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REVIEW

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 ectoptically 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*

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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 APP_{695} 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 APP_{695}, whereby the sequence identity is significantly higher in the C-terminal domain (51%) and in the E1 and E2 N-terminal, extra-cellular 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)

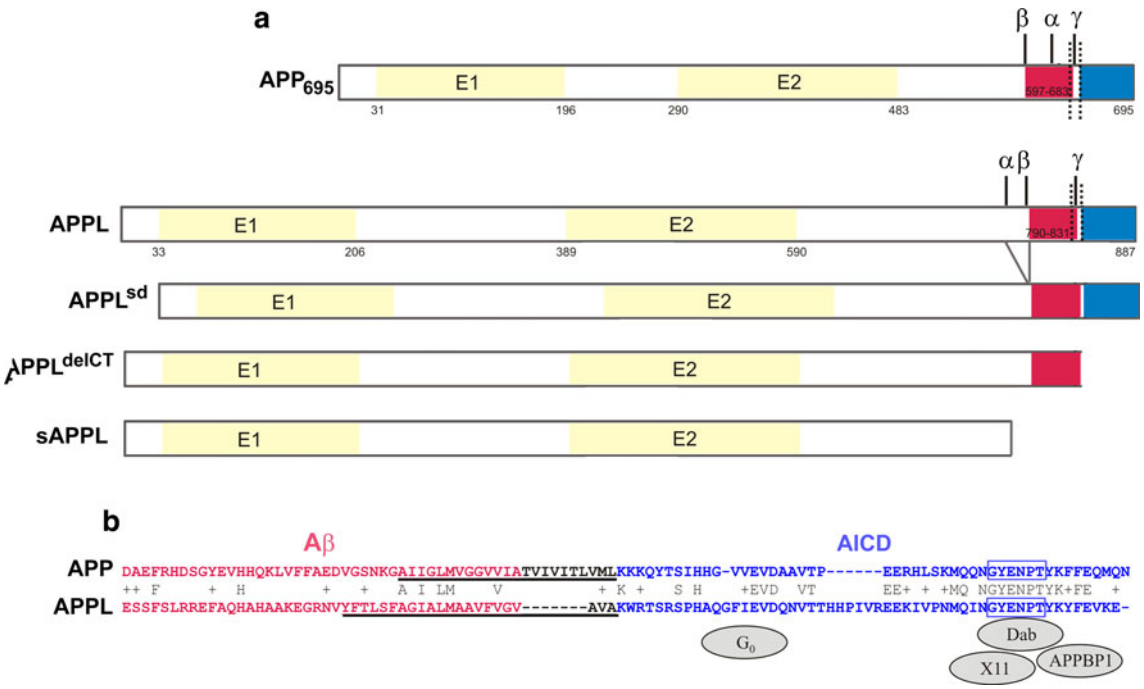

Fig. 1 Schematic drawing of human APP_{695} and APPL. **a** Overall structure of APP_{695} and APPL showing the conserved E1 and E2 domains in the extracellular part (yellow, 38 and 33% identity), the Aβ region (*red box*), and the C-terminal, intracellular AICD (*blue box*). The secretase cleavage sites 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 APP_{695} 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 $G_o\alpha$ 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 $A\beta_{42}$ 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, $G_o\alpha$, 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; Lichtenthaler 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 shown 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 ($Appl^d$) 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 $Appl^d$ 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 *Appl*^{d} 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 APP_{695}. Interestingly, this phenotype did also occur after the expression of a secretion-defective version of APPL (APPL^{sd}, see Fig. 1a), however, not by a mutant construct that in addition lacked the intracellular C-terminal domain (APPL^{delCT}; 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 G_0 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 MNJ 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 sAPPα 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 (APPL^{sd} and APPL^{delCT}) 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 (APPL^{sd}) 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 *APPL*^{d} 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 trans-cellular 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 overgrowth 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/α′ lobes project dorsally, while the β/β′ and y-lobes are horizontally orientated toward the midline (e.g., Aso et al. 2009). Continuous overexpression of APPL^{sd} 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 *Appl*^{d} null mutant and when overexpressing

