Review

The molecular machinery for secretion is conserved from yeast to neurons

Mark K. Bennett and Richard H. Scheller

Howard Hughes Medical Institute, Beckman Center B-155, Department of Molecular and Cellular Physiology, Stanford University, Stanford, CA 94305

ABSTRACT A variety of approaches have been utilized to identify and characterize the molecules that mediate vesicular trafficking along the secretory pathway. Two approaches that have been particularly fruitful include the genetic dissection of the yeast secretory pathway and the biochemical characterization of proteins involved in the synaptic vesicle membrane trafficking in the mammalian nerve terminal. The recent convergence of these approaches suggests that common mechanisms may underlie a wide variety of vesicle-mediated transport steps. We discuss the results that support this possibility and propose a model for synaptic vesicle docking and fusion that incorporates evolutionarily conserved elements that may be part of a constitutive fusion machinery and specialized elements that may mediate regulatory events that are specific to the process of neurotransmitter release.

Membrane trafficking along the exocytic and the endocytic pathways of eukaryotic cells is mediated by a series of vesicular intermediates. The fact that vesicular transport is vectorial suggests that each step is mediated by specific targeting, docking, and fusion events. These events are likely to involve protein components localized to the transport vesicle and target membrane as well as soluble factors. Although some components are expected to define the specificity of a particular transport step, others are likely to be common to multiple transport steps. An additional level of complexity is involved in the regulated secretory pathway, where the final step, exocytosis, is regulated by calcium or GTP. In recent years, a combination of approaches, which includes yeast genetics, in vitro reconstitution of transport, and biochemical characterization of vesicular intermediates, has led to the identification of a number of proteins that are involved in membrane trafficking. For example, NSF/sec18, a soluble protein required for membrane fusion at a number of steps along the exocytic and endocytic pathways, was identified by yeast genetics and in a mammalian in vitro assay (1–3).

In this review we will focus on several integral membrane proteins that may be involved in transport vesicle docking and fusion. Two approaches, the biochemical characterization of synaptic vesicles (the transport intermediate responsible for the regulated release of neurotransmitter from neurons) and yeast genetics, have provided results that suggest the mechanisms involved in transport vesicle docking and fusion may be evolutionarily conserved. The remarkable convergence of these two approaches provides great promise for future analysis of the machinery involved in membrane trafficking. We will briefly discuss these results and propose a model for synaptic vesicle docking and fusion that incorporates (i) common components that may be part of the constitutive machinery involved in many vesicular transport steps and (ii) a set of regulatory components that may confer calcium sensitivity to the specific processes involved in neurotransmitter release.

The relative abundance of synaptic vesicles in brain has made possible their biochemical characterization. Nearly all synaptic vesicle proteins so far identified are members of small gene families that are specific to neural or endocrine tissue. One exception is the protein VAMP (vesicle-associated membrane protein) (4–6) or synaptobrevin (7), an 18-kDa protein anchored to the cytoplasmic surface of the vesicle by a carboxy-terminal transmembrane domain. Although VAMP is predominantly expressed in the nervous system, a protein at least immunologically related to VAMP has been identified in adipocytes (8). In adipose cells, the VAMP-like protein is associated with vesicles that are responsible for the intracellular sequestration of the glucose transporter GLUT4. In response to insulin, these vesicles fuse with the plasma membrane, resulting in increased glucose transport into the cells. In Drosophila, one form of VAMP is expressed predominantly in the gut and malpighian tubules (9). Whether nonneuronal VAMP functions in membrane trafficking remains to be established. Although the extent of VAMP or VAMP-like protein expression outside the nervous system remains to be determined, these results raise the possibility that VAMP is a member of a family of proteins involved in different steps of vesicular transport.

