The amyloid precursor protein (APP) plays a central role in Alzheimer’s disease (AD) pathogenesis and in AD research. In large part, this is because APP is the precursor to the amyloid-β-protein (Aβ), the 40-42 amino acid residue peptide that is at the heart of the amyloid cascade hypothesis of AD. Consequently, intracellular trafficking and proteolytic processing of APP have been the focus of numerous investigations over the past two decades. Tremendous progress has been made since the initial identification of Aβ as the principal component of brain senile plaques of individuals with AD and the subsequent cloning of APP cDNA. Specifically, molecular characterization of the secretases involved in Aβ production has facilitated cell biological investigations on APP processing, and advanced efforts to model AD pathogenesis in animal models. In this chapter, we will review the recent developments in APP trafficking, discuss salient features of amyloidogenic processing of APP in organelles and membrane microdomains, and examine the putative biological functions of APP. The latter focus is essential because APP clearly plays physiological roles in the nervous system, some of which may contribute to neurodegeneration. Details concerning the pathways mediating production, aggregation, and degradation of Aβ will be covered extensively in Chapters 6, 8, 9 and 10, and will be mentioned here only in passing for the purposes of clarity.
1. **APP gene family**

The human *APP* gene was first identified in 1987 by several laboratories independently using partial protein sequence information obtained by the Glenner and Beyreuther/Masters laboratories several years earlier. The two *APP* homologues, *APLP1* and *APLP2*, were discovered several years later. The identification of *APP* led to several early surprises. First, *APP* is a type I membrane protein whereby two predicted cleavages, one in the extracellular domain (β-secretase cleavage) and the other in the transmembrane region (γ-secretase cleavage) are necessary to release Aβ from the precursor molecule. Second, *APP* is located on chromosome 21 (21q21.2-3). This provided an immediate connection to the almost invariant development of AD pathology in trisomy 21 (Down’s syndrome) individuals (see below). Finally, although no typical functional motifs were seen, it was speculated that *APP* might function as a cell surface receptor. Almost twenty years later, this prediction has yet to be definitively fulfilled. As an historical aside, it should be pointed out that the first mutations that were found to be causative in inherited forms of familial AD and a related inherited condition, hereditary cerebral haemorrhage with amyloid angiopathy, Dutch type, were found in the *APP* gene (Levy et al., 1990; Hardy, 1997). Although mutations in *APP* are rare in comparison to mutations in *PSEN* genes (which encode presenilin-1 and -2) (see Chapter 1), they are nevertheless important because they provided early and seminal evidence that *APP* plays a central role in AD pathogenesis.

*APP* is now known to be one of three members of a larger gene family. These include *APLP1* and *APLP2* in humans, *APPL* (fly), and *APL-1* (worm) (Coulson et al., 2000). All genes encode type I membrane proteins with a large extracellular domain and a short cytoplasmic region that undergo similar processing (see below). Importantly, only *APP*, but not any of the other *APP*-related genes, contains sequence encoding the Aβ domain. Therefore, *APLP1* and *APLP2* are not the precursors to Aβ and if these two genes contribute to AD pathogenesis, then their roles must be indirect. *APP* and *APLP2* are ubiquitously expressed although alternative splicing generates isoforms that may be expressed in a cell type specific manner; for example, APP695 (the 695 amino acid isoform) is neuron-specific. On the other hand, *APLP1* is expressed selectively in the nervous system.

2. **APP processing**

2.1 **APP secretases**

Full-length APP undergoes sequential proteolytic processing as outlined in Figure 1. APP is first cleaved by α-secretase (non-amyloidogenic pathway)
or β-secretase ( amyloidogenic pathway) within the luminal domain, resulting in the shedding of nearly the entire ectodomain and generation of membrane-tethered α- or β-C-terminal fragments (CTFs). The major neuronal β-secretase is a transmembrane aspartyl protease, termed BACE1 (β-site APP cleaving enzyme; also called Asp-2 and memapsin-2). BACE1 cleaves APP within the ectodomain, generating the N-terminus of Aβ (Vassar, 2004). However, the principal BACE (β’) cleavage site in native APP is between Glu +11 and Val +12 of the Aβ peptide. Several zinc metalloproteinases such as TACE/ADAM17, ADAM9, ADAM10 and MDC-9, and an aspartyl protease, BACE2, can cleave APP at, or near, the α-secretase site (Allinson et al., 2003), located within the Aβ domain (between residues Lys16 and Leu17 of the Aβ peptide), essentially precluding the generation of intact Aβ.

