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Regulated Proteolysis of APP and ApoE Receptors

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Abstract The β-amyloid precursor protein (APP) shares intracellular and extracellular-binding partners with the family of receptors for apolipoprotein E (apoE). Binding of APP and apoE receptors to specific extracellular matrix proteins (F-spondin and Reelin) promotes their presence on the cell surface and influences whether they will interact with specific cytoplasmic adaptor proteins. Cleavage of APP and apoE receptors at the cell surface occurs by α-secretase activities; thus, the processing of these proteins can be regulated by their trafficking either to or from the cell surface. Their cleavages can also be regulated by tissue inhibitor of metalloproteinase-3 (TIMP-3), a metalloprotease inhibitor in the extracellular matrix. ApoE receptors have functions in neuronal migration during development and in proper synaptic function in the adult. Thus, the functions of apoE receptors and by analogy of APP will be modified by the various extracellular and intracellular interactions reviewed in this paper.

Keywords Alzheimer's disease · Amyloid precursor protein · Apolipoprotein E · Secretase · Extracellualr matrix · Adaptor protein

Abbreviations

APP	β-amyloid precursor protein
apoE	apolipoprotein E
TIMP-3	tissue inhibitor of metalloproteinase-3
AD	Alzheimer's disease
CTF	C-terminal fragment
ADAM	A disintegrin and metalloproteinase
LDLr	low-density lipoprotein receptor
VLDLr	very low-density lipoprotein receptor
ApoEr2	apoE receptor 2
LRP	low-density lipoprotein receptor-related protein
ICD	intracellular domain
PSD	post-synaptic density
PDZ	PSD-95/Dlg/ZO-1

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Alzheimer's Disease and APP

Alzheimer's disease (AD) is caused by the abnormal accumulation in the brain of the Aβ peptide, a proteolytic product of the β-amyloid precursor protein (APP) [1]. APP and its two homologous family members, APLP1 and APLP2, are type I transmembrane proteins, whose extracellular domains contain two conserved sequences (E1 and E2) and whose cytoplasmic domains contain NPXY sequences important for regulated endocytosis. APP undergoes extracellular cleavage by one of two protease activities, α- or β-secretase, resulting in the release of large N-terminal extracellular fragments and smaller, membrane-bound C-terminal fragments (CTF). If the initial cleavage of APP occurs via β-secretase, then the subsequent cleavage of the CTF in the transmembrane region by γ-secretase results in the formation of Aβ [2]. Mutations in APP and presenilin genes cause familial, early-onset forms of AD apparently by altering production of Aβ [3].

The APP α-secretases include membrane-spanning proteins A disintegrin and metalloproteinase (ADAM)-10 and ADAM-17, and the majority of their activity is thought to occur at the cell surface [4]. In contrast, the APP β-secretases, BACE-1 and BACE-2, are mostly active in the low pH environment of endosomes [5]. Therefore, molecules

that affect APP trafficking between the cell membrane and endosomes would alter APP proteolysis and production of Aβ and thus may affect the long-term risk of AD. Understanding APP trafficking and processing in neurons may provide valuable information for developing interventions against AD.

Apolipoprotein E and ApoE Receptors

While rare mutations cause familial, early-onset AD, common polymorphisms in genes can affect the risk of late-onset AD. The strongest genetic risk factor for AD is APOE [6–8]. There are two polymorphic sites within APOE defining three APOE alleles: APOE-ε2, APOE-ε3, and APOE-ε4. Inheritance of the APOE-ε4 allele increases AD risk, while inheritance of APOE-ε2 is protective, compared to the most common ε3 allele. It is currently unknown how APOE genotype influences the pathogenic processes of AD.

APOE encodes the apolipoprotein E (apoE) protein, which binds to a family of cell surface receptors. This family was initially defined by the low-density lipoprotein receptor (LDLr) [9]. The family includes ApoE receptor 2 (ApoEr2) and the very low-density lipoprotein receptor (VLDLr) and the LDLr-related protein 1 (LRP1), LRP1b, and LRP2. Each of these receptors has a large N-terminal extracellular domain, with multiple ligand-binding repeats for binding of numerous other ligands in addition to apoE, and a small C-terminal cytoplasmic domain. Endocytosis and degradation or recycling of apoE occurs through this family of receptors [8, 10].

