From the Department of Neuroscience
Karolinska Institutet, Stockholm, Sweden

STUDIES OF AMYLOID PRECURSOR PROTEIN TRAFFICKING AND PROCESSING

Shaohua Xu

Stockholm 2018

Published by Karolinska Institutet.
Printed by E-Print AB

ISBN 978-91-7676-917-1

Studies of amyloid precursor protein trafficking and processing

THESIS FOR DOCTORAL DEGREE (Ph.D.)

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i **CMB Lecture Hall, Berzelius väg 21, Karolinska Institutet, Solna**

Torsdagen den 25 Januari, 2018, kl 10.00

By

Shaohua Xu

Principal Supervisor:
Professor Lennart Brodin
Karolinska Institutet
Department of Neuroscience

Co-supervisor:
Professor Oleg Shupliakov
Karolinska Institutet
Department of Neuroscience

Opponent:
Associate Professor Kina Höglund
University of Gothenburg
Sahlgrenska Academy
Department of Psychiatry and Neurochemistry

Examination Board:
Associate Professor Homira Behbahani
Karolinska Institutet
Department of Neurobiology, Care Sciences and Society
Division of Neurogeriatrics

Associate Professor Anna-Lena Ström
Stockholm University
Department of Neurochemistry

Associate Professor Mia Lindskog
Karolinska Institutet
Department of Neurobiology, Care Sciences and Society
Division of Translational Alzheimer Neurobiology

ABSTRACT

Alzheimer's disease (AD) is the most common form of neurodegenerative disease that slowly impairs memory and thinking abilities. Abnormal accumulation and aggregation of amyloid β peptides (Aβ), derived from proteolytic cleavage of the amyloid precursor protein (APP), is thought to be one of the critical triggers in the pathogenesis. The trafficking and processing of APP is a complex process which can be regulated by many proteins. This thesis aims to expand our understanding of mechanisms that regulate processing and trafficking of APP.

Study I aims to characterize the synaptic distribution of APP. We found that under normal conditions APP primarily distributed as fragments at synapses. Deletion of BACE1 led to an accumulation of APP C-terminal epitopes at synapses where APP N-terminal epitopes are present. A similar co-localization between APP N-terminals and C-terminals was also induced by pharmacological BACE1 inhibition. Moreover, proximity ligation assay revealed that the accumulation of APP C-terminals occurred in the presynaptic compartment. These results suggest that APP is primarily delivered to synapses as cleaved fragments, while full-length APP can be transported to the presynaptic compartment under conditions of reduced BACE1 activity.

Study II aims to evaluate mechanisms for how exercise and BDNF regulate α-cleavage of APP. It has been suggested that regular exercise can reduce Aβ production. Moreover, exercise was suggested to enhance cognitive capacity, in which BDNF may contribute. We found that while the production of Aβ was reduced, the levels of sAPPα and BDNF were increased by exercise in a transgenic AD mouse model. Moreover, BDNF treatment was found to reduce Aβ production by regulating α-secretase activity. These results suggest that exercise and BDNF reduce Aβ production by enhancing α-secretase processing of APP.

Study III and **IV** involve two members from the protein family of sorting nexins (SNXs), which have diverse functions in protein sorting and trafficking.

Study III aims to elucidate the role of SNX3 in APP trafficking and processing as well as in Aβ production. In this study, the expression of SNX3 was manipulated by overexpression. We found that overexpression of SNX3 reduced the formation of Aβ. Moreover, SNX3 overexpression disturbed the association between APP and BACE1, and reduced APP internalization. While no change in BACE1 levels was found, APP levels were significantly decreased by SNX3 overexpression. These results suggest that overexpression of SNX3 decreases Aβ production by reducing internalization of APP.

Study IV aims to test the involvement of SNX7 in Aβ production and APP processing. We found that overexpression of SNX7 decreased the levels of APP derived fragments, including Aβ. The cell surface and steady-state levels of APP, but not BACE1 were decreased by SNX7 overexpression. Moreover, the reduction of steady-state levels of APP was prevented by lysosomal inhibitors. Taken together, these results suggest that overexpression of SNX7 reduces Aβ production by enhancing lysosomal degradation of APP.

In summary, we have examined mechanisms that regulate APP trafficking and processing, which may help develop novel therapeutic strategies for AD.

LIST OF SCIENTIFIC PAPERS

I. Saket M. Nigam, **Shaohua Xu**, Frauke Ackermann, Joshua A. Gregory, Johan Lundkvist, Urban Lendahl, Lennart Brodin.
Endogenous APP accumulates in synapses after BACE1 inhibition. *Neurosci Res. 2016 Aug;109:9-15*.

II. Saket M. Nigam, **Shaohua Xu**, Joanna S. Kritikou, Krisztina Marosi, Lennart Brodin, Mark P. Mattson.
Exercise and BDNF reduce Aβ production by enhancing α-secretase processing of APP. *J Neurochem. 2017 Jul;142(2):286-296*.

III. **Shaohua Xu**, Saket M. Nigam, Lennart Brodin.
Overexpression of SNX3 decreases amyloid-β peptide production by reducing internalization of amyloid precursor protein. *Manuscript*.

IV. **Shaohua Xu**, Lu Zhang, Lennart Brodin.
Overexpression of SNX7 reduces Aβ production by enhancing lysosomal degradation of APP. *Biochem Biophys Res Commun. 2017 Nov 2*.

CONTENTS

LIST OF ABBREVIATIONS

AD	Alzheimer's disease
ADAM	A disintegrin and metalloproteinase
ADP	Adenosine diphosphate
AICD	APP intracellular domain
AP-2	Adaptor related protein complex 2
APBA	APP binding family A
APLP	APP‑like protein
ApoE	Apolipoprotein E
APP	Amyloid precursor protein
ARF6	ADP ribosylation factor 6
Aβ	Amyloid β
BACE1	Beta-site APP cleaving enzyme 1
BAR	Bin-Amphiphysin-Rvs
BBS	α-bungarotoxin-binding site
BDNF	Brain derived neurotrophic factor
BiFC	Bimolecular fluorescence complementation
CHL1	Neural cell adhesion molecule L1-like protein
CTF	C-terminal fragment
ER	Endoplasmic reticulum
GGA	Golgi-localized, γ-adaptin ear-containing, ADP ribosylation factor binding protein
GTPase	Guanosine triphosphate hydrolase
HEK293T	Human embryonic kidney cells 293T
L1	L1 cell adhesion molecule
LDLR	Low density lipoprotein receptor
LRP	Low density lipoprotein receptor related protein
NADPH	Nicotinamide adenine dinucleotide phosphate
NMDA	N-Methyl-D-aspartate
NRG1	Neuregulin 1
PI3P	Phosphatidylinositol 3-phosphate

PI(3,4,5)P3	Phosphatidylinositol (3,4,5)-trisphosphate
PI(3,5)P2	Phosphatidylinositol 3,5-bisphosphate
PI(4,5)P2	Phosphatidylinositol 4,5-bisphosphate
PLA	Proximity ligation assay
PX	Phox homology
RA	Retinoic acid
SAP97	Synapse-associated protein 97; also known as discs large MAGUK scaffold protein 1 (DLG1)
SH3	Src Homology 3
SNX	Sorting nexin
SV2	Synaptic vesicle glycoprotein 2
TGN	*Trans*-Golgi network

1 INTRODUCTION

1.1 ALZHEIMER'S DISEASE (AD)

Alzheimer's disease (AD) is the most common form of dementia in the elderly that slowly impairs memory and thinking abilities. Since its first description in 1906 by Dr. Alois Alzheimer, many efforts have been placed on the investigation of underlying mechanisms and on the development of therapeutic approaches. However, the pathological mechanisms of AD remain incompletely understood and no treatment is so far available to cure the disease.

AD is mainly featured by two pathological hallmarks: amyloid plaques and neurofibrillary tangles, as well as by synapse loss [1, 2]. Amyloid plaques are extracellular deposits largely composed of amyloid β peptides (Aβ), and neurofibrillary tangles are intracellular accumulations of abnormally phosphorylated tau protein. The relationships between the pathological features and the underlying mechanisms of AD have been controversial, and several hypotheses have been proposed, such as the amyloid cascade hypothesis, tau hypothesis, oxidative stress hypothesis and inflammation hypothesis [3]. Although no single hypothesis can fully explain the pathogenesis of AD, the amyloid cascade hypothesis is the most renowned proposal.

In the amyloid cascade hypothesis, abnormal accumulation and aggregation of amyloid β peptides (Aβ) is thought to be a critical trigger in the pathogenesis of AD [4, 5]. Amyloid β (Aβ) is generated through sequential cleavage of the amyloid precursor protein (APP). Multiple Aβ peptides with different lengths have been described. The predominant peptide has 40 amino acids (Aβ40 or Aβ1-40), whereas Aβ42 is more prone to aggregate. Aβ can exist in several different assembly forms, of which three major groups are monomers, soluble oligomers, and insoluble fibrils [6]. The mechanism of Aβ aggregation is not fully understood, but it has been suggested that this process can be modulated by Aβ concentration, Aβ mutations and lengths, chemical modifications, lipid composition of membrane, and

cofactors such as the cholesterol carrier protein ApoE and metals [7, 8]. Soluble oligomers are believed to be the most toxic form, and they can induce numerous toxicities. For instance, it was found that Aβ oligomers can induce hyperphosphorylation of tau [9]. Tau is a microtubule associated protein which can stabilize the microtubule network. Abnormal hyperphosphorylation of tau promotes the protein to dissociate from the microtubules and to aggregate into neurofibrillary tangles [10]. Moreover, it has been suggested that Aβ oligomers can disrupt the function of a number of receptors, such as the NMDA receptor, the NGF receptor, and the cellular prion protein (PrPC), leading to synaptic dysfunction and neuronal death [11]. Synapses are specialized structures in neurons that propagate signals, and synapse loss correlates strongly with the cognitive decline in AD [12].

It is also important to note that Aβ, which is present at a low concentration in healthy individuals [13], is a physiological product. Aβ has been ascribed several physiological functions such as regulation of synaptic activity and involvement in memory formation [14, 15]. In AD, because Aβ accumulates at a much higher level, it is prone to aggregate into deleterious oligomers.

AD is a multifactorial brain disorder. It has been suggested that genetic, environmental, and life-style factors can contribute to the development of disease. Inherited familial AD has been mainly linked with three genes: APP, PSEN1 and PSEN2; and an additional copy of chromosome 21, where the APP gene resides, can cause AD type pathology. In addition, several genetic risk factors have been linked with AD, including ApoE ε4 [16]. Exercise, among other life-style factors, was found to reduce the risk of developing AD [17]. Moreover, it has been suggested that exercise can enhance cognitive capacity by increasing the mRNA and protein levels of BDNF (brain derived neurotrophic factor), a neurotrophic protein which is involved in the regulation of long-term potentiation and synaptic plasticity [18].

1.2 AMYLOID PRECURSOR PROTEIN (APP)

Amyloid precursor protein (APP), together with APP-like proteins (APLPs) APLP1 and APLP2, belongs to an evolutionarily conserved protein family. These proteins have a similar domain structure, and share partially overlapping functions. They can also exhibit distinct functions: by analyzing different knockout mice (single knockout, double knockout, triple knockout), it has become clear that loss-of-function in one gene can not be fully compensated by others [19]. Notably, among them APP is the only one that can give rise to Aβ.

APP is a type I transmembrane protein with multiple functional domains. It has a large extracellular region, in which several domains have been described to be implicated in functions such as dimerization and cell adhesion [20]. In the short cytoplasmic tail of APP, there is a YENPTY motif which contributes to the internalization of APP and also to interactions with adaptor proteins [2]. APP has multiple isoforms, the three major ones being APP_{695}, APP_{751} and APP_{770}. APP_{695} is the one that is predominantly expressed in neurons [2]. APP can undergo sequential cleavages to produce several different APP fragments (see section 1.2.1).

In addition to the functions mediated by its fragments, APP, as a full-length protein, has been ascribed a number of functions. It was found that APP at the cell surface can form *trans*-dimers with APP or its homologs to promote cell-cell adhesion [21], suggesting that full-length APP may serve as a cell adhesion molecule. It has been proposed that APP may resemble a cell surface receptor [22, 23], and that the potential transsynaptic interaction between presynaptic and postsynaptic APP may promote synaptogenesis [24]. Moreover, fibrillar Aβ can bind to the cell surface APP [25], and the homodimerization of full-length APP can be induced by soluble Aβ [26], indicating that full-length APP may contribute to the signaling of Aβ toxicity. These findings suggest that APP can exhibit certain important functions as a full-length protein. However, its physiological functions largely remain unclear.

1.2.1 APP processing

APP can undergo sequential cleavages in two routes: the non-amyloidogenic pathway and the amyloidogenic pathway [27, 28]. APP processing mainly involves three secretases: α-, β, and γ-secretase. As shown in Fig. 1, in the non-amyloidogenic pathway, APP is initially cleaved by α-secretase within the extracellular region to release a soluble N-terminal fragment, sAPPα; the remaining membrane-tethered C-terminal fragment, CTFα, can be further cleaved by γ-secretase to produce P3 peptides and an APP intracellular domain (AICD). Alternatively, in the amyloidogenic pathway, APP cleavage by β-secretase yields sAPPβ and CTFβ, the latter subsequently being cleaved by γ-secretase to generate Aβ and AICD.

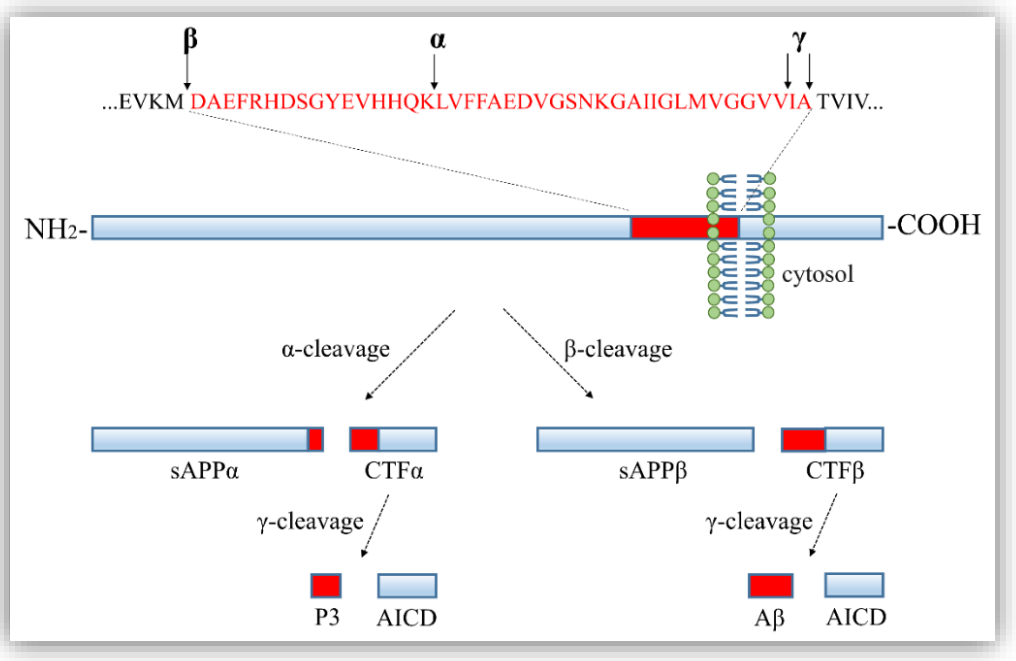


Fig. 1. Schematic diagram of APP processing. APP is a single-pass transmembrane protein with a large extracellular region and a short cytoplasmic tail. APP processing by α-, β-, and γ-secretase can occur in two routes to generate different APP derived fragments [27, 28]. The Aβ region is highlighted in red, and the secretase cleavage sites are shown along with the amino acid sequence of Aβ.

1.2.2 APP derived fragments

sAPPα and sAPPβ

sAPPα is generally thought to be beneficial to neurons [29]. It was found that sAPPα can facilitate neuritogenesis and LTP (long-term potentiation) [30]. Moreover, it has been shown that sAPPα can attenuate most of the deficits of APP knockout mice [31]. It may also be involved in cell proliferation [29, 30] and some other functions [32]. Recently it was found that sAPPα can inhibit β-cleavage of APP and Aβ production [33], suggesting that the neuroprotective functions of sAPPα may be partially dependent on pathways which are controlled by the APP β-cleaved fragments. sAPPβ is short of the C-terminal 16 amino acids in sAPPα. Similar to sAPPα it may also exhibit neuroprotective functions, but the effect is much less prominent [30] and may even be absent [29].

CTFα and CTFβ

Both CTFα and CTFβ are intermediate products during APP processing. CTFα can be derived by α-cleavage of APP as well as of CTFβ [34]. At present, the physiological function of CTFα is not clear. CTFβ was suggested to be neurotoxic [35]. In addition to being the precursor of Aβ, it has been implicated in endocytic dysfunction [36] and lysosomal pathology [37] independent of Aβ.

P3 and AICD

Possibly P3 can exhibit certain toxicity [38], but its physiological functions remain to be explored. AICD is considered to be involved in the regulation of transcriptional activity, in which the YENPTY motif and interactions with adaptor proteins may be essential [35, 39]. Importantly, it has been suggested that AICD targets genes including APP and BACE1 [39], suggesting that there may be a feedback mechanism for the regulation of APP processing.

