2015-03-01

Rab Proteins and Alzheimer's: A Current Review of Their Involvement in Amyloid Beta Generation with Focus on Rab10 Expression in N2A-695 Cells

Ivan Arano Rodriguez
Brigham Young University - Provo

Rab Proteins and Alzheimer's: A Current Review of Their Involvement in Amyloid Beta Generation with Focus on Rab10 Expression in N2A-695 Cells

Ivan Arano Rodriguez

A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Master of Science

John S. K. Kauwe, Chair
Perry G. Ridge
Paul R. Reynolds

Department of Biology

Brigham Young University

March 2015

ABSTRACT

Rab Proteins and Alzheimers: A Current Review of Their Involvement in Amyloid Beta Generation with Focus on Rab10 Expression in N2A-695 Cells

Ivan Arano Rodriguez
Department of Biology, BYU
Master of Science

This thesis work describes the role of Rab proteins in amyloid processing and clearance in different cell pathways. It also describes an experimental approach used to analyze the expression effects of Rab10 in amyloid beta production. Since the main theory behind neurodegeneration in Alzheimer's disease claims that high levels of amyloid beta 42 (Aβ42) molecules trigger widespread neuronal death, control of Aβ42 has been a main target in Alzheimer's disease research. In addition, several studies show increased levels of particular Rab proteins in Alzheimer's pathogenesis. However, no review consolidates current findings in neurodegeneration of Alzheimer's with Rab protein dysfunction. The first chapter of this thesis aims to address this need by providing a current review of Rab proteins associated with APP and neurodegeneration. The second chapter constitutes an experimental approach used to characterize the effects of Rab10 and Sar1A GTPases in APP and amyloid processing. We found that Rab10 expression does not affect APP production but significantly changes Aβ generation, particularly the toxic Aβ42 and Aβ42:40 ratio. On the other hand, we found no significant effect of Sar1A expression on either APP or amyloid beta generation. These findings partially confirm the work done by Kauwe et al (2015) and provide preliminary evidence for two potential targets for protective effects in neurodegeneration.

Keywords: Rab proteins, GTPases, Alzheimer's disease, gene, genetic variants, 3' UTR, amyloid beta, neurodegeneration, endocytosis, anterograde transport, autophagy, amyloid precursor protein, transient transfection, overexpression, knockdown

ACKNOWLEDGMENTS

This thesis work is the result of many efforts combined. I would not have been able to finish this work without the guidance of my committee members, friends and collaborators. Specifically, I would like to offer acknowledgement of gratitude towards the contributions of the following individuals:

Dr. John Kauwe for being an excellent mentor end example during these two years and for providing a fantastic atmosphere for continuous learning and discovery. It has been a fun, challenging and a bounteous ride and this work would never have been accomplished without his guidance and vision.

Dr. Perry Ridge and Dr. Paul Reynolds for kindly providing their experience and laboratory resources for my learning and presentation of the results in this thesis. Their insights towards writing this thesis and presenting these results have been extremely valuable.

I am very grateful for the members of both the Kauwe and Ridge labs, since they are always willing to explain and help in difficult concepts related to the research they are doing. Their input has been a great aid in my learning in these two years.

Dr. Celeste Karch for her mentorship in teaching me essential concepts and skills in her laboratory as well as for her help in completing the experimental part of this thesis. The experimental part of this thesis would not have been accomplished without her and her laboratory.

My lovely wife Christianna Arano and my family for their unconditional love and support in every step of the way in good and difficult times throughout my time at BYU.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER 1: Rab Gtpases in Alzheimer's Disease: A Review on Main Players That Influence Amyloid Production

Alzheimer's disease is the most common form of dementia and it is the sixth leading cause of death in the United States. In 2014, an estimated 5.2 million Americans developed Alzheimer's disease and the cost for caregiving to be $214 billion (Plassman, Langa et al. 2007). Amyloid production resulting by processing of APP is the main hypothesis associated with triggering the development of this disease. Rab GTPases are small proteins belonging to the Ras superfamily. Due to their major role in intracellular and membrane trafficking, some Rab GTPases have been identified as important players in regulating APP transport, processing and clearance. Consistent with these roles, AD brains present expression changes of Rab proteins involved in these processes. This review aims to provide a framework to understand those Rab GTPases associated with APP and neurodegeneration and to propose targets for future Alzheimer's disease research relating to this group of proteins.

APP Function and Amyloid Production

The most accepted theory for neurodegeneration in Alzheimer's disease is the widespread neuronal death triggered by high levels of a molecule called amyloid beta 42 (Aβ-42), which is produced by the cleaving of trans membrane amyloid precursor protein (APP) (Masters, Multhaup et al. 1985, Glenner and Wong 2012). This is commonly known as the amyloid cascade hypothesis (Hardy and Higgins 1992). The human APP gene is located in chromosome 21 and produces a type I protein with a large extracellular N-terminal, transmembrane and short C-terminal cytoplasmic domain (Reinhard, Hebert et al. 2005). APP is highly expressed in the brain and produces eight isoforms that range from 365 – 770 amino acids. The 695 isoform is the most expressed in the central neural system while the other isoforms can be ubiquitously

expressed in all tissues (Placido, Pereira et al. 2014). Several studies have proposed a variety of functions for APP in the brain; the most supported is the trophic effect on formation and regulation of synapses as well as neuritic outgrowth (Turner, O'Connor et al. 2003, Lee, Moussa et al. 2010). Additional evidence suggests APP is involved in synaptic plasticity (Hung, Koo et al. 1992, Oh, Savonenko et al. 2009), cell adhesion (Yamazaki, Koo et al. 1997, Sosa, Bergman et al. 2013), migration (Young-Pearse, Bai et al. 2007), axonal transport (Sisodia 2002), cell surface receptor (Selkoe 2001, Hashimoto and Matsuoka 2014) among others.

After expression, newly formed APP undergoes the constitutive secretory pathway where is subsequently internalized to the cell via clathrin mediated endocytosis (here referred as endocytosis) for processing. As part of the secretory pathway, APP undergoes several post-translation modifications in the ER and in the trans-Golgi network (TGN) including N- and O-glycosylation, cytoplasmic phosphorylation and sulfation (Selkoe 2001, Bhattacharyya, Barren et al. 2013) before being trafficked to the plasma membrane. In neurons, APP is mainly transported to the cell membrane of axons and dendrites using the axonal transport system with kinesin-1 as the main motor protein (Kins, Lauther et al. 2006). Although APP is mainly processed via endocytosis, evidence suggests that nascent APP molecules can be processed by intracellular transport from the TGN to the endosome, resulting on increased Aβ-42 production in these organelles (Capell, Meyn et al. 2002, Tam, Seah et al. 2014)

Different studies estimate that about 10% of APP is present on the plasma membrane at all times while the rest is carried into the cell via endocytosis for processing (Thinakaran and Koo 2008). APP has three main proteolytic sites (alpha, beta & gamma) and different enzymes termed secretases are used to cleave APP on each site. APP is processed in two pathways: the non-amyloidogenic pathway and the toxic or amyloidogenic pathway. The non-amyloidogenic

pathway is carried in the plasma membrane where APP is first cleaved in the N- terminus by alpha-secretase to produce sAPPα. The C- terminal is then cleaved by gamma-secretase to produce a short p3 and APP intracellular domain fragments (Reinhard, Hebert et al. 2005, Dawkins and Small 2014). In the amyloidogenic pathway, APP molecules not cleaved by alpha-secretase are internalized and processed by beta- secretase (BACE1) and the gamma- secretase complex (PSEN1 or PSEN2, nicastrin, APH1 & PEN-2) to produce sAPPβ and AB fragments ranging from 38-42 amino acids (Kamenetz, Tomita et al. 2003, Dawkins and Small 2014). This process is carried by the secretory and recycling pathways, where some APP fragments can be recycled back to the cell membrane along with sAPP-B and AB fragments. Most of the secreted amyloid beta produced contains 40 amino acids (Aβ40), however, the smaller portion of amyloid beta that contains 42 amino acids (Aβ42) tends to form extracellular aggregates (Haass, Kaether et al. 2012). The processing, aggregation and subsequent neuronal death triggered by Aβ42 fibrils are the center of the amyloid cascade hypothesis. Extensive reviews of amyloid processing and function can be accessed in references (Kamenetz, Tomita et al. 2003, Dawkins and Small 2014). Since APP is internalized to produce Aβ42, the main steps in this process, which include endocytosis and proteolytic processing via recycling and autophagy pathways will be analyzed next.

Endocytosis Pathway in Alzheimer's Pathogenesis

Endocytosis is a process by which many molecules enter the cytoplasm via cell membrane receptors. Specific cell membrane receptors contain signal domains that trigger the recruitment of clathrin molecules and the endocytic response, which engulfs the portion of the cell membrane containing the cell receptor along with other molecules around it. The intracellular and C- terminal domains of APP contain a YEMPTY motif that has been

demonstrated to be involved in the signal for endocytosis (Nordstedt, Caporaso et al. 1993, Ring, Weyer et al. 2007). Consistent with this observation, mutations on the YEMPTY domain inhibit APP internalization and Aβ42 generation, suggesting that endocytosis is critical for AB production (Perez, Soriano et al. 1999). The YEMPTY domain also serves as the binding site for other cytosolic adaptors including Mint1, Mint2, Mint3, Fe65, Dab1 and JNK protein members (Thinakaran and Koo 2008). Interestingly, Mint and Fe65 proteins also play an important role in APP endocytosis and Aβ42 regulation (Haass, Kaether et al. 2012, Perez, Soriano et al. 1999, Ring, Weyer et al. 2007). A more detailed review of the YEMPTY region of APP and predicted interactions can be accessed in these references (Kerr and Small 2005, Miller, McLoughlin et al. 2006, Schettini, Govoni et al. 2010).

In neurons, several regulatory proteins are involved in starting the endocytosis signal of APP; the most common are Rab5, Dynamin1 and, most recently, Mint1 and Mint2 proteins (Thomas, Lelos et al. 2011, Sullivan, Dillon et al. 2014). Newly formed APP vesicles are transported and fused to the first sorting station: the early endosome. Concurrently, BACE-1 is also trafficked to the early endosome from the plasma membrane where AB biogenesis occurs. Early endosomes are a major site for APP processing and Aβ42 production and gradually mature into late endosomes and subsequently into lysosomes (Cataldo, Peterhoff et al. 2000, Chia, Toh et al. 2013). Once on the early endosome, APP and AB containing vesicles can undergo different pathways: 1) early endosome to plasma membrane 2) early endosome to recycling endosome 3) early endosome to TGN or 4) early endosome to late endosome and lysosomes. Many studies show evidence of high activity of BACE-1 and gamma-secretases on these sorting pathways following endocytosis (Cataldo, Peterhoff et al. 2000, Chia, Toh et al. 2013, Lu and Hong 2014). In addition, newly formed APP molecules that are processed in intracellular fashion are also

incorporated into the TGN or into the lysosomes where are processed into AB peptides (Tam, Seah et al. 2014). Consequently, AB peptides can be measured in higher quantities along these pathways, particularly on the TGN and endosomes. Thus, keeping track of AB peptides have become a good indicator for APP processing cycle in the cell. Finally, the autophagy mechanism in the cell completes the APP processing cycle by recycling and secreting AB peptides to the extracellular space.

Macroautophagy (here referred as autophagy) serves as the main clearance mechanism for APP processing and AB clearance. During autophagy, double membrane vesicles that contain waste components and hydrolases are fused into large vesicles called autophagosomes. Autophagosomes then fuse with late endosomes, which eventually turn into lysosomes to degrade the original autophagosome components (Nixon 2007). The resulting products can be either recycled back as an amino acid source or secreted via exocytosis. A complete review of autophagy and its involvement in amyloidogenesis can be found in reference (Nixon 2007). In neurons, autophagosomes and endosomes are formed near the synapses and require retrograde transport to the lysosomes for efficient degradation (Nixon 2007). Briefly, as APP is processed in the endocytosis and intracellular pathways, the resulting AB peptides are mostly degraded by lysosomes. Upon reaching to the lysosomes, large pools of gamma-secretase components and other hydrolases complete the degradation process of APP and AB peptides, in which some Aβ42 is secreted via exocytosis (LeBlanc and Goodyer 1999, Rajendran, Honsho et al. 2006) Additional experiments show that Aβ42 was reduced when autophagy was suppressed, highlighting the role of autophagy on Aβ clearance (Lee, Yu et al. 2010).

Although endocytosis and autophagy are vital processes in APP turnover cycle, their disruption can also facilitate intracellular Aβ aggregation and apoptosis in the brain. In a model

by Yu et al. (2005), autophagy vacuoles containing full length APP and Aβ peptides accumulated in significant numbers in axons and neurites in Alzheimer's disease brains. These vacuoles were enriched in gamma-secretase activity inducing intracellular Aβ accumulation (Yu, Cuervo et al. 2005). A different study by Lee et al (2010)) showed that PSEN1 mutations in Familiar AD cases affected the autophagy turnover of proteins (Lee, Yu et al. 2010). In familiar AD, mutations along APP & gamma-secretase complex (PSEN1 & PSEN2) increase the ratio of Aβ40 to Aβ42 (Lee, Yu et al. 2010). In late-onset AD, multiple genes that regulate endocytosis or Aβ clearance have been, unsurprisingly associated with AD (Wu and Yao 2009, Ridge, Ebbert et al. 2013). Examples of these genes involve *BIN1, PICALM, CR1, CDAP2*, and some Rab GTPases. This paper will focus solely on the role of Rab GTPases involved in APP trafficking, processing and clearance.

Rab GTPases in Alzheimer's Disease

Rab GTPases are small proteins member of the Ras superfamily of monomeric proteins. They are involved in every step of the intracellular vesicle transport in the cell. Approximately 60 human Rab GTPases have been identified, being this family the largest and more heterogeneous of the Ras superfamily (Stenmark and Olkkonen 2001, Zerial and McBride 2001). Rab proteins are present in all eukaryotic cells, ranging from the most evolutionary conserved which are ubiquitously expressed for normal vesicle transport to the most specialized types that are only found on certain mammalian cell types (Seabra, Mules et al. 2002, Kaddai, Le Marchand-Brustel et al. 2008). Rab proteins switch between an inactive (GDP-bound) cytosol state and active (GTP-bound) membrane bound state. To switch from the GDP to GTP state, Rabs require activator proteins called GEFs (Guanine nucleotide exchange factors). Once in their active state, Rab-GTP recruits effector proteins that enable them to control different steps in

vesicle trafficking including cargo selection, tethering, movement and docking (Stenmark and Olkkonen 2001, Seabra, Mules et al. 2002, Grosshans, Ortiz et al. 2006, Kaddai, Le Marchand-Brustel et al. 2008). Once vesicle transport is complete, Rab-GTP is hydrolyzed by GAPs (GTPase activating proteins) and return to its inactive GDP-bound state. At this point, Rab-GDP is recycled back to the membrane of origin by binding to RabGDIs (GDP disassociation inhibitors), which determine the proper localization and activity of Rab proteins (Alory and Balch 2001, Markgraf, Peplowska et al. 2007).

The high number of components of the Rab family reflects the complexity of the intracellular transport system, thus Rab proteins can be assigned to a single or multiple sites. For instance, Rab5 plays a substantial role in endocytosis by transporting cargoes to multiple organelles (Markgraf, Peplowska et al. 2007, Ishikura, Koshkina et al. 2008), whereas Rab23 is a negative regulator in the Sonic hedgehog-signaling pathway (Eggenschwiler, Bulgakov et al. 2006). Because of their key role in vesicle transport, endocytosis and autophagy, Rab proteins have also been studied as part of the amyloid hypothesis of AB deposition. Disruptions of these pathways have been extensively documented in Alzheimer's disease (Perez, Squazzo et al. 1996, Nixon 2007, Wu and Yao 2009, Ridge, Ebbert et al. 2013, Tam, Seah et al. 2014). Specifically, this review will cover the main Rab proteins associated with APP and AB production: Rab3, Rab5, Rab6, Rab7, Rab11, Rab4 & Rab8.