The second proteolytic event in APP processing involves intramembranous cleavage of α- and β-CTFs by γ-secretase, that liberates p3 (3 kDa) and Aβ (4 kDa) peptides, respectively, into the extracellular milieu. The minimal components of γ-secretase include presenilin-1 or -2 (PS1 or PS2), nicastrin, APH-1, and PEN-2 (Edbauer et al., 2003; Iwatsubo, 2004) (see Chapter 3). Protein subunits of the γ-secretase assemble early during biogenesis and co-operatively mature as they leave the endoplasmic reticulum. Biochemical and pharmacological evidence are consistent with PS1 (or PS2) as the catalytic subunit of the γ-secretase. A pair of conserved aspartate residues within the predicted transmembrane domains 6 and 7 of PS1 and PS2 is crucial for γ-secretase activity. APH-1 and PEN2 are thought to stabilize the γ-secretase complex and nicastrin to mediate the recruitment of APP CTF to the catalytic site of the γ-secretase. The major sites of γ-secretase cleavage correspond to positions 40 and 42 of Aβ. Greater than 90% of secreted Aβ ends in residue 40, and Aβ42 accounts for less than 10% of total Aβ. In addition, γ-secretase cleavage at a distal site generates a cytoplasmic polypeptide, termed APP intracellular domain (AICD). Familial AD-linked mutations in APP near the γ-secretase cleavage site affect cleavage specificity at Aβ40/42 sites, favouring cleavage at position 42. Intriguingly, familial AD-linked mutations in PS1 and PS2 influence γ-secretase cleavage by an elusive mechanism that also modulates the proteolysis of APP to selectively enhance the generation of Aβ42 peptides.

Amyloidogenic processing is the favoured pathway of APP metabolism in neurons largely due to the greater abundance of BACE1, and non-amyloidogenic pathway is predominant in all other cell types. Commitment of APP to these pathways can be differentially modulated by the activation of cell-surface receptors such as serotonin 5-hydroxytryptamine (5-HT4) receptor, metabotropic glutamate receptors, muscarinic acetylcholine receptors, and platelet-derived growth factor receptor. Signalling downstream of these receptors regulate APPsα and Aβ secretion by engaging intermediates including
Figure 1. Proteolytic processing of APP. A) Schematic structure of APP is shown with $A\beta$ domain shaded in red and enlarged. The major sites of cleavage by $\alpha$-, $\beta$-, and $\gamma$-secretases are indicated along with $A\beta$ numbering from the N-terminus of $A\beta$ (Asp1). B) Non-amyloidogenic processing of APP refers to sequential processing of APP by membrane-bound $\alpha$- and $\gamma$-secretases. $\alpha$-secretase cleaves within the $A\beta$ domain, thus precluding generation of intact $A\beta$ peptide. The fates of N-terminally truncated $A\beta$ (p3) and APP intracellular domain (AICD) are not fully resolved. C) Amyloidogenic processing of APP is carried out by sequential action of membrane-bound $\beta$- and $\gamma$-secretases.

PKC, PKA, phosphatidylinositol 3 kinase, mitogen-activated protein kinase kinase, extracellular signal-regulated kinase, Src tyrosine kinase, small GTPase Rac, inositol 1,4,5-trisphosphate, cAMP, and calcium. However, whether APP is a direct substrate of these intermediates has not been established. Whereas secreted APP$\alpha$ has been reported to have neurotrophic properties, $A\beta$ peptides have adverse effects on neuronal survival.
It appears that none of the aforementioned secretases have unique substrate specificity towards APP. Besides APP, several transmembrane proteins such as pro-TNFα and pro-TGFα undergo ectodomain shedding by enzymes with α-secretase activity. The relatively low affinity of BACE1 toward APP led to the suggestion that APP is not its sole physiological substrate. In support of this idea, α2,6-sialyltransferase and low density lipoprotein receptor-related protein (LRP) have been identified as additional substrates that are processed by BACE1. Similarly, PS1 and PS2 play a crucial role in intramembranous γ-secretase cleavage of several type I membrane proteins other than APP, including the Notch1 receptor and its ligands, Delta and Jagged2, cell-surface adhesion protein CD44, the receptor tyrosine kinase ErbB4, netrin receptor DCC, LRP, lipoprotein receptor ApoER2, cell adhesion molecules N- and E-cadherins, synaptic adhesion protein nectin-1α, cell surface heparin sulphate proteoglycan syndecan-3, p75 neurotrophin receptor etc (Koo and Kopan, 2004). Like APP, a signature of γ-secretase cleavage of these additional substrates is the requirement of an ectodomain shedding event.