1.2.3 Subcellular trafficking of APP

APP trafficking is a complex process which is spatially and temporally regulated [28, 40-43]. After synthesis and initial modifications in the ER (endoplasmic reticulum), APP is transported to the Golgi apparatus and *trans*-Golgi network (TGN) where it undergoes full maturation involving multiple posttranslational modifications. A fraction of APP is delivered from the TGN to the plasma membrane where α-cleavage can take place. Non-cleaved APP is internalized via clathrin-mediated endocytosis to endosomes where β-cleavage predominantly occurs. From endosomes, remaining APP can be sorted into three routes [42]: retrograde transport to the TGN, recycling back to the cell surface, and delivery to lysosomes.

The retrograde transport of APP can be regulated by many proteins, one significant factor of which is the retromer complex. The retromer is a multiprotein complex that regulates protein retrieval from endosomes to the TGN. Interestingly, it has also been implicated in protein recycling back to the cell surface [44]. It is believed that in mammals the retromer complex contains a cargo recognition subunit composed of VPS26, VPS29 and VPS35, and a SNX dimer subunit formed by different dimeric combinations of SNX1 or SNX2 with SNX5 or SNX6 [45]. In addition, at least two alternative retromer complexes may exist: SNX27-retromer and SNX3-retromer [44, 46]. It has been found that these retromers are involved in retrograde trafficking of different cargos [46], and some of them have been implicated in APP trafficking [47, 48]. The recycling of APP back to the cell surface is not fully understood, and recently it was found that SNX15, in addition to SNX27 [47], may be involved in this process [49]. The degradation of APP occurs in lysosomes [50].

The trafficking of APP can be regulated by many other proteins, such as proteins in the Rab GTPase family, in the LDLR (low density lipoprotein receptor) family and in the APBA/MINT family [41, 43]. Importantly, several SNXs (sorting nexins) have been implicated in APP trafficking by different mechanisms (see section 1.4.4).

1.3 SECRETASES FOR APP

1.3.1 α-secretase

It was proposed that in neurons the α-secretase cleavage of APP is constitutively executed by ADAM10 [51], a member of the ADAM (A disintegrin and metalloproteinase) family [52]. In addition, α-secretase cleavage of APP may be induced above the basal level by stimuli, which is referred to as regulated α-secretase cleavage [53]. Although not fully examined, several ADAMs have been implicated in regulated α-secretase cleavage. For instance, ADAM17 was found to contribute to the phorbol ester PMA stimulated APP α-cleavage [54]; and interestingly ADAM10 has also been involved in regulated APP α-cleavage by stimulation with the neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) [55], and the drug acitretin [56]. The above findings indicate that ADAM10 plays a significant role in the α-secretase cleavage of APP. It has been suggested that regulated α-secretase cleavage of APP primarily occurs in the TGN, while constitutive α-cleavage takes place at the cell surface [57-59].

Although little is known about the intracellular trafficking of ADAM10, several proteins have been found to be involved in α-cleavage of APP. For instance, SAP97 (synapse-associated protein 97) was found to regulate ADAM10 trafficking and α-secretase activity by a protein interaction through its SH3 (Src Homology 3) domain [60]. Moreover, it was revealed that the intracellular region of ADAM10 can interact with many SH3 domain-containing proteins, such as SNXs and PACSINs [61], suggesting that these proteins may also influence ADAM10-medicated cleavage. Indeed, for example, one of these proteins, SNX33, was found to promote α-cleavage of APP [62]. However, the functions of these proteins in ADAM10 trafficking and in ADAM10 cleavage of APP remain to be explored.

1.3.2 β-secretase

With its β-secretase activity first reported in 1999 [63-66], BACE1 is now widely accepted as the principal β-secretase in the brain. BACE1 is a type I transmembrane aspartic protease with multiple domains including a cytosolic domain which contains an acidic cluster-dileucine (ACDL) motif (D*XX*LL, where X denotes a nonconserved residue).

After synthesis BACE1 is delivered to the plasma membrane. BACE1 can be internalized from the cell surface to early endosomes, and from endosomes it can undergo retrograde transport to the TGN, recycling to the cell surface, and delivery to lysosomes [67].

It was found that internalization of BACE1 to early endosomes is controlled by the small GTPase ARF6 (ADP ribosylation factor 6) [68], which is clathrin-independent. Moreover, it has been proposed that BACE1 internalization can also occur through clathrin/AP-2-dependent endocytosis [69, 70]. The ACDL in BACE1 (DISLL), which has been implicated in the internalization of BACE1 [71], appears to be mainly responsible for the association between BACE1 and the AP-2 complex [69]. In both the above cases, the dileucine motif was revealed to be essential in targeting BACE1 to early endosomes [68-70]. In addition, the ACDL motif was found to be involved in the interactions with the VHS domains in GGAs (Golgi-localized, γ-adaptin ear-containing, ADP ribosylation factor binding proteins) [72], which are a group of clathrin adaptors implicated in protein trafficking between endosomes and the TGN [73]. Therefore, it is not surprising that the retrograde transport of BACE1 can be regulated by GGAs [74, 75]. The associations between the dileucine motif and GGAs are enhanced by phosphorylation on the serine residue in ACDL (DISLL) [72, 76]. And it was suggested that phosphorylation on the ACDL motif can promote the retrograde transport of BACE1, whereas non-phosphorylated BACE1 is sorted to the plasma membrane [74]. Recycling of BACE1 back to the cell surface was recently found to be regulated by the GTPase Rab11 [77]. It is thought that the degradation of BACE1 can occur via the lysosomal

pathway [78] as well as the proteasomal pathway [79]. It is important to note that the trafficking of BACE1 involves many other proteins, including SNXs. It has been shown that SNX6, SNX12 and SNX4 can regulate BACE1 trafficking by different mechanisms (see section 1.4.4).

The activity of β-secretase was found to be increased in the AD brain [80]. Given that BACE1 cleavage of APP is the initial and putatively rate-limiting step in Aβ production [81], it has been viewed as a prime drug target for AD therapy. However, besides APP, BACE1 has numerous substrates [82], including CHL1 (neural cell adhesion molecule L1-like protein), NRG1 (neuregulin 1) and SCN2B (sodium channel subunit β-2), suggesting that it can display many different physiological functions by processing its substrates. Indeed, it has been revealed that $BACE1^{(-/-)}$ mice exhibit complex neurological defects, such as axon guidance defects, hypomyelination and seizures, in which different BACE1 substrates may be involved [83, 84].

BACE1 activity has an acidic pH optimum, and it is primarily localized in acidic intracellular organelles, such as TGN and endosomes [84]. Endosomes are considered to be the site where β-cleavage of APP mainly takes place [77, 85]. Moreover, BACE1 cleavage of APP, but not NRG1 and L1 whose cleavage appears to be endocytosis-independent, was found to be selectively inhibited by an endosomally targeted β-secretase inhibitor [85]. Therefore, specifically targeting BACE1 activity in endosomes could be a promising therapeutic strategy.

After the identification of BACE1, a homolog, BACE2 [86], was described. BACE2 has a low neuronal expression and preferred cleavage site within the Aβ sequence, making it unlikely to be a major β-secretase in the brain [83]. Instead, it was suggested to be involved in Down syndrome (DS) given that the gene is located on chromosome 21 and its expression is increased in DS patients [84].

1.3.3 γ-secretase

The γ-secretase is a multiprotein complex which is composed of presenilin (PSEN1 or PSEN2), nicastrin (NCT), anterior pharynx defective-1 (APH-1), and presenilin enhancer-2 (PEN-2) [87]. The assembly, maturation and trafficking of γ-secretase is a very complex process [88, 89].

γ-secretase has been shown to be a challenging drug target for AD therapy in that it has a wide range of substrates [90]. APP can be cleaved by γ-secretase at multiple sites to generate Aβ species with different C-terminals, ranging in length from 34 to 43 residues. Another significant substrate is the Notch protein, which has been implicated in numerous developmental and homeostatic processes [91]. Clinical trials have shown that γ-secretase inhibitors can cause significant side effects [92], possibly due to interference of cleavage of non-APP substrates, such as Notch. The activity of γ-secretase can be regulated by many proteins [93], one of them is the γ-secretase activating protein (GSAP) [94]. It was found that GSAP can influence Aβ production without affecting the cleavage of Notch [94]. These results indicate that γ-secretase may cleave its substrates by different mechanisms, suggesting that γ-cleavage of APP can be selectively modulated. Indeed, it was reported that some compounds can selectively inhibit the activity of γ-secretase in Aβ production while leaving the proteolysis of Notch intact [95]. Moreover, it was found that a class of compounds, referred to as the γ-secretase modulators, can shift Aβ production from Aβ42 to less pathogenic forms without changing the total amount of Aβ [93, 96]. It has been proposed that two strategies based on modification of the γ-secretase activity can be applied to develop treatments for AD [59]: 1) to develop γ-secretase inhibitors which are substrate-selective for APP; and 2) to develop efficient γ-secretase modulators.

1.3.4 Interplay of the α-, β-, and γ-secretase

γ-cleavage occurs following α- or β- cleavage of APP. However, the activities of α-, β-, and γ-secretase are not absolutely separated. It was revealed that in neurons inhibition of BACE1 is coupled with increased ADAM10 cleavage of APP [97]. ADAM10 was found to interact with γ-secretase [98]. Moreover, it has been shown that NCT and PSEN1 can bind to BACE1, and possibly regulate its maturation and activity [99-101]. But no interaction between ADAM10 and BACE1 was found [98]. These findings suggest that α- and β-secretase can form different complexes with γ-secretase to regulate different sequential cleavages of APP.

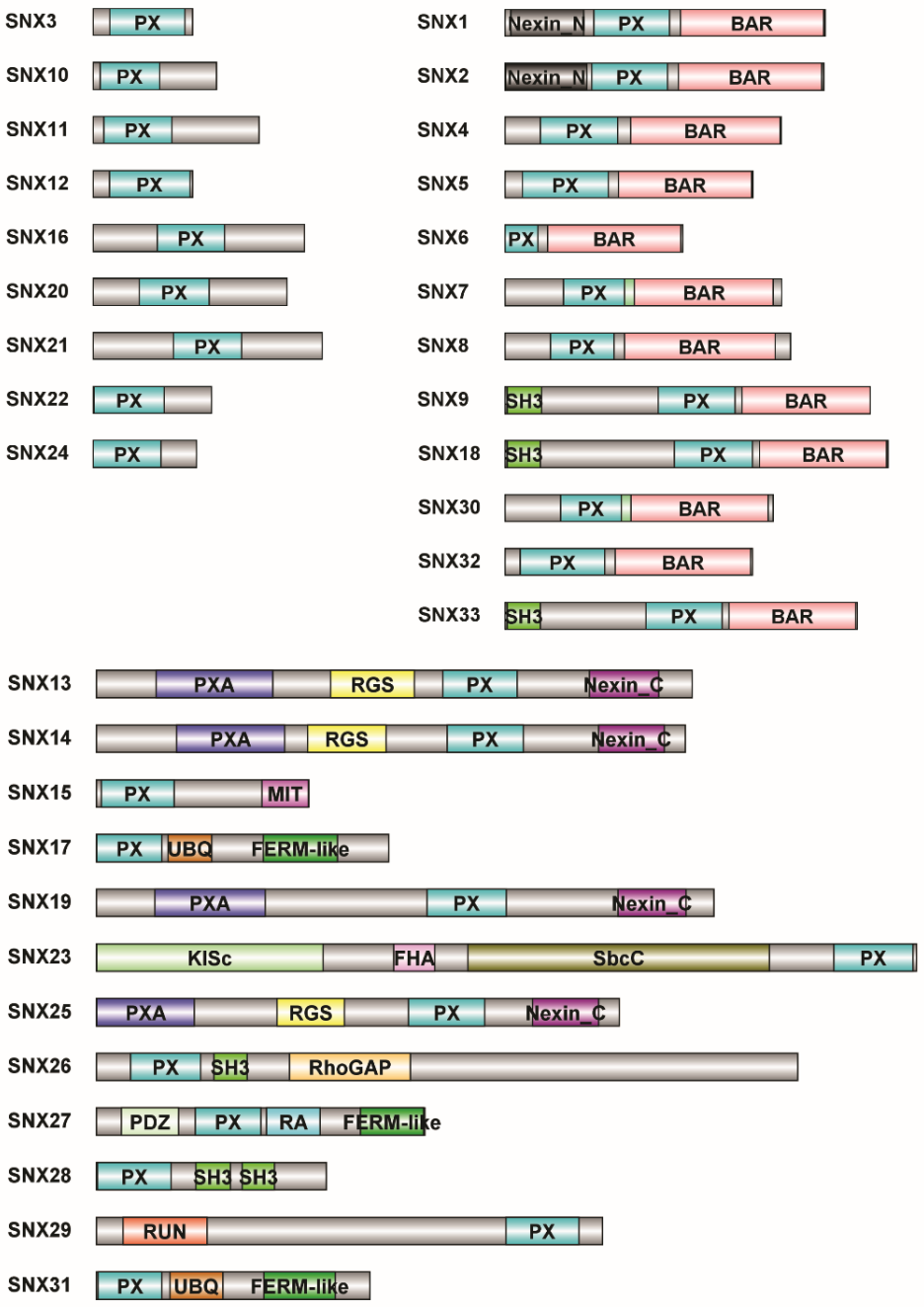
Therefore, it may be a good strategy to target the association between secretases while leaving the secretases activity unimpaired. Indeed, it has been reported that Aβ production and Aβ-related pathology can be reduced by blockade of the interaction between BACE1 and PSEN1 [102]. In addition, the association between APP and its secretases is an important prerequisite for APP cleavage, which may also serve as a target. Taken together, the associations between the secretases and secretase/substrate may represent a promising target for AD therapy.

Although not discussed in this thesis, it is interesting to note that there may be additional secretases for APP processing, such as the η-secretase [103] and the δ-secretase (asparagine endopeptidase) [104] which have been described recently.

1.4 SORTING NEXINS (SNXS)

Sorting nexins (SNXs) are a family of evolutionarily conserved proteins which are characterized by the presence of a PX (phox homology) domain. The PX domain was first described in $p47^{phox}$ and $p40^{phox}$, two subunits of the NADPH oxidase (nicotinamide adenine dinucleotide phosphate-oxidase) complex [105]. It is now clear that the PX domain is also present in many other proteins, including members of the PLD (phospholipase D) superfamily, CISK (cytokine-independent survival kinase), and SNXs [106]. The PX domain, approximately 120 residues long, is a phosphoinositide-binding module [106]. It has been shown that the PX domain primarily interacts with PI3P, but can also bind to other phospholipids, such as PI(3,5)P2, PI(4,5)P2 and PI(3,4,5)P3 [107]. The different phosphoinositide-binding properties possibly allow targeting of PX domain-bearing proteins to distinct cellular membranes. Moreover, the PX domain has been implicated in interactions with other domains and proteins [108], suggesting that it may coordinate membrane recognition and protein complex assembly. Among the mammalian PX domain-bearing proteins, a group of homologous proteins are referred to as SNXs.

The original family member of SNXs, SNX1, was identified in 1996 [109] and since then more than 30 putative human SNXs have been described. SNXs have been implicated in diverse functions, many of which relate to vesicular trafficking and protein sorting [110]. Based on structural similarities, SNXs can be divided into three subfamilies [111]: 1) PX-only SNXs; 2) PX-BAR SNXs; and 3) PX-other SNXs (Fig. 2).



PX: Phosphoinositide binding Phox Homology domain
BAR: Bin/Amphiphysin/Rvs domain
FERM-like: band4.1/ezrin/radixin/moesin (FERM)-like domain
FHA: Forkhead-associated domain
KISc: Kinesin motor, catalytic domain
MIT: Domain contained within Microtubule Interacting and Trafficking molecules
Nexin_C: Sorting nexin C terminal
Nexin_N: Sorting nexin, N-terminal domain
PDZ: PSD 95/discs large/zonus occludens1
PXA: Domain associated with PX domains
RA: RAS-associated domain
RGS: Regulator of G protein signaling domain
RhoGAP: RhoGAP (GTPase-activator protein [GAP] for Rho-like small GTPases) domain
SbcC: DNA repair exonuclease SbcCD ATPase subunit
SH3: Src Homology 3 domain
UBQ: Ubiquitin-like proteins

Fig. 2. Schematic diagram of the domain organization of putative human SNXs. Domain structures of 33 putative human SNXs. SNXs are divided into three subfamilies [111]: 1) PX-only SNXs (upper left); 2) PX-BAR SNXs (upper right); and 3) PX-other SNXs (lower group).

1.4.1 PX-only SNXs

Proteins in this subfamily have only a PX domain without any other recognized domains. They are SNX3, SNX10, SNX11, SNX12, SNX16, SNX20, SNX21, SNX22 and SNX24.

SNX10 and SNX11

SNX10 was found to selectively interact with PI3P [112]. It can induce the formation of giant vacuoles in multiple mammalian cells, suggesting a fundamental role in the regulation of endosome homeostasis [113]. SNX11 can interact with PI3P and PI(3,5)P2 [114], and it has been implicated in protein trafficking from the plasma membrane to lysosomes [115].

SNX16

Although preferentially binding to PI3P [116], SNX16 was found to widely distribute in recycling endosomes, early endosomes, late endosomes and lysosomes [116, 117]. It has been shown that SNX16 is implicated in protein targeting to the lysosome [117] and recycling to the plasma membrane [118]. It is also involved in the regulation of late endosome dynamics [119] and synaptic growth signaling [120]. Moreover, SNX16 was proposed to be a negative regulator in cell migration and tumorigenesis [121].

