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# A Genome-Wide Search for Synaptic Vesicle Cycle Proteins in *Drosophila*

Viewpoint

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## Summary

**The recent completion of the *Drosophila* genome sequence opens new avenues for neurobiology research. We screened the fly genome sequence for homologs of mammalian genes implicated directly or indirectly in exocytosis and endocytosis of synaptic vesicles. We identified fly homologs for 93% of the vertebrate genes that were screened. These are on average 60% identical and 74% similar to their vertebrate counterparts. This high degree of conservation suggests that little protein diversification has been tolerated in the evolution of synaptic transmission. Finally, and perhaps most exciting for *Drosophila* neurobiologists, the genomic sequence allows us to identify P element transposon insertions in or near genes, thereby allowing rapid isolation of mutations in genes of interest. Analysis of the phenotypes of these mutants should accelerate our understanding of the role of numerous proteins implicated in synaptic transmission.**

Knowledge of the mechanisms that lead to formation, docking, fusion, and recycling of synaptic vesicles is critical to our understanding of neural communication. Although a large number of proteins have been identified that may play a role in synaptic vesicle exocytosis and endocytosis (Hazuka et al., 1999), relatively little is known about the in vivo function of the majority of these proteins. *Drosophila* is an excellent model system for studying the in vivo mechanisms of synaptic vesicle exo- and endocytosis, as sophisticated electrophysiological techniques can be combined with the genetic power of *Drosophila* to determine if and how specific genes function in synaptic transmission (Pennetta et al., 1999). With the recent completion of the sequence of the *Drosophila* genome, fly neurobiologists are now able to tackle these questions on a genomic scale. We believe that in vivo approaches based on genetic manipulations will play a very prominent role in the near future in this research area.

The exocytosis of synaptic vesicles can be divided into three steps: targeting and docking of synaptic vesicles at the presynaptic membrane, priming of vesicles to become fusion competent, and rapid fusion of vesicles in response to calcium influx. Synaptic vesicle targeting to active zones is believed to require the actin cytoskeleton, but the exact mechanisms are poorly understood. One candidate for localizing vesicles to the active zone is the exocyst complex (exo70, 84, P71, sec3, 5, 6, 8, 10, 15), which defines the site of polarized secretion in yeast (Hazuka et al., 1999). Alternatively, a complex comprised of neurexin, CASK, and MINT may serve to localize active zones to sites of synaptic contact (Butz et al., 1998). After docking, the synaptic vesicle must be primed for fusion by forming *trans*-SNARE complexes between a v-SNARE (VAMP) on the vesicle and t-SNAREs (SNAP-25 and Syntaxin) on the presynaptic membrane (Robinson and Martin, 1998). NSF and SNAP are believed to play a critical role in this process by dissociating nonproductive and/or previously used *cis*-SNARE complexes. A large number of proteins have been identified which bind to SNARE proteins and have been implicated in regulating SNARE complex formation: complexins, Munc-13, Munc-18, syncollin, syntaphilin, and tomosyn bind to syntaxin; HRS-2 and snapin bind to SNAP-25; and VAP-33 binds to VAMP (Skehel, 1995; Bean et al., 1997; Edwardson, 1997; Mochida et al., 2000). Munc-13 and Munc-18 have been shown to play essential roles in neurotransmitter release in *Drosophila* and in mouse, but the in vivo role of most of the remaining proteins is largely unknown (Harrison et al., 1994; Augustin et al., 1999; Aravamudan et al., 1999; Verhage et al., 2000). Finally, in response to a calcium signal, the stable formation of a SNARE complex has been proposed to mediate the fusion of synaptic vesicles with the presynaptic membrane (Jahn and Hanson, 1998). The synaptic vesicle protein synaptotagmin is believed to be the primary fast calcium sensor, but several other calcium-binding proteins (e.g., DOC2, CAPS, rabphilin, calcineurin, Munc-13) may also regulate vesicle fusion in response to calcium (Nonet, 1999).