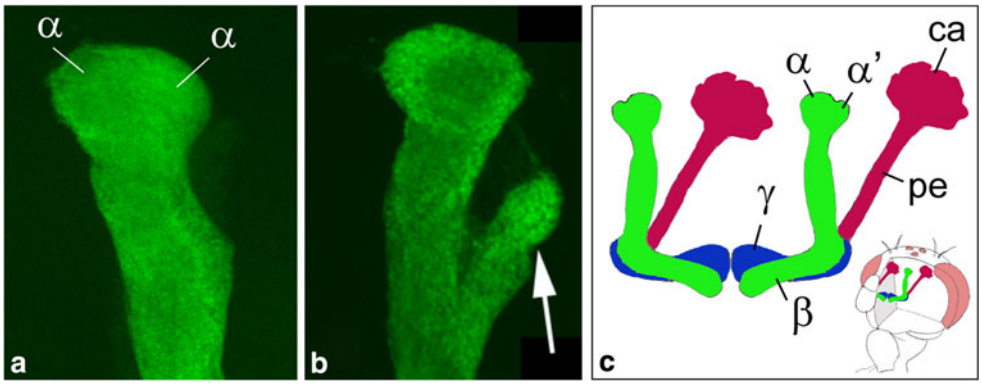

Fig. 2 APP induces projection defects in the *Drosophila* mushroom bodies. **a** Confocal image of the mushroom body α-lobe in a wild-type female. **b** Expression of sAPPβ in the α neurons during mushroom body development (via mb247-GAL4 + UAS-GFP) causes a de-fasciculation of α-lobe projections resulting 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

APPL^{delCT} (Li et al. 2004) suggesting that membrane-bound APPL can induce de-fasciculation. However, a de-fasciculation 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). *Appl*^{d} 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 linage 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 *Appl*^{d} 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 *Appl*^{d} 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* (*loe*; Tschäpe et al. 2002); *loe* 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

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 $Appl^{d}$ 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 $Appl^{d}$, *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 $Appl^{d}$ 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 APP_{695}, but not by the secretion-deficient form $APPL^{sd}$. 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 $Appl^{d}$ 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 sAPPL 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 sometimes 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.

References

Araki W, Kitaguchi N, Tokushima Y, Ishii K, Aratake H, Shimohama S, Nakamura S, Kimura J (1991) Trophic effect of beta-amyloid precursor protein on cerebral cortical neurons in culture. Biochem Biophys Res Commun 181(1):265–271

Ashley J, Packard M, Ataman B, Budnik V (2005) Fasciclin II signals new synapse formation through amyloid precursor protein and the scaffolding protein dX11/Mint. J Neurosci 25(25):5943–5955

Aso Y, Grübel K, Busch S, Friedrich AB, Siwanowicz I, Tanimoto H (2009) The mushroom body of adult *Drosophila* characterized by GAL4 drivers. J Neurogenet 23(1–2):156–172

Benzer S (1967) Behavioral mutants of *Drosophila* isolated by countercurrent distribution. Proc Natl Acad Sci USA 58(3):1112–1119

Bilen J, Bonini NM (2005) *Drosophila* as a model for human neurodegenerative disease. Annu Rev Genet 39:153–171

Boulianne GL, Livne-Bar I, Humphreys JM, Liang Y, Lin C, Rogaev E, St. George-Hyslop P (1997) Cloning and characterization of the *Drosophila presenilin* homologue. Neuroreport 8(4):1025–1029

Brou C, Logeat F, Gupta N, Bessia C, LeBail O, Doedens JR, Cumano A, Roux P, Black RA, Israël A (2000) A novel proteolytic cleavage involved in Notch signaling: the role of the disintegrin-metalloprotease TACE. Mol Cell 5:207–216

Cao X, Südhof TC (2001) A transcriptionally active complex of APP with Fe65 and histone acetyltransferase Tip60. Science 293(5527):115–120

Carmine-Simmen K, Proctor T, Tschäpe J, Poeck B, Triphan T, Strauss R, Kretzschmar D (2009) Neurotoxic effects induced by the *Drosophila* amyloid-beta peptide suggest a conserved toxic function. Neurobiol Dis 33(2):274–281

Clark RF, Hutton M, Fuldner M, Froelich S, Karran E, Alzheimer's disease collaborative group et al (1995) The structure of the presenilin 1 (S182) gene and identification of six novel mutations in early onset AD families. Nat Genet 11(2):219–222

Coulson EJ, Paliga K, Beyreuther K, Masters CL (2000) What the evolution of the amyloid protein precursor supergene family tells us about its function. Neurochem Int 36(3):175–184

Daigle I, Li C (1993) apl-1, a *Caenorhabditis elegans* gene encoding a protein related to the human beta-amyloid protein precursor. Proc Natl Acad Sci USA 90(24):12045–12049

