Analysis

A Genomic Analysis of Membrane Trafficking and Neurotransmitter Release in Drosophila

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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 (A dam 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 metazoa. 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 1).

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 ER, Pepl1p 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 ER to Golgi. Drosophila, like mammals, contains 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 syntaxin 2 and 3 (CTD 1). A n analysis of the Drosophila genome reveals that unlike the large syntaxin family, only three Drosophila and C. elegans gene products encode P25-related proteins. These include two homologues of P25 and one homologue of P29 (Fig. 2). The mammalian P25 and related P23 family 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 SNARE proteins membrin, Gos28, and Vti1p. M issing from the fly genome are homologues of the yeast SNAREs Bos1p and Sft1p. In general, the relative number of \( \nu \)-t-SNAREs 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 SNARE assortment present in multicellular organisms and provides the required framework for a genetic dissection of intracellular SNARE-mediated vesicular transport in Drosophila. Further analysis will provide insights into how SNAREs 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 SNARE assembly by binding to syntaxin, and controlling SNARE complex formation. Like yeast, Drosophila contains four Sec1 homologues, including ROP, Vps33p, Vps33p, and Sly1p. In addition, Drosophila contains homologues of proteins found in the yeast EXCST and TRAPP complexes, which are thought to function in vesicle targeting and docking before SNARE complex for-

### Table I. Drosophila Vesicle Trafficking Proteins

<table>
<thead>
<tr>
<th>Functional classification</th>
<th>Drosophila homologue</th>
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<th>Drosophila homologue</th>
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<tbody>
<tr>
<td>t-SNAREs</td>
<td>Syntaxin 1</td>
<td>( \nu )-SNAREs</td>
<td>n-Syb</td>
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<tr>
<td>Syntaxin 4</td>
<td></td>
<td>c-Syb</td>
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<td>Syntaxin 5</td>
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<td>Sec22</td>
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<tr>
<td>Syntaxin 6</td>
<td></td>
<td>Synaptobrevin-1 like</td>
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<td>Syntaxin 7</td>
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<td>Ykt6</td>
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<td>Syntaxin 8</td>
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</tr>
<tr>
<td>Syntaxin 13</td>
<td>Syntaxin 17</td>
<td>Other SNAREs</td>
<td>GOS28, membrin, and ViI1</td>
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<td>Syntaxin 16</td>
<td>SNAP-25</td>
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<tr>
<td>SNAP-25b</td>
<td>SNAP-29</td>
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<tr>
<td>Ufe1/Syntaxin 18</td>
<td>Bet1</td>
<td>TRAPP complex</td>
<td>Bet3</td>
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<td></td>
<td></td>
<td></td>
<td>Bet5</td>
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<tr>
<td>EXOCYST Complex</td>
<td>Sec5</td>
<td></td>
<td>Trs20</td>
</tr>
<tr>
<td>Sec6</td>
<td>Sec8</td>
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<td>Trs31</td>
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<td>Sem1</td>
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<td>HRS-2</td>
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<td>Synaptotagmin I</td>
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<td>SNAPIN</td>
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<td>Synaptotagmin VII</td>
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<td>Complexin</td>
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<td>Synaptotagmin V</td>
<td>Other Synaptotagmins (3 homologues)</td>
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<tr>
<td>Munc-13</td>
<td>Munc-13 related (2 homologues)</td>
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<tr>
<td>Otoferlin</td>
<td>Granulophin</td>
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<tr>
<td>Tricalbin (homologue of yeast C2 domain proteins)</td>
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<tr>
<td>Novel C2 domain proteins (4 homologues)</td>
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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, early endosome and lysosome. Syntaxins 7, 13, and 16 show homology 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 fulfill 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 SNA P-25 superfamily reveals three distinct subclasses (the SNA P-23, SNA P-25, and SNA P-29 subfamilies) that are conserved from Drosophila 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, SCAMPs, synapsin, CSP, SV2, CA PS, V A P-33, Rabphilin, H R-S-2, tomosyn, complexin, R im, 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, M unc-13, R im, Rabphilin, and D O C 2 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 that incorporates a neuroligin/neurexin junction in syn-

fied as neurexin ligands in mammals, generating a model
lar receptor family, the neuroligins, has also been identi-
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neurexins. Neurexins form a family of cell surface proteins
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tocadherin genes are present. Thus, although cadherins
likely to play an important role in synapse formation and
assembly. Among these are the integrin,
cadherin, and neurexin family of extracellular adhesion
components have been suggested to function in synapse
formation and assembly. A mong 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 n analysis 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 mam-
mals includes a number of synaptic isoforms, including
three large gene clusters encoding >50 protocadherin
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 spe-
cific subsets of neurons may provide a large and unique
synaptic targeting mechanism to establish the complex
connectivity in mammalian brain. In Drosophila, no pro-
tocadherin genes are present. Thus, although cadherins
may play a general role in synapse formation, they are un-
likely to underlie targeting specificity in the fly brain. A n
other protein family implicated in synapse formation is the
neurexins. Neurexins form a family of cell surface proteins
encoded by three genes in mammals. A lternative 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 extracellu-
lar receptor family, the neureligins, has also been identi-
fied as neurexin ligands in mammals, generating a model
that incorporates a neurexin/neurexin junction in syn-
aptic scaffolding. The Drosophila genome encodes three
neurexin-like genes. T wo of these, nrx IV and axotactin,
are not expressed in neurons, but rather in glia. T he third,
a homologue of neurexin III, may be expressed in neu-
rons, but like cadherins, neurexins are unlikely to play a
role in differential synaptic targeting for a wide array of
neurons. H owever, the fly genome encodes four neureli-
gin-like genes, suggesting that a neurexin III-neurexin
complex might play a more general role in synaptic scaf-
folding. Intracellular binding of PSD-95 to mammalian
neuroligins postsynaptically, and CA SK (a M A G U K-
related PDZ containing protein) binding to neurexins pre-
synaptically, may provide a substrate upon which further
synaptic macromolecular complexes are assembled. In-
deed, CA SK is known to form an additional complex with
the PDZ-containing proteins Velis and Mint, which subse-
quent to components of the synaptic exocytotic ma-
nery. Drosophila contains homologues of Mint, Velis,
PSD-95, and CA SK, providing the potential for a broadly
conserved synaptic assembly complex. A dditional synaptic
scaffolding proteins conserved in Drosophila include spe-
cific adapters for anchoring glutamate, GABA and acetyl-
choline receptors to specific synaptic subdomains. A
discussion of these proteins can be found in Littleton and
G anetzky (2000).

In conclusion, the rapid accumulation of genomic se-
quence data form multiple species is providing important
insights into the potential conservation of membrane traf-
ficking 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 exo-
cytosis. In many instances, a single gene encodes the
Drosophila homologue, making flies an attractive model
system for genetic dissection of the function of these pro-
teins in exocytosis. G enetic dissection of the larger protein
families such as the synaptotagmins will prove more diffi-
cult, given the potential for redundancy among similar
family members. H owever, the conservation of the indi-
vidual isoforms across species indicates they are likely to
have unique functions that have been selected for and con-
served through evolution. The analysis of the genome se-
quence of Drosophila has provided a basic framework to
begin to explore a large array of new ideas in membrane
trafficking. H owever, it is clear that the sequence repre-
sents the beginning of this analysis. G enetic and biochem-
ical approaches can now be employed to address the in
vivo functions of the known proteins components sug-
gested to underlie vesicular trafficking. Perhaps even more
importantly, the genomic sequence will facilitate the dis-
covery of novel components of the trafficking machinery
through the multitude of genetic tools available in Dro-
sophila.

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