microRNA networks surrounding APP and amyloid-β metabolism – Implications for Alzheimer's disease

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Abstract

MicroRNAs (miRNAs) are small non-coding RNA regulators of protein synthesis that function as "fine-tuning" tools of gene expression in development and tissue homeostasis. Their profiles are significantly altered in neurodegenerative diseases such as Alzheimer's disease (AD) that is characterized by both amyloid-β (Aβ) and tau deposition in brain. A key challenge remains in determining how changes in miRNA profiles translate into biological function in a physiological and pathological context. The key lies in identifying specific target genes for deregulated miRNAs and understanding which pathogenic factors trigger their deregulation. Here we review the literature about the intricate network of miRNAs surrounding the regulation of the amyloid precursor protein (APP) from which Aβ is derived by proteolytic cleavage. Normal brain function is highly sensitive to any changes in APP metabolism and miRNAs function at several steps to ensure the correct APP end product is produced and in the right form and abundance. Disruptions in this miRNA regulatory network may therefore alter Aβ production, which in turn can affect miRNA expression.

Key words: Alzheimer; Aβ (amyloid-β); APP (amyloid precursor protein); animal model; BACE1; β-secretase; microRNA; neurodegeneration; splicing; tau

Introduction

Over 30 million people suffer from dementia worldwide, with numbers firstly dramatically increasing and secondly, with no cure in sight (Ballard et al., 2011). Of all dementing disorders, Alzheimer's disease (AD) is the most prevalent. In the AD brain there is a widespread synaptic and neuronal loss that causes a progressive decline in memory and other cognitive functions ultimately leading to dementia.

The AD brain is histopathologically characterized by two types of deposits, extracellular amyloid-β (Aβ) plaques and intraneuronal neurofibrillary tangles (NFTs), both of which can be visualized with specific dyes and impregnation methods (Gotz and Ittner, 2008). Aβ is derived from the Amyloid Precursor Protein (APP). It is the major constituent of plaques, while hyperphosphorylated (i.e. abnormally phosphorylated) forms of tau constitute the NFTs (Selkoe, 1997). The majority of AD cases are sporadic (SAD), with familial (FAD) cases likely accounting for less than 1%. Here, autosomal dominant mutations have been identified in three genes, in *APP* itself, as well as in *presenilin 1 (PSEN1)* and *2 (PSEN2)* both of which encode a component of the enzyme complex that is required to generate Aβ (Bertram and Tanzi, 2008). While Aβ deposition is closely associated with the onset of AD, it is the tau pathology that correlates better with the severity of dementia (Braak and Braak, 1995). More specifically, studies in transgenic animals revealed that for Aβ to exert its toxic effects tau is required (Ittner and Gotz, 2011, Ittner et al., 2010, Roberson et al., 2007).

The currently prescribed drugs do not halt the neurodegenerative process in AD. In fact, their effect on cognition is moderate (Ballard et al., 2011): the cholinesterase inhibitors donepezil, rivastigmine and galantamine are licensed for mild-to-moderate AD, while memantine is an NMDA receptor antagonist prescribed for moderate-to-severe AD. At present alternative therapeutic strategies are being tested that aim to prevent the formation and aggregation of the above mentioned aggregation-prone peptides and proteins, or to facilitate their clearance (Pasic et al., 2011). Several of these newer strategies have their foundation in transgenic animal models that express familial mutant forms of the proteins that form the aggregates in human neurodegenerative disease, such as tau or APP (Gotz and Ittner, 2008). These models reproduce the biochemical, histopathological and clinical features of the human conditions. Transcriptomic and proteomic approaches have been applied to

these models, and again, the functional validation included a side-by-side comparison with the human AD brain (David et al., 2005, Hoerndli et al., 2005, Hoerndli et al., 2004). Regulation of APP is complex, with *APP* RNA levels and isoforms as well as proteolytic processing of APP protein affecting normal brain function and disease pathogenesis. Here, we will be focusing on the role of miRNAs and discuss how these small RNA species have been incorporated into the complex regulatory network surrounding the many aspects of APP metabolism and how a major product of APP proteolysis, Aβ, is itself regulating miRNAs.

APP isoforms, APP processing and Aβ species

In humans, APP is expressed as three major isoforms that arise from alternative splicing (Figure 1) (Zhang et al., 2011). APP751 and APP770 contain the 56 amino acid Kunitz Protease Inhibitor (KPI) domain; both isoforms are widely expressed. In contrast, APP695 lacks the Kunitz domain and is found predominantly in neurons. APP770 has an additional putative glycosylation domain, OX2, that is not present in the two other isoforms (Kitazume et al., 2010). APP is a member of a gene family that includes APP-like protein 1 (APLP1) and 2 (APLP2). All three are type I transmembrane proteins that are processed in a similar manner. What differentiates APP from its two homologues is the presence of the Aβ domain. APP's physiological function has remained largely undetermined although there is accumulating evidence for a role in neurite outgrowth and synaptogenesis, axonal transport, transmembrane signal transduction, cell adhesion, and calcium metabolism. Together with APLP2, APP is synergistically required to mediate neuromuscular transmission, spatial learning and synaptic plasticity (Weyer et al., 2011).

Full-length APP is synthesized in the endoplasmic reticulum (ER) and then transported through the Golgi apparatus to the trans-Golgi-network (TGN), where in neurons at steady state the highest concentration of APP is found (Greenfield et al., 1999, Hartmann et al., 1997, Xu et al., 1997). The Aβ peptide is generated by proteolytic cleavage in the ER and Golgi/TGN (Wilquet and De Strooper, 2004). While cleavage of APP by α-secretase (the 'non-amyloidogenic' pathway) precludes Aβ generation as the cleavage site is within the Aβ domain, cleavage first by β- and

then by γ-secretase generates the Aβ peptide (the 'amyloidogenic' pathway) (Figure 1).

Cleavage of APP by α-secretase generates sAPPα plus the c83 fragment. Several members of the ADAM (a disintegrin and metalloproteinase) family possess α-secretase-like activity and three of them, ADAM9, ADAM10, and ADAM17, have been suggested as the α-secretase. Like APP, they are type I transmembrane proteins (Zhang et al., 2011). Upon β-cleavage by the enzyme BACE1, c99 is generated and the ectodomain of APP is released as soluble APPβ (sAPPβ). Although only differing from sAPPα by lacking the Aβ1-16 region at its carboxy-terminus, sAPPβ has been reported to function as a death receptor 6 ligand and to mediate axonal pruning and neuronal cell death (Nikolaev et al., 2009). c99 is subsequently cleaved by the γ-secretase complex (that is composed of at least four components; presenilin, nicastrin, anterior pharynx-defective-1 (APH-1), and presenilin enhancer-2 (PEN-2)), generating Aβ. γ-cleavage generates both Aβ40, the major species, and Aβ42, the more amyloidogenic species, as well as releases the intracellular domain of APP (AICD). γ-Secretase further mediates ζ-site cleavage (Aβ46) (Zhao et al., 2004) as well as ε-site cleavage (Aβ49) (Sastre et al., 2001, Weidemann et al., 2002), suggesting a sequential cleavage model where cleavage at the ε-site is followed by the ζ-site and γ-site (Figure 1).

Aβ has been implicated in both physiological and pathological functions (Gotz et al., 2008). While little is known about its physiological role, a plethora of data are available on its aggregation in AD brains where it forms oligomers and fibrils that eventually deposit in the extracellular space as amyloid plaques (Gotz et al., 2011, Ono et al., 2009, Tomiyama et al., 2010). In sporadic cases of AD, the levels of Aβ and in particular of its major amyloidogenic form, Aβ42, are increased either because of an increased production or an impaired clearance, by mechanisms that again are only poorly understood (Gotz et al., 2008). In familial AD (FAD), increased Aβ levels are due to mutations either in the *APP* gene itself or in the *PSEN1* and *2* genes that encode subunits of the APP processing machinery (Selkoe and Podlisny, 2002). While increased Aβ levels characterize AD pathology, the precise mechanisms and signaling cascades that Aβ uses to exert its toxicity are only partly understood (da Cruz e Silva et al., 2010, Lim et al., 2010, Palop and Mucke, 2010). With regards to toxicity, an additional level of complexity has been added with the identification of species such

as Aβ43 (Saito et al., 2011) or pyroglutamate-modified Aβ42 (Aβ (3(pE)-42) that are believed to be even more toxic than Aβ42 itself, and by the fact that Aβ exists in different aggregation states that includes oligomeric species (Glabe, 2008, Walsh et al., 2002) (Figure 2). As far as down-stream toxicity is concerned, in some experimental paradigms such as mitochondrial function, oligomeric and fibrillar Aβ were both found to cause a similar degree of toxicity, while monomeric Aβ was not toxic (Eckert et al., 2008, Eckert et al., 2008).