De Strooper B, Annaert W (2010) Novel research horizons for presenilins and γ-secretases in cell biology and disease. Annu Rev Cell Dev Biol 26:235–260

De Strooper B, Saftig P, Craessaerts K, Vanderstichele H, Guhde G, Annaert W, Von Figura K, Van Leuven F (1989) Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. Nature 391(6665):387–390

Fossgreen A, Brückner B, Czech C, Masters CL, Beyreuther K, Paro R (1998) Transgenic *Drosophila* expressing human amyloid precursor protein show gamma-secretase activity and a blistered-wing phenotype. Proc Natl Acad Sci USA 95(23):13703–13708

Francis R, McGrath G, Zhang J, Ruddy DA, Sym M, Apfeld J, Nicoll M, Maxwell M, Hai B, Ellis MC, Parks AL, Xu W, Li J, Gurney M, Myers RL, Himes CS, Hiebsch R, Ruble C, Nye JS, Curtis D (2002) aph-1 and pen-2 are required for Notch pathway signaling, gamma-secretase cleavage of betaAPP, and presenilin protein accumulation. Dev Cell 3(1):85–97

Furukawa K, Barger SW, Blalock EM, Mattson MP (1996) Activation of K^+ channels and suppression of neuronal activity by secreted beta-amyloid-precursor protein. Nature 379(6560):74–78

Glenner GG, Wong CW (1984) Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem Biophys Res Commun 120(3):885–890

Goguel V, Belair AL, Ayaz D, Lampin-Saint-Amaux A, Scaplehorn N, Hassan BA, Preat T (2011) *Drosophila* amyloid precursor protein-like is required for long-term memory. J Neurosci 31(3):1032–1037

Goldgaber D, Lerman MI, McBride OW, Saffiotti U, Gajdusek DC (1987) Characterization and chromosomal localization of a cDNA encoding brain amyloid of Alzheimer's disease. Science 235(4791):877–880

Goodman Y, Mattson MP (1994) Secreted forms of beta-amyloid precursor protein protect hippocampal neurons against amyloid beta-peptide-induced oxidative injury. Exp Neurol 128(1):1–12

Goutte C, Hepler W, Mickey KM, Priess JR (2000) aph-2 encodes a novel extracellular protein required for GLP-1-mediated signaling. Development 127(11):2481–2492

Gramates LS, Budnik V (1999) Assembly and maturation of the *Drosophila* larval neuromuscular junction. Int Rev Neurobiol 43:93–117

Greeve I, Kretzschmar D, Tschäpe JA, Beyn A, Brellinger C, Schweizer M, Nitsch RM, Reifegerste R (2004) Age-dependent neurodegeneration and Alzheimer-amyloid plaque formation in transgenic *Drosophila*. J Neurosci 24(16):3899–3906

Guo M, Hong EJ, Fernandes J, Zipursky SL, Hay BA (2003) A reporter for amyloid precursor protein gamma-secretase activity in *Drosophila*. Hum Mol Genet 12(20):2669–2678

Hase M, Yagi Y, Taru H, Tomita S, Sumioka A, Hori K, Miyamoto K, Sasamura T, Nakamura M, Matsuno K, Suzuki T (2002) Expression and characterization of the *Drosophila* X11-like/mint protein during neural development. J Neurochem 81(6):1223–1232

Heisenberg M (2003) Mushroom body memoir: from maps to models. Nat Rev Neurosci 4(4):266–275

Hong CS, Koo EH (1997) Isolation and characterization of *Drosophila presenilin* homolog. Neuroreport 8(3):665–668

Hornsten A, Lieberthal J, Fadia S, Malins R, Ha L, Xu X, Daigle I, Markowitz M, O'Connor G, Plasterk R, Li C (2007) APL-1, a *Caenorhabditis elegans* protein related to the human beta-amyloid precursor protein, is essential for viability. Proc Natl Acad Sci USA 104(6):1971–1976

Hussain I, Powell D, Howlett DR, Tew DG, Meek TD, Chapman C, Gloger IS, Murphy KE, Southan CD, Ryan DM, Smith TS, Simmons DL, Walsh FS, Dingwall C, Christie G (1999) Identification of a novel aspartic protease (Asp 2) as beta-secretase. Mol Cell Neurosci 14:419–427

Jacobsen KT, Iverfeldt K (2009) Amyloid precursor protein and its homologues: a family of proteolysis-dependent receptors. Cell Mol Life Sci 66:2299–2318

