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 SNARE adapter proteins is also required during neuronal vesicle cycling to recycle SNAREs for additional rounds of fusion (Littleton et al., 1998; Tolias 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 SNA P-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 SNA P-25–related proteins. These include two homologues of SNA P-25 and one homologue of SNA P-29 (Fig. 2). The mammalian SNA P-25 and related SNA P-23 families are required for Golgi to plasma mem-

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A genomic analysis of membrane trafficking and neurotransmitter release in Drosophila

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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 α-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 v-SNARE family in yeast consists of 10 v-SNAREs, while 5 v-SNAREs can be easily identified in Drosophila. These include three homologues of synaptobrevin and single homologues of Ykt6p and Sec22p. Also present are homologues of the S N A R E proteins membrin, Gos28, and Vti1p. Missing from the fly genome are homologues of the yeast S N A R Es Bos1p and Sft1p. In general, the relative number of v-/t-S N A R Es 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 S N A R E assortment present in multicellular organisms and provides the required framework for a genetic dissection of intracellular S N A R E-mediated vesicular transport in Drosophila. Further analysis will provide insights into how S N A R Es 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 S N A R E assembly by binding to syntaxin, and controlling S N A R E 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 S N A R E complex for-
membrane–associated t-SNAREs and form a subfamily with yeast plasma membrane proteins Sso1p/Sso2p. Synaptophysin is found in 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 Syntaxins 7, 13, and 16 show homologies with yeast Pep12p and Tlg2p, components of 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 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 superfamily reveals three distinct subclasses (the SNAP-23, SNAP-25, and SNAP-29 subfamilies) that are conserved from yeast to mammals.

required only in multicellular eukaryotes. Based on these evolutionary comparisons, it is evident that the mechanisms for membrane trafficking between intracellular compartments has been extremely conserved, making yeast and invertebrates excellent model systems for dissecting the role of these protein families in vesicle transport.

**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, SCA MPs, synapsin, CSP, SV2, CA PS, VA P-33, Rabphilin, HRS-2, tomosyn, complexin, RIM, and SNA PIN. Surprisingly, although synaptophysin is found in C. elegans, it is lacking 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 ubiquitin ligase/Nedd4-like molecule

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neurexin ligands in mammals, generating a model for differential synaptic targeting. A postsynaptic extracellular adhesion molecule has been shown to generate thousands of unique neurexin isoforms. Neurexins form a family of cell surface proteins that 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 neuroligin-like genes, suggesting that a neurexin III-neuroligin complex might play a more general role in synaptic scaffolding. Intracellular binding of PSD-95 to mammalian neuroligins 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 Mint, V elis, and CA SK, providing the potential for a broadly conserved synaptic assembly complex. A dditional 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 form 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 protein 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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