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The role of presynaptic proteins in maintaining neuronal viability

Cerveira Tavares dos Santos, T.

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General discussion

Aims and summary of the main findings

Neurodegenerative diseases are characterized by dysfunction of the nervous system with often a gradual neuronal cell loss. Understanding the mechanisms of neuronal cell loss is a central aim in neuroscience. The general aim of this thesis was to understand the molecular mechanism(s) of neuronal cell loss triggered by the absence of presynaptic proteins. We specifically focused on the presynaptic protein Munc18-1, its role in neuronal survival and, to a larger extent, the role of this protein in neurodegenerative diseases such as Alzheimer's disease (AD).

In **chapter 2**, we depleted three presynaptic proteins from cultured neurons and systematically compared the similarities and differences in neuronal cell death. We have observed that t-SNAREs (syntaxin-1 and SNAP-25) and Munc18-1 are crucial for neuronal survival, while v-SNAREs (VAMPs/synaptobrevins) are dispensable. We provided additional support for the fact that in Munc18-1 knockout (KO) neurons, lack of synaptic transmission is not causally related to cell death, since Munc18-2 and -3, non-neuronal isoforms of Munc18-1, do not rescue synaptic transmission, but do rescue cell death. Furthermore, we have linked the condensed *cis*-Golgi to the atypical cell death pathway caused by the loss of these presynaptic proteins.

Next, in **chapter 3**, we set out to determine the causes of the condensed *cis*-Golgi phenotype. We have excluded vacuolar protein sorting 45 (VPS45)-, Golgi Phosphoprotein 3 (GOLPH3)- and phosphatidylinositol 4-kinase IIIα (PI4KIIIα)-dysfunction as single causes of the *cis*-Golgi abnormalities in Munc18-1 KO neurons. A decrease in phosphatidylinositol 4-phosphate (PI(4)P), the major phosphoinositide at the Golgi, was detected in Munc18-1 KO neurons. Our data suggest that the phosphoinositide metabolism might contribute to the condensed *cis*-Golgi observed in Munc18-1 KO neurons.

Following up on phosphoinositide metabolism, in **chapter 4** we studied a possible link between phosphatidylinositol 4,5-biphosphate (PI(4,5)P2) and phosphatidylinositol 3,4,5-trisphosphate (PI(3,4,5)P3) and cell death in Munc18-1 KO neurons. We have found a decrease in the PI(4,5)P2 pool in Munc18-1 KO neurons. However, phosphatidylinositol 4-phosphate 5-kinase (PIP5K) overexpression did not rescue neuronal survival, suggesting that Munc18-1 KO cell loss is not causal to PI(4,5)P2. In contrast, an increase in PI(3,4,5)P3 levels was detected in Munc18-1 KO neurons together with changes in proteins from the PI3K/Akt pathway (phosphoinositide 3-kinase (PI3K) and protein kinase B (PKB)/Akt). Thus, our data suggest a possible involvement of PI3K/Akt pathway in neuronal cell death in absence of the presynaptic protein Munc18-1.

Finally, in **chapter 5** we investigated the role of Munc18-1 in AD. We started by verifying a previously described interaction between the amyloid precursor protein (APP) and Munc18-1. We studied this interaction and detected an increase in APP levels in the absence of Munc18-1 protein in mouse brain. Furthermore, we have identified an interaction between Munc18-1 and beta-site APP cleaving enzyme 1 (BACE1) and a complex of APP/BACE1/Munc18-1/Mint-1. Hence, our data suggest that Munc18-1 plays a role in the amyloidogenic processing of APP, which may impact the development of Alzheimer's disease.

A summary of the main differences between WT and Munc18-1 KO described in this

thesis are presented in table 6.1.

	Wild-type	*Munc18-1 KO*		
Survival cortical neurons	14 DIV	3-7 DIV	↓	Chapter 2
Survival DRG	21 DIV	21 DIV	↔	Chapter 2
Golgi area	24.0 μm^2	11.1 μm^2	↓	Chapter 2
Golgi shape	0.4	0.7	↑	Chapter 2
PI(4)P Golgi	1215.2 A.U.	977.2 A.U.	↑	Chapter 3
PI(4)P soma	965.0 A.U.	831.0 A.U.	↑	Chapter 4
PI(4,5)P2	779.2 A.U.	638.0 A.U.	↓	Chapter 4
PI(3,4,5)P3	420.5 A.U.	481.3 A.U.	↑	Chapter 4
pAKT Thr308	100%	51%	↓	Chapter 4
Dynamins	100%	9%	↓	Chapter 4
APP	100%	147%	↑	Chapter 5

Table 6.1 – Summary of the main findings WT *vs.* Munc18-1 KO.

