Intracellular compartments are maintained via an organized system of transport pathways that traffic lipids and proteins in vesicular organelles in a specific and regulated manner (Bennett and Scheller, 1993; Ferro-Novick and Jahn, 1994). The recent completion of the Drosophila genome (Adams et al., 2000; Rubin et al., 2000) allows us to analyze the ∼14,000 genes that are encoded and begin to make evolutionary comparisons of mechanisms underlying membrane trafficking in metazoans. Models for intracellular trafficking have built upon the original SNARE hypothesis proposed by Söllner et al. (1993). In current models, the assembly and disassembly of a ternary complex composed of SNARE proteins is predicted to play a key role in vesicle-target membrane fusion. The neuronal SNARE complex, which is required for synaptic vesicle exocytosis at nerve terminals (Schulze et al., 1995; Deitcher et al., 1998; Littleton et al., 1998), is one of the best-characterized systems for intracellular fusion. The vesicle membrane v-SNARE, synaptobrevin, forms an SDS-resistant complex with the presynaptic membrane t-SNAREs, SNAP-25, and syntaxin 1. Within this complex, synaptobrevin and syntaxin each contribute one α-helix, while SNAP-25 contributes two α-helices (Sutton et al., 1998). These helices assemble to form a four-helix bundle which is thought to be characteristic of all cellular SNARE complexes throughout phylogeny. A assembly of the SNARE complex is required at a late post-docking stage in synaptic exocytosis (Littleton et al., 1998) and has been suggested to directly mediate bilayer membrane fusion (Weber et al., 1998). Disassembly of the SNARE complex by NSF and the SNAP adapter proteins is also required during neuronal vesicle cycling to recycle SNAREs for additional rounds of fusion (Littleton et al., 1998; Tolar and Pallanck, 1998). The regulation of SNARE assembly and disassembly, as well as the mechanisms for targeting vesicles to sites of SNARE fusion, are key processes that are likely conserved, but for which we know little about. A n analysis of the proteins predicted by the Drosophila genome reveals a broad conservation of many trafficking proteins and several relatively large protein families involved in vesicle trafficking. Indeed, mammals, Drosophila, C. elegans, and yeast share a conserved core set of proteins involved in intracellular trafficking (Table I).

The SNARE Superfamily

Given the central role of SNARE proteins in vesicle trafficking, knowledge of the complete set of SNAREs provides important information into the conservation of SNARE-mediated trafficking and the potential ability of SNAREs to specify intracellular compartmental identity. The yeast genome contains eight syntaxin t-SNAREs distributed in distinct compartments along the secretory pathway. These SNAREs include Sso1p/Sso2p at the plasma membrane, Ufe1p in the E.R., Pep12p on endosomes/lysosomes, Vam3p on vacuoles, Sed5p in the intermediate compartment and cis-Golgi, and Tlg1p/Tlg2p in the trans-Golgi network and early endosomes. A analysis of the Drosophila genome reveals 11 syntaxin family members, while the C. elegans genome encodes ∼9 syntaxins. A dendrogram of the syntaxin superfamily is shown in Fig. 1. Whereas Drosophila contains two members of the syntaxin 1 subfamily (syx 1 and syx 4), C. elegans contains six proteins related to syntaxin 1. Both Drosophila and C. elegans contain homologues of Ufe1, Sed5p, and Tlg2p, indicating the potential for broad conservation of membrane trafficking from the E.R to Golgi. Drosophila, like mammals, contain a number of additional putative endosomal/lysosomal SNAREs lacking in C. elegans and yeast, indicating the potential for a more elaborate endosomal trafficking system in these species. The large number of syntaxin t-SNAREs in Drosophila suggests that vesicular trafficking between individual cellular compartments may indeed be specified by the distribution of unique syntaxin isoforms. A analysis of individual v-/t-SNARE binding specificity and subcellular localization of the known t-SNAREs in Drosophila should provide further clues into SNARE-mediated trafficking models.

The remaining t-SNARE superfamily includes SNAREs related to SNAP-25/Sec9p. A n analysis of the Drosophila and C. elegans genomes reveals that unlike the large syntaxin family, only three Drosophila and C. elegans gene products encode SNAP-25-related proteins. These include two homologues of SNAP-25 and one homologue of SNAP-29 (Fig. 2). The mammalian SNAP-25 and related SNAP-23 families are required for Golgi to plasma mem-
brane trafficking, while SNAP-29 is present on intracellular membranes and likely functions in trafficking between intracellular compartments. Analysis at the primary sequence level demonstrates that the SNAP-29 subfamily, like yeast Sec9p, lacks the conserved palmitoylated cysteine residues that anchor SNAP-25 to the plasma membrane. Given the prediction that SNARE complexes from yeast to mammals form four-stranded parallel \( \alpha \)-helical bundles, one would predict either that SNARE complexes exist that lack helices contributed by a SNAP-25/29 homologue and/or that members of the SNAP-25 superfamily may be promiscuous in their interactions with various syntaxins. The lack of a membrane-anchoring site on SNAP-29 suggests this isoform might be capable of interacting with multiple syntaxins as a cytosolic protein.

