Synaptic function*

Janet Richmond[§], Department of Biological Sciences, University of Illinois, Chicago, IL 60612 USA

Table of Contents

1. Overview 1
2. Exocytosis 3
 2.1. Docking 3
 2.2. Priming 5
 2.3. Ca-sensing 6
 2.4. Fusion 6
3. Endocytosis 7
 3.1. Recruitment 7
 3.2. Budding 7
 3.3. Fission 8
 3.4. Uncoating 8
4. Summary 9
5. References 9

Abstract

***C. elegans* has emerged as a powerful genetic model organism in which to study synaptic function. Most synaptic proteins in the *C. elegans* genome are highly conserved and mutants can be readily generated by forward and reverse genetics. Most *C. elegans* synaptic protein mutants are viable affording an opportunity to study the functional consequences *in vivo*. Recent advances in electrophysiological approaches permit functional analysis of mutant synapses *in situ*. This has contributed to an already powerful arsenal of techniques available to study synaptic function in *C. elegans*. This review highlights *C. elegans* mutants affecting specific stages of the synaptic vesicle cycle, with emphasis on studies conducted at the neuromuscular junction.**

1. Overview

C. elegans is a powerful genetic system to study the molecular basis of synaptic function. Using behavioral and pharmacological selection criteria, genetic screens have identified many key proteins required for synaptic transmission (Brenner, 1974). With few exceptions synaptic proteins identified in *C. elegans* are highly conserved offering an opportunity to study universally important proteins in a relatively simple system. One of the great

*Edited by Erik M. Jorgensen and Joshua M. Kaplan. Last revised April 19, 2005. Published December 7, 2007. This chapter should be cited as: Richmond, J. Synaptic function (December 7, 2007), *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.69.1, http://www.wormbook.org.

Copyright: © 2007 Janet Richmond. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

§To whom correspondence should be addressed. E-mail: jer@uic.edu

advantages of *C. elegans* over other organisms is that most mutants affecting synaptic transmission are viable and can be propagated by self-fertilization. This provides an opportunity to study the functional consequences of eliminating individual synaptic proteins in an intact organism.

The *C. elegans* hermaphrodite nervous system contains only 302 neurons, which form about 7000 chemical synapses, 2000 of which occur at neuromuscular junctions (NMJs) (White et al., 1986). At excitatory NMJs acetylcholine (ACh) is released leading to muscle contraction, whereas inhibitory NMJs release γ-aminobutyric acid (GABA) and cause muscle relaxation. During locomotion, the command circuitry driving NMJ exocytosis pairs muscle contraction on one side of the body with simultaneous relaxation in contralateral muscles to establish sinusoidal body bends. *C. elegans* mutants defective in synaptic transmission often exhibit a locomotory phenotype resulting from disrupted signaling at the NMJs. Recent advances in electrophysiological approaches permit functional analysis of individual NMJs in *C. elegans* (Richmond and Jorgensen, 1999; Richmond et al., 1999) (Francis et al., 2003). This has contributed to an already powerful arsenal of techniques available to study synaptic function. This review highlights *C. elegans* mutants affecting specific stages of the synaptic vesicle cycle (Table 1), with emphasis on studies conducted at the NMJ.

Table 1. *C. elegans* mutants affecting specific stages of the synaptic vesicle cycle.

Gene	Molecular description	Putative function in *C. elegans*	References
dgk-1	Diacylglycerol (DAG) Kinase	Converts DAG to phosphatidic acid, reducing *unc-13* activity and inhibiting exocytosis	Nurrish et al., 1999
dyn-1	Dynamin	Fission of vesicles during endocytosis	Clark et al., 1997
eat-11	GBP-2, a β_5 homolog	Regulator of RGSs EGL-10 and EAT-16	Robatzek et al., 2001; van der Linden et al., 2001
eat-16	RGS (regulator of G-protein signaling)	Negatively regulates EGL-30 activity	Hajdu-Cronin et al., 1999
egl-8	Phospholipase C	Produces DAG from PIP_2 leading to increased *unc-13* activity	Lackner et al., 1999
egl-10	RGS (regulator of G-protein signaling)	Negatively regulates GOA-1 activity	Koelle and Horvitz, 1996
egl-30	G-protein alpha subunit, $G\alpha_q$	Activates PLC, increasing DAG leading to *unc-13*-dependent priming	Brundage et al., 1996; Lackner et al., 1999
ehs-1	Eps15 homolog	Functions in endocytosis, precise role unknown	Salcini et al., 2001
goa-1	G-protein alpha subunit, $G\alpha_o$	Mediates serotonin inhibition of exocyotsis by decreasing *unc-13* activity	Mendel et al., 1995; Ségalat et al., 1995; Nurrish et al., 1999
lin-10	MINT (Munc18-interacting molecule)	Unknown function	Whitfield et al., 1999
rab-3	Rab3, small GTPase	Unknown function, vesicle localization	Nonet et al., 1997
ric-4	SNAP-25, SNARE protein	Vesicle fusion	Nguyen et al., 1995; Nonet, 1999
snb-1	Synaptobrevin, SNARE protein	Vesicle fusion	Nonet et al., 1998
snt-1	Synaptotagmin	Ca^{2+}-sensor in exocytosis/AP-2 binding partner in endocytosis	Jorgensen et al., 1995; Nonet et al., 1993

Gene	Molecular description	Putative function in *C. elegans*	References
unc-2	α subunit of voltage-gated Ca^{2+} channel	Evoked release, Ca^{2+} influx at terminals	Nonet et al., 1998; Schafer and Kenyon, 1995; Richmond et al., 1999
unc-10	Rim (rab (and Munc-13) interacting protein)	Priming	Koushika et al., 2001; Nguyen et al., 1995
unc-11	AP180 adaptor protein	Clathrin recruitment	Nonet et al., 1999
unc-13	Phorbol ester/DAG binding protein	Priming, promoting open syntaxin	Maruyama and Brenner, 1991; Richmond et al., 1999; Richmond et al., 2001
unc-18	Syntaxin binding protein of the sec-1/Munc18 family	Role controversial, involved in vesicle docking but also implicated in priming and fusion	Gengyo-Ando et al., 1993; Gengyo-Ando et al., 1996; Hosono et al., 1992; Weimer et al., 2003
unc-26	Synaptojanin, a phosphoinositide phosphatase	Endocytic budding, fission, clathrin uncoating	Harris et al., 2000; Nguyen et al., 1995; Schuske et al., 2003
unc-31	CAPS (calcium activated protein for secretion)	Required for peptidergic granule exocytosis	Avery et al., 1993
unc-57	endophilin	Required to target synaptojanin (UNC-26) to vesicles, implicated in vesicle endocytic budding, fission and clathrin uncoating	Schuske et al., 2003
unc-64	Syntaxin, SNARE protein	Vesicle fusion	Saifee et al., 1998

2. Exocytosis

The life cycle of a synaptic vesicle is illustrated in Figure 1. During exocytosis neurotransmitter filled vesicles are mobilized and dock with the plasma membrane, where they undergo a priming process in which they become fusion competent. A calcium-signal then triggers vesicle fusion with the plasma membrane and neurotransmitter is released into the synaptic cleft. In the following sections, *C. elegans* proteins implicated in each stage of the exocytotic process will be discussed.

2.1. Docking

Synaptic vesicle docking is perhaps the least understood stage of the vesicle cycle in part due to the difficulties of assaying the docking process. In cultured vertebrate neurons, fluorescently labeled synaptic vesicles have been observed to approach the plasma membrane, and become stationary for periods of time, prior to either detaching or fusing (Murthy and Stevens, 1999; Zenisek et al., 2000). Thus docking may represent a reversible step of membrane association prior to priming. FM-dye uptake studies at the frog neuromuscular junction suggest vesicles that contribute to the readily releasable pool are mobilized to the active zone from disparate regions of the terminal immediately prior to release (Rizzoli and Betz, 2004). These data challenge the widely held notion that vesicles in the readily releasable pool, reside at the active zone in a docked and primed state awaiting a calcium signal to fuse. In *C. elegans* and other systems attempts to define the docked vesicle pool have relied on conventional electron microscopy (EM). At the EM level, a subset of synaptic vesicles are observed to be contacting the plasma membrane in the vicinity of the presynaptic specialization (reviewed in Zucker, 1996; Robinson and Martin, 1998). It is unclear which vesicles in the vicinity of the active zone represent docked vesicles, which have transitioned to a primed state and whether these vesicles do indeed constitute the releasable pool of vesicles. In this regard, it is hoped that efforts to better define the functional relevance of vesicle position will emerge by applying recently improved high pressure freeze EM and immunoEM in *C. elegans* (Rostaing et al., 2004) to various mutants implicated in docking and priming.