Ever increasing number of transmembrane substrates and intracellular domains released by the proteolytic cleavage of these substrates indicate that in addition to being a modulator of many cell signaling paradigms via cleavage of proteins such as Notch, γ-secretase could simply be a proteasome or secretosome that catabolises membrane-bound protein “stubs” of type I membrane proteins (Kopan and Ilagan, 2004). Outcome of γ-secretase cleavage of substrates can either be activation of signaling as is the case in Notch receptor cleavage and the release of Notch intracellular domain, or termination of signaling as described for intramembranous cleavage of DCC (Parent et al., 2005). Apart from its essential role in the proteolytic function of the γ-secretase, PS1 and PS2 have been shown to participate in fundamental physiological functions including calcium homeostasis, neuronal signaling, protein trafficking, protein degradation, fine-tuning of immune system, neurite outgrowth, apoptosis, memory and synaptic plasticity (Sisodia et al., 1999; Koo and Kopan, 2004; Thinakaran and Parent, 2004). Hence, although APP secretases and factors regulating their activity in amyloidogenic pathway have long been considered as therapeutic targets for the treatment of AD, it is unclear whether secretase inhibitors will be free from serious side effects.

2.2 Intracellular itinerary and processing of APP

During its transit from the ER to the plasma membrane through the constitutive secretory pathway (Figure 2), nascent APP undergoes post-translational modification by N- and O-glycosylation, ectodomain and cytoplasmic phosphorylation, and tyrosine sulphation. In cultured cells, it is estimated that only about 10% of nascent APP molecules are successfully delivered to the
plasma membrane, based primarily on overexpression systems. APP can be proteolytically processed at the cell surface mainly by $\alpha$-secretases, resulting in the shedding of APP$\alpha$ ectodomain (Sisodia, 1992). Activation of protein kinase C increases APP$\alpha$ secretion by mechanisms involving the formation and release of secretary vesicles from the trans-Golgi network, thus enhancing APP (and possibly $\alpha$-secretase) trafficking to the cell surface.

Unlike many cell surface receptors, full-length APP does not reside for considerable length of time at the cell surface. Approximately 70% of surface-bound APP is internalized within minutes of arriving at the plasma membrane. A “YENPTY” internalization motif located near the C-terminus of APP (residues 682-687 of APP695 isoform) is responsible for this efficient internalization. Following endocytosis, APP is delivered to late endosomes and a fraction of endocytosed molecules is recycled to the cell surface. Measurable amounts of internalized APP also undergo degradation in the lysosome.

At steady-state, the majority of BACE1 localizes to late Golgi/TGN and endosomes, consistent with amyloidogenic cleavage of wild-type APP during endocytic/recycling steps (Koo and Squazzo, 1994) (Figure 2). BACE1 activity is optimal at acidic pH in vitro, supporting the notion that BACE1 likely cleaves APP during transit in acidic endocytic compartments. Available data indicate the presence of $\gamma$-secretase complex and enzyme activity in multiple compartments including the ER, late-Golgi/TGN, endosomes and the plasma membrane (Cook et al., 1997; Xu et al., 1997; Greenfield et al., 1999; Kaether et al., 2002; Takahashi et al., 2002; Chyung et al., 2004; Vetrivel et al., 2004). Recent estimates suggest only a minor presence of $\gamma$-secretase activity at the cell-surface, whereas the majority of the mature components of $\gamma$-secretase complex are found, and shown to be enzymatically active, in intracellular organelles such as ERGIC, Golgi apparatus, the TGN, and late endosomes.

As discussed below, in neurons APP is trafficked anterogradely along peripheral and central axons, and proteolytically processed during transit (Koo et al., 1990; Buxbaum et al., 1998). Reduced A$\beta$ deposition in BACE1 transgenic mice illustrates how subcellular site of amyloidogenic processing in neurons can greatly influence A$\beta$ production and deposition in vivo (Lee et al., 2005). Nevertheless, the intracellular organelles/transport vesicles where A$\beta$ is generated in neurons are not fully characterized.

Studies conducted in non-neuronal and neuroblastoma cell lines show that A$\beta$ is mainly generated in TGN as APP is trafficked through the secretory and recycling pathways (Figure 2). Attempts to address the role of endocytic APP trafficking by expression of dominant-negative mutant of dynamin, an important component of the endocytic machinery, resulted in discrepant findings. This is not surprising, since overexpression of mutant dynamin causes pleiotropic effects on endocytic trafficking of numerous proteins including APP secretases. Nevertheless, mutations within the APP cytosolic YENPTY
Intracellular trafficking of APP. Nascent APP molecules mature through the constitutive secretory pathway. Once APP reaches the cell surface, it is rapidly internalized and subsequently trafficked through endocytic and recycling compartments back to the cell surface or degraded in the lysosome. Non-amyloidogenic processing mainly occurs at the cell surface where \( \alpha \)-secretases are present. Amyloidogenic processing involves transit through the endocytic organelles where APP encounters \( \beta \)- and \( \gamma \)-secretases.