SNX20, SNX21, SNX22 and SNX24

The phospholipid-binding property of SNX20 is elusive, in that some researchers showed its interactions with PI4P, PI5P and PI(3,5)P2 [122] whereas others indicated its bindings to PI3P and PI(4,5)P2 [123]. It was found that SNX20 can directly interact with the glycoprotein PSGL-1 and affect its subcellular localization [122]. The function of SNX21 is poorly studied, although its predominant expression in liver suggests an involvement in the development of this organ [124]. SNX22 was found to be involved in the CD4 downregulation by HIV-1 in T cells [125], while SNX24 was shown to be a regulator of vascular inflammation [126].

1.4.2 PX-BAR SNXs

SNXs in this subfamily have, in addition to a PX domain, a C-terminal BAR domain, which can sense and induce membrane curvature [127, 128]. This subfamily includes SNX1, SNX2, SNX4, SNX5, SNX6, SNX7, SNX8, SNX9, SNX18, SNX30, SNX32, and SNX33.

SNX1, SNX2, SNX5 and SNX6

SNX1 was first identified in a yeast two-hybrid screen for the binding partners of EGFR [109]. It has an N-terminal Nexin_N domain which appears to be specific to SNX1 and SNX2. SNX1 and SNX2 share some redundant functions [129], but they can play distinct roles in protein trafficking [130]. SNX5 was found to interact with SNX1 [131], and SNX6 can form heterodimers with SNX1 and SNX2 [132], suggesting that these proteins may coordinately regulate intracellular protein trafficking. SNX1, SNX2, SNX5 and SNX6 have a variety of functions, one of which is that they control protein transport by serving as subunits of the retromer complex [45].

SNX8

SNX8 was suggested to regulate retrograde protein transport [133], and cholesterol homeostasis [134]. Its C. elegans homolog was found to be a modifier of Aβ toxicity [135]. However, whether human SNX8 can exhibit similar effects, and if so by which mechanisms remain unknown.

SNX9, SNX18 and SNX33

Besides a PX and a BAR domain, these three SNXs have an additional N-terminal SH3 domain, which is a protein interaction domain that can bind proline-rich ligands.

SNX9 was found to have a variety of interaction proteins, including the AP-2 complex, clathrin and dynamin, and was suggested to play an important role in clathrin-mediated endocytosis [136-138]. SNX9 was also shown to be involved in the regulation of actin

assembly and membrane remodeling by its interactions with N-WASP and the Arp2/3 complex [139]. Moreover, it has been implicated in cell invasion and cell division [140].

Like SNX9, SNX18 can interact with dynamin and N-WASP [141], and was found to be involved in membrane remodeling [142]. In addition, SNX18 was shown to have an important role in the formation of autophagosomes [143]. SNX33 has been implicated in endocytosis and shedding of the cellular prion protein [144].

SNX9, SNX18 and SNX33 may share some redundant functions, as evidenced by that SNX9 and SNX18 can compensate the loss-of-function of each other in clathrin-mediated endocytosis [141]. However, they also have irreplaceable functions, as supported by, for example, that all of them are essential for cell progression and completion of mitosis [145].

SNX30 and SNX32

The functions of SNX30 and SNX32 are presently unclear.

1.4.3 PX-other SNXs

The rest of the SNXs are grouped into the PX-other subfamily, and they contain different domains (other than BAR domains) in addition to a PX domain. This subfamily consists of 12 members: SNX13, SNX14, SNX15, SNX17, SNX19, SNX23, SNX25, SNX26, SNX27, SNX28, SNX29 and SNX31.

SNX13, SNX14, SNX19 and SNX25

These SNXs have a PX domain, a PX associated domain (PXA) and a conserved C-terminal region (Nexin_C); an additional Regulator of G protein signaling (RGS) domain was identified in SNX13, SNX14 and SNX25. The functions of PXA and Nexin_C domains are unclear, and some RGS proteins have been implicated in G protein signaling by serving as GTPase activating proteins [146]. The domain organization of SNX13, SNX14 and SNX25 suggests that they may provide a link between protein sorting and G protein signaling.

The PX domain in SNX13 can bind to PI3P [147]. SNX13 can interact with the G protein subunit Gαs and regulate Gαs-mediated signaling [148], and it has been implicated in lysosomal protein degradation [148, 149]. In addition, SNX13 was found to play an essential role in embryonic development [150].

The PX domain in SNX14 mainly associates with PI(3,5)P2 [151], but not with PI3P [147]. SNX14 was found to be localized in lysosomes, suggesting that it plays a role in the regulation of lysosomal function. Indeed, it was shown that mutations in SNX14 were associated with lysosomal dysfunction [151]. Moreover, SNX14 has been implicated in the regulation of normal neuronal excitability and synaptic transmission [152], and genetically linked with intellectual disability [151, 153].

SNX19 has broad phospholipid-binding properties [154] and it has been suggested to be involved in the regulation of lysosomal function and formation of membrane vesicles [155].

Furthermore, it was found that SNX19 is implicated in the regulation of cell proliferation [156] and apoptosis [154].

SNX25 has been implicated in lysosomal degradation [157], and its expression was found to be increased in temporal lobe epilepsy [158]. Interestingly, SNX25 was suggested to be associated with age at onset in AD [159], but its functions remain largely unknown.

SNX23

SNX23 is also known as kinesin-like protein KIF16B. Transport of membranous vesicles and organelles along microtubules is driven by motor proteins, one type of which is the kinesin superfamily proteins (KIF). SNX23 is a member of the kinesin superfamily, and has been implicated in the relocation of early endosomes [160, 161], possibly by direct interaction between the PX domain and early endosome-enriched PI3P [162].

SNX26

SNX26 is also known as Rho GTPase-activating protein 33. Rho GTPase-activating (GAP) proteins can inactivate Rho GTPases by inducing GTP hydrolysis. SNX33 exhibits GAP activity in vitro [163], suggesting it may play a role in Rho GTPase signaling. SNX26 was found to be involved in the trafficking of the BDNF receptor TrkB [164]. Moreover, it can interact with PSD-95 [165], and has been implicated in neurite outgrowth [166] and synapse development [167].

SNX28

SNX28 is also known as NADPH oxidase organizer 1 (NOXO1), which is a regulatory subunit of the NADPH oxidase complex. NADPH oxidase (NOX) is an important source of reactive oxygen species (ROS). Human NOX has seven members: NOX1, NOX2, NOX3, NOX4, NOX5, as well as two dual oxidases DUOX1 and DUOX2 [168]. Each member is different in their membrane-bound and cytosolic regulatory subunits, which are composed of

p22phox, p40phox, p47phox, p67phox, Rac, NOXA1, and NOXO1 [169]. It was found that NOXO1 can induce the activity of NOX1 [170] and NOX3 [171]. The PX domain in NOXO1 can interact with PI4P, PI5P and PI(3,5)P2, and is involved in the activation of NOX1 [172].

SNX29

SNX29 is also known as RUN domain containing 2A (RUNDC2A). It has, in addition to a PX domain, an N-terminal RUN domain. Many RUN domain proteins have been proposed to be involved in Ras-like GTPase signaling and vesicle traffic [173, 174]. Although SNX29 has been associated with some diseases including schizophrenia [175], its function remains to be explored.

SNX31

SNX31, as well as SNX17 and SNX27, has a C-terminal 4.1/ezrin/radixin/moesin (FERM)-like domain. A Ras GTPase-binding property has been attributed to the FERM-line domain, with which many proteins were proposed as adaptors in the endosomal trafficking and signaling [176, 177]. SNX31 preferentially binds to PI3P [178], and has been implicated in protein distribution at the cell surface [179].

1.4.4 SNXs in APP trafficking and processing

An association between SNXs and APP processing was first supported by the observation that SNX17 regulates APP turnover. Subsequently, several studies have linked other SNXs with different aspects of APP processing.

SNX17 and SNX15

SNX17 was identified as an intracellular adaptor for APP [180]. It has been found that SNX17 can interact with the NP*X*Y motif of several proteins, including LDLR [181] and LRP [182]. Therefore, it is not surprising that SNX17 was found to directly bind to the Y*X*NP*X*Y motif of the APP cytoplasmic domain. Moreover, it was shown to colocalize with APP in early endosomes [180]. The gene expression of SNX17 was found to be decreased in an established APP transgenic mouse model [183], and it was revealed that SNX17, which can regulate Aβ production, influences APP turnover [180].

SNX15 is widely expressed in rodent tissues [49]. It has been shown that SNX15 regulates the morphology of several endosomal compartments [184]. SNX15 can directly bind to clathrin [185], and it has been implicated in endocytosis of several proteins, including transferrin [184]. Unlike SNX17, SNX15 was shown to regulate Aβ generation by influencing APP recycling back to the cell surface [49].

SNX33

SNX33, which primarily promotes α-cleavage, inhibits the endocytosis and increases the cell surface levels of APP [62]. These results suggest that SNX33 may facilitate APP α-cleavage by increasing the association between APP and α-secretase at the cell surface. SNX9, a homolog of SNX33, was shown to also increase α-cleavage of APP [62]. However, it is also important to note that SNX33 knockdown did not alter APP cleavage, possibly due to compensatory functions of the homologous SNX9 and SNX18, as proposed in the study.

Although SNX33 can influence APP endocytosis and shedding, it is unclear whether Aβ production is altered.

SNX6, SNX12 and SNX4

SNX6, which can reduce the production of sAPPβ and Aβ, was demonstrated to serve as a negative regulator of the retrograde transport of BACE1 [186]. SNX6 can directly interact with BACE1, and it has been suggested as a component of the multiprotein retromer complex [187]. Therefore, it is possible that SNX6 controls the retrograde transport of BACE1 by linking it to the retromer complex.

Compared to SNX6, SNX12, which can also interact with BACE1, regulates Aβ production by a different mechanism in that SNX12 influences the endocytosis of BACE1 [188].

SNX4 was shown to be a positive regulator of Aβ production by influencing BACE1 degradation [189]. It can interact with BACE1, and direct BACE1 away from the lysosomal degradation pathway, thereby increasing the half-life and steady-state levels of BACE1. Moreover, SNX4 can also increase BACE1 levels at the plasma membrane. It has been previously reported that SNX4 can interact with clathrin [190] and is involved in endocytosis [191]. But it remains unclear whether the effect of SNX4 on BACE1 also involves the process of endocytosis. Interestingly, SNX4 protein levels were found to be reduced in brain samples of AD patients [189], which may reflect a rescue strategy to reduce Aβ production. However, the mechanisms that regulate SNX4 levels in AD remain unknown.

The above results suggest that different SNXs may control different steps of BACE1 intracellular trafficking.

SNX27

SNX27 is distinct from other SNXs in that it bears a unique PDZ (postsynaptic density 95/discs large/zonus occludens 1) domain, which is a protein interaction module present in

many scaffold proteins [192]. SNX27 was suggested to be a subunit of the SNX27-retromer complex [193]. It has been mainly involved in endosome-to-cell surface protein trafficking [193, 194]. Moreover, SNX27 has recently been suggested to be implicated in endocytosis [195]. It has been shown that SNX27, which reduces Aβ production, influences the assembly and activity of γ-secretase complex by interaction with PSEN1 [196]. Furthermore, it was demonstrated to affect APP cleavage by regulating the endosome-to-cell surface recycling of APP [47]. These studies suggest that SNX27 can affect APP processing by multiple mechanisms.

SNX3 and SNX7

SNX3 is primarily localized in early endosomes. SNX3 can interact with VPS35 [197, 198], and clathrin [190], suggesting that it may be involved in several stages of endocytic trafficking. Indeed, SNX3 has been shown to be able to prevent protein delivery to lysosomes [199, 200], and to enhance protein transport to recycling endosomes [199] and the TGN [200]. It may also participate in endocytosis as it can regulate cell surface protein levels [197, 199, 201]. SNX3 mRNA is expressed in several vulnerable brain regions in AD, such as the cerebral cortex and hippocampus [202]. Moreover, SNX3 was found to be enriched in the rodent postsynaptic density fraction [203], and it has been implicated in neurite outgrowth [204], a process that is compromised in AD [205]. In addition, it was found that single nucleotide polymorphisms (SNPs) in SNX3 are associated with AD [206].

SNX7 preferentially interacts with PI3P [199]. It was reported that SNX7 downregulation can induce the activation of caspase 8 [207], an alteration observed in AD brain [208]. Moreover, it has been shown that SNX7 is genetically associated with AD [209], and expression of the SNX7 gene was found to be decreased in AD subjects [210].

It becomes clear that SNXs have emerged as key regulators in APP processing and trafficking. SNX3 and SNX7 have been linked with AD, but whether they can influence APP

processing and Aβ production remains unknown. In Study III and IV, we have examined the roles of SNX3 and SNX7 at the cellular level.

2 AIMS

The general aim of this thesis is to characterize the synaptic distribution of APP, and to investigate mechanisms that regulate APP trafficking and processing.

Specific aims of the constituent papers are:

1) to examine the fate of APP at synapses.

2) to evaluate mechanisms for how exercise and BDNF regulate α-cleavage of APP.

3) to elucidate the role of SNX3 in APP trafficking and processing as well as in Aβ production.

4) to test the involvement of SNX7 in Aβ production and APP processing.

3 RESULTS AND DISCUSSION

3.1 ENDOGENOUS APP ACCUMULATES IN SYNAPSES AFTER BACE1 INHIBITION (PAPER I)

Synapse loss is one of the key features in AD which correlates strongly with the cognitive decline in this disease [12]. Although the exact mechanism for the synaptic deficits is unclear, accumulating evidence indicates that Aβ derived from the proteolytic cleavage of APP plays a critical role [5]. APP has a number of functions in addition to giving rise to Aβ. It has been proposed that APP, as a full-length protein, is implicated in several synaptic functions, such as synaptogenesis [2, 24]. While the trafficking and processing of APP has been extensively studied in non-neuronal cells, the fate of APP in neurons and especially at synapses remains incompletely understood. The major β-secretase, BACE1, is an initial and putatively rate-limiting enzyme in Aβ production. In study I, we combined immunocytochemistry and perturbation of BACE1 activity to examine the synaptic distribution of APP.

Using antibodies directed to APP N-terminal epitopes (N-t1 and N-t2) and C-terminal epitopes (C-t1 and C-t2), we performed immunocytochemical characterization of APP on primary hippocampal neurons. In neurons derived from wild-type ($BACE1^{(+/+)}$) mice, APP N-terminal labeling predominantly overlapped with synaptic markers, whereas the APP C-terminal labeling was largely separated from synapses, suggesting that APP primarily exists as fragments at synapses under normal conditions (Fig. 3). However, in neurons from $BACE1^{(-/-)}$ mice, while the N-terminal labeling remained similar to that in $BACE1^{(+/+)}$ neurons, C-terminal labeling showed a significant increase of co-localization with synaptic markers. These results suggest that more APP may distribute as a full-length protein at synapses when the activity of BACE1 is deficient. This idea was supported by the results that full-length APP levels were increased in the brains from $BACE1^{(-/-)}$ mice as compared to those from $BACE1^{(+/+)}$ mice.

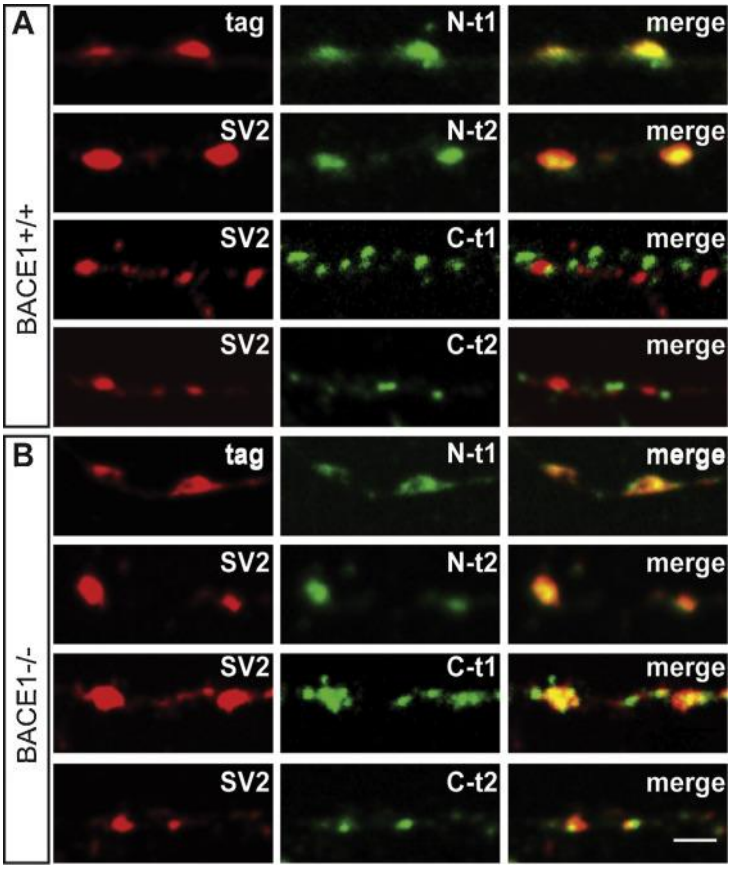


Fig. 3. Deletion of BACE1 leads to co-accumulation of APP N-terminal and C-terminal epitopes in synaptic regions [211]. (A, B) Double staining was performed with synaptotagmin (tag) or SV2 antibodies and antibodies against the N-terminus of APP (N-t1, N-t2, upper panels) and C-terminus of APP (C-t1, C-t2, lower panels) on primary hippocampal neurons from BACE1$^{(+/+)}$ mice (A) and in BACE1$^{(-/-)}$ mice (B). Scale bar = 2 μm.

To confirm these results, we applied a pharmacological approach to inhibit BACE1 activity in rat hippocampal neurons. Similarly, APP N-terminal labeling showed a strong co-localization with synaptic markers under both normal and the reduced BACE1 activity conditions. BACE1 inhibition led to a significant increase of the synaptic distribution of APP C-terminal epitopes, and thus more co-localization was found between the APP N-terminals and C-terminals after BACE1 inhibition. Taken together, these results indicate that full-length APP accumulates at synapses after inhibition of BACE1.

