J. Membrane Biol. 146, 113–122 (1995)

The Journal of
Membrane
Biology

Topical Review

Molecular Mechanisms in Exocytosis

J.M. Edwardson, S.J. Marciniak
Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QJ, United Kingdom

Received: 30 June 1994/Revised: 27 September 1994

Introduction

Exocytosis, the last stage in the secretory pathway, involves the fusion of the membranes of secretory vesicles with the plasma membrane. It results in the release of the vesicle contents from the cell and also the delivery of vesicle membrane proteins into the plasma membrane. Exocytosis may either be constitutive, where vesicles budding from the *trans*-Golgi network fuse rapidly with the plasma membrane, or regulated, where specialized secretory vesicles accumulate in the cell and fuse with the plasma membrane only when the cell receives an appropriate stimulus. The last few years have seen significant advances in our understanding of the mechanisms involved in exocytosis, and the identification of many proteins that play crucial roles. Here we review some of these advances.

Exocytosis Shares Features with other Membrane Fusion Events

Several different strategies have been used to identify proteins involved in membrane docking and fusion during intracellular traffic. Recently, a convergence of these lines of research has pointed to a highly conserved mechanism of membrane fusion and made a major impact on our thinking about exocytosis. A genetic approach to the problem of intracellular protein transport in yeast, begun in the late 1970s, led to the identification of a number of mutants (the *sec* mutants) in which transport was blocked at various stages (Novick et al., 1980, 1981). More recently, the normal counterparts of some of the mutated genes have been cloned and sequenced (reviewed by Schekman, 1992). A parallel biochemical approach involved the development of an in vitro assay for the transport of proteins between *cis* and *medial* compartments of the Golgi complex of CHO cells (Balch et al., 1984*a*). Transport was found to require ATP and cytosolic proteins, and to be inhibited by treatment with the sulfhydryl reagent N-ethylmaleimide (NEM; Balch et al., 1984b). The in vitro system was used to screen cytosolic fractions for the NEM-sensitive factor, NSF (Block et al., 1988). When NSF was purified and sequenced (Wilson et al., 1989), it turned out to be highly homologous to Sec18p, one of the proteins identified in the screen for secretory mutants in yeast (Novick et al., 1981). In fact, Sec18p was able to substitute for NSF in the mammalian in vitro transport assay (Wilson et al., 1989). Furthermore, NSF was found to be required for membrane fusion at various stages in the intracellular transport pathway, including ER-Golgi transport (Beckers et al., 1989), endocytosis (Diaz et al., 1989) and constitutive exocytosis (Sztul et al., 1993). These were the first clues that the machinery of membrane traffic might be highly conserved. NSF exists as a homotrimer of 76 kDa subunits, each of which has two ATP binding sites (Whiteheart et al., 1994). Binding of NSF to Golgi membranes requires other cytosolic proteins—soluble NSF-attachment proteins, or SNAPs (Weidman et al., 1989; Clary & Rothman, 1990; Clary et al., 1990). SNAPs come in three isoforms, α (35 kDa), β (36 kDa) and γ (39 kDa). α- and β-SNAPs are in fact interchangeable, since β-SNAP is a brain-specific isoform with an almost identical sequence to α-SNAP (Whiteheart et al., 1993). Like NSF, α-SNAP has a yeast counterpart, Sec17p (Griff et al., 1992), and α-SNAP can

Key words: Exocytosis — Membrane fusion — Neurotransmitter release — GTP-binding proteins — Fusion pore
Correspondence to: J.M. Edwardson

restore activity in the mammalian Golgi transport assay to cytosol prepared from *sec17* mutant yeast.

SNAPs bind to integral membrane proteins (SNAP receptors, or SNAREs), and this binding is necessary for NSF attachment (Whiteheart et al., 1992). Detergent solubilization of the NSF/α-SNAP/γ-SNAP/SNARE complex produces a multisubunit 20S particle, which has been proposed to act as a ubiquitous fusion machine (Wilson et al., 1992). ATP hydrolysis by NSF causes disassembly of the particle, suggesting that cycles of assembly/disassembly might be responsible for generating successive rounds of vesicle fusion. In an attempt to search for SNAREs, 20S particles were prepared from detergent extracts of bovine brain and attached via NSF to a solid support. When the particles were provided with ATP, the resulting disassembly released the SNAREs (Söllner et al., 1993*a*). Remarkably, the proteins released were synaptobrevin-2 (also known as VAMP-2), syntaxin (both A and B isoforms) and SNAP-25 (synaptosome-associated protein, 25 kDa), all of which had been implicated previously in neurotransmitter release. Synaptobrevin is a small integral protein of the synaptic vesicle membrane, with a single membrane spanning domain, a short intravesicular carboxyl terminus and a hydrophilic cytoplasmic amino terminus (Trimble et al., 1988; Baumert et al., 1989). Synaptobrevin-2 is the major isoform in brain. Syntaxins are integral proteins of the presynaptic membrane, which again have the bulk of their mass in the cytoplasm (Bennett et al., 1992). SNAP-25 is a hydrophilic protein that is bound mainly to the presynaptic membrane by palmitoylation of a cysteine cluster (Oyler et al., 1989). According to the 'SNARE hypothesis', the NSF/SNAP complex interacts with synaptobrevin (the vesicle—or v-SNARE) and with syntaxin and SNAP-25 (the target or t-SNAREs) in a docking reaction, which is then followed by fusion.

Confirmation that synaptobrevin, syntaxin and SNAP-25 are crucial to the process of regulated exocytosis in neurones has been provided recently by the demonstration of their sensitivity to tetanus toxin (TeTx) or one of the botulinum toxins (BoNTs). These agents, produced by bacteria of the genus *Clostridium,* are among the most powerful toxins known, and they act specifically to block neurotransmitter release. The toxins are all zinc proteases, and they each proteolyse one of the neuronal SNAREs. Synaptobrevin is cleaved by TeTx, BoNT/B, BoNT/D, BoNT/F and BoNT/G (Schiavo et al., 1992*a,b;* Link et al., 1992; Schiavo et al., 1993*a,b;* Yamasaki et al., 1994), syntaxin by BoNT/C (Blasi et al., 1993*a*) and SNAP-25 by BoNT/A and BoNT/E (Schiavo et al., 1993*a,c;* Blasi et al., 1993*b;* Binz et al., 1994). The fact that the toxins, between them, attack all three neuronal SNAREs adds weight to the contention that the NSF/SNAP/SNARE complex might indeed represent the core of a fusion machine.