Several cytosolic adaptors with phosphotyrosine-binding domains, including Fe65, Fe65L1, Fe65L2, Mint 1 (also called X11\( \alpha \)), Mint 2, Mint3, and Dab1 bind to the APP cytoplasmic tail at or near the YENPTY motif, and regulate APP trafficking and processing (King and Turner, 2004). Mint proteins (so named for their ability to interact with Munc18) can directly bind ADP-ribosylation factors, raising the intriguing possibility that vesicular trafficking of APP may be regulated by Mints serving as coat proteins (Hill et al., 2003). Interestingly, Fe65 acts as a functional linker between APP and LRP (another type I membrane protein containing two NPXY endocytosis motifs) in

**Figure 2.** Intracellular trafficking of APP. Nascent APP molecules mature through the constitutive secretory pathway. Once APP reaches the cell surface, it is rapidly internalized and subsequently trafficked through endocytic and recycling compartments back to the cell surface or degraded in the lysosome. Non-amyloidogenic processing mainly occurs at the cell surface where \( \alpha \)-secretases are present. Amyloidogenic processing involves transit through the endocytic organelles where APP encounters \( \beta \)- and \( \gamma \)-secretases.
modulating endocytic APP trafficking and amyloidogenic processing (Pietrzik et al., 2004). A conformational change introduced by phosphorylation at Thr-668 (14 amino acids proximal to the YENPTY motif) interferes with Fe65 binding to APP, and facilitates BACE1 and γ-secretase cleavage of APP (Ando et al., 2001; Lee et al., 2003). In addition, Fe65 stabilizes the highly labile AICD, which may serve as a regulatory step in modulating the physiological function of AICD (see below). Despite the elaborate regulatory mechanisms that modulate cell surface transport and endocytic trafficking of APP, transit through these compartments are not essential for generation of Aβ as shown by amyloidogenic processing of APP in cells expressing syntaxin 1A mutants defective in exocytosis (Khvotchev and Sudhof, 2004). Still, the overexpression of Mint 1, Mint 2 or Fe65 causes reduction in Aβ generation and deposition in the brains of transgenic mice, suggesting a physiological role for these adaptors in regulating amyloidogenic processing of APP in the nervous system.

2.3 Axonal transport

Neurons are unique in their morphology with a long axonal compartment and a rich dendritic arbor that have to be sustained bioenergetically almost entirely from the perikaryon. Protein processing and trafficking are therefore often modified in neurons, just as can happen in polarized epithelial cells. Indeed, the axonal compartment has been compared to the apical compartment while the somatodendritic compartment may be functionally analogous to the basolateral compartment in epithelial cells. Accordingly, APP trafficking in neurons and epithelial cells take on an extra layer of complexity (Haass et al., 1994). Further, neurons are believed to be the major source of Aβ in brain, an idea supported by the observation that APP expression is highest in neurons. Therefore, if amyloid deposits are deposited at sites removed from the neuronal cell body, then APP or Aβ must be axonally transported form the perikaryon to distal processes. Indeed, APP is transported in axons via the fast anterograde transport machinery such that at least one documented source of amyloid deposits originate from synaptically released Aβ pool (Koo et al., 1990; Lazarov et al., 2002). Because anterograde transport of APP requires conventional kinesin, it is not surprising that APP has been found in complexes with kinesin light chain (KLC) subunit, a component of the kinesin-1 transport machinery (Kamal et al., 2000). Indeed, it has been shown that overexpression of the Drosophila APP homolog, APPL, in Drosophila neurons disrupts axonal transport, a phenotype similar to that seen in flies lacking components of the kinesin motor (Torroja et al., 1999). Taken together, these findings led to the hypothesis that APP may represent a kinesin cargo receptor, linking kinesin-1 to a unique subset of transport vesicles because different motor proteins are known to carry different membranous cargos. This model is consistent with
the observation that the microtubules that carry APP anterogradely in axons are different from the transport carrier of synaptophysin (Kaether et al., 2000). However, enrichment of APP in Rab 5-positive vesicles from synaptosomal preparations, but not in synaptic vesicles, likely reflects APP sorting after internalization from the axonal plasmalemma and is probably not indicative of anterograde transport (Marquez-Sterling et al., 1997). APP was reported to interact directly with KLC but recent evidence is more consistent with the view that the interaction is mediated indirectly through adaptor proteins, of which JIP-1, a member of the JNK-interacting protein family (JIP), is a likely candidate as it is known to interact with both KLC and APP (King and Turner, 2004). Taken together, the data suggest that while still embedded within the membrane of a cargo vesicle, APP interacts with KLC either directly or more likely indirectly, to facilitate the anterograde movement of the membranous cargo along the axon. Unresolved by this model is how APP is initially sorted into a particular class of vesicles. The potential importance of the initial sorting of APP is underscored by the report that BACE1 and presenilins are contained within the same kinesin-1 dependent APP transport vesicles (Kamal et al., 2001). This finding led to the suggestion that not only is APP required for the delivery of the enzymatic machinery necessary for Aβ production, but Aβ generation also occurs enroute from the cell body to the nerve terminals within the transport cargo that is carried by APP. However, the report that APP is a kinesin-1 receptor and a common vesicular compartment carried all the processing machinery necessary for Aβ generation has not been confirmed by others (Lazarov et al., 2005). Nevertheless, KLC deficient animals, when crossed with APP transgenic mice, showed axonal pathology manifested by axonal swellings and increased amyloid levels and deposits in brain (Stokin et al., 2005). The latter argue that perturbations of axonal transport during aging may predispose to the development of AD pathology. This suggestion is in line with observations that disruption of slow axonal transport is associated with neuronal death in animal models of motor neuron disease (LaMonte et al., 2002).

