Analysis of amyloid precursor protein function in Drosophila melanogaster

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Abstract Amyloid precursor proteins (APPs) are evolutionary conserved from nematodes to man (Jacobsen and Iverfeldt in Cell Mol Life Sci 66:2299–2318, 2009) suggesting an important physiological function of these proteins. Human APP is a key factor in the pathogenesis of Alzheimer’s Disease because its proteolytic processing results in the production of the neurotoxic Aβ-peptide, which accumulates in the amyloid plaques characteristic for this disease (Selkoe in Physiol Rev 81(2):741–766, 2001). However, the processing also leads to the production of several other fragments and the role of these products, as well as the function of the full-length protein is so far not well understood. The functional analysis of APP in vertebrates has been hampered by the fact that two close relatives, APLP1 and APLP2, exist and that knock-out mice for APP only show subtle defects. In contrast, invertebrates like Caenorhabditis elegans and Drosophila express only one APP-like protein but whereas a null mutation in the C. elegans APL-1 protein is lethal, flies lacking APPL (Amyloid Precursor Protein-like) are viable but show synaptic defects and behavioral abnormalities. Together with the analyses of flies that express APP proteins ectopically or xenotopically, these studies show that APP proteins are involved in neuronal differentiation, neuritic outgrowth, and synapse formation. In addition, they play a role in long-term memory formation and maintaining brain integrity in adult flies.

Keywords APP proteins · Drosophila

APP and its processing is conserved in Drosophila

The human Amyloid Precursor Protein (APP) has first gained interest due to its connection with Alzheimer’s Disease (AD). Two small peptides, Aβ40 and Aβ42, were identified as major components of the amyloid plaques which are characteristic for AD (Glenner and Wong 1984; Masters et al. 1985) Subsequently the full-length APP gene was identified by four groups (Kang et al. 1987; Goldgaber et al. 1987; Tanzi et al. 1987; Robakis et al. 1987). APP is a type-one membrane-spanning protein with a large extracellular domain, the Aβ40/42 peptide that is located adjacent to the membrane domain, and a small intracellular C-terminal domain (Kang et al. 1987). There are three major isoforms (695aa, 751aa, and 770aa) produced by alternative splicing that are widely expressed, with APP695 being the predominant form in neurons (e.g., Tanaka et al. 1989; Lorent et al. 1995). Shortly after the identification of the human gene, the Drosophila ortholog APPL (Amyloid Precursor Protein-like) was isolated in the laboratory of Kalpana White in a search for neuronally enriched mRNAs (Rosen et al. 1989). The APPL gene is localized near the tip of the X-chromosome and due to a non-conserved stretch in the extracellular part the fly protein is larger (887aa) than its human counterpart. APPL shows an overall identity of 25.5% and a similarity of 39.5% to human APP695, whereby the sequence identity is significantly higher in the C-terminal domain (51%) and in the E1 and E2 N-terminal, extracellular domains (Fig. 1a).
In contrast, the region comprising the human β-peptide appears to lack conservation, which initially led to the assumption that a comparable neurotoxic peptide cannot be produced from fly APPL (Coulson et al. 2000; Bilen and Bonini 2005; Link 2005). APPL is pan-neuronally expressed starting from stage 13 of Drosophila embryogenesis, which correlates with the onset of axonal outgrowth. During development, the protein is especially abundant in growing axons and in areas of synapse formation (Martin-Morris and White 1990; Luo et al. 1990). The expression persists throughout the development and in the adult brain APPL expression is particularly prominent in the mushroom bodies (Torroja et al. 1996) which are involved in learning and memory (Heisenberg 2003). In contrast to the human protein, APPL expression appears to be restricted to the nervous system.