Rab3

Rab3 is a small GTPase that is localized in synaptic vesicles in neural cells and thought to be highly associated with regulating vesicle fusion in exocytosis. Rab3 has four isoforms (Rab3A, Rab3B, Rab3C & Rab3D) that contain a 77-85% amino acid homology (Baldini, Hohl et al. 1992). Although deletion of Rab3 causes a lethal phenotype in mice, experiments suggest

that the role of the four isoforms might be redundant in synaptic vesicle transport (Li, Jahn et al. 1996). Rab3A is the most common of the four and localizes on the surface of synaptic vesicles of various type of neurons in the rat brain including motor, sensory, adrenelergic and cholinergic neurons[61]. More specifically Rab3A has a key role in vesicle docking in the Ca^{2+}- dependent exocytosis of neurotransmitters in synapses (Komuro, Sasaki et al. 1996, Coleman, Bill et al. 2007). In neurons, Rab3 protein associates with motor protein Kinesin-1 in anterograde transport of synaptic vesicles to the neuritic axons (Schluter, Schmitz et al. 2004, Coleman, Bill et al. 2007, Kimura, Okabayashi et al. 2012). Another role of Rab3A is the association with actively recycling vesicles carried to synapse terminals. Star et al (2007) demonstrated that vesicles that are actively recycling contain higher Rab3A than vesicles that are stationary (Star, Newton et al. 2005). Taken together, these studies suggest that Rab3 is involved in anterograde transport and exocytosis of synaptic vesicles in neurons.

Rab3A is required for anterograde transport of APP and is associated with regulating APP and AB peptide levels via the secretory pathway. A study by Szodorai et al (2009) showed that the complex required for anterograde transport of APP vesicles contained Rab3A and Kinesin-1C. Szodorai argues that Rab3A is more important for initial recruitment of APP and kinesin-1C rather than for the actual vesicle fusion to the axons (Szodorai, Kuan et al. 2009). A separate study showed that a disruption of the anterograde transport increases levels of Rab3 and it is correlated with age-dependent impartment of cognitive function (Kimura, Okabayashi et al. 2012). Rab3A was found to be significantly downregulated, among other presynaptic proteins in the frontal and parietal cortex of AD brains (Reddy, Mani et al. 2005). Consistent with these findings, a recent study by recent study by Tan et al (2014) showed that a Rab3A effector Rabphilin3A (RBP3A) is also reduced in AD post-mortem brains; this reduction was correlated

with dementia progression, cholinergic deaferrentiation and high Aβ42 aggregation (Tan, Lee et al. 2014). In a different study, a Western Blot screening of lysosomal network proteins identified Rab3 and Rab7 as significantly increased in CSF of AD patients and was validated using a second cohort. This study suggested that both Rab3 and Rab7 are upregulated in the endosomal and autophagy pathways early in disease progression (Armstrong, Mattsson et al. 2014). These studies support the observation that Rab3 expression might be reduced in the brain and upregulated in the CSF of Azheimer's disease patients. Finally, a high-throughput RNAi screening of all human Rab GTPAses confirmed the role of Rab3 in the trafficking and maintenance of APP levels in neurons. In this same study, knockdown of all isoforms of Rab3, except for Rab3C, decreased both Aβ42 and sAPP-β levels (Udayar, Buggia-Prevot et al. 2013).

Rab5

Rab5 is by far the most characterized member of the Rab GTPase proteins and the most associated with AD progression. Rab5 has three known isoforms (Rab5A, Rab5B & Rab5C), similar to Rab3; all isoforms have a similar function. Although Rab5 is widely expressed in the cell, it is mainly localized in the early endosome, cell membrane, clathrin-coated vesicles and in recycling endosomes (Gorvel, Chavrier et al. 1991, Bucci, Parton et al. 1992, Li and Stahl 1993, Somsel Rodman and Wandinger-Ness 2000). The most established functions for Rab5 are the fusion of endocytic vesicles to the early endosomes and the vesicle docking events in recycling and endocytosis. Reviews can be accessed in references (Gorvel, Chavrier et al. 1991, Somsel Rodman and Wandinger-Ness 2000, Woodman 2000). Additional functions for Rab5 include promoting cell migration and integrin signaling pathways (Mendoza, Diaz et al. 2014). Overexpression of Rab5 causes an increase of endocytosis as well as a formation of large early endosomes whereas the opposite effects applied upon down regulation of Rab5 (Stenmark,

Parton et al. 1994, Horiuchi, Lippe et al. 1997). Due to the critical role of Rab5 in endocytosis and recycling pathways, there is much evidence that links Rab5 and its effectors with neuronal degeneration and Alzheimer's disease.

Characterization of Rab5 and its effectors in endocytosis and recycling have provided important insights towards APP processing and AD pathogenesis. Disruptions of the afore mentioned pathways are one of the earliest changes observed in AD and could be one of the main mechanisms involved in the progression of this disease. Increased endocytosis markers and endosome enlargement are one of the first responses in AD, independently of Aβ42 accumulation (Cataldo, Rebeck et al. 2001, Grbovic, Mathews et al. 2003, Nixon 2005, Ginsberg, Mufson et al. 2011). Rab5 overexpression replicates many of these observations in early endosomes and dramatically increases secreted Aβ40 & Aβ42 (Cataldo, Peterhoff et al. 2000, Grbovic, Mathews et al. 2003, Nixon 2005, Ginsberg, Mufson et al. 2011). A possible mechanism is that APP functions as an endocytosis receptor for Rab5, which could trigger overexpression of Rab5 and its correspondent downstream effects in endosomal dysfunction. A study by Laifenfeld et al (2007) showed that Rab5 binds to APP-B1 and initiates a signaling pathway that leads to apoptosis in a FAD model (Laifenfeld, Patzek et al. 2007). In addition, microarray analyses show that Rab5 and Rab7 are selectively up regulated in cholinergic and hippocampal neurons of MCI and AD individuals (Ginsberg, Mufson et al. 2010, Ginsberg, Mufson et al. 2011). Along with these observations, the majority of Aβ production occurs in in Rab5-endocytic vesicles in the early endosome and lysosomes. Overexpression of Rab5 and Rab7 showed to increase Aβ42 trafficking and processing in lysosomes. This effect is further increased in *APOEe4* when compared to *APOEe3* carriers (Li, Kanekiyo et al. 2012). Interestingly, Rab5B isoform is associated with neuroprotection by providing neuronal resistance

to exocytosis injury and synaptic plasticity (Baskys, Bayazitov et al. 2007). Similarly, silencing of only Rab5C isoform increased significantly APP, sAPPβ and Aβ42 cellular levels (Li and Stahl 1993). Together, these reports highlight the role of Rab5 in neurodegeneration and as a therapeutic candidate for AD progression.

Rab6

Rab6 is another well-characterized GTPase, which is involved in anterograde and retrograde transport of APP and Aβ peptides. Rab6 has four different isoforms: Rab6A, Rab6A', Rab6B and Rab6C. Rab6A' is a splice variant generated by alternative splicing on the Rab6A gene and only differs from Rab6A by three amino acids (Opdam, Echard et al. 2000). Rab6 GTP is commonly found in the TGN and regulates retrograde and anterograde intra-Golgi pathways (Martinez, Schmidt et al. 1994, Darchen and Goud 2000). In contrast to the previous Rab GTPases, Rab6 isoforms have differential tissue expressions and functions. RabA is a dynamic GTPase that regulates retrograde transport from the TGN to the ER in a COP-I independent mechanism and from endosomes (early endosomes and recycling endosomes) to the TGN or vice versa (Martinez, Schmidt et al. 1994, Young, Stauber et al. 2005). On the other hand, Rab6A' regulates anterograde transport from the TGN to the plasma membrane(Siniossoglou and Pelham 2001, Mallard, Tang et al. 2002). Rab6B is also involved in retrograde transport in the TGN and ER but it is expressed specifically in brain cells such as neurons, microglia and Purkinje cells (Opdam, Echard et al. 2000, Wanschers, van de Vorstenbosch et al. 2007). Finally, Rab6C is the least characterized of the four isoforms and it has been associated with the cell cycle progression and drug resistance to particular cancer types (Shan, Mason et al. 2000, Young, Menetrey et al. 2010).

In neurons, Rab6B GTP requires Mint effector proteins to bind to APP. As described earlier, Mint proteins bind to the YENPTY motif of APP, facilitating anterograde transport. Studies show that Rab6 can bind to Mint1, Mint2 and Mint3 to form a complex with APP (Teber, Nagano et al. 2005, Thyrock, Ossendorf et al. 2013). Evidence suggests that Rab6 is involved in retrograde trafficking of APP from the endosomes to the TGN and from the TGN to the ER. However, there is not a consensus of the actual role of Rab6 in APP processing and AD. A study by Elfrink et al (2011) showed that Rab6 is increased in non-tangle bearing neurons. This increase might occur as a protective response to reduce ER stress neurotoxicity (Elfrink, Zwart et al. 2012). Failure to recover from ER stress also contributes to intracellular Aβ42 accumulation and neurodegeneration in early AD pathology (Soejima, Ohyagi et al. 2013). Rab6 can also promote sAPPa production and decrease intracellular Aβ42 accumulation. A recent study suggests that Rab6 selectively facilitates anterograde transport of APP to the plasma membrane to be processed by alpha-secretase (McConlogue, Castellano et al. 1996). Moreover, a recent RNAi screen showed that silencing of Rab6 decreased Aβ42 production; the authors concluded that this effect could be a result of an alteration of the Gamma-secretase cleavage or the secretion of Aβ (Udayar, Buggia-Prevot et al. 2013). This conclusion is in line with previous results in fibroblasts where Rab6 is dependent on PSEN1 and protein kinase C for membrane association (Scheper, Zwart et al. 2004). These observations suggest of a protective role of Rab6 for neurodegeneration, however, further experiments are needed to assess this specific connection.

Rab7

Rab7 is a GPTase required in the late stages of the recycling and autophagy pathways. Rab7 is ubiquitously expressed and it comes in one isoform. However, a new Rab GTPase that

contains 65% identity with Rab7 has been named Rab7b. This GTPase is not coded by the same gene and seems to have different functions than Rab7 (Progida, Cogli et al. 2010). Rab7 has a critical role in regulating the recycling pathway, specifically in late steps in endocytosis and the maturation of endosomes and autophagosomes (Feng, Press et al. 1995, Gutierrez, Munafo et al. 2004, Jager, Bucci et al. 2004, Rink, Ghigo et al. 2005, Hyttinen, Niittykoski et al. 2013). In late endocytic trafficking, Rab7 acts downstream of Rab5 in transporting cargoes from early to late endosomes (Feng, Press et al. 1995). In the process of maturation of early endosomes to late endosomes, a crucial step is the replacement of Rab5 compartments with Rab7 via GEF effectors and other vesicle fusion events (Rink, Ghigo et al. 2005, Hyttinen, Niittykoski et al. 2013). After late-endosome maturation, cytoplasmic vesicles or other late-endosomes containing Rab7 are fused together and aid to the formation of lysosomes according to the "kiss and run model" (Storrie and Desjardins 1996, Bucci, Thomsen et al. 2000). Lastly, Rab7 is also required for the final maturation of autophagic vacuoles and their final step in fusing with lysosomes. Rab7 deficient cells experience mayor failures on lysosome function and autophagy (Gutierrez, Munafo et al. 2004, Jager, Bucci et al. 2004). The role of Rab7 in recycling and autophagy makes it an attractive target in connection to the AB aggregation hypothesis in Alzheimer's disease.

As explained earlier, changes in the endocytosis and recycling pathways are one of the earliest clinical changes in Alzheimer's. In these pathways, Rab5 and Rab7 are critical in maintaining normal cellular functioning. Alzheimer's disease brains exhibit increased endocytosis and vesicle fusion to produce enlarged early endosomes, which will later turn into enlarged late endosomes and lysosomes with impaired functions. Rab5 and Rab7 are common markers used to visualize each step on these events (Cataldo, Mathews et al. 2008, Ginsberg,

Alldred et al. 2010) and consequently, these seem to be upregulated in mild cognitive impaired and Alzheimer's disease patients (Yuyama and Yanagisawa 2009, Ginsberg, Mufson et al. 2010, Ginsberg, Mufson et al. 2011). A recent study by Armstrong et al (2014) identified Rab7 as one of six lysosomal proteins that could be used for potential Alzheimer's disease biomarkers in cerebrospinal fluid (Armstrong, Mattsson et al. 2014). Indeed, in Alzheimer's disease brains, an excessive number of autophagic vacuoles accumulate in neurites and synaptic terminals as a result of disruption of autophagy and lysosome pathways (Nixon, Wegiel et al. 2005). Moreover, autophagic vacuoles in axons fuse with Rab7 vesicles to form lysosomes via retrograde transport to the cell for degradation. Lee et al (2011) demonstrated that disruption of this pathway in mouse cortical neurons produced AD-like axonal dystrophy (Lee, Sato et al. 2011). An interesting study concluded that amyloid fibrils are formed as a result of Rab7 suppression, whereas Rab5 knockdown did not seem to affect amyloid aggregation. The authors concluded that dysfunction of the late endocytosis pathway; controlled by Rab7, contribute to amyloid aggregation in neuronal cells (Yuyama and Yanagisawa 2009). A follow-up study showed that inhibiting Aβ42 endocytosis reversed the increased levels of Rab7 and Rab5 and prevented neurodegeneration in mice neurons treated with Aβ42 (Song, Baker et al. 2011). Overall, the role Rab7 in the late endocytosis pathway provides important insights into Aβ42 production and could be a useful therapeutic target in Alzheimer's disease.

Rab11

Rab11 is a very dynamic player in different cell functions ranging from cell adhesion to endocytosis. Rab11 comes in three isoforms: Rab11A is ubiquitously expressed in mammalian cells, Rab11B which is mainly expressed in brain cells and Rab11C, which is known as Rab25 (Goldenring, Shen et al. 1993, Lai, Stubbs et al. 1994, Hales, Griner et al. 2001). Rab11 has a

fundamental role in the recycling endosome by controlling trafficking of proteins to and from the recycling endosome to other organelles in the recycling pathway (Urbe, Huber et al. 1993, Ullrich, Reinsch et al. 1996). Rab11 is also localized in compartments of the TGN, post-TGN vesicles and in vesicles for exocytosis, suggesting Rab11 as an important link between endocytosis and exocytosis (Urbe, Huber et al. 1993, Chen, Feng et al. 1998, Wilcke, Johannes et al. 2000). Recent studies also show that Rab11 promotes ciliogenesis, cell adhesion and cytokinesis functions by binding to several cell receptors and adhesion proteins (Knodler, Feng et al. 2010, Kelly, Horgan et al. 2012). Finally, Rab11 and its effector proteins associate with motor proteins such as myosin VA and dynein-1 in regulating transport along microtubule tracks (Welz, Wellbourne-Wood et al. 2014). Due to its importance in cell adhesion, Rab11C (Rab25) has been more characterized in Cancer biology; however, several new studies provide important insights towards the relation of Rab11 with neurodegeneration in AD.

Rab11 regulates Aβ42 production by associating with the beta- and gamma- secretases but not directly to APP. Ubayar et al (2013) performed RNAi screenning of all human Rab GTPases and identified Rab11 as a key regulator of sAPP-B and Aβ42 production by controlling trafficking of BACE-1 in endocytic compartments (Udayar, Buggia-Prevot et al. 2013). This observation is supported by other studies that confirm the trafficking of BACE-1 from axons and dendrites to the early endosomes in a Rab11-dependent manner (Chia, Toh et al. 2013, Buggia-Prevot, Fernandez et al. 2014). After internalized in endocytosis, APP and BACE-1 converge in the early endosome where Aβ42 production is initiated. Rab11 impairment decreases BACE-1 endocytosis and has the same effect in sAPP-β and secreted Aβ42 (Udayar, Buggia-Prevot et al. 2013, Buggia-Prevot, Fernandez et al. 2014). However, other studies used HEK293 cells expressing the Swedish mutation to show that sAPP-β and Aβ42 are not affected by Rab11

expression (Lopez-Perez, Dumanchin et al. 2000, Bulloj, Leal et al. 2010). Whether these contrasting observations are obtained because of the different cell type used remains to be elucidated. In the gamma-secretase complex, hydrophilic loops in PSEN1 and PSEN2 proteins interact with Rab11; however, this association did not seem to influence APP transport or processing in a negative way (Dumanchin, Czech et al. 1999, Lopez-Perez, Dumanchin et al. 2000). Altogether, Rab11 is a newly identified target that is associated with increased LOAD risk likely by the regulation of BACE-1 and Aβ42. Further studies of Rab11 regulation could provide important insights into therapeutic mechanisms in neurodegeneration.

