The molecular role of Serf2 in development and misfolded protein aggregation

Stroo, Esther

Chapter 6

General Discussion and Future Perspectives

The aim of the studies described in this thesis is to unravel the molecular role of Serf2 in protein aggregation, but also to learn more about the endogenous function of this small protein. Mouse and cell models, with and without the Serf2 protein, enabled us to investigate the molecular function of Serf2. In this chapter I discuss how the work presented in this thesis contributes to the knowledge of the Serf2 protein in relation to neurodegenerative diseases and its biological function. I will also suggest future directions for research into the Serf proteins

1. Full-body deletion of *Serf2* causes growth retardation, fetal atelectasis and neonatal death in mice

In Chapter 3 we describe the investigation into the endogenous function of Serf2 in mice, by studying the mechanisms behind the embryonic lethality of full body depletion. While worms without the worm homolog MOAG-4 are viable and show no effect on lifespan (van Ham et al., 2010), only one *Serf2*$^{-/-}$ mouse was born, where we expected over 40 according to Mendelian ratios. Therefore, we next investigated the origin of this embryonic lethality, in order to gain more knowledge on the biological function of Serf2. We found that neonatal *Serf2*$^{-/-}$ pups die shortly after birth, due to respiratory problems that resulted from fetal atelectasis. All *Serf2*$^{-/-}$ embryos and pups had a reduced body size and weight that corresponded with the observed upregulation of many cell cycle related genes in *Serf2*$^{-/-}$ MEFs. These results give rise to several questions:

Is the fetal atelectasis a cause or consequence of loss of Serf2?

In the *Serf2*$^{-/--}$ animals we observed a reduction in embryo size and weight starting at embryonic day 15.5, that eventually led to a delay in development of neonatal *Serf2*$^{-/-}$ pups at birth. Delayed development can lead to death at birth, particularly if the lung maturation is not completed to term. Differentiation of alveolar type I and II epithelial cells is one of the essential processes of prenatal lung maturation (Xu et al., 2008; Zhang et al., 2010). In the *Serf2*$^{-/-}$ pups delayed lung maturation was observed by the higher number of cells that are still proliferating and the high number of immature type II epithelial cells.

In the newborn *Serf2*$^{-/-}$ pups that die directly or soon after birth, (partial) fetal atelectasis was observed. Fetal atelectasis has been the cause of neonatal lethality in various knockout mouse models, such as these genes involved in general cellular pathways ERK3 a member of the MAP kinase family (Klinger et al., 2009), STK40 a activator of ERK/MAPK signaling (Yu et al., 2013) and β-arrestin 1 and 2 classical regulators of the G-protein-coupled receptors (Zhang et al., 2010). Loss of these genes during embryogenesis leads to fetal atelectasis with reduced lung maturation. Furthermore, the embryos also have a reduced size and weight compared to wild type littermates. Together indicating that loss of *Serf2* results in a slight delay in development, resulting in immature lung maturation at birth and as a consequence fetal atelectasis occurs and *Serf2*$^{-/-}$ pups die at birth because they are not able to breathe.

How does Serf2 affect cell growth?

In order to get a better understanding on how Serf2 influences embryonic growth, we isolated mouse embryonic fibroblasts (MEFs) from E13.5 embryos and investigated cellular growth. We observed that cellular growth was slightly reduced in *Serf2*$^{-/-}$ MEF cell lines, while observing variation between different MEF lines for both *Serf2*$^{-/-}$ and *Serf2*$^{+/+}$. Nevertheless, the small growth delay in the *Serf2*$^{-/-}$ embryos corresponds to the slight delay in cellular growth in the MEFs.