The targeting of transport vesicles to particular membrane compartments occurs with remarkable fidelity, and it is possible that the required specificity of the various membrane fusion events resides in particular combinations of v-SNAREs and t-SNAREs. In support of this idea, several SNARE homologues have been identified in yeast. For example, Bos1p, a synaptobrevin homologue, is localized to ER-derived transport vesicles, and in its absence the vesicles bud but do not fuse (Lian & Ferro-Novick, 1993). Sed5p, a syntaxin homologue, is also necessary for ER-Golgi transport (Hardwick & Pelham, 1992), and Sec9p, a SNAP-25 homologue, is required for the fusion of vesicles with the plasma membrane (reviewed by Niemann et al., 1994). SNARE homologues have also been found in non-neuronal mammalian tissues. The syntaxin family has a wide tissue distribution (Bennett et al., 1993). The synaptobrevin homologue, cellubrevin, is present in all cells tested and is localized to the constitutive endocytic/recycling pathway (McMahon et al., 1993). Cellubrevin, like synaptobrevin, is proteolysed by tetanus toxin. The toxin does not affect endosome/endosome fusion in vitro (Link et al., 1993), but does inhibit the delivery of transferrin-containing vesicles to the plasma membrane, suggesting that it might play a role in constitutive fusion of recycling vesicles with the plasma membrane (Galli et al., 1994). The demonstration that SNAP-25 is involved in axonal growth (Osen-Sand et al., 1993) represents a further potential connection of SNAREs with constitutive exocytosis. Synaptobrevin isoforms have also been found in the membranes of both adipocyte glucose transporter-containing vesicles (Cain et al., 1992), and pancreatic zymogen granules (Braun et al., 1994), suggesting an involvement of SNAREs in regulated exocytosis in non-neuronal tissues. The isoform in zymogen granule membranes (apparently synaptobrevin-2) is completely proteolysed by TeTx, whereas exocytosis in permeabilized pancreatic acini is only partially inhibited by toxin treatment (Gaisano et al., 1994), suggesting the existence of alternative mechanisms of exocytosis. Whether these involve further TeTx-insensitive isoforms of synaptobrevin remains to be determined.

Proteins that are likely to be additional components of the fusion complex in nerve terminals are still being identified. Recently, for example, a 67-kDa protein was isolated from rat brain on the basis of its ability to bind stably to the amino terminus of the t-SNARE, syntaxin (Hata et al., 1993*a*). This protein, Munc-18, is encoded by the mammalian homologue of the *Caenorhabditis elegans* gene *unc-18.* In *C. elegans,* mutations in unc-18 cause neuromuscular paralysis associated with accumulations of acetylcholine. It would appear, therefore, that unc-18 (and its mammalian homologue Munc-18) are involved in neurotransmitter release. The precise role played by Munc-18 in the exocytotic process has not been established, but the fact that it also has a yeast

homologue (sec1p) that is involved in exocytosis suggests that its function might be highly conserved.

Regulated Exocytosis Requires Controlled Membrane Fusion

Ca^{2+}-dependent Inactivation of a Fusion Clamp

If regulated exocytosis uses the same basic molecular machinery as other membrane fusion events, then additional components must exist that inhibit fusion until the cell receives an appropriate stimulus. It is well established that in most cells undergoing regulated exocytosis cell stimulation results in a rise in intracellular Ca^{2+} concentration (Knight et al., 1990). One likely effect of the Ca^{2+} is to switch off the fusion clamp, allowing exocytosis to proceed. In contrast, constitutive exocytosis appears to be Ca^{2+}-independent (Miller & Moore, 1991; Turner et al., 1992; Edwardson & Daniels-Holgate, 1992) presumably because there is no need to inactivate a fusion clamp.

Exocytosis at the synapse is extremely rapid, with a delay of approximately 100 μs between Ca^{2+} entry into the nerve terminal and the fusion of vesicles with the presynaptic membrane (Almers, 1990). The impressive speed of neurotransmitter release depends on the prior docking of the vesicles at a specialized region of the plasma membrane known as the active zone, which contains a dense array of voltage-sensitive Ca^{2+} channels. The most likely candidate for the Ca^{2+}-sensitive fusion clamp at the nerve terminal is synaptotagmin. This is a membrane protein of the synaptic vesicle that appears to exist as a homotetramer (Perin et al., 1990; 1991). Its carboxyl-terminal domain, which represents the bulk of the protein, projects into the cytoplasm. This domain contains two repeats with homology to the C2 domain of protein kinase C (PKC), and binds Ca^{2+} and phospholipids in a ternary complex (Brose et al., 1992). Synaptotagmin binds to both syntaxin (De Bello et al., 1993) and, in a Ca^{2+}-independent manner, to the conserved carboxyl-terminal domain of the receptor for α-latrotoxin, a component of black widow spider venom that triggers explosive exocytosis (Petrenko et al., 1991). The α-latrotoxin receptor is a plasma membrane protein that belongs to the neurexin family (Hata et al., 1993*b*). Finally, syntaxin binds to N-type Ca^{2+} channels in the presynaptic membrane (Bennett et al., 1992) which points to a mechanism for localizing synaptic vesicles immediately adjacent to the sites of Ca^{2+} entry. It is likely that docking and fusion of synaptic vesicles with the plasma membrane involves the interaction of several proteins in a large multimeric complex, as shown in Fig. 1. In the absence of NSF and SNAPs, the three SNAREs can be recovered bound to synaptotagmin in a 7S complex (Söllner et al., 1993*b*). It appears that synaptotagmin and α-SNAP compete for binding to a single site in the SNARE complex. Ca^{2+} is thought to promote the dissociation of synaptotagmin, which allows α-SNAP and then NSF to bind and form the 20S complex. Hydrolysis of ATP by NSF then causes the disruption of the 20S complex and leads eventually to membrane fusion. Attempts to determine the function of synaptotagmin during exocytosis have yielded confusing results. In synaptotagmin-deficient PC12 cell lines, secretion was actually greater than in normal cells (Shoji-Kasai et al., 1992). On the other hand, exocytosis in PC12 cells was reduced following injection of antibodies against synaptotagmin; surprisingly, antibodies against synaptobrevin, rab3a and synaptophysin (*see below*) were without effect (Elferink et al., 1993). Exocytosis was also inhibited by injection of peptides corresponding to the synaptotagmin C2 domains into the nerve terminals of the giant synapse of *Loligo pealei* (Bommert et al., 1993).

Other Ca^{2+}-dependent Processes

In non-neuronal cells, where exocytosis typically occurs several orders of magnitude more slowly than in neurones, there is evidence for the presence of soluble inhibitors of exocytosis that are switched off by Ca^{2+}. In mast cells, for example, exocytosis can be triggered by microinjection of GTPγS (Tatham & Gomperts, 1991), provided that micromolar Ca^{2+} is also present. However, when the same cells are held in the whole-cell patch configuration, so that the cytosol is dialyzed away, GTPγS triggers exocytosis in the absence of Ca^{2+}, albeit more slowly (Fernandez et al., 1984), suggesting that a cytosolic inhibitor of exocytosis must normally be switched off by Ca^{2+}.