If this is the case, one might expect to find VAMP-like proteins in simple eukaryotes, such as yeast. Indeed, several proteins have been identified in yeast that have domain structures similar to, and sequence homology with, VAMP. The yeast protein most like VAMP is SNC1. It shares 40% sequence identity with VAMP (10). SNC1 was isolated by its ability to suppress loss of one of the functions of CAP (cyclase-associated protein). CAP is a 526-amino acid protein component of the RAS-regulated adenylate cyclase complex in yeast. The amino-terminal 168 residues of CAP are sufficient for RAS to activate cyclase, whereas the carboxy-terminal 160 amino acids of CAP are required for survival in nutrient extremes. SNC1 suppresses only the loss of the carboxy-terminal function of CAP and only in yeast expressing an activated form of RAS2. Disruption of the SNC1 locus results in no apparent phenotype. However, a second VAMP homologue, SNC2, has been identified. Recent studies, including disruption of both SNC genes, suggest that the SNC proteins are both localized to post-Golgi vesicles and required for Golgi to plasma membrane trafficking (J. Gerst, personal communication). The relationship between the SNC proteins and RAS is not clear at this time.

It is clear however that part of the RAS superfamily, members of the rab family of GTP-binding proteins, is involved in the regulation of vesicular trafficking in yeast and higher eukaryotes (11). The rab family is now known to contain >20 members. Many of these molecules are localized to specific intracellular membrane compartments (12), including rab3 on the synaptic vesicle (13). Biochemical and genetic studies suggest that the rab family of low molecular weight GTP binding proteins may mediate vesicular membrane trafficking. For example, rab5, which is localized to the early endosome, regulates early endosome fusion in vitro (14) and in vivo (15). In yeast, two members of the rab family, YPT1 (16) and

Abbreviations: VAMP, vesicle-associated membrane protein; CAP, cyclase-associated protein; ER, endoplasmic reticulum.
SEC4 (17), have been identified. YPT1 and SEC4 are required for vesicular transport from the endoplasmic reticulum (ER) to the Golgi apparatus and from the Golgi apparatus to the plasma membrane, respectively. Several other proteins, involved in the regulation of vesicular trafficking in the early secretory pathway in yeast, interact genetically with YPT1. Two of these proteins, SLY12/BET1 (18, 19) and SLY2/SEC22 (19, 20), have a domain structure similar to, and limited sequence homology with, VAMP.

These observations suggest that members of the rab and VAMP families are involved in membrane trafficking at two stages of the yeast secretory pathway and in the nerve terminal (Fig. 1 and Table 1). The colocalization of these protein families (SEC4 and SNC on post-Golgi vesicles, rab 3 and VAMP on synaptic vesicles) and their potential interaction (detected genetically for YPT1 and SLY12/SLY2 in ER to Golgi transport) suggest that a rab protein interacting either directly or indirectly with a VAMP-like protein may be components of a conserved machinery on the surface of vesicular transport intermediates that participates in docking or fusion. That VAMP plays an important role in synaptic vesicle docking or fusion is supported by the recent observation that cleavage of VAMP by the zinc-endoprotease associated with tetanus and botulinum toxin is correlated with a block of neurotransmitter release (21).

Components of the docking and fusion machinery localized to the target membrane have been more difficult to identify. A candidate vesicle docking protein, syntaxin, has recently been identified in the nerve terminal (22). Syntaxin, a 35-kDa protein with a carboxyl-terminal membrane anchor, was identified by its ability to interact with the synaptic vesicle protein synaptotagmin (p65). Antibodies generated against syntaxin labeled axonal membranes, particularly at synaptic sites. These syntaxin antibodies were capable of immunoprecipitating or-conotoxin binding sites, suggesting an interaction of syntaxin with neuronal voltage-gated calcium channels. Based on these properties it was proposed that syntaxin is involved in the docking of synaptic vesicles near voltage-gated calcium channels in the presynaptic plasma membrane.

