaptobrevin 1 and 2 are predominantly expressed in neurons (Baumert *et al.*, 1989; Elferink *et al.*, 1989; Chilcote *et al.*, 1995). In fully differentiated neurons the bulk of synaptobrevin 1 and 2 are localized on synaptic vesicles in nerve terminals (Baumert *et al.*, 1989). Cellubrevin has a widespread distribution in non-neuronal cells while it is undetectable in neurons (McMahon *et al.*, 1993; Galli *et al.*, 1994; Chilcote *et al.*, 1995). In fibroblastic cells, where its localization has been investigated, it is primarily localized on vesicles which constitutively recycle plasmalemma receptors (McMahon *et al.*, 1993; Galli *et al.*, 1994). These findings had raised the possibility that the different patterns of expression of synaptobrevin 1 and 2 and cellubrevin are directly related to the different properties of the exocytotic events in which they participate. However, recent studies carried out in neuroendocrine cells speak against this hypothesis (Chilcote *et al.*, 1995).

PC12 cells were found to express all three synaptobrevin isoforms (Chilcote *et al.*, 1995). When exposed to NGF, PC12 cells extend axonal-like neurites with numerous varicosities where synaptic-like microvesicles [the synaptic vesicle-like organelles of PC12 cells (Thomas-Reetz and De Camilli, 1994)] and large dense-core veiscles are greatly enriched (Tischler and Greene, 1978). These processes, like axons of neurons (Cameron *et al.*, 1991; Mundigl *et al.*, 1993), exclude the transferrin receptor (Chilcote *et al.*, 1995). Thus, if cellubrevin and the two synaptobrevins were the v-SNAREs of two distinct specialized types of vesicles, cellubrevin should either be absent from the axon-like processes, or should be excluded from synaptic-like microvesicles.

In contrast, cellubrevin and the two synaptobrevins

were found to be colocalized in PC12 cells. Cellubrevin is present on synaptic-like microvesicles and the same synaptobrevin molecules are present on both synaptic-like microvesicles and on large dense-core vesicles, although at much lower concentration on the latter organelles (Chilcote *et al.*, 1995). In agreement with these results, not only synaptobrevin 1 and 2, but also cellubrevin, were found to interact biochemically with the neuronal t-SNAREs syntaxin 1 and SNAP-25 (Hayashi *et al.*, 1994; Chilcote *et al.*, 1995; McMahon and Südhof, 1995). The colocalization and similarity of function of the three members of the synaptobrevin family is further demonstrated by the blocking effect of tetanus toxin on exocytosis of synaptic vesicles, large dense-core vesicles and endosome-derived constitutive recycling vesicles (Schiavo *et al.*, 1992; Ahnert-Hilger and Weller, 1993; Galli *et al.*, 1994; Chilcote *et al.*, 1995).

These results are further corroborated by the finding that synaptobrevin 1 and 2, originally reported to be neuron-specific proteins, have now also been detected at low levels in a variety of non-neuroendocrine cells (Ralston *et al.*, 1994). In adipocytes, glut 4-containing organelles are positive for both synaptobrevin 2 and cellubrevin, indicating that these v-SNAREs may have an overlapping function also in non-neuronal cells (Volchuk *et al.*, 1995).

In conclusion, the three known v-SNAREs of the synaptobrevin family do not appear to have a primary role in defining the properties of exocytosis of the vesicular carrier on which they reside. We note however, that following tetanus toxin-mediated cleavage of the synaptobrevins in developing neurons, axonal processes continue to grow, indicating that other v-SNAREs insensitive to the toxin may be expressed by neurons (S. Catsicas, personal communication).

T-SNARES ARE NOT RESTRICTED TO SITES OF EXOCYTOSIS

One way by which sites of exocytosis could be defined is a selective localization of specific t-SNAREs at certain sites of the plasmalemma. In the case of synaptic vesicles, their exocytosis at the active zones could correlate with the presence of the cognate t-SNAREs, SNAP-25 and syntaxin 1, only at the presynaptic plasmalemma. Contrary to these predictions, immunocytochemical and biochemical studies carried out on frozen sections of rat brain have demonstrated that both t-SNAREs are present along the entire axolemma, including regions surrounded