Kang J, Lemaire HG, Unterbeck A, Salbaum JM, Masters CL, Grzeschik KH, Multhaup G, Beyreuther K, Müller-Hill B (1987) The precursor of alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. Nature 325(6106):733–736

Keene AC, Waddell S (2007) *Drosophila* olfactory memory: single genes to complex neural circuits. Nat Rev Neurosci 8(5):341–354

Kemp BE, Mitchelhill KI, Stapleton D, Michell BJ, Chen ZP, Witters LA (1999) Dealing with energy demand: the AMP-activated protein kinase. Trends Biochem Sci 24(1):22–25

Kim HJ, Kim SH, Shim SO, Park E, Kim C, Kim K, Tanouye MA, Yim J (2007) *Drosophila* homolog of APP-BP1 (dAPP-BP1) interacts antagonistically with APPL during *Drosophila* development. Cell Death Differ 14(1):103–115

Kimberly WT, Zheng JB, Guénette SY, Selkoe DJ (2001) The intracellular domain of the beta-amyloid precursor protein is stabilized by Fe65 and translocates to the nucleus in a notch-like manner. J Biol Chem 276(43):40288–40292

Kotani N, Kitazume S, Kamimura K, Takeo S, Aigaki T, Nakato H, Hashimoto Y (2005) Characterization of *Drosophila* aspartic proteases that induce the secretion of a Golgi-resident transferase, heparan sulfate 6-O-sulfotransferase. J Biochem 137(3): 315–322

Krashes MJ, Keene AC, Leung B, Armstrong JD, Waddell S (2007) Sequential use of mushroom body neuron subsets during *Drosophila* odor memory processing. Neuron 53(1):103–115

Lai EC, Orgogozo V (2004) A hidden program in *Drosophila* peripheral neurogenesis revealed: fundamental principles underlying sensory organ diversity. Dev Biol 269(1):1–17

Lammich S, Kojro E, Postina R, Gilbert S, Pfeiffer R, Jasionowski M, Haass C, Fahrenholz F (1999) Constitutive and regulated alpha-secretase cleavage of alzheimer's amyloid precursor protein by a disintegrin metalloprotease. Proc Natl Acad Sci USA 96(7): 3922–3927

Leyssen M, Ayaz D, Hebert SS, Reeve S, De Strooper B, Hassan BA (2005) Amyloid precursor protein promotes post-developmental neurite arborization in the *Drosophila* brain. EMBO J 24(16):2944–2955

Li Y, Liu T, Peng Y, Yuan C, Guo A (2004) Specific functions of *Drosophila* amyloid precursor-like protein in the development of nervous system and non-neural tissues. J Neurobiol 61(3):343–358

Lichtenthaler SF (2011) Alpha-secretase in alzheimer's disease: molecular identity, regulation and therapeutic potential. J Neurochem 116(1):10–21

Link CD (2005) Invertebrate models of alzheimer's disease. Genes Brain Behav 4(3):147–156

Lorent K, Overbergh L, Moechars D, De Strooper B, Van Leuven F, Van den Berghe H (1995) Expression in mouse embryos and in adult mouse brain of three members of the amyloid precursor protein family, of the alpha-2-macroglobulin receptor/low density lipoprotein receptor-related protein and of its ligands apolipoprotein E, lipoprotein lipase, alpha-2-macroglobulin and the 40,000 molecular weight receptor-associated protein. Neuroscience 65(4):1009–1025

Luo LQ, Martin-Morris L, White K (1990) Identification, secretion, and neural expression of APPL, a *Drosophila* protein similar to human amyloid protein precursor. J Neurosci 10(12):3849–3861

Luo L, Tully T, White K (1992) Human amyloid precursor protein ameliorates behavioral deficit of flies deleted for *Appl* gene. Neuron 9:595–605

Martin-Morris L, White K (1990) The *Drosophila* transcript encoded by the β-amyloid protein precursor-like gene is restricted to the nervous system. Development 110(1):185–195

Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, Beyreuther K (1985) Amyloid plaque core protein in alzheimer disease and down syndrome. Proc Natl Acad Sci USA 82(12):4245–4249

Mattson MP, Cheng B, Culwell AR, Esch FS, Lieberburg I, Rydel RE (1993) Evidence for excitoprotective and intraneuronal calcium-regulating roles for secreted forms of the beta-amyloid precursor protein. Neuron 10(2):243–254