Similarities of APP and ApoE Receptors

APP shares a number of features with apoE receptors; indeed, APP was proposed to be a receptor when it was first cloned [11]. APP and apoE receptors are both type I transmembrane proteins, sharing three general characteristics: First, they are similarly processed. Many of the same proteolytic events that occur for APP also occur for ApoE receptors, including α-, β-, and γ-cleavages, leading to the release of extracellular domains (soluble receptors) and intracellular domains (ICD) [12–14]. Interestingly, proteolysis of apoE receptors is promoted by the binding of ligands, such as apoE, activated α_2-macroglobulin, or Reelin [12]. Binding of molecules to the extracellular domain of APP similarly promotes its cleavage (see below). This proteolysis of APP and apoE receptors could regulate signaling cascades promoted by the binding of ligands or cleave connections between the cell and the extracellular milieu [15]. The sAPP and the soluble apoE receptors generated by this cleavage could also act to inhibit the interactions of the full-length transmembrane proteins with extracellular-binding partners.

Second, APP and apoE receptors bind many of the same adaptor proteins via cytoplasmic domains. Disabled-1 (Dab1) binds APP and apoE receptors via their NPXY sequences [16]. FE65 interacts (via separate domains) with APP [17, 18] and the apoE receptors LRP1 [19–21] and ApoEr2 [22], also through domains including the NPXY sequences. X11 binds to APP via its NPXY sequences [23, 24] and binds to ApoEr2 via an alternatively spliced domain [25]. APP and apoE receptors could compete for binding of these intracellular adaptor molecules, or the adaptor molecules could link the transmembrane proteins together [26].

Third, APP and apoE receptors share an extracellular ligand, F-spondin [27, 28]. F-spondin is a developmentally regulated neuronal protein associated with the extracellular matrix [29, 30]. In culture, F-spondin affects trafficking and processing of both APP and ApoEr2 [27, 28].

There is also evidence of direct interactions between APP and LRP1, with binding of forms of APP containing the Kunitz protease inhibitor domain to the ligand-binding domain of LRP1 [31]. The biological significances of these various interactions, both extracellularly and intracellularly, are not known. However, these shared characteristics suggest that APP and apoE receptors are functionally related molecules, and we will explore these interactions in this review.

APP, ApoE Receptors, and Intracellular-Binding Partners

The cytoplasmic domains of APP and ApoEr2 contain NPXY sequences that serve as binding sites for adaptor proteins that possess a phosphotyrosine-binding domain (PTB), such as members of the Dab, FE65, and X11 protein families. The cytoplasmic domains of LRP1 and ApoEr2 have other domains that interact with other adaptor proteins, such as the post-synaptic density 95 (PSD-95) protein [16, 32–34]. Such adaptor proteins play critical roles in tyrosine kinase-mediated signal transduction, protein trafficking and processing, and neuronal development [23, 26]. In the following sections, we will highlight how these adaptor proteins affect APP and apoE receptors in similar ways. We suggest that these similarities provide information about how the functions of APP and apoE receptors could be related.

Dab1

The Dab family members, Dab1 and Dab2, are important for nervous system development in *Drosophila* and

mammals [35]. These cytoplasmic proteins possess a PTB domain and have been shown to interact with APP, ApoEr2, and LRP1 in yeast two hybrid and co-immunoprecipitation experiments [36, 37]. In mice, the Dab1 protein functions downstream of the extracellular matrix protein Reelin during neuronal positioning [35]. Reelin binds extracellularly to ApoEr2 and VLDLr [38, 39], promotes phosphorylation of Dab1 [40, 41], and induces downstream activation of Src and PKB kinases [42]. Binding of Reelin to these receptors results in their clustering on the cell surface, an important event in their signaling properties [43]. These processes are necessary for correct migration of cortical, hippocampal, and cerebellar neurons during development; knock-out mice lacking the ApoEr2 and VLDLr genes demonstrate the same neuronal migration defects as mice deficient in Reelin and Dab1 [44].

Dab1 interactions with APP [45, 46] and apoE receptors [37, 47] affect their trafficking and processing. We found that overexpression of Dab1 resulted in increased cell surface APP and ApoEr2 in COS7 cells and increased α-cleavage and decreased β-cleavage of APP [37] Others found in HEK cells that Dab1 increased surface APP and APP α-secretase cleavage but (in contrast to our findings) increased β-cleavage [47]. These differences could result from a competition between Dab1 and other cytoplasmic adaptor proteins binding to APP and ApoEr2. We also found that Reelin significantly increased co-immunoprecipitation between Dab1 and APP and between Dab1 and ApoEr2 [37]; ApoEr2-Dab1 co-localization was also increased after Reelin treatment of cells based on an immunocytochemical approach [48]. Reelin increased cleavage of both APP and ApoEr2 and significantly decreased Aβ in the presence of Dab1 [37]. These studies have begun to address the important question of how interactions of intracellular adaptor proteins with surface receptors are regulated by extracellular events.