Soluble proteins that operate in a Ca^{2+}-dependent manner to promote exocytosis have also been identified. Annexin II (also known as calpactin and p36) is bound to the cytoplasmic surface of plasma membranes of chromaffin cells (Nakata et al., 1990). This 36-kDa protein, associated with a smaller, 10-kDa protein (calpactin light chain), as a heterotetramer ($p36_2p10_2$) has been shown to aggregate chromaffin granules in a Ca^{2+}-dependent manner and to cause membrane fusion in response to added arachidonic acid (Drust & Creutz, 1988). Annexin II will partially restore the secretory activity of permeabilized chromaffin cells, in which exocytosis has been allowed to 'run down' by leakage of cytosolic proteins (Ali et al., 1989), and in addition a synthetic peptide corresponding to the most highly conserved domain partially inhibits exocytosis. Ultrastructural studies using immunogold labeling have revealed 6–10 nm filaments of annexin II linking granules to the plasma membrane, suggesting that part of the function of this protein is to tether granules to the membrane (Nakata et al., 1990). The

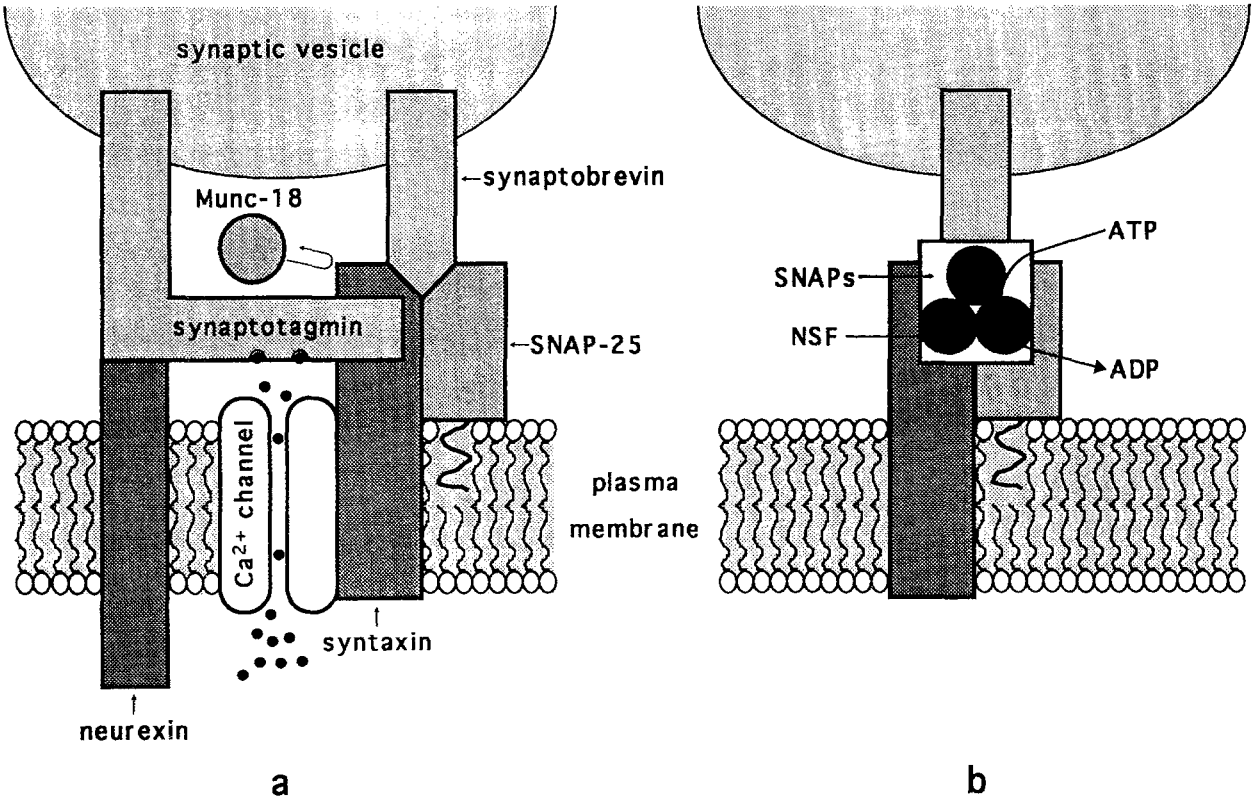

Fig. 1. Model of synaptic vesicle docking and fusion. (*a*) The v-SNARE synaptobrevin and the t-SNAREs syntaxin and SNAP-25 bind together to dock the synaptic vesicle at the plasma membrane. The SNAREs interact with the fusion clamp synaptotagmin to form a 7S complex. Synaptotagmin in turn binds to neurexin, the receptor for α-latrotoxin, and syntaxin binds to the N-type Ca^{2+} channel, so that the docking assembly is localized immediately adjacent to the site of Ca^{2+} entry. Ca^{2+} entering the nerve terminal binds to synaptotagmin at its two C2-like domains, causing it to dissociate from the SNARE complex. This allows the SNAPs and then NSF to bind in its place, forming a 20S complex (*b*). Hydrolysis of ATP by NSF causes disruption of the 20S complex, which eventually leads to membrane fusion. It is not clear at present what events occur between ATP hydrolysis and membrane fusion and what additional components are involved. The role played by Munc-18 is also unclear. Although it was isolated through its ability to bind to syntaxin, it is not found in either the 7S or the 20S complex. One possibility is that it regulates the formation of the 7S complex.

ability of annexin II to reconstitute exocytosis in permeabilized chromaffin cells declines as rundown proceeds. Two additional protein fractions, termed Exo1 and Exo2, that were able to rescue exocytosis in extensively rundown cells, were isolated from bovine brain (Morgan & Burgoyne, 1992). Exo1 was found to consist of a group of proteins of the 14-3-3 family, that share similarities with annexin II (Aitken et al., 1990), and Exo2 is now known to be the catalytic subunit of protein kinase A (Morgan et al., 1993).

GTP-binding Proteins, Large and Small

It is clear that vesicle budding and fusion events are both controlled by GTP-binding proteins. The evidence for this includes the ability of GTPγS to inhibit various transport steps, including ER-Golgi transport (Beckers & Balch, 1989), intra-Golgi transport (Melançon et al., 1987) and transport on the endocytic (Mayorga et al., 1989) and recycling (Goda & Pfeffer, 1988) pathways. The budding of both constitutive and regulated secretory vesicles from the *trans* Golgi network is also inhibited by GTPγS (Tooze et al., 1990; Leyte et al., 1992; Xu & Shields, 1993). Interestingly, its overall effect on the secretory event differs between the two types of exocytosis. Constitutive exocytosis is inhibited by GTPγS (Gravotta et al., 1990; Miller & Moore, 1991; Turner et al., 1992; Edwardson & Daniels-Holgate, 1992), whereas regulated exocytosis is usually stimulated (Fernandez et al., 1984; Barrowman et al., 1986; Cockcroft et al., 1987; reviewed by Gomperts, 1990), apparently through an effect on the ability of the pool of preformed secretory vesicles to fuse with the plasma membrane (Nadin et al., 1989; MacLean & Edwardson, 1992).

Small, monomeric GTP-binding proteins are known to be involved in intracellular membrane traffic. These proteins include Sec4p (Goud et al., 1988) and Ypt1p (Segev et al., 1988) in yeast, and the various rab proteins, which are localized to distinct membrane compartments

in mammalian cells (Zerial & Stenmark, 1993). Vesicular transport is inhibited by synthetic peptides of the 'effector domains' of rab proteins (Plutner et al., 1990) and by an anti-rab protein antibody (Plutner et al., 1991). These GTP-binding proteins are believed to act as molecular switches, with GTP hydrolysis driving the vectorial transport of proteins from one compartment to another (Bourne, 1988). GTPγS is thought to block transport by preventing GTP hydrolysis, whereas the synthetic effector domains act by binding to and inhibiting a GTPase-activating protein, or GAP.

At least two monomeric GTP-binding proteins have been implicated in the control of regulated exocytotic membrane fusion—rab3 and ADP-ribosylation factor. Secretory vesicles from several cell types that undergo regulated exocytosis have GTP-binding proteins of the rab3 subfamily on their cytoplasmic surfaces. In neurones, rab3a is localized specifically to synaptic vesicle membranes (Fischer von Mollard et al., 1990), suggesting that it might be involved in the control of exocytotic membrane fusion. A potential link between rab3a and the Ca^{2+}-sensitive membrane fusion machine described above is the protein rabphilin-3a, which binds specifically to the GTP-bound form of rab3a in neurones (Shiritaki et al., 1993). Rabphilin, like synaptotagmin, contains two copies of the Ca^{2+}-binding C2 domain, and it appears to compete with GAP for binding to rab3a.