Gene expression analyses revealed a significant proportion of the alternatively regulated genes to be related to cell cycle processes, most of them were upregulated. The upregulation of certain cell cycle genes was unexpected, such as E2F7 (Li et al., 2008), E2F8 (Li et al., 2008) and Cdk1 (Malumbres and Barbacid, 2009), it would be expected that a growth delay due to reduced cellular proliferation would be the result of down regulation of these cell cycle related genes. However, it is possible that the cells are stuck in the cell cycle which could be due to for example DNA damage, and thereby expression of these cell cycle regulatory genes can be upregulated (Dasika et al., 1999). Disruption of cell-cycle-dependent mRNA regulation often results in profound embryonic phenotypic consequences, like for some of the genes found in our study: the transcriptional repressors E2F7 and E2F8 (Li et al., 2008), the nuclear kinase Wee1 (Tominaga et al., 2006) and cyclin-dependent

kinase Cdk1 (Santamaría et al., 2007). Loss of these gene results in an embryonic phenotype whereas upregulation is often associated with cancer, and are therefore often investigated as therapeutic targets (Malumbres and Barbacid, 2009). As we do not see an increase in cellular growth, the upregulation of cell cycle genes seen in the *Serf2*$^{-/-}$ embryos and cells, would rather be the result of cells stuck in the cell cycle. To investigate if the *Serf2*$^{-/-}$ cells are indeed stuck in their cell cycle, it would be interesting to investigate the cell cycle profile and other signs of DNA damage in the *Serf2*$^{-/-}$ MEFs.

The most deregulated gene in the *Serf2*$^{-/-}$ MEFs, the matrix metallopeptidase 3 (MMP3), suggests another possible explanation for the reduced cellular growth. MMP3 is upregulated at the mRNA and protein level in senescent cells and one of the markers used to determine if cells or tissues are senescent (Parrinello et al., 2004). Senescence is a cellular mechanism that causes cellular arrest as a result of cellular stress, and has been implicated as a driver of aging and age-related diseases. Recent studies have shown that senescence also has an important function in tissue remodeling as it occurs during embryogenesis (Muñoz-Espín et al., 2013; Storer et al., 2013) and wound repair (Demaria et al., 2014). Senescence has also been observed in the aging brain, where senescence impairs the blood-brain barrier in aging mice (Yamazaki et al., 2016). In our transcriptomics dataset the 'senescence and autophagy pathway' was number three on wikipathways, which included upregulation of the well-known senescence genes: MMP3, P21, PLAU and PCNA. Preliminary experiments using high oxygen levels did not reveal an increase in senescence cells in our *Serf2*$^{-/-}$ MEF populations, further investigations are necessary to identify if Serf2 is somehow involved in cellular senescence.

Whether the upregulation of the specific cell cycle genes is a direct or indirect effect of Serf2' cellular function remains to be investigated. Based on the current results we hypothesize that the effect of Serf2 on cell cycle is a secondary effect of the endogenous function of Serf2. To further investigate Serf2 endogenous function in the cell, a few other genes are worth mentioning for future studies. For example the downregulated transmembrane glycoprotein non-metastatic b (GPNMB) which has high homology to the pmel17 gene, a highly aggregation-prone protein that forms functional amyloid

structures that are the main component of melanosome fibrils, membrane-bound organelles in pigment cells that store and synthesize melanin (McGlinchey et al., 2009). However, of the 25% amino acid homology with pmel17 the predicted amyloid fibril forming segment VSIVVLSGT (Louros and Iconomidou, 2016) was not conserved. Furthermore GPNMB has been described as a novel neuroprotective factor in ALS. The extracellular release of GPNMB by activated astrocytes resulted in prevention of neurotoxicity caused by SOD mutations and overexpression in an ALS mouse mode. This resulted in reduced motor function decline, delayed disease onset and increased survival (Tanaka et al., 2012).

Another interesting gene is the downregulated tribbles pseudo kinase 3 (Trib3), a protein kinase that was found to be upregulated in the dopaminergic neurons of PD patients, and causes cellular death when overexpressed in cellular PD models (Aime et al., 2015). Furthermore, Trib3 is suggested to be a inducer of insulin resistance in type 2 diabetes (Du et al., 2003) and silencing of Trib3 in diabetic mice results in less atherosclerosis (Wang et al., 2012).