MicroRNAs – post-transcriptional regulators of gene expression

MicroRNAs (miRNAs) add yet another level of complexity. They are "fine-tuning" tools of gene expression in development and tissue homeostasis. Evolutionary conserved, these 19-24 nucleotide-long non-coding RNAs negatively regulate expression of specific mRNA targets through base pairing between their "seed region" and sequences commonly located in the 3'UTR of their targets (Fabian et al., 2010, Siomi and Siomi, 2010). Whilst there is perfect to near perfect complementary base pairing in plants, in mammalian cells, only a pairing between the seed sequence of the miRNA and the 3′ UTR of the mRNA target is thought to be important (Hebert and De Strooper, 2009, Vilardo et al., 2010, Wang et al., 2010). Deep sequencing revealed that there are over 1400 miRNA genes in the human genome whereas in the mouse they are in the order of around 700 (miRBase.org). Of these, approximately 300 are expressed in mouse brain (Landgraf et al., 2007). It is estimated that each miRNA can target up to several hundred to a thousand transcripts, but unfortunately only a very few targets have been confirmed *in vivo* so far (Lau and de Strooper, 2010). The recent years have seen an explosion in studies linking miRNAs to pathological processes, and evidence is mounting that they have a role in neurodegenerative diseases ranging from AD to Parkinson's disease (Kim et al., 2007) and ALS (Williams et al., 2009).

How do miRNAs silence gene expression? The primary miRNA transcripts (pri-miRNAs) are transcribed by RNA polymerase II and can be several thousand bases in length (Lee et al., 2004). In the nucleus, these transcripts are processed by the Drosha/DGCR8 complex to produce so-called precursor miRNAs (pre-miRNAs), which are approximately 70 nucleotides in length and characterized by a stem-loop structure. After nuclear export by Exportin 5/Ran, pre-miRNAs are cleaved in the

cytoplasm by Dicer, an enzyme for which neuronal cell-type-specific knock-out mouse strains are available (Cuellar et al., 2008, Davis et al., 2008, Kim et al., 2007, Schaefer et al., 2007), to generate mature miRNAs. These bind to the 3′UTR of mRNA targets, recruiting the RNA induced silencing complex (RISC) that inhibits the expression of the bound mRNA target (Mathonnet et al., 2007, Wakiyama et al., 2007). While Dicer cleaves the pre-miRNAs, the Argonaute (Ago) proteins in the RISC bind the miRNAs and mediate gene silencing (O'Carroll et al., 2007).

Determining how changes in miRNAs expression levels translate into biological function remains a challenge; the key lies in identifying specific target genes for deregulated miRNAs and understanding which pathogenic factors trigger their deregulation (Schonrock et al., 2011). This is particularly true for neurodegenerative diseases where one finds a network of interactions, with miRNAs regulating key mRNAs such as APP, while one of APP's prime proteolytic products, Aβ, regulates a myriad of miRNAs which in turn can create a feed-back regulatory loop on *APP* transcript levels.

De-regulation of miRNAs in AD brain

It took seven years after discovering the first human miRNAs (Pasquinelli et al., 2000) until the first report of differential miRNA profiles was published for human AD tissue (Lukiw, 2007). Since then several groups have performed genome-wide profiling of AD tissue highlighting AD-specific changes in the miRNA regulatory system (Hebert et al., 2008, Nunez-Iglesias et al., 2010, Shioya et al., 2010, Wang et al., 2008). These may either involve neuronal or glial cells or both, as both cell types are affected in human 'neurodegenerative' conditions (Kurosinski and Gotz, 2002). miRNA profiling of human cortical tissue identified thirteen 'AD-specific' miRNAs that are down-regulated in human AD brain (Cogswell et al., 2008, Hebert et al., 2008). The subsequent generation of mice with a neuronal knockout of *Dicer* is a proof-of-principle that miRNAs are likely to play a role in neurodegeneration (Davis et al., 2008, Hebert et al., 2010, Shin et al., 2009). However, miRNA expression studies on AD patients have revealed either no or only very little overlap in miRNA changes (Hebert and De Strooper, 2009). To remove the complexity inherently associated with human studies tissue culture and animal model systems have been used to dissect pathomechanisms (see below). These studies have replicated many of

the changes observed in human AD brain, thus underscoring the validity of model systems in gaining a better understanding of the pathogenesis of AD.

Direct regulation of the APP mRNA by miRNAs

As miRNAs are known to have in the order of hundreds of putative mRNA target genes, which in addition tend to contain binding sites for more than one miRNA species adding to possible combinatorial effects, a major challenge is firstly to link distinct miRNAs to distinct mRNA targets *in vivo* and secondly to determine whether such a binding has an affect on protein levels. With regards to AD, a major question is whether miRNAs are capable of altering Aβ levels. Most miRNA studies involving AD-related genes rely on luciferase-based reporter gene assays using 3' UTR fusion constructs with intact (and mutated) miRNA binding sites. In the AD field there are a few obvious targets, which includes APP (Figure 2). Help comes from *C. elegans*, where the worm homolog of *APP*, *APL-1*, is developmentally regulated by the miRNA Let-7 (Niwa et al., 2008).

Several miRNAs have been identified *in vitro* to directly regulate APP. They include miR-106a, -520c (Patel et al., 2008) as well as members of the miR-20a family, such as -20a, -106a/b and -17 (previously referred to as miR-17-5p, http://www.mirbase.org) (Hebert et al., 2009), miR-16 and -101 (Long and Lahiri, 2011, Vilardo et al., 2010) and most recently miR-147, -655, -323-3p and -153 (Delay et al., 2011). Whilst miR-106b and miR-101 have been shown to be down-regulated in AD brain, therefore potentially contributing to increased APP expression and Aβ generation (Hebert et al., 2008, Nunez-Iglesias et al., 2010), it remains to be determined which of these miRNAs actually regulates APP *in vivo*. Interestingly, AD-specific polymorphisms identified in the APP 3'UTR (T171C and A454G) affect the APP-modulating activity of miR-147 and -20a, respectively, and could therefore affect AD risk via altering miRNA-mediated regulation of APP expression (Delay et al., 2011).

Whilst, the majority of observed miRNA regulatory binding sites are located in the 3'UTRs of mRNAs, effective miRNA binding sites have also been identified in open reading frames and 5'UTRs of target mRNAs (Duursma et al., 2008, Forman et al., 2008, Jopling et al., 2005, Orom et al., 2008, Tay et al., 2008). The APP 5'UTR plays fundamental roles in APP regulation and biogenesis (Lahiri et al., 2005,

Maloney et al., 2004, Rogers et al., 2002) and although no miRNAs have so far been reported to target the APP 5'UTR, it remains an interesting possibility.

Regulation of APP alternative splicing by miRNAs

Changes in neuronal APP isoform expression are associated with an increase in Aβ production (Donev et al., 2007), and increases of exon 7 and/or 8 containing APP isoforms have been reported in various regions of AD brain. A very recent study reveals a contribution of miRNAs to yet another level of APP regulation, alternative splicing (Figure 2) (Smith et al., 2011). Exon 7 of APP encodes the KPI domain, while exon 8 encodes the OX2 domain, with the neuron-enriched APP695 form lacking both domains (Figure 1). The new study found that the lack of miRNAs in post-mitotic neurons *in vivo* (achieved via a *Dicer* knockout) was associated with exon 7 and 8 inclusion (Smith et al., 2011). This indicates that miRNAs expressed in post-mitotic neurons *in vivo* participate in the physiological regulation of APP mRNA splicing. Given the potentially important role of miR-124 in neuronal maintenance and splicing (Makeyev et al., 2007, Papagiannakopoulos and Kosik, 2009), the authors suggest that loss of this miRNA could be responsible for the above effects possibly through the regulation of its target gene, polypyrimidine tract binding protein 1 (PTBP1).

They further found that ectopic expression of miR-124 reversed the above effects on APP splicing in cultured neurons. Interestingly, miR-124 levels are reduced in AD (Lukiw, 2007, Smith et al., 2011). Whether these reflect selective neuronal loss remains to be seen but it is intriguing to assume that specific neuronal miRNAs regulate APP splicing.

