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Regulation of AMPAR expression by microRNAs

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Abstract

AMPA receptors (AMPARs) are the major excitatory neurotransmitter receptor in the brain, and their expression at synapses is a critical determinant of synaptic transmission and therefore brain function. Synaptic plasticity involves increases or decreases in synaptic strength, caused by changes in the number or subunit-specific subtype of AMPARs expressed at synapses, and resulting in modifications of functional connectivity of neuronal circuits, a process which is thought to underpin learning and the formation or loss of memories. Furthermore, numerous neurological disorders involve dysregulation of excitatory synaptic transmission or aberrant recruitment of plasticity processes. MicroRNAs (miRNAs) repress the translation of target genes by partial complementary base pairing with mRNAs, and are the core component of a mechanism widely used in a range of cell processes for regulating protein translation. MiRNA-dependent translational repression can occur locally in neuronal dendrites, close to synapses, and can also result in relatively rapid changes in protein expression. MiRNAs are therefore well-placed to regulate synaptic plasticity via the local control of AMPAR subunit synthesis, and can also result in synaptic dysfunction in the event of dysregulation in disease. Here, I will review the miRNAs that have been identified as playing a role in physiological or pathological changes in AMPAR subunit expression at synapses, focussing on miRNAs that target mRNAs encoding AMPAR subunits, and on miRNAs that target AMPAR accessory proteins involved in AMPAR trafficking and hence the regulation of AMPAR synaptic localisation.

Introduction

MicroRNAs (miRNAs) are small noncoding endogenous RNA molecules that repress the translation of target mRNAs in a process that has emerged in the past decade as being fundamentally important for fine-tuning protein synthesis in a wide range of cellular processes. Numerous excellent reviews describe the mechanisms that underlie miRNA processing and function (Ha and Kim, 2014; Meister, 2013; Treiber et al., 2019; Wilson and Doudna, 2013), so only a summarised account will be presented here. miRNAs are transcribed as primary RNAs (pri-RNAs) and cleaved by the microprocessor complex in the nucleus. The resulting pre-miRNA is exported to the cytosol and further processed by Dicer to an intermediate miRNA duplex. The leading miRNA strand is loaded into the miRNA-induced silencing complex (miRISC), the core protein component of which is a member of the Argonaute (Ago) family. The miRNA guides the miRISC to target mRNAs via complementary, but imperfect, base pairing mainly in the 3' untranslated regions (UTRs) of the mRNA. This interaction leads to translational repression of the target mRNAs via various additional proteins that associate with the RISC via Argonaute. MiRNA expression is under tight control, and in neurons, their transcription has been shown to be regulated by synaptic activity (Aksoy-Aksel et al., 2014; Hu and Li, 2017). In addition, Dicer is active in dendrites and regulated by the stimulation of individual synapses to rapidly generate mature miRNAs in a precisely localised manner (Sambandan et al., 2017). Furthermore, NMDAR stimulation has been shown to regulate Ago2 function and binding to other RISC protein components to modulate specific miRNA-dependent silencing events (Rajgor et al., 2018).

Nearly half of all mammalian miRNAs are expressed at high levels in the brain, where they regulate neuronal development, dendritic spine morphogenesis and synaptic function (McNeill and Van Vactor, 2012; Rajman and Schratt, 2017). Numerous miRNAs have been shown to be involved in regulating protein synthesis for specific forms of learning and memory, and

dysfunction of miRNA systems is implicated in neurological and neuropsychiatric diseases including Alzheimer's, Multiple Sclerosis, Schizophrenia and drug addiction, amongst others (Aksoy-Aksel et al., 2014; Hu and Li, 2017; Thomas et al., 2018; Wang et al., 2012b). Furthermore, a large proportion of neuronal miRNAs are enriched in dendrites, and several have been assigned roles in modulating the local translation of specific proteins involved in excitatory synaptic transmission or in regulating the actin cytoskeleton to control the morphology of dendritic spines (Weiss et al., 2015).