To examine whether the above accumulation can occur in the presynaptic compartment, we took advantage of proximity ligation assay (PLA) which has a spatial resolution of about 40 nm. Antibodies against the APP C-terminal and the synaptic vesicle marker SV2 were used for detection. A significant increase in PLA signals was found after BACE1 inhibition, suggesting that the above accumulation takes place in the presynaptic compartment [211].

Our results indicate that at synapses APP mainly occurs as cleaved fragments, while a fraction of full-length APP can be transported to synapses, the latter process being enhanced

by reducing BACE1 activity. These results are in line with previous findings that APP secretases can be found in synaptic vesicles [212]. However, the role of the presynaptic compartment in APP processing remains to be elucidated.

3.2 EXERCISE AND BDNF REDUCE AB PRODUCTION BY ENHANCING A-SECRETASE PROCESSING OF APP (PAPER II)

It has been suggested that regular exercise, among other life-style factors, can reduce the risk of developing AD [17], and running wheel exercise can decrease Aβ production in AD transgenic models [18, 213]. Moreover, exercise has been suggested to enhance cognitive capacity by increasing the mRNA and protein levels of BDNF (brain derived neurotrophic factor) [18], a protein which has been implicated in APP processing and sAPPα production [214]. However, the exact mechanisms by which exercise can decrease Aβ production remain incompletely understood. In study II, we examined the relationship between exercise, BDNF and APP processing.

Consistent with previous results showing that exercise can reduce Aβ levels in other AD mice models [213, 215], we found that runner mice showed a reduction of Aβ levels in hippocampi compared with sedentary control mice in a 2xTg AD (APP/PS1 double-mutant transgenic) mouse model. Moreover, the levels of both sAPPα and BDNF were increased in runner mice as compared to control mice.

Previous studies indicate that BDNF can regulate sAPPα production [214]. To test whether BDNF may alter Aβ production by a mechanism involving α-cleavage of APP, we treated the differentiated SH-SY5Y cells with BDNF and/or the α-secretase inhibitor batimastat. Inhibition of the α-secretase activity led to an increase of both Aβ40 and Aβ42. BDNF treatment caused a significant reduction of Aβ levels. However, the effect of BDNF was occluded by batimastat, suggesting that the BDNF-induced reduction of Aβ levels was dependent on the α-cleavage.

Since ADAM10 has been suggested to be the constitutive α-secretase, we examined whether BDNF treatment can alter its levels and distribution. Protein levels of ADAM10 were revealed by Western blot, and no change was found. However, the cell surface levels of ADAM10 were significantly reduced upon BDNF treatment. These results together suggest that BDNF treatment may shift the distribution of ADAM10 from the cell surface to intracellular compartments where regulated α-secretase activity mainly takes place [216].

Taken together, these results show that exercise can decrease Aβ production and increase sAPPα as well as BDNF levels, and suggest that BDNF plays an important role in the production of Aβ by regulating α-secretase activity. However, it is still unclear whether exercise regulates Aβ production through the pathways medicated by BDNF. In future studies, it will be of interest to examine whether exercise can still regulate Aβ generation when the function of BDNF is removed.

3.3 OVEREXPRESSION OF SNX3 DECREASES AMYLOID-B PEPTIDE PRODUCTION BY REDUCING INTERNALIZATION OF AMYLOID PRECURSOR PROTEIN (PAPER III)

The trafficking and processing of APP is a complex process which can be regulated by many proteins. Among them sorting nexins (SNXs) have emerged as key players [41]. SNXs, characterized by a PX (phox homology) domain, is a family of evolutionarily conserved proteins [108, 111]. SNXs have diverse cellular functions many of which relate to protein trafficking and sorting [110]. SNX3, a member of the SNXs family, has a number of functions in protein sorting and trafficking [199, 200]. It was recently found that single nucleotide polymorphisms (SNPs) in SNX3 are associated with AD [206]. However, whether SNX3 directly affects APP processing and Aβ production remains unclear. In this study we provide a first test of this possibility.

Overexpression of SNX3 together with APP in HEK293T cells significantly decreased the levels of both Aβ40, Aβ42 and sAPPβ as compared to empty vector control, whereas

overexpression of another SNX family protein, SNX29, did not lead to any alteration. Moreover, BACE1 levels were not altered by SNX3 overexpression. These results suggest that SNX3 can decrease amyloidogenic cleavage of APP.

The association of APP with BACE1 is an important prerequisite for its β-cleavage. To test whether SNX3 can disrupt this association, we employed a bimolecular fluorescence complementation (BiFC) assay [217]. In this assay, APP is tagged with the non-fluorescent N-terminal fragment of the Venus protein (VN), while BACE1 is tagged with the non-fluorescent C-terminal fragment of the Venus protein (VC) (Fig. 4). When APP and BACE1 are in proximity, a functional Venus protein that produces a fluorescent signal is reconstituted. We found that overexpression of SNX3 resulted in a small but significant reduction in the association between APP and BACE1. These results suggest that SNX3 overexpression may decrease β-cleavage of APP by interference with the association between APP and BACE1.

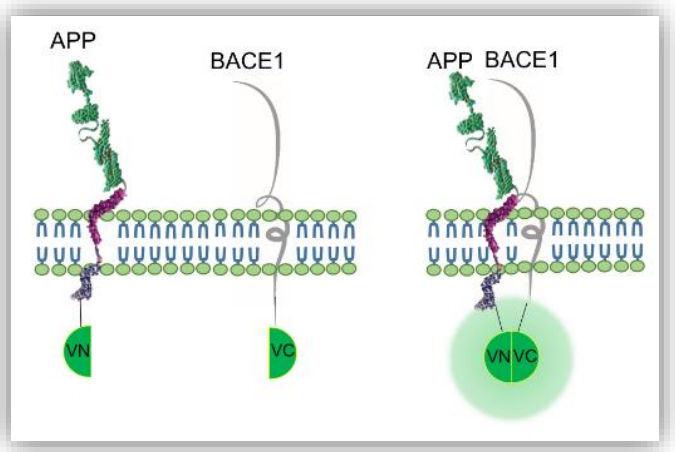


Fig. 4. Principle of BiFC for the detection of association between APP and BACE1. APP and BACE1 are C-terminally tagged with VN and VC fragment, respectively. Association between APP and BACE1 leads to formation of a functional Venus protein that produces a fluorescent signal. Adapted from Ref. [217].

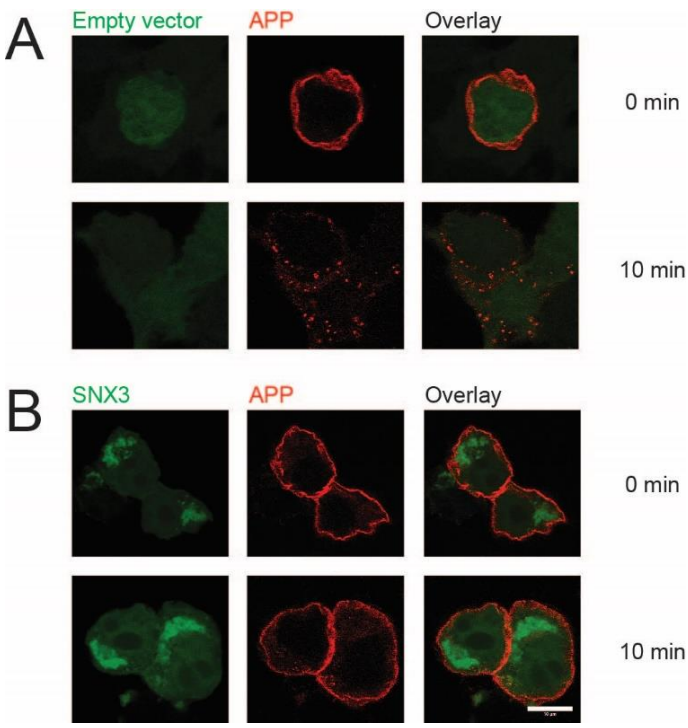


Fig. 5. SNX3 overexpression inhibits APP internalization. HEK293T cells were co-transfected with empty vector (pEGFP-C1) or pEGFP-C1_SNX3 and BBS-APP, and internalization of cell surface APP was examined. Scale bar = 10 μm.

Since many lines of evidence indicate that APP encounters BACE1 in endosomes following its internalization [218, 219], we sought to test whether SNX3 affects the internalization of APP by using a well-established internalization assay [220]. APP was N-terminally tagged with an α-bungarotoxin-binding site (BBS). Internalization of APP was examined by monitoring cell surface BBS-APP labeled by incubation with α-bungarotoxin conjugated with Alexa Fluor® 555. We observed that overexpression of SNX3 decreased APP internalization (Fig. 5).

Therefore, it would be expected that the cell surface distribution of APP would be enhanced by SNX3. Indeed, we found that the cell surface levels of APP, measured by flow cytometry, were significantly increased by SNX3 overexpression. These results together suggest that overexpression of SNX3 decreases Aβ production by reducing internalization of APP.

These results coincide with previous findings suggesting that SNX3 may be involved in endocytosis of other cargo molecules [197, 201]. The precise role of SNX3 in endocytosis is unclear. However, it is interesting to note that that SNX3 can interact with the heavy chain of clathrin [200], suggesting that it may directly regulate the clathrin machinery. Our results suggest that SNX3 overexpression can affect Aβ production by influencing the association between APP and BACE1 as well as APP internalization. Therefore, modulation of SNX3

may be an interesting approach to regulate amyloidogenic processing of APP while leaving the β-secretase activity unimpaired.

3.4 OVEREXPRESSION OF SNX7 REDUCES AB PRODUCTION BY ENHANCING LYSOSOMAL DEGRADATION OF APP (PAPER IV)

Many SNXs have been implicated in APP trafficking and processing. In this study, we extended the investigation to another protein, SNX7. SNX7 has, in addition to a PX domain, a C-terminal BAR domain, which can sense and induce membrane curvature [127, 128]. It was found that SNX7 downregulation can induce the activation of caspase 8 [207], an alteration observed in AD brain [208]. Moreover, it has been shown that SNX7 is genetically associated with AD [209], and expression of the SNX7 gene was found to be decreased in AD subjects [210]. However, it is still unclear whether SNX7 can influence APP processing and Aβ production.

We found that overexpression of SNX7 in HEK293T cells decreased the levels of both Aβ40 and Aβ42 as compared to empty vector control. Overexpression of another protein, SNX29, did not cause any change. Since β-secretase processing of APP is the putatively rate-limiting step in Aβ production [221], we next examined whether overexpression of SNX7 altered β-cleavage. We found that SNX7 overexpression reduced the production of sAPPβ, suggesting that it may alter the β-cleavage of APP. The other β-cleavage product, CTFβ, was also examined. We found that SNX7 overexpression caused a significant decrease of both CTFβ and CTFα.

It has been shown that SNX12 [222] and SNX4 [189] which influence processing of APP, can affect the cell surface levels of BACE1. To test whether SNX7 has a similar effect, we examined the cell surface distribution of BACE1 by confocal microscopy and flow cytometry. We found that SNX7 overexpression did not alter the cell surface levels of BACE1. In addition, no change of the steady-state levels of BACE1 could be detected by

overexpression of SNX7. These results suggest that overexpression of SNX7 does not influence Aβ production by affecting the cellular trafficking of BACE1.

We then examined whether SNX7 can influence the trafficking of APP. As in the experiments above, the cell surface distribution of APP was examined by confocal microscopy and flow cytometry. We found that the cell surface levels of APP were decreased by SNX7 overexpression. Moreover, the steady-state levels of APP were also prominently reduced by overexpression of SNX7. These results suggest that SNX7 may alter Aβ production by influencing APP trafficking and processing.

It has been shown that SNX4, which has a similar domain organization to SNX7, can influence protein trafficking to the lysosomal degradative pathway [189]. Therefore, it is possible that SNX7 overexpression affects APP levels by altering its degradation. To test this possibility, we used two compounds, NH_4Cl [157, 223] and Bafilomycin A1 (Baf) [50, 189] to prevent protein degradation. As shown in Fig. 6, NH_4Cl and Baf treatment both caused a significant increase of the APP levels, as expected by the known dependence of APP degradation on lysosomal pH [50, 224]. Consistent with the above results, SNX7 overexpression reduced the APP levels. However, when NH_4Cl or Baf was added, the effect of SNX7 overexpression was occluded, suggesting that the SNX7-induced reduction of APP was dependent on the degradative pathway [225].

This study has provided a first examination of the role of SNX7 in APP processing and Aβ production. We found that the levels of Aβ, sAPPβ, CTFs, cell surface APP and steady-state APP were decreased by SNX7 overexpression, which can all be explained by increased APP degradation. We do not, however, exclude the possibility that SNX7 additionally takes part in internalization of APP from the cell surface, a role linked with SNX33 [62] and SNX3 (observations in Paper III). Taken together, these results suggest that overexpression of SNX7 enhances APP degradation, thereby reducing Aβ production.

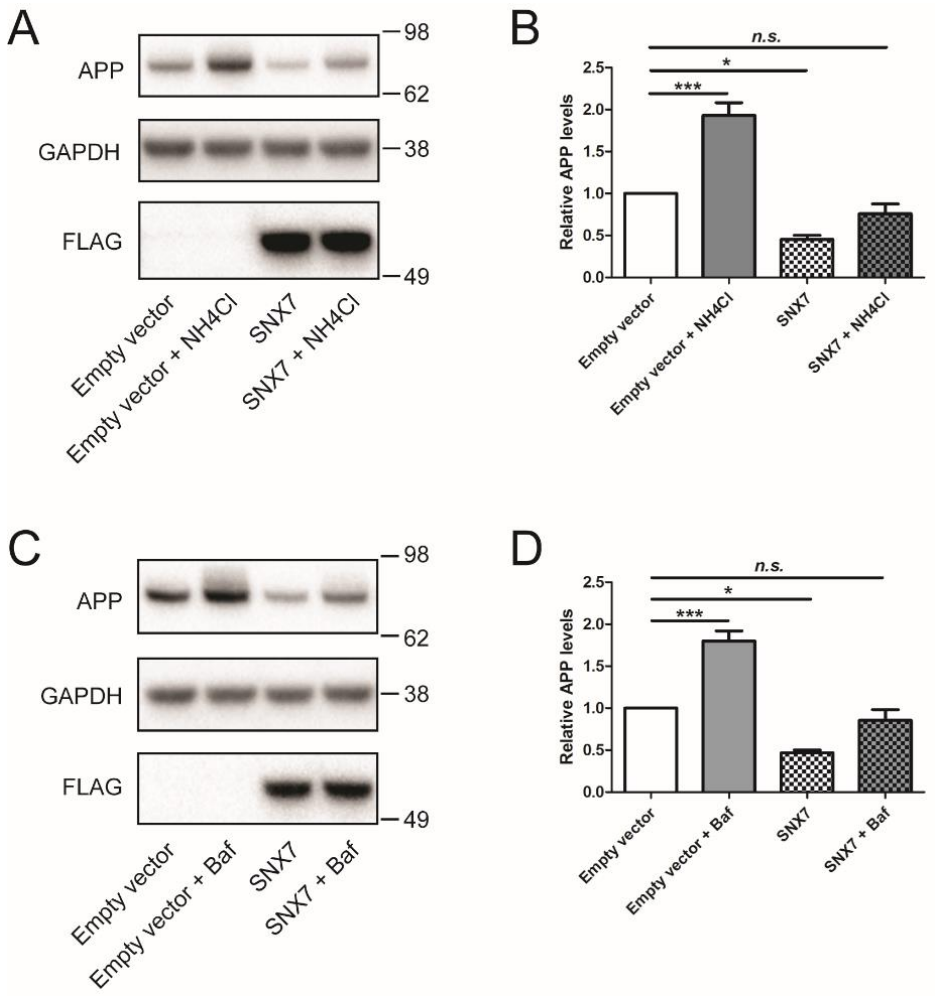


Fig. 6. Lysosomal inhibitors prevent the reduction of APP levels by SNX7 overexpression [225].

A-D. HEK293T cells were co-transfected with either empty vector or FLAG-SNX7 together with Myc-APP. The cells were cultured for a total of 48 h after transfection. NH_4Cl or Bafilomycin A1 (Baf) was applied to the cultures 6 h before harvest. APP levels were examined by Western blot with anti-Myc antibody. Representative blots from NH_4Cl treatment (**A**) and Baf treatment (**C**) experiments are shown. **B.** Quantification of APP levels in the NH_4Cl treatment experiment. **D.** Quantification of APP levels in the Baf treatment experiment.

4 SUMMARY AND FUTURE PERSPECTIVES

The trafficking and processing of APP is a complex process which is spatially and temporally regulated. Interference with the trafficking and subsequent localization of APP strongly influences APP processing, and thus Aβ production. This thesis aims to expand our understanding of mechanisms that regulate processing and trafficking of APP. We found that: 1) APP mainly distributes as cleaved fragments at synapses under normal conditions. However, a pathway for the transport of full-length APP also exists, which can be revealed by reduction of BACE1 activity. 2) Exercise and BDNF can reduce Aβ production by enhancing α-cleavage of APP, and these effects were possibly regulated by ADAM10 distribution and activity. 3) Overexpression of SNX3 can disturb the association between APP and BACE1, as well as APP internalization, thereby reducing Aβ production. 4) SNX7 overexpression can decrease Aβ production by enhancing APP degradation.