Recent studies of the yeast secretory pathway have raised the possibility that syntaxin is a member of a family of proteins involved in vesicular trafficking. Three genes have been identified in yeast that encode proteins with a carboxyl-terminal membrane anchor and displaying significant homology to syntaxin, primarily over a 70-amino acid segment near the membrane anchor. The first of these is SED5, a multicopy suppressor of loss of ERD2 function (23). ERD2 is the ER retention receptor that is thought to recognize the amino acid sequence HDEL and function in retrieval of ER resident proteins from the Golgi (24). SED5 is itself required for a functional secretory pathway. Another yeast protein with homology to syntaxin is PEP12 (25) (K. Becherer and E. Jones, GenBank sequence YSCPEP12P), which is required for proper targeting of proteins to the yeast vacuole, the functional equivalent of the lysosome in the secretory pathway of higher eukaryotes. The yeast protein with the highest level of homology to syntaxin is an open reading frame near the 5' end of the gene for fatty acid synthase (26). Genetic studies have revealed that this open reading frame is one of two genes, SSO1 and SSO2, that are capable of acting as multicopy suppressors of a temperature-sensitive allele of SEC1 (S. Keranen, personal communication). SEC1 is a hydrophilic protein required for proper transport from the Golgi to the plasma membrane (27). SEC1 is itself a member of a family of proteins involved in membrane trafficking (28). SLY1 (19) and SLP1 (29), two proteins with sequence similarity to SEC1, are required for transport from the ER to the Golgi and from the Golgi to the vacuole, respectively. No mammalian homologues in the SEC1 family have yet been described.

These findings suggest that members of several families of molecules (VAMP, rab, syntaxin, and SEC1) may be required for membrane transport in the nerve terminal and at several stages of the secretory pathway in yeast (Fig. 1 and Table 1). That the three syntaxin-like yeast proteins are involved at distinct stages of the secretory pathway, coupled with the proposed role of syntaxin in synaptic vesicle docking, raises the possibility that these proteins function as target membrane-specific receptors for transport vesicles. Based on these results we suggest that members of membrane protein families localized to the transport vesicle (VAMP and rab) and target membrane (syntaxin) are components of the molecular machinery that mediates specific docking and fusion reactions in

Fig. 1. Yeast proteins required at distinct positions in the secretory pathway. The members of four protein families involved in the yeast secretory pathway (syntaxin, VAMP, rab, and SEC1), and their proposed sites of action, are indicated.
many vesicular trafficking processes. Although it is attractive to envisage a direct or indirect connection between the two membranes involving these components, such a connection has not been demonstrated. It is of interest that as one moves through the yeast secretory pathway toward the plasma membrane, the vesicular trafficking components become more homologous to the nerve terminal components. Thus VAMP is most like SNC, rab3 is most like SEC4, and syntaxin is most like SSO. This suggests the interesting possibility that the machinery responsible for secretion of neurotransmitter is likely to have evolved directly from the trafficking machinery that mediates delivery of vesicles from the Golgi to the plasma membrane.

Our current model for synaptic vesicle docking and fusion is presented in Fig. 2. This model incorporates the molecules that have counterparts in the yeast secretory pathway as well as potential regulatory elements likely to be specific to neurotransmitter release. The central elements of the model are the rab, VAMP, and syntaxin proteins, which, as discussed above, may comprise a portion of a conserved constitutive docking or fusion machinery. However, the process of synaptic vesicle fusion is not constitutive but rather is tightly regulated by calcium. One candidate for the calcium-sensitive regulator is the synaptic vesicle protein synaptotagmin (30, 31). Synaptotagmin is composed of an amino-terminal intravesicular domain, a single transmembrane domain, and a cytoplasmic domain made up of two repeats homologous to the C2 regulatory domain of protein kinase C. These C2-homologous domains have been implicated in the calcium-dependent interaction of synaptotagmin with membrane phospholipids (32). Thus, it has been proposed that synaptotagmin acts as a calcium sensor on the surface of the synaptic vesicle. Indeed, the interaction of synaptotagmin with syntaxin coupled with the interaction of syntaxin with voltage-gated calcium channels (22) place synaptotagmin in an ideal position to respond to calcium. The observation that catecholamine release from PC12 cells can be blocked by microinjection of either antibodies against synaptotagmin or soluble fragments of synaptotagmin supports the possibility that this protein is an important regulator of neurotransmitter release (33).