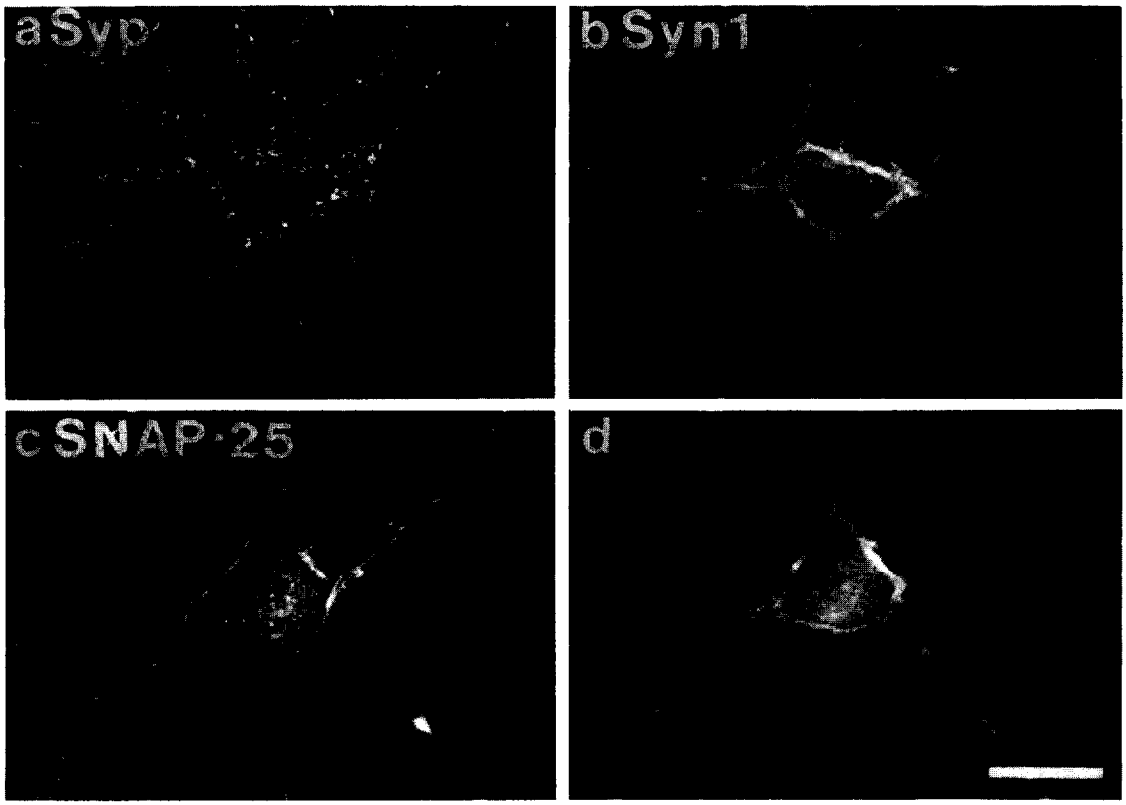

Fig. 1. Widespread distribution of t-SNAREs on the axonal plasmalemma of hippocampal neurons in primary culture after synapse formation. Double immunofluorescence of mature hippocampal neurons in primary culture stained for synaptophysin (a) and syntaxin 1 (b) and for SNAP-25 (c) and syntaxin 1 (d). Synaptophysin immunoreactivity has the typical presynaptic nerve terminal pattern while the t-SNAREs syntaxin 1 and SNAP-25 have a widespread localization all along the plasma membrane, primarily the axonal plasmamembrane. Bar, 36.6 μm.

by the myelin sheath (Garcia *et al.*, 1995). Further support to this conclusion is provided by studies on hippocampal neurons in primary culture. As shown by the double immunofluorescence images of Fig. 1, syntaxin 1 immunoreactivity decorates the whole axonal arbour [Fig. 1 (b,d)] with no obvious concentration at synaptic sites, which are indicated by the presence of synaptophysin immunoreactivity [Fig. 1 (a)]. The distribution of syntaxin immunoreactivity, in turn, is identical to that of SNAP-25 immunoreactivity [Fig. 1 (c)]. An even more striking example of the non-restricted distribution of syntaxin 1 and SNAP-25 at the regions of the axonal surface where synaptic vesicle exocytosis takes place, is the localization of these two proteins in neurons developing in isolation. Figure 2 (a) shows that syntaxin 1 is localized both in dendrites and axons at a developmental stage when synaptic vesicles [Fig. 2 (b)] are already primarily concentrated in axons. In the axons of these young neurons syntaxin 1 is present along the entire axolemma, including the axolemma of growth cone filopodia [inset of Fig. 2 (a) and (c)], a cell region from which synaptic vesicles are excluded [inset of Fig. 2 (b) and (d)] (Kraszewski *et al.*, 1995). Both in dendrites and in axons, the localization of syntaxin 1 [Fig. 2 (e)] is nearly identical to the localization of SNAP-25 [Fig. 2 (f)].

These results obtained in neurons are in good agreement with results obtained in yeast. In these unicellular organisms exocytosis is primarily restricted to a well defined region of the cell surface within the bud (Novick *et al.*, 1980). Yet, both SSO1/2 (yeast homolog of syntaxin) and SEC9 (yeast homolog of SNAP-25) are not restricted to the bud but instead are localized throughout the yeast plasma membrane (Brennwald *et al.*, 1994).

In conclusion, the t-SNAREs involved in exocytosis of synaptic vesicles are localized along the entire plasma membrane. Therefore they cannot be responsible for defining sites of exocytosis of synaptic vesicles or secretory granules.

COLOCALIZATION OF NEURONAL SEC1 WITH SYNTAXIN 1 THROUGHOUT THE AXON

Genetic studies in yeast have demonstrated that several other genes, in addition to the SNAREs, are required for membrane fusion events including exocytosis. Some of these genes may contribute an additional level of specificity to the docking and fusion machinery. One yeast gene which interacts with the yeast syntaxin genes (SSO genes) is Sec1. The family of Sec1 proteins in yeast comprises several members which function at distinct stations of the secretory pathway. These include Sly1 (Ossig *et al.*, 1991; Dascher *et al.*, 1991), which participates in ER to Golgi transport and Slp1 (Wada *et al.*, 1990) and Vsp45 (Piper *et al.*, 1994; Cowles *et al.*, 1994) which participate in Golgi to vacuole transport. Homologues of Sec1 have been identified in a variety of species and found to have a general role in exocytosis. In mammals, alternatively spliced neuron-specific isoforms (Hata *et al.*, 1993; Garcia *et al.*, 1994; Pevsner *et al.*, 1994b; Garcia *et al.*, 1995) and isoforms with widespread tissue distribution (Hodel *et al.*, 1994; Tellam *et al.*, 1995) have been identified. Additionally, biochemical studies carried out with mammalian proteins have demonstrated a direct biochemical interaction between Sec1 family members and syntaxin family members. Studies in *C. elegans* (Hosono *et al.*, 1992) and *Drosophila* (Harrison *et al.*, 1994; Schulze *et al.*, 1994) have shown that neuronal Sec1 (Unc18) participates in synaptic vesicle exocytosis. Accordingly, mammalian neuronal Sec1 (referred to as Munc18, rbSec1 and N-Sec1), binds syntaxin 1. Immunofluorescence staining of frozen sections of rat brain demonstrated a widespread localization of rbSec1 throughout the axons of most neurons, with no selective accumulation in axon terminals (Garcia *et al.*, 1995). Similar results were obtained in hippocampal neurons in primary culture, where, at the light microscopic level of resolution, the distribution of rbSec1 [Fig. 3 (d)] was very similar to that of SNAP-25 [Fig. 3 (b)] and syntaxin 1 [cf. Figs. 1 (c) with 3 (d)] and very different from the distribution of the synaptic vesicle marker synaptobrevin 2 [Fig. 3 (a) and (c)].