The Rab GTPases reviewed above are the most associated with amyloid processing and neurodegeneration in AD. However, there are other Rab proteins which association with neurodegeneration is less documented. These Rab proteins are often associated with some of the Rabs reviewed here either by localizing to the same organelle or by regulating the same pathways. A quick overview of such less documented Rab proteins follows:

Rab4

Rab4 is localized to the early endosome along with Rab5 and Rab11 (Sonnichsen, De Renzis et al. 2000). However, Rab4 only affects trafficking to recycling and autophagy pathways and facilitates the formation of late endosomes and recycling endosomes (Vandersluijs, Hull et al. 1991, Vandersluijs, Hull et al. 1992, McCaffrey, Bielli et al. 2001). Rab4 initiates a small cascade of adaptor proteins that direct trafficking to other organelles and that also triggers the formation of endosomes (D'Souza, Semus et al. 2014). Rab4 overexpression does not influence endocytosis but changes the accumulation of recycling markers (TfRs) that could have an effect on the recycling pathway (Vandersluijs, Hull et al. 1992). Due to its involvement in the

endocytic and recycling pathways, Rab4 has also been associated with APP processing in neurons.

Rab4 is upregulated in forebrain neurons from mild cognitive impairment and AD patients, which suggests a role with AB accumulation (Ginsberg, Mufson et al. 2011, Soejima, Ohyagi et al. 2013). Indeed, independent studies found accumulation of exogenous Aβ42 in Rab4 compartments in the early endosome (Arriagada, Astorga et al. 2007, Arriagada, Bustamante et al. 2010). Enlargement of early endosomes, which is a hallmark of Rab5 overexpression, was also associated with increase of Rab4; suggesting that Rab4 and Rab5 might have similar functions in early endosomes and endocytosis. Finally, Udayar et al (2013) reports that silencing of Rab4 decreased production of Aβ42 and sAPP-B (Udayar, Buggia-Prevot et al. 2013). These studies agree that Rab4 and Rab5 are affected in early AD pathology and that Rab4 and Aβ42 accumulation in the early endosome leads to endocytosis and recycling dysfunction. However, further studies are needed to confirm this association.

Rab8

Rab8 is ubiquitously expressed in most cells and localizes to the TGN, vesicular structures and basolateral plasma membrane. In trafficking mechanisms, Rab8 regulates retrograde and anterograde transport between these structures (Huber, Pimplikar et al. 1993). Interestingly, Rab8 has a key role in regulating cell shape by the interaction of actin and microtubules with membrane trafficking. Rab8 also plays an important role in other cell processes such as ciliogenesis, cell migration, exocytosis and receptor exchange in the basal body (Peranen, Auvinen et al. 1996, Sato, Mushiake et al. 2007, Knodler, Feng et al. 2010, Peränen 2011).

In neurons, Rab8 regulates anterograde trafficking to the dendrites but not to the axons and it is required for neurite outgrowth (Huber, Dupree et al. 1995). This is supported by the observation that Rab8 depletion inhibits neuritic growth and mediates polarized membrane transport (Hattula and Peranen 2005). It is possible that Rab8 serves a similar function as Rab6 and Rab3 in anterograde transport of APP to the dendrites. This is supported by the observation that mutations in PSEN1 disturb APP transport and down regulates Rab8 levels in PC12 cells (Kametani, Usami et al. 2004). Analysis of small G proteins (Ras, Ral & Rab) from AD brains identified a significant increase of Rab8 only in the cell membrane whereas other Ras and Rab GTPases decreased in the cytoplasmic region (Shimohama, Kamiya et al. 1999). A similar observation showed that a PSEN1 mutation increased Aβ42 levels in vesicles containing Rab8. These vesicles accumulated in the late stages of the secretory pathway between the TGN and cell membrane (Petanceska, Seeger et al. 2000). Interestingly, previous studies presented here do not show the same observation in upregulated Rab GTPases in AD brains (Ginsberg, Alldred et al. 2010, Ginsberg, Mufson et al. 2010). Finally, RNAi screening for GTPases showed that silencing of Rab8 increased intracellular Aβ42 levels, indicating a possible neuroprotective effect of Rab8 (Udayar, Buggia-Prevot et al. 2013). These reports suggest a likely interaction of Rab8 with PSEN1 and APP via the secretory pathway as well as a possible protective effect on Alzheimer's disease pathogenesis. Further studies need to confirm this association and establish a more relevant role of Rab8 in neurodegeneration.

Rab10

Rab10 is another dynamic GTPase involved mainly in anterograde transport and in the recycling pathway. Rab10 belongs to a Rab subfamily that includes Rab8 and Rab13 and shares about 66% homology with Rab8; however, both localize to different organelles in the cell (Chen,

Holcomb et al. 1993). Similar to Rab8, Rab10 is involved in numerous cell processes and functions including endocytosis (Babbey, Ahktar et al. 2006), ciliary transport (Babbey, Bacallao et al. 2010), phagosome maturation (Cardoso, Jordao et al. 2010), cell polarization (Schuck, Gerl et al. 2007) and insulin GLUT4 transport (Sano, Eguez et al. 2007, Chen, Wang et al. 2012). Even though Rab10 shares similar homology and functions to Rab8, most literature suggests a complimentary role to Rab11 in the recycling pathway. In *C elegans*, Rab10 is required for proper recycling function and trafficking from the early endosome to the recycling endosome and from there to the basolateral membrane (Chen, Schweinsberg et al. 2006, Schuck, Gerl et al. 2007). Finally, Rab10 seems to be a main regulator of organelle shape and morphology by associating with microtubules. A recent study shows that Rab10 has given a key role in the regulation of ER dynamic growth and morphology by associating with microtubules (Chang and Blackstone 2013, English and Voeltz 2013). A separate study shows that Rab10 is necessary for microtubule network growth in the basolateral recycling pathway in endosomes (Chen, Li et al. 2014).

In neurons, Rab10 is associated with anterograde transport of receptors that promote axon development and neuronal polarization. Rab10 binds to a specific number of effectors including Myosyn Vb, Lgl1, JIP1 and MARCKS in post-TGN vesicles that carry axonal receptors responsive to axonal growth factors (Wang, Liu et al. 2011, Liu, Xu et al. 2013, Deng, Lei et al. 2014, Xu, Deng et al. 2014). Despite its involvement in anterograde and recycling mechanisms, only one study links Rab10 with APP and amyloid processing. In the previously mentioned RNAi expression screen, silencing of Rab10 decreased Aβ42 production without affecting sAPP-B levels. This study also suggested of an interaction of Rab10, Rab23 & Rab25 in regulating the same trafficking pathways (Udayar, Buggia-Prevot et al. 2013). In adipocytes, Rab10

knockdown resulted in an attenuation of Glut4 redistribution to the plasma membrane and a two-fold decrease of Glut4 exocytosis rate (Sano, Eguez et al. 2007). Together, these studies underscore a role of Rab10 in anterograde transport of important cell receptors and exocytosis of recycled peptides. It is possible that Rab10 regulates transport of APP molecules and Aβ42 to the cell membrane via the constitutive secretory and recycling pathways. However, further studies are needed to confirm this association, making Rab10 a promising marker in AD pathogenesis.

Conclusion

Rab GTPases are involved in most intracellular trafficking, endocytosis, recycling and exocytosis in the cell. They are also involved in numerous processes such as organelle formation, response to stimuli, cell migration, etc. Due to their important role in these processes, Rab GTPases provide important insight into mechanisms involved in disease progression and signaling such as Cancer, diabetes, anemia and neurological diseases. In this review, we focused on Rab GTPases that have been mainly associated with Alzheimer's pathogenesis in endocytosis, recycling and degradation pathways of APP and Aβ42. In addition, we presented Rab proteins with limited association with neurodegeneration and how these could provide future insights in this process. Future directions could be finding mechanisms mediated by Rab proteins that offer protective effects towards neurotoxicity. In addition, therapeutic approaches that could rescue processes such as endocytosis and recycling pathways in early stages of neurodegeneration could prove to be effective in controlling cascade of events that lead to AD pathology.

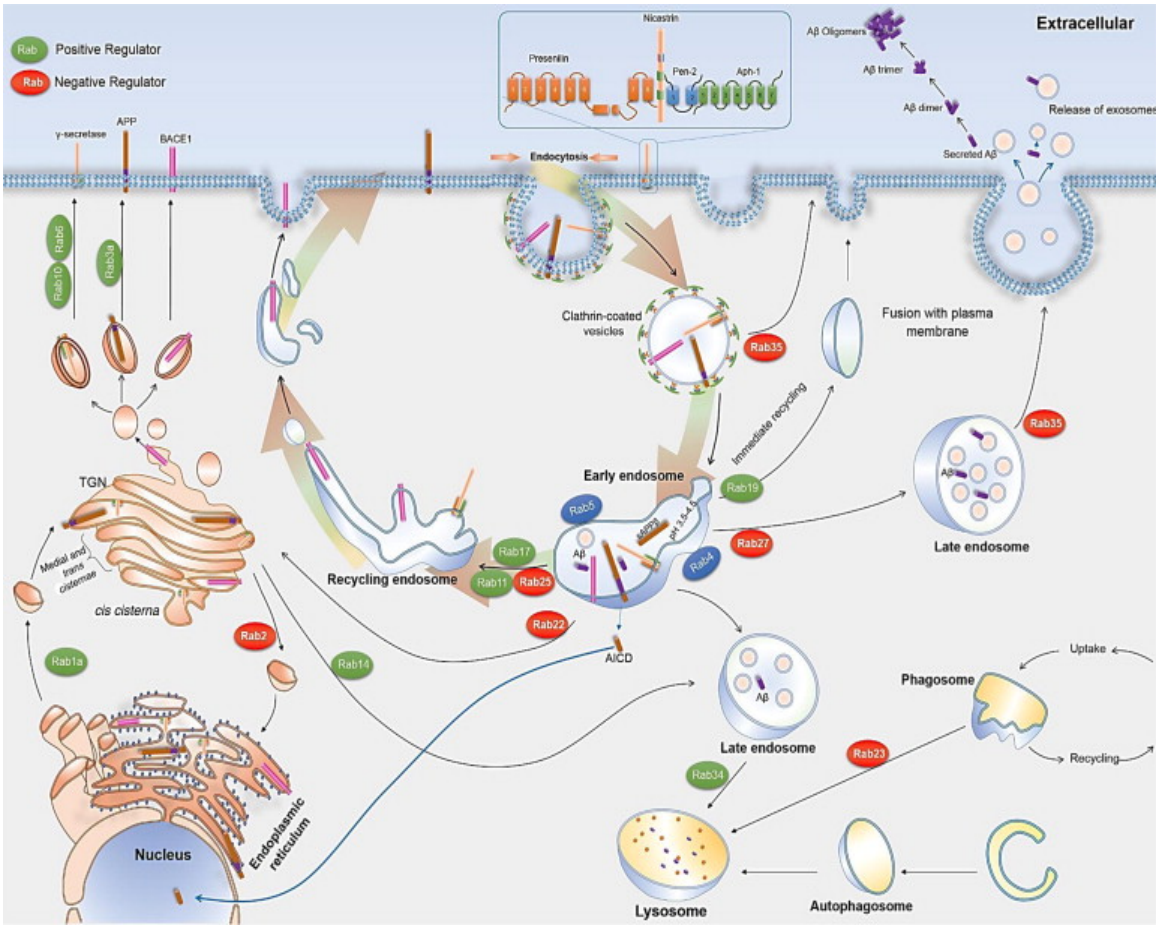

Figure 1 – Schematic of Rab GTPases involved in APP trafficking in the cell. This figure represents a likely model for the role of specific Rab proteins in APP and AB transport in the cell. Retrieved from (Udayar, Buggia-Prevot et al. 2013).

References:

Alzheimer's Association, 2014 Alzheimer's Disease Facts and Figures, Alzheimer's and Dementia. Volume 10, issue 2.

Alory, C. and W. E. Balch (2001). "Organization of the Rab-GDI/CHM superfamily: the functional basis for choroideremia disease." Traffic **2**(8): 532-543.

Armstrong, A., N. Mattsson, H. Appelqvist, C. Janefjord, L. Sandin, L. Agholme, B. Olsson, S. Svensson, K. Blennow, H. Zetterberg and K. Kagedal (2014). "Lysosomal Network Proteins as Potential Novel CSF Biomarkers for Alzheimer's Disease." Neuromolecular Medicine **16**(1): 150-160.

Armstrong, A., N. Mattsson, H. Appelqvist, C. Janefjord, L. Sandin, L. Agholme, B. Olsson, S. Svensson, K. Blennow, H. Zetterberg and K. Kagedal (2014). "Lysosomal network proteins as potential novel CSF biomarkers for Alzheimer's disease." Neuromolecular Med **16**(1): 150-160.

Arriagada, C., C. Astorga, I. Atwater, E. Rojas, D. Mears, R. Caviedes and P. Caviedes (2007). "Endosomal abnormalities related to amyloid precursor protein in cholesterol treated cerebral cortex neuronal cells derived from trisomy 16 mice, an animal model of Down syndrome." Neuroscience Letters **423**(2): 172-177.

Arriagada, C., M. Bustamante, I. Atwater, E. Rojas, R. Caviedes and P. Caviedes (2010). "Apoptosis is directly related to intracellular amyloid accumulation in a cell line derived from the cerebral cortex of a trisomy 16 mouse, an animal model of Down syndrome." Neuroscience Letters **470**(1): 81-85.

Babbey, C. M., N. Ahktar, E. Wang, C. C. H. Chen, B. D. Grant and K. W. Dunn (2006). "Rab10 regulates membrane transport through early endosomes of polarized Madin-Darby Canine Kidney cells." Molecular Biology of the Cell **17**(7): 3156-3175.

Babbey, C. M., R. L. Bacallao and K. W. Dunn (2010). "Rab10 associates with primary cilia and the exocyst complex in renal epithelial cells." American Journal of Physiology-Renal Physiology **299**(3): F495-F506.

Baldini, G., T. Hohl, H. Y. Lin and H. F. Lodish (1992). "Cloning of a Rab3 isotype predominantly expressed in adipocytes." Proc Natl Acad Sci U S A **89**(11): 5049-5052.

Baskys, A., I. Bayazitov, E. Zhu, L. Fang and R. Wang (2007). "Rab-mediated endocytosis: linking neurodegeneration, neuroprotection, and synaptic plasticity?" Ann N Y Acad Sci **1122**: 313-329.

Bhattacharyya, R., C. Barren and D. M. Kovacs (2013). "Palmitoylation of amyloid precursor protein regulates amyloidogenic processing in lipid rafts." J Neurosci **33**(27): 11169-11183.

Bucci, C., R. G. Parton, I. H. Mather, H. Stunnenberg, K. Simons, B. Hoflack and M. Zerial (1992). "The small GTPase rab5 functions as a regulatory factor in the early endocytic pathway." Cell **70**(5): 715-728.

Bucci, C., P. Thomsen, P. Nicoziani, J. McCarthy and B. van Deurs (2000). "Rab7: A key to lysosome biogenesis." Molecular Biology of the Cell **11**(2): 467-480.

Buggia-Prevot, V., C. G. Fernandez, S. Riordan, K. S. Vetrivel, J. Roseman, J. Waters, V. P. Bindokas, R. Vassar and G. Thinakaran (2014). "Axonal BACE1 dynamics and targeting in hippocampal neurons: a role for Rab11 GTPase." Molecular Neurodegeneration **9**.

Bulloj, A., M. C. Leal, H. Xu, E. M. Castaño and L. Morellia (2010). "Insulin-Degrading Enzyme Sorting in Exosomes: A Secretory Pathway for a Key Brain Amyloid-β Degrading Protease." Journal of Alzheimer's Disease **19**(1): 79-95.

Capell, A., L. Meyn, R. Fluhrer, D. B. Teplow, J. Walter and C. Haass (2002). "Apical sorting of beta-secretase limits amyloid beta-peptide production." J Biol Chem **277**(7): 5637-5643.