As mentioned above, APP can be cleaved into several fragments by the so-called secretases. The processing occurs by two alternative pathways; the amyloidogenic pathway, which leads to Aβ (red region in Fig. 1) production, and the non-amyloidogenic pathway (for example, Turner et al. 2003). In the former, APP is sequentially cleaved by the β- and γ-secretase, and besides the Aβ peptide, this also results in the production of a large soluble N-terminal fragment (sAPPβ) and a small intracellular C-terminal fragment (AICD, shown in blue Fig. 1). In the predominant non-amyloidogenic pathway, the N-terminus is cleaved by the α-secretase, creating a slightly larger secreted fragment (sAPPα). The remaining part of APP is then again processed by γ-secretase cleavage, which results in the same AICD. Whereas the AICD was proposed to relocate to the nucleus and play a role in transcriptional regulation (Cao and Südhof 2001; Kimberly et al. 2001; Müller et al. 2007), both sAPPs have been implicated in multiple cell biological processes (for example, Jacobsen and Iverfeldt 2009) although their function can be different. For instance, it has been suggested that sAPPα has a neuroprotective function (Araki et al. 1991; Mattson et al. 1993; Goodman and Mattson 1994) whereas sAPPβ might be deleterious for neuronal survival (Nakagawa et al. 2006; Nikolaev et al. 2009). Already White and co-workers showed that also Drosophila APPL can be cleaved at the N-terminus, resulting in the production of a soluble sAPPL (Fig. 1a; Luo et al. 1990). Although a fly AICD (dAICD)

![Fig. 1](https://example.com/diagram.png)

**Fig. 1** Schematic drawing of human APP695 and APPL. a Overall structure of APP695 and APPL showing the conserved E1 and E2 domains in the extracellular part (yellow, 38 and 33% identity), the Aβ region (red box), and the C-terminal, intracellular AICD (blue box). The secretase cleavage sites are indicated (note that the α- and β-cleavage sites appear reversed in APPL although their exact locations are still unknown), and the transmembrane domain is outlined by the dotted lines. b A Blastp alignment of the C-termini of APP695 and APPL containing the Aβ region (red) and the AICD (blue). The AICDs are highly conserved with 51% sequence identity, including the completely conserved GYENPTY (boxed) that are required for binding to the multidomain adaptor proteins Dab (disabled) and X11 (X11/mint family protein). This region is also required for binding to APP-BP1 (the β-Amyloid precursor protein binding protein) which is a subunit of the ubiquitin-like protein NEDD8. The G-protein Gαi binds to a region upstream of the GYENPTY site but still within the AICD. In contrast to the highly conserved AICD, the fly dAβ peptide and the human Aβ42 peptide (red) show only a limited conservation of 28%. Underlined sequences indicate the membrane-spanning domains.
has not been demonstrated directly, the existence of a γ-secretase complex and the high homology in this region suggest that a similar C-terminal fragment is produced from APPL. In addition, the conservation of binding site for the interaction partners like X11, Gαz, and APP-BP in this region (Fig. 1b; e.g., Jacobsen and Iverfeldt 2009) suggests that also the proposed function of this fragment in transcriptional regulation might be conserved.

The existence of a soluble APPL fragment and the structural homology to APP suggested that at least the non-amyloidogenic processing is conserved in flies and that corresponding secretases are expressed in Drosophila. In vertebrates, several laboratories have focused on the ADAM (A Disintegrin And Metalloproteinase) protein family as candidates for α-secretase, with ADAM10 being the best established member (Roberts et al. 1994; Lammich et al. 1999; Lichtenhaler 2011). In Drosophila, an ADAM-like protease is encoded by the kuzbanian gene (kuz; Rooke et al. 1996), which was originally identified due to its role in lateral inhibition during neurogenesis. Subsequently it was shown that KUZ can cleave Notch (Pan and Rubin 1997), which is also a target of ADAM10 in vertebrates (Brou et al. 2000). More recently, it was confirmed that KUZ does indeed exhibit α-secretase activity and is able to cleave APPL (Carmine-Simmen et al. 2009).

The vertebrate γ-secretase is a multi-protein complex that consists minimally of Presenilin 1 (PS-1; De Strooper et al. 1989), Anterior Pharyx Defective 1 (APH-1; Goutte et al. 2000), nicastrin (NCT; Yu et al. 2000), and Presenilin Enhancer-2 (PSEN-2; Francis et al. 2002). The catalytic activity is most likely provided by Presenilin 1 (Wolfe et al. 1999) that shows partial redundancy with a second family member, Presenilin 2 (PS-2; Clark et al. 1995). γ-Secretase is an aspartyl protease that, like ADAM10, can also cleave Notch in addition to a variety of other substrates (for a current review see De Strooper and Annaert 2010). Drosophila Presenilin (PSN) has been identified by two groups (Hong and Koo 1997; Boulianne et al. 1997) and it was shown in several publications (e.g., Ye et al. 1999; Struhl and Greenwald 1999) that it processes Notch. Moreover, Drosophila PSN was shown to promote APPL cleavage (Carmine-Simmen et al. 2009) as well as human APP cleavage when expressed in flies (Guo et al. 2003; Greeve et al. 2004). Like vertebrate γ-secretase, the fly version is a complex containing PSN, NCT, APH1, PEN2, and recently, γ-secretase activity against APP and APPL has been reconstituted by expressing tagged versions of the four Drosophila orthologs in transgenic flies (Stempfle et al. 2010).