Merdes G, Soba P, Loewer A, Bilic MV, Beyreuther K, Paro R (2004) Interference of human and *Drosophila* APP and APP-like proteins with PNS development in *Drosophila*. EMBO J 23(20):4082–4095

Müller U, Cristina N, Li ZW, Wolfer DP, Lipp HP, Rülicke T, Brandner S, Aguzzi A, Weissmann C (1994) Behavioral and anatomical deficits in mice homozygous for a modified beta-amyloid precursor protein gene. Cell 179(5):7557–7565

Müller T, Concannon CG, Ward MW, Walsh CM, Tirniceriu AL, Tribl F, Kögel D, Prehn JH, Egensperger R (2007) Modulation of gene expression and cytoskeletal dynamics by the amyloid precursor protein intracellular domain (AICD). Mol Biol Cell 18(1):201–210

Nakagawa K, Kitazume S, Oka R, Maruyama K, Saido TC, Sato Y, Endo T, Hashimoto Y (2006) Sialylation enhances the secretion of neurotoxic amyloid-beta peptides. J Neurochem 96(4):924–933

Nikolaev A, McLaughlin T, O'Leary DD, Tessier-Lavigne M (2009) APP binds DR6 to trigger axon pruning and neuron death via distinct caspases. Nature 457(7232):981–989

Pan D, Rubin GM (1997) Kuzbanian controls proteolytic processing of Notch and mediates lateral inhibition during *Drosophila* and vertebrate neurogenesis. Cell 90(2):271–280

Reid PC, Urano Y, Kodama T, Hamakubo T (2007) Alzheimer's disease: cholesterol, membrane rafts, isoprenoids and statins. J Cell Mol Med 11(3):383–392

Robakis NK, Ramakrishna N, Wolfe G, Wisniewski HM (1987) Molecular cloning and characterization of a cDNA encoding the cerebrovascular and the neuritic plaque amyloid peptides. Proc Natl Acad Sci USA 84(12):4190–4194

Roberts SB, Ripellino JA, Ingalls KM, Robakis NK, Felsenstein KM (1994) Non-amyloidogenic cleavage of the beta-amyloid precursor protein by an integral membrane metalloendopeptidase. J Biol Chem 269(4):3111–3116

Rooke J, Pan D, Xu T, Rubin GM (1996) KUZ, a conserved metalloprotease-disintegrin protein with two roles in *Drosophila* neurogenesis. Science 273(5279):1227–1231

Rosen DR, Martin-Morris L, Luo LQ, White K (1989) A *Drosophila* gene encoding a protein resembling the human beta-amyloid protein precursor. Proc Natl Acad Sci USA 86(7):2478–2482

Selkoe DJ (2001) Alzheimer's disease: genes, proteins, and therapy. Physiol Rev 81(2):741–766

Senechal Y, Larmet Y, Dev KK (2006) Unraveling in vivo functions of amyloid precursor protein: insights from knockout and knockdown studies. Neurodegener Dis 3(3):134–147

Sinha S, Anderson JP, Barbour R, Basi GS, Caccavello R, Davis D, Doan M, Dovey HF, Frigon N, Hong J, Jacobson-Croak K, Jewett N, Keim P, Knops J, Lieberburg I, Power M, Tan H, Tatsuno G, Tung J, Schenk D, Seubert P, Suomensaari SM, Wang S, Walker D, Zhao J, McConlogue L, John V (1999) Purification and cloning of amyloid precursor protein beta-secretase from human brain. Nature 402(6761):537–540

Soba P, Eggert S, Wagner K, Zentgraf H, Siehl K, Kreger S, Löwer A, Langer A, Merdes G, Paro R, Masters CL, Müller U, Kins S, Beyreuther K (2005) Homo- and heterodimerization of APP family members promotes intercellular adhesion. EMBO J 24(20):3624–3634

Solans A, Estivill X, de La Luna S (2001) A new aspartyl protease on 21q22.3, BACE2, is highly similar to Alzheimer's amyloid precursor protein beta-secretase. Cytogenet Cell Genet 89(3–4): 177–184

Sone M, Uchida A, Komatsu A, Suzuki E, Ibuki I, Asada M, Shiwaku H, Tamura T, Hoshino M, Okazawa H, Nabeshima Y (2009) Loss of *yata*, a novel gene regulating the subcellular localization of APPL, induces deterioration of neural tissues and lifespan shortening. PLoS ONE 4(2):e4466