It has been suggested that rab3 proteins also control regulated exocytosis in non-neuronal cells. The best evidence for this is the inhibition of exocytosis seen in anterior pituitary cells when rab3b expression was abolished by injection of an antisense oligonucleotide (Lledo et al., 1993). The effect is specific for rab3b, since a rab3a antisense oligonucleotide was without effect. The ability of rab3 effector domain peptides to stimulate exocytosis in permeabilized pancreatic acini (Padfield et al., 1992), chromaffin cells (Senyshyn et al., 1992) and mast cells (Oberhauser et al., 1992) has been taken as further support for the general involvement of rab3 in regulated exocytosis. The stimulatory effect of the effector peptides contrasts with their inhibitory effects elsewhere on the secretory pathway (Plutner et al., 1990). To account for their behavior, it was proposed that the peptides either keep rab3 in its GTP-bound state by binding to and inhibiting its GAP or activate the normal (unidentified) downstream effector protein in place of rab3. It has been shown recently, however, that although the peptides stimulate fusion between pancreatic zymogen granules and plasma membranes in vitro (Edwardson et al., 1993), even peptides with a completely scrambled sequence retain activity (MacLean et al., 1993*a*), casting considerable doubt on the idea that they act by mimicking endogenous rab3. It is probably significant that the rab3 effector peptide is polycationic, since other polycationic peptides, such as mastoparan and substance P also stimulate exocytotic membrane fusion in vitro (S. Marciniak & J. Edwardson, *unpublished results*). In fact, it is now known that not only mastoparan and substance P (Mousli et al., 1989) but also the rab3 effector peptides (Law et al., 1993) trigger mast cell degranulation through a pertussis toxin-sensitive mechanism, indicating the involvement of the heterotrimeric GTP-binding protein, G_i/G_o.

ADP-ribosylation factor (ARF) is a monomeric GTP-binding protein which is known to participate in intracellular membrane traffic. Although its best-understood function is in the formation of the nonclathrin coat on Golgi membranes, which is a prerequisite of vesicle budding (reviewed by Rothman & Orci, 1992), it has recently been identified on the membrane of secretory vesicles in hepatocytes, suggesting an additional role in exocytosis (Nickel et al., 1994). This role may relate to its ability to activate phospholipase D (Cockcroft et al., 1994), which hydrolyses phosphatidylcholine to produce choline and phosphatidic acid, a known fusogen for phospholipid vesicles (Simmonds & Halsey, 1985).

A question that remains to be answered is whether monomeric GTP-binding proteins mediate the stimulatory effect of GTPγS on regulated exocytosis. If this is so, then they must operate in a different way from the other members of the family, with the GTP-bound form promoting fusion. However, the observation that GTPγS inhibits neurotransmitter release at the squid giant synapse, apparently via a small GTP-binding protein, argues against this (Hess et al., 1993). In fact, a model based upon the involvement of a heterotrimeric GTP-binding protein would more easily accommodate the stimulatory effect of GTPγS, since heterotrimers are known to be active in their GTP-bound form. Evidence for the involvement of heterotrimeric GTP-binding proteins in the control of exocytosis in a number of cell types has emerged recently. For example, exocytosis in chromaffin cells can be inhibited via G_0 (Vitale et al., 1993) and G_{i3} is required for exocytosis in a mast cell line (Aridor et al., 1993).

It is likely, in fact, that both large and small GTP-binding proteins are involved in the control of regulated exocytosis. Both types of protein are found on secretory vesicles; the pancreatic zymogen granule, for instance, has G_i and up to 30 small GTP-binding proteins associated with it (Padfield & Jamieson, 1991; Schnefel et al., 1992). Further, rab3a dissociates from synaptic vesicles during exocytosis and reassociates later (Fischer von Mollard et al., 1991), and during stimulation of the pancreatic acinar cell rab3 redistributes from the zymogen granules to vesicles in the region of the Golgi complex (Jena et al., 1994). A clue as to the likely role of rab3a in neurotransmitter release has been provided recently by a study of synaptic transmission in mice in which *rab3a* gene expression had been deleted (Geppert et al., 1994). It was found that most of the characteristics of synaptic transmission were normal, although synaptic depression

after short trains of stimuli was increased, suggesting that rab3a is not essential for exocytotic membrane fusion, but plays a role in synaptic vesicle recruitment. The mechanism of operation of the heterotrimeric GTP-binding proteins is unclear. In particular, it would be interesting to know whether they are controlled by an upstream regulator, in the same way as their counterparts involved in signal transduction at the plasma membrane.

Protein Phosphorylation/Dephosphorylation

A change in protein phosphorylation in synchrony with regulated exocytosis has been seen in a number of cell types, suggesting that phosphorylation/dephosphorylation is involved in its control. In nerve terminals, phosphorylation of the vesicle protein synapsin I appears to trigger the release of synaptic vesicles from the cytoskeleton (Südhof et al., 1989). Synapsin I binds to synaptic vesicles by interacting both with the phospholipids and with proteins, including Ca^{2+}/calmodulin-dependent kinase II (CaMKII; Benfenati et al., 1992*a*). It also binds cytoskeletal elements, including spectrin and actin (Valtorta et al., 1992). The affinity of this latter interaction is reduced 5-fold once synapsin I is phosphorylated by CaMKII (Valtorta et al., 1992). It has been suggested that phosphorylation of synapsin I in response to a Ca^{2+} signal might release bound vesicles from cytoskeletal constraints, enabling them to move to the plasma membrane to dock and fuse (Benfenati et al., 1992*b*). Hence in neurones, Ca^{2+} would have not only a direct disinhibiting effect on exocytotic membrane fusion, but also an 'upstream' effect on vesicle-cytoskeleton interaction. The action of the highly-homologous synapsin II remains unclear. Synapsin II also possesses synaptic vesicle and cytoskeletal binding activity, but does not contain a CaMKII phosphorylation site; further, phosphorylation of either its CaMKI or its PKA sites does not appear to alter its activity.

In many cells there does not appear to be an immediate requirement for ATP (Cockcroft et al., 1987), although a run-down is often seen following its removal (Vilmart-Seuwen et al., 1986). One interpretation of these findings is that dephosphorylation of a 'primed' (phosphorylated) protein may drive exocytosis. Protein phosphatases have been implicated in the control of exocytosis in the exocrine pancreas. For example, introduction of a recombinant tyrosine phosphatase into permeabilized pancreatic acini was found to enhance Ca^{2+}-stimulated amylase secretion (Jena et al., 1991). Conversely, exocytosis in pancreatic acini is reduced by the immunosuppressants cyclosporin A and FK506, which are known to inhibit the Ca^{2+}/calmodulin-dependent phosphatase, calcineurin (Waschulewski et al., 1993). Okadaic acid, at high concentrations required to inhibit protein phosphatase type 2B (Wagner et al., 1992), also blocks exocytosis, as well as preventing the dephosphorylation of an unidentified 19-kDa protein.