Functional cooperation between Munc18-1 and syntaxin-1 is not sufficient for neuronal survival

A functional cooperation between the co-chaperones Munc18-1 and syntaxin-1 was suggested as an important mechanism to maintain neuronal viability in Munc18-1 KO neurons[29]. Supporting this idea, overexpressing syntaxin-1 delays Munc18-1 KO cell loss[29]; syntaxin-1 levels are reduced in Munc18-1 KO animals[174] and Munc18-1 is crucial for proper syntaxin-1 targeting (chapter 2). However, our data provide evidence to suggest distinct aspects for the two proteins in maintaining neuronal viability.

First, cell loss in Munc18-1 KO is less severe and slower than in syntaxin-1 KO neurons[29; chapter 2,221]. Second, Munc18-3, which has a very low affinity for syntaxin-1, rescues cell loss in Munc18-1 KO neurons (chapter 2). Third, to completely abolish binding between Munc18-3 and syntaxin-1, a syntaxin-1 truncation with deleted N-terminal domain was used. Overexpression of Munc18-3 in combination with this syntaxin-1 truncation also restored neuronal survival. Finally, we have previously shown that a Munc18-1 mutant (D34N, M38V) unable to bind syntaxin-1, rescues neuronal cell loss[350]. Taken together, these data argue for distinct roles of Munc18-1 (and syntaxin-1) in addition to the functional cooperation between Munc18-1 and syntaxin-1, as a key mechanism for neuronal survival.

Loss of synaptic transmission cannot explain neuronal loss

Munc18-1 KO neurons are synaptically silent and degenerate in culture[26,27] and therefore a causal link between cell loss and synaptic transmission was initially suggested. However, later evidence showed that in Munc18-1 KO neurons, neuronal cell loss occurs before synaptogenesis[26]. In addition, we show here that depletion of syntaxin-1 and SNAP-25 also leads to cell death before

synaptogenesis (chapter 2). In Munc13-1/-2 (double knockout (DKO) as well as VAMP2 KO or TeNT-treated neurons, synaptic transmission is also lost, but in these cases no neuronal loss is observed[30,31,223]. Furthermore, we show that overexpressing non-neuronal isoforms (Munc18-2 or -3) in Munc18-1 KO neurons rescues neuronal survival, but not synaptic transmission. Similarly, syntaxin-1A/B DKO neurons are also synaptically silent, but overexpression of a syntaxin-1 mutant (A240V, V244A) rescues neuronal survival, but not synaptic transmission[29]. Hence, loss of synaptic transmission is not causal to cell loss in neurons lacking Munc18-1 or syntaxin-1.

Likewise, synaptic transmission is lost in SNAP-25 KO and neurons degenerate in culture[25,28]. In neurons treated with BoNT/E, a neurotoxin that cleaves SNAP-25, neuronal loss is also observed[221]. In contrast, BoNT/A also cleaves SNAP-25, albeit at a different site in the protein[218] and abolishes synaptic transmission, but does not cause neuronal cell loss[221]. Thus, these data show distinct roles of SNAP-25 in neuronal viability and in synaptic transmission.

While the last 9 amino acids (AA) of SNAP-25 are crucial to maintain synaptic function, but not survival, the last 26 AA are crucial for both functions[351]. This suggests that a short truncation (9 AA), but not a larger truncation (26 AA) still supports SNAP-25's role in neuronal survival. This also suggests that this unknown, SNARE-dependent survival process is less tightly regulated than synaptic transmission, as it tolerates the 9 AA truncations, whereas synaptic transmission does not. Furthermore, SNAP-25 stability may be different after being cleaved by BoNT/A or BoNT/E and a different half-life may have a different impact on neuronal viability and synaptic transmission.

In the synaptically silent neurons that degenerate in culture, immature synapses are still formed in syntaxin-1A[185,186], syntaxin-1B[186], SNAP-25[25] and Munc18-1 KO animals[27,184,200] in vivo. This suggests that basic synaptogenesis still takes place in neurons lacking these presynaptic proteins. In summary, the role of Munc18-1, syntaxin-1 and SNAP-25 in synaptic transmission is distinct from their role in neuronal survival and unrelated to synaptogenesis.