Like γ-secretase, β-secretase is an aspartyl protease, and two closely related members, BACE1 (β-site APP-cleaving enzyme; Hussain et al. 1999; Sinha et al. 1999; Vassar et al. 1999; Yan et al. 1999) and BACE2 (Solans et al. 2001), have been identified in vertebrates. Initially, it had been suggested that Drosophila might lack β-secretase activity (for example; Fossgreen et al. 1998) and although two aspartic proteases, identified by their homology to human BACE (DASP1 and DASP2), were identified in 2005 they did not show β-secretase activity toward human APP or APPL in cell culture experiments (Kotani et al. 2005). However, another study using flies overexpressing human APP and BACE1 in Drosophila indicated that an endogenous β-secretase-like enzyme might exist in flies (Greeve et al. 2004). As expected, the co-expression of APP and BACE1 resulted in the production of the Aβ-peptide surprisingly however a toxic Aβ-peptide was also produced in flies that only expressed APP, although this peptide was slightly larger. Similarly, determining the production of the β-CTF (C-Terminal Fragment), which results from APP processing by BACE prior to γ-secretase cleavage, revealed a slightly longer fragment in flies expressing APP alone and a β-CTF of the expected length in double transgenic animals. Based on these results, we identified a fly BACE ortholog (dBACE, which turned out to correspond to DASP2) and showed that it can cleave Drosophila APPL, resulting in a slightly smaller CTF than observed after KUZ cleavage (Carmine-Simmen et al. 2009). Although this suggested that the sites for α- and β-cleavage are reversed in APPL compared to human APP (Fig. 1), we showed that nevertheless a neurotoxic Aβ-like peptide (dAβ) is produced. Because the conservation between the primary sequences of human Aβ and fly dAβ is low, the toxicity might be due to a similar secondary structure. Indeed, as indicted by Thioflavin-S staining dAβ appears to form β-sheets like the human Aβ. The evolutionary conservation of APP and APPL, the similarities in their processing, and the comparable detrimental effects caused by their Aβ peptides strongly suggest that also their normal physiological functions are conserved. Therefore, studies in Drosophila might also provide valuable insights into the normal function of human APP proteins.

The role of APPL in neuronal development

Flies lacking APPL (Appld) are viable and fertile with no obvious structural defects in the adult central nervous system (Luo et al. 1992). This parallels knock-out mice for the APP gene that display only minor neuronal deficits that vary in different genetic backgrounds (Müller et al. 1994; Zheng et al. 1995; review in Senechal et al. 2006). In contrast, mutants for the apl-1 gene in C. elegans (Daigle and Li 1993) show an early developmental arrest and are lethal (Hornsten et al. 2007). Although APPL is not an essential gene for the fly’s development, its loss is not without consequences because Appld mutants reveal
defects at the larval neuromuscular junction (NMJ). The *Drosophila* NMJs are stereotypically shaped at each individual body wall muscle, so developmental changes can be observed easily. Different types of synaptic boutons, the neuro-transmitter releasing sites, are added along the axonal terminus during larval development (Gramates and Budnik 1999), and ApplΔ mutant larvae showed a significant reduction in bouton numbers (Torroja et al. 1999a, b). In contrast, overexpression of APPL induced excessive formation of boutons of different sizes, i.e., large “parent” and small “satellite” boutons that are connected to the former and especially these satellite boutons were also induced by the xenotopic expression of human APPΔ95s. Interestingly, this phenotype did also occur after the expression of a secretion-defective version of APPL (APPLΔd, see Fig. 1a), however, not by a mutant construct that in addition lacked the intracellular C-terminal domain (APPLΔeICT; Fig. 1a). Further analysis of mutant isoforms revealed that satellite-bouton formation required the conserved internalization sequence (GYENPTY) in the cytoplasmic domain, whereas the induction of additional parent boutons depended on the presence of the G0 protein binding site (Fig. 1b). Together these results indicated that APPL acts as a receptor that mediates cell-cell communication at the larval NMJ, thereby promoting synapse formation (Torroja et al. 1999a, b). To fulfill this function, APPL interacts with the cell adhesion molecule Fasciclin II and the PDZ-domain containing dX11/Mint protein (Fig. 1b) via its intracellular domain (Hase et al. 2002; Ashley et al. 2005).