Rab proteins are small GTPases that are believed to play a critical role in all stages of vesicle transport, but their precise role in the exocytic process is not known (Chavrier and Goud, 1999). Rab3 is the Rab family member implicated in synaptic vesicle exocytosis, and a large number of Rab3 regulators and effectors have been identified, including Rab3-GAP, Rab3-GEF, Rab-GDI, Rabphilin-3A, and Rim (Geppert and Südhof, 1998). Similarly, several proteins have been found to be abundant on synaptic vesicles and have been proposed to regulate exocytosis, including SCAMP, SV2/SVOP, synapsin, synaptogyrin, and synaptophysin (Janz et al., 1998; Fernandez-Chacon et al., 1999; Lloyd and Bellen, 1999), but the role of most of these proteins is unclear.

Following synaptic vesicle exocytosis, synaptic vesicles are reformed by the process of endocytosis (Zhang and Ramaswami, 1999). Synaptotagmin and probably other synaptic vesicle proteins recruit members of the AP-2 adaptor protein complex, which in turn recruits clathrin. The AP-3 complex may also form synaptic vesicles from the endosome, and the AP-1 complex forms vesicles from the Golgi. The final pinching off of the

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Table 1. *Drosophila* Homologs of Synaptic Vesicle Cycle Proteins

| Proposed Function | Vertebrate Protein | Number of Known Vertebrate Genes | *Drosophila* Homolog(s) | Blast Score ($e^x$) | Percent Alignment | Percent Identity/ Percent Similarity | Cytological Location | P Element in or Near Gene |
|---|---|---|---|---|---|---|---|---|
| Targeting/ docking | CASK | 2 | CASK | −54 | 50 | | 93F | |
| | | | **CAKI** | −47 | 55 | 45/64 | 42D-E | |
| | exo70 | 1 | exo70 | −26 | 85 | | no cyto | |
| | exo84 | 1 | exo84 | −40 | 82 | | 69F | |
| | MINT | 3 | MINT | −104 | 45 | | 15F-16A | |
| | Neurexin | 4 | 3 homologs | −32 to −92 | 33 to 58 | 32/50 | 36D | |
| | P71 | 1 | P71 | −146 | 97 | | 95E | |
| | rsec5 | 1 | dsec5 | −115 | 99 | | 23F-24A | |
| | rsec6 | 1 | dsec6 | −79 | 98 | | 55E-F | EP(2)2021 |
| | rsec8 | 1 | dsec8 | −115 | 90 | | 60E | |
| | rsec10 | 1 | **dsec10** | −148 | 99 | 43/65 | 95E | |
| | rsec15 | 1 | dsec15 | −163 | 92 | | no cyto | |
| Priming/ regulation | α/β-SNAP | 2 | **SNAP** | −86 | 85 | 61/80 | 77B | |
| | γ-SNAP | 1 | γ-**SNAP** | −32 | 83 | 38/60 | 85F | |
| | | | γ-SNAP-2 | −25 | 83 | | 60B | |
| | Complexin | 2 | none (see text) | | | | | |