The precise mechanism by which the interaction of rbSec1 with syntaxin 1 control exocytosis remains to be elucidated. The high affinity interaction between rbSec1 and syntaxin 1 was found to inhibit the interaction of syntaxin 1 with synaptobrevin and SNAP-25 (Pevsner *et al.*, 1994a), suggesting that rbSec1 may serve as negative clamp to formation of the SNARE complex, and therefore to fusion (Schulze *et al.*, 1994; Pevsner *et al.*, 1994a). However, genetic data in yeast have shown that the function of Sec1 is essential for fusion (Novick *et al.*, 1980; Aalto *et al.*, 1993).

Electron microscopy and subcellular fractionation studies demonstrated presence of soluble and membrane bound pools of rbSec1 but failed to demonstrated the occurrence of a significant pool of rbSec1 in a complex with syntaxin 1. This was in contrast to the recovery of a large fraction of syntaxin 1 in association with SNAP-25 or with both SNAP-25 and synaptobrevin (Garcia *et al.*, 1995). Thus, the interaction of rbSec1 with syntaxin may be very transient *in situ*. Such interaction may be needed to make syntaxin competent for SNARE complex formation, rather than to inhibit its formation. Irrespective of whether rbSec1 has an inhibitory or facilitatory role in fusion, clearly the localization of rbSec1 throughout the axon speaks against a major role of this protein in defining sites where synaptic vesicle are allowed to fuse. The recent demonstration that rbSec1 may regulate the function of Cdk5 (Shetty *et al.*, 1995), a protein kinase which phosphorylates neurofilament proteins and which is present throughout the axons