At last, the Huntington interacting protein (Hypk) was downregulated in the *Serf2*$^{-/-}$ MEFs. This gene is located downstream of Serf2, and is suggested to be cotranscribed with Hypk. This gene was identified in a yeast-two hybrid screen to interact with HTT, and has a protective role when overexpressed in polyQ cell models. Like MOAG-4 (van Ham et al., 2010) and SERF2, the protein does not colocalize with the polyQ aggregates. Furthermore, knockdown results in increased apoptosis and cell cycle arrest (Arnesen et al., 2010). The downregulation of this gene as a result of SERF2 depletion might therefore also affect protein aggregation, as HYPK has the opposite effect of SERF2. HYPK protein-interaction studies identified interaction between several proteins involved in cell growth and cell cycle regulation, and knockdown of HYPK resulted in reduced neuronal growth (Choudhury et al., 2012). Together indicating a possible important role for this protein in the *Serf2*$^{-/-}$ MEFs and mice, further experiments will have to rule out effects of Hypk downregulation in our experiments.

One other interesting future experiment is to analyze the gene expression profile of the *Serf2* conditional brain knockout mice, which will give more

insight in the effect of *Serf2* depletion on the whole organ level. In these mice we observe a small reduction of brain size in comparison with the wild type mice, this could be due to a cell growth phenotype early in development similar to the *Serf2*$^{-/-}$ embryos. We observed a loss of approximately 10 percent of brain weight at 1, 3 and 11 months, indicating that there is no degeneration of brain tissue during adulthood or recovery of the growth reduction with aging. It would therefore be interesting to investigate the gene expression profile at different time points, e.g. early in development and in adulthood, to determine the effect of Serf2 on cellular growth on the whole organ level.

In conclusion, the function of Serf2 on cellular and embryonic growth is important in mouse development. Nevertheless, it is still unclear if Serf2 is directly involved in the cell cycle pathways or that it is influencing the process indirectly, for example as a result of protein stress or DNA damage. However, with the generation of the *Serf2*$^{-/-}$ MEF cell lines we created a useful tool to further investigate the cellular role of Serf2.

Is there a relation between SMA and Serf2?

Many genetic studies have shown that SERF1A is a possible modifier of spinal muscular atrophy (SMA), as SERF1A is located on the same genetic locus and deletion of the SMA genes is often accompanied by deletion of SERF1A (Amara et al., 2012; Kesari et al., 2005; Scharf et al., 1998). Interestingly, death in SMA patients is usually the result of respiratory failure due to diffuse respiratory muscle weakness of the intercostal muscles and the diaphragm. Muscles are weakened as a result of degeneration of the lower motor neurons (Lunn and Wang, 2008). In the *Serf2*$^{-/-}$ pups that died directly after birth the intercostal muscles and diaphragm were thinner and showed mild to moderate muscle degeneration. This phenotype was not observed in the *Serf2*$^{-/-}$ pups that were alive at birth, but showed had trouble breathing. Further research is necessary to determine the role of Serf2 in the muscle and diaphragm muscle degeneration, and into of the role of Serf1 in this process.

2. Serf2 in an Alzheimer's mouse model changes the Aβ plaque morphotype

In Chapter 5 we describe three major findings, (1) Serf2 can directly drive amyloid formation of aggregation prone proteins in a test tube, (2) loss of Serf2 does not affect the amount of insoluble Aβ or Aβ plaque load in a mouse model for Alzheimer's disease (AD) and (3) loss of Serf2 alters the amyloid structure of the Aβ plaques in the brain of AD mice. Below I discuss the findings of Serf2 loss in an AD mouse model based on the results of chapter 5 in more detail.

SERF2 directly drives amyloid formation of disease proteins.