| | HRS-2 | 1 | HRS | −39 | 70 | 41/54 | 23A | |
| | Munc-13 | 4 | **dunc-13** | −132 | 69 | 58/73 | 102D | |
| | | | dunc-13-4A | −45 | 63 | | 65F | |
| | | | dunc-13-4B | −24 | 75 | | 96B | |
| | Munc-18 | 2 | **ROP** | 0 | 97 | 61/77 | 64A-B | |
| | | | VPS45 | −20 | 91 | | 85D-E | EP(3)3122 [3] |
| | NSF | 1 | **comatose** | 0 | 84 | 62/78 | 11D-E | |
| | | | **NSF-2** | −134 | 98 | 63/78 | 87F | |
| | Snapin | 1 | snapin | −16 | 76 | | 23A | |
| | Syncollin | 1 | none | | | | | |
| | Syntaphilin | 1 | none | | | | | |
| | Tomosyn | 1 | tomosyn | −55, −33 | 83 | 48/57 | 11B | |
| | | | **L(2)GL** | −56 | 56 | 37/56 | 21A | |
| | VAP-33 | 1 | VAP-33-1 | −20 | 38 | | no cyto | |
| | | | VAP-33-2 | −19 | 80 | | 101F-102F | |
| Fusion/ calcium sensing | Calcineurin A | 2 | 3 homologs | −65 to −124 | 51 to 93 | 70/81 | 14C | |
| | Calcineurin B | 1 | **calcineurinB** | −81 | 78 | 86/94 | 4F | |
| | | | **calcineurinB2** | −70 | 73 | 87/93 | 43E | EP(2)0774 |
| | CIRL/latrophilin | 3 | CIRL | −34 | 33 | | 44D-E | |
| | CAPS | 1 | **SDCAP** | −89, −58 | 81 | 57/71 | 101F-102F | |
| | CSP | 1 | **CSP** | −35 | 46 | 65/79 | 79E | **EP(3)0659** [2] |
| | DOC2 | 2 | none | | | | | |
| | Frequenin | 3+ | 5 homologs | −15 to −55 | 74 to 97 | 71/83 | 76D | |
| | SNAP-25 | 3 | **SNAP-25** | −12, −11 | 56 | 63/78 | 64E-65B | |
| | | | SNAP-24 | −65 | 96 | | 85F | |
| | Synaptotagmin | 12 | **SYT1** | −42 | 47 | 61/74 | 23B | |
| | | | SYT3 | −38 | 63 | | 36D | |
| | | | **SYT4** | −58 | 63 | 50/67 | 84D | |
| | | | SYT7 | −29 | 67 | | 101F-102 | |
| | | | 3 others | −23 to −26 | 38 to 62 | | 71A | |
| | Syntaxin | 16 | **SYX1A** | −101 | 91 | 68/82 | 95E | |
| | | | **SED5** | −77 | 95 | 56/76 | 35F | **EP(2)2313** |
| | | | SYX6/10 | −16 | 89 | | 78F | |
| | | | SYX7 | −21 | 68 | | 47E-F | |
| | | | SYX16 | −36 | 71 | | no cyto | |
| | VAMP | 8 | **synaptobrevin** | −28 | 70 | 69/83 | 47A-B | |
| | | | **n-SYB** | −20 | 63 | 64/74 | 62A-B | |
| Rabs/ regulators and effectors | Rab1-14 | 36 | 26 homologs | −18 to −158 | 76 to 100 | | 35A | P's in 4 genes |
| | Rab3 | 4 | **drab3** | −77 | 100 | 77/89 | 47B | |
| | Rab3-GAP | 1 | rab3-GAP | −36 | 69 | | 33B-C | |
| | Rab3-GEF | 1 | rab3-GEF | −81 | 63 | | 13A-B | |
| | RabGDI | 1 | **GDI** | −93 | 98 | 67/81 | 30B | EP(2)2080 |
| | | | rab escort protein | −17 | 67 | | 56D-E | |
| | Rabphilin-3A | 1 | rabphilin-3A | −32 | 63 | | 9E | |
| | RIM | 1 | RIM | −40 | 32 | | 90C-D | |

(*continued*)