Considering that all the major secretases for APP have a number of substrates, it would possibly be very challenging to target them without any side-effects. In this thesis, we have shown that increased expression of SNX3 can decrease toxic Aβ production. Interestingly, while BACE1 levels were not altered, SNX3 can disturb the association between APP and BACE1, as well as APP internalization. The association between APP and BACE1 is an important prerequisite for β-cleavage and Aβ production. In principle specific targeting of the association would reduce Aβ production while leaving BACE1 activity intact. Therefore, the association between APP and BACE1 may be a promising therapeutic target.

We provided a first examination of the role of SNX3 and SNX7 in APP processing and Aβ production using HEK293T as a cell model. In future studies it will be of interest to examine whether these results apply also to other cell types, including primary neurons.

5 ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to all the people who have helped make this thesis possible.

In particular I would like to thank:

Prof. **Lennart Brodin**, my main supervisor, for providing me with the great opportunity to conduct a challenging and exciting project at Karolinska Institutet. Thank you for sharing your knowledge and guiding me how to conduct and present scientific research. I truly appreciate the instruction, encouragement and freedom that you have given throughout my PhD studies. It has been a great pleasure working with you. Thank you for your patience in my English writing, and for having always been supportive and nice.

Prof. **Oleg Shupliakov**, for being my co-supervisor. Thank you for your inspiration and insightful discussions, and for sharing the advice: "*Do not look for a dark thing in a dark room, especially if you do not know whether it is there*."

Ingeborg van der Ploeg, my mentor. Many thanks for your continued support and warm encouragement from the very beginning to the very end of my PhD studies. Thank you for sharing your experience, knowledge and vision. Nice to have known you!

Present and former members in Brodin/Shupliakov groups:
Saket M. Nigam, for sharing the PhD experience, and I have really learned a lot from you. Best of luck for the future! Dr. **Frauke Ackermann**, Dr. **Joshua A. Gregory**, for showing me the hippocampus dissection and immunocytochemical staining. Dr. **Olga Vorontsova**, for the helpful suggestions on biochemistry and molecular cloning, and for taking care of everything in the lab! Dr. **Tuomas Näreoja**, for all the inspiring scientific ideas and image analysis support. **Gianvito Arpino**, for sharing a positive attitude towards scientific research and personal life. Dr. **Åsa Winther**, Dr. **Kathryn Rees**, and **Elena Sopova**, for all the wonderful "*fika*" and nice chats.

Colleagues in the **Department of Neuroscience** & the **Department of Cell and Molecular Biology**:
Xiaofei Li, **Yang Xuan**, **Yiqiao Wang**, for the helpful discussions on research and other things. Dr. **Shaobo Jin**, for the nice advice and warm help in both my research and life. Nice to have known you! Dr. **Aldwin Suryo Rahmanto**, for the inspiration and critical evaluation of my thoughts on research. Dr. **Hideaki Nakamura**, for showing me how to

perform real-time PCR, and helping me solve different problems in my experiments. Dr. **Benjamin Ka-Cheuk Liu**, Dr. **Lianhe Chu**, Dr. **Jeremie Charbord**, **Christos Karampelias**, Dr. **Xiaochuan Ma** and Dr. **Jing Lu**, for sharing a wonderful lab corridor, and for all the nice coffee and tea time. Dr. **Florian Salomons**, **Annika Pfeiffer**, **Thibaud Richard**, **Tatiana Álvarez**, **Laura Herzog**, Dr. **Eike-Benjamin Braune,** for sharing a nice experience in cell culture rooms and for all the nice chats. Dr. **María Díaz Moreno**, **Simona Hankeová**, **Nikola Vojnovic**, for all the help and encouragement.

I am very grateful to: Prof. **Gilberto Fisone**, **Karin Lagerman**, **Åsa Garmager**, **Axel Bergwik**, and **Elzbieta Holmberg**, for the help of administrative work. Professors at KI, **Anders Gustafsson**, **Jinjing Pei**, **Marianne Schultzberg**, **Jie Zhu**, **Eirikur Benedikz**, **Susanne Frykman** for all the kind support that you have given.

I would like to express my special thanks to my lovely friends in Sweden: **Rong Yu**, **Jia Guo**, **Murad Altmash**, **Jiyu Guan**, **Jian Zhu**, for the company during this long journey! **Bo Zhang** and **Yinghan Miao**, **Stefan Yang** and **Xuyue Yang**, **Xiaofei Ye** and **Yinjiang Long**, for the organization of exciting events. **Juan Du**, **Fuxiang Bao**, **Hongya Han** and **Xinyan Miao**, **Qiang Zhang** and **Fang Hu**, for the continued encouragement, and all the happy time that we had together. **Xiaotian Yuan**, **Min Guo**, **Yuanyuan Zhang**, **Lei Du**, **Anquan Liu**, **Yuan Xu**, for all the help and nice chats. **Chao Sun** and **Ying Lei**, for the memorable trips. **Bingnan Li** and **Xiaolu Zhang**, for all the help that you have kindly provided. **Xi Chen**, **Xinyun Lin**, for the wonderful corridor life. **Zhi Tang**, **Hongliang Zhang**, **Xiangyu Zheng** and **Yang Ruan**, **Mingqin Zhu**, **Xiaoke Wang**, **Jia Liu**, for helping me adapt to the new life in Sweden! **Chengjun Wu** and **Baofeng Yang**, for the warm encouragement and guidance. **Chengjun Sun** and **Ying Hu**, for the delicious Chinese food and enjoyable time. **Cuong Do Duy**, for all the help that you have offered, and for sharing beers and life stories. **Nina Gennebäck**, my "classmate", for sharing the defence experience and useful tips. **Hui Ding**, **Ying Sun**, **Wei Jiao**, for providing useful information when I began my life in Stockholm.

I would like to thank the **China Scholarship Council** for providing me a scholarship to study in Sweden. My most sincere thanks also go to: Prof. **Jinsheng He** and **Yanpeng Zheng** at Beijing Jiaotong University, for all the trust and support on me; my beloved parents, parents-in-law, sisters and brothers-in-law, and my wife **Lu**, for your understanding!

6 REFERENCES

[1] A. Serrano-Pozo, M.P. Frosch, E. Masliah, B.T. Hyman, Neuropathological alterations in Alzheimer disease, Cold Spring Harbor perspectives in medicine, 1 (2011) a006189.

[2] H. Zheng, E.H. Koo, Biology and pathophysiology of the amyloid precursor protein, Molecular neurodegeneration, 6 (2011) 27.

[3] Z. Liu, A. Zhang, H. Sun, Y. Han, L. Kong, X. Wang, Two decades of new drug discovery and development for Alzheimer's disease, RSC Advances, 7 (2017) 6046-6058.

[4] J. Hardy, D.J. Selkoe, The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics, Science, 297 (2002) 353-356.

[5] D.J. Selkoe, J. Hardy, The amyloid hypothesis of Alzheimer's disease at 25 years, EMBO molecular medicine, 8 (2016) 595-608.

[6] V.H. Finder, R. Glockshuber, Amyloid-beta aggregation, Neurodegener Dis, 4 (2007) 13-27.

[7] M.T. Fodero-Tavoletti, V.L. Villemagne, C.C. Rowe, C.L. Masters, K.J. Barnham, R. Cappai, Amyloid-beta: the seeds of darkness, The international journal of biochemistry & cell biology, 43 (2011) 1247-1251.

[8] F.M. LaFerla, K.N. Green, S. Oddo, Intracellular amyloid-[beta] in Alzheimer's disease, Nature reviews. Neuroscience, 8 (2007) 499-509.

[9] M. Jin, N. Shepardson, T. Yang, G. Chen, D. Walsh, D.J. Selkoe, Soluble amyloid beta-protein dimers isolated from Alzheimer cortex directly induce Tau hyperphosphorylation and neuritic degeneration, Proceedings of the National Academy of Sciences of the United States of America, 108 (2011) 5819-5824.

[10] K. Iqbal, F. Liu, C.X. Gong, I. Grundke-Iqbal, Tau in Alzheimer disease and related tauopathies, Curr Alzheimer Res, 7 (2010) 656-664.

[11] R. Kayed, C.A. Lasagna-Reeves, Molecular mechanisms of amyloid oligomers toxicity, Journal of Alzheimer's disease : JAD, 33 Suppl 1 (2013) S67-78.

[12] R.M. Koffie, B.T. Hyman, T.L. Spires-Jones, Alzheimer's disease: synapses gone cold, Molecular neurodegeneration, 6 (2011) 63.

[13] D.J. Selkoe, Toward a comprehensive theory for Alzheimer's disease. Hypothesis: Alzheimer's disease is caused by the cerebral accumulation and cytotoxicity of amyloid beta-protein, Annals of the New York Academy of Sciences, 924 (2000) 17-25.

[14] H.A. Pearson, C. Peers, Physiological roles for amyloid beta peptides, The Journal of physiology, 575 (2006) 5-10.

[15] E. Fedele, D. Rivera, B. Marengo, M.A. Pronzato, R. Ricciarelli, Amyloid beta: Walking on the dark side of the moon, Mechanisms of ageing and development, (2015).

[16] C.C. Liu, C.C. Liu, T. Kanekiyo, H. Xu, G. Bu, Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy, Nature reviews. Neurology, 9 (2013) 106-118.

[17] S. Norton, F.E. Matthews, D.E. Barnes, K. Yaffe, C. Brayne, Potential for primary prevention of Alzheimer's disease: an analysis of population-based data, The Lancet. Neurology, 13 (2014) 788-794.

[18] K.A. Intlekofer, C.W. Cotman, Exercise counteracts declining hippocampal function in aging and Alzheimer's disease, Neurobiology of disease, 57 (2013) 47-55.

[19] U.C. Muller, T. Deller, M. Korte, Not just amyloid: physiological functions of the amyloid precursor protein family, Nature reviews. Neuroscience, 18 (2017) 281-298.

[20] E. Dawkins, D.H. Small, Insights into the physiological function of the beta-amyloid precursor protein: beyond Alzheimer's disease, Journal of neurochemistry, 129 (2014) 756-769.

[21] P. Soba, S. Eggert, K. Wagner, H. Zentgraf, K. Siehl, S. Kreger, A. Lower, A. Langer, G. Merdes, R. Paro, C.L. Masters, U. Muller, S. Kins, K. Beyreuther, Homo- and heterodimerization of APP family members promotes intercellular adhesion, The EMBO journal, 24 (2005) 3624-3634.

[22] A. Ho, T.C. Sudhof, Binding of F-spondin to amyloid-beta precursor protein: a candidate amyloid-beta precursor protein ligand that modulates amyloid-beta precursor protein cleavage, Proceedings of the National Academy of Sciences of the United States of America, 101 (2004) 2548-2553.

[23] C. Deyts, G. Thinakaran, A.T. Parent, APP Receptor? To Be or Not To Be, Trends in pharmacological sciences, 37 (2016) 390-411.

[24] Z. Wang, B. Wang, L. Yang, Q. Guo, N. Aithmitti, Z. Songyang, H. Zheng, Presynaptic and postsynaptic interaction of the amyloid precursor protein promotes peripheral and central synaptogenesis, The Journal of neuroscience : the official journal of the Society for Neuroscience, 29 (2009) 10788-10801.

[25] A. Lorenzo, M. Yuan, Z. Zhang, P.A. Paganetti, C. Sturchler-Pierrat, M. Staufenbiel, J. Mautino, F.S. Vigo, B. Sommer, B.A. Yankner, Amyloid beta interacts with the amyloid precursor protein: a potential toxic mechanism in Alzheimer's disease, Nature neuroscience, 3 (2000) 460-464.

[26] D.C. Lu, G.M. Shaked, E. Masliah, D.E. Bredesen, E.H. Koo, Amyloid beta protein toxicity mediated by the formation of amyloid-beta protein precursor complexes, Annals of neurology, 54 (2003) 781-789.

[27] R.J. O'Brien, P.C. Wong, Amyloid precursor protein processing and Alzheimer's disease, Annual review of neuroscience, 34 (2011) 185-204.

[28] G. Thinakaran, E.H. Koo, Amyloid precursor protein trafficking, processing, and function, The Journal of biological chemistry, 283 (2008) 29615-29619.

[29] V.W. Chow, M.P. Mattson, P.C. Wong, M. Gleichmann, An overview of APP processing enzymes and products, Neuromolecular medicine, 12 (2010) 1-12.

[30] S. Chasseigneaux, B. Allinquant, Functions of Abeta, sAPPalpha and sAPPbeta : similarities and differences, Journal of neurochemistry, 120 Suppl 1 (2012) 99-108.

[31] S. Ring, S.W. Weyer, S.B. Kilian, E. Waldron, C.U. Pietrzik, M.A. Filippov, J. Herms, C. Buchholz, C.B. Eckman, M. Korte, D.P. Wolfer, U.C. Muller, The secreted beta-amyloid precursor protein ectodomain APPs alpha is sufficient to rescue the anatomical, behavioral, and electrophysiological abnormalities of APP-deficient mice, The Journal of neuroscience : the official journal of the Society for Neuroscience, 27 (2007) 7817-7826.

[32] A. Habib, D. Sawmiller, J. Tan, Restoring Soluble Amyloid Precursor Protein alpha Functions as a Potential Treatment for Alzheimer's Disease, Journal of neuroscience research, (2016).

[33] D. Obregon, H. Hou, J. Deng, B. Giunta, J. Tian, D. Darlington, M. Shahaduzzaman, Y. Zhu, T. Mori, M.P. Mattson, J. Tan, Soluble amyloid precursor protein-alpha modulates beta-secretase activity and amyloid-beta generation, Nature communications, 3 (2012) 777.

[34] S. Jager, S. Leuchtenberger, A. Martin, E. Czirr, J. Wesselowski, M. Dieckmann, E. Waldron, C. Korth, E.H. Koo, M. Heneka, S. Weggen, C.U. Pietrzik, alpha-secretase mediated conversion of the amyloid precursor protein derived membrane stub C99 to C83 limits Abeta generation, Journal of neurochemistry, 111 (2009) 1369-1382.

[35] H.S. Nhan, K. Chiang, E.H. Koo, The multifaceted nature of amyloid precursor protein and its proteolytic fragments: friends and foes, Acta Neuropathol, 129 (2015) 1-19.

[36] Y. Jiang, K.A. Mullaney, C.M. Peterhoff, S. Che, S.D. Schmidt, A. Boyer-Boiteau, S.D. Ginsberg, A.M. Cataldo, P.M. Mathews, R.A. Nixon, Alzheimer's-related endosome dysfunction in Down syndrome is Abeta-independent but requires APP and is reversed by BACE-1 inhibition, Proceedings of the National Academy of Sciences of the United States of America, 107 (2010) 1630-1635.

[37] I. Lauritzen, R. Pardossi-Piquard, A. Bourgeois, S. Pagnotta, M.G. Biferi, M. Barkats, P. Lacor, W. Klein, C. Bauer, F. Checler, Intraneuronal aggregation of the beta-CTF fragment of APP (C99) induces Abeta-independent lysosomal-autophagic pathology, Acta Neuropathol, 132 (2016) 257-276.

[38] W. Wei, D.D. Norton, X. Wang, J.W. Kusiak, Abeta 17-42 in Alzheimer's disease activates JNK and caspase-8 leading to neuronal apoptosis, Brain : a journal of neurology, 125 (2002) 2036-2043.

[39] R. Pardossi-Piquard, F. Checler, The physiology of the beta-amyloid precursor protein intracellular domain AICD, Journal of neurochemistry, 120 Suppl 1 (2012) 109-124.

[40] C. Haass, C. Kaether, G. Thinakaran, S. Sisodia, Trafficking and proteolytic processing of APP, Cold Spring Harbor perspectives in medicine, 2 (2012) a006270.

[41] S. Jiang, Y. Li, X. Zhang, G. Bu, H. Xu, Y.W. Zhang, Trafficking regulation of proteins in Alzheimer's disease, Molecular neurodegeneration, 9 (2014) 6.

[42] X. Wang, X. Zhou, G. Li, Y. Zhang, Y. Wu, W. Song, Modifications and Trafficking of APP in the Pathogenesis of Alzheimer's Disease, Frontiers in molecular neuroscience, 10 (2017) 294.

[43] P. Agostinho, A. Pliassova, C.R. Oliveira, R.A. Cunha, Localization and Trafficking of Amyloid-beta Protein Precursor and Secretases: Impact on Alzheimer's Disease, Journal of Alzheimer's disease : JAD, (2015).

[44] S.A. Small, G.A. Petsko, Retromer in Alzheimer disease, Parkinson disease and other neurological disorders, Nature reviews. Neuroscience, 16 (2015) 126-132.

[45] J.S. Bonifacino, J.H. Hurley, Retromer, Current opinion in cell biology, 20 (2008) 427-436.

[46] J.R. van Weering, P.J. Cullen, Membrane-associated cargo recycling by tubule-based endosomal sorting, Seminars in cell & developmental biology, 31 (2014) 40-47.

[47] T.Y. Huang, Y. Zhao, X. Li, X. Wang, I.C. Tseng, R. Thompson, S. Tu, T.E. Willnow, Y.W. Zhang, H. Xu, SNX27 and SORLA Interact to Reduce Amyloidogenic Subcellular Distribution and Processing of Amyloid Precursor Protein, The Journal of neuroscience : the official journal of the Society for Neuroscience, 36 (2016) 7996-8011.

[48] S.I. Vieira, S. Rebelo, H. Esselmann, J. Wiltfang, J. Lah, R. Lane, S.A. Small, S. Gandy, E.S.E.F. da Cruz, E.S.O.A. da Cruz, Retrieval of the Alzheimer's amyloid precursor protein from the endosome to the TGN is S655 phosphorylation state-dependent and retromer-mediated, Molecular neurodegeneration, 5 (2010) 40.