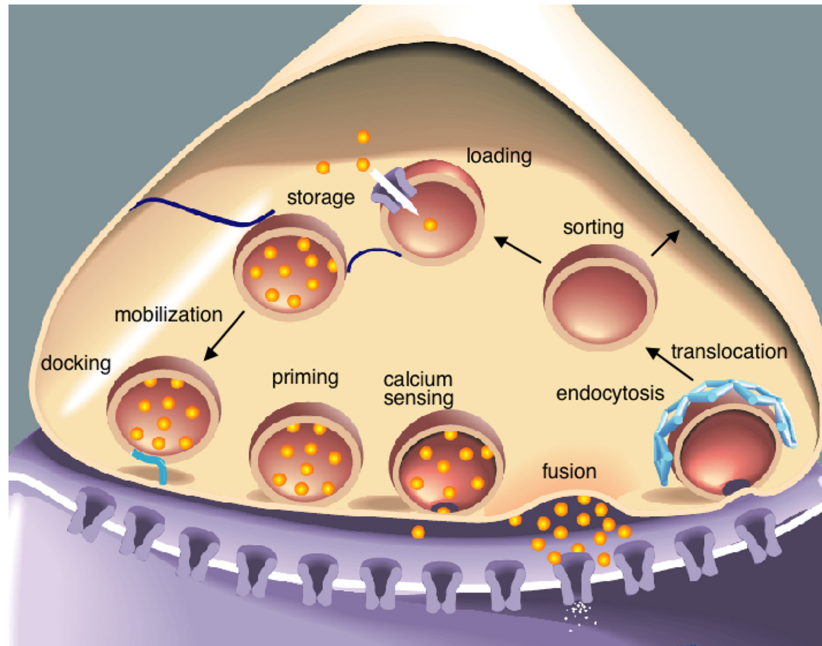

Figure 1. The synaptic vesicle cycle. Synaptic vesicles contain neurotransmitter transporters that load vesicles with neurotransmitter. Mobilized vesicles translocate to the terminal plasma membrane where they selectively dock close to the active zone. Docked vesicles then undergo a priming step, during which vesicles become fusion competent. A rise in intracellular calcium binds to calcium sensors triggering vesicle fusion and release of neurotransmitter into the synaptic cleft. Neurotransmitter can then bind and activate receptors. Following full-fusion, vesicle proteins and membrane are retrieved by clathrin-mediated endocytosis.

The requirement for membrane-associated vesicles to dock near active zones implies that docking is mediated via molecular recognition machinery. It was originally proposed that the SNARE (soluble NSF attachment protein receptors) proteins synaptobrevin, syntaxin and SNAP-25 could constitute this docking complex (Sollner et al., 1993). In *C. elegans* the vesicle associated SNARE synaptobrevin is encoded by *snb-1*, and the plasma membrane associated SNAREs syntaxin and SNAP-25 are encoded by *unc-64* and *ric-4*, respectively. SNARE proteins are essential for *C. elegans* viability as mutants in all three arrest at the L1 larval stage, limiting the characterization of the synaptic phenotype (Nonet et al., 1998; Saifee et al., 1998). Therefore, it remains to be determined whether these mutants have a docking defect. However, perturbation of SNARE function in *Drosophila*, squid or mice fails to block vesicle docking (Hunt et al., 1994; Broadie et al., 1995; Deak et al., 2004).

One of the first vesicle-associated proteins to be implicated in vesicle docking was synaptotagmin (Reist et al., 1998). A detailed morphometric analysis of *Drosophila* synaptotagmin mutants revealed a dramatic decrease in the number of vesicles associating with the plasma membrane near the presynaptic specialization compared to wildtype synapses. A similar requirement for synaptotagmin was demonstrated in an *in vitro* docking assay of pituitary cell dense core granules. Intriguingly, the interaction between synaptotagmin and plasma membrane associated SNAP-25 was implicated in this docking mechanism (Chieregatti et al., 2002). Mutants in *C. elegans* synaptotagmin1 (*snt-1*) have not been reported to have a vesicle docking defect although these mutants clearly are defective in synaptic function. Later in the chapter we will discuss evidence implicating synaptotagmin in other stages of the vesicle cycle. namely calcium-sensing and endocytosis (Jorgensen et al., 1995).

Another contender for a vesicle docking factor is Rab3, a small GTPase that associates with synaptic vesicles in *C. elegans* (Nonet et al., 1997). *rab-3* mutants are only mildly uncoordinated and result in subtle synaptic defects based on electropharyngeogram recordings. At the EM level *rab-3* mutants exhibit vesicle dispersal at synapses, suggesting that *rab-3* could be involved in vesicle localization or docking. The latter possibility is supported by the observation that overexpression of mammalian Rab3A in PC12 cells enhances granule docking (Martelli et al., 2000). If vesicle associated Rab3 plays a role in docking, it must interact with proteins associated with the active zone plasma membrane. One candidate for a docking partner is Rim.

Rim, first identified in mammals as a Rab3 interacting protein (Rab3 interacting molecule; Wang et al., 1997) is encoded by *unc-10* in *C. elegans*. *unc-10* localizes to a subdomain of the presynaptic terminal, suggesting that like vertebrate Rim, *unc-10* is associated with the presynaptic specialization. Consistent with a synaptic role, *unc-10*

mutants exhibit sluggish locomotion (Brenner, 1974) aldicarb-resistance (Nguyen et al., 1995) and decreased exocytosis. Both *rab-3* and *unc-10* mutants exhibit a similar reduction in vesicle density at the synapse (Nonet et al., 1997). However, in *rab-3* mutants the vesicles are dispersed to distal regions of the synaptic varicosity, whereas in *unc-10* mutants there appear to be just fewer vesicles. In addition, the synaptic defects of *unc-10* mutants are more severe than those of *rab-3* mutants (Nonet et al., 1997), as well as mutants in the Rab3 nucleotide exchange factor (AEX-3; Iwasaki et al., 1997) and the Rab3 effector rabphilin (Staunton et al., 2001). These observations suggest *unc-10* plays a wider role than Rab3 for example, a putative role in vesicle priming is discussed below.

Recently, *unc-18* a panneuronal, cytosolic protein homologous to yeast Sec1p, and mammalian Munc18 (hence referred to as the SM protein family) (Gengyo-Ando et al., 1993) has been implicated in vesicle docking. In wild-type *C. elegans*, acetylcholinesterase inhibitors such as aldicarb cause ACh to accumulate in the synaptic cleft resulting in muscle hypercontraction and ultimately death. *unc-18* mutants are uncoordinated and resistant to aldicarb suggesting that ACh release is defective (Brenner, 1974; Hosono et al., 1992). This defect can be rescued by expressing either *unc-18* or Munc18-1 in *C. elegans* (Gengyo-Ando et al., 1996). Electrophysiological recordings from *unc-18* mutant NMJs demonstrate an 80% loss of evoked and endogenous synaptic activity. In Munc18-1 knockout mice, the synaptic phenotype is even more severe, with complete loss of synaptic activity and neuronal cell death (Verhage et al., 2000). However, chromaffin cells lacking Munc18-1 exhibit reduced release similar to *unc-18* mutants. In chromaffin cells, this exocytotic defect corresponds to fewer docked secretory granules (Voets et al., 2001). A similar reduction in the proportion of docked vesicles has been observed in *unc-18* mutants (Weimer et al., 2003), thus SM proteins may serve a docking function.