Cardoso, C. M. P., L. Jordao and O. V. Vieira (2010). "Rab10 Regulates Phagosome Maturation and Its Overexpression Rescues Mycobacterium-Containing Phagosomes Maturation." Traffic **11**(2): 221-235.

Cataldo, A., G. W. Rebeck, B. Ghetri, C. Hulette, C. Lippa, C. Van Broeckhoven, C. van Duijn, P. Cras, N. Bogdanovic, T. Bird, C. Peterhoff and R. Nixon (2001). "Endocytic disturbances distinguish among subtypes of Alzheimer's disease and related disorders." Ann Neurol **50**(5): 661-665.

Cataldo, A. M., P. M. Mathews, A. B. Boiteau, L. C. Hassinger, C. M. Peterhoff, Y. Jiang, K. Mullaney, R. L. Neve, J. Gruenberg and R. A. Nixon (2008). "Down syndrome fibroblast model of Alzheimer-related endosome pathology - Accelerated endocytosis promotes late endocytic defects." American Journal of Pathology **173**(2): 370-384.

Cataldo, A. M., C. M. Peterhoff, J. C. Troncosco, T. Gomez-Isla, B. T. Hyman and R. A. Nixon (2000). "Endocytic pathway abnormalities precede amyloid beta deposition in sporadic Alzheimer's disease and Down syndrome - Differential effects of APOE genotype and presenilin mutations." American Journal of Pathology **157**(1): 277-286.

Cataldo, A. M., C. M. Peterhoff, J. C. Troncoso, T. Gomez-Isla, B. T. Hyman and R. A. Nixon (2000). "Endocytic pathway abnormalities precede amyloid beta deposition in sporadic Alzheimer's disease and Down syndrome: differential effects of APOE genotype and presenilin mutations." Am J Pathol **157**(1): 277-286.

Chang, J. and C. Blackstone (2013). "Rab10 joins the ER social network." Nat Cell Biol **15**(2): 135-136.

Chen, C. C.-H., P. J. Schweinsberg, S. Vashist, D. P. Mareiniss, E. J. Lambie and B. D. Grant (2006). "RAB-10 Is Required for Endocytic Recycling in the Caenorhabditis elegans Intestine." Molecular Biology of the Cell **17**(3): 1286-1297.

Chen, S., L. Li, J. Li, B. Liu, X. Zhu, L. Zheng, R. Zhang and T. Xu (2014). "SEC-10 and RAB-10 coordinate basolateral recycling of clathrin-independent cargo through endosomal tubules in Caenorhabditis elegans." Proceedings of the National Academy of Sciences **111**(43): 15432-15437.

Chen, W., Y. Feng, D. Y. Chen and A. Wandinger-Ness (1998). "Rab11 is required for trans-Golgi network to plasma membrane transport and a preferential target for GDP dissociation inhibitor." Molecular Biology of the Cell **9**(11): 3241-3257.

Chen, Y., Y. Wang, J. Z. Zhang, Y. Q. Deng, L. Jiang, E. Song, X. F. S. Wu, J. A. Hammer, T. Xu and J. Lippincott-Schwartz (2012). "Rab10 and myosin-Va mediate insulin-stimulated GLUT4 storage vesicle translocation in adipocytes." Journal of Cell Biology **198**(4): 545-560.

Chen, Y. T., C. Holcomb and H. P. H. Moore (1993). "EXPRESSION AND LOCALIZATION OF 2 LOW-MOLECULAR-WEIGHT GTP-BINDING PROTEINS, RAB8 AND RAB10, BY EPITOPE TAG." Proceedings of the National Academy of Sciences of the United States of America **90**(14): 6508-6512.

Chia, P. Z. C., W. H. Toh, R. Sharples, I. Gasnereau, A. F. Hill and P. A. Gleeson (2013). "Intracellular Itinerary of Internalised beta-Secretase, BACE1, and Its Potential Impact on beta-Amyloid Peptide Biogenesis." Traffic **14**(9): 997-1013.

Coleman, W. L., C. A. Bill and M. Bykhovskaia (2007). "Rab3a deletion reduces vesicle docking and transmitter release at the mouse diaphragm synapse." Neuroscience **148**(1): 1-6.

D'Souza, Ryan S., R. Semus, Emily A. Billings, Corey B. Meyer, K. Conger and James E. Casanova (2014). "Rab4 Orchestrates a Small GTPase Cascade for Recruitment of Adaptor Proteins to Early Endosomes." Current Biology **24**(11): 1187-1198.

Darchen, F. and B. Goud (2000). "Multiple aspects of Rab protein action in the secretory pathway: focus on Rab3 and Rab6." Biochimie **82**(4): 375-384.

Dawkins, E. and D. H. Small (2014). "Insights into the physiological function of the beta-amyloid precursor protein: beyond Alzheimer's disease." J Neurochem **129**(5): 756-769.

Deng, C. Y., W. L. Lei, X. H. Xu, X. C. Ju, Y. Liu and Z. G. Luo (2014). "JIP1 Mediates Anterograde Transport of Rab10 Cargos during Neuronal Polarization." Journal of Neuroscience **34**(5): 1710-1723.

Dumanchin, C., C. Czech, D. Campion, M. H. Cuif, T. Poyot, C. Martin, F. Charbonnier, B. Goud, L. Pradier and T. Frebourg (1999). "Presenilins interact with Rab11, a small GTPase involved in the regulation of vesicular transport." Human Molecular Genetics **8**(7): 1263-1269.

Eggenschwiler, J. T., O. V. Bulgakov, J. Qin, T. Li and K. V. Anderson (2006). "Mouse Rab23 regulates hedgehog signaling from smoothened to Gli proteins." Dev Biol **290**(1): 1-12.

Elfrink, H. L., R. Zwart, M. L. Cavanillas, A. J. Schindler, F. Baas and W. Scheper (2012). "Rab6 is a modulator of the unfolded protein response: implications for Alzheimer's disease." J Alzheimers Dis **28**(4): 917-929.

English, A. R. and G. K. Voeltz (2013). "Rab10 GTPase regulates ER dynamics and morphology." Nature Cell Biology **15**(2): 169-178.

Feng, Y., B. Press and A. Wandingerness (1995). "rab-7 - an important regulator of late endocytic membrane traffic." Journal of Cell Biology **131**(6): 1435-1452.

Ginsberg, S. D., M. J. Alldred, S. E. Counts, A. M. Cataldo, R. L. Neve, Y. Jiang, J. Wuu, M. V. Chao, E. J. Mufson, R. A. Nixon and S. L. Che (2010). "Microarray Analysis of Hippocampal CA1 Neurons Implicates Early Endosomal Dysfunction During Alzheimer's Disease Progression." Biological Psychiatry **68**(10): 885-893.

Ginsberg, S. D., E. J. Mufson, M. J. Alldred, S. E. Counts, J. Wuu, R. A. Nixon and S. Che (2011). "Upregulation of select rab GTPases in cholinergic basal forebrain neurons in mild cognitive impairment and Alzheimer's disease." J Chem Neuroanat **42**(2): 102-110.

Ginsberg, S. D., E. J. Mufson, M. J. Alldred, S. E. Counts, J. Wuu, R. A. Nixon and S. L. Che (2011). "Upregulation of select rab GTPases in cholinergic basal forebrain neurons in mild cognitive impairment and Alzheimer's disease." Journal of Chemical Neuroanatomy **42**(2): 102-110.

Ginsberg, S. D., E. J. Mufson, S. E. Counts, J. Wuu, M. J. Alldred, R. A. Nixon and S. Che (2010). "Regional selectivity of rab5 and rab7 protein upregulation in mild cognitive impairment and Alzheimer's disease." J Alzheimers Dis **22**(2): 631-639.

Glenner, G. G. and C. W. Wong (2012). "alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein (reprinted from biochemical and biophysical research COMMUNICATIONS, vol 120, pg 885-890, 1984)." Biochemical and Biophysical Research Communications **425**(3): 534-539.

Goldenring, J. R., K. R. Shen, H. D. Vaughan and I. M. Modlin (1993). "Identification of a small GTP-binding protein, Rab25, expressed in the gastrointestinal mucosa, kidney, and lung." J Biol Chem **268**(25): 18419-18422.

Gorvel, J. P., P. Chavrier, M. Zerial and J. Gruenberg (1991). "rab5 controls early endosome fusion in vitro." Cell **64**(5): 915-925.

Grbovic, O. M., P. M. Mathews, Y. Jiang, S. D. Schmidt, R. Dinakar, N. B. Summers-Terio, B. P. Ceresa, R. A. Nixon and A. M. Cataldo (2003). "Rab5-stimulated up-regulation of the endocytic pathway increases intracellular beta-cleaved amyloid precursor protein carboxyl-terminal fragment levels and Abeta production." J Biol Chem **278**(33): 31261-31268.

Grosshans, B. L., D. Ortiz and P. Novick (2006). "Rabs and their effectors: achieving specificity in membrane traffic." Proc Natl Acad Sci U S A **103**(32): 11821-11827.

Gutierrez, M. G., D. B. Munafo, W. Beron and M. I. Colombo (2004). "Rab7 is required for the normal progression of the autophagic pathway in mammalian cells." Journal of Cell Science **117**(13): 2687-2697.

Haass, C., C. Kaether, G. Thinakaran and S. Sisodia (2012). "Trafficking and proteolytic processing of APP." Cold Spring Harb Perspect Med **2**(5): a006270.

Hales, C. M., R. Griner, K. C. Hobdy-Henderson, M. C. Dorn, D. Hardy, R. Kumar, J. Navarre, E. K. L. Chan, L. A. Lapierre and J. R. Goldenring (2001). "Identification and characterization of a family of Rab11-interacting proteins." Journal of Biological Chemistry **276**(42): 39067-39075.

Hardy, J. A. and G. A. Higgins (1992). "Alzheimer's disease: the amyloid cascade hypothesis." Science **256**(5054): 184-185.

Hashimoto, Y. and M. Matsuoka (2014). "A mutation protective against Alzheimer's disease renders amyloid beta precursor protein incapable of mediating neurotoxicity." J Neurochem **130**(2): 291-300.

Hattula, K. and J. Peranen (2005). "Purification and functional properties of a Rab8-specific GEF (Rabin3) in action remodeling and polarized transport." Methods Enzymol **403**: 284-295.

Horiuchi, H., R. Lippe, H. M. McBride, M. Rubino, P. Woodman, H. Stenmark, V. Rybin, M. Wilm, K. Ashman, M. Mann and M. Zerial (1997). "A novel Rab5 GDP/GTP exchange factor complexed to Rabaptin-5 links nucleotide exchange to effector recruitment and function." Cell **90**(6): 1149-1159.

Huber, L. A., P. Dupree and C. G. Dotti (1995). "a deficiency of the small gtpase rab8 inhibits membrane traffic in developing neurons." Molecular and Cellular Biology **15**(2): 918-924.

Huber, L. A., S. Pimplikar, R. G. Parton, H. Virta, M. Zerial and K. Simons (1993). "rab8, a small gtpase involved in vesicular traffic between the tgn and the basolateral plasma-membrane." Journal of Cell Biology **123**(1): 35-45.

Hung, A. Y., E. H. Koo, C. Haass and D. J. Selkoe (1992). "Increased expression of beta-amyloid precursor protein during neuronal differentiation is not accompanied by secretory cleavage." Proc Natl Acad Sci U S A **89**(20): 9439-9443.

Hyttinen, J. M. T., M. Niittykoski, A. Salminen and K. Kaarniranta (2013). "Maturation of autophagosomes and endosomes: A key role for Rab7." Biochimica Et Biophysica Acta-Molecular Cell Research **1833**(3): 503-510.

Ishikura, S., A. Koshkina and A. Klip (2008). "Small G proteins in insulin action: Rab and Rho families at the crossroads of signal transduction and GLUT4 vesicle traffic." Acta Physiol (Oxf) **192**(1): 61-74.

Jager, S., C. Bucci, I. Tanida, T. Ueno, E. Kominami, P. Saftig and E. L. Eskelinen (2004). "Role for Rab7 in maturation of late autophagic vacuoles." Journal of Cell Science **117**(20): 4837-4848.

Kaddai, V., Y. Le Marchand-Brustel and M. Cormont (2008). "Rab proteins in endocytosis and Glut4 trafficking." Acta Physiol (Oxf) **192**(1): 75-88.

Kamenetz, F., T. Tomita, H. Hsieh, G. Seabrook, D. Borchelt, T. Iwatsubo, S. Sisodia and R. Malinow (2003). "APP processing and synaptic function." Neuron **37**(6): 925-937.

Kametani, F., M. Usami, K. Tanaka, H. Kume and H. Mori (2004). "Mutant presenilin (A260V) affects Rab8 in PC12D cell." Neurochemistry International **44**(5): 313-320.

Kelly, E. E., C. P. Horgan and M. W. McCaffrey (2012). "Rab11 proteins in health and disease." Biochem Soc Trans **40**(6): 1360-1367.

Kerr, M. L. and D. H. Small (2005). "Cytoplasmic domain of the beta-amyloid protein precursor of Alzheimer's disease: function, regulation of proteolysis, and implications for drug development." J Neurosci Res **80**(2): 151-159.

Kimura, N., S. Okabayashi and F. Ono (2012). "Dynein dysfunction disrupts intracellular vesicle trafficking bidirectionally and perturbs synaptic vesicle docking via endocytic disturbances a potential mechanism underlying age-dependent impairment of cognitive function." Am J Pathol **180**(2): 550-561.

Kins, S., N. Lauther, A. Szodorai and K. Beyreuther (2006). "Subcellular trafficking of the amyloid precursor protein gene family and its pathogenic role in Alzheimer's disease." Neurodegener Dis **3**(4-5): 218-226.

Knodler, A., S. Feng, J. Zhang, X. Zhang, A. Das, J. Peranen and W. Guo (2010). "Coordination of Rab8 and Rab11 in primary ciliogenesis." Proc Natl Acad Sci U S A **107**(14): 6346-6351.

Komuro, R., T. Sasaki, S. Orita, M. Maeda and Y. Takai (1996). "Involvement of rabphilin-3A in Ca2+-dependent exocytosis from PC12 cells." Biochem Biophys Res Commun **219**(2): 435-440.

Lai, F., L. Stubbs and K. Artzt (1994). "Molecular analysis of mouse Rab11b: a new type of mammalian YPT/Rab protein." Genomics **22**(3): 610-616.

Laifenfeld, D., L. J. Patzek, D. L. McPhie, Y. Chen, Y. Levites, A. M. Cataldo and R. L. Neve (2007). "Rab5 mediates an amyloid precursor protein signaling pathway that leads to apoptosis." J Neurosci **27**(27): 7141-7153.

LeBlanc, A. C. and C. G. Goodyer (1999). "Role of endoplasmic reticulum, endosomal-lysosomal compartments, and microtubules in amyloid precursor protein metabolism of human neurons." J Neurochem **72**(5): 1832-1842.

Lee, J. H., W. H. Yu, A. Kumar, S. Lee, P. S. Mohan, C. M. Peterhoff, D. M. Wolfe, M. Martinez-Vicente, A. C. Massey, G. Sovak, Y. Uchiyama, D. Westaway, A. M. Cuervo and R. A. Nixon (2010). "Lysosomal proteolysis and autophagy require presenilin 1 and are disrupted by Alzheimer-related PS1 mutations." Cell **141**(7): 1146-1158.

Lee, K. J., C. E. Moussa, Y. Lee, Y. Sung, B. W. Howell, R. S. Turner, D. T. Pak and H. S. Hoe (2010). "Beta amyloid-independent role of amyloid precursor protein in generation and maintenance of dendritic spines." Neuroscience **169**(1): 344-356.

Lee, S., Y. Sato and R. A. Nixon (2011). "Lysosomal Proteolysis Inhibition Selectively Disrupts Axonal Transport of Degradative Organelles and Causes an Alzheimer's-Like Axonal Dystrophy." Journal of Neuroscience **31**(21): 7817-7830.

Li, G. and P. D. Stahl (1993). "Structure-function relationship of the small GTPase rab5." J Biol Chem **268**(32): 24475-24480.