[49] T. Feng, M. Niu, C. Ji, Y. Gao, J. Wen, G. Bu, H. Xu, Y.W. Zhang, SNX15 Regulates Cell Surface Recycling of APP and Abeta Generation, Molecular neurobiology, (2015).

[50] Q. Xiao, P. Yan, X. Ma, H. Liu, R. Perez, A. Zhu, E. Gonzales, D.L. Tripoli, L. Czerniewski, A. Ballabio, J.R. Cirrito, A. Diwan, J.M. Lee, Neuronal-Targeted TFEB Accelerates Lysosomal Degradation of APP, Reducing Abeta Generation and Amyloid Plaque Pathogenesis, The Journal of neuroscience : the official journal of the Society for Neuroscience, 35 (2015) 12137-12151.

[51] P.H. Kuhn, H. Wang, B. Dislich, A. Colombo, U. Zeitschel, J.W. Ellwart, E. Kremmer, S. Rossner, S.F. Lichtenthaler, ADAM10 is the physiologically relevant, constitutive alpha-secretase of the amyloid precursor protein in primary neurons, The EMBO journal, 29 (2010) 3020-3032.

[52] K. Reiss, P. Saftig, The "a disintegrin and metalloprotease" (ADAM) family of sheddases: physiological and cellular functions, Seminars in cell & developmental biology, 20 (2009) 126-137.

[53] S.F. Lichtenthaler, alpha-secretase in Alzheimer's disease: molecular identity, regulation and therapeutic potential, Journal of neurochemistry, 116 (2011) 10-21.

[54] J.D. Buxbaum, K.N. Liu, Y. Luo, J.L. Slack, K.L. Stocking, J.J. Peschon, R.S. Johnson, B.J. Castner, D.P. Cerretti, R.A. Black, Evidence that tumor necrosis factor alpha converting enzyme is involved in regulated alpha-secretase cleavage of the Alzheimer amyloid protein precursor, The Journal of biological chemistry, 273 (1998) 27765-27767.

[55] E. Kojro, R. Postina, C. Buro, C. Meiringer, K. Gehrig-Burger, F. Fahrenholz, The neuropeptide PACAP promotes the alpha-secretase pathway for processing the Alzheimer amyloid precursor protein, Faseb J, 20 (2006) 512-514.

[56] F. Tippmann, J. Hundt, A. Schneider, K. Endres, F. Fahrenholz, Up-regulation of the alpha-secretase ADAM10 by retinoic acid receptors and acitretin, Faseb J, 23 (2009) 1643-1654.

[57] S. Parvathy, I. Hussain, E.H. Karran, A.J. Turner, N.M. Hooper, Cleavage of Alzheimer's amyloid precursor protein by alpha-secretase occurs at the surface of neuronal cells, Biochemistry, 38 (1999) 9728-9734.

[58] D.M. Skovronsky, D.B. Moore, M.E. Milla, R.W. Doms, V.M. Lee, Protein kinase C-dependent alpha-secretase competes with beta-secretase for cleavage of amyloid-beta precursor protein in the trans-golgi network, The Journal of biological chemistry, 275 (2000) 2568-2575.

[59] R. MacLeod, E.K. Hillert, R.T. Cameron, G.S. Baillie, The role and therapeutic targeting of alpha-, beta- and gamma-secretase in Alzheimer's disease, Future science OA, 1 (2015) Fso11.

[60] E. Marcello, F. Gardoni, D. Mauceri, S. Romorini, A. Jeromin, R. Epis, B. Borroni, F. Cattabeni, C. Sala, A. Padovani, M. Di Luca, Synapse-associated protein-97 mediates alpha-secretase ADAM10 trafficking and promotes its activity, The Journal of neuroscience : the official journal of the Society for Neuroscience, 27 (2007) 1682-1691.

[61] H. Ebsen, M. Lettau, D. Kabelitz, O. Janssen, Identification of SH3 domain proteins interacting with the cytoplasmic tail of the a disintegrin and metalloprotease 10 (ADAM10), PloS one, 9 (2014) e102899.

[62] S. Schobel, S. Neumann, M. Hertweck, B. Dislich, P.H. Kuhn, E. Kremmer, B. Seed, R. Baumeister, C. Haass, S.F. Lichtenthaler, A novel sorting nexin modulates endocytic trafficking and alpha-secretase cleavage of the amyloid precursor protein, The Journal of biological chemistry, 283 (2008) 14257-14268.

[63] R. Vassar, B.D. Bennett, S. Babu-Khan, S. Kahn, E.A. Mendiaz, P. Denis, D.B. Teplow, S. Ross, P. Amarante, R. Loeloff, Y. Luo, S. Fisher, J. Fuller, S. Edenson, J. Lile, M.A. Jarosinski, A.L. Biere, E. Curran, T. Burgess, J.C. Louis, F. Collins, J. Treanor, G. Rogers, M. Citron, Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE, Science, 286 (1999) 735-741.

[64] R. Yan, M.J. Bienkowski, M.E. Shuck, H. Miao, M.C. Tory, A.M. Pauley, J.R. Brashier, N.C. Stratman, W.R. Mathews, A.E. Buhl, D.B. Carter, A.G. Tomasselli, L.A. Parodi, R.L. Heinrikson, M.E. Gurney, Membrane-anchored aspartyl protease with Alzheimer's disease beta-secretase activity, Nature, 402 (1999) 533-537.

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

[66] I. Hussain, D. Powell, D.R. Howlett, D.G. Tew, T.D. Meek, C. Chapman, I.S. Gloger, K.E. Murphy, C.D. Southan, D.M. Ryan, T.S. Smith, D.L. Simmons, F.S. Walsh, C. Dingwall, G. Christie, Identification of a novel aspartic protease (Asp 2) as beta-secretase, Molecular and cellular neurosciences, 14 (1999) 419-427.

[67] W. Araki, Post-translational regulation of the beta-secretase BACE1, Brain research bulletin, 126 (2016) 170-177.

[68] R. Sannerud, I. Declerck, A. Peric, T. Raemaekers, G. Menendez, L. Zhou, B. Veerle, K. Coen, S. Munck, B. De Strooper, G. Schiavo, W. Annaert, ADP ribosylation factor 6 (ARF6) controls amyloid precursor protein (APP) processing by mediating the endosomal sorting of BACE1, Proceedings of the National Academy of Sciences of the United States of America, 108 (2011) E559-568.

[69] Y. Prabhu, P.V. Burgos, C. Schindler, G.G. Farias, J.G. Magadan, J.S. Bonifacino, Adaptor protein 2-mediated endocytosis of the beta-secretase BACE1 is dispensable for amyloid precursor protein processing, Molecular biology of the cell, 23 (2012) 2339-2351.

[70] P.Z. Chia, W.H. Toh, R. Sharples, I. Gasnereau, A.F. Hill, P.A. Gleeson, Intracellular itinerary of internalised beta-secretase, BACE1, and its potential impact on beta-amyloid peptide biogenesis, Traffic, 14 (2013) 997-1013.

[71] L. Pastorino, A.F. Ikin, A.C. Nairn, A. Pursnani, J.D. Buxbaum, The carboxyl-terminus of BACE contains a sorting signal that regulates BACE trafficking but not the formation of total A(beta), Molecular and cellular neurosciences, 19 (2002) 175-185.

[72] X. He, G. Zhu, G. Koelsch, K.K. Rodgers, X.C. Zhang, J. Tang, Biochemical and structural characterization of the interaction of memapsin 2 (beta-secretase) cytosolic domain with the VHS domain of GGA proteins, Biochemistry, 42 (2003) 12174-12180.

[73] J.S. Bonifacino, The GGA proteins: adaptors on the move, Nature reviews. Molecular cell biology, 5 (2004) 23-32.

[74] T. Wahle, K. Prager, N. Raffler, C. Haass, M. Famulok, J. Walter, GGA proteins regulate retrograde transport of BACE1 from endosomes to the trans-Golgi network, Molecular and cellular neurosciences, 29 (2005) 453-461.

[75] X. He, F. Li, W.P. Chang, J. Tang, GGA proteins mediate the recycling pathway of memapsin 2 (BACE), The Journal of biological chemistry, 280 (2005) 11696-11703.

[76] C.A. von Arnim, M.M. Tangredi, I.D. Peltan, B.M. Lee, M.C. Irizarry, A. Kinoshita, B.T. Hyman, Demonstration of BACE (beta-secretase) phosphorylation and its interaction with GGA1 in cells by fluorescence-lifetime imaging microscopy, J Cell Sci, 117 (2004) 5437-5445.

[77] V. Udayar, V. Buggia-Prevot, R.L. Guerreiro, G. Siegel, N. Rambabu, A.L. Soohoo, M. Ponnusamy, B. Siegenthaler, J. Bali, M. Simons, J. Ries, M.A. Puthenveedu, J. Hardy, G. Thinakaran, L. Rajendran, Aesg, A Paired RNAi and RabGAP Overexpression Screen Identifies Rab11 as a Regulator of beta-Amyloid Production, Cell Rep, 5 (2013) 1536-1551.

[78] Y.H. Koh, C.A. von Arnim, B.T. Hyman, R.E. Tanzi, G. Tesco, BACE is degraded via the lysosomal pathway, The Journal of biological chemistry, 280 (2005) 32499-32504.

[79] H. Qing, W. Zhou, M.A. Christensen, X. Sun, Y. Tong, W. Song, Degradation of BACE by the ubiquitin-proteasome pathway, Faseb J, 18 (2004) 1571-1573.

[80] J.H. Stockley, C. O'Neill, The proteins BACE1 and BACE2 and beta-secretase activity in normal and Alzheimer's disease brain, Biochem Soc Trans, 35 (2007) 574-576.

[81] S.L. Cole, R. Vassar, The Alzheimer's disease beta-secretase enzyme, BACE1, Molecular neurodegeneration, 2 (2007) 22.

[82] B. Dislich, S.F. Lichtenthaler, The Membrane-Bound Aspartyl Protease BACE1: Molecular and Functional Properties in Alzheimer's Disease and Beyond, Frontiers in physiology, 3 (2012) 8.

[83] R. Yan, R. Vassar, Targeting the beta secretase BACE1 for Alzheimer's disease therapy, The Lancet. Neurology, 13 (2014) 319-329.

[84] P.C. Kandalepas, R. Vassar, The normal and pathologic roles of the Alzheimer's beta-secretase, BACE1, Curr Alzheimer Res, 11 (2014) 441-449.

[85] S. Ben Halima, S. Mishra, K.M. Raja, M. Willem, A. Baici, K. Simons, O. Brustle, P. Koch, C. Haass, A. Caflisch, L. Rajendran, Specific Inhibition of beta-Secretase Processing of the Alzheimer Disease Amyloid Precursor Protein, Cell Rep, (2016).

[86] A.J. Saunders, T.-W. Kim, R.E. Tanzi, BACE maps to chromosome 11 and a BACE homolog, BACE2, reside in the obligate Down syndrome region of chromosome 21, Science, 286 (1999) 1255-1255.

[87] W.T. Kimberly, M.J. LaVoie, B.L. Ostaszewski, W. Ye, M.S. Wolfe, D.J. Selkoe, Gamma-secretase is a membrane protein complex comprised of presenilin, nicastrin, Aph-1, and Pen-2, Proceedings of the National Academy of Sciences of the United States of America, 100 (2003) 6382-6387.

[88] D.R. Dries, G. Yu, Assembly, maturation, and trafficking of the gamma-secretase complex in Alzheimer's disease, Curr Alzheimer Res, 5 (2008) 132-146.

[89] C.M. Carroll, Y.M. Li, Physiological and pathological roles of the gamma-secretase complex, Brain research bulletin, (2016).

[90] A. Haapasalo, D.M. Kovacs, The many substrates of presenilin/gamma-secretase, Journal of Alzheimer's disease : JAD, 25 (2011) 3-28.

[91] E.R. Andersson, R. Sandberg, U. Lendahl, Notch signaling: simplicity in design, versatility in function, Development (Cambridge, England), 138 (2011) 3593-3612.

[92] B.P. Imbimbo, G.A. Giardina, gamma-secretase inhibitors and modulators for the treatment of Alzheimer's disease: disappointments and hopes, Current topics in medicinal chemistry, 11 (2011) 1555-1570.

[93] C.J. Crump, D.S. Johnson, Y.M. Li, Development and mechanism of gamma-secretase modulators for Alzheimer's disease, Biochemistry, 52 (2013) 3197-3216.

[94] G. He, W. Luo, P. Li, C. Remmers, W.J. Netzer, J. Hendrick, K. Bettayeb, M. Flajolet, F. Gorelick, L.P. Wennogle, P. Greengard, Gamma-secretase activating protein is a therapeutic target for Alzheimer's disease, Nature, 467 (2010) 95-98.

[95] C.E. Augelli-Szafran, H.X. Wei, D. Lu, J. Zhang, Y. Gu, T. Yang, P. Osenkowski, W. Ye, M.S. Wolfe, Discovery of notch-sparing gamma-secretase inhibitors, Curr Alzheimer Res, 7 (2010) 207-209.

[96] T.E. Golde, E.H. Koo, K.M. Felsenstein, B.A. Osborne, L. Miele, gamma-Secretase inhibitors and modulators, Biochimica et biophysica acta, 1828 (2013) 2898-2907.

[97] A. Colombo, H. Wang, P.H. Kuhn, R. Page, E. Kremmer, P.J. Dempsey, H.C. Crawford, S.F. Lichtenthaler, Constitutive alpha- and beta-secretase cleavages of the amyloid precursor protein are partially coupled in neurons, but not in frequently used cell lines, Neurobiology of disease, 49 (2013) 137-147.

[98] A.C. Chen, S. Kim, N. Shepardson, S. Patel, S. Hong, D.J. Selkoe, Physical and functional interaction between the alpha- and gamma-secretases: A new model of regulated intramembrane proteolysis, The Journal of cell biology, 211 (2015) 1157-1176.

[99] C. Hattori, M. Asai, Y. Oma, Y. Kino, N. Sasagawa, T.C. Saido, K. Maruyama, S. Ishiura, BACE1 interacts with nicastrin, Biochemical and biophysical research communications, 293 (2002) 1228-1232.

[100] S.S. Hebert, V. Bourdages, C. Godin, M. Ferland, M. Carreau, G. Levesque, Presenilin-1 interacts directly with the beta-site amyloid protein precursor cleaving enzyme (BACE1), Neurobiology of disease, 13 (2003) 238-245.

[101] A. Kuzuya, K. Uemura, N. Kitagawa, N. Aoyagi, T. Kihara, H. Ninomiya, S. Ishiura, R. Takahashi, S. Shimohama, Presenilin 1 is involved in the maturation of beta-site amyloid precursor protein-cleaving enzyme 1 (BACE1), Journal of neuroscience research, 85 (2007) 153-165.

[102] J. Cui, X. Wang, X. Li, X. Wang, C. Zhang, W. Li, Y. Zhang, H. Gu, X. Xie, F. Nan, J. Zhao, G. Pei, Targeting the γ-/β-secretase interaction reduces β-amyloid generation and ameliorates Alzheimer’s disease-related pathogenesis, Cell Discovery, 1 (2015) 15021.

[103] M. Willem, S. Tahirovic, M.A. Busche, S.V. Ovsepian, M. Chafai, S. Kootar, D. Hornburg, L.D. Evans, S. Moore, A. Daria, H. Hampel, V. Muller, C. Giudici, B. Nuscher, A. Wenninger-Weinzierl, E. Kremmer, M.T. Heneka, D.R. Thal, V. Giedraitis, L. Lannfelt, U. Muller, F.J. Livesey, F. Meissner, J. Herms, A. Konnerth, H. Marie, C. Haass, eta-Secretase processing of APP inhibits neuronal activity in the hippocampus, Nature, (2015).

[104] Z. Zhang, M. Song, X. Liu, S. Su Kang, D.M. Duong, N.T. Seyfried, X. Cao, L. Cheng, Y.E. Sun, S. Ping Yu, J. Jia, A.I. Levey, K. Ye, Delta-secretase cleaves amyloid precursor protein and regulates the pathogenesis in Alzheimer's disease, Nature communications, 6 (2015) 8762.

[105] C.P. Ponting, Novel domains in NADPH oxidase subunits, sorting nexins, and PtdIns 3-kinases: binding partners of SH3 domains?, Protein science : a publication of the Protein Society, 5 (1996) 2353-2357.

[106] C.D. Ellson, S. Andrews, L.R. Stephens, P.T. Hawkins, The PX domain: a new phosphoinositide-binding module, J Cell Sci, 115 (2002) 1099-1105.

[107] T.K. Sato, M. Overduin, S.D. Emr, Location, location, location: membrane targeting directed by PX domains, Science, 294 (2001) 1881-1885.

[108] L.F. Seet, W. Hong, The Phox (PX) domain proteins and membrane traffic, Biochimica et biophysica acta, 1761 (2006) 878-896.

[109] R.C. Kurten, D.L. Cadena, G.N. Gill, Enhanced degradation of EGF receptors by a sorting nexin, SNX1, Science, 272 (1996) 1008-1010.

[110] C.A. Worby, J.E. Dixon, Sorting out the cellular functions of sorting nexins, Nature reviews. Molecular cell biology, 3 (2002) 919-931.

[111] P.J. Cullen, Endosomal sorting and signalling: an emerging role for sorting nexins, Nature reviews. Molecular cell biology, 9 (2008) 574-582.

[112] D. Yao, B. Wu, B. Qin, D. Pei, PX domain and CD domain play different roles in localization and vacuolation of Sorting Nexin 10, Chinese Science Bulletin, 54 (2009) 3965.