Table 1. Continued

| Proposed Function | Vertebrate Protein | Number of Known Vertebrate Genes | *Drosophila* Homolog(s) | Blast Score ($e^x$) | Percent Alignment | Percent Identity/ Percent Similarity | Cytological Location | P Element in or Near Gene |
|---|---|---|---|---|---|---|---|---|
| SV phospho- | SCAMP | 3 | SCAMP | −15 | 62 | | 13C-E | EP(X)1593 [2] |
| proteins | SV2/SVOP | 8+ | 8 homologs | −16 to −43 | 40 to 90 | | 60B | EP(2)2077 |
| | Synapsin | 3 | **synapsin** | −18 | 34 | 42/59 | 86A | |
| | Synaptogyrin | 4 | synaptogyrin | −14 | 47 | | 50C | |
| | Synaptophysin | 2 | none | | | | | |
| Endocytosis | AP-1γ | 2 | AP-1γ | −95 | 73 | | no cyto | |
| adaptor | AP-1μ | 2 | **AP-47** | 0 | 99 | 82/92 | 85D | **EP(3)3436** [2] |
| proteins | AP-1σ | 1 | AP-1σ | −55 | 94 | | 95C-D | |
| | AP-2α | 2 | **α adaptin** | −150 | 99 | 70/82 | 21C | **l(2)06694** [4] |
| | AP-2β | 1 | **AP-2β** | 0 | 100 | 74/84 | 18E-F | |
| | AP-2γ | 1 | **AP-2γ** | −65 | 50 | 70/82 | no cyto | |
| | AP-2μ | 1 | **AP-50** | 0 | 99 | 87/94 | 94B | |
| | AP-2σ | 2 | AP-2σ | −55 | 97 | | 93D-E | |
| | AP-3β | 3 | **AP-3β** | 0 | 84 | 87/94 | 4C | |
| | AP-3δ | 1 | **garnet** | −120 | 55 | 50/65 | 12B | EP(X)0514 |
| | AP-3μ | 1 | **carmine** | −147 | 100 | 70/86 | 6E | |
| | AP-3σ | 3 | AP-3σ | −65 | 96 | | 60B | |
| | AP-180 | 1 | **LAP** | −55 | 33 | 52/67 | 84C-D | l(3)neo34 |
| Endocytosis | Amphiphysin | 2 | **DAMP** | −25 | 42 | 52/67 | 49B | EP(2)2175 [2] |
| | ARF | 6 | 10 Arf genes | −15 to −78 | 64 to 100 | 87/92 | 51F | **EP(2)2126** |
| | Auxillin | 1 | auxillin | −34 | 74 | | 82A-B | |
| | Clathrin heavy | 1 | **CHC** | 0 | 98 | 80/91 | 13F | |
| | Clathrin light | 2 | **CLC** | −27 | 86 | 34/52 | 77A | |
| | Dynamin | 3 | **shibire** | 0 | 86 | 67/80 | 14A | |
| | | | dynamin-2 | −77 | 63 | | 22F-23A | |
| | EHSH1 | 1 | **DAP-160** | −37 | 60 | 39/56 | 39A | EP(2)2543 |
| | Endophilin | 3 | endophilin | −97 | 99 | | 91D-F | **EP(3)0927** [4] |
| | Eps15 | 1 | EPS-15 | −32 | 40 | | 60E | EP(2)2513 |
| | Epsin | 2 | **liquid facets** | −70 | 72 | 45/61 | 66A | |
| | | | epsin-2 | −30 | 30 | | 94B | l(3)03685 |
| | STAM | 1 | **STAM** | −88 | 83 | 39/54 | 32A | |
| | Synaptojanin | 2 | synaptojanin | 0 | 82 | | 58A-B | |
| | | | 4 others | −29 to −44 | 32 to 55 | | 61F-62A | |
| | Syndapin | 2 | syndapin | −49 | 73 | | 92E-93A | EP(3)0877 [2] |

Vertebrate protein families have been grouped roughly according to their proposed function in the synaptic vesicle cycle and listed alphabetically. Vertebrate protein sequences were found by searching GenBank, and the number of known members of each vertebrate protein family is listed. *Drosophila* homologs were identified using a tblastn search of all vertebrate protein sequences against the finished, unfinished, and nonannotated *Drosophila* genomic sequence databases. Genes were considered homologs if the blast score was less than $e^{-15}$ and aligned over >30% of the vertebrate protein sequence. In some cases, two genomic sequences for a single gene were identified due to a gap in the genomic sequence, in which case the blast scores for both genomic sequences are listed. When known *Drosophila* genes were previously identified, the *Drosophila* name is in bold, and the percent identity/similarity was determined using the Smith-Waterman algorithm (http://www.timelogic.com/). Genes with a published mutant phenotype in *Drosophila* are underlined. The cytological location was determined by comparing Celera genomic sequence with sequence from BAC and P1 clones, which have been mapped by the BDGP (FlyBase Consortium, 1999). In some cases, multiple homologs were identified, and only the cytology for the most homologous gene is listed. "no cyto" refers to rare cases in which the Celera genomic sequence did not align with BAC or P1 sequence. P element insertions in genes were found using a blastn search of the P element STS database with *Drosophila* genomic sequence within 1 kb of the ends of the presumptive 5′ and 3′ ends of each *Drosophila* gene. Brackets denote the total number of P elements found in each gene, and lethal P element lines are in bold.