Li, J., T. Kanekiyo, M. Shinohara, Y. Zhang, M. J. LaDu, H. Xu and G. Bu (2012). "Differential regulation of amyloid-beta endocytic trafficking and lysosomal degradation by apolipoprotein E isoforms." J Biol Chem **287**(53): 44593-44601.

Li, J. Y., R. Jahn, X. E. Hou, A. Kling-Petersen and A. Dahlstrom (1996). "Distribution of Rab3a in rat nervous system: comparison with other synaptic vesicle proteins and neuropeptides." Brain Res **706**(1): 103-112.

Liu, Y., X. H. Xu, Q. Chen, T. Wang, C. Y. Deng, B. L. Song, J. L. Du and Z. G. Luo (2013). "Myosin Vb controls biogenesis of post-Golgi Rab10 carriers during axon development." Nature Communications **4**.

Lopez-Perez, E., C. Dumanchin, C. Czech, D. Campion, B. Goud, L. Pradier, T. Frebourg and F. Checler (2000). "Overexpression of Rab11 or constitutively active Rab11 does not affect sAPP alpha and A beta secretions by wild-type and Swedish mutated beta APP-expressing HEK293 cells." Biochemical and Biophysical Research Communications **275**(3): 910-915.

Lu, L. and W. Hong (2014). "From endosomes to the trans-Golgi network." Semin Cell Dev Biol **31**: 30-39.

Mallard, F., B. L. Tang, T. Galli, D. Tenza, A. Saint-Pol, X. Yue, C. Antony, W. Hong, B. Goud and L. Johannes (2002). "Early/recycling endosomes-to-TGN transport involves two SNARE complexes and a Rab6 isoform." The Journal of Cell Biology **156**(4): 653-664.

Markgraf, D. F., K. Peplowska and C. Ungermann (2007). "Rab cascades and tethering factors in the endomembrane system." FEBS Lett **581**(11): 2125-2130.

Martinez, O., A. Schmidt, J. Salamero, B. Hoflack, M. Roa and B. Goud (1994). "The small GTP-binding protein rab6 functions in intra-Golgi transport." J Cell Biol **127**(6 Pt 1): 1575-1588.

Masters, C. L., G. Multhaup, G. Simms, J. Pottgiesser, R. N. Martins and K. Beyreuther (1985). "Neuronal origin of a cerebral amyloid: neurofibrillary tangles of Alzheimer's disease contain the same protein as the amyloid of plaque cores and blood vessels." The EMBO Journal **4**(11): 2757-2763.

McCaffrey, M. W., A. Bielli, G. Cantalupo, S. Mora, V. Roberti, M. Santillo, F. Drummond and C. Bucci (2001). "Rab4 affects both recycling and degradative endosomal trafficking." Febs Letters **495**(1-2): 21-30.

McConlogue, L., F. Castellano, C. deWit, D. Schenk and W. A. Maltese (1996). "Differential effects of a Rab6 mutant on secretory versus amyloidogenic processing of Alzheimer's beta-amyloid precursor protein." Journal of Biological Chemistry **271**(3): 1343-1348.

Mendoza, P., J. Diaz and V. A. Torres (2014). "On the role of Rab5 in cell migration." Curr Mol Med **14**(2): 235-245.

Miller, C. C., D. M. McLoughlin, K. F. Lau, M. E. Tennant and B. Rogelj (2006). "The X11 proteins, Abeta production and Alzheimer's disease." Trends Neurosci **29**(5): 280-285.

Nixon, R. A. (2005). "Endosome function and dysfunction in Alzheimer's disease and other neurodegenerative diseases." Neurobiol Aging **26**(3): 373-382.

Nixon, R. A. (2007). "Autophagy, amyloidogenesis and Alzheimer disease." J Cell Sci **120**(Pt 23): 4081-4091.

Nixon, R. A., J. Wegiel, A. Kumar, W. H. Yu, C. Peterhoff, A. Cataldo and A. M. Cuervo (2005). "Extensive involvement of autophagy in Alzheimer disease: an immuno-electron microscopy study." J Neuropathol Exp Neurol **64**(2): 113-122.

Nordstedt, C., G. L. Caporaso, J. Thyberg, S. E. Gandy and P. Greengard (1993). "Identification of the Alzheimer beta/A4 amyloid precursor protein in clathrin-coated vesicles purified from PC12 cells." J Biol Chem **268**(1): 608-612.

Oh, E. S., A. V. Savonenko, J. F. King, S. M. Fangmark Tucker, G. L. Rudow, G. Xu, D. R. Borchelt and J. C. Troncoso (2009). "Amyloid precursor protein increases cortical neuron size in transgenic mice." Neurobiol Aging **30**(8): 1238-1244.

Opdam, F. J., A. Echard, H. J. Croes, J. A. van den Hurk, R. A. van de Vorstenbosch, L. A. Ginsel, B. Goud and J. A. Fransen (2000). "The small GTPase Rab6B, a novel Rab6 subfamily member, is cell-type specifically expressed and localised to the Golgi apparatus." Journal of Cell Science **113**(15): 2725-2735.

Opdam, F. J. M., A. Echard, H. J. E. Croes, J. van den Hurk, R. A. van de Vorstenbosch, L. A. Ginsel, B. Goud and J. A. M. Fransen (2000). "The small GTPase Rab6B, a novel Rab6 subfamily member, is cell-type specifically expressed and localised to the Golgi apparatus." Journal of Cell Science **113**(15): 2725-2735.

Peränen, J. (2011). "Rab8 GTPase as a regulator of cell shape." Cytoskeleton **68**(10): 527-539.

Peranen, J., P. Auvinen, H. Virta, R. Wepf and K. Simons (1996). "Rab8 promotes polarized membrane transport through reorganization of actin and microtubules in fibroblasts." Journal of Cell Biology **135**(1): 153-167.

Perez, R. G., S. Soriano, J. D. Hayes, B. Ostaszewski, W. Xia, D. J. Selkoe, X. Chen, G. B. Stokin and E. H. Koo (1999). "Mutagenesis identifies new signals for beta-amyloid precursor protein endocytosis, turnover, and the generation of secreted fragments, including Abeta42." J Biol Chem **274**(27): 18851-18856.

Perez, R. G., S. L. Squazzo and E. H. Koo (1996). "Enhanced release of amyloid beta-protein from codon 670/671 "Swedish" mutant beta-amyloid precursor protein occurs in both secretory and endocytic pathways." J Biol Chem **271**(15): 9100-9107.

Petanceska, S. S., M. Seeger, F. Checler and S. Gandy (2000). "Mutant presenilin 1 increases the levels of Alzheimer amyloid beta-peptide A beta 42 in late compartments of the constitutive secretory pathway." Journal of Neurochemistry **74**(5): 1878-1884.

Placido, A. I., C. M. Pereira, A. I. Duarte, E. Candeias, S. C. Correia, R. X. Santos, C. Carvalho, S. Cardoso, C. R. Oliveira and P. I. Moreira (2014). "The role of endoplasmic reticulum in amyloid precursor protein processing and trafficking: implications for Alzheimer's disease." Biochim Biophys Acta **1842**(9): 1444-1453.

Plassman, B. L., K. M. Langa, G. G. Fisher, S. G. Heeringa, D. R. Weir, M. B. Ofstedal, J. R. Burke, M. D. Hurd, G. G. Potter, W. L. Rodgers, D. C. Steffens, R. J. Willis and R. B. Wallace (2007). "Prevalence of dementia in the united states: The aging, demographics, and memory study." Neuroepidemiology **29**(1-2): 125-132.

Progida, C., L. Cogli, F. Piro, A. De Luca, O. Bakke and C. Bucci (2010). "Rab7b controls trafficking from endosomes to the TGN." Journal of Cell Science **123**(9): 1480-1491.

Rajendran, L., M. Honsho, T. R. Zahn, P. Keller, K. D. Geiger, P. Verkade and K. Simons (2006). "Alzheimer's disease beta-amyloid peptides are released in association with exosomes." Proc Natl Acad Sci U S A **103**(30): 11172-11177.

Reddy, P. H., G. Mani, B. S. Park, J. Jacques, G. Murdoch, W. Whetsell, Jr., J. Kaye and M. Manczak (2005). "Differential loss of synaptic proteins in Alzheimer's disease: implications for synaptic dysfunction." J Alzheimers Dis **7**(2): 103-117; discussion 173-180.

Reinhard, C., S. S. Hebert and B. De Strooper (2005). "The amyloid-beta precursor protein: integrating structure with biological function." Embo Journal **24**(23): 3996-4006.

Ridge, P. G., M. T. Ebbert and J. S. Kauwe (2013). "Genetics of Alzheimer's disease." Biomed Res Int **2013**: 254954.

Ring, S., 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 and U. C. Muller (2007). "The secreted beta-amyloid precursor protein ectodomain APPs alpha is sufficient to rescue the anatomical, behavioral, and electrophysiological abnormalities of APP-deficient mice." J Neurosci **27**(29): 7817-7826.

Rink, J., E. Ghigo, Y. Kalaidzidis and M. Zerial (2005). "Rab conversion as a mechanism of progression from early to late endosomes." Cell **122**(5): 735-749.

Sano, H., L. Eguez, M. N. Teruel, M. Fukuda, T. D. Chuang, J. A. Chavez, G. E. Lienhard and T. E. McGrau (2007). "Rab10, a target of the AS160 rab GAP, is required for insulin-stimulated translocation of GLUT4 to the adipocyte plasma membrane." Cell Metabolism **5**(4): 293-303.

Sato, T., S. Mushiake, Y. Kato, K. Sato, M. Sato, N. Takeda, K. Ozono, K. Miki, Y. Kubo, A. Tsuji, R. Harada and A. Harada (2007). "The Rab8 GTPase regulates apical protein localization in intestinal cells." Nature **448**(7151): 366-369.

Scheper, W., R. Zwart and F. Baas (2004). "Rab6 membrane association is dependent of Presenilin 1 and cellular phosphorylation events." Molecular Brain Research **122**(1): 17-23.

Schettini, G., S. Govoni, M. Racchi and G. Rodriguez (2010). "Phosphorylation of APP-CTF-AICD domains and interaction with adaptor proteins: signal transduction and/or transcriptional role--relevance for Alzheimer pathology." J Neurochem **115**(6): 1299-1308.

Schluter, O. M., F. Schmitz, R. Jahn, C. Rosenmund and T. C. Sudhof (2004). "A complete genetic analysis of neuronal Rab3 function." J Neurosci **24**(29): 6629-6637.

Schuck, S., M. J. Gerl, A. Ang, A. Manninen, P. Keller, I. Mellman and K. Simons (2007). "Rab10 is involved in basolateral transport in polarized madin-darby canine kidney cells." Traffic **8**(1): 47-60.

Seabra, M. C., E. H. Mules and A. N. Hume (2002). "Rab GTPases, intracellular traffic and disease." Trends Mol Med **8**(1): 23-30.

Selkoe, D. J. (2001). "Alzheimer's disease: genes, proteins, and therapy." Physiol Rev **81**(2): 741-766.

Shan, J., J. M. Mason, L. Yuan, M. Barcia, D. Porti, A. Calabro, D. Budman, V. Vinciguerra and H.-p. Xu (2000). "Rab6c, a new member of the Rab gene family, is involved in drug resistance in MCF7/AdrR cells." Gene **257**(1): 67-75.

Shimohama, S., S. Kamiya, T. Taniguchi, Y. Sumida and S. Fujimoto (1999). "Differential involvement of small G proteins in Alzheimer's disease." International Journal of Molecular Medicine **3**(6): 597-600.

Siniossoglou, S. and H. R. B. Pelham (2001). An effector of Ypt6p binds the SNARE Tlg1p and mediates selective fusion of vesicles with late Golgi membranes.

Sisodia, S. S. (2002). "Biomedicine. A cargo receptor mystery APParently solved?" Science **295**(5556): 805-807.

Soejima, N., Y. Ohyagi, N. Nakamura, E. Himeno, K. M. Iinuma, N. Sakae, R. Yamasaki, T. Tabira, K. Murakami, K. Irie, N. Kinoshita, F. M. LaFerla, Y. Kiyohara, T. Iwaki and J. I. Kira (2013). "Intracellular Accumulation of Toxic Turn Amyloid-beta is Associated with Endoplasmic Reticulum Stress in Alzheimer's Disease." Current Alzheimer Research **10**(1): 11-20.

Somsel Rodman, J. and A. Wandinger-Ness (2000). "Rab GTPases coordinate endocytosis." J Cell Sci **113 Pt 2**: 183-192.

Song, M. S., G. B. Baker, K. G. Todd and S. Kar (2011). "INHIBITION OF beta-AMYLOID(1-42) INTERNALIZATION ATTENUATES NEURONAL DEATH BY STABILIZING THE ENDOSOMAL-LYSOSOMAL SYSTEM IN RAT CORTICAL CULTURED NEURONS." Neuroscience **178**: 181-188.

Sonnichsen, B., S. De Renzis, E. Nielsen, J. Rietdorf and M. Zerial (2000). "Distinct membrane domains on endosomes in the recycling pathway visualized by multicolor imaging of Rab4, Rab5 and Rab11." Journal of Cell Biology **149**(4): 901-913.

Sosa, L. J., J. Bergman, A. Estrada-Bernal, T. J. Glorioso, J. M. Kittelson and K. H. Pfenninger (2013). "Amyloid precursor protein is an autonomous growth cone adhesion molecule engaged in contact guidance." PLoS One **8**(5): e64521.

Star, E. N., A. J. Newton and V. N. Murthy (2005). "Real-time imaging of Rab3a and Rab5a reveals differential roles in presynaptic function." J Physiol **569**(Pt 1): 103-117.

Stenmark, H. and V. M. Olkkonen (2001). "The Rab GTPase family." Genome Biol **2**(5): Reviews3007.

Stenmark, H., R. G. Parton, O. Steele-Mortimer, A. Lutcke, J. Gruenberg and M. Zerial (1994). "Inhibition of rab5 GTPase activity stimulates membrane fusion in endocytosis." Embo j **13**(6): 1287-1296.

Storrie, B. and M. Desjardins (1996). "The biogenesis of lysosomes: is it a kiss and run, continuous fusion and fission process?" Bioessays **18**(11): 895-903.

Sullivan, S. E., G. M. Dillon, J. M. Sullivan and A. Ho (2014). "Mint proteins are required for synaptic activity-dependent amyloid precursor protein (APP) trafficking and amyloid beta generation." J Biol Chem **289**(22): 15374-15383.

Szodorai, A., Y. H. Kuan, S. Hunzelmann, U. Engel, A. Sakane, T. Sasaki, Y. Takai, J. Kirsch, U. Muller, K. Beyreuther, S. Brady, G. Morfini and S. Kins (2009). "APP anterograde

transport requires Rab3A GTPase activity for assembly of the transport vesicle." J Neurosci **29**(46): 14534-14544.

Tam, J. H., C. Seah and S. H. Pasternak (2014). "The Amyloid Precursor Protein is rapidly transported from the Golgi apparatus to the lysosome and where it is processed into beta-amyloid." Mol Brain **7**: 54.

Tan, M. G., C. Lee, J. H. Lee, P. T. Francis, R. J. Williams, M. J. Ramirez, C. P. Chen, P. T. Wong and M. K. Lai (2014). "Decreased rabphilin 3A immunoreactivity in Alzheimer's disease is associated with Abeta burden." Neurochem Int **64**: 29-36.

Teber, I., F. Nagano, J. Kremerskothen, K. Bilbilis, B. Goud and A. Barnekow (2005). "Rab6 interacts with the mint3 adaptor protein." Biological Chemistry **386**(7): 671-677.

Thinakaran, G. and E. H. Koo (2008). "Amyloid precursor protein trafficking, processing, and function." J Biol Chem **283**(44): 29615-29619.

Thomas, R. S., M. J. Lelos, M. A. Good and E. J. Kidd (2011). "Clathrin-mediated endocytic proteins are upregulated in the cortex of the Tg2576 mouse model of Alzheimer's disease-like amyloid pathology." Biochem Biophys Res Commun **415**(4): 656-661.

Thyrock, A., E. Ossendorf, M. Stehling, M. Kail, T. Kurtz, G. Pohlentz, D. Waschbusch, S. Eggert, E. Formstecher, J. Muthing, K. Dreisewerd, S. Kins, B. Goud and A. Barnekow (2013). "A New Mint1 Isoform, but Not the Conventional Mint1, Interacts with the Small GTPase Rab6." Plos One **8**(5).