These APPL-induced structural changes at the NMJ also had physiological consequences. Performing intracellular recordings from body wall muscles, it was shown that the loss of APPL resulted in a reduction in the amplitude of evoked excitatory junctional potentials (EJPs). This was in part compensated by an elevation of miniature EJP amplitude and frequency of release (Ashley et al. 2005). Electrophysiological studies on embryonic cells in culture supported a role of APPL in modulating synaptic function because whole-cell patch clamp measurements of these neurons revealed that the loss and overexpression of APPL both increased A-type K⁺ currents. A more detailed analysis, using different mutant forms of APPL, indicated that the secreted APPL fragment (sAPPL) was necessary to modulate K⁺-channel activation (Li et al. 2004). A similar function has also been suggested in vertebrates by findings that sAPPLα activation can activate K⁺-channels in hippocampal cells (Furukawa et al. 1996).

Studies on neuronal cells cultured from embryonic neuroblasts revealed a role of APPL in regulating neuronal outgrowth and synaptic function. Surprisingly, the lack of APPL as well as overexpression of full-length APPL resulted in reduced neurite outgrowth. In contrast, the expression of secretion-defective and therefore membrane-bound forms (APPLΔd and APPLΔeICT) induced excessive neurite growth. Further analysis revealed that the expression of the secreted sAPPL again reduced neurite length whereas the combined expression of sAPPL and the secretion-defective form (APPLΔd) resulted in nearly wild-type neurite outgrowth (Li et al. 2004). Together these experiments suggest that the full-length membrane-bound APPL is acting as a receptor that promotes neurite growth, whereas the secreted ectodomain, presumably acting as a ligand, inhibits growth. In this scenario, overexpression of APPL could lead to increased levels of the secreted ectodomain, thus resulting in neurite shortening. In the case of the APPLΔ mutant, a similar phenotype would result from lacking the growth promoting function of the full-length APPL receptor. Interestingly, in contrast to the function of APPL in synaptic bouton formation, an intact C-terminal domain appears not to be required for the function in neurite outgrowth (Li et al. 2004). So far, no receptor has been identified for sAPPL, but it has been shown that APP family members can form homo-dimers and promote transcellular adhesion (Soba et al. 2005) but whether sAPPL can bind to full-length APPL remains to be determined.

The influence of APPL on neurite growth is not restricted to embryonic cells in culture. In line with the results described above, overexpression of APPL or APP in neuropeptide secreting neurons during pupal development or in the adult resulted in increased axonal arborisation (Leyssen et al. 2005). In contrast to the cell culture study, in these in vivo experiments, an intact C-terminus is required, at least for APP. Specifically the YENPTY motif of APP, which mediates the binding to adaptor proteins like X11/Mint and Dab1, had to be intact to induce these phenotypes. Moreover, genetic interaction studies revealed that the axonal outgrowth is dependent on the activated dAbl kinase that appears to interact with APP via the *Drosophila* adaptor protein Dab (Fig. 1b; Leyssen et al. 2005). Excessive axonal sprouting might also explain phenotypes observed in a small, but significant number of flies after overexpression of APPL in the developing mushroom bodies (Li et al. 2004). This prominent paired structure within the central brain (Fig. 2c) consists of the posterior located calyx, containing dendrites that receive olfactory input, and a ventral-anterior axonal projection called peduncle which splits into five mushroom body lobes. The α/β lobes project dorsally, while the β′/β′ lobes and γ-lobes are horizontally orientated toward the midline (e.g., Aso et al. 2009). Continuous overexpression of APPLΔd in the developing mushroom body neurons resulted in the fusion of the β′ lobes at the midline and a loosened fasciculation of the axons within the β/β′ lobes causing a fuzzy appearance. The latter phenotype was also observed in the ApplΔ null mutant and when overexpressing...
APPLΔCCT (Li et al. 2004) suggesting that membrane-bound APPL can induce de-fasciculation. However, a defasciculation phenotype in the α-lobes was also induced when sAPPβ was expressed in the mushroom bodies (Fig. 2b). It should be noted that in these studies, the endogenous Appl gene was present allowing possible interactions between fragments and/or the full-length proteins.