### 2.4 Raft association of secretases and amyloidogenic processing

Growing evidence indicates a functional relationship between cellular cholesterol level and efficiency of amyloidogenic processing. Cholesterol depletion of cultured cells by lovastatin treatment and methyl-β-cyclodextrin extraction inhibits APP processing by BACE1 and lowers Aβ production (Simons et al., 1998; Ehehalt et al., 2003). Furthermore, the above treatments stimulate non-amyloidogenic processing of APP by α-secretase ADAM10 (Kojro et al., 2001), raising the intriguing possibility that cholesterol levels may determine the balance between amyloidogenic and non-amyloidogenic
processing of APP. However, moderate, but not complete, reduction of cholesterol leads to increased amyloidogenesis in neuronal cells (Abad-Rodriguez et al., 2004). Thus, cholesterol regulation of APP secretases may be more complex than previously understood.

Evidence from a variety of \textit{in vitro} and \textit{in vivo} studies indicates that specialized membrane microdomains termed lipid rafts, which are rich in cholesterol and sphingolipids, might be the critical link between cholesterol levels and amyloidogenic processing of APP. Lipid rafts function in the trafficking of proteins in the secretory and endocytic pathways in epithelial cells and neurons, and participate in a number of important biological functions (Simons and Toomre, 2000). Disruption of rafts by depletion of cellular sphingolipids increases secretion of APP\textsubscript{\textalpha} and A\textbeta\textsubscript{42}, but not A\textbeta\textsubscript{40} (Sawamura et al., 2004), suggesting that at least certain aspects of amyloidogenic processing of APP can be modulated by raft microdomains. High versus low cholesterol or sphingolipid depletion may cause selective alterations in the association of APP and secretases with cholesterol-rich membrane domains (discussed below), thus causing the apparent discrepancy in the outcome of APP processing.

Lipid rafts are biochemically defined as detergent-insoluble membrane (DIM) domains that resist extraction with cold non-ionic detergents such as Triton X100, Brij-96, and Lubrol WX. These microdomains are formed by lateral association of sphingolipids and cholesterol in the Golgi, and are present in the plasma membrane and other intracellular organelles such as endosomes and the TGN. As mentioned above, the BACE1 cytoplasmic tail undergoes palmitoylation, a post-translational modification that targets proteins to lipid rafts. Indeed, a significant fraction of BACE1 is localized in lipid raft microdomains in a cholesterol-dependent manner, and addition of a GPI-anchor to target BACE1 exclusively to lipid rafts increases APP processing at the \textbeta-cleavage site (Riddell et al., 2001; Cordy et al., 2003). Elegant studies by Simons and colleagues showed antibody-mediated co-patching of cell surface APP and BACE1 as well as provided evidence for amyloidogenic processing of APP in raft microdomains (Ehehalt et al., 2003). These observations are consistent with the paucity of full-length APP in raft microdomains at steady state, and the preferential accumulation of APP CTFs in adult brain and cultured cells in raft microdomains, until they can be further processed. Indeed, all four components of the \gamma-secretase complex (PS1 derived N- and C-terminal fragments, nicastrin, APH-1, and PEN-2) also associate with DIM fractions enriched in lipid raft markers such as caveolin, flotillin, PrP, and ganglioside GM1 (Vetrivel et al., 2004). Consistent with the typical behaviour of \textit{bona fide} raft-resident proteins, association of \gamma-secretase components with DIM is also sensitive to cholesterol depletion from cellular membranes. In contrast to BACE1 and the \gamma-secretase complex, \alpha-secretases have not been linked to raft microdomains based on cholesterol depletion/loading studies.
(Ehehalt et al., 2003). Thus, mounting evidence suggest that lipid rafts may be the principal membrane platforms where amyloidogenic processing of APP occurs.