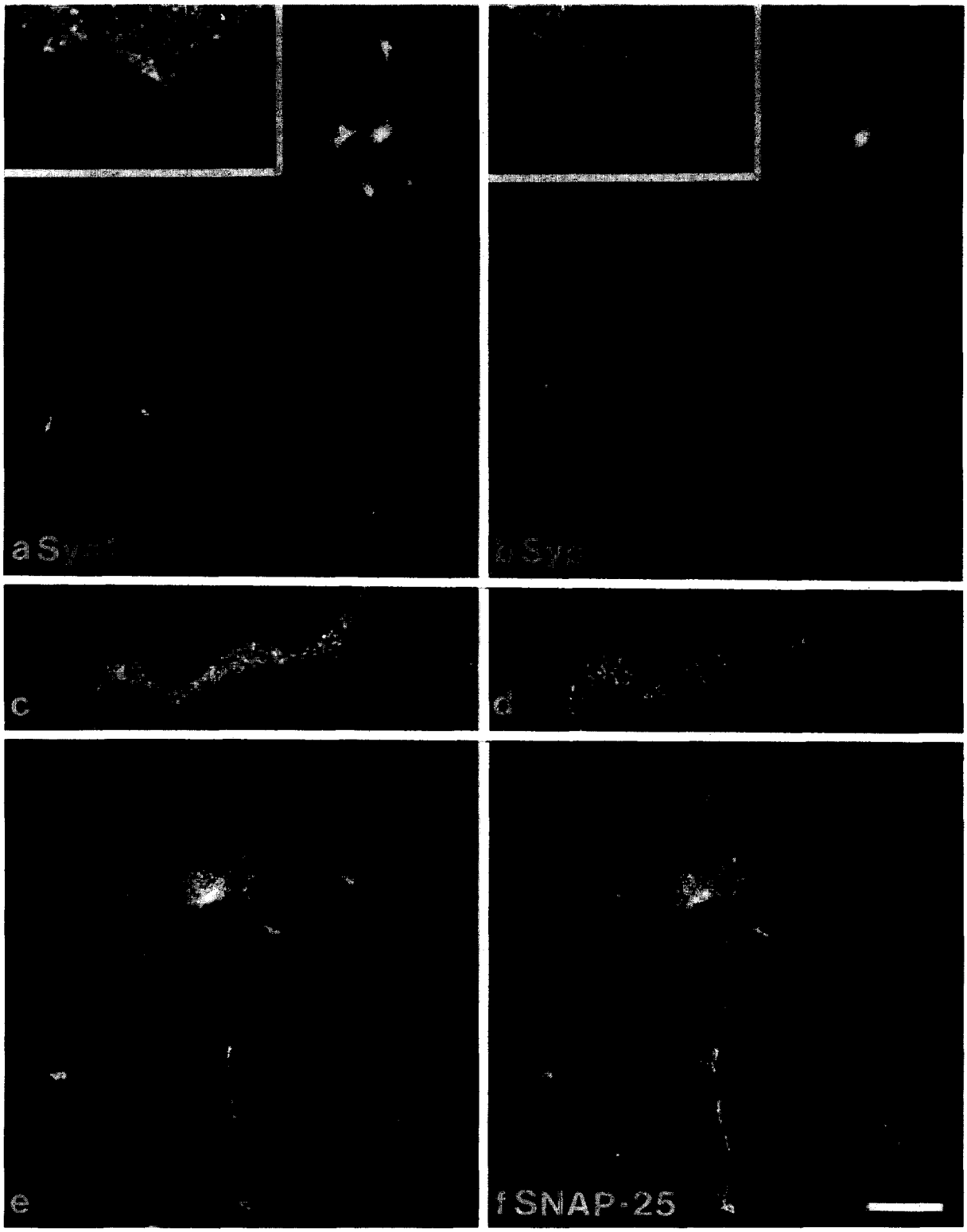

Fig. 2. Widespread distribution of t-SNAREs on the plasmalemma of hippocampal neurons developing in isolation in primary culture. Double immunofluorescence of neurons stained for syntaxin 1 (a, c, e), synaptophysin (b, d) or SNAP-25 (f). Syntaxin 1 is localized both in dendrites and axons while synaptophysin immunoreactivity is concentrated in axons. In axons syntaxin 1 is present along the entire axolemma, including the axolemma of growth cone filopodia (2a, inset and 2c), a cell region from which synaptic vesicles are excluded (2b, inset and 2d). Both in dendrites and in axons, the localization of syntaxin 1 is nearly identical to the localization of SNAP25. Bar, 7.1 μm (insets a, b), 37.5 μm (a, b), 14.4 μm (c, d), 27.3 μm (e, f).

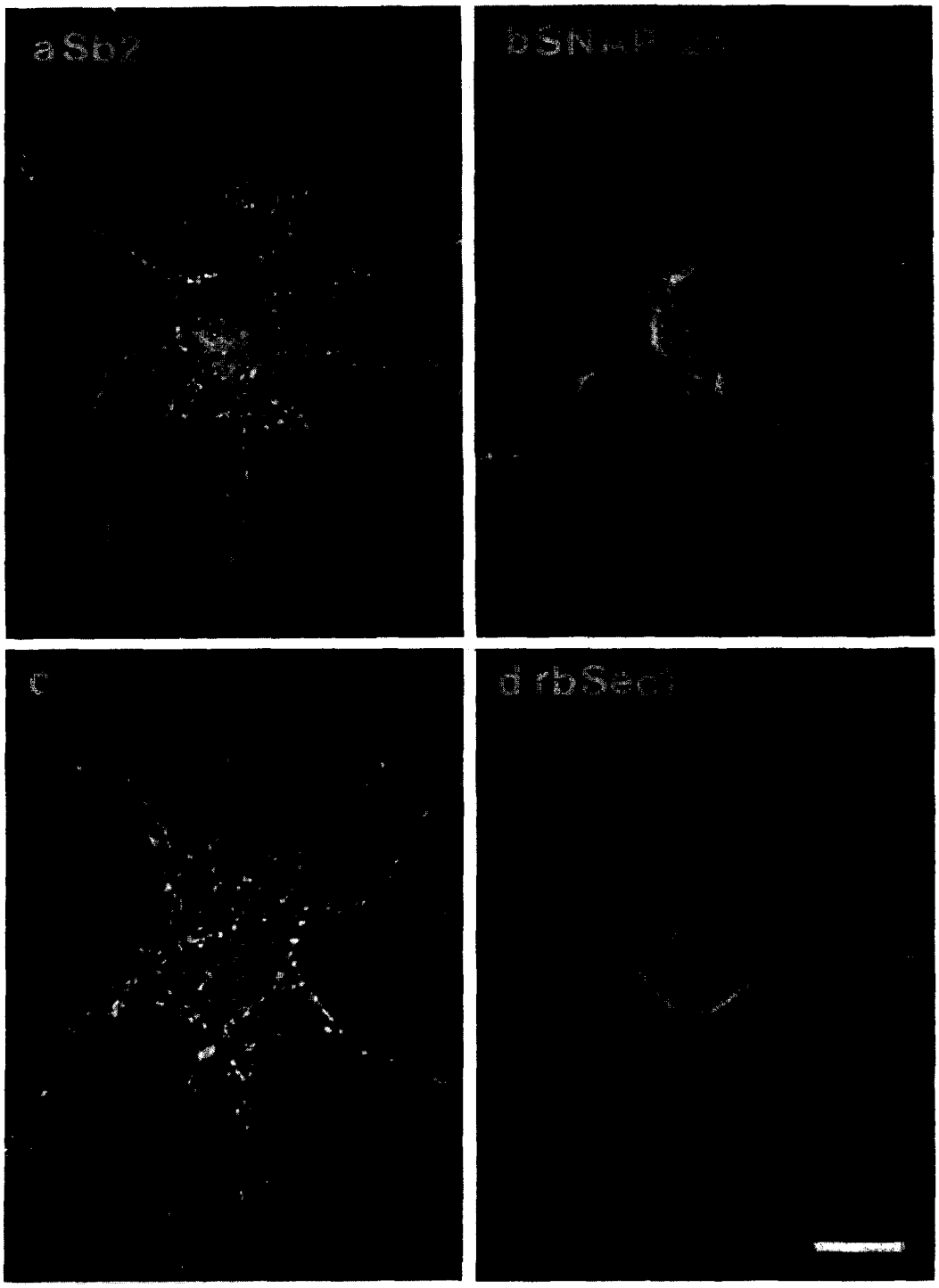

Fig. 3. Widespread distribution of rbSec1 along the axons of hippocampal neurons in primary culture after synapse formation. Double immunofluorescence of neurons stained for synaptobrevin 2 (a, c), SNAP-25 (b) or rbSec1 (d). Synaptobrevin 2 immunoreactivity has the typical presynaptic nerve terminal pattern while SNAP-25 and rbSec1 have a similar widespread localization along the entire axon. Bar, 19.8 μm.

(Shetty *et al.*, 1995), raise the possibility that rbSec1 may have multiple functions.