Finally, APPL was shown to be required during the development of the peripheral nervous system, specifically the development of the mechano-sensory organs (MSO; Merdes et al. 2004). The mechano-sensory bristles on the adult thorax are derived from a sensory organ precursor cell (SOP) which is singled out of a group of neuro-ectodermal cells by lateral inhibition mediated by the Notch receptor. Notch signaling is also responsible for determining the cell fate of the progeny of the SOP, which finally build the MSO: shaft, socket, a sheath cell, the sensory neuron, and a supporting glia cell (Lai and Orgogozo 2004). ApplΔ mutant flies have a significant reduction in scutellar and sternopleural MSOs on their thorax and a ubiquitous knockdown of Appl mRNA during the development via RNA-interference can phenocopy this defect. Surprisingly, not only the sensory neuron but also the external cell types of the MSO are missing, suggesting a role of APPL in SOP lineage formation (Merdes et al. 2004). This implies that in the peripheral nervous system, APPL is expressed in neuronal precursor cells, whereas in the developing central nervous system, it is restricted to differentiated neurons. The loss of MSOs in the ApplΔ mutant can be suppressed by reducing the copy number of the Drosophila ortholog of the β-Amyloid precursor protein binding protein 1 (dAPP-BP1). dAPP-BP1 binds to the C-terminal domain of APPL (Fig. 1b) and accumulates in an Appl minus background. Further developmental studies on dAPP-BP1 suggested that the elevated levels of dAPP-BP1 in ApplΔ induce apoptosis and subsequent loss of macrochaete due to excessive cell death. Surprisingly, strong overexpression of APPL during the development can also result in the loss of MSOs via apoptosis, which is further enhanced when the dAPP-BP1 copy number is reduced (Kim et al. 2007). Although more experiments have to be done to fully understand the function of APPL, these experiments already demonstrate the complex functional requirements for APPL and its fragments in the development of the central as well as peripheral nervous system.

**The role of APPL in neuronal function and maintenance**

Besides a role in the development of the nervous system, several studies have also indicated a function of APPL in the adult nervous system of the fly. One study found that injury-induced lesions in different areas of the adult brain increased APPL expression in the cell bodies and axonal projections of neurons next to those directly affected by the injury (Leyssen et al. 2005). This result suggests that APPL could play a role in neuronal repair and/or compensatory axonal sprouting of neighboring neurons after neuronal loss. That APPL might support neuronal integrity of the adult brain also comes from a genetic interaction study with the neuro-degenerative mutant löchrig (loε; Tschaep et al. 2002); loε encodes the γ-subunit of the AMP-activated protein kinase (AMPK), which is involved in numerous signaling pathways regulating energy homeostasis in all eukaryotes (Kemp et al. 1999). In particular, cholesterol synthesis is blocked via the action of activated

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**Fig. 2** APP induces projection defects in the Drosophila mushroom bodies. a Confocal image of the mushroom body α-lobe in a wild-type female. b Expression of sAPPβ in the α neurons during mushroom body development (via mb247-GAL4 + UAS-GFP) causes a de-fasciculation of α-lobe projections resulting in two separate lobes in this female (arrow; de-fasciculation was observed in six out of 23 preparations, while none of the 14 control flies showed such a phenotype). c Schematic drawing of the mushroom body and its localization in the fly head. The α-lobe consists of two closely associated lobes, α and α′, which are formed by distinct populations of neurons. ca calyx, pe peduncle
AMPK, and interestingly, APP processing is altered in response to changes in cholesterol levels (Reid et al. 2007). Although flies do not synthesize cholesterol de novo, loe flies showed reduced sAPPL levels, suggesting that AMPK can interfere with APPL processing independent from its effect on cholesterol. Combining loe with the Appld mutation enhanced the neurodegenerative phenotype in the adult brain (Tschäpe et al. 2002) and a similar effect was later found with another degenerative mutant, yata (Sone et al. 2009). yata-Mutant flies display progressive neurodegeneration of the adult brain and, even more prominent, of the eye. The degeneration of the brain was enhanced in the Appld, yata double mutant, whereas it was suppressed by neuronal overexpression of APPL. Although the biochemical function of the YATA protein is unknown, it might affect APPL transport because its localization at the NMJ and in pupal axons and dendrites is severely disrupted in the yata mutant (Sone et al. 2009). Together these experiments suggest that a major function of APPL may be maintaining the integrity of the nervous system, especially after injury or when otherwise challenged.