Using *in vitro* assays with purified mouse Serf2 we demonstrated that Serf2, like SERF1A (Falsone et al., 2012) and MOAG4 (Yoshimura et al.) can directly drive the amyloid formation of disease proteins in a test tube. Whereas, the aggregation of the non-amyloidogenic proteins citrate synthase and insulin could not be promoted by Serf2, as was previously observed for SERF1A (Falsone et al., 2012). The direct effect of Serf2 on amyloid formation could explain why Serf2 works independently from known protein quality control pathways as autophagy (van Ham et al., 2010) and chaperones (unpublished data) in cell culture experiments. The knowledge of the direct effect on amyloid formation opens up opportunities for future experiments to identify how Serf2 binds and drives the transition of these aggregation-prone proteins into amyloids.

In vitro aggregation studies are an effective method to give insights on how proteins influence amyloid formation of disease proteins and is therefore often used to investigate the direct interaction between disease proteins (Månsson et al., 2013) or the effect of certain domains, mutations or charges (Kakkar et al., 2016). *In vitro* studies with SERF1A identified that interaction with the C-terminal region of α-synuclein causes the conformation to change into a nucleation-active amyloid intermediate (Falsone et al., 2012). SERF1A and SERF2 have high similarity in their amino acid sequence, specifically in the first two exons of the protein. Further *in vitro* studies could identify the

interaction between Serf2 and disease proteins to identify the crucial regions of the protein for the formation of amyloids.

However, these *in vitro* studies only give insights in possible direct interaction between these two proteins, while in the normal situation many more factors affect the protein functioning and interaction. Therefore, it remains to be established how Serf2 drives amyloid formation in a living cell, where processes such as molecular crowding, pH and salt concentrations influence protein functioning. It would therefore be interesting to add cell or brain lysate with and without Serf2 to purified disease proteins to investigate if Serf2, in a more 'natural' environment, still drives amyloid formation. Another option is to add AD brain lysate with and without Serf2 to the Aβ assays in order to investigate seeding-competent Aβ in absence of Serf2. This experiment was done with lysates of MOAG-4 deletion Aβ worms were deletion resulted in lower amyloidogenic seeding (van Ham et al., 2010). More interesting would be to investigate the exact role of Serf2 in amyloid kinetics using live-cell imaging. Using FRET (Forster Resonance Energy Transfer) labeled intra- and intermolecular mutant HTT and fast temperature jump-induced kinetics (Ebbinghaus et al., 2010), we tried to investigate the early aggregation kinetics in single living cells. Using this technique no changes in early aggregation kinetics could be observed in SERF2 and SERF1A double mutant human embryonic kidney cells (HEK293T). The settings of this experiments were not ideal, future experiments should include the temperature at 37°C, increase of sample size and more comparable cell lines using SERF2 overexpression or knock out. Nevertheless, these results could also indicate that SERF does not affect the early aggregation kinetics in a living cell, future studies are necessary to establish the molecular role of SERF in the early steps of amyloid kinetics in the living cell.

Loss of Serf2 does not affect Aβ plaque load or insoluble Aβ in an AD mouse model

Next we aimed to translate *in vitro* and cellular findings to an AD mouse model. To do so, we used the APPPS1-21 mouse model which shows Aβ aggregation and plaque formation after only two months (Radde et al., 2006). We found that loss of Serf2 in the brain of these AD mice resulted in no change

on insoluble Aβ levels and Aβ plaque load or gliosis in the cortex. Whereas deletion of MOAG-4 in a *C. elegans* AD model, overexpressing Aβ42 in the body wall muscle, resulted in a reduction of paralysis and a decrease of high and low weight molecular species (van Ham et al., 2010). Changes in the molecular species of Aβ were not observed in our study, however closer examination of Thioflavin-S stained Aβ plaques revealed less organized and less structured Aβ plaques in the AD*;Serf2*$^{-/-}$ mice. In order to zoom in on the amyloid structures of the Aβ plaques, we used electron microscopy. Here we observed more compact forms of the Aβ plaques in the AD;*Serf2*$^{-/-}$ brains, with thicker and shorter fibrils. Similar changes in amyloid compactness were observed in a different AD mouse model heterozygous for the insulin growth factor 1 (Igf1) gene, where more densely packed Aβ plaques were shown to have beneficial effects on cognitive performance (Cohen et al., 2009).