Detailed biochemical fractionation and magnetic immunoisolation studies indicate that active and mature components of $\gamma$-secretase complex co-reside in lipid raft microdomains with SNARES such as VAMP-4 (TGN), syntaxin 6 (TGN and vesicles) and syntaxin 13 (early endosomes) (Vetrivel et al., 2004). These findings strongly implicate lipid raft microdomains of intracellular organelles as the preferred sites of amyloidogenic processing. Interestingly, the cell-surface raft associated protein, SNAP-23 does not co-reside with mature components of $\gamma$-secretase complex (Vetrivel et al., 2004), raising the possibility that the relatively low level of active $\gamma$-secretase complex at the cell surface could be associated with non-raft membrane domains and also explain the apparent low level of activity at the cell surface. Such spatially distinct localization of the $\gamma$-secretase allows for intramembrane processing of diverse substrates. Indeed, unlike APP, CTFs derived from several other $\gamma$-secretase substrates such as Notch1, Jagged2, N-cadherin, and DCC largely remain in non-raft membranes (Vetrivel et al., 2005). Taken together, these findings are consistent with the prediction that $\gamma$-secretase cleavage of APP occurs in lipid rafts. Further investigations are needed to address how the components of the $\gamma$-secretase and APP are recruited into raft microdomains, and clarify whether genetic mutations in APP, PS1, and PS2 modulate A$\beta$42 production by affecting the localization and processing of APP in lipid rafts.

3. APP function

3.1 Trophic properties

While a number of physiological roles have been attributed to APP, some unique to certain isoforms, the in vivo function(s) of the molecule remain unclear. The literature covering APP function is extensive and cannot be reviewed comprehensively (Mattson, 1997). Suffice to say that a number of functional domains have since been mapped to the extra- and intracellular region of APP. These include metal (copper and zinc) binding, extracellular matrix components (heparin, collagen, and laminin), neurotrophic and adhesion domains, and protease inhibition (Kunitz protease inhibitor domain present in APP751 and APP770 isoforms). One of the earliest indication of APP function came from assessing growth pattern of fibroblasts where APP levels were decreased by expression of an antisense APP construct (Saitoh et al., 1989). These cells grew slowly but the growth retardation can be restored by treatment with secreted APPs. The active domain was subsequently mapped to a pentapeptide domain “RERMS” near the middle of the extracellular domain (positions 403-407)
(Ninomiya et al., 1993). The activity is not limited to fibroblasts as infusion of this pentapeptide as well as APPs into brain resulted in increased synaptic density and improved memory retention in animals (Roch et al., 1994; Meziane et al., 1998). Because, as mentioned above, APPs is constitutively released from cells following \( \alpha \)-secretase cleavage, these findings indicated that APP has autocrine and paracrine functions in growth regulation.

In all, a trophic role for APP has been perhaps the most consistently and arguably the best established function for the molecule. APP has been shown to stimulate neurite outgrowth in a variety of experimental settings. This phenotype is compatible with the upregulation of APP expression during neuronal maturation (Hung et al., 1992). The N-terminal heparin-binding domain of APP (residues 28-123), just upstream from the “RERMS” sequence, also stimulates neurite outgrowth and promotes synaptogenesis. Interestingly, the crystal structure of this domain shows similarities to known cysteine-rich growth factors (Rossjohn et al., 1999). Conversely, injection of APP antibodies directly into the brain led to impairment in behavioral tasks in adult rat (Meziane et al., 1998). Finally, a recent report indicated the presence of binding sites for APPs in epidermal growth factor (EGF)-responsive neural stem cells in the subventricular zone in the adult rodent brain (Caille et al., 2004). In this context, APPs\( \alpha \) acts in concert with EGF to stimulate the proliferation of these cells both in neurospheres in culture and \textit{in vivo}. However, APPs is necessary, but not sufficient, for full activity, as it appears to act as a co-factor with EGF. If these findings are true in human brain, then the reduction in APPs levels in cerebrospinal fluid of individuals with AD may indicate the loss of additional trophic activity in AD, together with the reduction of other growth factors in brain (see Chapter 15).