Indirect regulation of APP processing by miRNAs

In addition to APP, the β-secretase BACE1 has been identified as a miRNA target (Figure 2). Loss of the miRNA cluster containing miR-29a, -29b1 and -9 in SAD has been found to correlate with an increased BACE1 expression (and by extension, increased Aβ levels) (Hebert et al., 2008). More recently, miR-29c was shown to lower BACE1 protein levels *in vitro*, and miR-29c-overexpressing mice down-regulated BACE1 levels suggesting that miR-29c might be an endogenous BACE1

regulator (Zong et al., 2011). FAD5 mice are a transgenic mouse strain with elevated BACE1 levels and early-onset plaque formation; in these mice the above-mentioned three miRNAs are not increased (O'Connor et al., 2008). Likewise, when a second APP mutant strain, Tg2576, was energy-deprived this caused an increase in miRNAs -29a and –b1, which would have been expected to cause a concomitant decrease in BACE1 expression, which however was not found (O'Connor et al., 2008). Yet, the final word on BACE1 regulation is not spoken, as energy deprivation could affect additional miRNAs that could either directly or indirectly feed-back on BACE1 expression levels. In fact this example avidly demonstrates the difficulties one encounters in studying a system as complex as the miRNA network.

Another study in an APP/PS1 mutant mouse model of AD revealed an inverse correlation between BACE1 protein levels and two miRNAs (miR-298 and -328) and showed these two miRNAs directly interacted with the BACE1 3'UTR in mouse cell lines (Boissonneault et al., 2009). Whether additional miRNAs are deregulated remains to be determined. Finally, in APP mutant Tg19959 mice, BACE1 levels were increased while miR-103 and -107 levels were decreased (Faghihi et al., 2008), however whether there is a direct relationship or not remains to be elucidated.

Down-stream effects of Aβ on miRNA expression

An interesting finding of both transcriptomic and proteomic studies is the complementarities of cellular and transgenic mouse models as they often reveal a significant overlap in deregulated genes and proteins, respectively (David et al., 2006). We analysed the effect of Aβ itself on miRNA expression in different experimental systems and found again a remarkable overlap. When primary hippocampal neurons were incubated with fibrillar preparations of Aβ42, we found that this invoked a strong change in miRNA profiles with a substantial proportion of miRNAs being down-regulated (Schonrock et al., 2010). This response was rapid. We validated nine miRNAs (miR-9, -181c, -148b, -30c, -20b, -361, -21, -409-3p and Let-7i) as being down-regulated by aged Aβ42 (Schonrock et al., 2010).

Interestingly, the miRNA down-regulation in Aβ-treated hippocampal neurons was paralleled in the hippocampus of Aβ-plaque forming APP23 mice at the onset of plaque formation. Some of the deregulated miRNAs in our study became affected prior to Aβ plaque formation (such as miR-409-3p and Let-7i) similar to what is seen

in a related study (Wang et al., 2009). Furthermore, the expression of certain miRNAs changed over time (from up- to down-regulated or *vice versa*), supporting the transient effect on miRNA expression during AD development. Biological pathways affected by predicted miRNA target genes such as *axon guidance*, *mitogen-activated protein kinase (MAPK) signaling*, *TGFβ signaling*, *glutamate metabolism*, *long term potentiation*, and *regulation of the actin cytoskeleton* are intricately associated with proper brain function relevant to AD. miRNAs -181c, -9, -30c, -148b, -20b and Let7i are particularly interesting as these are also down-regulated in human AD brain (Cogswell et al., 2008, Hebert et al., 2008).

miR-9 is the most abundant human brain miRNA (Mattick and Makunin, 2005) and a recurring candidate from several AD profiling studies. Studies performed in zebrafish and mice revealed that it is essential in patterning, neurogenesis and differentiation and thus ideally placed to impact various aspects of brain function. Over-expression of miR-9 accelerates neuronal differentiation, while its inhibition in the medial pallium of embryonic day 11.5 mouse embryos results in defective differentiation of Cajal-Retzius cells, the first neurons to populate the embryonic cortex. Similarly, loss of miR-9 in zebrafish embryos decreases the relative numbers of differentiated neurons in the anterior hindbrain (Leucht et al., 2008, Shibata et al., 2008, Zhao et al., 2009). Neurogenesis is not only important in the developing brain but is a process which continues in the adult hippocampus, a region heavily affected by Aβ pathology in AD (Hallbergson et al., 2003). Interestingly, AD patients exhibit altered expression of early neuronal markers in the hippocampus, which has been attributed to increased neurogenesis (Jin et al., 2004). Decreased expression of miR-9 may therefore impact adult brain function.

Global miRNA profiles have also been established from APP mutant mice that both over-express human APP and accumulate Aβ. Compared with the use of synthetic Aβ, there is a confounding effect of increased AICD production. In an APP/PS1 double mutant mouse model, of 37 differently expressed miRNAs, several (miR-20a, -29a, -125b, -128a, and -106b) were down-regulated, while others (miR-34a, let-7, miR-28 and -98) were up-regulated, with miR-29a and 106b showing the same direction of changes as seen in AD brain (Wang et al., 2009). miRNA quantitative RT-PCR showed that miR-106b is up-regulated in 3-month-old APP/PS1 mice and down-regulated at 6 months (Wang et al., 2010). While these studies suggest a possible transient effect of Aβ plaque pathology on miRNA (miR-106b) expression,

miR-106b also regulates APP mRNA levels (Hebert et al., 2009) thus creating a possible regulatory feedback loop.

Role of miRNAs in AD – fine-tuning versus decisive role

A major challenge in general and in neurodegeneration in particular is in linking deregulated miRNAs to their mRNA targets, as so far only a few selected targets have been identified that include *APP*, *BACE1* and the α-synuclein-encoding *SNCA* (Lau and de Strooper, 2010). At present, target prediction relies on algorithms such as miRBase, PicTar, miRanda, PITA or TargetScan with the latter showing the most accurate predictions upon target validation (Baek et al., 2008, Selbach et al., 2008). One approach to circumvent this inherent problem is to over-express or inhibit individual miRNAs and analyze protein levels by using proteomics approaches such as stable-isotope labeling with amino acid in cell culture (SILAC) (Baek et al., 2008, Selbach et al., 2008). Another option is co-immunoprecipitating mRNA targets with the Argonaute protein Ago2 combined with high-throughput sequencing of RNAs isolated by crosslinking immunoprecipitation (HITS-CLIP) (Chi et al., 2009). Furthermore, with the advent of the Zinc Finger Nuclease technology it is now possible to directly dissect the role of miRNAs in neuronal function and neurodegenerative disorders, by manipulating specific miRNA binding sites *in situ*. An ultimate challenge however will be an integrated view of APP/Aβ regulation that takes into account the role of miRNAs, mRNA transcription, translation, posttranslational modifications, subcellular compartmentalization, brain regional differences and changes over time, both under physiological and pathological conditions. This will determine whether miRNAs are merely fine-tuning instruments as far as the major players in AD are concerned or whether they indeed play a decisive role. In addition it seems that the miRNA network in AD is tightly regulated by feedback loops.

Exploiting miRNAs as diagnostic tools in AD

The diagnosis of AD, while only 90% accurate, typically includes an assessment of the medical and medication history, a physical examination, blood tests to rule out

diabetes, cancer and other conditions, a neuropsychological examination, and increasingly, imaging techniques (such as magnetic resonance imaging, MRI, or positron emission tomography, PET) as well as measuring biomarkers in either CSF (cerebrospinal fluid) or blood. Especially CSF biomarkers are currently being considered for inclusion in revised diagnostic criteria for research and/or clinical purposes to increase the certainty of *ante mortem* diagnosis of AD. These include platforms such as the INNOTEST enzyme-linked immunosorbent assay or the INNO-BIA AlzBio3, for measurement of CSF Aβ and tau proteins, both total and phosphorylated at epitope Thr181 (AT270) (Fagan et al., 2011). As lumbar punctures are quite invasive there is a trend in the field to move into plasma as the changes in Aβ levels reported for CSF are also reflected by according changes in plasma (Lewczuk et al., 2010). With regards to miRNAs these are readably detectable in bodily fluids, making them attractive biological markers. In addition, they are in general quite stable. So far however only a few groups have explored the role of miRNAs in blood and CSF, with the deregulated brain miRNAs having roles in known and novel pathways in AD pathogenesis related to amyloid processing, neurogenesis, insulin resistance, and innate immunity (Cogswell et al., 2008). By analyzing blood mononuclear cells of patients with SAD, miRNAs -34a and -181b were found to be significantly up-regulated in AD subjects and confirmed by quantitative PCR, however more work needs to be invested and the finding replicated using a bigger sample size (Schipper et al., 2007).