SM proteins have a high affinity interaction with the membrane bound protein, syntaxin. If this interaction were important for vesicle docking, one would predict that *C. elegans* syntaxin nulls (*unc-64* mutants) would exhibit a similar docking defect. However, the L1 larval arrest of *unc-64* mutants makes EM challenging and the possibility of remaining maternal syntaxin a possibility.

Exactly how the plasma membrane docking platform links to incoming synaptic vesicles is unknown. The Munc18-interacting protein, Mint, has been postulated to act as a bridging protein between Munc18 and the vesicle (Okamoto and Sudhof, 1997). Mints could interact with the vesicle via a PIP2 binding domain. *C. elegans* Mint, encoded by the *lin-10* gene, is expressed in neurons. *lin-10* null mutants have been shown to disrupt the post-synaptic localization of neuronal glutamate receptors (*glr-1*; Rongo et al., 1998) however they do not exhibit any obvious neuromuscular defects based on behavioral criteria (Whitfield et al., 1999).

2.2. Priming

Priming refers to the molecular events following vesicle docking that lead to vesicle fusion competence. During priming SNARE proteins resident on the plasma membrane (syntaxin and SNAP-25) and on the vesicle membrane (synaptobrevin) form a tight complex that brings the vesicle into close apposition with the plasma membrane (Chen and Scheller, 2001). SNARE complex formation occurs between α-helices of the three SNARE proteins, SNAP-25 contributing two of the fours helices in the α-helical bundle (Sutton et al., 1998). Formation of SNARE complexes is considered an essential priming reaction, as evidenced by the correlation between the size of the readily releasable pool and level of SNARE complex assembly (Lonart and Sudhof, 2000).

The presynaptic protein *unc-13* has emerged as a key priming factor. *unc-13* mutants were originally identified based on paralyzed phenotypes (Brenner, 1974; Rose and Baillie, 1980). *unc-13* mutants are also aldicarb-resistant (Nguyen et al., 1995) but have normal responses to the muscle ACh receptor agonist levamisole, indicating the neurotransmission defect is presynaptic. Consistent with a synaptic role, *unc-13* is highly enriched at presynaptic terminals (Kohn et al., 2000). Electrophysiological recordings of evoked and endogenous synaptic activity at *unc-13* mutant NMJs confirm the behavioral observations (Richmond et al., 1999). Evoked responses in the most severe allele *unc-13*(*s69*) are completely abolished and endogenous synaptic events are virtually eliminated. This synaptic defect appears to be downstream of docking because vesicles in contact with the membrane accumulate in *unc-13* mutant terminals. Reduced hyperosmotic saline responses, a measure of the readily releasable pool of vesicles, also indicate that vesicles are fusion incompetent in *unc-13* mutants. Mutants of the mouse and *Drosophila* homologs of *unc-13*, Munc13-1 and Dunc-13 show similar priming defects (Aravamudan et al., 1999; Augustin et al., 1999), although in the case of mammalian synapses there is partial redundancy between Munc13-1 and Munc13-2 (Varoqueaux et al., 2002).

The observation that Munc13 binds to the N-terminal of syntaxin led to the proposal that *unc-13* may promote the open configuration of syntaxin that is required for SNARE complex formation (Betz et al., 1997; Brose et al.,

2000). Syntaxin adopts a default closed conformation in solution, in which the SNARE binding motif (H3 domain) is occluded (Dulubova et al., 1999). This model was tested by introducing point mutations into *C. elegans* syntaxin (*unc-64*) that render syntaxin constitutively open (Dulubova et al., 1999) and assessing whether open syntaxin could bypass the requirement for *unc-13*. Constitutively open syntaxin was able to rescue evoked release in *unc-13* mutants, supporting a role for *unc-13* in promoting SNARE complex formation (Richmond et al., 2001).

unc-13 has C2 domains implicated in calcium and protein binding reactions as well as a C1 diacylglycerol (DAG) binding domain (Maruyama and Brenner, 1991; Richmond et al., 1999). Binding of phorbol ester to the C1 domain of Munc13 has been shown to cause Munc13 translocation to the plasma membrane suggesting that the C1 domain is involved in localization and possibly activation of Munc13 (Betz et al., 1998). In *C. elegans*, several G-protein cascades that regulate the level of synaptic transmission (Miller et al., 1999) target the *unc-13* C1 domain. Specifically, DAG production through a Gαq (EGL-30) and phospholipase Cβ (EGL-8) pathway leads to the accumulation of *unc-13* at *C. elegans* synapses and an enhancement of release (Lackner et al., 1999). Furthermore, phorbol ester stimulated neurotransmission assessed by aldicarb sensitivity, is largely blocked by a point mutation in the *unc-13* C1 domain that prevents DAG binding. Conversely, Gαo (GOA-1) activated by serotonin causes a reduction in neurotransmitter release, which acts by limiting DAG levels and functions in concert with a DAG kinase (*dgk-1*) that converts DAG to phosphatidic acid, thereby reducing *unc-13* activity (Nurrish et al., 1999). The activity of both EGL-30 and GOA-1 are themselves controlled by the RGS (regulators of G-protein signaling) proteins, EAT-16 (Hajdu-Cronin et al., 1999) and EGL-10 (Koelle and Horvitz, 1996) respectively, interacting with the G-beta subunit EAT-11 (Robatzek et al., 2001; van der Linden et al., 2001).

A supporting role for the *unc-13* interacting protein Rim in priming has also been demonstrated (Koushika et al., 2001). Rim synaptic function is completely restored in *unc-10* mutants expressing open syntaxin suggesting that *unc-10* acts in the *unc-13* pathway (Koushika et al., 2001). Although the precise mechanism for Rim function remains to be elucidated these data suggest a model in which Rim may signal the arrival of a synaptic vesicle through a Rab interaction and promote priming via *unc-13*.

2.3. Ca-sensing

Vesicle fusion occurs in response to a local rise in intracellular calcium within nerve terminals. The source of calcium at *C. elegans* NMJs appears to be, in part, provided via voltage-gated calcium channels containing the UNC-2 alpha subunit, as *unc-2* mutants have reduced evoked synaptic responses (Richmond et al., 2001). Other calcium channel subunits that may also contribute to the calcium signaling at the synapse include EGL-19, an L-type alpha1 subunit expressed in both neurons and muscle (Lee et al., 1997), UNC-36 which based on genetic evidence is likely to be an auxiliary subunit of the UNC-2 channel (Schafer et al., 1996) and two novel calcium channel alpha subunits (NCA-1 and NCA-2) that are neuronally expressed in *C. elegans* (Hamming et al, 2002., WCWM abstract 203). The *C. elegans* rynaodine receptor UNC-68 may also act as a source of calcium for vesicle exocytosis (Wang et al, 2003 IWM abstract 181). A large body of evidence points to a calcium-sensing role for the vesicle associated protein synaptotagmin (Geppert et al., 1994; Littleton et al., 1993; Littleton et al., 1994). In *C. elegans* mutants in the gene encoding synaptotagmin *snt-1*, are severely uncoordinated and exhibit synaptic transmission defects (Nonet et al., 1993), however *snt-1* appears to have an additional role in vesicle endocytosis (see below), complicating the interpretation of the exocytotic defect (Jorgensen et al., 1995).

2.4. Fusion

Within milliseconds of calcium entry, primed synaptic vesicles fuse with the plasma membrane releasing neurotransmitter into the synaptic cleft. Evidence suggests that the SNARE proteins, syntaxin, SNAP-25 and synaptobrevin mediate vesicle fusion. These proteins form a ternary SNARE complex via a four alpha helical bundle. Vesicle associated synaptobrevin contributes one alpha helix, two alpha helices are contributed by the plasma membrane associated protein SNAP-25 and the integral plasma membrane protein syntaxin provides the fourth alpha helix. SNARE complex assembly brings the vesicle membrane into close apposition with the plasma membrane (Hanson et al., 1997) promoting lipid mixing leading to vesicle fusion. In other systems, perturbations that affect any of the SNARE proteins, such as cleavage by clostridial toxins or antibodies directed against SNAREs, inhibit fusion (Chen et al., 1999; Chen et al., 2001; Xu et al., 1999). Likewise, vesicle fusion is completely abolished in *Drosophila* syntaxin null mutants (Aravamudan et al., 1999). Furthermore, reconstituted vesicles containing only SNARE proteins are capable of fusion (Weber et al., 1998). Likewise *C. elegans* mutants that disrupt any of the three SNARE proteins (*snb-1*, *unc-64* and *ric-4*) produce early larval lethality, suggesting that as in other organisms there is an essential role for SNARE proteins in synaptic release (Nonet et al., 1998; Saifee et al., 1998).