vesicle is believed to be mediated by the GTPase dynamin and the phospholipid acyl-transferase endophilin, and auxillin and ARF family members are required to remove the clathrin coat (Ringstad et al., 1999; Zhang and Ramaswami, 1999). Finally, a large number of proteins have been implicated in regulating synaptic vesicle endocytosis through interactions with the adaptor protein complex or dynamin, including amphiphysin, synaptojanin, eps15, and epsin, but their precise function is unclear.

We have attempted to identify all *Drosophila* homologs of vertebrate proteins implicated in neurotransmitter release to initiate systematic genetic analyses. We therefore screened the recently completed Celera genomic sequence (Adams et al., 2000; Rubin et al., 2000) for homologs of vertebrate proteins that have been implicated in synaptic vesicle cycle biology based on biochemical or functional studies. A search of 196 vertebrate proteins representing 70 gene families allowed us to identify 144 fly homologs corresponding to 65 gene families (see Table 1). Out of 144 fly genes, we found 84 (60%) novel homologs. We surmise that we have identified almost all homologous *Drosophila* genes, as our search of the Celera genomic database alone allowed us to identify 59 out of 60 previously characterized fly homologs. Only a single gene was not identified in

the Celera database search, even though it was present in the database, because a small gap in the genomic sequence led to a blast score just below our cutoff for the two pieces. This finding further suggests that the 1300 gaps reported in the Celera genomic sequence cannot be extensive (Adams et al., 2000). In addition, the homologs of vertebrate genes that were not identified in the Celera database were also not present in the *C. elegans* genome (*C. elegans* Sequencing Consortium, 1998). Based on these and other data, we estimate that we have identified more than 95% of the *Drosophila* homologs implicated in synaptic vesicle biology.

Since the entire protein sequence is available for all previously characterized fly homologs (40% of the genes), we calculated the average similarity and identity for these proteins. Known *Drosophila* homologs are on average 74% ± 13% (SD) similar and 60% ± 16% (SD) identical to their vertebrate counterparts over their full length. The fully sequenced fly genes encode proteins that align over 74% of their length with the vertebrate homologs, while the new homologs line up over 70% of the length of the vertebrate protein sequence. Hence, the proteins implicated in the biology of synaptic vesicle exo- and endocytosis are highly conserved over most of their length. This is in sharp contrast to orthologous proteins involved in developmental pathways. Many of these proteins are transcription factors and secreted proteins, and their similarity and identity is often confined to relatively short functional domains, many of which correspond to DNA or protein interaction domains. The data therefore suggest that many synaptic vesicle cycle proteins interact with several other proteins implicated in synaptic vesicle exo- and endocytosis, causing strong evolutionary constraints (Mochida, 2000). In addition, it is interesting to note that while the central players in exo- and endocytosis are well conserved, putative regulators exhibit more variation.

Although almost all protein families searched are present in *Drosophila*, as stated previously, some protein families are absent. No homologs were found for DOC2, syncollin, synaptophysin, and syntaphilin. Similarly, we did not identify homologs of these proteins in the *C. elegans* genome either (*C. elegans* Sequencing Consortium, 1998). This suggests that these proteins are not essential for neurotransmitter release in *Drosophila* and *C. elegans*, but rather that they evolved relatively recently and likely play an accessory regulatory function. This nonessential role is further supported by loss-of-function studies of synaptophysin (McMahon et al., 1996) and DOC2 (Sakaguchi et al., 1999) in mice, in which removal of either protein shows no or very mild phenotypes. Although complexins were not found in our initial tblastn search because they are such small proteins, a more sensitive search of the genome identified a putative homolog that is ~50% identical.