Using miRNAs in AD therapy

In addition to diagnostics, miRNAs might also be used in therapy. As discussed above, there are at present only four FDA-approved drugs available whose efficacy however is modest. Patients are further treated with antipsychotic drugs although the benefits are moderate and are accompanied by severe side effects such as sedation and Parkinsonism (Ballard et al., 2009, Ballard and Howard, 2006). To combat depression in people with AD antidepressant therapies are pursued (Weintraub et al., 2010). Finally, anticonvulsants are used off license as an effective treatment of agitation and aggression (Ballard et al., 2009). There is therefore a need for alternative strategies. Among the proposed disease-modifying treatments are immunotherapy, along with

secretase inhibitors, amyloid and tau aggregation inhibitors, copper/zinc modulators, lithium as an inhibitor of the tau kinase GSK3 as well as natural products and vitamins (Ballard et al., 2011). Given the role of miRNAs in regulating APP levels, splicing and Aβ metabolism it is well possible that they may emerge as therapeutic reagents. As tau is also subjected to alternative splicing in addition to a high degree of posttranslational modifications (Chen et al., 2004), it would not be surprising that this protein, that constitutes the second hallmark lesion of AD, the NFTs, would also be regulated by miRNAs. Indeed, tau hyperphosphorylation is affected in part by the kinase Erk1, which in turn is directly regulated by the miR-15 family, which is down-regulated in AD (Hebert et al., 2010). The near future will determine which role miRNAs play in neurodegeneration and to which extent they can be exploited as diagnostic and therapeutic tools.

Acknowledgements

This work is supported by grants from the National Health & Medical Research Council, the Australian Research Council, and the J.O. & J.R. Wicking Trust (JG). Postgraduate scholarship support for the laboratory of JG has been provided by the Wenkart Foundation and by Alzheimer's Australia. NS is supported by the Human Frontier Science Program (HFSP), and MM by an EMBO Long-Term Fellowship.

References

Baek, D., Villen, J., Shin, C., Camargo, F. D., Gygi, S. P., and Bartel, D. P., 2008. The impact of microRNAs on protein output. Nature 455**,** 64-71.

Ballard, C., Gauthier, S., Corbett, A., Brayne, C., Aarsland, D., and Jones, E., 2011. Alzheimer's disease. Lancet 377**,** 1019-1031.

Ballard, C., Hanney, M. L., Theodoulou, M., Douglas, S., McShane, R., Kossakowski, K., Gill, R., Juszczak, E., Yu, L. M., and Jacoby, R., 2009. The dementia antipsychotic withdrawal trial (DART-AD): long-term follow-up of a randomised placebo-controlled trial. Lancet Neurol 8**,** 151-157.

Ballard, C., and Howard, R., 2006. Neuroleptic drugs in dementia: benefits and harm. Nat Rev Neurosci 7**,** 492-500.

Ballard, C. G., Gauthier, S., Cummings, J. L., Brodaty, H., Grossberg, G. T., Robert, P., and Lyketsos, C. G., 2009. Management of agitation and aggression associated with Alzheimer disease. Nat Rev Neurol 5**,** 245-255.

Bertram, L., and Tanzi, R. E., 2008. Thirty years of Alzheimer's disease genetics: the implications of systematic meta-analyses. Nat Rev Neurosci 9**,** 768-778.

Boissonneault, V., Plante, I., Rivest, S., and Provost, P., 2009. MicroRNA-298 and microRNA-328 regulate expression of mouse beta-amyloid precursor protein-converting enzyme 1. J Biol Chem 284**,** 1971-1981.

Braak, H., and Braak, E., 1995. Staging of Alzheimer's disease-related neurofibrillary changes. Neurobiol Aging 16**,** 271-278; discussion 278-284.

Chen, F., David, D., Ferrari, A., and Gotz, J., 2004. Posttranslational modifications of tau - Role in human tauopathies and modeling in transgenic animals. Curr Drug Targets 5**,** 503-515.

Chi, S. W., Zang, J. B., Mele, A., and Darnell, R. B., 2009. Argonaute HITS-CLIP decodes microRNA-mRNA interaction maps. Nature 460**,** 479-486.

Cogswell, J. P., Ward, J., Taylor, I. A., Waters, M., Shi, Y., Cannon, B., Kelnar, K., Kemppainen, J., Brown, D., Chen, C., Prinjha, R. K., Richardson, J. C., Saunders, A. M., Roses, A. D., and Richards, C. A., 2008. Identification of miRNA changes in Alzheimer's disease brain and CSF yields putative biomarkers and insights into disease pathways. J Alzheimers Dis 14**,** 27-41.

Cuellar, T. L., Davis, T. H., Nelson, P. T., Loeb, G. B., Harfe, B. D., Ullian, E., and McManus, M. T., 2008. Dicer loss in striatal neurons produces behavioral and neuroanatomical phenotypes in the absence of neurodegeneration. Proc Natl Acad Sci U S A 105**,** 5614-5619.

da Cruz e Silva, O. A., Henriques, A. G., Domingues, S. C., and da Cruz e Silva, E. F., 2010. Wnt signalling is a relevant pathway contributing to amyloid beta-peptide-mediated neuropathology in Alzheimer's disease. CNS Neurol Disord Drug Targets 9**,** 720-726.

David, D., Hoerndli, F., and Gotz, J., 2005. Functional Genomics meets neurodegenerative disorders Part I: Transcriptomic and proteomic technology. Prog Neurobiol 76**,** 153-168.

David, D. C., Ittner, L. M., Gehrig, P., Nergenau, D., Shepherd, C., Halliday, G., and Gotz, J., 2006. ß-Amyloid treatment of two complementary P301L tau-expressing Alzheimer's disease models reveals similar deregulated cellular processes. Proteomics 6**,** 6566-6577.

Davis, T. H., Cuellar, T. L., Koch, S. M., Barker, A. J., Harfe, B. D., McManus, M. T., and Ullian, E. M., 2008. Conditional loss of Dicer disrupts cellular and tissue morphogenesis in the cortex and hippocampus. J Neurosci 28**,** 4322-4330.

Delay, C., Calon, F., Mathews, P., and Hebert, S. S., 2011. Alzheimer-specific variants in the 3'UTR of Amyloid precursor protein affect microRNA function. Mol Neurodegener 6**,** 70.

Donev, R., Newall, A., Thome, J., and Sheer, D., 2007. A role for SC35 and hnRNPA1 in the determination of amyloid precursor protein isoforms. Mol Psychiatry 12**,** 681-690.

Duursma, A. M., Kedde, M., Schrier, M., le Sage, C., and Agami, R., 2008. miR-148 targets human DNMT3b protein coding region. RNA 14**,** 872-877.

Eckert, A., Hauptmann, S., Scherping, I., Meinhardt, J., Rhein, V., Drose, S., Brandt, U., Fandrich, M., Muller, W. E., and Gotz, J., 2008. Oligomeric and fibrillar species of β-amyloid (Aβ42) both impair mitochondrial function in P301L tau transgenic mice. J Mol Med 86**,** 1255-1267.

Eckert, A., Hauptmann, S., Scherping, I., Rhein, V., Muller-Spahn, F., Gotz, J., and Muller, W. E., 2008. Soluble beta-amyloid leads to mitochondrial defects in amyloid precursor protein and tau transgenic mice. Neurodegener Dis 5**,** 157-159.

Fabian, M. R., Sonenberg, N., and Filipowicz, W., 2010. Regulation of mRNA translation and stability by microRNAs. Annu Rev Biochem 79**,** 351-379.

Fagan, A. M., Shaw, L. M., Xiong, C., Vanderstichele, H., Mintun, M. A., Trojanowski, J. Q., Coart, E., Morris, J. C., and Holtzman, D. M., 2011. Comparison of Analytical Platforms for Cerebrospinal Fluid Measures of {beta}-Amyloid 1-42, Total tau, and P-tau181 for Identifying Alzheimer Disease Amyloid Plaque Pathology. Arch Neurol 68**,** 1137-1144.

Faghihi, M. A., Modarresi, F., Khalil, A. M., Wood, D. E., Sahagan, B. G., Morgan, T. E., Finch, C. E., St Laurent, G., 3rd, Kenny, P. J., and Wahlestedt, C., 2008. Expression of a noncoding RNA is elevated in Alzheimer's disease and drives rapid feed-forward regulation of beta-secretase. Nat Med 14**,** 723-730.