We have examined the effects of agents that affect secretion in the pancreatic acinar cell on the phosphorylation states of proteins on the zymogen granule membrane. We have shown (MacLean et al., 1993*b*) that Ca^{2+} and GTPγS, which stimulate secretion, also stimulate phosphorylation of a protein of molecular mass 45 kDa (p45) on isolated granules. On the other hand, the protein kinase inhibitor genistein inhibits both secretion and p45 phosphorylation. The effective concentration ranges of all three agents is identical to those that affect secretion. The stimulatory effect of GTPγS and the inhibitory effect of genistein are also seen when the phosphorylation state of p45 on granules within permeabilized cells is examined. Ca^{2+}, however, now causes dephosphorylation of p45 over the same time course as that of Ca^{2+}-triggered secretion. There is good circumstantial evidence, therefore, that p45 is involved in the control of exocytosis in the pancreas, with dephosphorylation acting as a trigger for exocytosis. Exocytosis in permeabilized acini requires Ca^{2+} (Edwardson et al., 1990), while exocytotic membrane fusion in vitro does not (Nadin et al., 1989; MacLean & Edwardson, 1992). It appears, then, that Ca^{2+} might have a disinhibitory effect through a cytosolic protein, and it is tempting to speculate that the cytosolic target might be a Ca^{2+}-dependent phosphatase, such as calcineurin. Recently, we have found that p45 is also present on parotid secretory granules (Marciniak & Edwardson, *unpublished results*), which suggests that it might play a general role, at least in exocrine tissue.

Membrane Lipids Influence Exocytotic Membrane Fusion

The lipid composition of secretory vesicle membranes and plasma membranes is known to be asymmetric (reviewed by Zimmerberg et al., 1993). For example, cholesterol, which enhances secretory granule fusion with liposomes, is asymmetrically distributed in zymogen granules of the exocrine pancreas (Orci et al., 1980). As the granules mature and acquire the capacity to fuse, there is a change in the polarity of cholesterol distribution, with a transfer of this lipid from the cytoplasmic leaflet to the inner leaflet, which might increase the fluidity and fusogenic properties of the cytoplasmic surface of the granules (reviewed by Beaudoin & Grondin, 1992). In contrast, lysolipids have been shown to be potent reversible inhibitors of fusion (Chernomordik et al., 1993). They may act by inhibiting the formation of curved membrane intermediates or by interfering with hydrophobic regions of fusion proteins.

The metabolism of membrane lipids also appears to play a role in exocytotic membrane fusion. The potential significance of the ability of ARF to control phospholi-

pase D activity, for example, has already been discussed. Regulated exocytosis in PC12 cells has been shown to involve sequential priming and triggering steps which depend on ATP and Ca^{2+}, respectively, and require different cytosolic proteins (Hay & Martin, 1992). One of the priming factors, PEP3, is now known to be identical to phosphatidylinositol transfer protein, or PITP (Hay & Martin, 1993), which catalyzes the transfer of phosphatidylinositol between donor and acceptor membranes. The importance of this enzyme in vesicle traffic is underlined by the discovery that Sec14p, the yeast form of PITP, is essential for protein transport from the Golgi to the plasma membrane. It has been suggested that PITP might act either by increasing the fusability of the membranes involved or by facilitating the binding of transport vesicles to cytoskeletal elements.

Exocytosis Proceeds via the Formation of a Fusion Pore

Exocytotic membrane fusion begins with the formation of a fusion pore, a channel linking vesicle interior with cell exterior. These pores were first visualized in electron micrographs of freeze-fractured samples (Chandler & Heuser, 1980) and have since been investigated electrophysiologically by the use of whole-cell capacitance measurement (Neher & Marty, 1982). The fusion of the vesicle membrane with the plasma membrane results in an increase in cell surface area and thus an increased capacitance. It was shown originally in rat mast cells that exocytosis involves both the irreversible incorporation of vesicle membrane into the plasma membrane, seen as stepwise increases of capacitance, and transient flickering increases of capacitance, believed to represent opening and closing of a fusion pore (Fernandez et al., 1984; Breckenridge & Almers, 1987). Investigation of the release of catecholamines from chromaffin cells by amperometry revealed a small and relatively long-lasting 'foot' before the major secretory signal (Chow et al., 1992), and it was proposed that this foot represented the leak of catecholamines through the fusion pore. This proposal has been confirmed by the use of simultaneous patch clamp measurement and amperometry (Alvarez de Toledo et al., 1993). The flickering pore has a conductance which varies between 0.2 and 1.5 nS (radius 0.5–2 nm). Prior to complete fusion, the pore expands, and the time required for this to happen depends on the size of the secretory vesicle. Vesicles in beige mouse mast cells, which are unusually large (mean radius 1.2 μm), take on average 410 msec to open fully, during which time they release 2.1% of their contents. Wild-type mast cell vesicles (radius 0.35 μm), on the other hand, take 66 msec to open, releasing 3.2% of their contents while doing so. From these data, it can be predicted that synaptic vesicles (radius 24 nm) would open in only 0.55 msec and release 8% of their contents. The true figure for release might actually be larger than this, since the contents of synaptic vesicles are freely diffusible, unlike serotonin and histamine in mast cell vesicles which are contained in a dense-core gel. These results have resurrected on old debate about whether neurotransmitter release can occur without full vesicle fusion. There is in fact considerable evidence to support the idea that reversible fusion accounts for a significant proportion of transmitter release at the nerve terminal (reviewed by Fesce et al., 1994), and such a mechanism has the advantage of being 'economical' in that it does not require membrane recycling to sustain it.

Both 'protein' and 'lipid' models have been advanced to explain the process of fusion pore formation. According to the former model, a protein channel spans both vesicle and plasma membranes, and its disassembly leads to the fusion of the membranes (Almers, 1990). Advocates of the latter model propose that protein-protein-interactions are involved only in bringing the lipid bilayers close enough together to allow fusion, through the formation of a lipid pore (Monck & Fernandez, 1992). This might be the role, for example, of the annexins (reviewed by Creutz, 1992). In a recent study of lipid flux during fusion of erythrocytes with fibroblasts expressing the fusogenic protein influenza haemagglutinin (Tse et al., 1993), it was found that lipids did not leak around the pore when its conductance was less that 0.5 pS, indicating that the initial pore was lined with protein. Influenza haemagglutinin is in fact the best-characterized fusion protein (reviewed by White, 1992). It exists as a trimer of identical subunits, each of which contains a fusion peptide, a conserved sequence containing many hydrophobic amino acids. The tips of the molecule are responsible for its binding to sialic acid residues on the target membrane. At neutral pH, the fusion peptides are buried in the stem of the molecule, close to the viral membrane. When the pH falls to around 5.0 (usually in endosomes), the peptides are rapidly exposed and interact with the target membrane. Several trimers are then thought to aggregate and form a fusion pore. Interestingly, PH-30, a protein on the surface of sperm, which mediates sperm-egg fusion, contains a fusion peptide of similar structure to viral fusion proteins, suggesting a common mode of operation (Blobel et al., 1992). Of course, viral penetration into cells and sperm-egg fusion involve interaction between the extracellular faces of membranes. Whether the results discussed above have any bearing on exocytotic membrane fusion, where cytoplasmic faces of membranes interact, remains to be seen.