COMBINATORIAL MATCHES OF SNARES AND ACCESSORY PROTEINS

Studies with protein from cell and tissue extracts, as well as with recombinant proteins, have demonstrated that the interaction between synaptobrevin and syntaxin isoforms as well as of syntaxin isoforms with rbSec1 are both promiscuous and specific. RbSec1 interacts with some but not all known syntaxins (Pevsner *et al.*, 1994a). The same applies to synaptobrevin 1 and 2 (Calakos *et al.*, 1994). These combinatorial matches may contribute

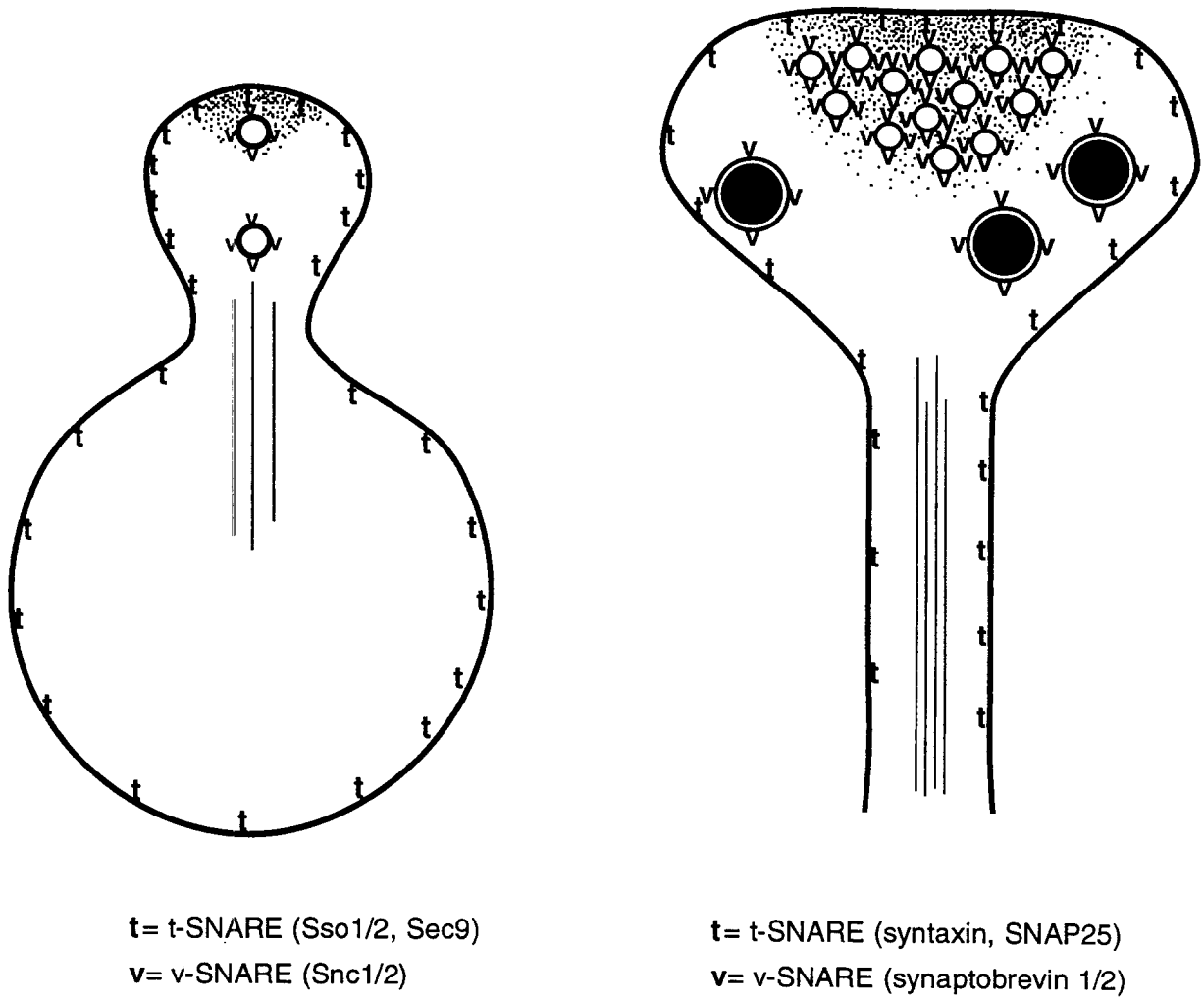

Fig. 4. v- and t-SNAREs are not responsible for the specificity of the site of release in yeast and in neurons. Model depicting the widespread localization of t-SNAREs all along the plasma membrane of a yeast cell and the axon of a neuron, the presence of multiple isoforms of v-SNAREs on the same secretory vesicles (in yeast and in neurons) and of identical v-SNAREs both on synaptic vesicles and large dense-core granules (in the neuron). The site of exocytosis of secretory vesicles in yeast and synaptic vesicle in neurons is highly specific and this specificity probably results from the presence of a specific set of proteins of the cytomatrix (dots around the vesicles).

specificity to fusion reactions. However, this hypothesis awaits experimental support from localization studies on various members of the syntaxin family. Given the information already available on syntaxin 1, which is not restricted to active zones of nerve terminals, at most the distribution of other syntaxin isoforms may define general cell regions where exocytosis may occur, +but not precise sites.

RAB PROTEIN AND PROTEINS OF THE PERIPHERAL CYTOMATRIX

One mechanism by which the level of specificity accounted for by the SNAREs could be further fine-tuned, is the 'activation' of SNAREs only at certain cellular sites. Rab proteins may have such a function. Studies in yeast have suggested that Rab proteins may have a catalytic role on the formation of SNARE complex (Lian *et al.*, 1994; Søgaard *et al.*, 1994). A regulatory role of Rab3 on the formation of the synaptic SNARE complex is suggested by studies on neuroendocrine cells and on mice lacking a functional Rab3a gene (Holz *et al.*, 1994; Johannes *et al.*, 1994; Geppert *et al.*, 1994).