3. Endocytosis

Following fusion, vesicle proteins and membrane are retrieved, sorted and reformed into synaptic vesicles for subsequent rounds of exocytosis. A major pathway for the retrieval of fused synaptic vesicles is thought to be clathrin-mediated endocytosis (Figure 1). This process requires vesicle membrane to be targeted for internalization through the recruitment of clathrin adaptors (AP2 and AP180) which in turn bind clathrin, resulting in formation of a clathrin-coated bud, fission of the coated vesicle, uncoating and sorting back to the vesicle pool. Proteins implicated in clathrin-mediated endocytosis have been identified through a combination of genetic screens and biochemical studies for interacting proteins (De Camilli and Takei, 1996; Harris et al., 2001).

3.1. Recruitment

During recruitment, adaptor proteins including AP-2 and AP180 are recruited to the fused vesicle membrane via interactions with phosphoinositides and specific vesicle-associated proteins such as synaptotagmin and synaptobrevin. For example, biochemical experiments have established that the μ2 and α-adaptin subunits of the heterotetrameric AP-2 complex bind to the C2B domain of synaptotagmin (Fukuda et al., 1995; Haucke and De Camilli, 1999; Haucke et al., 2000; Zhang et al., 1994). In *C. elegans* studies of the synaptotagmin mutant *snt-1* support a role for synaptotagmin in vesicle recycling (Jorgensen et al., 1995). *snt-1* mutants are uncoordinated and exhibit impaired synaptic transmission (Nonet et al., 1993). At the ultrastructural level, the number of vesicles is depleted at synaptic terminals, suggesting vesicle recycling is impaired. Consistent with this interpretation the vesicle associated protein, synaptobrevin becomes trapped in the plasma membrane in *snt-1* mutants. Elegant experiments photoinactivating *Drosophila* synaptotagmin following exocytosis also demonstrate that synaptotagmin is required for vesicle endocytosis (Poskanzer et al., 2003). Similarly, dissection of exocytosis and endocytosis in mouse synaptotagmin1 mutants, reveals a defect in endocytosis (Nicholson-Tomishima and Ryan, 2004). The lack of recycled vesicles and the mislocalization of the vesicle SNARE synaptobrevin are both likely to contribute to the loss of synaptic transmission in *snt-1* mutants although this does not preclude an additional role for *snt-1* in the calcium-sensing step of exocytosis.

AP180 is also implicated in the recruitment of endocytic cargo proteins. UNC-11, the *C. elegans* AP180 homolog is enriched at synapses and *unc-11* mutants exhibit synaptic defects and the specific mislocalization of synaptobrevin (Nonet et al., 1999). Thus, UNC-11 appears to recruit synaptobrevin for endocytosis, although a direct interaction between the two proteins has not been demonstrated. Unlike *snt-1* mutants, endocytosis still occurs in *unc-11* mutants based on the continued presence of vesicles, although vesicle diameters are increased. Therefore UNC-11 may regulate endocytosis of specific cargo proteins such as SNB-1.

Evidence that the AP2 complex is required for endocytosis in *C. elegans* is less well substantiated. Perturbations of the AP-2 subunits α-adaptin and β-adaptin by RNAi result in embryonic lethality. RNAi against the σ2 (AP17) subunit gene produces a dumpy morphology similar to a mutation in the μ2 (AP50) subunit encoding gene *dpy-23* (Grant and Hirsh, 1999; Harris et al., 2001). Unpublished ultrastructural analysis of *dpy-23* mutant synapses shows a severe depletion of synaptic vesicles consistent with a required role in synaptic vesicle endocytosis (Davis et al., 1999 IWM Abstract 262).

3.2. Budding

Following clathrin recruitment to the fused vesicle membrane, rearrangement of clathrin triskelions from a flat hexagonal matrix to the inclusion of pentagons is thought to initiate curvature of the clathrin cage and membrane indentation or early budding (Kirchhausen, 2000). The role of clathrin in *C. elegans* endocytosis has been probed by RNAi and results in an embryonic lethal phenotype (Grant and Hirsh, 1999) predictable for a protein required in all clathrin-mediated vesicle budding events.

AP180 has also been implicated in the regulation of budding based on the in vitro studies in which AP180 can recruit clathrin to liposome membrane and create uniform clathrin cages (Ahle and Ungewickell, 1986). The enlarged non-uniform vesicle diameters observed in *unc-11* mutants are consistent with this role (Nonet et al., 1999). A similar phenotype is observed in mutants of the *Drosophila* AP180 homolog LAP (Zhang et al., 1998), as well as the *Drosophila* Stoned A/B adaptors, suggesting that several monomeric adaptor proteins, may regulate vesicle size either during endocytosis or at some subsequent vesicle sorting step (Fergestad and Broadie, 2001; Fergestad et al., 1999).

Endophilin has been postulated to act in the conversion of shallow pits to deeply invaginated pits ready for fission. Based on reported lysophosphatidic-acid transferase activity, endophilin is proposed to catalyze the production of wedge shaped phosphatidic acid within the membrane that promotes membrane curvature resulting in invagination (Huttner and Schmidt, 2000; Schmidt et al., 1999). This model is supported by the appearance of arrested shallow pits at neuromuscular synapses in *Drosophila* endophilin mutants (Guichet et al., 2002; Verstreken et al., 2002). A similar arrest of endocytic pits and depletion of vesicles is observed in mutants of *C. elegans* endophilin, which is encoded by *unc-57* (Schuske et al., 2003) as well as it's binding partner the lipid phosphatase, synaptojanin encoded by *unc-26* (Harris et al., 2000). However, both *unc-57* and *unc-26* mutants exhibit other alterations indicative of additional roles later in endocytosis (see below).

3.3. Fission

The final separation of the coated pits from the plasma membrane requires dynamin, a small GTPase that forms a collar around the neck of the budding vesicle and drives vesicle fission (Sweitzer and Hinshaw, 1998). The *Drosophila* dynamin mutant, shibire exhibits a lethal temperature-sensitive blockade of endocytosis resulting in extended invaginations (Koenig and Ikeda, 1989). Similarly, temperature-sensitive mutants in the *C. elegans* dynamin gene, *dyn-1* become uncoordinated, assuming a kinked posture at the restrictive temperature (25° C) and fail to propagate (Clark et al., 1997). GTP-bound dynamin appears to be rate limiting for endocytosis as increasing the GTP-bound form of dynamin, enhances rates of endocytosis (Sever et al., 2000). It is therefore expected that dynamin is under tight regulation. Dynamin interacts with several proteins, including Eps15 an EH-domain containing protein implicated in several vesicle sorting pathways including endocytosis. A knockout of the *C. elegans* Eps15 homolog, *ehs-1* supports a non-essential accessory role for EHS-1 in endocytosis (Salcini et al., 2001), in that these mutants exhibit locomotory defects, aldicarb resistance and vesicle depletion at raised temperatures arguing for an underlying endocytic defect. Dynamin also interacts with endophilin, an SH3 domain-containing protein that interacts with proline rich domains of both dynamin and synaptojanin (Ringstad et al., 1997). *C. elegans* endophilin (UNC-57) localizes to synapses and *unc-57* mutants are uncoordinated and at the ultrastructural level contain ~ 70% fewer synaptic vesicles than wild-type (Schuske et al., 2003). A possible role for endophilin in fission is implied by the accumulation of coated and uncoated invaginated pits in *unc-57* mutants, which are rarely observed in wild-type worms. Recent work in *C. elegans* suggests that endophilin may function as an adaptor protein for the phosphoinositide phosphatase, synaptojanin (UNC-26). Specifically, *unc-57* mutants cause UNC-26 to be mislocalized, appearing diffusely within neurons instead of targeted to synapses. Furthermore, the phenotypes of *unc-57* and *unc-26* mutants are strikingly similar and are not exacerbated when combined (Harris et al., 2000; Schuske et al., 2003). Both mutants are uncoordinated and exhibit the same loss of synaptic vesicles and accumulation of endocytic intermediates. Electrophysiologically, *unc-57* and *unc-26* mutants show identical synaptic defects, including reduced evoked amplitudes, fewer endogenous synaptic events and enhanced fatigue, all consistent with defective endocytosis. Thus endophilin may act to target synaptojanin to sites of endocytosis, a conclusion also reached for *Drosophila* homologs.(Verstreken et al., 2002). Precisely how loss of these functional partners affects fission is unclear, either the lipid shaping function of endophilin or the degradation of PIP2 by synaptojanin may regulate the fission step.