Is the alteration in amyloid structure of the AD; Serf2$^{-/-}$ *animals beneficial?*

Whether the ability of Serf2 to influence the amyloid structure is beneficial for the AD;*Serf2*$^{-/-}$ mice remains to be investigated. It will be important to learn if the changes in amyloid structure caused by loss of Serf2 affect the cognitive performance of the AD;*Serf2*$^{-/-}$ mice. In most AD mouse models memory deficiency and impairment of orientation and locomotion are associated with the Aβ production in the cortex and the hippocampus (Jankowsky et al., 2005; Oakley et al., 2006; Radde et al., 2006). These behavioral phenotypes can be examined by different behavioral tasks such as the Morris water maze to test cognitive performance, or the open field and elevated plus maze to test anxiety-like behavior (Webster et al., 2014).

The APPPS1-21 mouse model we choose in this thesis, shows fast Aβ production and Aβ pathology, starting after two months of age. This is caused by expression of the human APP gene with the Swedish mutation (KM670/671NL) in the β-secretase cleavage site that increases the total Aβ levels (Cai et al., 1993; Citron et al., 1992) and the PS1 gene with the L166P mutation that increases the Aβ42 generation, thereby shifting the Aβ40/42 ratio (Moehlmann et al., 2002), both genes are regulated under the Thy1 promoter (Radde et al., 2006). The combination of these transgenes, the mutations and expression levels results in active transcription of the

transgenes starting approximately 2 weeks after birth which leads to high Aβ42 levels already at two months of age (Radde et al., 2006). Even though the AD pathology starts early, the behavioral phenotypes of this mouse model are only found in the Morris water maze starting after 6/7 months (Dionísio et al., 2015; Montarolo et al., 2013; Psotta et al., 2015), reversed learning using the food-rewarded four-arm spatial maze task starting at 8 months (Radde et al., 2006) and anxiety-like behavior in open field and elevated plus maze tasks starting at 12 months (Psotta et al., 2015). Because we expected Serf2 to affect the early stages of Aβ pathology we did not include behavior assays for this mouse model but focused on the effect of Serf2 on the biochemistry of Aβ aggregation.

Given the promising results in the APPPS1-21 mice, we will investigate if loss of Serf2 in an AD mouse model is beneficial. However, to examine if the changes on amyloid structure has an effect on AD related behavior, another AD mouse model is preferred. The mouse model we chose is the APP/PS1 mouse model (Jankowsky et al., 2004), this mouse model expresses the mouse APP gene with a human Aβ domain, including the Swedish mutation (KM670/671NL), and the human PS1 transgene with the exon-9 deletion variant (Perez-Tur et al., 1995), that is known to prefer the Aβ42 splicing and thereby increasing the Aβ40/42 ratio (Woodruff et al., 2013). Both transgenes are expressed under the mouse prion protein promoter that results in high expression in brain and heart (Borchelt et al., 1996). The first Aβ plaques can be found around 6 months in the hippocampus and cortex (Jankowsky et al., 2004), significant effects in the Morris water maze start between 6-9 months of age (Cohen et al., 2009; Cramer et al., 2012). Furthermore, neuronal loss has been described in 12 month old animals in the hippocampus and frontal cortex (Cohen et al., 2009). As this model is less aggressive and the AD pathology and behavior are more synchronized, this will give more insights in the effect of Serf2 depletion in AD pathology.