Mechanisms may exist to concentrate Rab3a in the cytomatrix of the nerve terminal. Such a mechanism, in turn, may be responsible for binding of Rab3 only to synaptic vesicles in this cellular region, thereby allowing formation of a synaptic SNARE complex only in proximity of the presynaptic plasmalemma. Recruitment of rabphilin to the vesicle surface may be part of this process (Geppert *et al.*, 1994; Li *et al.*, 1994). An interaction between Rab3 proteins and the peripheral cytomatrix is further supported by the close similarity between Rabs and other Rab-like GTPases, which function in the control of the peripheral cytoskeleton (Nobes and Hall, 1995).

Clearly, proteins of the cytoskeleton and of the peripheral cytomatrix must play an important role in defining sites of exocytosis. In the case of synaptic vesicles, one protein with this function is synapsin. Strong evidence indicates that synapsin acts as a link between the vesicle surface and the actin cytoskeleton and that it plays a direct role in the clustering of synaptic vesicles at the presynaptic plasmalemma (De Camilli *et al.*, 1983; Greengard *et al.*, 1994). In addition to synapsin I, many other proteins of the peripheral cell cytoskeleton or proteins which regulate the function of the peripheral cytoskeleton may play positive and negative roles on exocytosis (Cheek and Burgoyne, 1992; Martin, 1994; Vitale *et al.*, 1995; Muallem *et al.*, 1995). It is of interest, in this context, that rabphilin was reported to interact with adducin, a protein of the peripheral submembranous cytoskeleton (Miyazaki *et al.*, 1994). An interesting avenue for future investigation is the elucidation of the functional link between proteins of the cell periphery and the SNAREs.

Concluding remarks

Clearly, v- and t-SNAREs play a fundamental role in membrane recognition and fusion, but their role must be complemented by many other factors to account for the temporal and spatial specificity typical of fusion reactions in the living cell. v- and t-SNARE combinations may generally define compartments which can become continuous with each other and forbid fusion of membranes which delimit incompatible compartments. In the case of neuronal exocytosis, the presence of potential docking sites throughout the plasmalemma may allow for rapid plastic changes when fusion of post-Golgi vesicles must be redirected from one site to another. It is also possible that v- and t-SNAREs, in addition to play a fundamental role in docking and fusion, may also participate in other key and general properties of the host membrane.

REFERENCES

Aalto M. K., Ronne H. and Keranen S. (1993) Yeast syntaxins Sso1p and Sso2p belong to a family of related membrane proteins that function in vesicular transport. *EMBO J.* **12:** 4095–4104.

Ahnert-Hilger G. and Weller U. (1993) Comparison of the intracellular effects of clostridial neurotoxins on exocytosis from streptolysin O-permeabilized rat pheochromocytoma (PC 12) and bovine adrenal chromaffin cells. *Neuroscience* **53:** 547–552.

Baumert M., Maycox P. R., Navone F., De Camilli P. and Jahn R. (1989) Synaptobrevin: an integral membrane protein of 18,000 daltons present in small synaptic vesicle of rat brain. *EMBO J.* **8:** 379–384.

Bennett M. K. and Scheller R. H. (1993) The molecular machinery for secretion is conserved from yeast to neurons. *Proc. Natn. Acad. Sci. U.S.A.* **90:** 2559–2563.

Bennett M. K., Garcia-Arraras J. E., Elferink L. A., Peterson K., Fleming A. M., Hazuka C. D. and Scheller R. H. (1993) The syntaxin family of vesicular transport receptors. *Cell* **74:** 863–873.

Brennwald P., Kearns B., Champion K., Keränen S., Bankaitis V. and Novick P. (1994) Sec9 is a SNAP-25-like component of a yeast SNARE complex that may be the effector of Sec4 function in exocytosis. *Cell* **79:** 245–258.

Burgoyne R. D. and Morgan A. (1995) Ca^{2+} and secretory-vesicle dynamics. *TINS* **18:** 191–196.

Cain C. C., Trimble W. S. and Lienhard G. E. (1992) Members of the VAMP family of synaptic vesicle proteins are components of glucose transporter-containing vesicles from rat adipocytes. *J. Biol. Chem.* **267:** 11,681–11,684.

Calakos N., Bennett M. K., Peterson K. E. and Scheller R. H. (1994) Protein-protein interactions contributing to the specificity of intracellular vesicular trafficking. *Science* **263:** 1146–1149.

Cameron P. L., Südhof T. C., Jahn R. and De Camilli P. (1991) Colocalization of Synaptophysin with transferrin receptors: implications for synaptic vesicle biogenesis. *J. Cell Biol.* **115:** 151–164.

Cheek T. R. and Burgoyne R. D. (1992) Cytoskeleton in secretion and neurotransmitter release. In: *The Neuronal Cytoskeleton* (Burgoyne, R. D., Ed.) pp. 309–325. Wiley-Liss, New-York.

Chilcote T. J., Galli T., Mundigl O., Edelmann L., McPherson P. S., Takei K. and De Camilli P. (1995) Cellubrevin and synaptobrevins: similar subcellular localization and biochemical properties in PC12 cells. *J. Cell Biol.* **129:** 219–231.

Cowles C. R., Emr S. D. and Havazdovsky B. F. (1994) Mutations in the *VPS45* gene, a *SEC1* homologue, results in vacuolar protein sorting defects and accumulation of membrane vesicles. *J. Cell Sci.* **107:** 3449–3459.

Dascher C., Ossig R., Gallwitz D. and Schmitt H. D. (1991) Identification and structure of four yeast genes (SLY) that are able to suppress the functional loss of YPT1, a member of the RAS superfamily. *Molec. Cell. Biol.* **11:** 872–885.

De Camilli P. (1993) Secretion: exocytosis goes with a SNAP. *Nature* **364:** 387–388.

De Camilli P. and Jahn R. (1990) Pathways to regulated exocytosis in neurons. *A. Rev. Physiol.* **52:** 625–645.