[113] B. Qin, M. He, X. Chen, D. Pei, Sorting nexin 10 induces giant vacuoles in mammalian cells, The Journal of biological chemistry, 281 (2006) 36891-36896.

[114] J. Xu, T. Xu, B. Wu, Y. Ye, X. You, X. Shu, D. Pei, J. Liu, Structure of sorting nexin 11 (SNX11) reveals a novel extended phox homology (PX) domain critical for inhibition of SNX10-induced vacuolation, The Journal of biological chemistry, 288 (2013) 16598-16605.

[115] C. Li, W. Ma, S. Yin, X. Liang, X. Shu, D. Pei, T.M. Egan, J. Huang, A. Pan, Z. Li, Sorting Nexin 11 Regulates Lysosomal Degradation of Plasma Membrane TRPV3, Traffic, (2016).

[116] B.J. Hanson, W. Hong, Evidence for a role of SNX16 in regulating traffic between the early and later endosomal compartments, The Journal of biological chemistry, 278 (2003) 34617-34630.

[117] J.H. Choi, W.P. Hong, M.J. Kim, J.H. Kim, S.H. Ryu, P.G. Suh, Sorting nexin 16 regulates EGF receptor trafficking by phosphatidylinositol-3-phosphate interaction with the Phox domain, J Cell Sci, 117 (2004) 4209-4218.

[118] J. Xu, L. Zhang, Y. Ye, Y. Shan, C. Wan, J. Wang, D. Pei, X. Shu, J. Liu, SNX16 Regulates the Recycling of E-Cadherin through a Unique Mechanism of Coordinated Membrane and Cargo Binding, Structure (London, England : 1993), 25 (2017) 1251-1263.e1255.

[119] B. Brankatschk, V. Pons, R.G. Parton, J. Gruenberg, Role of SNX16 in the dynamics of tubulo-cisternal membrane domains of late endosomes, PloS one, 6 (2011) e21771.

[120] A.A. Rodal, A.D. Blunk, Y. Akbergenova, R.A. Jorquera, L.K. Buhl, J.T. Littleton, A presynaptic endosomal trafficking pathway controls synaptic growth signaling, The Journal of cell biology, 193 (2011) 201-217.

[121] L. Zhang, D. Qin, C. Hao, X. Shu, D. Pei, SNX16 negatively regulates the migration and tumorigenesis of MCF-7 cells, Cell regeneration (London, England), 2 (2013) 3.

[122] U.Y. Schaff, H.H. Shih, M. Lorenz, D. Sako, R. Kriz, K. Milarski, B. Bates, B. Tchernychev, G.D. Shaw, S.I. Simon, SLIC-1/sorting nexin 20: a novel sorting nexin that directs subcellular distribution of PSGL-1, European journal of immunology, 38 (2008) 550-564.

[123] T. Clairfeuille, S.J. Norwood, X. Qi, R.D. Teasdale, B.M. Collins, Structure and Membrane Binding Properties of the Endosomal Tetratricopeptide Repeat (TPR) Domain-containing Sorting Nexins SNX20 and SNX21, The Journal of biological chemistry, 290 (2015) 14504-14517.

[124] W. Zeng, W. Yuan, Y. Wang, W. Jiao, Y. Zhu, C. Huang, D. Li, Y. Li, C. Zhu, X. Wu, M. Liu, Expression of a novel member of sorting nexin gene family, SNX-L, in human liver development, Biochemical and biophysical research communications, 299 (2002) 542-548.

[125] A. Landi, J. Vermeire, V. Iannucci, H. Vanderstraeten, E. Naessens, M. Bentahir, B. Verhasselt, Genome-wide shRNA screening identifies host factors involved in early endocytic events for HIV-1-induced CD4 down-regulation, Retrovirology, 11 (2014) 118.

[126] Y.J. Lin, J.S. Chang, X. Liu, T.H. Lin, S.M. Huang, C.C. Liao, C.W. Lin, W.K. Chien, J.H. Chen, J.Y. Wu, C.H. Chen, L.C. Chang, H. Tsang, K.T. Jeang, C.Y. Chen, F.J. Tsai, Sorting nexin 24 genetic variation associates with coronary artery aneurysm severity in Kawasaki disease patients, Cell & bioscience, 3 (2013) 44.

[127] C. Mim, V.M. Unger, Membrane curvature and its generation by BAR proteins, Trends in biochemical sciences, 37 (2012) 526-533.

[128] A. Frost, V.M. Unger, P. De Camilli, The BAR domain superfamily: membrane-molding macromolecules, Cell, 137 (2009) 191-196.

[129] D.G. Schwarz, C.T. Griffin, E.A. Schneider, D. Yee, T. Magnuson, Genetic analysis of sorting nexins 1 and 2 reveals a redundant and essential function in mice, Molecular biology of the cell, 13 (2002) 3588-3600.

[130] A. Gullapalli, T.A. Garrett, M.M. Paing, C.T. Griffin, Y. Yang, J. Trejo, A role for sorting nexin 2 in epidermal growth factor receptor down-regulation: evidence for distinct functions of sorting nexin 1 and 2 in protein trafficking, Molecular biology of the cell, 15 (2004) 2143-2155.

[131] H. Liu, Z.Q. Liu, C.X. Chen, S. Magill, Y. Jiang, Y.J. Liu, Inhibitory regulation of EGF receptor degradation by sorting nexin 5, Biochemical and biophysical research communications, 342 (2006) 537-546.

[132] W.T. Parks, D.B. Frank, C. Huff, C. Renfrew Haft, J. Martin, X. Meng, M.P. de Caestecker, J.G. McNally, A. Reddi, S.I. Taylor, A.B. Roberts, T. Wang, R.J. Lechleider, Sorting nexin 6, a novel SNX, interacts with the transforming growth factor-beta family of receptor serine-threonine kinases, The Journal of biological chemistry, 276 (2001) 19332-19339.

[133] A.B. Dyve, J. Bergan, A. Utskarpen, K. Sandvig, Sorting nexin 8 regulates endosome-to-Golgi transport, Biochemical and biophysical research communications, 390 (2009) 109-114.

[134] G. Muirhead, K.K. Dev, The expression of neuronal sorting nexin 8 (SNX8) exacerbates abnormal cholesterol levels, Journal of molecular neuroscience : MN, 53 (2014) 125-134.

[135] S. Treusch, S. Hamamichi, J.L. Goodman, K.E. Matlack, C.Y. Chung, V. Baru, J.M. Shulman, A. Parrado, B.J. Bevis, J.S. Valastyan, H. Han, M. Lindhagen-Persson, E.M. Reiman, D.A. Evans, D.A. Bennett, A. Olofsson, P.L. DeJager, R.E. Tanzi, K.A. Caldwell, G.A. Caldwell, S. Lindquist, Functional links between Abeta toxicity, endocytic trafficking, and Alzheimer's disease risk factors in yeast, Science, 334 (2011) 1241-1245.

[136] R. Lundmark, S.R. Carlsson, Sorting nexin 9 participates in clathrin-mediated endocytosis through interactions with the core components, The Journal of biological chemistry, 278 (2003) 46772-46781.

[137] F. Soulet, D. Yarar, M. Leonard, S.L. Schmid, SNX9 regulates dynamin assembly and is required for efficient clathrin-mediated endocytosis, Molecular biology of the cell, 16 (2005) 2058-2067.

[138] N. Shin, S. Lee, N. Ahn, S.A. Kim, S.G. Ahn, Z. YongPark, S. Chang, Sorting nexin 9 interacts with dynamin 1 and N-WASP and coordinates synaptic vesicle endocytosis, The Journal of biological chemistry, 282 (2007) 28939-28950.

[139] N. Shin, N. Ahn, B. Chang-Ileto, J. Park, K. Takei, S.G. Ahn, S.A. Kim, G. Di Paolo, S. Chang, SNX9 regulates tubular invagination of the plasma membrane through interaction with actin cytoskeleton and dynamin 2, J Cell Sci, 121 (2008) 1252-1263.

[140] N. Bendris, S.L. Schmid, Endocytosis, Metastasis and Beyond: Multiple Facets of SNX9, Trends in cell biology, (2016).

[141] J. Park, Y. Kim, S. Lee, J.J. Park, Z.Y. Park, W. Sun, H. Kim, S. Chang, SNX18 shares a redundant role with SNX9 and modulates endocytic trafficking at the plasma membrane, J Cell Sci, 123 (2010) 1742-1750.

[142] K. Haberg, R. Lundmark, S.R. Carlsson, SNX18 is an SNX9 paralog that acts as a membrane tubulator in AP-1-positive endosomal trafficking, J Cell Sci, 121 (2008) 1495-1505.

[143] H. Knaevelsrud, S.R. Carlsson, A. Simonsen, SNX18 tubulates recycling endosomes for autophagosome biogenesis, Autophagy, 9 (2013) 1639-1641.

[144] A. Heiseke, S. Schobel, S.F. Lichtenthaler, I. Vorberg, M.H. Groschup, H. Kretzschmar, H.M. Schatzl, M. Nunziante, The novel sorting nexin SNX33 interferes with cellular PrP formation by modulation of PrP shedding, Traffic, 9 (2008) 1116-1129.

[145] M.P. Ma, M. Chircop, SNX9, SNX18 and SNX33 are required for progression through and completion of mitosis, J Cell Sci, 125 (2012) 4372-4382.

[146] G.B. Willars, Mammalian RGS proteins: multifunctional regulators of cellular signalling, Seminars in cell & developmental biology, 17 (2006) 363-376.

[147] C. Mas, S.J. Norwood, A. Bugarcic, G. Kinna, N. Leneva, O. Kovtun, R. Ghai, L.E. Ona Yanez, J.L. Davis, R.D. Teasdale, B.M. Collins, Structural basis for different phosphoinositide specificities of the PX domains of sorting nexins regulating G-protein signaling, The Journal of biological chemistry, 289 (2014) 28554-28568.

[148] B. Zheng, Y.C. Ma, R.S. Ostrom, C. Lavoie, G.N. Gill, P.A. Insel, X.Y. Huang, M.G. Farquhar, RGS-PX1, a GAP for GalphaS and sorting nexin in vesicular trafficking, Science, 294 (2001) 1939-1942.

[149] J. Li, C. Li, D. Zhang, D. Shi, M. Qi, J. Feng, T. Yuan, X. Xu, D. Liang, L. Xu, H. Zhang, Y. Liu, J. Chen, J. Ye, W. Jiang, Y. Cui, Y. Zhang, L. Peng, Z. Zhou, Y.H. Chen, SNX13 reduction mediates heart failure through degradative sorting of apoptosis repressor with caspase recruitment domain, Nature communications, 5 (2014) 5177.

[150] B. Zheng, T. Tang, N. Tang, K. Kudlicka, K. Ohtsubo, P. Ma, J.D. Marth, M.G. Farquhar, E. Lehtonen, Essential role of RGS-PX1/sorting nexin 13 in mouse development and regulation of endocytosis dynamics, Proceedings of the National Academy of Sciences of the United States of America, 103 (2006) 16776-16781.

[151] N. Akizu, V. Cantagrel, M.S. Zaki, L. Al-Gazali, X. Wang, R.O. Rosti, E. Dikoglu, A.B. Gelot, B. Rosti, K.K. Vaux, E.M. Scott, J.L. Silhavy, J. Schroth, B. Copeland, A.E. Schaffer, P.L. Gordts, J.D. Esko, M.D. Buschman, S.J. Field, G. Napolitano, G.M. Abdel-Salam, R.K. Ozgul, M.S. Sagiroglu, M. Azam, S. Ismail, M. Aglan, L. Selim, I.G. Mahmoud, S. Abdel-Hadi, A.E. Badawy, A.A. Sadek, F. Mojahedi, H. Kayserili, A. Masri, L. Bastaki, S. Temtamy, U. Muller, I. Desguerre, J.L. Casanova, A. Dursun, M. Gunel, S.B. Gabriel, P. de Lonlay, J.G. Gleeson, Biallelic mutations in SNX14 cause a syndromic form of cerebellar atrophy and lysosome-autophagosome dysfunction, Nat Genet, 47 (2015) 528-534.

[152] H.S. Huang, B.J. Yoon, S. Brooks, R. Bakal, J. Berrios, R.S. Larsen, M.L. Wallace, J.E. Han, E.H. Chung, M.J. Zylka, B.D. Philpot, Snx14 regulates neuronal excitability, promotes synaptic transmission, and is imprinted in the brain of mice, PloS one, 9 (2014) e98383.

[153] A.C. Thomas, H. Williams, N. Seto-Salvia, C. Bacchelli, D. Jenkins, M. O'Sullivan, K. Mengrelis, M. Ishida, L. Ocaka, E. Chanudet, C. James, F. Lescai, G. Anderson, D. Morrogh, M. Ryten, A.J. Duncan, Y.J. Pai, J.M. Saraiva, F. Ramos, B. Farren, D. Saunders, B. Vernay, P. Gissen, A. Straatmaan-Iwanowska, F. Baas, N.W. Wood, J. Hersheson, H. Houlden, J. Hurst, R. Scott, M. Bitner-Glindzicz, G.E. Moore, S.B. Sousa, P. Stanier, Mutations in SNX14 cause a distinctive autosomal-recessive cerebellar ataxia and intellectual disability syndrome, American journal of human genetics, 95 (2014) 611-621.

[154] S.I. Harashima, C. Harashima, T. Nishimura, Y. Hu, A.L. Notkins, Overexpression of the autoantigen IA-2 puts beta cells into a pre-apoptotic state: autoantigen-induced, but non-autoimmune-mediated, tissue destruction, Clinical and experimental immunology, 150 (2007) 49-60.

[155] S. Harashima, T. Horiuchi, Y. Wang, A.L. Notkins, Y. Seino, N. Inagaki, Sorting nexin 19 regulates the number of dense core vesicles in pancreatic beta-cells, Journal of diabetes investigation, 3 (2012) 52-61.

[156] A.H. Olsson, P. Volkov, K. Bacos, T. Dayeh, E. Hall, E.A. Nilsson, C. Ladenvall, T. Ronn, C. Ling, Genome-wide associations between genetic and epigenetic variation influence mRNA expression and insulin secretion in human pancreatic islets, PLoS genetics, 10 (2014) e1004735.

[157] X. Hao, Y. Wang, F. Ren, S. Zhu, Y. Ren, B. Jia, Y.P. Li, Y. Shi, Z. Chang, SNX25 regulates TGF-beta signaling by enhancing the receptor degradation, Cellular signalling, 23 (2011) 935-946.

[158] Y. Du, Y. Zou, W. Yu, R. Shi, M. Zhang, W. Yang, J. Duan, Y. Deng, X. Wang, Y. Lu, Expression pattern of sorting Nexin 25 in temporal lobe epilepsy: a study on patients and pilocarpine-induced rats, Brain research, 1509 (2013) 79-85.

[159] J.H. Lee, R. Cheng, B. Vardarajan, R. Lantigua, D. Reyes-Dumeyer, W. Ortmann, R.R. Graham, T. Bhangale, T.W. Behrens, M. Medrano, I.Z. Jimenez-Velazquez, R. Mayeux, Genetic Modifiers of Age at Onset in Carriers of the G206A Mutation in PSEN1 With

Familial Alzheimer Disease Among Caribbean Hispanics, JAMA neurology, 72 (2015) 1043-1051.

[160] S. Hoepfner, F. Severin, A. Cabezas, B. Habermann, A. Runge, D. Gillooly, H. Stenmark, M. Zerial, Modulation of receptor recycling and degradation by the endosomal kinesin KIF16B, Cell, 121 (2005) 437-450.

[161] A. Farkhondeh, S. Niwa, Y. Takei, N. Hirokawa, Characterizing KIF16B in neurons reveals a novel intramolecular "stalk inhibition" mechanism that regulates its capacity to potentiate the selective somatodendritic localization of early endosomes, The Journal of neuroscience : the official journal of the Society for Neuroscience, 35 (2015) 5067-5086.

[162] N.R. Blatner, M.I. Wilson, C. Lei, W. Hong, D. Murray, R.L. Williams, W. Cho, The structural basis of novel endosome anchoring activity of KIF16B kinesin, The EMBO journal, 26 (2007) 3709-3719.

[163] S.H. Chiang, J. Hwang, M. Legendre, M. Zhang, A. Kimura, A.R. Saltiel, TCGAP, a multidomain Rho GTPase-activating protein involved in insulin-stimulated glucose transport, The EMBO journal, 22 (2003) 2679-2691.

[164] T. Nakazawa, R. Hashimoto, K. Sakoori, Y. Sugaya, A. Tanimura, Y. Hashimotodani, K. Ohi, H. Yamamori, Y. Yasuda, S. Umeda-Yano, Y. Kiyama, K. Konno, T. Inoue, K. Yokoyama, T. Inoue, S. Numata, T. Ohnuma, N. Iwata, N. Ozaki, H. Hashimoto, M. Watanabe, T. Manabe, T. Yamamoto, M. Takeda, M. Kano, Emerging roles of ARHGAP33 in intracellular trafficking of TrkB and pathophysiology of neuropsychiatric disorders, Nature communications, 7 (2016) 10594.

[165] Y. Kim, C.M. Ha, S. Chang, SNX26, a GTPase-activating protein for Cdc42, interacts with PSD-95 protein and is involved in activity-dependent dendritic spine formation in mature neurons, The Journal of biological chemistry, 288 (2013) 29453-29466.

[166] H. Liu, T. Nakazawa, T. Tezuka, T. Yamamoto, Physical and functional interaction of Fyn tyrosine kinase with a brain-enriched Rho GTPase-activating protein TCGAP, The Journal of biological chemistry, 281 (2006) 23611-23619.