### 3.2 Cell adhesion

An “RHDS” motif near the extralumenal portion of APP or at the C-terminus of APPs that is contained within the \( \text{A}\beta \) region appear to promote cell adhesion. It is believed that this region acts in an integrin-like manner and can, accordingly, be blocked by RGDS peptide sequence derived from the fibronectin-binding domain (Ghiso et al., 1992). Similarly, APP colocalizes with integrins on the surface of axons and at sites of adhesion (Storey et al., 1996; Yamazaki et al., 1997). Evidence of interaction with laminin and collagen provides further evidence of adhesion promoting properties. Interestingly, because the RHDS sequence is contained within the N-terminus of \( \text{A}\beta \) (residues 4-7), similar cell adhesive promoting properties have also been attributed to \( \text{A}\beta \) peptide itself. This latter property, however, is difficult to tease out in view of the cytotoxicity of \( \text{A}\beta \) peptide when tested in a variety cell systems \textit{in vitro}. Furthermore, it is difficult to separate the cell adhesive from the neurite...
outgrowth promoting roles of APP. Clearly, these are probably somewhat inseparable, as neuronal migration, neurite outgrowth, and even synaptogenesis would involve substrate adhesion. The phenotype of APP and APLP-deficient animals are certainly in agreement with these proposed physiological activity of these molecules (see below).

3.3 Is APP a receptor?

Although APP was initially proposed to act as a cell surface receptor, the evidence supporting this idea has been unconvincing. Aside from interactions with extracellular matrix proteins, only recently has a candidate ligand been proposed. It was reported that F-spondin, a neuronally secreted signaling glycoprotein that may function in neuronal development and repair, binds to the extracellular domain of APP as well as APLP1 and APLP2 (Ho and Sudhof, 2004). This binding reduces β-secretase cleavage of APP and nuclear transactivation of AICD (see below), suggesting therefore that F-spondin may be a ligand that regulates APP processing.

As mentioned above, γ-secretase processing of APP also releases an intracellular domain of APP, termed AICD (Figure 1). This processing step is not unique for APP, and indeed may be a rather generalized phenomenon whereby membrane anchored proteins are cleaved to either release cytosolic fragments that participate in cell signaling, as in the Notch receptor, or for degradation. Because APP undergoes the same γ-secretase membrane proteolysis as Notch, the analogy to Notch is simply too tempting or obvious, even though the evidence that APP is itself a cofactor for transcriptional activation within the nucleus, remains to be firmly established. Using a heterologous signalling reporter system, AICD can form a transcriptionally active complex together with two other molecules, Fe65 and Tip60 (Cao and Sudhof, 2001). Although it was initially felt that AICD must enter the nucleus with Fe65, subsequent study showed that nuclear translocation of AICD is not required but may be indirect through Fe65 (Cao and Sudhof, 2004). An alternative approach to address this question is to look for AICD activated candidate genes. In this regards, two genes have been proposed to date, KAI1, a tumour suppressor gene, and nepriylisin, a neutral endopeptidase with Aβ degrading activity (Baek et al., 2002; Pardossi-Piquard et al., 2005). The latter pathway is particularly interesting because it suggests that γ-secretase release of AICD can regulate the degradation of Aβ in the extracellular space. If this is true, it will be important to know the feedback pathways that modulate γ-secretase activity to regulate neprilysin expression.
3.4 APP-deficient animals

In view of the above discussion, it is perhaps a little surprising then that with so many functions attributed to APP, the initial phenotype of APP-deficient mice obtained by gene-targeting was rather unappealing (Zheng et al., 1995). These mice were lighter in body mass and with age; there was weakness in the extremities. Examination of the brain revealed gliosis only, a rather non-specific astrocytic reaction. Postnatal growth deficit was also noted in the APLP1-deficient mice but APLP2-deficient mice demonstrated no apparent phenotype (von Koch et al., 1997; Heber et al., 2000). Interestingly, \( \text{APLP2}^{-/-}/\text{APLP1}^{-/-} \) and \( \text{APP}^{-/-}/\text{APLP2}^{-/-} \) double mutants, but not \( \text{APP}^{-/-}/\text{APLP1}^{-/-} \) animals, showed early postnatal lethality, indicating that members of the APP gene family are essential genes that exhibit partial overlapping functions. Curiously, the histopathological phenotype of the animals that displayed early lethality was rather bland by initial descriptions. Similarly, neurons cultured from these animals were unaltered in their basal growth rates or response to excitotoxicity. However, in the peripheral nervous system, \( \text{APP}^{-/-}/\text{APLP2}^{-/-} \) double knockout animals exhibited poorly formed neuromuscular junction with reduced apposition of pre- and postsynaptic elements of the junctional synapses (Wang et al., 2005). The number of synaptic vesicles at the presynaptic terminals were also reduced, a finding confirmed by defective neurotransmitter release. With knowledge of the neuromuscular junction phenotypes of \( \text{APP}^{-/-}/\text{APLP2}^{-/-} \) double knockout mice in mind, examination of the parasympathetic submandibular ganglia of these animals also showed a reduction in active zone size, synaptic vesicle density, and number of docked vesicles per active zone (Yang et al., 2005). This function of APP/APLP is evolutionarily conserved, as evidenced by the decreased number of synaptic boutons in neuromuscular junction of \textit{Drosophila} larvae lacking \textit{APPL}, and involves interaction of \textit{APPL} with the cytosolic adaptor Mint and a transmembrane cell adhesion molecule named Fasciclin II (Ashley et al., 2005).