De Camilli P., Cameron R. and Greengard P. (1983) Synapsin I (protein I) a nerve terminal specific phosphoprotein. I. Its general distribution in synapses of the central and peripheral nervous system demonstrated by immunofluorescence in frozen and plastic sections. *J. Cell Biol.* **96:** 1337–1354.

Elferink L. A., Trimble W. S. and Scheller R. H. (1989) Two vesicle-associated membrane protein genes are differentially expressed in the rat central nervous system. *J. Biol. Chem.* **264:** 11,061–11,064.

Ferro-Novick, S. and Jahn R. (1994) Vesicle fusion from yeast to man. *Nature* **370:** 191–193.

Galli T., Chilcote T., Mundigl O., Binz T., Niemann H. and De Camilli P. (1994) Tetanus toxin-mediated cleavage of cellubrevin impairs exocytosis of transferrin receptor-containing vesicles in CHO cells. *J. Cell Biol.* **125:** 1015–1024.

Garcia E. P., Gatti E., Butler M., Burton J. and De Camilli P. (1994) A rat brain Sec1 homologue related to Rop and UNC18 interacts with syntaxin. *Proc. Natn. Acad. Sci. U.S.A.* **91:** 2003–2007.

Garcia E. P., McPherson P. S., Chilcote T. J., Takei K. and De Camilli P. (1995) rbSec1A and B colocalize with syntaxin 1

and SNAP-25 throughout the axon, but are not in a stable complex with syntaxin. *J. Cell Biol.* **129:** 105–120.

Geppert M., Bolshakov V. Y., Siegelbaum S. A., Takei K., De Camilli P., Hammer R. E. and Südhof T. C. (1994) The role of Rab3a in neurotransmitter release. *Nature* **369:** 493–497.

Greengard P., Benfenati F. and Valtorta F. (1994) Synapsin I, an actin-binding protein regulating synaptic vesicle traffic in the nerve terminal. *Adv. Second Messenger Phosphoprotein Res.* **29:** 31–45.

Harrison S. D., Broadie K., van de Goor J. and Rubin G. M. (1994) Mutations in the Drosophila Rop gene suggest a function in general secretion and synaptic transmission. *Neuron* **13:** 555–566.

Hata Y., Slaughter C. A. and Südhof T. (1993) Synaptic vesicle fusion complex contains unc-18 homologue bound to syntaxin. *Nature* **366:** 347–351.

Hayashi T., McMahon H., Yamasaki S., Binz T., Hata Y., Südhof T. C. and Niemann H. (1994) Synaptic vesicle membrane fusion complex: action of clostridial neurotoxins on assembly. *EMBO J.* **13:** 5051–5061.

Hodel A., Schafer T., Gerosa D. and Burger M. M. (1994) In chromaffin cells, the mammalian Sec1p homologue is a syntaxin 1A-binding protein associated with chromaffin granules. *J. Biol. Chem.* **269:** 8623–8626.

Holz R. W., Brondyk W. H., Senter R. A., Kuizon L. and Macara I. G. (1994) Evidence for the involvement of Rab3A in Ca^{2+}-dependent exocytosis from adrenal chromaffin cells. *J. Biol. Chem.* **269:** 10,229–10,234.

Hosono R., Hekimi S., Kamiya Y., Sassa T., Murakami S., Nishiwaki K., Miwa J., Taketo A. and Kodaira K. I. (1992) The unc-18 gene encodes a novel protein affecting the kinetics of actylcholine metabolism in the nematode *Caenorhabditis elegans*. *J. Neurochem.* **58:** 1517–1525.

Jo I., Harris H. W., Amendt-Raduege A. M., Majewski R. R. and Hammond T. G. (1995) Rat kidney papilla contains abundant synaptobrevin protein that participates in the fusion of antidiuretic hormone-regulated water channel-containing endosomes *in vitro*. *Proc. Natn. Acad. Sci. U.S.A.* **92:** 1876–1880.

Johannes L., Lledo P.-M., Roa M., Vincent J.-D., Henry J.-P. and Darchen F. (1994) The GTPase Rab3a negatively controls calcium-dependent exocytosis in neuroendocrine cells. *EMBO J.* **13:** 2029–2037.

Kraszewski K., Mundigl O., Daniell L., Verderio C., Matteoli M. and De Camilli P. (1995) Synaptic vesicle dynamics in living cultured hippocampal neurons visualized with Cy3-conjugated antibodies directed against the lumenal domain of synaptotagmin. *J. Neurosci.* **15:** 4328–4342.

Li C., Takei K., Geppert M., Daniell L., Stenius K., Chapman E. R., Jahn R., De Camilli P. and Südhof T. C. (1994) Synaptic targeting of rabphilin-3A, a synaptic vesicle Ca^{2+}/phospholipid-binding protein, depends on rab3A/3C. *Neuron* **13:** 885–898.

Lian J. P., Stone S., Jiang Y., Lyons P. and Ferro-Novick S. (1994) Ypt1p implicated in v-SNARE activation. *Nature* **372:** 698–701.

Martin T. F. J. (1994) The molecular machinery for fast and slow neurosecretion. *Curr. Opin. Neurobiol.* **4:** 626–632.

McMahon H. T. and Südhof T. C. (1995) Synaptic core complex of synaptobrevin, syntaxin, and SNAP25 forms high affinity -SNAP binding site. *J. Biol. Chem.* **270:** 2213–2217.

McMahon H. T., Ushkaryov Y. A., Edelmann L., Link E., Binz T., Niemann H., Jahn R. and Südhof T. C. (1993) Cellubrevin is a ubiquitous tetanus-toxin substrate homologous to a putative synaptic vesicle fusion protein. *Nature* **364:** 346–349.