Synaptophysin, a transmembrane glycoprotein of synaptic vesicles, is the main candidate for a fusion pore-forming protein at the nerve terminal. It has been shown to form oligomers which have ion channel activity when reconstituted into black lipid membranes (Thomas et al.,

1988), and there is some evidence for its involvement in exocytotic membrane fusion. For instance, when rat cerebellar mRNA is injected into *Xenopus* oocytes, a Ca^{2+}-dependent release of the neurotransmitter glutamate can be detected (Alder et al., 1992). Removal of synaptophysin from this system by addition of either an antisense oligonucleotide or antisynaptophysin antibodies, causes a reduction in the extent of glutamate release. It has yet to be shown, however, that the function of synaptophysin being disrupted in these experiments is the formation of the fusion pore.

The Next Steps

Further advances in our understanding of the mechanisms of exocytosis in the near future will probably center around the SNARE hypothesis. Identification of candidate SNAREs in non-neuronal tissues has already begun, and it will be important to test whether the Rothman fusion machine operates in all exocytotic membrane fusion events. The precise roles of the proteins located at the synapse remain to be determined. In particular, it is necessary now to separate the molecular requirements for docking from those for fusion. In addition, components such as the GTP-binding proteins need to be placed into the basic fusion mechanism in order to explain observations such as the ability of GTPγS under some circumstances to trigger exocytosis in the absence of Ca^{2+}. It is clear that some of the factors that appear to play a role in the control of exocytosis are not found in all tissues, and it will be necessary to distinguish the features of the exocytotic mechanism that are universal from those that are tissue specific.

Many of the crucial components involved in exocytotic membrane fusion have now been identified. It is not unreasonable to speculate, therefore, that it might be possible to reconstitute fusion in a fully defined system. At the present rate of progress, it is unlikely to be too long before this feat is accomplished.

We are grateful to Prof. R.D. Burgoyne of the Physiological Laboratory, University of Liverpool, U.K. for a helpful discussion. Work in our laboratory is supported by the Wellcome Trust and the Medical Research Council (MRC). SJM is a member of the University of Cambridge M.B./Ph.D. Programme, and is supported by an MRC studentship.

References

Aitken, A., Ellis, C.A., Harris, A., Sellers, L.A., Toker, A.. 1990. *Nature* **344**:594

Alder, J., Lu, B., Valtorta, F., Greengard, P., Poo, M., 1992. *Science* **257**:657–661

Ali, S.M., Geisow, M.J., Burgoyne, R.D. 1989. *Nature* **340**:313–315

Almers, W. 1990. *Annu. Rev. Physiol.* **52**:607–624

Alvarez de Toledo, G., Fernandez-Chacon, R., Fernandez, J.M. 1993. *Nature* **363**:554–558

Aridor, M., Rajmilevich, G., Beavan, M.A., Sagi-Eisenberg, R. 1993. *Science* **262**:1569–1572

Balch, W.E., Dunphy, D.W., Braell, W.A., Rothman, J.E. 1984*a*. *Cell* **39**:405–416

Balch, W.E., Glick, B.S., Rothman, J.E. 1984*b*. *Cell* **39**:525–536

Barrowman, M.M., Cockcroft, S., Gomperts, B.D. 1986. *Nature* **319**:504–507

Baumert, M., Maycox, P.R., Navone, F., De Camilli, P., Jahn, R. 1989. *EMBO J.* **8**:379–384

Beaudoin, A.R., Grondin, G. 1992. *Int. Rev. Cytol.* **132**:177–221

Beckers, C.J.M., Balch, W.E. 1989. *J. Cell Biol.* **108**:1245–1256

Beckers, C.J.M., Block, M.R., Glick, B.S., Rothman, J.E., Balch, W.E. 1989. *Nature* **339**:397–398

Benfenati, F., Valtorta, F., Rubenstein, J.L., Gorelick, F.S., Greengard, P., Czernik, A.J. 1992*a*. *Nature* **359**:417–420

Benfenati, F., Valtorta, F., Chieregatti, E., Greengard, P. 1992*b*. *Neuron* **8**:377–386

Bennett, M.K., Calakos, N., Scheller, R.H. 1992. *Science* **257**:255–259

Bennett, M.K., Garcia-Arraras, J.E., Elferink, L.A., Peterson, K., Fleming, A.M., Hazuka, C.D., Scheller, R.H. 1993. *Cell* **74**:863–873

Blasi, J., Chapman, E.R., Link, E., Binz, T., Yamasaki, S., De Camilli, P., Südhof, T.C., Niemann, H., Jahn, R. 1993*a*. *Nature* **365**:160–163

Blasi, J., Chapman, E.R., Yamasaki, S., Binz, T., Niemann, H., Jahn, R. 1993*b*. *EMBO J.* **12**:4821–4828

Binz, T., Blasi, J., Yamasaki, S., Baumeister, A., Link, E., Südhof, T.C., Jahn, R., Niemann, H. 1994. *J. Biol. Chem.* **269**:1617–1620

Blobel, C.P., Wolfsberg, T.G., Turck, C.W., Myles, D.G., Primakoff, P., White, J.M. 1992. *Nature* **356**:248–252

Block, M.R., Glick, B.S., Wilcox, C.A., Wieland, F.T., Rothman, J.E. 1988. *Proc. Natl. Acad. Sci. USA* **85**:7852–7856

Bommert, K., Charlton, M.P., DeBello, W.M., Chin, G.J., Betz, H., Augustine, G.J. 1993. *Nature* **363**:163–165

Bourne, H.R. 1988. *Cell* **53**:669–671

Braun, J.E.A., Fritz, B.A., Wong, S.M.E., Lowe, A.W. 1994. *J. Biol. Chem.* **269**:5328–5335

Breckenridge, L., Almers, W. 1987. *Nature* **328**:814–817

Brose, N., Petrenko, A.G., Südhof, T.C., Jahn, R. 1992. *Science* **256**:1021–1025

Cain, C.C., Trimble, W.S., Lienhard, G.E. 1992. *J. Biol. Chem.* **267**:11681–11684

Chandler, D.E., Heuser, J.E. 1980. *J. Cell Biol.* **86**:666–674

Chernomordik, L.V., Vogel, S.S., Sokoloff, A., Onaron, H.O., Leikina, E.A., Zimmerberg, J. 1993. *FEBS Lett* **318**:71–76

Chow, R.H., von Rüden, L., Neher, E. 1992. *Nature* **356**:60–63

Clary, D.O., Griff, I.C., Rothman, J.E. 1990. *Cell* **61**:709–721

Clary, D.O., Rothman, J.E. 1990. *J. Biol. Chem.* **265**:10109–10117

Cockcroft, S., Howell, T.W., Gomperts, B.D. 1987. *J. Cell Biol.* **105**:2745–2750

Cockcroft, S., Thomas, G.M.H., Fensome, A., Geny, B., Cunningham, E., Gout, I., Hiles, I., Totty, N.F., Truong, O., Hsuan, J.J. 1994. *Science* **263**:523–526

Creutz, C.E. 1992. *Science* **258**:924–931

DeBello, W.M., Betz, H., Augustine, G.J. 1993. *Cell* **74**:947–950

Diaz, R., Mayorga, L.S., Weidman, P.J., Rothman, J.E., Stahl, P.D. 1989. *Nature* **339**:398–400