Deficiency of all three \textit{APP} genes led to death shortly after birth. The majority of the animals showed cortical dysplasia suggestive of migrational abnormalities of the neuroblasts and partial loss of cortical Cajal Retzius cells (Herms et al., 2004). Taken together, the recent findings presented a convincing picture that members of the \textit{APP} gene family play essential roles in the development of the nervous system relating to synapse structure and function, as well as in neuronal migration. Whether these abnormalities underlie the early postnatal survival of the animals remain to be established. Further, whether these activities are due to mechanical properties or mediated by activating signaling pathways, or both, are interesting questions that remain to be elucidated.
In view of the trophic properties of APP, it would be natural to predict that overexpression of APP would demonstrate phenotypes related to the enhanced neurite outgrowth, enhanced cell growth, etc. Indeed, many studies have reported such findings (Leyssen et al., 2005). Surprisingly and more interestingly, however, convincing negative phenotypes have also been reported. Overexpression of APP in cells induced to differentiate into neurons led to cell death (Yoshikawa et al., 1992). In vivo, genetic engineering to overexpress APP carrying various familial AD mutations in transgenic mice resulted in the development of amyloid deposition and amyloid associated changes in brain, including loss of synaptic markers, confirming the pathogenic nature of these mutations. Careful examination also showed axonal swellings and varicosities, months before any evidence of amyloid deposition or amyloid associated pathology in brain (Stokin et al., 2005). Perhaps the best example of the consequences of APP overexpression is trisomy 21 in humans and trisomy 16 in mice, the latter containing many of the cognate human chromosome 21 orthologous genes. Individuals with Down’s syndrome (DS) who live beyond the 3rd decade of life almost invariably develop histopathology indistinguishable from AD (Burger and Vogel, 1973). APP is present in three copies in trisomy 21 and this excess gene dosage leads to early elevation of Aβ levels, even in brains of fetuses (Teller et al., 1996). Several lines of evidence support the concept that APP gene triplication is necessary and possibly even sufficient to cause the AD histopathology in DS individuals. First, fine mapping of genes duplicated in several individuals with partial trisomy, where some but not all chromosome 21 genes are triplicated, excluded APP and SOD1 genes in generating classical features of DS (Korenberg et al., 1990). Second, a remarkable case report of a 78 year-old woman with DS features due to partial trisomy 21 who at postmortem examination did not have any of the expected AD pathological changes in brain (Prasher et al., 1998). The segment of the chromosome that was triplicated in this individual excluded the APP gene, thereby confirming that APP or possibly genes immediately adjacent to APP is necessary for the development of AD histopathology. Third, the segmental trisomy 16 mouse (Ts65Dn), a genetic model of DS, shows physiological and structural abnormalities that are in common with human DS. For example, vesicular enlargements in neuronal perikarya containing endosomal markers (Rab5, EEA1, etc.) present in AD and DS individuals are also seen in this mouse model. Interestingly, these changes can be reversed if the APP gene dosage was reduced back to the euploid state when the Ts65Dn mice were crossed to the APP-deficient animals, showing that these changes are uniquely due to APP gene dosage (Cataldo et al., 2003). Finally, the studies from DS cases have led to some to suggest that AD may be caused by triplication of APP.
in brain. However, generalized APP gene triplication appears to be excluded in AD but somatic aneuploidy remains a distinct possibility (Yang et al., 2003; Rehen et al., 2005). If true, then this intriguing idea can certainly provide a plausible mechanism for the development of sporadic AD.

5. Summary

This review has covered some of the salient aspects of APP biology, concentrating on the recent advances in processing, trafficking, and function of APP and the related APLP1 and APLP2 members. The importance of APP in AD clearly lies in its role as precursor to the Aβ peptide that plays a central role in the amyloid hypothesis. However, APP has a number of additional biological activities, some of which impact neuronal development and function. Growing evidence suggests that perturbations of some of these activities may also contribute to AD pathogenesis and neurodegeneration. As such, it will be important to continue to investigate the normal function of APP.

References

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