Forman, J. J., Legesse-Miller, A., and Coller, H. A., 2008. A search for conserved sequences in coding regions reveals that the let-7 microRNA targets Dicer within its coding sequence. Proc Natl Acad Sci U S A 105**,** 14879-14884.

Glabe, C. G., 2008. Structural classification of toxic amyloid oligomers. J Biol Chem 283**,** 29639-29643.

Gotz, J., Eckert, A., Matamales, M., Ittner, L. M., and Liu, X., 2011. Modes of Abeta toxicity in Alzheimer's disease. Cell Mol Life Sci 68**,** 3359-3375.

Gotz, J., and Ittner, L. M., 2008. Animal models of Alzheimer's disease and frontotemporal dementia. Nat Rev Neurosci 9**,** 532-544.

Gotz, J., Ittner, L. M., Schonrock, N., and Cappai, R., 2008. An update on the toxicity of Abeta in Alzheimer's disease. Neuropsychiatr Dis Treat 4**,** 1033-1042.

Greenfield, J. P., Tsai, J., Gouras, G. K., Hai, B., Thinakaran, G., Checler, F., Sisodia, S. S., Greengard, P., and Xu, H., 1999. Endoplasmic reticulum and trans-Golgi network generate distinct populations of Alzheimer beta-amyloid peptides. Proc Natl Acad Sci U S A 96**,** 742-747.

Hallbergson, A. F., Gnatenco, C., and Peterson, D. A., 2003. Neurogenesis and brain injury: managing a renewable resource for repair. J Clin Invest 112**,** 1128-1133.

Hartmann, T., Bieger, S. C., Bruhl, B., Tienari, P. J., Ida, N., Allsop, D., Roberts, G. W., Masters, C. L., Dotti, C. G., Unsicker, K., and Beyreuther, K., 1997. Distinct sites of intracellular production for Alzheimer's disease A beta40/42 amyloid peptides. Nat Med 3**,** 1016-1020.

Hebert, S. S., and De Strooper, B., 2009. Alterations of the microRNA network cause neurodegenerative disease. Trends Neurosci 32**,** 199-206.

Hebert, S. S., Horre, K., Nicolai, L., Bergmans, B., Papadopoulou, A. S., Delacourte, A., and De Strooper, B., 2009. MicroRNA regulation of Alzheimer's Amyloid precursor protein expression. Neurobiol Dis 33**,** 422-428.

Hebert, S. S., Horre, K., Nicolai, L., Papadopoulou, A. S., Mandemakers, W., Silahtaroglu, A. N., Kauppinen, S., Delacourte, A., and De Strooper, B., 2008. Loss of microRNA cluster miR-29a/b-1 in sporadic Alzheimer's disease correlates with increased BACE1/beta-secretase expression. Proc Natl Acad Sci U S A 105**,** 6415-6420.

Hebert, S. S., Papadopoulou, A. S., Smith, P., Galas, M. C., Planel, E., Silahtaroglu, A. N., Sergeant, N., Buee, L., and De Strooper, B., 2010. Genetic ablation of Dicer in adult forebrain neurons results in abnormal tau hyperphosphorylation and neurodegeneration. Hum Mol Genet 19**,** 3959-3969.

Hoerndli, F., David, D., and Gotz, J., 2005. Functional genomics meets neurodegenerative disorders. Part II: Application and data integration. Prog Neurobiol 76**,** 169-188.

Hoerndli, F. J., Toigo, M., Schild, A., Gotz, J., and Day, P. J., 2004. Reference genes identified in SH-SY5Y cells using custom-made gene arrays with validation by quantitative polymerase chain reaction. Anal Biochem 335**,** 30-41.

Ittner, L. M., and Gotz, J., 2011. Amyloid-beta and tau - a toxic pas de deux in Alzheimer's disease. Nat Rev Neurosci 12**,** 65-72.

Ittner, L. M., Ke, Y. D., Delerue, F., Bi, M., Gladbach, A., van Eersel, J., Wolfing, H., Chieng, B. C., Christie, M. J., Napier, I. A., Eckert, A., Staufenbiel, M., Hardeman, E., and Gotz, J., 2010. Dendritic function of tau mediates amyloid-beta toxicity in Alzheimer's disease mouse models. Cell 142**,** 387-397.

Jin, K., Peel, A. L., Mao, X. O., Xie, L., Cottrell, B. A., Henshall, D. C., and Greenberg, D. A., 2004. Increased hippocampal neurogenesis in Alzheimer's disease. Proc Natl Acad Sci U S A 101**,** 343-347.

Jopling, C. L., Yi, M., Lancaster, A. M., Lemon, S. M., and Sarnow, P., 2005. Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA. Science 309**,** 1577-1581.

Kim, J., Inoue, K., Ishii, J., Vanti, W. B., Voronov, S. V., Murchison, E., Hannon, G., and Abeliovich, A., 2007. A MicroRNA feedback circuit in midbrain dopamine neurons. Science 317**,** 1220-1224.

Kitazume, S., Tachida, Y., Kato, M., Yamaguchi, Y., Honda, T., Hashimoto, Y., Wada, Y., Saito, T., Iwata, N., Saido, T., and Taniguchi, N., 2010. Brain endothelial cells produce amyloid {beta} from amyloid precursor protein 770 and preferentially secrete the O-glycosylated form. J Biol Chem 285**,** 40097-40103.

Kurosinski, P., and Gotz, J., 2002. Glial cells under physiologic and pathological conditions. Arch Neurol 59**,** 1524-1528.

Lagos-Quintana, M., Rauhut, R., Lendeckel, W., and Tuschl, T., 2001. Identification of novel genes coding for small expressed RNAs. Science 294**,** 853-858.

Lahiri, D. K., Ge, Y. W., and Maloney, B., 2005. Characterization of the APP proximal promoter and 5'-untranslated regions: identification of cell type-specific domains and implications in APP gene expression and Alzheimer's disease. FASEB J 19**,** 653-655.

Landgraf, P., Rusu, M., Sheridan, R., Sewer, A., Iovino, N., Aravin, A., Pfeffer, S., Rice, A., Kamphorst, A. O., Landthaler, M., Lin, C., Socci, N. D., Hermida, L., Fulci, V., Chiaretti, S., Foa, R., Schliwka, J., Fuchs, U., Novosel, A., Muller, R. U., Schermer, B., Bissels, U., Inman, J., Phan, Q., Chien, M., Weir, D. B., Choksi, R., De Vita, G., Frezzetti, D., Trompeter, H. I., Hornung, V., Teng, G., Hartmann, G., Palkovits, M., Di Lauro, R., Wernet, P., Macino, G., Rogler, C. E., Nagle, J. W., Ju, J., Papavasiliou, F. N., Benzing, T., Lichter, P., Tam, W., Brownstein, M. J., Bosio, A., Borkhardt, A., Russo, J. J., Sander, C., Zavolan, M., and Tuschl, T., 2007. A mammalian microRNA expression atlas based on small RNA library sequencing. Cell 129**,** 1401-1414.

Lau, P., and de Strooper, B., 2010. Dysregulated microRNAs in neurodegenerative disorders. Semin Cell Dev Biol 21**,** 768-773.

Lee, Y., Kim, M., Han, J., Yeom, K. H., Lee, S., Baek, S. H., and Kim, V. N., 2004. MicroRNA genes are transcribed by RNA polymerase II. EMBO J 23**,** 4051-4060.

Leucht, C., Stigloher, C., Wizenmann, A., Klafke, R., Folchert, A., and Bally-Cuif, L., 2008. MicroRNA-9 directs late organizer activity of the midbrain-hindbrain boundary. Nat Neurosci 11**,** 641-648.

Lewczuk, P., Kornhuber, J., Vanmechelen, E., Peters, O., Heuser, I., Maier, W., Jessen, F., Burger, K., Hampel, H., Frolich, L., Henn, F., Falkai, P., Ruther, E., Jahn, H., Luckhaus, C., Perneczky, R., Schmidtke, K., Schroder, J., Kessler, H., Pantel, J., Gertz, H. J., Vanderstichele, H., de Meyer, G., Shapiro, F., Wolf, S., Bibl, M., and Wiltfang, J., 2010. Amyloid beta peptides in plasma in early diagnosis of Alzheimer's disease: A multicenter study with multiplexing. Exp Neurol 223**,** 366-370.

Lim, Y.-A., Rhein, V., Baysang, G., Meier, F., Poljak, A., Raftery, M., Ittner, L. M., Guilhaus, M., Eckert, A., and Gotz, J., 2010. Abeta and human amylin share a common toxicity pathway via mitochondrial dysfunction. Proteomics 10**,** 1621-1633.