Turner, P. R., K. O'Connor, W. P. Tate and W. C. Abraham (2003). "Roles of amyloid precursor protein and its fragments in regulating neural activity, plasticity and memory." Prog Neurobiol **70**(1): 1-32.

Udayar, V., V. Buggia-Prevot, R. L. Guerreiro, G. Siegel, N. Rambabu, A. L. Soohoo, M. Ponnusamy, B. Siegenthaler, J. Bali, Aesg, M. Simons, J. Ries, M. A. Puthenveedu, J. Hardy, G. Thinakaran and L. Rajendran (2013). "A paired RNAi and RabGAP overexpression screen identifies Rab11 as a regulator of beta-amyloid production." Cell Rep **5**(6): 1536-1551.

Ullrich, O., S. Reinsch, S. Urbe, M. Zerial and R. G. Parton (1996). "Rab11 regulates recycling through the pericentriolar recycling endosome." Journal of Cell Biology **135**(4): 913-924.

Urbe, S., L. A. Huber, M. Zerial, S. A. Tooze and R. G. Parton (1993). "rab11, a small gtpase associated with both constitutive and regulated secretory pathways in pc12-cells." Febs Letters **334**(2): 175-182.

Vandersluijs, P., M. Hull, P. Webster, P. Male, B. Goud and I. Mellman (1992). "THE SMALL GTP-BINDING PROTEIN RAB4 CONTROLS AN EARLY SORTING EVENT ON THE ENDOCYTIC PATHWAY." Cell **70**(5): 729-740.

Vandersluijs, P., M. Hull, A. Zahraoui, A. Tavitian, B. Goud and I. Mellman (1991). "the small gtp-binding protein rab4 is associated with early endosomes." Proceedings of the National Academy of Sciences of the United States of America **88**(14): 6313-6317.

Wang, T., Y. Liu, X. H. Xu, C. Y. Deng, K. Y. Wu, J. Zhu, X. Q. Fu, M. He and Z. G. Luo (2011). "Lgl1 Activation of Rab10 Promotes Axonal Membrane Trafficking Underlying Neuronal Polarization." Developmental Cell **21**(3): 431-444.

Wanschers, B. F., R. van de Vorstenbosch, M. A. Schlager, D. Splinter, A. Akhmanova, C. C. Hoogenraad, B. Wieringa and J. A. Fransen (2007). "A role for the Rab6B Bicaudal-D1 interaction in retrograde transport in neuronal cells." Exp Cell Res **313**(16): 3408-3420.

Welz, T., J. Wellbourne-Wood and E. Kerkhoff (2014). "Orchestration of cell surface proteins by Rab11." Trends in Cell Biology **24**(7): 407-415.

Wilcke, M., L. Johannes, T. Galli, V. Mayau, B. Goud and J. Salamero (2000). "Rab11 regulates the compartmentalization of early endosomes required for efficient transport from early endosomes to the trans-golgi network." Journal of Cell Biology **151**(6): 1207-1220.

Woodman, P. G. (2000). "Biogenesis of the sorting endosome: the role of Rab5." Traffic **1**(9): 695-701.

Wu, F. and P. J. Yao (2009). "Clathrin-mediated endocytosis and Alzheimer's disease: an update." Ageing Res Rev **8**(3): 147-149.

Xu, X. H., C. Y. Deng, Y. Liu, M. He, J. Peng, T. Wang, L. Yuan, Z. S. Zheng, P. J. Blackshear and Z. G. Luo (2014). "MARCKS regulates membrane targeting of Rab10 vesicles to promote axon development." Cell Research **24**(5): 576-594.

Yamazaki, T., E. H. Koo and D. J. Selkoe (1997). "Cell surface amyloid beta-protein precursor colocalizes with beta 1 integrins at substrate contact sites in neural cells." J Neurosci **17**(3): 1004-1010.

Young, J., J. Menetrey and B. Goud (2010). "RAB6C is a retrogene that encodes a centrosomal protein involved in cell cycle progression." J Mol Biol **397**(1): 69-88.

Young, J., T. Stauber, E. del Nery, I. Vernos, R. Pepperkok and T. Nilsson (2005). "Regulation of microtubule-dependent recycling at the trans-Golgi network by Rab6A and Rab6A'." Mol Biol Cell **16**(1): 162-177.

Young-Pearse, T. L., J. Bai, R. Chang, J. B. Zheng, J. J. LoTurco and D. J. Selkoe (2007). "A critical function for beta-amyloid precursor protein in neuronal migration revealed by in utero RNA interference." J Neurosci **27**(52): 14459-14469.

Yu, W. H., A. M. Cuervo, A. Kumar, C. M. Peterhoff, S. D. Schmidt, J. H. Lee, P. S. Mohan, M. Mercken, M. R. Farmery, L. O. Tjernberg, Y. Jiang, K. Duff, Y. Uchiyama, J. Naslund, P. M. Mathews, A. M. Cataldo and R. A. Nixon (2005). "Macroautophagy--a novel

Beta-amyloid peptide-generating pathway activated in Alzheimer's disease." J Cell Biol **171**(1): 87-98.

Yuyama, K. and K. Yanagisawa (2009). "Late endocytic dysfunction as a putative cause of amyloid fibril formation in Alzheimer's disease." Journal of Neurochemistry **109**(5): 1250-1260.

Zerial, M. and H. McBride (2001). "Rab proteins as membrane organizers." Nat Rev Mol Cell Biol **2**(2): 107-117.

Chapter 2: Expression Effects of Rab10 and Sar1A GTPases in APP and Aβ Levels in N2A-695 Cells

Abstract

Rab proteins are members of a large family of monomeric GTP binding proteins that are key players in numerous steps of cellular transport ranging from intracellular transport to endocytosis and autophagy. Several studies show increased levels of particular Rab proteins in Alzheimer's pathogenesis. Previous experiments indicate an association with Rab10 with amyloid generation and Alzheimer's disease risk. Here we show an experimental approach used to characterize the effects of Rab10 and Sar1A GTPases in APP and amyloid processing. N2A cells were transient transfected with overexpression and shRNA plasmids and their effects in APP expression and Aβ40 and Aβ42 were measured using sandwich ELISA. We found that Rab10 expression does not affect APP production but significantly changes AB generation, particularly the toxic Aβ42. On the other hand, we found no significant effect of Sar1A expression on either APP or amyloid beta generation. These findings partially confirm the work done by Kauwe et al (2015) and provide preliminary evidence for two potential targets for protective effects in neurodegeneration.

Introduction

Alzheimer's disease is the 6th most prevalent cause of death in US with an estimated 500,000 fatalities each year due to the disease (Alz.org retrieved 2014). A major neurological hallmark of the disease is the accumulation of senile plaques composed of amyloid beta molecules. The most accepted theory behind the pathogenesis of Alzheimer's is known as the amyloid cascade hypothesis (Hardy and Higgins 1992, Ridge, Ebbert et al. 2013). Briefly, amyloid beta molecules are produced by cleavage of APP by beta and gamma secretases

producing fragments ranging from 39-43 amino acids. Aβ42 is the peptide most susceptible to aggregation and it is the main component of the senile plaques (Thinakaran and Koo 2008, Tam, Seah et al. 2014). Accumulation of senile plaques in the brain disrupts neuronal connections and synapses, causing oxidative stress on neurons that form neurofibrillary tangles and apoptosis that lead to widespread neurodegeneration (Masters, Multhaup et al. 1985, Hung, Koo et al. 1992, Selkoe 2001, Ring, Weyer et al. 2007, Oh, Savonenko et al. 2009, Hashimoto and Matsuoka 2014). Interestingly, Aβ40 is by far the most common product from APP cleavage but does not aggregate into senile plaques. The change in Aβ40:42 ratio is generally considered to contribute to Aβ42 deposition and neurotoxicity.

Transmembrane APP undergoes the constitutive secretory pathway in the cell where it is post-translationally modified in the ER and TGN to be transported via anterograde transport to the cell membrane. APP is then internalized via endocytosis to the cell where it is processed by the recycling and autophagy machineries (Kins, Lauther et al. 2006, Nixon 2007, Thinakaran and Koo 2008, Thomas, Lelos et al. 2011, Haass, Kaether et al. 2012). Aβ production occurs mainly on the endosomal – lysosomal systems and has also been detected in compartments of the TGN (LeBlanc and Goodyer 1999, Haass, Kaether et al. 2012, Lu and Hong 2014). The majority of AB fragments are then secreted via exocytosis where Aβ42 aggregates in the extracellular space (Yu, Cuervo et al. 2005, Nixon 2007). However, small quantities of Aβ42 stay in the cell where they contribute in disrupting cellular pathways. Polymorphisms in proteins involved in the transporting and processing of APP have been associated with differential Aβ-42 deposition and a change in the Aβ40:42 ratio (Citron, Westaway et al. 1997, Cataldo, Peterhoff et al. 2000, Jankowsky, Fadale et al. 2004, Lee, Yu et al. 2010, Ridge, Ebbert et al. 2013). In this context, GTPases are small proteins involved in processes such as vesicle formation, transport, signal

transduction, translocation and processing of membrane proteins (Zerial and McBride 2001, Seabra, Mules et al. 2002, Grosshans, Ortiz et al. 2006). Expectedly, various studies show that APP and Aβ production is influenced by specific GTPases (Cataldo, Peterhoff et al. 2000, Reddy, Mani et al. 2005, Baskys, Bayazitov et al. 2007, Udayar, Buggia-Prevot et al. 2013, Armstrong, Mattsson et al. 2014, Buggia-Prevot, Fernandez et al. 2014, Tan, Lee et al. 2014). This study focused on two small GTPases not previously associated with AB generation: Rab10 and Sar1A.

Rab10

Rab10 is a member of the Ras superfamily of small GTPases. In general, Rab GTPases are involved in every step of the intracellular vesicle transport in the cell (Stenmark and Olkkonen 2001, Seabra, Mules et al. 2002). In addition to trafficking, some Rab proteins seem to play an important role in organelle shape and growth by associating with microtubules (Zerial and McBride 2001, Seabra, Mules et al. 2002, Grosshans, Ortiz et al. 2006). Rab proteins switch between an inactive (GDP-bound) cytosolic state and active (GTP-bound) membrane bound state. Among other cellular processes, Rab10 has two main roles: regulating ER dynamics and anterograde transport to the plasma membrane (Schuck, Gerl et al. 2007, English and Voeltz 2013, Liu, Xu et al. 2013, Deng, Lei et al. 2014). English et al (2013) showed that Rab10 localizes in the ER and associates with microtubules to direct ER growth. Mutant Rab10 show an altered ER morphology and reduced ability to fuse with adjacent ER tubules (English and Voeltz 2013). Independent studies show that Rab10 has an active role in transporting cell receptors to and from the TGN to the plasma membrane (Sano, Eguez et al. 2007, Schuck, Gerl et al. 2007, Lerner, McCoy et al. 2013, Deng, Lei et al. 2014). In neurons, Rab10 promotes axon development and neuronal polarization by transporting axonal receptors that interact with

extracellular growth factors (Wang, Liu et al. 2011, Liu, Xu et al. 2013, Xu, Deng et al. 2014). Additional evidence suggests a particular role of Rab10 in the endosome – recycling pathways (Babbey, Ahktar et al. 2006, Chen, Schweinsberg et al. 2006, Chen, Li et al. 2014). In a recent RNAi screen of all human Rab GTPases, Rab10 was found to decrease Aβ42 production and to affect sAPP-B levels in neural cells (Udayar, Buggia-Prevot et al. 2013).

Sar1A

Sar1A is another member of the Ras supefamily of small GTPases, involved in vesicle transport and membrane rigidity. In mammals, the two Sar1 isoforms (Sar1A and Sar1B) contain 89% homology. However, Sar1A is commonly referenced as Sar1 (Bi, Corpina et al. 2002, Loftus, Hsieh et al. 2012). Unlike Rab10, which localizes to vesicles throughout the secretory and recycling pathways, Sar1A is only localized to the ER, particularly in COPII vesicles. COPII transport is the first step of the secretory pathway for most secreted proteins starting with the ER (Wang and Wu 2012, Zanetti, Pahuja et al. 2012, Cutrona, Beznoussenko et al. 2013). Sar1A is a principal component of COPII vesicle formation and transport from the ER to the Golgi (Barlowe, d'Enfert et al. 1993, Kuge, Dascher et al. 1994, Long, Yamamoto et al. 2010). In its active GTP form, Sar1A triggers vesicle formation by binding with the ER membrane to recruit other COPII components. Depletion of Sar1A severely disrupts COPII assembly and transport from the ER to Golgi (Cutrona, Beznoussenko et al. 2013). Sar1A also decreases rigidity of the membrane that is bound, either by associating with lipid bilayers or with microtubules (Long, Yamamoto et al. 2010, Loftus, Hsieh et al. 2012, Riggs, Bergman et al. 2012, Fokin, Brodsky et al. 2014). This observation is supported by recent studies that link polymorphisms along Sar1A with the development of membrane structure deficiencies such as sickle cell and Anderson disease (Pepperkok, Lowe et al. 1998, Kumkhaek, Zhu et al. 2007, Kumkhaek, Taylor et al.

2008, Georges, Bonneau et al. 2011). Interestingly, both Rab10 and Sar1A have an important role in regulating anterograde transport and ER structure by interacting with microtubules. However, a specific link with neurodegenerative diseases has not been previously established.

Recent unpublished work by Kauwe (2015) and collaborators discovered two rare variants in Rab10 (rs1427874) and Sar1A (rs7653) that influence Alzheimer's disease risk. Rab10 and Sar1A have not previously been associated with APP transport or amyloid production. We sought to characterize the effects of differential expression of Rab10 and Sar1A on APP and Aβ production in murine N2A/APP695 cells. We found that differential expression of Rab10 change Aβ42/Aβ40 ratio and influences Aβ42 production.

Results

Rab10 & Sar1A expression does not affect full-length APP

We sought to first test the effects of cellular Rab10 and Sar1A overexpression on full length APP. We transfected pCMV6-Rab10 and pCMV6-Sar1A overexpression plasmids in N2A/APP695 mouse cells. At 48 h after transfection, cells were harvested and protein expression was measured using 6E10 and 9E10 antibodies as described in methods section. Fig 1 shows that full-length APP expression is the same in GFP control lanes with Rab10 and Sar1A transfected cells. We further tested whether this observation by immunoblotting with three different gradients of protein aliquots (25 ug/uL, 37.5 ug/uL and 50 ug/uL). The results were the same as before, indicating that APP, Rab10 and Sar1A exposure increase proportionally to the total protein immunoblotted (not shown). Similarly, we measured APP expression of transfected N2A/APP695 cells with shRNA plasmids that produced a 62% knockdown of Rab10 and a 26% knockdown of Sar1A. Fig 1 also shows no visible change in full length APP between the

scrambled plasmid and the knockdown plasmids. Together, these results show that full length APP is not affected by overexpression of Rab10 and Sar1A.

Rab10 expression alters Aβ whereas Sar1A does not change Aβ levels.

Next, we sought to quantify the effect of varying expression of Rab10 and Sar1A on Aβ levels. A sandwich ELISA measuring Aβ40 and Aβ42 levels was performed from cell media obtained from transient transfected cells with overexpression and shRNA plasmids for Rab10 and Sar1A. We observed increased levels of Aβ42 levels and an increased Aβ40:42 ratio (Fig 2A, $p = 0.0017$) in cells overexpressing Rab10. Conversely, we observed the opposite effect in knockdown Rab10 cells, resulting in decreased Aβ42 levels and a decreased Aβ40:42 ratio (Fig 2B), however, the p-value was verily significant $p = 0.048$. On the other hand, no change in AB42 levels and AB40:42 ratios were observed in both overexpressed and knockdown Sar1A cells (Fig 3, p-value not shown). Together, these results show that Rab10 plays a role in APP processing but not on full-length expression whereas the expression effects of Sar1A were did not significantly influence APP expression and processing.