FE65

FE65 family members [FE65, FE65-like 1 (FE65L1), and FE65L2] interact with APP, as defined by yeast two hybrid, pull-down, and co-localization assays [49–53]. Mice deficient in FE65 and FE65L1 share developmental abnormalities with mice deficient in the three members of the APP family [54] in that each display prominent lissencephaly [55]. FE65 family members are expressed at high levels in neurons [56] and possess three protein-binding domains: a WW domain and two PTB domains. The WW domain binds the mammalian-enabled protein, which binds actin and thus links FE65 and APP to cytoskeletal dynamics and cellular motility and morphology [57, 58].

FE65 interaction with APP and apoE receptors affects their trafficking and processing. FE65 increased surface APP in Madin–Darby canine kidney cells and H4 cells [49, 59]. In addition, FE65 stabilized immature APP and inhibited sAPP generation and Aβ production in HEK cells [60] and FE65L1 increased Aβ production [52]. FE65 family members reduced cell surface LRP [51] and increased cell surface ApoEr2 [37], suggesting that FE65 alters APP and apoE receptor trafficking.

The PTB1 domain of FE65 binds LRP1 [20] and ApoEr2 [22], and the PTB2 domain of FE65 binds APP, providing a potential intracellular link between APP and apoE receptors [19]. We found that FE65 containing both the PTB1 and PTB2 domains increased cleavage of APP and ApoEr2, whereas constructs containing only single PTB domains did not affect their processing [22]. These data suggest that FE65 may bind APP and ApoEr2 at the same time (forming the APP–FE65–ApoEr2 complex), and the presence of this complex could affect the processing of both APP and ApoEr2. We also hypothesized that LRP could compete with ApoEr2 for binding to FE65 and APP. Using CHO $LRP^{+/+}$ and CHO $LRP^{-/-}$ cells, we observed that the $LRP^{+/+}$ cells showed lower levels of co-precipitation of APP and ApoEr2 in the presence of FE65 [22], suggesting that the presence of different apoE receptors could alter APP trafficking involving FE65.

In addition to binding to full-length proteins, FE65 also may bind to the intracellular fragments of APP. FE65 complexes with the APP ICD have been suggested to alter nuclear translocation and gene transcription after γ-cleavage of APP [61, 62]. The binding of FE65 to the ICD of membrane bound forms of apoE receptors could alter whether the FE65 can transverse from the cell surface to the nucleus complexed with the APP ICD.

X11

Members of the Mint/X11 family, X11α, β, and γ, are adaptor proteins with divergent N-termini but highly conserved C-termini that contain a PTB domain and two PDZ (PSD-95/Dlg/ZO-1) domains. The X11α PTB domain interacts with APP, APLP1, and APLP2 [23, 63]. X11α is a synaptic protein, and the N-terminal domains of X11α and β interact with Munc18-1, a protein essential for synaptic vesicle docking and exocytosis [64]. Co-expression of X11α and APP slows cellular APP processing and reduces Aβ secretion. Aβ secretion may be inhibited by X11α via impaired trafficking of APP to subcellular compartments containing active γ-secretase complex [24]. In addition, X11β reduces Aβ levels and amyloid plaque formation in the brains of transgenic mice [65]. In contrast, knockdown of X11α and X11β decreased Aβ production [66]. Interestingly, a recent study has shown that X11α and β also interact with ApoEr2 [25], and this interaction may affect the processing of APP. These data suggest that the

X11 family affects APP trafficking and processing, either directly or in concert with other adaptor proteins.

Post-Synaptic Density 95

ApoE receptors are synaptic proteins that affect the activity of glutamate receptors in the post-synaptic density [32, 34, 67–70] and modulate learning, memory, and long-term potentiation (LTP) [32, 40]. One of the major organizers of the post-synaptic density is PSD-95, an adaptor protein consisting of three PDZ domains and one Guanylate kinase-like domain. One PSD-95 PDZ domain interacts with subunits of the NMDA receptor [71], and the third PDZ domain of PSD-95 interacts with Neuroligin, important for synapse formation and maturation [72]. PSD-95 also associates with ApoEr2, specifically through the alternatively spliced, intracellular exon of ApoEr2 [32, 33]. This interaction between ApoEr2 and PSD-95 was altered by an ApoEr2 ligand [33]. APP also is a synaptic protein and expressed both pre-and post-synaptic densities [73]. However, it is unknown whether APP also interacts with PSD-95 and how the interaction between APP and PSD-95 might affect APP trafficking.