Long, J. M., and Lahiri, D. K., 2011. MicroRNA-101 downregulates Alzheimer's amyloid-beta precursor protein levels in human cell cultures and is differentially expressed. Biochem Biophys Res Commun 404**,** 889-895.

Lukiw, W. J., 2007. Micro-RNA speciation in fetal, adult and Alzheimer's disease hippocampus. Neuroreport 18**,** 297-300.

Makeyev, E. V., Zhang, J., Carrasco, M. A., and Maniatis, T., 2007. The MicroRNA miR-124 promotes neuronal differentiation by triggering brain-specific alternative pre-mRNA splicing. Mol Cell 27**,** 435-448.

Maloney, B., Ge, Y. W., Greig, N., and Lahiri, D. K., 2004. Presence of a "CAGA box" in the APP gene unique to amyloid plaque-forming species and absent in all APLP-1/2 genes: implications in Alzheimer's disease. FASEB J 18**,** 1288-1290.

Mathonnet, G., Fabian, M. R., Svitkin, Y. V., Parsyan, A., Huck, L., Murata, T., Biffo, S., Merrick, W. C., Darzynkiewicz, E., Pillai, R. S., Filipowicz, W., Duchaine, T. F., and Sonenberg, N., 2007. MicroRNA inhibition of translation initiation in vitro by targeting the cap-binding complex eIF4F. Science 317**,** 1764-1767.

Mattick, J. S., and Makunin, I. V., 2005. Small regulatory RNAs in mammals. Hum Mol Genet 14 Spec No 1**,** R121-132.

Nikolaev, A., McLaughlin, T., O'Leary, D. D., and Tessier-Lavigne, M., 2009. APP binds DR6 to trigger axon pruning and neuron death via distinct caspases. Nature 457**,** 981-989.

Niwa, R., Zhou, F., Li, C., and Slack, F. J., 2008. The expression of the Alzheimer's amyloid precursor protein-like gene is regulated by developmental timing microRNAs and their targets in Caenorhabditis elegans. Dev Biol 315**,** 418-425.

Nunez-Iglesias, J., Liu, C. C., Morgan, T. E., Finch, C. E., and Zhou, X. J., 2010. Joint genome-wide profiling of miRNA and mRNA expression in Alzheimer's disease cortex reveals altered miRNA regulation. PLoS ONE 5**,** e8898.

O'Carroll, D., Mecklenbrauker, I., Das, P. P., Santana, A., Koenig, U., Enright, A. J., Miska, E. A., and Tarakhovsky, A., 2007. A Slicer-independent role for Argonaute 2 in hematopoiesis and the microRNA pathway. Genes Dev 21**,** 1999-2004.

O'Connor, T., Sadleir, K. R., Maus, E., Velliquette, R. A., Zhao, J., Cole, S. L., Eimer, W. A., Hitt, B., Bembinster, L. A., Lammich, S., Lichtenthaler, S. F., Hebert, S. S., De Strooper, B., Haass, C., Bennett, D. A., and Vassar, R., 2008. Phosphorylation of the translation initiation factor eIF2alpha increases BACE1 levels and promotes amyloidogenesis. Neuron 60**,** 988-1009.

Ono, K., Condron, M. M., and Teplow, D. B., 2009. Structure-neurotoxicity relationships of amyloid beta-protein oligomers. Proc Natl Acad Sci U S A 106**,** 14745-14750.

Orom, U. A., Nielsen, F. C., and Lund, A. H., 2008. MicroRNA-10a binds the 5'UTR of ribosomal protein mRNAs and enhances their translation. Mol Cell 30**,** 460-471.

Palop, J. J., and Mucke, L., 2010. Amyloid-beta-induced neuronal dysfunction in Alzheimer's disease: from synapses toward neural networks. Nat Neurosci 13**,** 812-818.

Papagiannakopoulos, T., and Kosik, K. S., 2009. MicroRNA-124: micromanager of neurogenesis. Cell Stem Cell 4**,** 375-376.

Pasic, M. D., Diamandis, E. P., McLaurin, J., Holtzman, D. M., Schmitt-Ulms, G., and Quirion, R., 2011. Alzheimer disease: advances in pathogenesis, diagnosis, and therapy. Clin Chem 57**,** 664-669.

Pasquinelli, A. E., Reinhart, B. J., Slack, F., Martindale, M. Q., Kuroda, M. I., Maller, B., Hayward, D. C., Ball, E. E., Degnan, B., Muller, P., Spring, J., Srinivasan, A., Fishman, M., Finnerty, J., Corbo, J., Levine, M., Leahy, P., Davidson, E., and Ruvkun, G., 2000. Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. Nature 408**,** 86-89.

Patel, N., Hoang, D., Miller, N., Ansaloni, S., Huang, Q., Rogers, J. T., Lee, J. C., and Saunders, A. J., 2008. MicroRNAs can regulate human APP levels. Mol Neurodegener 3**,** 10.

Roberson, E. D., Scearce-Levie, K., Palop, J. J., Yan, F., Cheng, I. H., Wu, T., Gerstein, H., Yu, G. Q., and Mucke, L., 2007. Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model. Science 316**,** 750-754.

Rogers, J. T., Randall, J. D., Cahill, C. M., Eder, P. S., Huang, X., Gunshin, H., Leiter, L., McPhee, J., Sarang, S. S., Utsuki, T., Greig, N. H., Lahiri, D. K., Tanzi, R. E., Bush, A. I., Giordano, T., and Gullans, S. R., 2002. An iron-responsive element type II in the 5'-untranslated region of the Alzheimer's amyloid precursor protein transcript. J Biol Chem 277**,** 45518-45528.

Saito, T., Suemoto, T., Brouwers, N., Sleegers, K., Funamoto, S., Mihira, N., Matsuba, Y., Yamada, K., Nilsson, P., Takano, J., Nishimura, M., Iwata, N., Van Broeckhoven, C., Ihara, Y., and Saido, T. C., 2011. Potent amyloidogenicity and pathogenicity of Abeta43. Nat Neurosci 14**,** 1023-1032.

Sastre, M., Steiner, H., Fuchs, K., Capell, A., Multhaup, G., Condron, M. M., Teplow, D. B., and Haass, C., 2001. Presenilin-dependent gamma-secretase processing of beta-amyloid precursor protein at a site corresponding to the S3 cleavage of Notch. EMBO Rep 2**,** 835-841.

Schaefer, A., O'Carroll, D., Tan, C. L., Hillman, D., Sugimori, M., Llinas, R., and Greengard, P., 2007. Cerebellar neurodegeneration in the absence of microRNAs. J Exp Med 204**,** 1553-1558.

Schipper, H. M., Maes, O. C., Chertkow, H. M., and Wang, E., 2007. MicroRNA expression in Alzheimer blood mononuclear cells. Gene Regul Syst Bio 1**,** 263-274.

Schonrock, N., Humphreys, D. T., Preiss, T., and Gotz, J., 2011. Target Gene Repression Mediated by miRNAs miR-181c and miR-9 Both of Which Are Down-regulated by Amyloid-beta. J Mol Neurosci., in press [Epub ahead of print]

Schonrock, N., Ke, Y. D., Humphreys, D., Staufenbiel, M., Ittner, L. M., Preiss, T., and Gotz, J., 2010. Neuronal microRNA deregulation in response to Alzheimer's disease amyloid-beta. PLoS ONE 5**,** e11070.

Selbach, M., Schwanhausser, B., Thierfelder, N., Fang, Z., Khanin, R., and Rajewsky, N., 2008. Widespread changes in protein synthesis induced by microRNAs. Nature 455**,** 58-63.

Selkoe, D. J., 1997. Alzheimer's disease: genotypes, phenotypes, and treatments. Science 275**,** 630-631.

Selkoe, D. J., and Podlisny, M. B., 2002. Deciphering the genetic basis of Alzheimer's disease. Annu Rev Genomics Hum Genet 3**,** 67-99.

Shibata, M., Kurokawa, D., Nakao, H., Ohmura, T., and Aizawa, S., 2008. MicroRNA-9 modulates Cajal-Retzius cell differentiation by suppressing Foxg1 expression in mouse medial pallium. J Neurosci 28**,** 10415-10421.

Shin, D., Shin, J. Y., McManus, M. T., Ptacek, L. J., and Fu, Y. H., 2009. Dicer ablation in oligodendrocytes provokes neuronal impairment in mice. Ann Neurol 66**,** 843-857.