Dysregulation of actin and actin-binding proteins is not the single cause for Golgi abnormalities and neurodegeneration

Our data produce evidence for a role of actin in Golgi morphology. First, depolymerization of actin by Latrunculin B (LatB) produces a condensed Golgi phenotype[125] similar to the *cis*-Golgi phenotype in Munc18-1 KO neurons (chapter 2). Second, we previously showed that Munc18-1 regulates actin in adrenal chromaffin cells, where cells lacking Munc18-1 show an increased cortical F-actin network at the plasma membrane[167]. However, in cultured neurons, no changes in F-actin, including the region adjacent to the Golgi, were detected in Munc18-1 KO neurons (chapter 2).

The Golgi apparatus requires interplay between many proteins and lipids to keep its structure and function[352]. Recently, Dippold and colleagues presented a Golgi model where GOLPH3 binds PI(4)P in the Golgi membrane and myosin 18A (MYO18A), which binds F-actin, connects the Golgi apparatus to the cytoskeleton and promotes Golgi elongation[127]. Interestingly, knockdown of GOLPH3 or the SM-protein VPS45 leads to a condensed Golgi

phenotype similar to the Golgi phenotype in Munc18-1 KO neurons[127,259]. However, the data presented in chapter 3 exclude VPS45- or GOLPH3-dysfunction as single causes of the *cis*-Golgi abnormalities. Furthermore, GOLPH3 binds to the *cis*-Golgi pool of PI(4)P, which is reduced in Munc18-1 KO neurons (chapter 3). Overexpression of PI4KIIIα in Munc18-1 KO neurons, which generates PI(4)P, did not rescue *cis*-Golgi morphology nor neuronal loss (chapter 3). Thus, although our data suggest that Golgi defects might be caused by actin dysregulation we did not find causality, but instead excluded dysregulation of GOLPH3, VPS45 and PI4KIIIα as single causes.

In the secretory pathway, the transport from the ER to the Golgi apparatus (anterograde traffic) is mediated by COPII-coated vesicles[353,354]. The retrograde transport, from the Golgi to the ER, is mediated by COPI-coated vesicles[355,356]. In cells treated with Latrunculin B (LatB), anterograde traffic is not affected while retrograde traffic and Golgi structure (as mentioned above) are affected[126]. Since the Golgi of Munc18-1 KO neurons mimics the Golgi of WT neurons treated with LatB, it is plausible that in Munc18-1 KO neurons retrograde transport is hampered. Although dysregulation of actin is not the single cause to explain the Golgi abnormalities, dysregulation of retrograde transport, possibly in combination with dysregulation of actin, may be an important aspect that contributes to Golgi abnormalities and neuronal loss in Munc18-1 KO neurons.

Munc18-1 controls dynamin levels in neurons, but the link with neurodegeneration remains elusive

Recent evidence suggests that loss of the endocytosis protein dynamin might be involved in a similar type of neurodegeneration as observed for Munc18-1 or syntaxin-1 depletion. First, dynamin 1/3 DKO mice show neurodegeneration[154]. However, this degeneration occurs later in development than in Munc18-1 or syntaxin-1A/B DKO mice and only *in vivo*[26,27,29,154]. Second, de novo mutations in dynamin-1 and Munc18-1 lead to the same syndrome in humans: epileptic encephalopathies[357-359]. Third, viability of BoNT/C-treated neurons is rescued by a dominant negative dynamin (K44A)[221]. Finally, dynamin levels are severely reduced in Munc18-1 KO brains (chapter 4). Peng and colleagues suggested that survival in BoNT/C-treated neurons rescued with dynamin dominant negative is supported by a temporary block of endocytosis that compensates for the exocytosis defects in the plasma membrane recycling process[221]. However, it is not known which dynamin isoform was used in this study. An attempt to rescue or delay neuronal loss in Munc18-1 KO neurons by overexpressing a dominant negative dynamin-1 (K44A) was not successful (data not shown in this thesis). Thus, although several observations suggest that loss of dynamin and Munc18-1 may lead to similar and interconnected cellular deficits, a causal link between dynamin-1 dysregulation and neuronal loss in Munc18-1 KO neurons has not been established yet.