Already the initial characterization of the Appld mutant included behavioral assays to reveal a potential role in neuronal function and indeed these mutants showed a reduce performance index (PI) in the fast-phototaxis assay (Luo et al. 1992). In this paradigm, startled flies are scored by their escape response toward a light source (Benzer 1967), providing quantitative measurements for general fitness, mobility, and orientation ability of the fly. The deficits in the performance could be partially rescued by ubiquitous expression of APPL or APP695, but not by the secretion-deficient form APPLsd. The latter result indicates that especially the secreted sAPPL might play a role in neuronal function in the adult, possibly by modulating potassium currents as shown in cell culture (Li et al. 2004). Interestingly, our own published data revealed that pan-neuronal overexpression of APPL also reduced the PI in the fast-phototaxis assay in an age-dependent manner. Moreover, overexpression of the Drosophila β-secretase dBACE caused a similar phenotype (Carmine-Simmen et al. 2009), whereas flies heterozygous for a recently identified mutant in Drosophila dBace showed a suppression of the fast phototaxis deficits induced by APPL overexpression (our own unpublished results). This implies that the deficits in fast phototaxis induced by overexpression of APPL are based on elevated levels of neurotoxic dAβ and/or sAPPLβ. These observations suggest that, similar to the action of APPL during development, an imbalance in APPL processing leads to neuronal dysfunctions that result in behavioral changes.

In addition to the defects in fast phototaxis, White and colleagues (Luo et al. 1992) also noted a reduced response to electric shocks in Appld mutant flies, further supporting a requirement of APPL for neuronal function in the adult fly. Unfortunately, this phenotype prevented the analysis of APPL’s possible role in learning and memory formation, as assessed by the Tully-Quinn paradigm for aversive associative olfactory learning. In this assay, flies are trained in a single trial to associate an odor with an electric shock, leading to an avoidance of the punished odor in subsequent tests (Tully and Quinn 1985). This learning paradigm can be used to measure short-term memory (lasting for an hour), or long-term memory that requires repeated training trials and de novo protein synthesis and lasts for a couple of days. Olfactory-memory formation and retrieval depend on the neurons of the mushroom bodies where odor-shock association is integrated and the memory to avoid the punished odor is stored (review by Keene and Waddell 2007). Notable, APPL expression is especially strong in the adult mushroom bodies (Torroja et al. 1996). In a recent publication, Goguel et al. (2011) made use of an inducible expression system to knock down Appl mRNA only in the adult mushroom bodies, thus avoiding effects by the general loss of APPL. Indeed, these flies were sensitive to the electrical shock and could be trained to avoid the punished odor, indicating an intact short-term memory. In contrast, long-term memory, which was assessed after 24 h, was significantly impaired. That only the long-term memory, which requires protein synthesis, was disrupted suggests a role of APPL in synaptic plasticity which is required to consolidate the liable shorter forms of memory into a long lasting one. Whether APPL actually promotes de novo or elevated expression of proteins involved in synaptic plasticity remains to be determined. Interestingly, overexpression of the human protein in the mushroom bodies in the wild-type background destroyed long-term memory, indicating that the levels of APP proteins have to be tightly regulated to fulfill their role in learning and memory.

**Conclusion**

The studies summarized in this review show that APPL plays a role in developmental processes and in the maintenance and function of the adult brain. Interestingly, in both stages, APPL appears to affect synaptic function; bouton formation and excitability at the NMJ during development and synaptic plasticity and long-term memory formation in the adult. Whether the developmental function and the adult function are mediated by the same interaction pathways still remains to be determined. Interestingly, Fasciclin II that is required for the function of APPL at the larval NMJ (Ashley et al. 2005) is also enriched in α/β neurons of the mushroom bodies (Krashes et al. 2007). These neurons appear to be necessary to store the long-term memory trace (Keene and Waddell 2007) which raises...
the possibility that the FASII–APPL interaction required for bouton formation is also utilized to modulate synapses during long-term memory formation. Another possible partner of APPL in synaptic function could be APPL itself. As described above, both overexpression and loss of APPL lead to neurite shortening and this could be explained by sAPP acting as a negative signaling molecule on the full-length APPL receptor. These experiments suggest that, as in vertebrates, distinct cleavage fragments of APPL could play different stages even opposing roles. Although some experiments have been performed to address this aspect in the development, unfortunately the function of different fragments in long-term memory formation and/or neuronal integrity has not been tested yet. Hopefully, future studies in Drosophila as well as in other model systems will eventually lead to a comprehensive understanding of the functions of APP proteins and their various fragments.

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