Miyazaki M., Shirataki H., Kohno H., Kaibuchi K., Tsugita A. and Takai Y. (1994) Identification as β-adducin of a protein interacting with Rabphilin-3A in the presence of Ca^{2+} and phosphatidylserine. *Biochem. Biophys. Res. Commun.* **205:** 460–466.

Muallem S., Kwiatkowska K., Xu X. and Yin H. L. (1995) Actin filament disassembly is a sufficient final trigger for exocytosis in nonexcitable cells. *J. Cell Biol.* **128:** 589–598.

Mundigl O., Matteoli M., Daniell L., Thomas-Reetz A., Metcalf A., Jahn R. and De Camilli P. (1993) Synaptic vesicle proteins and early endosomes in cultured hippocampal neurons: differential effects of brefeldin A in axon and dendrites. *J. Cell Biol.* **122:** 1207–1221.

Nobes C. D. and Hall A. (1995) Rho, Rac, and Cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. *Cell* **81:** 53–62.

Novick P., Field C. and Schekman R. (1980) Identification of 23 complementation groups required for post-translational events in the yeast secretory pathway. *Cell* **21:** 205–215.

Ossig R., Dascher C., Trepte H. H., Schmitt H. D. and Gallwitz D. (1991) The yeast SLY gene products, suppressor of defects in the essential GTP-binding Ypt1 protein, may act in endoplasmic reticulum-to-Golgi transport. *Molec. Cell. Biol.* **10:** 2214–2223.

Pevsner J., Hsu S.-C., Braun J. E. A., Calakos N., Ting A. E., Bennett M. K. and Scheller R. H. (1994a) Specificity and regulation of a synaptic vesicle docking complex. *Neuron* **13:** 353–361.

Pevsner J., Shu-Chan H. and Scheller R. H. (1994b) N-sec1: a neural-specific syntaxin-binding protein. *Proc. Natn. Acad. Sci. U.S.A.* **91:** 1445–1449.

Piper R. C., Whitters E. A. and Stevens T. H. (1994) Yeast Vps45p is a Sec1p-like protein required for the consumption of vacuole-targeted, post-Golgi transport vesicles. *Eur. J. Cell Biol.* **65:** 305–318.

Ralston E., Beushausen S. and Ploug T. (1994) Expression of the synaptic vesicle proteins VAMPs/synaptobrevins 1 and 2 in non-neural tissues. *J. Biol. Chem.* **269:** 15,403–15,406.

Rothman J. E. (1994) Mechanisms of intracellular protein transport. *Nature* **372:** 55–63.

Schiavo G., Benfenati F., Poulain B., Rossetto O., Polverino de Laureto P., DasGupta B. R. and Montecucco C. (1992) Tetanus and botulinum-B neurotoxins block neurotransmitter release by proteolytic cleavage of synaptobrevin. *Nature* **359:** 832–835.

Schiavo G., Rossetto O., Benfenati F., Poulain B. and Montecucco C. (1994) Tetanus and botulinum neurotoxins are zinc proteases specific for components of the neuroexocytosis apparatus. *Ann. NY Acad. Sci.* **710:** 65–75.

Schulze K. L., Littleton J. T., Salzberg A., Halachmi N., Stern M., Lev Z. and Bellen H. J. (1994) rop, a Drosophila homolog of yeast Sec1 and vertebrate n-Sec1/Munc-18 proteins, is a negative regulator of neurotransmitter release *in vivo*. *Neuron* **13:** 1099–1108.

Shetty K. T., Kaech S., Link W. T., Jaffe H., Flores C. M., Wray S., Pant H. C. and Beushausen S. (1995) Molecular characterization of a neuronal-specific protein that stimulates the activity of Cdk5. *J. Neurochem.* **64:** 1988–1995.

Söllner T. and Rothman J. E. (1994) Neurotransmission: harnessing fusion machinery at the synapse. *TINS* **17:** 344–348.

Söllner T., Whiteheart S. W., Brunner M., Erdjument-Bromage H., Geromanos S., Tempst P. and Rothman J. E. (1993) SNAP receptors implicated in vesicle targeting and fusion. *Nature* **362:** 318–324.

Südhof T. C., De Camilli P., Niemann H. and Jahn R. (1993) Membrane fusion machinery: insights from synaptic proteins. *Cell* **75:** 1–4.

Søgaard M., Tani K., Ye R. R., Geromanos S., Tempst P., Kirchhausen T., Rothman J. E. and Söllner T. (1994) A rab protein is required for the assembly of SNARE complexes in the docking of transport vesicles. *Cell* **78:** 937–948.

Tellam J. T., McIntosh S. and James D. E. (1995) Molecular identification of two novel Munc-18 isoforms expressed in non-neuronal tissues. *J. Biol. Chem.* **270:** 5857–5863.

Thomas-Reetz A. and De Camilli P. (1994) A role for synaptic vesicles in non-neuronal cells: clues from pancreatic β cells and from chromaffin cells. *FASEB J.* **8:** 209–216.

Tischler A. S. and Greene L. A. (1978) Morphologic and cytochemical properties of a clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. *Lab. Invest.* **2:** 77–89.

Vitale M. L., Seward E. P. and Trifaró J.-M. (1995) Chromaffin cell cortical actin network dynamics control the size of the release-ready vesicle pool and the initial rate of exocytosis. *Neuron* **14:** 353–363.

Volchuk A., Sargeant R., Sumitani S., Liu Z., He L. and Klip A. (1995) Cellubrevin is a resident protein of insulin-sensitive GLUT4 glucose transporter vesicles in 3T3-L1 adipocytes. *J. Biol. Chem.* **270:** 8233–8240.

Wada Y., Kitamoto K., Kanbe T., Tanaka K. and Anraku Y. (1990) The SLP1 gene of Saccharomyces cerevisiae is essential for vacuolar morphogenesis and function. *Molec. Cell. Biol.* **10:** 2214–223.