The \( \nu \)-SNARE family in yeast consists of 10 \( \nu \)-SNAREs, while 5 \( \nu \)-SNAREs can be easily identified in Drosophila. These include three homologues of synaptobrevin and single homologues of Ykt6p and Sec22p. A iso present are homologues of the SNA RE proteins membrin, Gos28, and Vti1p. Missing from the fly genome are homologues of the yeast SNA REs Bos1p and Sft1p. In general, the relative number of \( \nu \)-t-SNA REs has changed little from yeast to Drosophila, suggesting basic subcellular compartmentalization has been conserved from unicellular to multicellular eukaryotes. Genomic sequencing and analysis has defined the minimal SNA RE assortment present in multicellular organisms and provides the required framework for a genetic dissection of intracellular SNA RE-mediated vesicular transport in Drosophila. Further analysis will provide insights into how SNA REs are differentially distributed on intracellular membranes and how they function in vesicle fusion.

### Constitutive Trafficking Proteins

Other conserved components of the intracellular trafficking machinery encoded by the Drosophila genome include homologues of the yeast Sec1p family, which are predicted to regulate SNA RE assembly by binding to syntaxin, and controlling SNA RE complex formation. Like yeast, Drosophila contains four Sec1 homologues, including ROP, Vps45p, Vps33p, and Sly1p. In addition, Drosophila contain homologues of proteins found in the yeast EXOCYST and TRAPP complexes, which are thought to function in vesicle targeting and docking before SNA RE complex for-
roles in several compartments. Alternatively, the invertebrate/vertebrate syntaxins noted above trafficking proteins may have evolved to fulfil the role of Vam3p. Drosophila cis-Golgi. No obvious homologues of yeast Vam3p was found in shown to be associated with the intermediate compartment and the TGN and endosome. The syntaxin 5/Sed5p family has been shown homologies with yeast Pep12p and Tlg2p, components of TGN, early endosome and lysosome. Syntaxins 7, 13, and 16 have been identified in late sorting compartments including the taxins 6, 8, and 10 show the most similarity to yeast Tlg1p, and ily with the yeast plasma membrane proteins Sso1p/Sso2p. Syn- be plasma membrane–associated t-SNAREs and form a subfam-

Figure 1. Family tree for the syntaxin superfamily from yeast, C. elegans, Drosophila, and mammals. Nearest neighbor dendrograms were generated for the syntaxin superfamily (syntaxin 17 was not included in the analysis). Based on the relationship of the invertebrate/vertebrate syntaxins to their yeast counterparts, several general findings emerge. First, syntaxins 1–4 are predicted to be plasma membrane–associated t-SNAREs and form a subfamily with the yeast plasma membrane proteins Sso1p/Sso2p. Syntaxins 6, 8, and 10 show the most similarity to yeast Tlg1p, and have been identified in late sorting compartments including the TGN, early endosome and lysosome. Syntaxins 7, 13, and 16 show homologies with yeast Pep12p and Tlg2p, components of the TGN and endosome. The syntaxin 5/Sed5p family has been shown to be associated with the intermediate compartment and cis-Golgi. No obvious homologues of yeast Vam3p was found in Drosophila or in C. elegans, suggesting that other more divergent trafficking proteins may have evolved to fulfil the role of Vam3p. Alternatively, the invertebrate/vertebrate syntaxins noted above may have expanded their distribution to subserve trafficking roles in several compartments.

Figure 2. Nearest neighbor dendrograms of the SNAP-25 superfam-

Synaptic Trafficking Proteins

Neurotransmitter release has evolved as a specialized form of membrane trafficking in neurons that is calcium-regulated and extremely rapid. In addition, synaptic vesicles undergo numerous rounds of local recycling at nerve terminals. The basic fusion machinery that mediates intracellular trafficking is also present at synapses. However, the additional specializations of synaptic membrane trafficking require several novel protein families not found in yeast. Among the group of synaptic proteins thought to play a role in exocytosis at nerve terminals in mammals, both Drosophila and C. elegans contain homologues of synaptotagmin, synaptogyrin, Munc-13, SCAMPs, synapsin, CSP, SV2, CAPS, VAP-33, Rabphilin, HRS-2, to-