Another protein that interacts with synaptotagmin is the α-latrotoxin receptor (34). α-Latrotoxin, a component of black widow spider venom, binds to its plasma membrane-localized receptor and induces the efficient and calcium-independent fusion of synaptic vesicles with the presynaptic plasma membrane (35). The α-latrotoxin receptor is a member of a family of proteins known as the neurexins (36). The neurexins have a large extracellular domain, a single transmem-

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**Table 1. Families of membrane proteins involved in the regulation of vesicular trafficking**

<table>
<thead>
<tr>
<th>Family</th>
<th>Yeast homologue and transport step</th>
<th>Nerve terminal homologue</th>
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</thead>
<tbody>
<tr>
<td>rab</td>
<td>YPT1</td>
<td>ER to Golgi</td>
</tr>
<tr>
<td>SEC4</td>
<td></td>
<td>Golgi to plasma membrane</td>
</tr>
<tr>
<td>VAMP</td>
<td>BET1/SLY12</td>
<td>ER to Golgi</td>
</tr>
<tr>
<td>SEC22/SLY2</td>
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<tr>
<td>SNC1 and SNC2</td>
<td></td>
<td>Golgi to plasma membrane</td>
</tr>
<tr>
<td>Syntaxin</td>
<td>SED5</td>
<td>ER to Golgi</td>
</tr>
<tr>
<td></td>
<td>PEP12</td>
<td>Golgi to vacuole</td>
</tr>
<tr>
<td></td>
<td>SSO1 and SSO2*</td>
<td>Golgi to plasma membrane</td>
</tr>
</tbody>
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*Open reading frame near FAS2.

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**Fig. 2.** Proposed model for the molecular machinery involved in synaptic vesicle docking and fusion. Components that have conserved counterparts in yeast (syntaxin, VAMP, and rab3) form a constitutive machinery that is regulated by nerve terminal specific components (synaptotagmin, neurexin, and calcium channels). For a detailed discussion of the model, see the text. Interactions between the proteins (indicated by double-headed arrows) either have been detected in biochemical studies (syntaxin–synaptotagmin, syntaxin–calcium channel, and synaptotagmin–neurexin) or are inferred from genetic interactions in yeast (VAMP–rab3). The interactions may be either direct or mediated through additional molecules. The potential sites of action of α-latrotoxin and tetanus and botulinum toxins are indicated.
brane domain, and a short intracellular region. At least three neurexin genes undergo extensive alternative RNA splicing, resulting in a large number of isoforms. The interaction between synaptotagmin and neurexins would be predicted to involve the cytoplasmic domains of the two proteins. We propose that a neurexin, upon binding of α-latrotoxin, induces a change in the conformation of synaptotagmin or other proteins that mimics that induced by an influx of calcium.

The release of neurotransmitter from the presynaptic nerve terminal occurs very rapidly following the influx of calcium (37). This suggests that the machinery responsible for synaptic vesicle fusion may be preassembled or "primed" in a prefusion complex. The conserved components depicted in Fig. 2 may represent a portion of such a complex. We suggest that the synaptotagmin/neurexin complex may function as a negative regulator (or inhibitor) of the constitutive fusion machinery. This inhibition could be relieved either by the influx of calcium during nerve stimulation or by α-latrotoxin binding. PC12 cells selected for a lack of synaptotagmin by complement-mediated cell killing are still capable of regulated release of neurotransmitter (38). This result is consistent with the idea that synaptotagmin is a negative regulator and that it is not absolutely required for membrane fusion. If this is the case, an additional calcium-dependent step would be required to explain the observation that secretion from the synaptotagmin-depleted PC12 cells is still regulated rather than constitutive.