Drust, D.S., Creutz, C.E. 1988. *Nature* **331**:88–91

Edwardson, J.M., Daniels-Holgate, P.U. 1992. *Biochem. J.* **285**:383–385

Edwardson, J.M., MacLean, C.M., Law, G.J. 1993. *FEBS Lett.* **320**:52–56

Edwardson, J.M., Vickery, C., Christy, L.J. 1990. *Biochim. Biophys. Acta* **1053**:32–36

Elferink, L.A., Peterson, M.R., Scheller, R.H. 1993. *Cell* **72**:153–159

Fernandez, J.M., Neher, E., Gomperts, B.D. 1984. *Nature* **312**:453–455

Fesce, R., Grohavaz, F., Valtorta, F., Meldolesi, J. 1994. *Trends Cell Biol.* **4**:1–4

Fischer von Mollard, G., Mignery, G.A., Baumert, M., Perin, M.S., Hanson, T.J., Burger, P.M., Jahn, R., Südhof, T.C. 1990. *Proc. Natl. Acad. Sci.* USA **87**:1988–1992

Fischer von Mollard, G., Südhof, T.C., Jahn, R. 1991. *Nature* **349**:79–81

Gaisano, H.Y., Sheu, L., Foskett, J.K., Trimble, W.S. 1994. *J. Biol. Chem.* **269**:17062–17066

Galli, T., Chilcote, T., Mundigl, O., Binz, T., Niemann, H., De Camilli, P. 1994. *J. Cell Biol.* **125**:1015–1024

Geppert, M., Bolshakov, V.Y., Siegelbaum, S.A., Takei, K., De Camilli, P., Hammer, R.E., Südhof, T.C. 1994. *Nature* **369**:493–497

Goda, Y., Pfeffer, S.R. 1988. *Cell* **55**:309–320

Gomperts, B.D. 1990. *Annu. Rev. Physiol.* **52**:591–605

Goud, B., Salminen, A., Walworth, N., Novick, P. 1988. *Cell* **53**:753–768

Gravotta, D., Adesnik, M., Sabatini, D.D. 1990. *J. Cell Biol.* **111**:2893–2908

Griff, I.C., Schekman, R., Rothman, J.E., Kaiser, C.A. 1992. *J. Biol. Chem.* **267**:12106–12115

Hardwick, K.G., Pelham, H.R.B. 1992. *J. Cell Biol.* **119**:513–521

Hata, Y., Davletov, B., Petrenko, A.G., Jahn, R., Südhof, T.C. 1993*b*. *Neuron* **10**:307–315

Hata, Y., Slaughter, C.A., Südhof, T.C. 1993*a*. *Nature* **366**:347–351

Hay, J.C., Martin, T.F.J. 1992. *J. Cell Biol.* **119**:139–151

Hay, J.C., Martin, T.F.J. 1993. *Nature* **366**:572–575

Hess, S.D., Doroshenko, P.A., Augustine, G.J. 1993. *Science* **259**:1169–1172

Jena, B.P., Gumkowski, F.D., Konieczko, E.M., Fischer von Mollard, G., Jahn, R., Jamieson, J.D. 1994. *J. Cell Biol.* **124**:43–53

Jena, B.P., Padfield, P.J., Ingebritsen, T.S., Jamieson, J.D. 1991. *J. Biol. Chem.* **266**:17744–17746

Knight, D.E., von Grafenstein, H., Athayde, C.M. 1990. *Trends Neurosci.* **12**:451–458

Law, G.J., Northrup, A.J., Mason, W.T. 1993. *FEBS Lett.* **333**:56–60

Leyte, A., Barr, F.A., Kehlenbach, R.H., Huttner, W.B. 1992. *EMBO J.* **11**:4795–4804

Lian, J.P., Ferro-Novick, S. 1993. *Cell* **73**:735–745

Link, E., Edelmann, L., Chou, J.H., Binz, T., Yamasaki, S., Eisel, U., Baumert, M., Südhof, T.C., Niemann, H., Jahn, R. 1992. *Biochem. Biophys. Res. Commun.* **189**:1017–1023

Link, E., McMahon, H., Fischer von Mollard, G., Yamasaki, S., Niemann, H., Südhof, T.C., Jahn, R. 1993. *J. Biol. Chem.* **268**:18423–18426

Lledo, P.M., Vernier, P., Vincent, J.-D., Mason, W.T., Zorec, R. 1993. *Nature* **364**:540–544

MacLean, C.M., Edwardson, J.M. 1992. *Biochem. J.* **286**:747–753

MacLean, C.M., Law, G.J., Edwardson, J.M. 1993*a*. *Biochem. J.* **294**:325–328

MacLean, C.M., Marciniak, S.J., Hall, D.V., Edwardson, J.M. 1993b. *J. Cell Sci.* **106**:663–670

Mayorga, L.S., Diaz, R., Stahl, P.D. 1989. *Science* **244**:1475–1477

McMahon, H.T., Ushkaryov, Y.A., Edelmann, L., Link, E., Binz, T., Niemann, H., Jahn, R., Südhof, T.C. 1993. *Nature* **364**:346–349

Melançon, P., Glick, B.S., Malhotra, V., Weidman, P.J., Serafini, T., Gleason, M.J., Orci, L., Rothman, J.E. 1987. *Cell* **51**:1053–1062

Miller, S.G., Moore, H.-P.H. 1991. *J. Cell Biol.* **112**:39–54

Monck, J.R., Fernandez, J.M. 1992. *J. Cell Biol.* **119**:1395–1404

Morgan, A., Burgoyne, R.D. 1992. *Nature* **355**:833–836

Morgan, A., Wilkinson, M., Burgoyne, R.D. 1993. *EMBO J.* **12**:3747–3752

Mousli, M., Bronner, C., Bueb, J.-L., Tschirhart, E., Glies, J.-P., Landry, Y. 1989. *J. Pharmacol. Exp. Therap.* **250**:329–335

Nadin, C.Y., Rogers, J., Tomlinson, S., Edwardson, J.M. 1989. *J. Cell Biol.* **109**:2801–2808

Nakata, T., Sobue, K., Hirokawa, N. 1990. *J. Cell Biol.* **110**:13–25

Neher, E., Marty, A. 1982. *Proc. Natl. Acad. Sci. USA* **79**:6712–6716

Nickel, W., Huber, L.A., Kahn, R.A., Kipper, N., Barthel, A., Fasshauer, D., Söling, H.-D. 1994. *J. Cell Biol.* **125**:721–732

Niemann, H., Blasi, J., Jahn, R. 1994. *Trends Cell Biol.* **4**:179–185

Novick, P., Ferro, S., Schekman, R. 1981. *Cell* **25**:461–469

Novick, P., Field, C., Schekman, R. 1980. *Cell* **21**:205–215

Oberhauser, A.F., Monck, J.R., Balch, W.E., Fernandez, J.M. 1992. *Nature* **360**:270–273

Orci, L., Miller, R.G., Montesano, R., Perrelet, A., Amherdt, M., Vassali, P. 1980. *Science* **210**:1019–1021

Osen-Sand, A., Catsicas, M., Staple, J.K., Jones, K.A., Ayala, G., Knowles, J., Greeningloh, G., Catsicas, S. 1993. *Nature* **364**:445–448

Oyler, G.A., Higgins, G.A., Hart, R.A., Battenberg, E., Billingsley, M., Bloom, F.E., Wilson, M.C. 1989. *J. Cell Biol.* **109**:3039–3052

Padfield, P.J., Balch, W.E., Jamieson, J.D. 1992. *Proc. Natl. Acad. Sci. USA* **89**:1656–1660