The PI3K/Akt pathway is involved in Munc18-1 KO neuronal cell loss

Several lines of evidence suggest the involvement of PI3K/Akt pathway in Munc18-1 KO neuronal loss. First, a decrease in pAkt threonine 308 (Thr308) levels (but not pPDK1) was

observed in Munc18-1 KO brains (chapter 4). Second, neuronal cell loss is delayed *in vitro* by neurotrophic factors such as brain-derived neurotrophic factor (BDNF) or insulin in Munc18-1 KO[26]. In syntaxin-1B KO, BDNF and neurotrophin-3 (NT-3) also delayed cell loss, however insulin did not[186]. Extracellular signaling by BDNF and insulin-like growth factor I (IGFI) activates the PI3K/Akt pathway by tropomyosin receptor kinase B (TrkB) dimerization[51,53]. TrkB stimulates PI3K leading to conversion of PI(4,5)P2 into PI(3,4,5)P3[54] and activation of effector proteins such as Akt and PDK1[55,56]. Third, in spinal cord neurons of Munc18-1 KO, TrkB, but not TrkA or TrkC, accumulates in the soma[360]. Thus, the decrease in pAkt, the delay in cell death when neurotrophic factors are added and the accumulation of TrkB all point towards the involvement of the PI3K/Akt pathway in Munc18-1 KO neuronal loss.

If neurotrophic factor signaling and/or TrkB activity would be reduced in the absence of Munc18-1 (or syntaxin-1), a reduction in PI3K activity and PI(3,4,5)P3 levels would be expected in Munc18-1 KO neurons, as PI3K is a central downstream effector in this pathway[286]. However, we observed the opposite: an increase in PI(3,4,5)P3 and a decrease in PI(4,5)P2 levels (suggesting increased PI3K activity). Furthermore, MARCKS protein is known to sequester plasma membrane PI(4,5)P2 and to promote PI(4,5)P2 clustering[304]. In Munc18-1 KO animals a decrease in MARCKS protein was detected (chapter 4). Low levels of PI(4,5)P2 have been linked to low levels of MARCKS[361] and it was suggested that the lack of membrane clustering of PI(4,5)P2 increases PI3K availability and PI(3,4,5)P3 levels. Thus, the increase in PI(3,4,5)P3 might be explained by the hyperactivity of PI3K, rather than lack of neurotrophic factors and/or TrkB. In summary, the delay observed in Munc18-1 KO cell loss by neurotrophic factors, together with the decrease in pAkt, suggest the involvement of PI3K/Akt pathway in Munc18-1 KO cell loss, although a discrepancy in the phosphoinositides levels, mainly the increase in PI(3,4,5)P3, is not in line with this.

Implication of Munc18-1 in the diseases

Heterozygous mutations in *munc18-1* (Syntaxin-binding protein-1 (STXBP1)) gene have been reported in patients with early onset epileptic encephalopathy (EOEE), presenting epileptic seizures that begin within the first three months of life[358]. Within EOEE, different syndromes have been described, the most common, Ohtahara Syndrome (OS) also known as early infantile epileptic encephalopathy (EIEE), is one of the most severe and earlier forms of epilepsy[333,358,362]. OS is characterized by early onset of tonic spasms, seizures intractability, suppression-burst pattern on the electroencephalogram (EEG) and severe psychomotor retardation[363,364]. With patients aging, the epileptic seizures often change and a transition to West Syndrome, characterized by tonic spasms with clustering, arrest of psychomotor development and hypsarrhythmia on EEG, occurs in 75% of the patients[363-365]. The defects in neurotransmitter release have been suggested as a cause of epileptic seizures in patients[358]. However, data in this thesis suggest that other Munc18-1 dependent pathways in neuronal maintenance may provide alternative or additional explanations for the pathogenesis in these patients.

Cellular characterization of induced neurons from patients has been used as a human neuronal model to study haploinsufficiency in STXBP1[201,366]. Neurons from Munc18-1 KO

mice degenerate in culture while Munc18-1 heterozygous (HZ) neurons survive at least until day in vitro (DIV) 14[26,367,chapter 2]. Similarly, in human induced neurons, homozygous STXBP1-mutant neurons (Munc18-1$^{-/-}$) degenerate fast in culture[201]. Heterozygous STXBP1-mutant neurons (Munc18-1$^{+/-}$) do not degenerate in culture and present normal neuronal development and synaptogenesis[201]. However, synaptic transmission is impaired in heterozygous STXBP1-mutant neurons[201], in a more severe way than in mouse Munc18-1 HZ neurons[367]. Thus, while neuronal survival is similar between mice and human induced neurons, synaptic transmission defects are more severe in the human induced neurons compared to mouse neurons.