3.4. Uncoating

The final stage after the vesicle has been severed from the plasma membrane is the uncoating process, during which both the clathrin cage and adaptor proteins disengage from the vesicle. *In vitro*, the clathrin lattice is thought to be disassembled via the recruitment of the ATPase, Hsc70 via auxilin (Rothman and Schmid, 1986; Ungewickell et al., 1995). RNAi inhibition of the single *C. elegans* auxilin homolog results in defective receptor-mediated endocytosis in oocytes, altered clathrin dynamics based on FRAP and larval arrest (Greener et al., 2001). This phenotype is likely caused by the loss of unbound clathrin as overexpression of clathrin can rescue viability in RNAi treated worms, supporting the notion that clathrin is rate limiting.

Adaptor complexes can remain associated with synaptic vesicles after clathrin has been removed suggesting that other mechanisms are responsible for the dissociation of AP2 and AP180 (Greene and Eisenberg, 1990). The binding of AP2 and AP180 is regulated by the phospholipid composition of the membrane. Therefore, the dephosphorylation of PI(4,5)P on the vesicle by the phosphoinositide phosphatase, UNC-26, may reduced the affinity of the adaptors for the vesicle membrane and promote adaptor dissociation. Consistent with this model, the failure to recruit UNC-26 in *unc-57* mutants may explain the accumulation of clathrin-coated vesicles in both *unc-57* and *unc-26* mutants (Harris et al., 2000; Schuske et al., 2003). In this model, the stabilization of adaptors on the vesicle in the absence of UNC-26, also results in the retention of the clathrin coat. In the wild type, following

uncoating, vesicles may either directly re-enter the vesicle pool or fuse with early endosomes for protein sorting and vesicle budding into new synaptic vesicles, thereby completing the vesicle cycle.

4. Summary

Using the power of *C. elegans* genetics, the research community has identified founding members of several protein families that play key roles in synaptic transmission (e.g., UNC-13, UNC-18; the vesicular ACh transporter UNC-17, UNC-26). Through the development of behavioral tests imaging tools and electrophysiological assays *C. elegans* researchers have stayed at the leading edge of the synaptic function field. The challenge now is to continue to use the strengths of *C. elegans* in the gene discovery process, but also to develop new techniques to probe the function of existing proteins in the different stages of the synaptic vesicle cycle outlined above. This will require the development of new optical tools to study vesicle dynamics, calcium handling, voltage-changes and possibly protein interactions. Several reagents have already shown great promise in this regard including: 1) Functional analyses using calcium-indictor constructs such as cameleon to study calcium dynamics in neurons and muscles (Kerr et al., 2000). 2) The use of styryl dyes such as FM1-43 to study vesicle cycling in sectioned worms (Kay et al., 1999) and cell cultures (Fields et al, IWM 2003 abstract 561). 3) Recent improvements in EM preservation using high pressure freeze fixation allowing a greater understanding of the morphological correlates of vesicle functional states (Rostaing et al., 2004). 4) Advances in techniques to perform immunoEM allowing better resolution of protein localization within the synapse (Bosher et al., 2003). 5) The possibility of combining FM-dye uptake with EM in cultured neurons also holds great promise. These nascent technologies combined with an enormous and growing molecular, biochemical and genetic arsenal in *C. elegans* will provide new avenues of exploration to further our understanding of the very dynamic events underlying exocytosis and endocytosis, keeping the *C. elegans* research community at the forefront of the neuroscience field.

5. References

Ahle, S., and Ungewickell, E. (1986). Purification and properties of a new clathrin assembly protein. EMBO J. *5*, 3143–3149. Abstract

Aravamudan, B., Fergestad, T., Davis, W.S., Rodesch, C.K., and Broadie, K. (1999). *Drosophila* UNC-13 is essential for synaptic transmission. Nat. Neurosci. *2*, 965–971. Abstract Article

Augustin, I., Rosenmund, C., Sudhof, T.C., and Brose, N. (1999). Munc13-1 is essential for fusion competence of glutamatergic synaptic vesicles. Nature *400*, 457–461. Abstract Article

Avery, L., Bargmann, C.I., and Horvitz, H.R. (1993). The *Caenorhabditis elegans unc-31* gene affects multiple nervous system-controlled functions. Genetics *134*, 455–464. Abstract

Betz, A., Ashery, U., Rickmann, M., Augustin, I., Neher, E., Sudhof, T.C., Rettig, J., and Brose, N. (1998). Munc13-1 is a presynaptic phorbol ester receptor that enhances neurotransmitter release. Neuron *21*, 123–136. Abstract Article

Betz, A., Okamoto, M., Benseler, F., and Brose, N. (1997). Direct interaction of the rat *unc-13* homologue Munc13-1 with the N terminus of syntaxin. J. Biol. Chem. *272*, 2520–2526. Abstract Article

Bosher, J.M., Hahn, B.S., Legouis, R., Sookhareea, S., Weimer, R.M., Gansmuller, A., Chisholm, A.D., Rose, A.M., Bessereau, J.L., and Labouesse, M. (2003). The *Caenorhabditis elegans vab-10* spectraplakin isoforms protect the epidermis against internal and external forces. J. Cell Biol. *161*, 757–768. Abstract Article

Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. Genetics *77*, 71–94. Abstract

Broadie, K., Prokop, A., Bellen, H.J., O'Kane, C.J., Schulze, K.L., and Sweeney, S.T. (1995). Syntaxin and synaptobrevin function downstream of vesicle docking in Drosophila. Neuron *15*, 663–673. Abstract Article

Brose, N., Rosenmund, C., and Rettig, J. (2000). Regulation of transmitter release by Unc-13 and its homologues. Curr. Opin. Neurobiol. *10*, 303–311. Abstract Article

Brundage, L., Avery, L., Katz, A., Kim, U.J., Mendel, J.E., Sternberg, P.W., and Simon, M.I. (1996). Mutations in a *C. elegans* Gqalpha gene disrupt movement, egg laying, and viability. Neuron *16*, 999–1009. Abstract Article

Chen, Y.A., Scales, S.J., Patel, S.M., Doung, Y.C., and Scheller, R.H. (1999). SNARE complex formation is triggered by Ca^{2+} and drives membrane fusion. Cell *97*, 165–174. Abstract Article

Chen, Y.A., Scales, S.J., and Scheller, R.H. (2001). Sequential SNARE assembly underlies priming and triggering of exocytosis. Neuron *30*, 161–170. Abstract Article

Chen, Y.A., and Scheller, R.H. (2001). SNARE-mediated membrane fusion. Nat. Rev. Mol. Cell Biol. *2*, 98–106. Abstract Article

Chieregatti, E., Witkin, J.W., and Baldini, G. (2002). SNAP-25 and synaptotagmin 1 function in Ca^{2+}-dependent reversible docking of granules to the plasma membrane. Traffic *3*, 496–511. Abstract Article