While nearly all vertebrate gene families are present in *Drosophila*, they are typically represented by fewer gene members. For example, well-characterized genes such as the SNARE proteins have on average three vertebrate homologs for every *Drosophila* gene. When taken as a whole, on the other hand, there is on average a 1:1 relationship between the number of known vertebrate genes and *Drosophila* homologs. Given the size and complexity of vertebrate genomes, this strongly suggests that a large number of homologous vertebrate genes remain to be identified. Nevertheless, the fact that most genes are present in a single copy in *Drosophila* will make the phenotypes of genetic lesions much easier to interpret.

While most *Drosophila* gene families have only one or two members, some have many more. We have found only two SNAP-25 and two VAMP homologs, but there are five syntaxin and seven synaptotagmins in *Drosophila*. Furthermore, we have identified at least 26 Rab family members and 10 ARFs in *Drosophila*, which is not surprising considering more than 35 Rab members and 6 ARFs involved in different stages of vesicle trafficking have been identified in vertebrates (Chavrier and Goud, 1999). When our blast searches identified multiple potential homologs, a blastx search was performed on each homologous genomic sequence to exclude those homologs that were more similar to other well-characterized vertebrate proteins. In several cases, a surprisingly large number of homologs remained. For example, we found at least eight SV2-like organic cation transporters, five synaptojanin-like inositol polyphosphate 5-phosphatases, five members of the frequenin/neurocalcin family, and five calcineurin family members. Such a large number of related genes suggests that these gene families either play multiple roles in vesicular trafficking and synaptic transmission or that they are also involved in other processes.

One of the primary goals of the postgenomic era will be to assign a function to newly discovered genes, primarily by analyzing the effects of the loss of each gene. The first step in identifying mutations in *Drosophila* is to map the gene cytologically to identify rearrangements in or near the gene of interest. Since many BAC and P1 clones have been mapped as part of the Berkeley *Drosophila* Genome Project (BDGP), the majority of genes can be assigned a cytological position (FlyBase Consortium, 1999). Analysis of these cytological positions suggests that although most genes listed in Table 1 are randomly distributed, some are clustered. For example, the cytological interval 22F–23B contains four putative synaptic vesicle cycle genes within a 160 kb region: *dynamin-2*, *SYT1*, *snapin*, and *HRS*. Similar clusters are present at 47A–B (three genes), 60B (three genes), 94B (three genes), 95E-F (five genes), and 101F–102F (seven genes) (Table 1). Furthermore, SV2-related genes (organic cation transporters) and frequenin genes appear to be duplicated in some areas of the genome. The cytological interval 95F–96A contains three organic transporters within 15 kb, and two other regions each have two genes within 30 kb. This clustering may facilitate their genetic analysis, as small deletions may remove many interesting genes, allowing a single saturation mutagenesis to identify mutations in numerous genes implicated in synaptic vesicle trafficking.

One of the most powerful tools used to create mutations in *Drosophila* is the P element transposon. These transposable elements can be mobilized in the genome and preferentially insert into the 5′ end of genes (Bellen, 1999). Thanks to a large-scale effort headed by BDGP and P element screens carried out in the US and Europe, about 25% of all essential fly genes have now been associated with a homozygous lethal P element insertion (Spradling et al., 1999). One of the advantages of

P elements is the ability to determine the exact genomic position of the P element by sequencing the flanking genomic DNA. A search of the BDGP database for P element STS insertion sites in or near (<1 kb) the genes listed in Table 1 allowed us to identify 35 independent P element insertion lines in 21 different genes. Eighteen of these genes, including *sec6*, *CIRL/latrophilin*, *amphiphysin*, *endophilin*, and *eps15*, have at present not been genetically characterized in any animal species. Furthermore, the vast majority of these P elements are EP P elements (Rorth, 1996), which contain UAS sites, enabling the flanking gene to be overexpressed when driven by an appropriate GAL4 driver line. Hence, it should be possible to generate relatively quickly gain- and loss-of-function phenotypes for many of the genes listed in Table 1.