Syntaxin-1 levels are decreased in Munc18-1 KO but no changes are observed in Munc18-1 HZ[174]. However, human heterozygous STXBP1-mutant neurons show a decrease of ~ 40%[201] and in a heterozygous SXTBP1 mutant (c.1099C>T; p.R367X) syntaxin-1 levels are also reduced[366]. In chapter 2, we have demonstrated that depletion of Munc18-1 or insufficient binding of syntaxin-1 to another Munc18 isoform - Munc18-3 - led to syntaxin-1 targeting defects. Mislocalization of syntaxin-1 was also reported in heterozygous SXTBP1 mutant (c.1099C>T; p.R367X) induced human neurons[366]. Though the human induced neurons studies were performed in heterozygous STXBP1, and the mouse study in homozygous, it is interesting to verify that mislocalization of syntaxin-1 might have implications in human diseases. Furthermore, although in mice the Munc18-1 heterozygous mutation might not have a large effect, it can cause severe disorders in humans, but not necessarily by defects in synaptic transmission but rather by an alternative (neuronal survival) pathway.

Several other mutations in Munc18-1 have been linked to EOEE, such as C180Y, V84D, G544D, M443R and T574P[333,358]. Munc18-1 C180Y is more instable than WT Munc18-1 and binding with syntaxin-1 is hampered[362]. Munc18-1 WT and C180Y were identified in Lewy body-like structures, a Parkinson's disease (PD) hallmark, with Munc18-1 C180Y forming aggregates with α-synuclein[368]. Knocking out Munc18-1 led to α-synuclein aggregation in neurosecretory cells[368], however in spinal cord neurons α-synuclein was not changed[360]. Thus, although a Munc18-1 mutation linked to EOEE (Munc18-1 C180Y) was shown to aggregate α-synuclein and to be related to PD, the involvement of Munc18-1 WT as chaperone for α-synuclein and its role in controlling α-synuclein aggregation is still controversial and needs further research. For the Munc18-1 T574P mutation, the three newborns studied presented epileptic seizures from day 1 until 1 year old[333]. This Munc18-1 mutation affects a cyclin-dependent kinase 5 (cdk5) phosphorylation site, leading to lack of modulation[333]. Activation of cdk5 has been linked to AD[369], leading to tau hyperphosphorylation[330,370] and cleavage of APP, while cdk5 inhibition leads to a decrease in Aβ42[371]. Phosphorylation of Munc18-1 by cdk5 reduces APP-syntaxin-1 association, and increases APP-BACE1 co-localization[24]. Thus, a Munc18-1 mutation known to cause EOEE may also be involved in AD via the cdk5 pathway.

In chapter 5, we presented evidence that Munc18-1 is important to promote APP processing, by showing an increase in APP levels in the absence of Munc18-1. In AD post-mortem brains, Munc18-1 and cdk5 levels are increased but syntaxin-1 levels are unchanged[22], while another study reported a decrease in Munc18-1 and an increase in syntaxin-1 levels[21]. Furthermore, Munc18-1a levels were decreased while Munc18-1b levels were not changed[332].

The differences in Munc18 levels observed between these studies might be due to post-mortem delays and/or the use of different techniques. Additionally, in postmortem brains from AD patients the levels of PI(4)P and PI(4,5)P2 were reduced[372]. It has also been reported that increasing Aβ42 levels led to a decrease in PI(4,5)P2[373] and that decreasing PI(4,5)P2 levels led to an increase of Aβ42[374]. A mutation in presenilin causing familiar AD led to an imbalance of PI(4,5)P2, either by diminished synthesis or enhanced breakdown[374]. In our study we showed a decrease in PI(4,5)P2 in Munc18-1 KO neurons and an increase in APP together with a new interaction between BACE1 and Munc18-1. Although more information is needed regarding Aβ40/42 levels in Munc18-1 KO and a consensus does not exist regarding Munc18-1 changes in AD brains, one possible hypothesis is that Munc18-1 plays a role in AD pathogenesis via an alternative pathway that involves PI(4,5)P2.