As the major excitatory neurotransmitter receptor in the central nervous system, the regulation of AMPAR number or subunit-specific subtype expression at synapses is essential for brain function. In particular, increases or decreases in synaptic strength (synaptic plasticity) modify the functional connectivity of neuronal circuits and consequently underpin processes of learning and the formation or loss of memories (Diering and Huganir, 2018; Hardt et al., 2014; Huganir and Nicoll, 2013; Kessels and Malinow, 2009). Research into the mechanisms of synaptic plasticity has predominantly been focussed on AMPAR trafficking, which is well-established as a major process that underlies such plasticity. Exocytosis, endocytosis and endosomal sorting of AMPAR complexes are highly regulated to bring about rapid changes in synaptic strength in response to specific patterns of synaptic or network activity. Synapse-specific ("Hebbian") forms of plasticity include Long-Term Potentiation (LTP) and Long-Term Depression (LTD), which are triggered typically by NMDAR stimulation, or in some forms of LTD, by mGluR stimulation, both of which activate various signalling pathways that result in changes in AMPAR trafficking (Anggono and Huganir, 2012; Chater and Goda, 2014). In addition, a form of plasticity known as homeostatic synaptic plasticity or synaptic scaling involves up- or down-regulating the activity of all of a neuron's synapses in response to a network-wide change in activity (Chowdhury and Hell, 2018; Fernandes and Carvalho, 2016). AMPARs are tetrameric complexes composed of subunits GluA1-GluA4, encoded by genes *Gria1*-*Gria4*, respectively. The majority of AMPARs in the brain are GluA1/2 heteromers,

followed by GluA2/3 (Lu et al., 2009; Wenthold et al., 1996). Nevertheless, a small, yet functionally highly significant proportion of AMPARs lack GluA2 subunit (GluA1 homomers or GluA1/3 heteromers). Since GluA2 confers Ca^{2+} impermeability, these GluA2-lacking AMPARs are Ca^{2+} permeable (CP-AMPARs) (Hanley, 2014; Henley and Wilkinson, 2016).

In addition to trafficking mechanisms, it has more recently emerged that miRNAs play critical roles in regulating the translation and hence expression levels of AMPAR subunits or of proteins involved in AMPAR trafficking, to contribute to plasticity. Here, I will review the current knowledge of miRNA-dependent mechanisms involved in regulating AMPAR expression at synapses in LTD, LTP and synaptic scaling. In addition, I will discuss recent reports of related mechanisms that dysfunction in some important neurological disorders.

MiRNA-dependent regulation of AMPARs in physiological synaptic plasticity

- Regulation of AMPARs in Homeostatic Synaptic Scaling by miR-92a, miR-124, miR-186-5p, miR-218 and miR-134.

Homeostatic plasticity or synaptic scaling has a longer time course compared to LTD and LTP, and therefore is more intuitively suited to control by translational regulation of AMPAR subunits. Synaptic scaling is typically studied *in vitro* by bath application of agents to increase or decrease network activity. Picrotoxin or Bicuculline block inhibitory GABA(A) receptors and hence cause an increase in excitatory network activity. The neuronal response is to downregulate synaptic transmission, i.e. synaptic downscaling. In contrast, decreases in network activity caused by TTX to block evoked neurotransmitter release, or by AMPAR and NMDAR antagonists to reduce postsynaptic excitation, stimulate neurons to increase synaptic transmission, i.e. synaptic upscaling (Turrigiano et al., 1998). The first demonstration of a role for miRNAs in such a mechanism came with the observation that a 4 h application of TTX