Clark, S.G., Shurland, D.L., Meyerowitz, E.M., Bargmann, C.I., and van der Bliek, A.M. (1997). A dynamin GTPase mutation causes a rapid and reversible temperature-inducible locomotion defect in *C. elegans*. Proc. Natl. Acad. Sci. USA *94*, 10438–10443. Abstract Article

De Camilli, P., and Takei, K. (1996). Molecular mechanisms in synaptic vesicle endocytosis and recycling. Neuron *16*, 481–486. Abstract Article

Deak, F., Schoch, S., Liu, X., Sudhof, T.C., and Kavalali, E.T. (2004). Synaptobrevin is essential for fast synaptic-vesicle endocytosis. Nat. Cell Biol. *6*, 1102–1108. Abstract Article

Dulubova, I., Sugita, S., Hill, S., Hosaka, M., Fernandez, I., Sudhof, T.C., and Rizo, J. (1999). A conformational switch in syntaxin during exocytosis: role of munc18. EMBO J. *18*, 4372–4382. Abstract Article

Fergestad, T., and Broadie, K. (2001). Interaction of stoned and synaptotagmin in synaptic vesicle endocytosis. J. Neurosci. *21*, 1218–1227. Abstract

Fergestad, T., Davis, W.S., and Broadie, K. (1999). The stoned proteins regulate synaptic vesicle recycling in the presynaptic terminal. J. Neurosci. *19*, 5847–5860. Abstract

Francis, M.M., Mellem, J.E., and Maricq, A.V. (2003). Bridging the gap between genes and behavior: recent advances in the electrophysiological analysis of neural function in *Caenorhabditis elegans*. Trends Neurosci. *26*, 90–99. Abstract Article

Fukuda, M., Moreira, J.E., Lewis, F.M., Sugimori, M., Niinobe, M., Mikoshiba, K., and Llinas, R. (1995). Role of the C2B domain of synaptotagmin in vesicular release and recycling as determined by specific antibody injection into the squid giant synapse preterminal. Proc. Natl. Acad. Sci. USA *92*, 10708–10712. Abstract

Gengyo-Ando, K., Kamiya, Y., Yamakawa, A., Kodaira, K., Nishiwaki, K., Miwa, J., Hori, I., and Hosono, R. (1993). The *C. elegans unc-18* gene encodes a protein expressed in motor neurons. Neuron *11*, 703–711. Article

Gengyo-Ando, K., Kitayama, H., Mukaida, M., and Ikawa, Y. (1996). A murine neural-specific homolog corrects cholinergic defects in *Caenorhabditis elegans unc-18* mutants. J. Neurosci. *16*, 6695–6702.

Geppert, M., Goda, Y., Hammer, R.E., Li, C., Rosahl, T.W., Stevens, C.F., and Sudhof, T.C. (1994). Synaptotagmin I: a major Ca^{2+} sensor for transmitter release at a central synapse. Cell *79*, 717–727. Abstract Article

Grant, B., and Hirsh, D. (1999). Receptor-mediated endocytosis in the *Caenorhabditis elegans* oocyte. Mol. Biol. Cell *10*, 4311–4326. Abstract

Greene, L.E., and Eisenberg, E. (1990). Dissociation of clathrin from coated vesicles by the uncoating ATPase. J. Biol. Chem. *265*, 6682–6687. Abstract

Greener, T., Grant, B., Zhang, Y., Wu, X., Greene, L.E., Hirsh, D., and Eisenberg, E. (2001). *Caenorhabditis elegans* auxilin: a J-domain protein essential for clathrin-mediated endocytosis in vivo. Nat. Cell Biol. *3*, 215–219. Abstract Article

Guichet, A., Wucherpfennig, T., Dudu, V., Etter, S., Wilsch-Brauniger, M., Hellwig, A., Gonzalez-Gaitan, M., Huttner, W.B., and Schmidt, A.A. (2002). Essential role of endophilin A in synaptic vesicle budding at the Drosophila neuromuscular junction. EMBO J. *21*, 1661–1672. Abstract Article

Hajdu-Cronin, Y.M., Chen, W.J., Patikoglou, G., Koelle, M.R., and Sternberg, P.W. (1999). Antagonism between G(o)α and G(q)α in *Caenorhabditis elegans*: the RGS protein EAT-16 is necessary for G(o)α signaling and regulates G(q)α activity. Genes Dev. *13*, 1780–1793. Abstract

Hanson, P.I., Roth, R., Morisaki, H., Jahn, R., and Heuser, J.E. (1997). Structure and conformational changes in NSF and its membrane receptor complexes visualized by quick-freeze/deep-etch electron microscopy. Cell *90*, 523–535. Abstract Article

Harris, T.W., Hartwieg, E., Horvitz, H.R., and Jorgensen, E.M. (2000). Mutations in synaptojanin disrupt synaptic vesicle recycling. J. Cell Biol. *150*, 589–600. Abstract Article

Harris, T.W., Schuske, K., and Jorgensen, E.M. (2001). Studies of synaptic vesicle endocytosis in the nematode *C. elegans*. Traffic *2*, 597–605. Abstract Article

Haucke, V., and De Camilli, P. (1999). AP-2 recruitment to synaptotagmin stimulated by tyrosine-based endocytic motifs. Science *285*, 1268–1271. Abstract Article

Haucke, V., Wenk, M.R., Chapman, E.R., Farsad, K., and De Camilli, P. (2000). Dual interaction of synaptotagmin with mu2- and α-adaptin facilitates clathrin-coated pit nucleation. EMBO J. *19*, 6011–6019. Abstract Article

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 acetylcholine metabolism in the nematode *Caenorhabditis elegans*. J. Neurochem. *58*, 1517–1525. Abstract

Hunt, J.M., Bommert, K., Charlton, M.P., Kistner, A., Habermann, E., Augustine, G.J., and Betz, H. (1994). A post-docking role for synaptobrevin in synaptic vesicle fusion. Neuron *12*, 1269–1279. Abstract Article

Huttner, W.B., and Schmidt, A. (2000). Lipids, lipid modification and lipid-protein interaction in membrane budding and fission -- insights from the roles of endophilin A1 and synaptophysin in synaptic vesicle endocytosis. Curr. Opin. Neurobiol. *10*, 543–551. Abstract Article

Iwasaki, K., Staunton, J., Saifee, O., Nonet, M., and Thomas, J.H. (1997). *aex-3* encodes a novel regulator of presynaptic activity in *C. elegans*. Neuron *18*, 613–622. Abstract Article

Jorgensen, E.M., Hartwieg, E., Schuske, K., Nonet, M.L., Jin, Y., and Horvitz, H.R. (1995). Defective recycling of synaptic vesicles in synaptotagmin mutants of *Caenorhabditis elegans*. Nature *378*, 196–199. Abstract Article

Kay, A.R., Alfonso, A., Alford, S., Cline, H.T., Holgado, A.M., Sakmann, B., Snitsarev, V.A., Stricker, T.P., Takahashi, M., and Wu, L.G. (1999). Imaging synaptic activity in intact brain and slices with FM1-43 in *C. elegans*, lamprey, and rat. Neuron *24*, 809–817. Abstract Article

Kerr, R., Lev-Ram, V., Baird, G., Vincent, P., Tsien, R.Y., and Schafer, W.R. (2000). Optical imaging of calcium transients in neurons and pharyngeal muscle of *C. elegans*. Neuron *26*, 583–594. Abstract Article

Kirchhausen, T. (2000). Clathrin. Annu. Rev. Biochem. *69*, 699–727. Abstract Article

Koelle, M.R., and Horvitz, H.R. (1996). EGL-10 regulates G protein signaling in the *C. elegans* nervous system and shares a conserved domain with many mammalian proteins. Cell *84*, 115–125. Abstract Article

Koenig, J.H., and Ikeda, K. (1989). Disappearance and reformation of synaptic vesicle membrane upon transmitter release observed under reversible blockage of membrane retrieval. J. Neurosci. *9*, 3844–3860. Abstract