The final step in neurotransmitter secretion, as in other vesicular transport events, is membrane fusion. The earliest detectable event in the fusion of biological membranes in other systems (viral spike protein-mediated cell fusion and mast cell degranulation) is the formation of a fusion pore (39). The molecular nature of this fusion pore, which may reflect a common fusion intermediate, remains to be established. Several components of the synaptic vesicle membrane may play a role in the fusion process. Synaptophysin (40, 41), and the related synaptoporin (42), has four transmembrane domains and is present as a homooligomeric complex (43, 44). Upon reconstitution in artificial bilayers, synaptophysin can form a transmembrane channel (45). It has therefore been proposed that synaptophysin, in conjunction with an as yet undefined protein in the plasma membrane with similar properties, forms a pore across both bilayers (similar to a gap junction) and that this pore represents the first step in membrane fusion. If synaptophysin functions in the formation of a fusion pore, other (related) proteins might fulfill this function in the fusion reaction at other vesicular trafficking steps. In spite of extensive screening, no synaptophysin homologues have yet been identified in other systems. The generality of synaptophysin's proposed function awaits confirmation in other systems.

Another component of the synaptic vesicle membrane is SV2 (42). cDNA clones encoding two forms of SV2 have been identified (47-49). The SV2 proteins are composed of 12 membrane-spanning domains and display homology to bacterial drug and nutrient transporters. It has been suggested that these proteins play a role in the movement of molecules, such as neurotransmitter, across the synaptic vesicle membrane. However, the wide distribution of SV2 expression in the nervous system suggests that its function is not limited to a particular class of neurotransmitter. Another possible function for a large protein with multiple membrane-spanning domains such as SV2 might be in the process of membrane fusion. As with synaptophysin, the role of SV2, if any, in synaptic vesicle fusion remains to be established.

The processes of synaptic vesicle docking and fusion are potential targets for the physiological modulation of synaptic transmission. Such modulation of synaptic efficacy is likely to contribute to such processes as learning and memory. A common mechanism by which protein function can be modulated is protein phosphorylation. Several components of the proposed synaptic vesicle docking and fusion complex are kinase substrates. For example, in vitro synapsin and synaptotagmin are substrates for casein kinase II (50). In addition, casein kinase II appears to interact with synaptotagmin. Interestingly, casein kinase II activity increases in hippocampal slices following the induction of long-term potentiation (51). A number of rab proteins have been shown to be phosphorylated in a cell cycle-dependent manner (52, 53), suggesting that the function of these proteins might be regulated by phosphorylation. Finally, synaptophysin is phosphorylated on a tyrosine residue by pp60v-src (54). The physiological consequences of these phosphorylation events have not been established.

The multiple interactions among the synaptic vesicle and plasma membrane proteins illustrated in Fig. 2 may provide a scaffold upon which the complete vesicle fusion machinery is assembled. Additional components might include soluble factors implicated in vesicle fusion reactions such as NSF (55), SNAPs (soluble NSF attachment proteins; ref. 56), annexins (57), and p145 (58) or membrane proteins, both on the synaptic vesicle and on the plasma membrane. A number of interactions among synaptic vesicle membrane proteins have been detected, including interactions between synaptophysin and VAMP and between synaptotagmin and SV2 (59). It is possible that such interactions contribute to the vesicle docking and fusion machinery. The challenge for the future will be to establish the precise role of each protein, whether as direct mediators or regulators of neurotransmitter release. The combination of yeast genetics, biochemistry of synaptic proteins, and in vitro reconstitution of vesicular transport should continue to provide insight into the conserved and unique components required for multiple membrane trafficking pathways.

Many of the fundamental biochemical processes essential for living organisms are conserved across most species. Energy metabolism, information flow through DNA and RNA, and protein synthesis are examples of this conservation. Thus, perhaps we should not be surprised that homologues of molecules that mediate membrane flow in one of the simplest organisms, yeast, are critical components of the most complex process exhibited by living organisms, that of mammalian brain function. We do not intend to imply that when we understand membrane flow in yeast that we will completely understand the molecular basis of synaptic transmission. Certainly all cellular processes have adapted a fundamental set of biochemical principles to suit specific evolutionary pressures. Since the conservation of molecules involved in vesicular trafficking in yeast has proven useful for analyzing synaptic vesicle trafficking in the mammalian nerve terminal, so the regulatory mechanisms that modulate protein transport in yeast may well provide clues as to molecular mechanisms by which higher organisms modulate neurotransmitter release for the purpose of learning and memory.

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