Discussion

Here we show that Rab10 expression significantly affects amyloid generation while maintaining stable levels of APP in N2A-695 cells. This is the first study that presents primary evidence linking Rab10 with APP and Aβ production following preliminary results from Udayar suggesting this effect on Rab10 knockdown (Udayar, Buggia-Prevot et al. 2013). A possible hypothesis surrounding these observations might be that Rab10 does not directly interact in the secretory pathway of APP. Even though Rab10 heavily regulates ER dynamics and morphology (English and Voeltz 2013), it could be possible that Rab10 does not directly transport APP in anterograde fashion to the axons and synapses. APP is prevalent in axonal cones and evidence

shows that one of the trophic functions of APP is to promote axon development (Kins, Lauther et al. 2006, Sosa, Bergman et al. 2013). Interestingly, Rab10 also promotes axon development and elongation by facilitating the transport of membrane receptors such as GLUT4 (Liu, Xu et al. 2013, Sano and Eguez 2007). Nascent APP molecules could be a specific cargo to another Rab GTPases such as Rab3 and Rab6, which have been shown to transport APP to axons and neurites (McConlogue, Castellano et al. 1996, Szodorai, Kuan et al. 2009).

The significant observation of Rab10 in amyloid beta generation suggests a downstream effect in the APP processing pathway. A second hypothesis explaining the results of this study is that Rab10 participates in AB processing following APP endocytosis. Rab10 also localizes to the recycling and sorting endosomes and mediates vesicle transport from basolateral endosomes to early and late endosomes (Babbey, Ahktar et al. 2006, Chen, Schweinsberg et al. 2006). This transport is part of endosomal pathways where proteins are taken to and from the plasma membrane to be degraded by authophagy or recycled back to the plasma membrane. Expression changes of Rab10 might accelerate or suppress transport between endosomes as part of recycling and autopaghy processes of APP. Future experiments specifically tracking endosomal transport will prove useful to determine the precise role of Rab10 in amyloid beta transport.

Conversely, we did not see a significant effect of Sar1A expression in both APP expression and amyloid processing. Since Sar1A only localizes to ER compartments and vesicles leading to the Golgi, it is possible that similar to Rab10, Sar1A is not directly involved in APP anterograde transport (Kuge, Dascher et al. 1994, Cutrona, Beznoussenko et al. 2013). Even though a direct role of Sar1A in amyloid beta production seems unlikely due to its localization in the cell, it should not be completely discarded. Our preliminary results support the former by not showing any interesting effects on Aβ. However, in N2A cells transfected with shRNA plasmids,

we only obtained a 26% total knockdown of Sar1A. We considered this knockdown percentage to be enough to obtain observable conclusions regarding Sar1A role in amyloid beta generation. Another factor that might influence in our results was the number of replicated used. We only used three replicates in our sandwich ELISA to evaluate total Aβ42 and Aβ40 production. It is possible that by increasing the number of replicates would and knockdown conditions by the use of other RNAi methods could influence these results. Further experiments including these recommendations and other cell imaging methods could be used to confirm these results.

In closing, these results support previous observations where silencing of Rab10 decreases only Aβ (Udayar, Buggia-Prevot et al. 2013) and show that the opposite also applies in higher Aβ generation. In addition, these results offer preliminary evidence supporting results by Kauwe et al (2015) where polymorphisms on the 3'UTR of Rab10 affect Alzheimer's disease risk. The next step will be to repeat the methods used in this study in N2A-695 cells expressing rs14278789 (Rab10) to further confirm the functional effect of this genetic variation in Alzheimer's.

Acknowledgements

This research was done in collaboration with Celeste Karch Ph. D at Washington University in St. Louis, who kindly provided N2A-695 cells, reagents needed for this experiment and valuable advice. The author also acknowledges Simon Hsu and Lubov Ezerkiy who provided great assistance in the experiments presented here.

Methods

Plasmids

The plasmids used for this study were the following: pCMV6-Rab10 (Origene # RC201464), pCMV6-Sar1A (Origene # RC201450) for the overexpression experiments. pGFP-V-RS-Rab10 shRNA (Origene #TG501823) and pGFP-V-RS-Sar1A shRNA (Origene #TG501970). Four shRNA versions per gene were tested to obtain greater knockdown levels.

Cell culture

Mouse neuroblastoma cells (N2A/APP695) expressing human APP-695 isoform were kindly given by Celeste Karch, Ph.D. N2A/APP695 are a mouse neuroblastoma line that express human APP695 isoform and is commonly used in functional APP studies (Thinakaran, Teplow et al. 1996, Rajendran, Honsho et al. 2006, Wang, Wang et al. 2006). N2A/APP695 cells were plated and grown in Dulbecco's modified eagle medium (DMEM) and Opti-MEM (1:1) supplemented with 1% L-glutamine, 5% FBS and 1% anti-mycotic solution. Cells were grown between 80% to 90% confluence for posterior analyses. Upon confluency, cells were transiently transfected using Lipofectamine 2000 (Invitrogen). Culture media was changed 24 h after transfection. Following 48 h after transfection, cell media was collected and centrifuged for 10 minutes at 4°C and protease inhibitor was added for peptide preservation. Cell pellets were collected, lysed and centrifuged with protease inhibitor to collect total protein. Protein concentration was measured using a BCA method in preparation for immunoblotting.

Transfection and reporter gene assays

Functional assays of reporter gene constructs were performed by transient transfection of N2A/APP695 cells using Lipofectamine 2000 reagent (Life Technologies). Cells were allow to confluence between 85 to 90% and transfected with and pCDNA control vector to bring total

DNA concentration 1.0 μg. Cells were growth for 48 h; following this time, media was collected for ELISA assays. Cells were washed and RNA was isolated from cells for RT-qPCR or lysed to assess protein concentration by Western blot analysis.

RNA isolation and RT-PCR analysis

In order to assess greatest knockdown, total RNA was isolated from N2A/APP695 cells after transfection with four plasmids containing specific shRNA for knockdown of either Rab10 or Sar1A genes. RNA was extracted from cells 48 h following transfection using RNeasy (Qiagen) following manufacturer protocol. RNA was converted to cDA using High-capacity cDNA reverse transcription (ABI). Following RT-PCR, Taqman real time PCR assays were used to observe the expression of Rab10 (Mm00489481_m1) and Sar1A (Mm01150424_m1) from ABI technologies. Total gene expression was quantified in triplicates using an ABI-7900 Real-Time PCR system. A housekeeping gene GAPDH was used for normalizing expression values using the CT method.

Protein analysis

N2A/APP695 cell lysates were used to assess Rab10, Sar1A and APP695 protein expression by SDS-PAGE and Western blot analysis with primary mouse 9E10 or 6E10 polyclonal antibody and goat-anti mouse polyclonal as secondary antibody. Briefly, equivalent amounts of total protein were evaluated by SDS-PAGE, blocked with 5% nonfat milk, and exposed to the primary antibody diluted at 1:5000 at 4°C overnight. Exposure to the secondary antibody was carried at 1:2000 for 2 h. Blots were exposed with enhanced chemiluminescence (Lumigen TMA-6).

Enzyme-linked immunosorbent assay

The levels of Aβ40 and Aβ42 were measured from collected cell culture media by sandwich ELISA as described by the manufacturer (Invitrogen). ELISA values were obtained (pg/mL) and corrected for total intracellular protein (ug/mL) based on BCA measurements.

Statistical analysis

Values are expressed as mean ± SD obtained from at least three separate experiments in each group. Data were assessed by one-way analysis of variance (ANOVA). When ANOVA indicated significant differences, the Student's t-test was used with Bonferroni correction for multiple comparisons. Results presented are representative and those with P values <0.05 were considered significant.

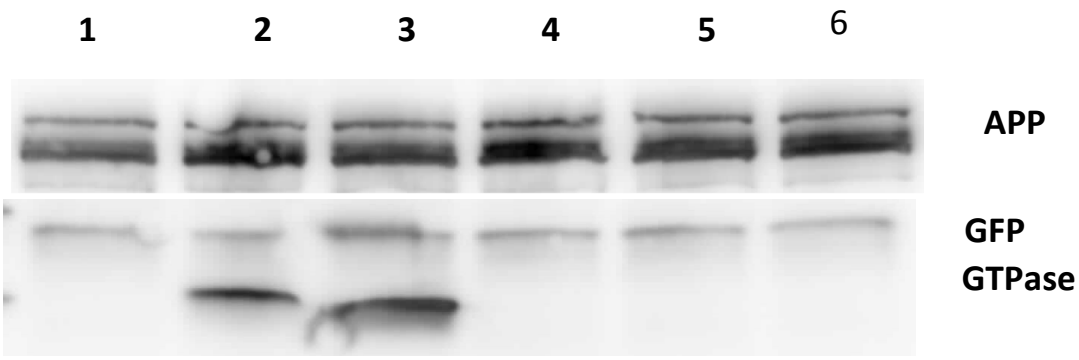

Figure 2: Expression of Rab10 and Sar1A does not affect full length APP. This figure shows an immunoblot using the following legends: lanes 1- GFP, lane 2 – GFP + Rab10, lane 3- GFP+Sar1A, lane 4 – Scrambled shRNA, lane 5 – scrambled + Rab10 shRNA, lane 6 – scrambled + Sar1A.

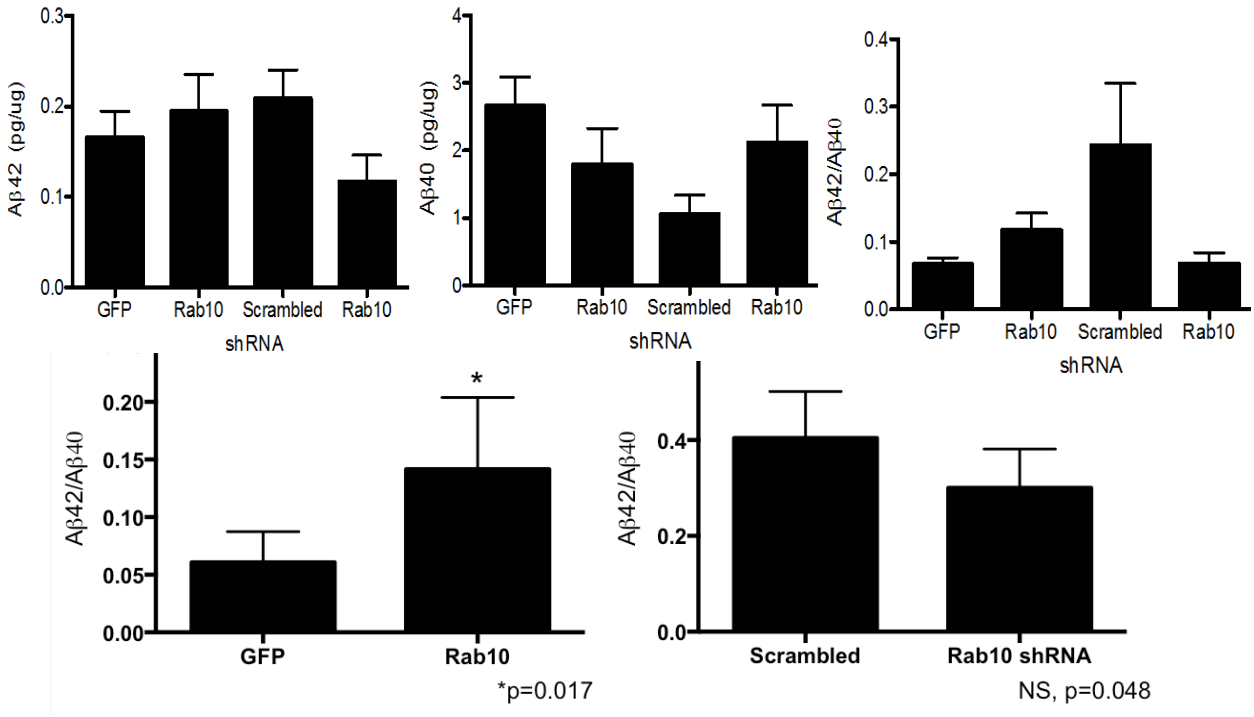

Figure 3: Rab10 expression influences Aβ42 and Aβ42/aβ40 ratio. Figure 3A shows the results from a sandwich ELISA in N2A-695 cells transfected with overexpression and shRNA plasmids. Experiments and their respective controls (GFP for overexpression and Scrambled for shRNA) are depicted on Aβ42 (left), Aβ40 (middle) and Aβ42/Aβ40 ratio (right) panels. Each bar represents the results from six replicates. Figure 2B shows the effects of Rab10 for Aβ42/Aβ40 ratio only. Each bar represents the results from 10 replicates.

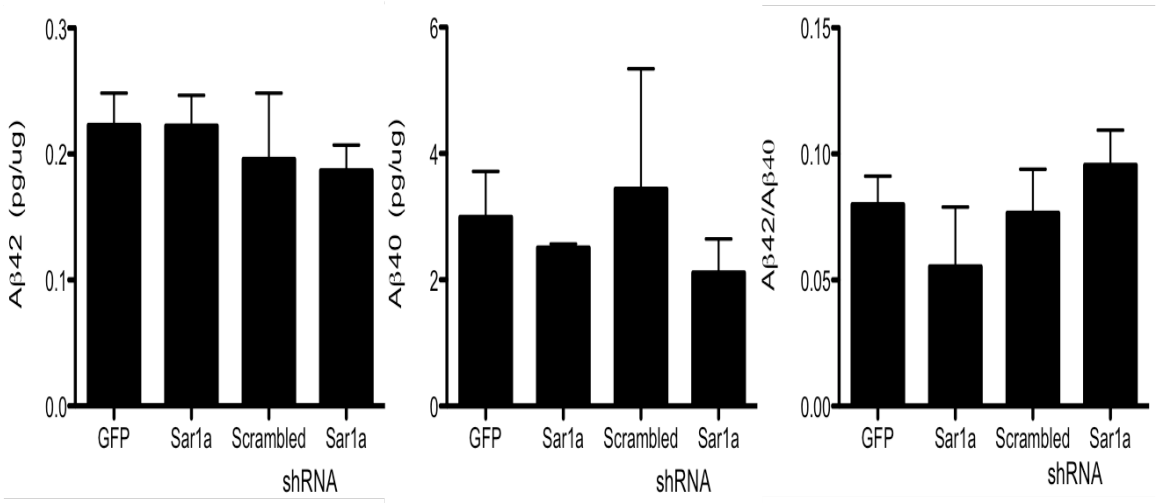

Figure 4: Sar1A expression does not influence Aβ42 and Aβ42/Aβ40 ratio. This figure shows the results from the sandwich ELISA in N2A-695 cells transfected with overexpression and shRNA plasmids. Experiments and their respective controls (GFP for overexpression and Scrambled for shRNA) are depicted on Aβ42 (left), Aβ40 (middle) and Aβ42/Aβ40 ratio (right) panels. Each bar represents the results from three replicates.

References:

Armstrong, A., N. Mattsson, H. Appelqvist, C. Janefjord, L. Sandin, L. Agholme, B. Olsson, S. Svensson, K. Blennow, H. Zetterberg and K. Kagedal (2014). "Lysosomal network proteins as potential novel CSF biomarkers for Alzheimer's disease." Neuromolecular Med **16**(1): 150-160.

Babbey, C. M., N. Ahktar, E. Wang, C. C. H. Chen, B. D. Grant and K. W. Dunn (2006). "Rab10 regulates membrane transport through early endosomes of polarized Madin-Darby Canine Kidney cells." Molecular Biology of the Cell **17**(7): 3156-3175.

Barlowe, C., C. d'Enfert and R. Schekman (1993). "Purification and characterization of SAR1p, a small GTP-binding protein required for transport vesicle formation from the endoplasmic reticulum." Journal of Biological Chemistry **268**(2): 873-879.

Baskys, A., I. Bayazitov, E. Zhu, L. Fang and R. Wang (2007). "Rab-mediated endocytosis: linking neurodegeneration, neuroprotection, and synaptic plasticity?" Ann N Y Acad Sci **1122**: 313-329.

Bi, X., R. A. Corpina and J. Goldberg (2002). "Structure of the Sec23/24-Sar1 pre-budding complex of the COPII vesicle coat." Nature **419**(6904): 271-277.