APP, ApoE Receptors, and Extracellular-Binding Partners

The intracellular interactions of adaptor proteins with APP and apoE receptors are regulated by extracellular interactions. In the following sections, we will review data that the extracellular domains of APP and ApoEr2 interact with the extracellular matrix proteins, F-spondin and Reelin. Such extracellular matrix proteins play critical roles in neurite outgrowth, neuronal migration, and neuronal development. Interactions between these extracellular matrix proteins and APP or ApoE receptors also alter their proteolysis, potentially representing a pathway for breaking the cellular interactions with the extracellular matrix. Another extracellular matrix protein, TIMP-3, regulates the surface levels of APP and apoE receptors, potentially modifying the interactions with the extracellular matrix and the functions of these transmembrane proteins.

F-Spondin

F-spondin is a component of the extracellular matrix with functions in neuronal migration and plasticity, both during development and in the adult brain [29, 30]. F-spondin contains three domains: reeler, spondin, and thrombospondin [74]. The thrombospondin domain of F-spondin promotes neurite outgrowth in a wide variety of neuron types [29, 74–76] and acts as a guidance molecule in the pathfinding of commissural axons during development [76], promoting or inhibiting axonal migration of specific types of neurons [77]. F-spondin is also important in the migration of olfactory cells [78]. F-spondin lacking the thrombospondin domain promoted neuronal differentiation and neurite outgrowth [79], suggesting that the other domains of F-spondin may be important to neuronal differentiation. These studies demonstrate that F-spondin regulates neuronal differentiation and migration, in both development and in the adult brain.

Interactions of F-spondin with cell surface proteins affect their trafficking and processing. The spondin domain of F-spondin binds APP via its E2 domain [27] and leads to decreased β-cleavage of APP [27, 28]. We found F-spondin increased surface levels of APP, increased α-cleavage of APP, and decreased Aβ production. We also found that the C-terminal thrombospondin domain of F-spondin bound ApoEr2, and this binding affected the processing of both ApoEr2 and APP, potentially through an extracellular linkage of APP and ApoEr2 [28]. F-spondin also interacts with several other members of the apoE receptor family, including LRP2 and LRP4, which are expressed in the floor plate [80]. Processing of APP and ApoE receptors may regulate these types of interactions with F-spondin, affecting any functions they have in neuronal migration and neurite outgrowth.

Reelin

As mentioned above, mice lacking functional Reelin, ApoEr2 and VLDLr, or Dab1 are strikingly similar in their phenotypes: neurons in the cortex, hippocampus, and cerebellum fail to migrate properly during development [38, 39]. In the cortex, for example, the neurons born in the subventricular zone normally migrate along processes of radial glia to form the cortical layers, such that the later-developing neurons travel the farthest and form the outer layers of the cortex. In the mutant mice, the newly formed neurons do not migrate past earlier layers of cells, and thus the cortical layers are inverted [81]. In addition to these functions in neuronal migration, Reelin and apoE receptors also may play important roles in synaptic plasticity in adult brain [70]. Reelin modulates learning and memory via ApoEr2 [32], affects maturation of CA1 glutamatergic synapses [82], increases NMDA and AMPA receptor activity in the adult hippocampus [83], and regulates NMDA receptor surface trafficking and synaptic subunit composition [84]. Questions remain about how the Reelin signal is transduced and regulated and the identity of potential co-receptors, but it has clear importance in neuronal migration and synaptic function.

Reelin binds to the ligand-binding domains of apoE receptors, affecting the phosphorylation of the cytoplasmic

Dab1 [38, 85]. Reelin binding to apoE receptors also leads to activation of kinases [40], increased processing of receptors [12], and receptor clustering on the cell surface [43]. Whether Reelin also interacts with APP is unknown, but several interesting connections exist. First, as noted above, Dab1 interacts with APP in addition to apoE receptors [37]. Second, Reelin protein and mRNA is decreased in APP 2576 transgenic mice [86] and increased in APP knockout mice (our unpublished data). Finally, both APP and Reelin affect hippocampal dendritic neurite outgrowth [87–91] and neuronal migration [93].

These studies lead to the hypothesis that cell surface APP and apoE receptors are both important in neuronal migration during development and in synaptic function in the adult brain. While APP knockout mice do not demonstrate the dramatic neuronal migration deficits seen in knock-outs of ApoEr2/VLDLR mice [78, 92], embryos lacking all three APP family members (APP, APLP1, and APLP2) do demonstrate type 2 lissencephaly [55]. Furthermore, APP small interfering RNA caused a complete cessation of neuronal migration based on an in utero electroporation approach [93]. This effect of APP reduction depended not only on the extracellular domain of APP but also the intracellular NPXY sequence, which interacts with Dab-1 and FE65. The reduced APP levels led to decreased migration of the neurons from the subventricular zone through the cortical layers but did not affect the tangential migration of the neurons [93]. Migration through the cortical mantle depends on cellular interactions with Reelin [81], while the tangential migration may depend on cellular interactions with F-spondin [78]. F-spondin also provides guidance cues through interactions with apoE receptors to affect axonal outgrowth [80]. Thus, members of the APP and apoE receptor families may mediate various extracellular signals to define the precise process of developmental migration of neurons.