Another possibility to investigate whether the amyloid structures in the AD; *Serf2*$^{-/-}$ animals are beneficial would be to examine neuronal or synapse loss, which is often used in other AD mouse models (Calhoun et al., 1998; Cohen et al., 2009; Wang et al., 2015). In the APPPS1-21 animals no reduction in neuronal numbers of the cortex has been observed up to 12 months

(Montarolo et al., 2013; Rupp et al., 2011). It was however shown, using live two-photon microscopy that after 3 months dendritic spine loss occurs in close proximity to the Aβ plaques. The loss of dendritic spines occurs approximately 4 weeks after the Aβ plaques are formed, whereas no alterations are observed in regions more than 50 µm from the Aβ plaques (Bittner et al., 2012). It would be interesting to see how the dendritic spines react around the Aβ plaques that have a different structure as a result of Serf2 depletion. But as this is a very specialized technique that involves living animals and a two-photon microscope, these experiments might be too challenging and costly. Therefore, it would be sensible to perform some analyses into dendritic and synaptic genes on immunostainings or on RNA and protein level, in order to get an indication if there would be loss of these important neuronal structures in the AD;*Serf2*$^{-/-}$ mice.

How do Serf2 and Aβ meet in the cell?

So far it is unclear where and if Serf2 interacts with Aβ in the cell in order to change the end stage Aβ plaques. Quantative RT-PCR in different mouse brain regions informed us that both Serf1 and Serf2 are expressed in all the different brain regions, including the cortex, the important brain regions for our study. However, this does not teach us anything about cell type specific expression or cellular localization of Serf1 and Serf2. The availability of the *Serf2*$^{-/-}$ mice made it possible to test the immunospecificity of several Serf2 antibodies. Unfortunately, we were not able to find a Serf2 antibody that showed a specific signal in the mouse brains. Putting more effort into finding or generating a working Serf2 antibody is important to learn how Serf2 behaves in the brain and in the cells in presence and absence of amyloidogenic proteins. This could have given us an idea of where and how Aβ and Serf2 meet in the brain in order to alter the amyloid structure we observe in the Aβ plaques.

One possibility is that Serf2 and Aβ interact inside the cells where it is known to be located in various intracellular membranes, such as lysosomes (Haass et al., 1992), endoplasmic reticulum (Bückig et al., 2002) and endosomes (Yang et al., 1998). Furthermore, the intracellular Aβ was found to have high seeding potency (Marzesco et al., 2016). It is therefore possible that

Serf2 interacts with Aβ in one of these compartments where it alters its conformation towards a more aggregation prone state, eventually resulting in a different Aβ plaque morphotype. Alternatively, Serf2 is located extracellularly and interacts with Aβ after it is secreted by the cell. In order to establish this it would be important to know more about the exact location of Serf2. Therefore it would be useful to isolate different cellular compartments and use immunoprecipitation to establish where Serf2 and Aβ interact.

A final possibility could be that Serf2 does not directly interact with Aβ but influences the aggregation process indirectly, for example through microglia functioning. Recently it was shown that loss of TREM2, a cell-surface receptor that is mainly expressed by microglia, disrupts the formation of the neuroprotective microglia barrier around the Aβ plaque and thereby affecting the amyloid structure, into more compact and less fibrillar Aβ plaques (Wang et al., 2016; Yuan et al., 2016). To investigate this possibility it would be useful to make confocal images of co-stainings with Iba-1 positive microglia and the Thioflavin dye or Aβ antibody, changes in microglia organization around the plaque would indicate changes in the neuroprotective microglia barrier and thus reason for further investigation.