Buggia-Prevot, V., C. G. Fernandez, S. Riordan, K. S. Vetrivel, J. Roseman, J. Waters, V. P. Bindokas, R. Vassar and G. Thinakaran (2014). "Axonal BACE1 dynamics and targeting in hippocampal neurons: a role for Rab11 GTPase." Molecular Neurodegeneration **9**.

Cataldo, A. M., C. M. Peterhoff, J. C. Troncoso, T. Gomez-Isla, B. T. Hyman and R. A. Nixon (2000). "Endocytic pathway abnormalities precede amyloid beta deposition in sporadic Alzheimer's disease and Down syndrome: differential effects of APOE genotype and presenilin mutations." Am J Pathol **157**(1): 277-286.

Chen, C. C.-H., P. J. Schweinsberg, S. Vashist, D. P. Mareiniss, E. J. Lambie and B. D. Grant (2006). "RAB-10 Is Required for Endocytic Recycling in the Caenorhabditis elegans Intestine." Molecular Biology of the Cell **17**(3): 1286-1297.

Chen, S., L. Li, J. Li, B. Liu, X. Zhu, L. Zheng, R. Zhang and T. Xu (2014). "SEC-10 and RAB-10 coordinate basolateral recycling of clathrin-independent cargo through endosomal tubules in Caenorhabditis elegans." Proceedings of the National Academy of Sciences **111**(43): 15432-15437.

Citron, M., D. Westaway, W. Xia, G. Carlson, T. Diehl, G. Levesque, K. Johnson-Wood, M. Lee, P. Seubert, A. Davis, D. Kholodenko, R. Motter, R. Sherrington, B. Perry, H. Yao, R. Strome, I. Lieberburg, J. Rommens, S. Kim, D. Schenk, P. Fraser, P. St George Hyslop and D. J. Selkoe (1997). "Mutant presenilins of Alzheimer's disease increase production of 42-residue amyloid beta-protein in both transfected cells and transgenic mice." Nat Med **3**(1): 67-72.

Cutrona, M. B., G. V. Beznoussenko, A. Fusella, O. Martella, P. Moral and A. A. Mironov (2013). "Silencing of Mammalian Sar1 Isoforms Reveals COPII-Independent Protein Sorting and Transport." Traffic **14**(6): 691-708.

Deng, C. Y., W. L. Lei, X. H. Xu, X. C. Ju, Y. Liu and Z. G. Luo (2014). "JIP1 Mediates Anterograde Transport of Rab10 Cargos during Neuronal Polarization." Journal of Neuroscience **34**(5): 1710-1723.

English, A. R. and G. K. Voeltz (2013). "Rab10 GTPase regulates ER dynamics and morphology." Nature Cell Biology **15**(2): 169-178.

Fokin, A. I., I. B. Brodsky, A. V. Burakov and E. S. Nadezhdina (2014). "Interaction of early secretory pathway and Golgi membranes with microtubules and microtubule motors." Biochemistry-Moscow **79**(9): 879-893.

Georges, A., J. Bonneau, D. Bonnefont-Rousselot, J. Champigneulle, J. P. Rabes, M. Abifadel, T. Aparicio, J. C. Guenedet, E. Bruckert, C. Boileau, A. Morali, M. Varret, L. P. Aggerbeck and M. E. Samson-Bouma (2011). "Molecular analysis and intestinal expression of SAR1 genes and proteins in Anderson's disease (Chylomicron retention disease)." Orphanet Journal of Rare Diseases **6**.

Grosshans, B. L., D. Ortiz and P. Novick (2006). "Rabs and their effectors: achieving specificity in membrane traffic." Proc Natl Acad Sci U S A **103**(32): 11821-11827.

Haass, C., C. Kaether, G. Thinakaran and S. Sisodia (2012). "Trafficking and proteolytic processing of APP." Cold Spring Harb Perspect Med **2**(5): a006270.

Hardy, J. A. and G. A. Higgins (1992). "Alzheimer's disease: the amyloid cascade hypothesis." Science **256**(5054): 184-185.

Hashimoto, Y. and M. Matsuoka (2014). "A mutation protective against Alzheimer's disease renders amyloid beta precursor protein incapable of mediating neurotoxicity." J Neurochem **130**(2): 291-300.

Hung, A. Y., E. H. Koo, C. Haass and D. J. Selkoe (1992). "Increased expression of beta-amyloid precursor protein during neuronal differentiation is not accompanied by secretory cleavage." Proc Natl Acad Sci U S A **89**(20): 9439-9443.

Jankowsky, J. L., D. J. Fadale, J. Anderson, G. M. Xu, V. Gonzales, N. A. Jenkins, N. G. Copeland, M. K. Lee, L. H. Younkin, S. L. Wagner, S. G. Younkin and D. R. Borchelt (2004). "Mutant presenilins specifically elevate the levels of the 42 residue β-amyloid peptide in vivo: evidence for augmentation of a 42-specific γ secretase." Human Molecular Genetics **13**(2): 159-170.

Kins, S., N. Lauther, A. Szodorai and K. Beyreuther (2006). "Subcellular trafficking of the amyloid precursor protein gene family and its pathogenic role in Alzheimer's disease." Neurodegener Dis **3**(4-5): 218-226.

Kuge, O., C. Dascher, L. Orci, T. Rowe, M. Amherdt, H. Plutner, M. Ravazzola, G. Tanigawa, J. E. Rothman and W. E. Balch (1994). "SAR1 PROMOTES VESICLE BUDDING FROM THE ENDOPLASMIC-RETICULUM BUT NOT GOLGI COMPARTMENTS." Journal of Cell Biology **125**(1): 51-65.

Kumkhaek, C., J. G. Taylor, J. Q. Zhu, C. Hoppe, G. J. Kato and G. P. Rodgers (2008). "Fetal haemoglobin response to hydroxycarbamide treatment and sar1a promoter polymorphisms in sickle cell anaemia." British Journal of Haematology **141**(2): 254-259.

Kumkhaek, C., J. Zhu, J. G. Taylor, C. Hoppe, G. J. Kato and G. P. Rodgers (2007). "Variation in the small guanosine triphosphate (GTP)-binding protein, secretion-associated and RAS-related (SAR1A) gene and response to hydroxyurea treatment in sickle cell disease." Blood **110**(11): 994A-994A.

LeBlanc, A. C. and C. G. Goodyer (1999). "Role of endoplasmic reticulum, endosomal-lysosomal compartments, and microtubules in amyloid precursor protein metabolism of human neurons." J Neurochem **72**(5): 1832-1842.

Lee, J. H., W. H. Yu, A. Kumar, S. Lee, P. S. Mohan, C. M. Peterhoff, D. M. Wolfe, M. Martinez-Vicente, A. C. Massey, G. Sovak, Y. Uchiyama, D. Westaway, A. M. Cuervo and R. A. Nixon (2010). "Lysosomal proteolysis and autophagy require presenilin 1 and are disrupted by Alzheimer-related PS1 mutations." Cell **141**(7): 1146-1158.

Lerner, D. W., D. McCoy, A. J. Isabella, A. P. Mahowald, G. F. Gerlach, T. A. Chaudhry and S. Horne-Badovinac (2013). "A Rab10-Dependent Mechanism for Polarized Basement Membrane Secretion during Organ Morphogenesis." Developmental Cell **24**(2): 159-168.

Liu, Y., X. H. Xu, Q. Chen, T. Wang, C. Y. Deng, B. L. Song, J. L. Du and Z. G. Luo (2013). "Myosin Vb controls biogenesis of post-Golgi Rab10 carriers during axon development." Nature Communications **4**.

Loftus, A. F., V. L. Hsieh and R. Parthasarathy (2012). "Modulation of membrane rigidity by the human vesicle trafficking proteins Sar1A and Sar1B." Biochemical and Biophysical Research Communications **426**(4): 585-589.

Long, K. R., Y. Yamamoto, A. L. Baker, S. C. Watkins, C. B. Coyne, J. F. Conway and M. Aridor (2010). "Sar1 assembly regulates membrane constriction and ER export." J Cell Biol **190**(1): 115-128.

Lu, L. and W. Hong (2014). "From endosomes to the trans-Golgi network." Semin Cell Dev Biol **31**: 30-39.

Masters, C. L., G. Multhaup, G. Simms, J. Pottgiesser, R. N. Martins and K. Beyreuther (1985). "Neuronal origin of a cerebral amyloid: neurofibrillary tangles of Alzheimer's disease contain the same protein as the amyloid of plaque cores and blood vessels." The EMBO Journal **4**(11): 2757-2763.

Nixon, R. A. (2007). "Autophagy, amyloidogenesis and Alzheimer disease." J Cell Sci **120**(Pt 23): 4081-4091.

Oh, E. S., A. V. Savonenko, J. F. King, S. M. Fangmark Tucker, G. L. Rudow, G. Xu, D. R. Borchelt and J. C. Troncoso (2009). "Amyloid precursor protein increases cortical neuron size in transgenic mice." Neurobiol Aging **30**(8): 1238-1244.

Pepperkok, R., M. Lowe, B. Burke and T. E. Kreis (1998). "Three distinct steps in transport of vesicular stomatitis virus glycoprotein from the ER to the cell surface in vivo with differential sensitivities to GTP gamma S." Journal of Cell Science **111**: 1877-1888.

Rajendran, L., M. Honsho, T. R. Zahn, P. Keller, K. D. Geiger, P. Verkade and K. Simons (2006). "Alzheimer's disease beta-amyloid peptides are released in association with exosomes." Proc Natl Acad Sci U S A **103**(30): 11172-11177.

Reddy, P. H., G. Mani, B. S. Park, J. Jacques, G. Murdoch, W. Whetsell, Jr., J. Kaye and M. Manczak (2005). "Differential loss of synaptic proteins in Alzheimer's disease: implications for synaptic dysfunction." J Alzheimers Dis **7**(2): 103-117; discussion 173-180.

Ridge, P. G., M. T. Ebbert and J. S. Kauwe (2013). "Genetics of Alzheimer's disease." Biomed Res Int **2013**: 254954.

Riggs, B., Z. J. Bergman and R. Heald (2012). "Altering membrane topology with Sar1 does not impair spindle assembly in Xenopus egg extracts." Cytoskeleton (Hoboken) **69**(8): 591-599.

Sano, H., L. Eguez, M. N. Teruel, M. Fukuda, T. D. Chuang, J. A. Chavez, G. E. Lienhard and T. E. McGrau (2007). "Rab10, a target of the AS160 rab GAP, is required for insulin-stimulated translocation of GLUT4 to the adipocyte plasma membrane." Cell Metabolism **5**(4): 293-303.

Schuck, S., M. J. Gerl, A. Ang, A. Manninen, P. Keller, I. Mellman and K. Simons (2007). "Rab10 is involved in basolateral transport in polarized madin-darby canine kidney cells." Traffic **8**(1): 47-60.

Seabra, M. C., E. H. Mules and A. N. Hume (2002). "Rab GTPases, intracellular traffic and disease." Trends Mol Med **8**(1): 23-30.

Selkoe, D. J. (2001). "Alzheimer's disease: genes, proteins, and therapy." Physiol Rev **81**(2): 741-766.

Stenmark, H. and V. M. Olkkonen (2001). "The Rab GTPase family." Genome Biol **2**(5): Reviews3007.

Tam, J. H., C. Seah and S. H. Pasternak (2014). "The Amyloid Precursor Protein is rapidly transported from the Golgi apparatus to the lysosome and where it is processed into beta-amyloid." Mol Brain **7**: 54.

Tan, M. G., C. Lee, J. H. Lee, P. T. Francis, R. J. Williams, M. J. Ramirez, C. P. Chen, P. T. Wong and M. K. Lai (2014). "Decreased rabphilin 3A immunoreactivity in Alzheimer's disease is associated with Abeta burden." Neurochem Int **64**: 29-36.

Thinakaran, G. and E. H. Koo (2008). "Amyloid precursor protein trafficking, processing, and function." J Biol Chem **283**(44): 29615-29619.

Thinakaran, G., D. B. Teplow, R. Siman, B. Greenberg and S. S. Sisodia (1996). "Metabolism of the Swedish Amyloid Precursor Protein Variant in Neuro2a (N2a) Cells: EVIDENCE THAT CLEAVAGE AT THE "β-SECRETASE" SITE OCCURS IN THE GOLGI APPARATUS." Journal of Biological Chemistry **271**(16): 9390-9397.

Thomas, R. S., M. J. Lelos, M. A. Good and E. J. Kidd (2011). "Clathrin-mediated endocytic proteins are upregulated in the cortex of the Tg2576 mouse model of Alzheimer's disease-like amyloid pathology." Biochem Biophys Res Commun **415**(4): 656-661.

Udayar, V., V. Buggia-Prevot, R. L. Guerreiro, G. Siegel, N. Rambabu, A. L. Soohoo, M. Ponnusamy, B. Siegenthaler, J. Bali, Aesg, M. Simons, J. Ries, M. A. Puthenveedu, J. Hardy, G. Thinakaran and L. Rajendran (2013). "A paired RNAi and RabGAP overexpression screen identifies Rab11 as a regulator of beta-amyloid production." Cell Rep **5**(6): 1536-1551.

Wang, G. and G. Wu (2012). "Small GTPase regulation of GPCR anterograde trafficking." Trends Pharmacol Sci **33**(1): 28-34.

Wang, T., Y. Liu, X. H. Xu, C. Y. Deng, K. Y. Wu, J. Zhu, X. Q. Fu, M. He and Z. G. Luo (2011). "Lgl1 Activation of Rab10 Promotes Axonal Membrane Trafficking Underlying Neuronal Polarization." Developmental Cell **21**(3): 431-444.

Wang, Y. P., X. C. Wang, Q. Tian, Y. Yang, Q. Zhang, J. Y. Zhang, Y. C. Zhang, Z. F. Wang, Q. Wang, H. Li and J. Z. Wang (2006). "Endogenous overproduction of beta-amyloid induces tau hyperphosphorylation and decreases the solubility of tau in N2a cells." J Neural Transm **113**(11): 1723-1732.

Xu, X. H., C. Y. Deng, Y. Liu, M. He, J. Peng, T. Wang, L. Yuan, Z. S. Zheng, P. J. Blackshear and Z. G. Luo (2014). "MARCKS regulates membrane targeting of Rab10 vesicles to promote axon development." Cell Research **24**(5): 576-594.

Yu, W. H., A. M. Cuervo, A. Kumar, C. M. Peterhoff, S. D. Schmidt, J. H. Lee, P. S. Mohan, M. Mercken, M. R. Farmery, L. O. Tjernberg, Y. Jiang, K. Duff, Y. Uchiyama, J. Naslund, P. M. Mathews, A. M. Cataldo and R. A. Nixon (2005). "Macroautophagy--a novel Beta-amyloid peptide-generating pathway activated in Alzheimer's disease." J Cell Biol **171**(1): 87-98.

Zanetti, G., K. B. Pahuja, S. Studer, S. Shim and R. Schekman (2012). "COPII and the regulation of protein sorting in mammals." Nat Cell Biol **14**(1): 20-28.

Zerial, M. and H. McBride (2001). "Rab proteins as membrane organizers." Nat Rev Mol Cell Biol **2**(2): 107-117.

Kins, S., N. Lauther, A. Szodorai and K. Beyreuther (2006). "Subcellular trafficking of the amyloid precursor protein gene family and its pathogenic role in Alzheimer's disease." Neurodegener Dis **3**(4-5): 218-226.

McConlogue, L., F. Castellano, C. deWit, D. Schenk and W. A. Maltese (1996). "Differential effects of a Rab6 mutant on secretory versus amyloidogenic processing of Alzheimer's beta-amyloid precursor protein." Journal of Biological Chemistry **271**(3): 1343-1348.

Sosa, L. J., J. Bergman, A. Estrada-Bernal, T. J. Glorioso, J. M. Kittelson and K. H. Pfenninger (2013). "Amyloid precursor protein is an autonomous growth cone adhesion molecule engaged in contact guidance." PLoS One **8**(5): e64521.

Szodorai, A., Y. H. Kuan, S. Hunzelmann, U. Engel, A. Sakane, T. Sasaki, Y. Takai, J. Kirsch, U. Muller, K. Beyreuther, S. Brady, G. Morfini and S. Kins (2009). "APP anterograde transport requires Rab3A GTPase activity for assembly of the transport vesicle." J Neurosci **29**(46): 14534-14544.