together with the NMDAR antagonist AP5 caused a decrease in expression of a number of miRNAs in hippocampal neurons, including miR-92a, which was shown to target the *Gria1* mRNA (Letellier et al., 2014). TTX/AP5 treatment caused a corresponding increase in expression of a reporter construct regulated by the *Gria1* 3'UTR. Overexpression of miR-92a blocked TTX/AP5-induced increases in endogenous GluA1 expression as measured by immunocytochemistry, and blocked upscaling of mEPSCs. Taken together, this study demonstrated that when circuit activity is globally down-regulated, miR-92a expression is reduced, releasing translational repression of *Gria1* to enhance synaptic AMPAR expression. It further suggests that GluA1 protein synthesis is tonically restricted by basal miR-92a activity. This study did not investigate changes in *Gria2* expression, indeed they reported that the levels of miRNAs known to target *Gria2* were unaffected by TTX/AP5 treatment (Letellier et al., 2014). This raises the question about subunit-specific changes in synaptic AMPARs, especially since it has been suggested in other reports that synaptic upscaling involves a transient expression of GluA2-lacking CP-AMPARs (Chowdhury and Hell, 2018).

While subunit-specific trafficking mechanisms have been shown to play a role in regulating the synaptic targeting of CP-AMPARs during synaptic upscaling (Wang et al., 2012a), it has also been demonstrated that miRNA activity represses GluA2 protein synthesis to favour CP-AMPAR expression in response to TTX/AP5 application (Hou et al., 2015). miR-124 is one of the most abundant miRNAs expressed in the brain, accounting for more than a quarter of all brain miRNAs (Lagos-Quintana et al., 2002). It plays a critical role in neurogenesis, and its deletion causes severe neurodevelopmental deficiencies, as a result of targeting numerous mRNAs (Sanuki et al., 2011). In mature neurons, the expression of miR-124 is activity-dependent, with 15 h of neuronal activity blockade by TTX/AP5 causing an increase in miR-124 expression to inhibit translation of *Gria2* (Hou et al., 2015). While miR-124 overexpression was sufficient to induce the formation of functional CP-AMPARs at synapses, it was not sufficient to induce synaptic upscaling in the absence of a reduction in neuronal activity. However, inhibition of miR-124 activity blocked the induction of upscaling in response to

TTX/AP5 application, indicating that it is necessary for this form of plasticity, and providing further support for the essential role of CP-AMPARs in homeostatic upscaling. This study also addressed the question of how miR-124 transcription is regulated to bring about these changes. Work in non-neuronal cells had previously established a role for the transcription factor EVI1 in supressing miR-124 expression (Vazquez et al., 2010). In agreement with these findings, knocking down EVI1 by siRNA in cultured neurons caused an upregulation of miR-124 expression, and overexpressing EVI1 blocked synaptic upscaling in response to TTX/AP5. Further mechanistic detail was provided by demonstrating that TTX/AP5 treatment caused a decrease in EV11 binding to the histone deacetylase HDAC1 (Hou et al., 2015).

A later study identified another miRNA, miR-186-5p, with an apparently similar function as miR-124 in regulating GluA2 expression as a critical component of synaptic upscaling (Silva et al., 2019). This was the result of screening 16 miRNAs for candidates that are up- or down-regulated by network inactivity that induces synaptic upscaling. Instead of TTX, this study used the AMPAR antagonist GYKI-52466 together with the NMDAR channel blocker MK-801 to induce network inactivity for a range of time periods. MiR-186-5p expression was dramatically decreased after just 2 h of synaptic activity blockade by GYKI-52466/MK-801. Surprisingly, neither miR-92a nor miR-124 showed significant changes in this study. One possible explanation for this discrepancy is the different methods used for blocking network activity; changes in miR-92a and miR-124 expression were observed after application of TTX/AP5, which would not affect AMPAR miniature EPSCs (Letellier et al., 2014; Hou et al., 2015). In contrast, downregulation of miR-186-5p was caused by GYKI-52466/MK-801, which would be expected to block all AMPAR and NMDAR activity (Silva et al., 2019). Evidence that miR-186-5p represses *Gria2* translation was provided by the observation that overexpression of pre-miR-186-5p in cortical neuronal cultures caused a reduction in the expression of luciferase reporters under the control of the *Gria2* 3’UTR. Neurons overexpressing pre-miR-186-5p also showed a down-regulation of endogenous GluA2 expression at synapses and an increase in sensitivity of EPSCs to the selective CP-AMPAR blocker NASPM, indicating the presence of