Padfield, P.J., Jamieson, J.D. 1991. *Biochem. Biophys. Res. Commun.* **174**:600–605

Perin, M.S., Brose, N., Jahn, R. & Südhof, T.C. 1991. *J. Biol. Chem.* **266**:623–629

Perin, M.S., Fried, V.A., Mignery, G.A., Jahn, R., Südhof, T.C. 1990. *Nature* **345**:260–263

Petrenko, A.G., Perin, M.S., Davletov, B.A., Ushkaryov, Y.A., Geppert, M., Südhof, T.C. 1991. *Nature* **353**:65–68

Plutner, H., Cox, A.D., Pind, S., Khosravi-Far, R., Bourne, J.R., Schwaninger, R., Der, C.J., Balch, W.E. 1991. *J. Cell Biol* **115**:31–43

Plutner, H., Schwaninger, R., Pind, S., Balch, W.E. 1990. *EMBO J.* **9**:2375–2383

Rothman, J.E., Orci, L. 1992. *Nature* **355**:409–416

Schekman, R. 1992. *Curr. Opin. Cell Biol.* **4**:587–592

Schiavo, G., Benfenati, F., Poulain, B., Rossetto, O., Polverino de Laureto, P., DasGupta, B.R., Montecuco, C. 1992b. *Nature* **359**:832–835

Schiavo, G., Poulain, B., Rossetto, O., Benfenati, F., Tauc, L., Montecucco, C. 1992a. *EMBO J.* **11**:3577–3583

Schiavo, G., Rossetto, O., Catsicas, S., Polverino de Laureto, P., DasGupta, B.R., Benfenati, F., Montecucco, C. 1993*a*. *J. Biol. Chem.* **268**:23784–23787

Schiavo, G., Santucci, A., Dasgupta, B.R., Mehta, P.P., Jontes, J., Benfenati, F., Wilson, M.C., Montecucco, C. 1993*c*. *FEBS Lett.* **353**:99–103

Schiavo, G., Shone, C.C., Rossetto, O., Alexander, F.C.G., Montecucco, C. 1993*b*. *J. Biol. Chem.* **268**:11516–11519

Schnefel, S., Zimmerman, P., Pröfrock, A., Jahn, R., Aktories, K., Zeuzem, S., Haase, W., Schulz, I. 1992. *Cell Physiol. Biochem.* **2**:77–89

Segev, N., Mulholland, J., Botstein, D. 1988. *Cell* **52**:915–924

Senyshyn, J., Balch, W.E., Holz, R.W. 1992. *FEBS Lett.* **309**:41–46

Shiritaki, H., Kaibuchi, K., Sakoda, T., Kishida, S., Yamaguchi, T., Wada, K., Miyazaki, M., Takai, Y. 1993. *Mol. Cell. Biol.* **13**:2061–2068

Shoji-Kasai, Y., Yoshida, A., Sato, K., Hoshino, T., Ogura, A., Kondo, S., Fujimoto, Y., Kuwahara, R., Kato, R., Takahashi, M. 1992. *Science* **256**:1820–1823

Simmonds, A.C., Halsey, M.J. 1985. *Biochim. Biophys. Acta* **813:**331–337

Söllner, T., Bennett, M.K., Whiteheart, S.W., Scheller, R.H., Rothman, J.E. 1993*b*. *Cell* **75:**409–418

Söllner, T., Whiteheart, S.W., Brunner, M., Erdjument-Bromage, H., Geromanos, S., Tempst, P., Rothman, J.E. 1993*a*. *Nature* **362:**318–324

Südhof, T.C., Czernik, A.J., Kao, H.-T., Takei, K., Johnston, P.A., Horiuchi, A., Kanazir, S.D., Wagner, M.A., Perin, M.S., De Camilli, P., Greengard, P. 1989. *Science* **245:**1474–1479

Sztul, E., Colombo, M., Stahl, P., Samanta, R. 1993. *J. Biol. Chem.* **268:**1876–1885

Tatham, P.E.R., Gomperts, B.D. 1991. *J. Cell Sci.* **98:**217–224

Thomas, L., Hartung, K., Langosch, D., Rehm, H., Bamberg, E., Franke, W.W., Betz, H. 1988. *Science* **242:**1050–1053

Tooze, S.A., Weiss, U., Huttner, W.B. 1990. *Nature* **347:**207–208

Trimble, W.S., Cowan, D.M., Scheller, R.H. 1988. *Proc. Natl. Acad. Sci. USA* **85:**4538–4542

Tse, F.W., Iwata, A., Almers, W. 1993. *J. Cell Biol.* **121:**543–552

Turner, M.D., Rennison, M.E., Handel, S.E., Wilde, C.J., Burgoyne, R.D. 1992. *J. Cell Biol.* **117:**269–278

Valtorta, F., Benfenati, F., Greengard, P. 1992. *J. Biol. Chem.* **267:**7195–7198

Vilmart-Seuwen, J., Kersken, H., Sturzl, R., Plattner, H. 1986. *J. Cell Biol.* **103:**1279–1288

Vitale, N., Mukai, H., Rouot, B., Thiersé, D., Aunis, D., Bader, M.-F. 1993. *J. Biol. Chem.* **268:**14715–14723

Wagner, A.C.C., Wishart, M.J., Yule, D.I., Williams, J.A. 1992. *Amer. J. Physiol.* **263:**C1172–C1180

Waschulewski, I.H., Hall, D.V., Kern, H.F., Edwardson, J.M. 1993. *Brit. J. Pharmacol.* **108:**892–900

Weidman, P.J., Melançon, P., Block, M.R., Rothman, J.E. 1989. *J. Cell Biol.* **108:**1589–1596

White, J. 1992. *Science* **258:**917–924

Whiteheart, S.W., Brunner, M., Wilson, D.W., Wiedmann, M., Rothman, J.E., 1992. *J. Biol. Chem.* **267:**12239–12243

Whiteheart, S.W., Griff, I.C., Brunner, M., Clary, D.O., Mayer, T., Buhrow, S.A., Rothman, J.E., 1993. *Nature* **362:**353–355

Whiteheart, S.W., Rossnagel, K., Buhrow, S.A., Brunner, M., Jaenicke, R., Rothman, J.E. 1994. *J. Cell Biol.* **126:**945–954

Wilson, D.W., Whiteheart, S.W., Wiedmann, M., Brunner, M., Rothman, J.E. 1992. *J. Cell Biol.* **117:**531–538

Wilson, D.W., Wilcox, C.A., Flynn, G.C., Chen, E., Kuang, W.-J., Henzel, W.J., Block, M.R., Ullrich, A., Rothman, J.E. 1989. *Nature* **399:**355–359

Xu, H., Shields, D. 1993. *J. Cell Biol.* **122:**1169–1184

Yamasaki, S., Baumeister, A., Binz, T., Blasi, J., Link, E., Cornille, F., Roques, B., Fyske, E.M., Südhof, T.C., Jahn, R., Niemann, H. 1994. *J. Biol. Chem.* **269:**12764–12772

Zerial, M., Stenmark, H. 1993. *Curr. Opin. Cell Biol.* **5:**613–620

Zimmerberg, J., Vogel, S.S., Chernomordik, L.V. 1993. *Annu. Rev. Biophys. Biomol. Struct.* **22:**433–466