The majority of P elements (~90%) in large-scale genetic screens are homozygous viable (Bellen, 1999). However, half (11 out of 22) of the lines we have received thus far are associated with homozygous lethality. Although some of the P elements may not cause this lethality, these chromosomes supposedly carry single P element insertions (Spradling et al., 1999). The data therefore suggest that many of the genes encoding proteins associated with the synaptic vesicle cycle may be essential, as shown previously for those that have already been characterized using reverse genetic approaches (Pennetta et al., 1999). Thus, analysis of these P element lines will provide valuable information on the loss-of-function and overexpression phenotypes of many genes implicated in synaptic transmission.

Finally, knowledge of the cytological positions of these *Drosophila* genes should vastly accelerate the cloning of genes mutated in forward genetic screens. For example, a large number of temperature-sensitive paralytic mutations are present throughout the *Drosophila* genome that have not yet been cloned (Littleton et al., 1998). Several synaptic vesicle cycle genes are present in the same cytological locations as these paralytic mutations, making these genes strong candidates for being responsible for these phenotypes. Furthermore, a new technique, combining the power of the FLP/FRT system with the ease of ERG recording in *Drosophila*, should allow for many more synaptic vesicle cycle genes to be mutated in genetic screens using this approach (Stowers and Schwarz, 1999). In summary, the completed genomic sequence of *Drosophila* has brought fly neurobiologists a tremendous boost to uncovering the mechanisms of neurotransmitter release.

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#### References

Adams, M.D., Celniker, S., Holt, R., Evans, C., Gocayne, J., Amanatides, P., Scherer, S., Li, P., Hoskins, R., Galle, R., et al. (2000). The genome sequence of *Drosophila melanogaster*. Science *287*, 2185–2195.

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.

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

Bean, A.J., Seifert, R., Chen, Y.A., Sacks, R., and Scheller, R.H. (1997). Hrs-2 is an ATPase implicated in calcium-regulated secretion. Nature *385*, 826–829.

Bellen, H.J. (1999). Ten years of enhancer detection: a fly viewpoint. Plant Cell *12*, 2271–2282.

Butz, S., Okamoto, M., and Südhof, T.C. (1998). A tripartite protein complex with the potential to couple synaptic vesicle exocytosis to cell adhesion in brain. Cell *94*, 773–782.

*C. elegans* Sequencing Consortium (1998). Genome sequencing of the nematode C. elegans: a platform for investigating biology. Science *282*, 2012–2018.

Chavrier, P., and Goud, B. (1999). The role of ARF and Rab GTPases in membrane transport. Curr. Opin. Cell Biol. *11*, 466–475.

Edwardson, J.M., An, S., and Jahn, R. (1997). The secretory granule protein syncollin binds to syntaxin in a $Ca^{2+}$-sensitive manner. Cell *25*, 325–333.

Fernandez-Chacon, R., de Toledo, G.A., Hammer, R.E., and Südhof, T.C. (1999). Analysis of SCAMP1 function in secretory vesicle exocytosis by means of gene targeting in mice. J. Biol. Chem. *274*, 32551–32554.

FlyBase Consortium (1999). The FlyBase Database of the *Drosophila* Genome Projects and community literature. Nucleic Acids Res. *27*, 85–855.

Geppert, M., and Südhof, T.C. (1998). RAB3 and synaptotagmin: the yin and yang of synaptic membrane fusion. Annu. Rev. Neurosci. *21*, 75–95.