The effect of variation between AD mouse models on Aβ plaque load outcome

Even though we do not see a significant effect on Aβ plaque load in the AD;*Serf2*$^{-/-}$ mice, we did observe a trend towards more Aβ plaques in the AD;*Serf2*$^{-/-}$ mice. Inconsistent outcomes between different AD mouse models are often observed. It was described for TREM2 haplodeficient AD mice, that while no significant effect of Aβ plaque load in the cortex was observed for the APPPS1-21 mice (Jay et al., 2015; Ulrich et al., 2015), a significant increase in Aβ plaque load was found in the cortex of 5xFAD mice (Wang et al., 2015). Next to the differences in the transgene promoter, mutations and genetic background, the brain region-specific expression levels of APP has huge variation in different AD models, that might explain differences between them (Höfling et al., 2016). The variation between AD mouse models makes it difficult to compare these kinds of studies.

Also variation within one model has been described, such as the significant gender differences in Aβ plaque onset in the APPPS1-21 mouse model (Ulrich et al., 2015). For the 5xFAD mouse model, it was found that the transgenic levels of APP are higher in the female animals, due to an estrogen response element in the Thy-1 promoter (Sadleir et al., 2015). As the APPPS1-21 also uses the Thy-1 promoter for transgene expression, this could be the reason for gender specific differences in Aβ plaque load.

Together, these studies shows the importance of transgene levels in the brain on the Aβ levels and Aβ plaques, therefore, differences between different AD mouse models can be problematic to interpret data. It would therefore be important to test the effect of loss of Serf2 in at least one other AD mouse model to see if we can replicate our results, and if we might see an effect on Aβ plaque load that was hypothesized for this study based on the results of MOAG-4 in an AD model in *C.elegans*.

The effect of Serf2 depletion and Cre expression on brain size

To establish the role of Serf2 in an AD mouse model we had to generate a brain specific knock out, as the full body knockout of *Serf2* results in neonatal death (Chapter 3). We therefore crossed the mice with the *loxP* sites around the Serf2 gene with a Cre mouse model that expresses Cre under a brain specific promoter. For our study we chose the Sox1-Cre mice (Takashima et al., 2007), as the widely used Nestin-Cre model is known for expression in non-neuronal tissues like kidney and pancreas (Delacour et al., 2004; Dubois et al., 2006). The average loss of 10% in brain weight in the *Serf* knockout animals did not result in any pathological brain abnormities. In Chapter 4 we describe a slight reduction on brain weight and size in the Sox1-Cre line. We initially observed this reduction in size in the *Serf2*$^{-/-}$ and AD; *Serf2*$^{-/-}$, however loss of Serf2 can only explains approximately 40-60 percent of the weight loss, the other 40-60 percent was the result of the Sox1-Cre background. The decrease in brain weight stays stable with aging in both the *Serf2*$^{-/-}$ and the Sox1-Cre mice.

In Chapter 5 we identified Serf2 as a modifier of Aβ plaques structure and organization in the APPPS1-21 mouse model. However, the effect of Cre

expression and brain size reduction on the structure of the Aβ plaques has to be ruled out. Therefore progeny of AD and Sox1-Cre crosses were aged to 12 weeks and analyzed on the Aβ plaque structure, in order to rule out possible effects of the Sox1-Cre background. Expression of Cre under the Sox1 promoter did not affect the Aβ plaque structure, confirming that Serf2 plays an important role in the formation of the amyloid in disease.

To avoid possible side effects of the reduced brain size, future studies into the role of Serf2 in mice could use a Cre inducible system. These mice express the Cre gene fused to mutated hormone-binding domains of the estrogen receptor, which can be activated by the synthetic estrogen receptor ligand 4-hydroxytamoxifin (Feil et al., 2009). This will enable us to delete *Serf2* at any given time. In case of our study, Cre should be activated after embryonic development, together with the activation of the AD transgenes.

3. Conclusions

Together, this thesis describes the first studies into the physiological function of Serf2 and provides a promising first step into uncovering the potential of Serf2 as a therapeutic target for neurodegenerative diseases. To this end, it will be important to further investigate the mechanisms by which Serf2 acts, as well as the behavioral consequences of *Serf2* depletion.

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