Stempfle D, Kanwar R, Loewer A, Fortini ME, Merdes G (2010) In vivo reconstitution of gamma-secretase in *Drosophila* results in substrate specificity. Mol Cell Biol 30(13):3165–3175

Struhl G, Greenwald I (1999) Presenilin is required for activity and nuclear access of Notch in *Drosophila*. Nature 398(6727): 522–525

Tanaka S, Shiojiri S, Takahashi Y, Kitaguchi N, Ito H, Kameyama M, Kimura J, Nakamura S, Ueda K (1989) Tissue-specific expression of three types of beta-protein precursor mRNA: enhancement of protease inhibitor-harboring types in Alzheimer's disease brain. Biochem Biophys Res Commun 165(3): 1406–1414

Tanzi RE, Gusella JF, Watkins PC, Bruns GA, St George-Hyslop P, Van Keuren ML, Patterson D, Pagan S, Kurnit DM, Neve RL (1987) Amyloid beta protein gene: cDNA, mRNA distribution, and genetic linkage near the alzheimer locus. Science 235(4791): 880–884

Torroja L, Luo L, White K (1996) APPL, the *Drosophila* member of the APP-family, exhibits differential trafficking and processing in CNS neurons. J Neurosci 16(15):4638–4650

Torroja L, Packard M, Gorczyca M, White K, Budnik V (1999a) The *Drosophila* beta-amyloid precursor protein homolog promotes synapse differentiation at the neuromuscular junction. J Neurosci 19(18):7793–7803

Torroja L, Chu H, Kotovsky I, White K (1999b) Neuronal overexpression of APPL, the *Drosophila* homologue of the amyloid precursor protein (APP), disrupts axonal transport. Curr Biol 9(9):489–492

Tschäpe JA, Hammerschmied C, Mühlig-Versen M, Athenstaedt K, Daum G, Kretzschmar D (2002) The neurodegeneration mutant *löchrig* interferes with cholesterol homeostasis and APPL processing. EMBO J 21(23):6367–6376

Tully T, Quinn WG (1985) Classical conditioning and retention in normal and mutant *Drosophila melanogaster*. J Comp Physiol A 157(2):263–277

Turner PR, O'Connor K, Tate WP, Abraham WC (2003) Roles of amyloid precursor protein and its fragments in regulating neural activity, plasticity and memory. Prog Neurobiol 70(1):1–32

Vassar R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, Denis P, Teplow DB, Ross S, Amarante P, Loeloff R, Luo Y, Fisher S, Fuller J, Edenson S, Lile J, Jarosinski MA, Biere AL, Curran E, Burgess T, Louis JC, Collins F, Treanor J, Rogers G, Citron M (1999) Beta-secretase cleavage of alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. Science 286(5440):735–741

Wolfe MS, Xia W, Ostaszewski BL, Diehl TS, Kimberly WT, Selkoe DJ (1999) Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and gamma-secretase activity. Nature 398(6727):513–517

Yan R, Bienkowski MJ, Shuck ME, Miao H, Tory MC, Pauley AM, Brashier JR, Stratman NC, Mathews WR, Buhl AE, Carter DB, Tomasselli AG, Parodi LA, Heinrikson RL, Gurney ME (1999) Membrane-anchored aspartyl protease with alzheimer's disease beta-secretase activity. Nature 402(6761):533–537

Ye Y, Lukinova N, Fortini ME (1999) Neurogenic phenotypes and altered Notch processing in *Drosophila* Presenilin mutants. Nature 398(6727):525–529

Yu G, Nishimura M, Arawaka S, Levitan D, Zhang L, Tandon A, Song YQ, Rogaeva E, Chen F, Kawarai T, Supala A, Levesque L, Yu H, Yang DS, Holmes E, Milman P, Liang Y, Zhang DM, Xu DH, Sato C, Rogaev E, Smith M, Janus C, Zhang Y, Aebersold R, Farrer LS, Sorbi S, Bruni A, Fraser P, St George-Hyslop P (2000) Nicastrin modulates presenilin-mediated *notch/glp-1* signal transduction and *βAPP* processing. Nature 407(6800):48–54

Zheng H, Jiang M, Trumbauer ME, Sirinathsinghji DJ, Hopkins R, Smith DW, Heavens RP, Dawson GR, Boyce S, Conner MW, Stevens KA, Slunt HH, Sisoda SS, Chen HY, Van der Ploeg LH (1995) beta-Amyloid precursor protein-deficient mice show reactive gliosis and decreased locomotor activity. Cell 81(4):525–531