Hazuka, C.D., Foletti, D.L., and Scheller, R.H. (1999). Nerve terminal membrane trafficking proteins: from discovery to function. In Neurotransmitter Release, H.J. Bellen, ed. (New York: Oxford University Press), pp. 81–125.

Harrison, S.D., Broadie, K., van de Goor, J., and Rubin, G.M. (1994). Mutations in the *Drosophila Rop* gene suggest a function in general secretion and synaptic transmission. Neuron *13*, 555–566.

Jahn, R., and Hanson, P.I. (1998). Membrane fusion. SNAREs line up in new environment. Nature *393*, 14–15.

Janz, R., Hofmann, K., and Südhof, T.C. (1998). SVOP, an evolutionarily conserved synaptic vesicle protein, suggests novel transport functions of synaptic vesicles. J. Neurosci. *18*, 9269–9281.

Littleton, J.T., Chapman, E.R., Kreber, R., Garment, M.B., Carlson, S.D., and Ganetzky, B. (1998). Temperature-sensitive paralytic mutations demonstrate that synaptic exocytosis requires SNARE complex assembly and disassembly. Neuron *21*, 401–413.

Lloyd, T.E., and Bellen, H.J. (1999). Genetic analysis of neurotransmitter release in mice and humans. In Neurotransmitter Release, H.J. Bellen, ed. (New York: Oxford University Press), pp. 352–388.

McMahon, H.T., Bolshakov, V.Y., Janz, R., Hammer, R.E., Siegelbaum, S.A., and Südhof, T.C. (1996). Synaptophysin, a major synaptic vesicle protein, is not essential for neurotransmitter release. Proc. Natl. Acad. Sci. USA *93*, 4760–4764.

Mochida, S. (2000). Protein-protein interactions in neurotransmitter release. Neurosci. Res. *36*, 175–182.

Nonet, M. (1999). Studying mutants that affect neurotransmitter release in *C. elegans*. In Neurotransmitter Release, H.J. Bellen, ed. (New York: Oxford University Press), pp. 265–303.

Pennetta, G., Wu, M.N., and Bellen, H.J. (1999). Dissecting the molecular mechanisms of neurotransmitter release in *Drosophila*. In Neurotransmitter Release, H.J. Bellen, ed. (New York: Oxford University Press), 304–351.

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

Rorth, P. (1996). A modular misexpression screen in *Drosophila* detecting tissue-specific phenotypes. Proc. Natl. Acad. Sci. USA *93*, 12418–12422.

Rubin, G.M., Yandell, M., Wortman, J., Miklos, G., Nelson, C., Hariharan, I., Fortini, M., Li, P., Apweiler, R., Fleischmann, W., et al.

(2000). Comparative genomics of the eukaryotes. Science *287*, 2204–2215.

Sakaguchi, G., Manabe, T., Kobayashi, K., Orita, S., Sasaki, T., Naito, A., Maeda, M., Igarashi, H., Katsuura, G., Nishioka, H., et al. (1999). Doc2α is an activity-dependent modulator of excitatory synaptic transmission. Eur. J. Neurosci. *12*, 4262–4268.

Skehel, P.A., Martin, K.C., Kandel, E.R., and Bartsch, D. (1995). A VAMP-binding protein from Aplysia required for neurotransmitter release. Science *269*, 1580–1583.

Spradling, A.C., Stern, D., Beaton, A., Rhem, E.J., Laverty, T., Mozden, N., Misra, S., and Rubin, G.M. (1999). The Berkeley *Drosophila* Genome Project gene disruption project. Single P-element insertions mutating 25% of vital *Drosophila* genes. Genetics *153*, 135–177.

Stowers, R.S., and Schwarz, T.L. (1999). A genetic method for generating *Drosophila* eyes composed exclusively of mitotic clones of a single genotype. Genetics *152*, 1631–1639.

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.

Zhang, B., and Ramaswami, M. (1999). Synaptic vesicle endocytosis and recycling. In Neurotransmitter Release, H.J. Bellen, ed. (New York: Oxford University Press), pp. 389–432.