Shioya, M., Obayashi, S., Tabunoki, H., Arima, K., Saito, Y., Ishida, T., and Satoh, J., 2010. Aberrant microRNA expression in the brains of neurodegenerative diseases: miR-29a decreased in Alzheimer disease brains targets neurone navigator 3. Neuropathol Appl Neurobiol 36**,** 320-330.

Siomi, H., and Siomi, M. C., 2010. Posttranscriptional regulation of microRNA biogenesis in animals. Mol Cell 38**,** 323-332.

Smith, P., Al Hashimi, A., Girard, J., Delay, C., and Hebert, S. S., 2011. In vivo regulation of amyloid precursor protein neuronal splicing by microRNAs. J Neurochem 116**,** 240-247.

Tay, Y., Zhang, J., Thomson, A. M., Lim, B., and Rigoutsos, I., 2008. MicroRNAs to Nanog, Oct4 and Sox2 coding regions modulate embryonic stem cell differentiation. Nature 455**,** 1124-1128.

Tomiyama, T., Matsuyama, S., Iso, H., Umeda, T., Takuma, H., Ohnishi, K., Ishibashi, K., Teraoka, R., Sakama, N., Yamashita, T., Nishitsuji, K., Ito, K., Shimada, H., Lambert, M. P., Klein, W. L., and Mori, H., 2010. A mouse model of amyloid beta oligomers: their contribution to synaptic alteration, abnormal tau phosphorylation, glial activation, and neuronal loss in vivo. J Neurosci 30**,** 4845-4856.

Vilardo, E., Barbato, C., Ciotti, M., Cogoni, C., and Ruberti, F., 2010. MicroRNA-101 regulates amyloid precursor protein expression in hippocampal neurons. J Biol Chem 285**,** 18344-18351.

Wakiyama, M., Takimoto, K., Ohara, O., and Yokoyama, S., 2007. Let-7 microRNA-mediated mRNA deadenylation and translational repression in a mammalian cell-free system. Genes Dev 21**,** 1857-1862.

Walsh, D. M., Klyubin, I., Fadeeva, J. V., Cullen, W. K., Anwyl, R., Wolfe, M. S., Rowan, M. J., and Selkoe, D. J., 2002. Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. Nature 416**,** 535-539.

Wang, H., Liu, J., Zong, Y., Xu, Y., Deng, W., Zhu, H., Liu, Y., Ma, C., Huang, L., Zhang, L., and Qin, C., 2010. miR-106b aberrantly expressed in a double transgenic mouse model for Alzheimer's disease targets TGF-beta type II receptor. Brain Res 1357**,** 166-174.

Wang, W. X., Rajeev, B. W., Stromberg, A. J., Ren, N., Tang, G., Huang, Q., Rigoutsos, I., and Nelson, P. T., 2008. The expression of microRNA miR-107

decreases early in Alzheimer's disease and may accelerate disease progression through regulation of beta-site amyloid precursor protein-cleaving enzyme 1. J Neurosci 28**,** 1213-1223.

Wang, W. X., Wilfred, B. R., Madathil, S. K., Tang, G., Hu, Y., Dimayuga, J., Stromberg, A. J., Huang, Q., Saatman, K. E., and Nelson, P. T., 2010. miR-107 Regulates Granulin/Progranulin with Implications for Traumatic Brain Injury and Neurodegenerative Disease. Am J Pathol 177**,** 334-345.

Wang, X., Liu, P., Zhu, H., Xu, Y., Ma, C., Dai, X., Huang, L., Liu, Y., Zhang, L., and Qin, C., 2009. miR-34a, a microRNA up-regulated in a double transgenic mouse model of Alzheimer's disease, inhibits bcl2 translation. Brain Res Bull 80**,** 268-273.

Weidemann, A., Eggert, S., Reinhard, F. B., Vogel, M., Paliga, K., Baier, G., Masters, C. L., Beyreuther, K., and Evin, G., 2002. A novel epsilon-cleavage within the transmembrane domain of the Alzheimer amyloid precursor protein demonstrates homology with Notch processing. Biochemistry 41**,** 2825-2835.

Weintraub, D., Rosenberg, P. B., Drye, L. T., Martin, B. K., Frangakis, C., Mintzer, J. E., Porsteinsson, A. P., Schneider, L. S., Rabins, P. V., Munro, C. A., Meinert, C. L., and Lyketsos, C. G., 2010. Sertraline for the treatment of depression in Alzheimer disease: week-24 outcomes. Am J Geriatr Psychiatry 18**,** 332-340.

Weyer, S. W., Klevanski, M., Delekate, A., Voikar, V., Aydin, D., Hick, M., Filippov, M., Drost, N., Schaller, K. L., Saar, M., Vogt, M. A., Gass, P., Samanta, A., Jaschke, A., Korte, M., Wolfer, D. P., Caldwell, J. H., and Muller, U. C., 2011. APP and APLP2 are essential at PNS and CNS synapses for transmission, spatial learning and LTP. EMBO J 30**,** 2266-2280.

Williams, A. H., Valdez, G., Moresi, V., Qi, X., McAnally, J., Elliott, J. L., Bassel-Duby, R., Sanes, J. R., and Olson, E. N., 2009. MicroRNA-206 delays ALS progression and promotes regeneration of neuromuscular synapses in mice. Science 326**,** 1549-1554.

Wilquet, V., and De Strooper, B., 2004. Amyloid-beta precursor protein processing in neurodegeneration. Curr Opin Neurobiol 14**,** 582-588.

Xu, H., Sweeney, D., Wang, R., Thinakaran, G., Lo, A. C., Sisodia, S. S., Greengard, P., and Gandy, S., 1997. Generation of Alzheimer beta-amyloid protein in the trans-Golgi network in the apparent absence of vesicle formation. Proc Natl Acad Sci U S A 94**,** 3748-3752.

Zhang, Y. W., Thompson, R., Zhang, H., and Xu, H., 2011. APP processing in Alzheimer's disease. Mol Brain 4**,** e3.

Zhao, C., Sun, G., Li, S., and Shi, Y., 2009. A feedback regulatory loop involving microRNA-9 and nuclear receptor TLX in neural stem cell fate determination. Nat Struct Mol Biol 16**,** 365-371.

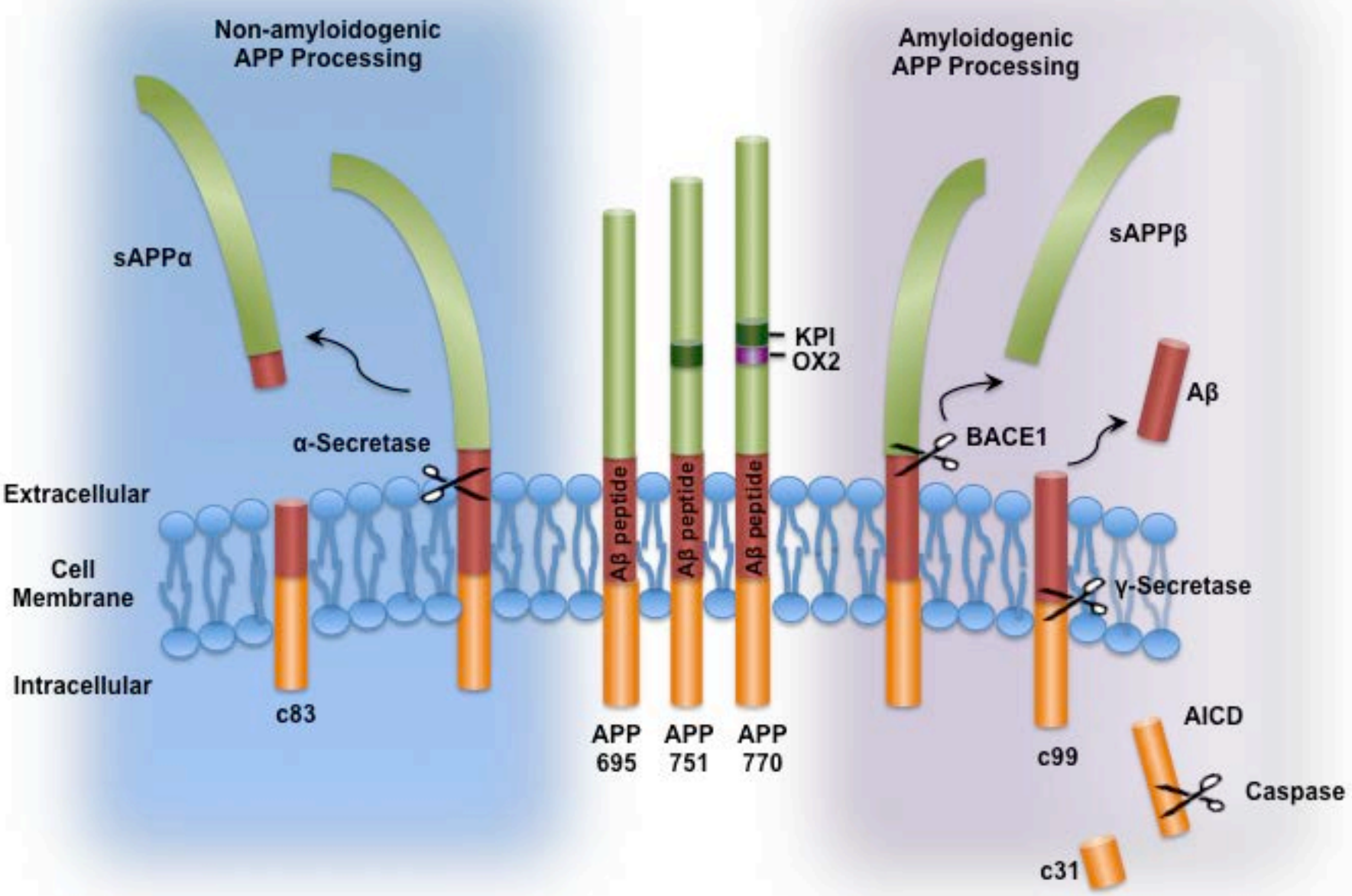
Zhao, G., Mao, G., Tan, J., Dong, Y., Cui, M. Z., Kim, S. H., and Xu, X., 2004. Identification of a new presenilin-dependent zeta-cleavage site within the transmembrane domain of amyloid precursor protein. J Biol Chem 279**,** 50647-50650.