[167] S. Schuster, M. Rivalan, U. Strauss, L. Stoenica, T. Trimbuch, N. Rademacher, S. Parthasarathy, D. Lajko, C. Rosenmund, S.A. Shoichet, Y. Winter, V. Tarabykin, M. Rosario, NOMA-GAP/ARHGAP33 regulates synapse development and autistic-like behavior in the mouse, Molecular psychiatry, 20 (2015) 1120-1131.

[168] B. Lassegue, A. San Martin, K.K. Griendling, Biochemistry, physiology, and pathophysiology of NADPH oxidases in the cardiovascular system, Circulation research, 110 (2012) 1364-1390.

[169] A. Panday, M.K. Sahoo, D. Osorio, S. Batra, NADPH oxidases: an overview from structure to innate immunity-associated pathologies, Cellular & molecular immunology, 12 (2015) 5-23.

[170] B. Banfi, R.A. Clark, K. Steger, K.H. Krause, Two novel proteins activate superoxide generation by the NADPH oxidase NOX1, The Journal of biological chemistry, 278 (2003) 3510-3513.

[171] G. Cheng, D. Ritsick, J.D. Lambeth, Nox3 regulation by NOXO1, p47phox, and p67phox, The Journal of biological chemistry, 279 (2004) 34250-34255.

[172] G. Cheng, J.D. Lambeth, NOXO1, regulation of lipid binding, localization, and activation of Nox1 by the Phox homology (PX) domain, The Journal of biological chemistry, 279 (2004) 4737-4742.

[173] I. Callebaut, J. de Gunzburg, B. Goud, J.P. Mornon, RUN domains: a new family of domains involved in Ras-like GTPase signaling, Trends in biochemical sciences, 26 (2001) 79-83.

[174] H. Yoshida, Y. Kitagishi, N. Okumura, M. Murakami, Y. Nishimura, S. Matsuda, How do you RUN on?, FEBS letters, 585 (2011) 1707-1710.

[175] A.D. Borglum, D. Demontis, J. Grove, J. Pallesen, M.V. Hollegaard, C.B. Pedersen, A. Hedemand, M. Mattheisen, A. Uitterlinden, M. Nyegaard, T. Orntoft, C. Wiuf, M. Didriksen, M. Nordentoft, M.M. Nothen, M. Rietschel, R.A. Ophoff, S. Cichon, R.H. Yolken, D.M. Hougaard, P.B. Mortensen, O. Mors, Genome-wide study of association and interaction with maternal cytomegalovirus infection suggests new schizophrenia loci, Molecular psychiatry, 19 (2014) 325-333.

[176] R. Ghai, B.M. Collins, PX-FERM proteins: A link between endosomal trafficking and signaling?, Small GTPases, 2 (2011) 259-263.

[177] R. Ghai, M. Mobli, S.J. Norwood, A. Bugarcic, R.D. Teasdale, G.F. King, B.M. Collins, Phox homology band 4.1/ezrin/radixin/moesin-like proteins function as molecular scaffolds that interact with cargo receptors and Ras GTPases, Proceedings of the National Academy of Sciences of the United States of America, 108 (2011) 7763-7768.

[178] N. Vieira, F.M. Deng, F.X. Liang, Y. Liao, J. Chang, G. Zhou, W. Zheng, J.P. Simon, M. Ding, X.R. Wu, R. Romih, G. Kreibich, T.T. Sun, SNX31: a novel sorting nexin associated with the uroplakin-degrading multivesicular bodies in terminally differentiated urothelial cells, PloS one, 9 (2014) e99644.

[179] H.Y. Tseng, N. Thorausch, T. Ziegler, A. Meves, R. Fassler, R.T. Bottcher, Sorting nexin 31 binds multiple beta integrin cytoplasmic domains and regulates beta1 integrin surface levels and stability, Journal of molecular biology, 426 (2014) 3180-3194.

[180] J. Lee, C. Retamal, L. Cuitino, A. Caruano-Yzermans, J.E. Shin, P. van Kerkhof, M.P. Marzolo, G. Bu, Adaptor protein sorting nexin 17 regulates amyloid precursor protein trafficking and processing in the early endosomes, The Journal of biological chemistry, 283 (2008) 11501-11508.

[181] J.J. Burden, X.M. Sun, A.B. Garcia, A.K. Soutar, Sorting motifs in the intracellular domain of the low density lipoprotein receptor interact with a novel domain of sorting nexin-17, The Journal of biological chemistry, 279 (2004) 16237-16245.

[182] P. van Kerkhof, J. Lee, L. McCormick, E. Tetrault, W. Lu, M. Schoenfish, V. Oorschot, G.J. Strous, J. Klumperman, G. Bu, Sorting nexin 17 facilitates LRP recycling in the early endosome, The EMBO journal, 24 (2005) 2851-2861.

[183] P.H. Reddy, S. McWeeney, B.S. Park, M. Manczak, R.V. Gutala, D. Partovi, Y. Jung, V. Yau, R. Searles, M. Mori, J. Quinn, Gene expression profiles of transcripts in amyloid precursor protein transgenic mice: up-regulation of mitochondrial metabolism and apoptotic genes is an early cellular change in Alzheimer's disease, Human molecular genetics, 13 (2004) 1225-1240.

[184] V.A. Barr, S.A. Phillips, S.I. Taylor, C.R. Haft, Overexpression of a novel sorting nexin, SNX15, affects endosome morphology and protein trafficking, Traffic, 1 (2000) 904-916.

[185] C. Danson, E. Brown, O.J. Hemmings, I.J. McGough, S. Yarwood, K.J. Heesom, J.G. Carlton, J. Martin-Serrano, M.T. May, P. Verkade, P.J. Cullen, SNX15 links clathrin

endocytosis to the PtdIns3P early endosome independently of the APPL1 endosome, J Cell Sci, 126 (2013) 4885-4899.

[186] H. Okada, W. Zhang, C. Peterhoff, J.C. Hwang, R.A. Nixon, S.H. Ryu, T.W. Kim, Proteomic identification of sorting nexin 6 as a negative regulator of BACE1-mediated APP processing, Faseb J, 24 (2010) 2783-2794.

[187] T. Wassmer, N. Attar, M.V. Bujny, J. Oakley, C.J. Traer, P.J. Cullen, A loss-of-function screen reveals SNX5 and SNX6 as potential components of the mammalian retromer, J Cell Sci, 120 (2007) 45-54.

[188] V. Pons, C. Ustunel, C. Rolland, E. Torti, R.G. Parton, J. Gruenberg, SNX12 role in endosome membrane transport, PloS one, 7 (2012) e38949.

[189] N.Y. Kim, M.H. Cho, S.H. Won, H.J. Kang, S.Y. Yoon, D.H. Kim, Sorting nexin-4 regulates beta-amyloid production by modulating beta-site-activating cleavage enzyme-1, Alzheimer's research & therapy, 9 (2017) 4.

[190] S.S. Skanland, S. Walchli, A. Brech, K. Sandvig, SNX4 in complex with clathrin and dynein: implications for endosome movement, PloS one, 4 (2009) e5935.

[191] C. Leprince, E. Le Scolan, B. Meunier, V. Fraisier, N. Brandon, J. De Gunzburg, J. Camonis, Sorting nexin 4 and amphiphysin 2, a new partnership between endocytosis and intracellular trafficking, J Cell Sci, 116 (2003) 1937-1948.

[192] H.J. Lee, J.J. Zheng, PDZ domains and their binding partners: structure, specificity, and modification, Cell communication and signaling : CCS, 8 (2010) 8.

[193] F. Steinberg, M. Gallon, M. Winfield, E.C. Thomas, A.J. Bell, K.J. Heesom, J.M. Tavare, P.J. Cullen, A global analysis of SNX27-retromer assembly and cargo specificity reveals a function in glucose and metal ion transport, Nature cell biology, 15 (2013) 461-471.

[194] P. Temkin, B. Lauffer, S. Jager, P. Cimermancic, N.J. Krogan, M. von Zastrow, SNX27 mediates retromer tubule entry and endosome-to-plasma membrane trafficking of signalling receptors, Nature cell biology, 13 (2011) 715-721.

[195] L. Sun, X. Hu, W. Chen, W. He, Z. Zhang, T. Wang, Sorting nexin 27 interacts with Fzd7 and mediates Wnt signalling, Bioscience reports, 36 (2016) e00296.

[196] X. Wang, T. Huang, Y. Zhao, Q. Zheng, R.C. Thompson, G. Bu, Y.W. Zhang, W. Hong, H. Xu, Sorting nexin 27 regulates Abeta production through modulating gamma-secretase activity, Cell Rep, 9 (2014) 1023-1033.

[197] C. Chen, D. Garcia-Santos, Y. Ishikawa, A. Seguin, L. Li, K.H. Fegan, G.J. Hildick-Smith, D.I. Shah, J.D. Cooney, W. Chen, M.J. King, Y.Y. Yien, I.J. Schultz, H. Anderson, A.J. Dalton, M.L. Freedman, P.D. Kingsley, J. Palis, S.M. Hattangadi, H.F. Lodish, D.M. Ward, J. Kaplan, T. Maeda, P. Ponka, B.H. Paw, Snx3 regulates recycling of the transferrin receptor and iron assimilation, Cell metabolism, 17 (2013) 343-352.

[198] M.S. Harrison, C.S. Hung, T.T. Liu, R. Christiano, T.C. Walther, C.G. Burd, A mechanism for retromer endosomal coat complex assembly with cargo, Proceedings of the National Academy of Sciences of the United States of America, 111 (2014) 267-272.

[199] Y. Xu, H. Hortsman, L. Seet, S.H. Wong, W. Hong, SNX3 regulates endosomal function through its PX-domain-mediated interaction with PtdIns(3)P, Nature cell biology, 3 (2001) 658-666.

[200] M. Harterink, F. Port, M.J. Lorenowicz, I.J. McGough, M. Silhankova, M.C. Betist, J.R. van Weering, R.G. van Heesbeen, T.C. Middelkoop, K. Basler, P.J. Cullen, H.C.

Korswagen, A SNX3-dependent retromer pathway mediates retrograde transport of the Wnt sorting receptor Wntless and is required for Wnt secretion, Nature cell biology, 13 (2011) 914-923.

[201] S. Boulkroun, D. Ruffieux-Daidie, J.J. Vitagliano, O. Poirot, R.P. Charles, D. Lagnaz, D. Firsov, S. Kellenberger, O. Staub, Vasopressin-inducible ubiquitin-specific protease 10 increases ENaC cell surface expression by deubiquitylating and stabilizing sorting nexin 3, American journal of physiology. Renal physiology, 295 (2008) F889-900.

[202] R. Mizutani, K. Nakamura, S. Yokoyama, A. Sanbe, S. Kusakawa, Y. Miyamoto, T. Torii, H. Asahara, H. Okado, J. Yamauchi, A. Tanoue, Developmental expression of sorting nexin 3 in the mouse central nervous system, Gene expression patterns : GEP, 11 (2011) 33-40.

[203] K.W. Li, M.P. Hornshaw, R.C. Van Der Schors, R. Watson, S. Tate, B. Casetta, C.R. Jimenez, Y. Gouwenberg, E.D. Gundelfinger, K.H. Smalla, A.B. Smit, Proteomics analysis of rat brain postsynaptic density. Implications of the diverse protein functional groups for the integration of synaptic physiology, The Journal of biological chemistry, 279 (2004) 987-1002.

[204] R. Mizutani, J. Yamauchi, S. Kusakawa, K. Nakamura, A. Sanbe, T. Torii, Y. Miyamoto, A. Tanoue, Sorting nexin 3, a protein upregulated by lithium, contains a novel phosphatidylinositol-binding sequence and mediates neurite outgrowth in N1E-115 cells, Cellular signalling, 21 (2009) 1586-1594.

[205] W.K. Dowjat, T. Wisniewski, S. Efthimiopoulos, H.M. Wisniewski, Inhibition of neurite outgrowth by familial Alzheimer's disease-linked presenilin-1 mutations, Neurosci Lett, 267 (1999) 141-144.

[206] B.N. Vardarajan, S.Y. Bruesegem, M.E. Harbour, R. Inzelberg, R. Friedland, P. St George-Hyslop, M.N. Seaman, L.A. Farrer, Identification of Alzheimer disease-associated variants in genes that regulate retromer function, Neurobiol Aging, 33 (2012) 2231.e2215-2231.e2230.

[207] L. Xu, W. Yin, J. Xia, M. Peng, S. Li, S. Lin, D. Pei, X. Shu, An antiapoptotic role of sorting nexin 7 is required for liver development in zebrafish, Hepatology (Baltimore, Md.), 55 (2012) 1985-1993.

[208] T.T. Rohn, E. Head, W.H. Nesse, C.W. Cotman, D.H. Cribbs, Activation of caspase-8 in the Alzheimer's disease brain, Neurobiology of disease, 8 (2001) 1006-1016.

[209] T. Raj, J.M. Shulman, B.T. Keenan, L.B. Chibnik, D.A. Evans, D.A. Bennett, B.E. Stranger, P.L. De Jager, Alzheimer disease susceptibility loci: evidence for a protein network under natural selection, American journal of human genetics, 90 (2012) 720-726.

[210] S. Sekar, J. McDonald, L. Cuyugan, J. Aldrich, A. Kurdoglu, J. Adkins, G. Serrano, T.G. Beach, D.W. Craig, J. Valla, E.M. Reiman, W.S. Liang, Alzheimer's disease is associated with altered expression of genes involved in immune response and mitochondrial processes in astrocytes, Neurobiol Aging, 36 (2015) 583-591.

[211] S.M. Nigam, S. Xu, F. Ackermann, J.A. Gregory, J. Lundkvist, U. Lendahl, L. Brodin, Endogenous APP accumulates in synapses after BACE1 inhibition, Neuroscience research, 109 (2016) 9-15.

[212] J.L. Lundgren, S. Ahmed, S. Schedin-Weiss, G.K. Gouras, B. Winblad, L.O. Tjernberg, S. Frykman, ADAM10 and BACE1 are localized to synaptic vesicles, Journal of neurochemistry, (2015).

[213] P.A. Adlard, V.M. Perreau, V. Pop, C.W. Cotman, Voluntary exercise decreases amyloid load in a transgenic model of Alzheimer's disease, The Journal of neuroscience : the official journal of the Society for Neuroscience, 25 (2005) 4217-4221.

[214] S. Holback, L. Adlerz, K. Iverfeldt, Increased processing of APLP2 and APP with concomitant formation of APP intracellular domains in BDNF and retinoic acid-differentiated human neuroblastoma cells, Journal of neurochemistry, 95 (2005) 1059-1068.

[215] H.S. Um, E.B. Kang, Y.H. Leem, I.H. Cho, C.H. Yang, K.R. Chae, D.Y. Hwang, J.Y. Cho, Exercise training acts as a therapeutic strategy for reduction of the pathogenic phenotypes for Alzheimer's disease in an NSE/APPsw-transgenic model, International journal of molecular medicine, 22 (2008) 529-539.

[216] S.M. Nigam, S. Xu, J.S. Kritikou, K. Marosi, L. Brodin, M.P. Mattson, Exercise and BDNF reduce Abeta production by enhancing alpha-secretase processing of APP, Journal of neurochemistry, 142 (2017) 286-296.

[217] U. Das, L. Wang, A. Ganguly, J.M. Saikia, S.L. Wagner, E.H. Koo, S. Roy, Visualizing APP and BACE-1 approximation in neurons yields insight into the amyloidogenic pathway, Nature neuroscience, 19 (2016) 55-64.

[218] J.R. Cirrito, J.E. Kang, J. Lee, F.R. Stewart, D.K. Verges, L.M. Silverio, G. Bu, S. Mennerick, D.M. Holtzman, Endocytosis is required for synaptic activity-dependent release of amyloid-beta in vivo, Neuron, 58 (2008) 42-51.

[219] U. Das, D.A. Scott, A. Ganguly, E.H. Koo, Y. Tang, S. Roy, Activity-induced convergence of APP and BACE-1 in acidic microdomains via an endocytosis-dependent pathway, Neuron, 79 (2013) 447-460.

[220] Y. Sekine-Aizawa, R.L. Huganir, Imaging of receptor trafficking by using alpha-bungarotoxin-binding-site-tagged receptors, Proceedings of the National Academy of Sciences of the United States of America, 101 (2004) 17114-17119.

[221] S.L. Cole, R. Vassar, The role of amyloid precursor protein processing by BACE1, the beta-secretase, in Alzheimer disease pathophysiology, The Journal of biological chemistry, 283 (2008) 29621-29625.

[222] Y. Zhao, Y. Wang, J. Yang, X. Wang, Y. Zhao, X. Zhang, Y.W. Zhang, Sorting nexin 12 interacts with BACE1 and regulates BACE1-mediated APP processing, Molecular neurodegeneration, 7 (2012) 30.

[223] H. Ling, P. Ardjomand, S. Samvakas, A. Simm, G.L. Busch, F. Lang, K. Sebekova, A. Heidland, Mesangial cell hypertrophy induced by NH4Cl: role of depressed activities of cathepsins due to elevated lysosomal pH, Kidney international, 53 (1998) 1706-1712.

[224] A.A. Golabek, E. Kida, M. Walus, W. Kaczmarski, M. Michalewski, K.E. Wisniewski, CLN3 protein regulates lysosomal pH and alters intracellular processing of Alzheimer's amyloid-beta protein precursor and cathepsin D in human cells, Molecular genetics and metabolism, 70 (2000) 203-213.

[225] S. Xu, L. Zhang, L. Brodin, Overexpression of SNX7 reduces Abeta production by enhancing lysosomal degradation of APP, Biochemical and biophysical research communications, (2017).