Future perspectives

Additional research is required to fully understand the mechanism of cell death in the absence of presynaptic proteins. In chapter 2, we show that depletion of t-SNAREs and Munc18-1 leads to early neuronal cell loss. In contrast, in dorsal root ganglion neurons (DRGs), depletion of Munc18-1 does not lead to degeneration. Therefore, it would be interesting to further study DRGs and their ability to survive in absence of Munc18-1: 1) comparing affected pathways in central nervous system (CNS) neurons with DRGs, such as the PI3K/Akt pathway and phosphoinositides; 2) are t-SNAREs as important for DRGs survival as for CNS neurons, or like Munc18-1, are t-SNAREs dispensable for DRGs survival? Additionally, a dominant negative dynamin (K44A) rescues neuronal survival of BoNT/C treated neurons[221]. Although dynamin-1 K44A did not rescue neuronal survival in Munc18-1 KO neurons, further experiments should test if a different dynamin isoform - dynamin-2 K44A - rescues neuronal survival in Munc18-1 and SNAP-25 KO neurons.

Performing a proteomic study in WT vs. Munc18-1 KO neurons would allow identification of proteins that are changed in the mutant and may point to a new pathway involved in neuronal cell death. Moreover, a proteomics screen could help to pinpoint which protein(s) are responsible for the Golgi morphology changes observed in chapter 2. Although it is plausible that the absence of Munc18-1 changes the link between the Golgi and the cytoskeleton indirectly, three questions still remain unanswered: 1) which protein/lipid interactions between the Golgi and the cytoskeleton are disrupted in Munc18-1 KO? 2) Does Munc18-1 regulate levels of proteins and lipids responsible for Golgi morphology? 3) Is Golgi retrograde transport affected in Munc18-1 KO neurons?

Phosphoinositides metabolism is altered in Munc18-1 KO neurons (chapter 4). PI(4)P and PI(4,5)P2 levels are reduced while PI(3,4,5)P3 levels are increased. These measurements were done using a live probe and quantified by immunofluorescence. Nevertheless, this method has some limitations, which are discussed extensively in chapter 4. Thus, more experiments are needed, like biochemical detection techniques such as radioactivity or non-radioactivity: thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) and mass spectrometry (MS) to validate the changes observed in phosphoinositides levels. Next,

it would be interesting to study, in live cells, changes in phosphoinositides localization at the synapses. To do so, the use of super resolution microscopy, which offers an increased spatial and temporal resolution, would be recommended. Finally, by inducible acute manipulations, changes of phosphoinositides in real time and its influences on other subspecies, can be studied. While our data suggest that the reduction in PI(4,5)P2 is not linked to cell death, further experiments are needed to understand if the unbalance between PI(4,5)P2/PI(3,4,5)P3 leads to cell death. Furthermore, syntaxin-1, the co-chaperone of Munc18-1, requires high PI(4,5)P2 accumulation to be sequestered[375]. Thus, it would be interesting to study, by overexpressing Munc18-1 and -3 in Munc18-1 KO neurons, whether changes in PI(4,5)P2 are due to lack of synaptic transmission and/or changes in syntaxin-1 localization.

In chapter 5, we studied the link between Munc18-1 and AD. However it is still not clear: 1) from existing literature, whether Munc18-1 is up or downregulated in AD patients. Thus, Munc18-1 levels should be studied in AD postmortem material; 2) how does Munc18-1 regulate APP levels, via vesicle release or microdomain switch? Additionally, the link between Munc18-1 and PI(4,5)P2 and its implication in AD should be further studied by: 1) measuring Aβ42 in cultured neurons; 2) manipulate PI(4,5)P2 and evaluate Aβ42 changes; 3) manipulate phosphoinositides by optogenetics *in vivo* in AD mouse models. It would also be interesting to follow up on the BACE1/Munc18-1 interaction and its molecular mechanism. Does this interaction influence Aβ40/42 production?

Munc18-1 was recently reported in PD as a chaperone for α-synuclein, together with the involvement of Munc18-1 C180Y mutant in Lewy bodies-like structures[368]. Although, in spinal cord neurons form Munc18-1 KO animals, no changes were observed in α-synuclein[360]. Thus, further experiments should test if depletion of Munc18-1 from neurons produces an increase in toxic α-synuclein oligomers in the brain. If yes, would clearance of these oligomers *in vitro* rescue Munc18-1 KO cell death? Furthermore, what is the molecular mechanism of Munc18-1 in PD? 1) How can Munc18-1 aggregate in PD? 2) What are the consequences of this aggregation? 3) What are the binding domains of Munc18-1 and α-synuclein?

Understanding the mechanism(s) of cell death in the absence of presynaptic proteins, as well as the role of Munc18-1, its mutants and its chaperone activity in AD and PD, could be of great value for new therapeutic studies for neurodegenerative diseases.