moxyn, complexin, Rim, and SNAP25. Surprisingly, although synaptophysin is found in C. elegans, it is missing in Drosophila. Among the group of conserved synaptic proteins, the C2 domain–containing protein family, including the synaptotagmins, stands out as an extremely large and diverse family potentially involved in membrane trafficking and neurotransmitter release in Drosophila. Synaptotagmins were originally identified as synaptic vesicle proteins containing a single transmembrane domain and two copies of a calcium-dependent phospholipid–binding motif known as the C2 domain (Perin et al., 1990). This family of proteins has received much attention for its potential role as a calcium sensor in synaptic exocytosis (Littleton and Bellen, 1995; Littleton et al., 1999). Subsequently, C2 domain–containing proteins, including synaptotagmin, Munc-13, Rim, Rabphilin, and DOC2 have been implicated in various aspects of membrane trafficking in invertebrates and mammals. The C2 domain family in yeast is quite small and includes three synaptotagmin–related molecules termed tricalbins, each containing three C2 domains, one ubiquitination ligase/Nedd4–like molecule
aptic scaffolding. The synaptic adhesion molecules encoded by the neurexin and neuroligin gene families, as well as other protein families such as the cadherins, have unique functions that have been selected for and conserved through evolution. The analysis of the genome sequence will facilitate the discovery of novel components of the trafficking machinery through the multitude of genetic tools available in Drosophila.

### Synapse Formation

Additional functions required by neurons for effective synaptic transmission include synapse formation and assembly of specialized pre- and postsynaptic structures that allow coupling of synaptic vesicle fusion to sites of postsynaptic receptor clustering. A number of mammalian components have been suggested to function in synapse formation and assembly. Among these are the integrin, cadherin, and neurexin family of extracellular adhesion molecules and associated adapter proteins. In Drosophila, the homotypic cell adhesion molecule fasciclin II is also likely to play an important role in synapse formation and stabilization (Schuster et al., 1996). A study of putative synaptic adhesion molecules encoded by the Drosophila genome reveals seven integrins (five α- and two β-sub-units) and three cadherins. The cadherin family in mammals includes a number of synaptic isoforms, including three large gene clusters encoding ~50 proteocadherin genes. These gene clusters are somewhat similar to the immunoglobulin and T cell receptor gene clusters, and hint at the possibility that differential use of these genes in specific subsets of neurons may provide a large and unique synaptic targeting mechanism to establish the complex connectivity in mammalian brain. In Drosophila, no proteocadherin genes are present. Thus, although cadherins may play a general role in synapse formation, they are unlikely to underlie targeting specificity in the fly brain. Another protein family implicated in synapse formation is the neurexins. Neurexins form a family of cell surface proteins encoded by three genes in mammals. Alternative splicing has been shown to generate thousands of unique neurexin isoforms, suggesting the possibility that a combinatorial expression of unique neurexin isoforms may participate in differential synaptic targeting. A postsynaptic extracellular receptor family, the neurelin, has also been identified as neurexin ligands in mammals, generating a model that incorporates a neureolin/neurexin junction in synaptic scaffolding. The Drosophila genome encodes three neurexin-like genes. Two of these, nrx IV and axotactin, are not expressed in neurons, but rather in glia. The third, a homologue of neurexin III, may be expressed in neurons, but like cadherins, neurexins are unlikely to play a role in differential synaptic targeting for a wide array of neurons. However, the fly genome encodes four neurelin-like genes, suggesting that a neurexin III-neurelin complex might play a more general role in synaptic scaffolding. Intracellular binding of PSD-95 to mammalian neurexins postsynaptically, and CA SK (a MAGUK-related PDZ containing protein) binding to neurexins presynaptically, may provide a substrate upon which further synaptic macromolecular complexes are assembled. Indeed, CA SK is known to form an additional complex with the PDZ-containing proteins V elis and M int, which subsequently link to components of the synaptic exocytotic machinery. Drosophila contains homologues of M int, V elis, PSD-95, and CA SK , providing the potential for a broadly conserved synaptic assembly complex. A differential synaptic scaffolding proteins conserved in Drosophila include specific adapters for anchoring glutamate, GABA and acetylcholine receptors to specific synaptic subdomains. A discussion of these proteins can be found in Littleton and Ganetzky (2000).

In conclusion, the rapid accumulation of genomic sequence data from multiple species is providing important insights into the potential conservation of membrane trafficking mechanisms. The broad conservation of the basic SNARE machinery makes it likely that this complex forms the core of the fusion machinery and that individual SNAREs may facilitate the specification of intracellular compartmental identity. In addition to the SNAREs, there is broad conservation of a large number of specialized components that are thought to function in synaptic exocytosis. In many instances, a single gene encodes the Drosophila homologue, making flies an attractive model system for genetic dissection of the function of these proteins in exocytosis. Genetic dissection of the larger protein families such as the synaptotagmins will prove more difficult, given the potential for redundancy among similar family members. However, the conservation of the individual isoforms across species indicates they are likely to have unique functions that have been selected for and conserved through evolution. The analysis of the genome sequence of Drosophila has provided a basic framework to begin to explore a large array of new ideas in membrane trafficking. However, it is clear that the sequence represents the beginning of this analysis. Genetic and biochemical approaches can now be employed to address the in vivo functions of the known proteins components suggested to underlie vesicular trafficking. Perhaps even more importantly, the genomic sequence will facilitate the discovery of novel components of the trafficking machinery through the multitude of genetic tools available in Drosophila.

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