In addition to the deficits in normal neuronal migration, both APP knockout mice and mice lacking specific apoE receptors demonstrate impaired synaptic activities. Specifically, both APP knockout mice and apoE receptor knockout mice have impaired LTP and memory [32, 70, 92, 94, 95]. Furthermore, Reelin cooperates with ApoE receptors to facilitate LTP and memory [70]. These data suggest that APP and ApoE receptors affect the function of synapses in vivo.

Tissue Inhibitor of Metalloproteinase-3

TIMP-3 belongs to a family of four secreted proteins (TIMP-1 to TIMP-4) that are found in the extracellular matrix. TIMPs were originally identified as inhibitors of the matrix metalloproteinases [96]. TIMP-3 also inhibits other zinc-dependent proteases, including members of the ADAM family [97, 98]. Specifically, TIMP-3 inhibits ADAM-10 and ADAM-17 activities [99, 100], two known APP α-secretases [101]. Recently, we found that TIMP-3 inhibited α-secretase cleavage of APP and ApoE receptors, leading to increased APP β-CTF and Aβ production [102].

We also found that TIMP-3 expression was regulated in an interesting way. We were examining genes altered by liver X receptors (LXR) while studying mechanisms of cholesterol homeostasis in the central nervous system. We observed the expected increase in genes known to be induced by LXR (ABCA1, ABCG1, and SREBP), as well as an unexpected decrease in TIMP-3 [102]. This decrease in TIMP-3 levels after LXR activation may partially explain effects of cellular cholesterol on APP processing and Aβ production [103, 104]. In vivo, TIMP-3 levels were increased in AD brains and brains of transgenic APP mice [102], suggesting that it may contribute to the accumulation of Aβ in the disease state.

Conclusions

Since TIMP-3 can regulate the levels of APP and apoE receptors on the cell surface, we hypothesize that it may regulate their effects on neuronal migration and synaptic functions. We have diagrammed the common interactions of APP and apoE receptors outlined in this review (Fig. 1). Much work over the last two decades has gone into defining the molecules responsible for the cleavages of APP, due to the interest in Aβ generation and its effects on AD pathogenesis. An important challenge remaining is to

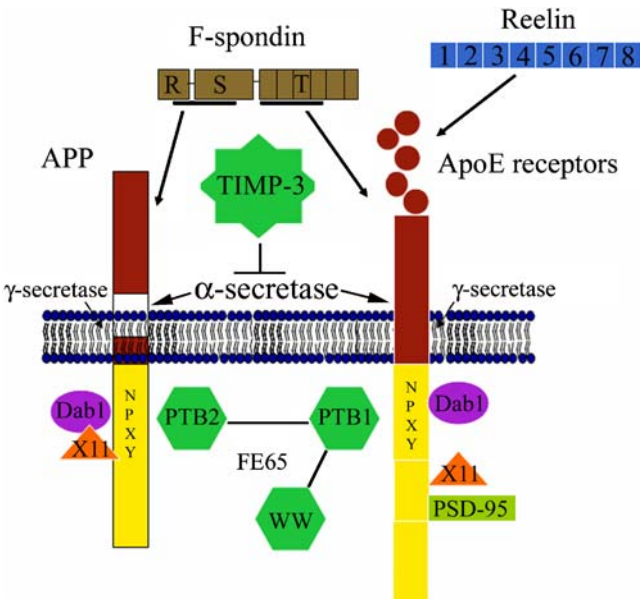

Fig. 1 APP and apoE receptor interactions. Cartoon of various similarities of intracellular and extracellualr interacting proteins. These commonalities suggest related functions of APP and apoE receptors

understand the normal regulation of these cleavage events. ApoE receptors share many characteristics with APP but have better-defined functions in ligand binding, endocytosis, and neuronal migration. The cleavage of apoE receptors and APP affects the cellular interaction with the extracellular matrix and the interactions of cytoplasmic adaptor proteins with transmembrane anchors. The regulated cleavages of APP and apoE receptors affect their intertwined functions and will likely provide avenues for altering the amyloidogenic processing of APP.

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