Kohn, R.E., Duerr, J.S., McManus, J.R., Duke, A., Rakow, T.L., Maruyama, H., Moulder, G., Maruyama, I.N., Barstead, R.J., and Rand, J.B. (2000). Expression of multiple UNC-13 proteins in the *Caenorhabditis elegans* nervous system. Mol. Biol. Cell *11*, 3441–3452. Abstract

Koushika, S.P., Richmond, J.E., Hadwiger, G., Weimer, R.M., Jorgensen, E.M., and Nonet, M.L. (2001). A post-docking role for active zone protein Rim. Nat. Neurosci. *4*, 997–1005. Abstract Article

Lackner, M.R., Nurrish, S.J., and Kaplan, J.M. (1999). Facilitation of synaptic transmission by EGL-30 Gqalpha and EGL-8 PLCbeta: DAG binding to UNC-13 is required to stimulate acetylcholine release. Neuron *24*, 335–346. Abstract Article

Lee, R.Y., Lobel, L., Hengartner, M., Horvitz, H.R., and Avery, L. (1997). Mutations in the alpha1 subunit of an L-type voltage-activated Ca^{2+} channel cause myotonia in *Caenorhabditis elegans*. EMBO J. *16*, 6066–6076. Abstract Article

Littleton, J.T., Stern, M., Perin, M., and Bellen, H.J. (1994). Calcium dependence of neurotransmitter release and rate of spontaneous vesicle fusions are altered in *Drosophila* synaptotagmin mutants. Proc. Natl. Acad. Sci. USA *91*, 10888–10892. Abstract

Littleton, J.T., Stern, M., Schulze, K., Perin, M., and Bellen, H.J. (1993). Mutational analysis of *Drosophila* synaptotagmin demonstrates its essential role in Ca^{2+}-activated neurotransmitter release. Cell *74*, 1125–1134. Abstract Article

Lonart, G., and Sudhof, T.C. (2000). Assembly of SNARE core complexes prior to neurotransmitter release sets the readily releasable pool of synaptic vesicles. J. Biol. Chem. *275*, 27703–27707. Abstract

Martelli, A.M., Baldini, G., Tabellini, G., Koticha, D., Bareggi, R., and Baldini, G. (2000). Rab3A and Rab3D control the total granule number and the fraction of granules docked at the plasma membrane in PC12 cells. Traffic *1*, 976–986. Abstract Article

Maruyama, I.N., and Brenner, S. (1991). A phorbol ester/diacylglycerol-binding protein encoded by the *unc-13* gene of *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA *88*, 5729–5733. Abstract

Mendel, J.E., Korswagen, H.C., Liu, K.S., Hajdu-Cronin, Y.M., Simon, M.I., Plasterk, R.H., and Sternberg, P.W. (1995). Participation of the protein Go in multiple aspects of behavior in *C. elegans*. Science *267*, 1652–1655. Abstract Article

Miller, K.G., Emerson, M.D., and Rand, J.B. (1999). Goalpha and diacylglycerol kinase negatively regulate the Gqalpha pathway in *C. elegans*. Neuron *24*, 323–333. Abstract Article

Murthy, V.N., and Stevens, C.F. (1999). Reversal of synaptic vesicle docking at central synapses. Nat. Neurosci. *2*, 503–507. Abstract Article

Nguyen, M., Alfonso, A., Johnson, C.D., and Rand, J.B. (1995). *Caenorhabditis elegans* mutants resistant to inhibitors of acetylcholinesterase. Genetics *140*, 527–535. Abstract

Nicholson-Tomishima, K., and Ryan, T.A. (2004). Kinetic efficiency of endocytosis at mammalian CNS synapses requires synaptotagmin I. Proc. Natl. Acad. Sci. USA *101*, 16648–16652. Abstract Article

Nonet, M.L. (1999). Visualization of synaptic specializations in live *C. elegans* with synaptic vesicle protein-GFP fusions. J. Neurosci. Methods *89*, 33–40. Abstract Article

Nonet, M.L., Grundahl, K., Meyer, B.J., and Rand, J.B. (1993). Synaptic function is impaired but not eliminated in *C. elegans* mutants lacking synaptotagmin. Cell *73*, 1291–1305. Abstract Article

Nonet, M.L., Holgado, A.M., Brewer, F., Serpe, C.J., Norbeck, B.A., Holleran, J., Wei, L., Hartwieg, E., Jorgensen, E.M., and Alfonso, A. (1999). UNC-11, a *Caenorhabditis elegans* AP180 homologue, regulates the size and protein composition of synaptic vesicles. Mol. Biol. Cell *10*, 2343–2360. Abstract

Nonet, M.L., Saifee, O., Zhao, H., Rand, J.B., and Wei, L. (1998). Synaptic transmission deficits in *Caenorhabditis elegans* synaptobrevin mutants. J. Neurosci. *18*, 70–80. Abstract

Nonet, M.L., Staunton, J.E., Kilgard, M.P., Fergestad, T., Hartwieg, E., Horvitz, H.R., Jorgensen, E.M., and Meyer, B.J. (1997). *Caenorhabditis elegans rab-3* mutant synapses exhibit impaired function and are partially depleted of vesicles. J. Neurosci. *17*, 8061–8073. Abstract

Nurrish, S., Segalat, L., and Kaplan, J.M. (1999). Serotonin inhibition of synaptic transmission: Galpha(0) decreases the abundance of UNC-13 at release sites. Neuron *24*, 231–242. Abstract Article

Okamoto, M., and Sudhof, T.C. (1997). Mints, Munc18-interacting proteins in synaptic vesicle exocytosis. J. Biol. Chem. *272*, 31459–31464. Abstract Article

Poskanzer, K.E., Marek, K.W., Sweeney, S.T., and Davis, G.W. (2003). Synaptotagmin I is necessary for compensatory synaptic vesicle endocytosis in vivo. Nature *426*, 559–563. Abstract Article

Reist, N.E., Buchanan, J., Li, J., DiAntonio, A., Buxton, E.M., and Schwarz, T.L. (1998). Morphologically docked synaptic vesicles are reduced in synaptotagmin mutants of Drosophila. J. Neurosci. *18*, 7662–7673. Abstract

Richmond, J.E., Davis, W.S., and Jorgensen, E.M. (1999). UNC-13 is required for synaptic vesicle fusion in *C. elegans*. Nat. Neurosci. *2*, 959–964. Abstract Article

Richmond, J.E., and Jorgensen, E.M. (1999). One GABA and two acetylcholine receptors function at the *C. elegans* neuromuscular junction. Nat. Neurosci. *2*, 791–797. Abstract Article

Richmond, J.E., Weimer, R.M., and Jorgensen, E.M. (2001). An open form of syntaxin bypasses the requirement for UNC-13 in vesicle priming. Nature *412*, 338–341. Abstract Article

Ringstad, N., Nemoto, Y., and De Camilli, P. (1997). The SH3p4/Sh3p8/SH3p13 protein family: binding partners for synaptojanin and dynamin via a Grb2-like Src homology 3 domain. Proc. Natl. Acad. Sci. USA *94*, 8569–8574. Abstract Article

Rizzoli, S.O., and Betz, W.J. (2004). The structural organization of the readily releasable pool of synaptic vesicles. Science *303*, 2037–2039. Abstract Article

Robatzek, M., Niacaris, T., Steger, K., Avery, L., and Thomas, J.H. (2001). *eat-11* encodes GPB-2, a Gβ(5) ortholog that interacts with G(o)α and G(q)α to regulate *C. elegans* behavior. Curr. Biol. *11*, 288–293. Abstract Article

Robinson, L.J., and Martin, T.F. (1998). Docking and fusion in neurosecretion. Curr. Opin. Cell. Biol. *10*, 483–492. Abstract Article

Rongo, C., Whitfield, C.W., Rodal, A., Kim, S.K., and Kaplan, J.M. (1998). LIN-10 is a shared component of the polarized protein localization pathways in neurons and epithelia. Cell *94*, 751–759. Abstract Article

Rose, A.M., and Baillie, D.L. (1980). Genetic organization of the region around UNC-15 (I), a gene affecting paramyosin in *Caenorhabditis elegans*. Genetics *96*, 639–648. Abstract