Zong, Y., Wang, H., Dong, W., Quan, X., Zhu, H., Xu, Y., Huang, L., Ma, C., and Qin, C., 2011. miR-29c regulates BACE1 protein expression. Brain Res 1395**,** 108-115.

Figure legends

Figure 1. Schematic drawing of the Amyloid Precursor Protein (APP) and its processing pathways. In humans, APP exists in three major isoforms that are generated by alternative splicing and differ mainly by the presence or absence of the Kunitz-type protease inhibitor (KPI) and OX2 homology domains. APP can be processed by α-secretase in a non-amyloidogenic pathway, which precludes the formation of Aβ and generates soluble APPα (sAPPα) and a C-terminal fragment (c83). Alternatively, in the amyloidogenic pathway, cleavage by the β-secretase BACE1 generates soluble APPβ (sAPPβ), which is secreted, and a C-terminal fragment (β-CTF or c99). Subsequent cleavage of c99 by the γ-secretase complex generates Aβ and the APP intracellular domain (AICD) which can be further cleaved by caspases to produce a c31 fragment.

Figure 2. The miRNA network surrounding APP has both upstream and downstream components which directly or indirectly affect APP (not drawn to scale) processing and Aβ metabolism. miR-124 affects the generation of APP mRNA isoforms via alternative splicing, whilst a range of miRNAs target APP mRNA directly via its 3'UTR thus impacting *APP* translation efficiency. APP is also regulated indirectly by a host of miRNAs, which affect APP processing via targeting the BACE1 (β-secretase) mRNA. This level of regulation affects the type of Aβ species produced, which differ in their degrees of toxicity. Aβ40 is the major species, whilst Aβ42 is the major amyloidogenic species. However, longer and shorter Aβ versions exist (such as Aβ38 and Aβ43) or forms that are pyroglutamate-modified at their amino-terminus (Aβ(3pE)). Furthermore, Aβ can exist as a monomer, as higher order aggregates (such as in oligomers or ADDLs, Amyloid β-Derived Diffusible Ligands) or as fibrils. Fibrillar Aβ is the major constituent of amyloid plaques. Aβ itself causes a deregulation of neuronal miRNAs, several of which have feedback loops, which in turn affect APP metabolism and Aβ generation.


Non-amyloidogenic
APP Processing
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Intracellular
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AICD
Caspase
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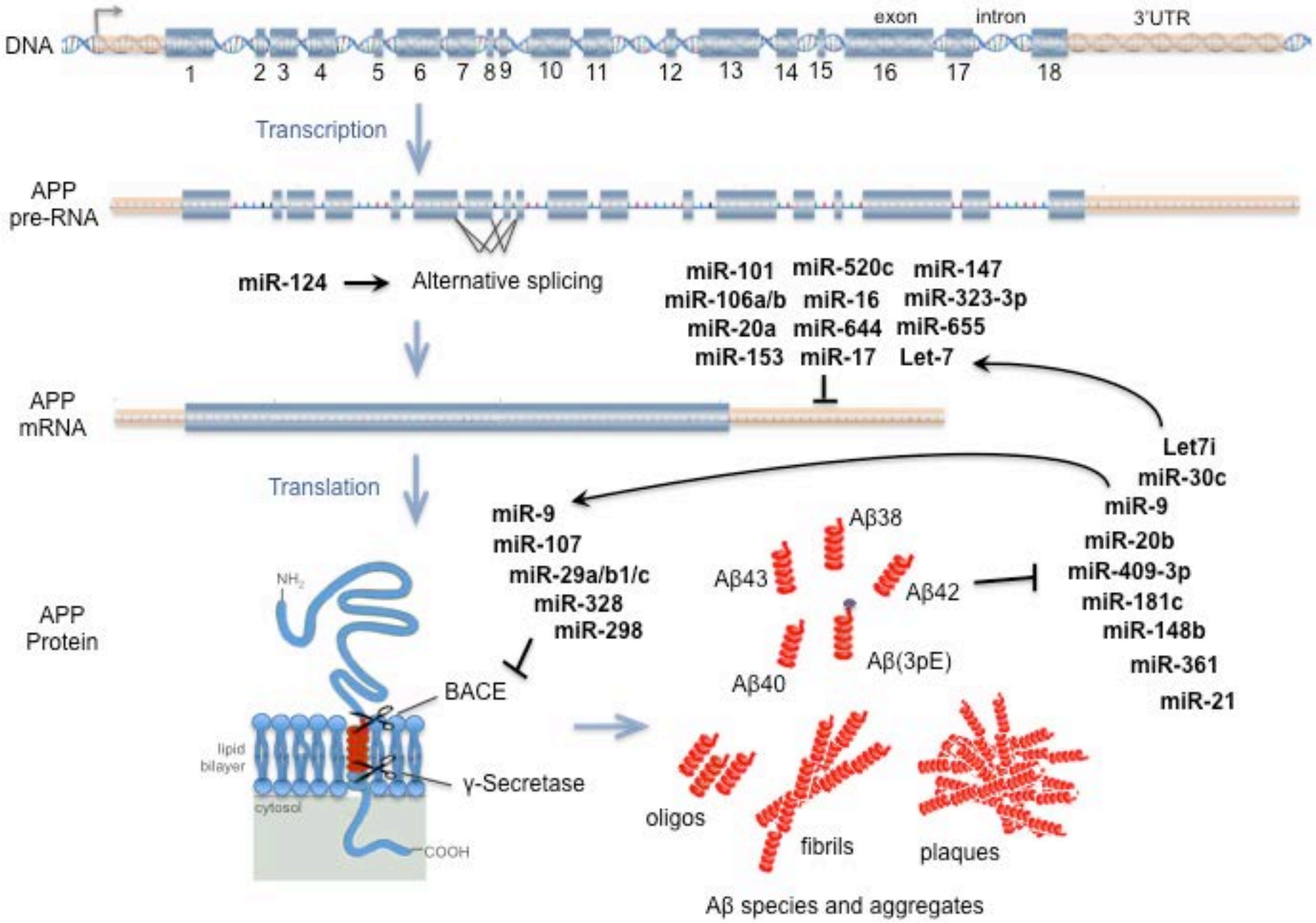
DNA
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Transcription
APP pre-RNA
miR-124
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miR-106a/b miR-16 miR-323-3p
miR-20a miR-644 miR-655
miR-153 miR-17 Let-7
APP mRNA
Translation
Let7i
miR-30c
miR-9
miR-20b
miR-409-3p
miR-181c
miR-148b
miR-361
miR-21
miR-9
miR-107
miR-29a/b1/c
miR-328
miR-298
APP Protein
NH2
BACE
lipid bilayer
γ-Secretase
cytosol
COOH
Aβ38
Aβ43
Aβ42
Aβ40
Aβ(3pE)
oligos
fibrils
plaques
Aβ species and aggregates