Rostaing, P., Weimer, R.M., Jorgensen, E.M., Triller, A., and Bessereau, J.L. (2004). Preservation of immunoreactivity and fine structure of adult *C. elegans* tissues using high-pressure freezing. J. Histochem. Cytochem. *52*, 1–12. Abstract

Rothman, J.E., and Schmid, S.L. (1986). Enzymatic recycling of clathrin from coated vesicles. Cell *46*, 5–9. Abstract Article

Saifee, O., Wei, L., and Nonet, M.L. (1998). The *Caenorhabditis elegans unc-64* locus encodes a syntaxin that interacts genetically with synaptobrevin. Mol. Biol. Cell *9*, 1235–1252. Abstract

Salcini, A.E., Hilliard, M.A., Croce, A., Arbucci, S., Luzzi, P., Tacchetti, C., Daniell, L., De Camilli, P., Pelicci, P.G., Di Fiore, P.P., and Bazzicalupo, P. (2001). The Eps15 *C. elegans* homologue EHS-1 is implicated in synaptic vesicle recycling. Nat. Cell Biol. *3*, 755–760. Abstract Article

Schafer, W.R., and Kenyon, C.J. (1995). A calcium-channel homologue required for adaptation to dopamine and serotonin in *Caenorhabditis elegans*. Nature *375*, 73–78. Abstract Article

Schafer, W.R., Sanchez, B.M., and Kenyon, C.J. (1996). Genes affecting sensitivity to serotonin in *Caenorhabditis elegans*. Genetics *143*, 1219–1230. Abstract

Schmidt, A., Wolde, M., Thiele, C., Fest, W., Kratzin, H., Podtelejnikov, A.V., Witke, W., Huttner, W.B., and Soling, H.D. (1999). Endophilin I mediates synaptic vesicle formation by transfer of arachidonate to lysophosphatidic acid. Nature *401*, 133–141. Abstract Article

Schuske, K.R., Richmond, J.E., Matthies, D.S., Davis, W.S., Runz, S., Rube, D.A., van der Bliek, A.M., and Jorgensen, E.M. (2003). Endophilin is required for synaptic vesicle endocytosis by localizing synaptojanin. Neuron *40*, 749–762. Abstract Article

Ségalat, L., Elkes, D.A., and Kaplan, J.M. (1995). Modulation of serotonin-controlled behaviors by Go in *Caenorhabditis elegans*. Science *267*, 1648–1651. Abstract Article

Sever, S., Damke, H., and Schmid, S.L. (2000). Dynamin:GTP controls the formation of constricted coated pits, the rate limiting step in clathrin-mediated endocytosis. J. Cell Biol. *150*, 1137–1148. Abstract Article

Sollner, T., Bennett, M.K., Whiteheart, S.W., Scheller, R.H., and Rothman, J. E. (1993). A protein assembly-disassembly pathway in vitro that may correspond to sequential steps of synaptic vesicle docking, activation, and fusion. Cell *75*, 409–418. Article

Staunton, J., Ganetzky, B., and Nonet, M.L. (2001). Rabphilin potentiates soluble N-ethylmaleimide sensitive factor attachment protein receptor function independently of *rab3*. J. Neurosci. *21*, 9255–9264. Abstract

Sutton, R.B., Fasshauer, D., Jahn, R., and Brunger, A.T. (1998). Crystal structure of a SNARE complex involved in synaptic exocytosis at 2.4 A resolution. Nature *395*, 347–353. Abstract Article

Sweitzer, S.M., and Hinshaw, J.E. (1998). Dynamin undergoes a GTP-dependent conformational change causing vesiculation. Cell *93*, 1021–1029. Abstract Article

Ungewickell, E., Ungewickell, H., Holstein, S.E., Lindner, R., Prasad, K., Barouch, W., Martin, B., Greene, L.E., and Eisenberg, E. (1995). Role of auxilin in uncoating clathrin-coated vesicles. Nature *378*, 632–635. Abstract Article

van der Linden, A.M., Simmer, F., Cuppen, E., and Plasterk, R.H. (2001). The G-proteinβ-subunit GPB-2 in *Caenorhabditis elegans* regulates the G(o)α-G(q)α signaling network through interactions with the regulator of G-protein signaling proteins EGL-10 and EAT-16. Genetics *158*, 221–235. Abstract

Varoqueaux, F., Sigler, A., Rhee, J.S., Brose, N., Enk, C., Reim, K., and Rosenmund, C. (2002). Total arrest of spontaneous and evoked synaptic transmission but normal synaptogenesis in the absence of Munc13-mediated vesicle priming. Proc. Natl. Acad. Sci. USA *99*, 9037–9042. Abstract Article

Verhage, M., Maia, A.S., Plomp, J.J., Brussaard, A.B., Heeroma, J.H., Vermeer, H., Toonen, R.F., Hammer, R.E., van den Berg, T.K., Missler, M., et al. (2000). Synaptic assembly of the brain in the absence of neurotransmitter secretion. Science *287*, 864–869. Abstract Article

Verstreken, P., Kjaerulff, O., Lloyd, T.E., Atkinson, R., Zhou, Y., Meinertzhagen, I.A., and Bellen, H.J. (2002). Endophilin mutations block clathrin-mediated endocytosis but not neurotransmitter release. Cell *109*, 101–112. Abstract Article

Voets, T., Toonen, R.F., Brian, E.C., de Wit, H., Moser, T., Rettig, J., Sudhof, T.C., Neher, E., and Verhage, M. (2001). Munc18-1 promotes large dense-core vesicle docking. Neuron *31*, 581–591. Abstract Article

Wang, Y., Okamoto, M., Schmitz, F., Hofmann, K., and Sudhof, T.C. (1997). Rim is a putative Rab3 effector in regulating synaptic-vesicle fusion. Nature *388*, 593–598. Abstract Article

Weber, T., Zemelman, B.V., McNew, J.A., Westermann, B., Gmachl, M., Parlati, F., Sollner, T.H., and Rothman, J.E. (1998). SNAREpins: minimal machinery for membrane fusion. Cell *92*, 759–772. Abstract Article

Weimer, R.M., Richmond, J.E., Davis, W.S., Hadwiger, G., Nonet, M.L., and Jorgensen, E.M. (2003). Defects in synaptic vesicle docking in *unc-18* mutants. Nat. Neurosci. *6*, 1023–1030. Abstract Article

White, J.G., Southgate, E., Thomson, J.N., and Brenner, S. (1986). The structure of the nervous system of the nematode *Caenorhabditis elegans*. Phil. Trans. R. Soc. Lond. B *314*, 1–340.

Whitfield, C.W., Benard, C., Barnes, T., Hekimi, S., and Kim, S.K. (1999). Basolateral localization of the *Caenorhabditis elegans* epidermal growth factor receptor in epithelial cells by the PDZ protein LIN-10. Mol. Biol. Cell *10*, 2087–2100. Abstract

Xu, T., Rammner, B., Margittai, M., Artalejo, A.R., Neher, E., and Jahn, R. (1999). Inhibition of SNARE complex assembly differentially affects kinetic components of exocytosis. Cell *99*, 713–722. Abstract Article

Zenisek, D., Steyer, J.A., and Almers, W. (2000). Transport, capture and exocytosis of single synaptic vesicles at active zones. Nature *406*, 849–854. Abstract Article

Zhang, B., Koh, Y.H., Beckstead, R.B., Budnik, V., Ganetzky, B., and Bellen, H.J. (1998). Synaptic vesicle size and number are regulated by a clathrin adaptor protein required for endocytosis. Neuron *21*, 1465–1475. Abstract Article

Zhang, J.Z., Davletov, B.A., Sudhof, T.C., and Anderson, R.G. (1994). Synaptotagmin I is a high affinity receptor for clathrin AP-2: implications for membrane recycling. Cell *78*, 751–760. Abstract Article

Zucker, R.S. (1996). Exocytosis: a molecular and physiological perspective. Neuron *17*, 1049–1055. Abstract Article

All WormBook content, except where otherwise noted, is licensed under a Creative Commons Attribution License.