synaptic CP-AMPARs. Importantly, pre-miR-186-5p overexpression blocked synaptic upscaling in response to GYKI-52466/MK-801, and the opposite intervention, namely inhibiting miR-186-5p, mimicked synaptic upscaling in the absence of these drugs (Silva et al., 2019). These results suggest a mechanism whereby synaptic inactivity causes a rapid reduction in miR-186-5p expression, by an as yet undefined mechanism, resulting in an increase in GluA2 protein synthesis to scale up synaptic transmission. Apart from differences in which miRNAs are reported to change in response to synaptic activity blockade, there appears to be some conflict between Hou et al. and Silva et al. from the point of view of whether GluA2 expression is up- or down-regulated during synaptic upscaling. Timing is possibly important here; perhaps an initial increase in CP-AMPAR expression caused by miR-124-mediated downregulation of *Gria2* translation is later followed by an increase in *Gria2* translation as a result of reduced miR-186-5p expression, returning the normal complement of GluA2-containing AMPARs. Interestingly, miR-186-5p has also been suggested to target NSF (Moore et al., 2015), which is involved in restricting GluA2 endocytosis to promote surface expression of GluA2-containing AMPARs (Hanley, 2018). Hence, while not tested experimentally, this theoretically represents an additional influence of miR-186-5p on increasing synaptic AMPARs.

The miRNAs discussed above were reported to have a specific role in up-scaling after suppressing network activity. An interesting question is whether they are bidirectionally regulated to mediate up- or down-scaling, depending on the change in network activity. While there appear to be no reports of miR-92a, miR-124 or miR-186-5p levels being affected by increased network activity, another miRNA targeting *Gria2* has been suggested to play a role in both up- and down-scaling forms of plasticity. A recent report identified miR-218 as a regulator of GluA2 translation, overexpression of which caused an increase in translation via the *Gria2* 3'UTR in luciferase reporter assays, and also caused an increase in total and synaptic levels of GluA2 and a consequent increase in mEPSC amplitude in hippocampal neurons (Rocchi et al., 2019). While upregulating translation is an unusual role for miRNAs, it

has been reported previously (Vasudevan and Steitz, 2007). The expression level of miR-218 itself was upregulated by 48 h of TTX treatment, but downregulated by an increase in network activity following bicuculline application, thereby generating the appropriate changes in GluA2 protein expression for bidirectional homeostatic plasticity (Rocchi et al., 2019). While miR-218 is mechanistically well-positioned to mediate both forms of homeostatic synaptic plasticity, this report did not directly demonstrate a requirement for miR-218 activity in the expression of synaptic up- or down-scaling. Interestingly, miR-218 also targets and inhibits translation of *REST* (RE-1-silencing transcription factor) mRNA (Liu et al., 2016). REST controls *Gria2* transcription (Myers et al., 1998), suggesting multiple levels that miR-218 could control GluA2 protein synthesis.

An indirect effect of miRNA activity on homeostatic plasticity, and a miRNA activity specifically involved in synaptic downscaling, is seen via *Pum2*, which is a target for miR-134 and codes for the RNA-binding protein Pumilio-2 (Fiore et al., 2014). MiR-134 was the first miRNA found to be localised to dendrites and to play a role in regulating dendritic spine morphogenesis in an activity-dependent manner (Schratt et al., 2006). An increase in excitatory network activity induced by picrotoxin caused an increase in miR-134 levels, and miR-134 inhibitors blocked picrotoxin-induced downscaling of synaptic GluA2 expression, AMPAR EPSC frequency and also downscaling of dendritic spine size (Fiore et al., 2014). MiR-134 does not target AMPAR subunit mRNA, indeed this study demonstrated that synaptic down-scaling after picrotoxin does not require a decrease in total GluA2 expression, but instead involves a decrease in surface levels of GluA2, suggesting an effect on receptor trafficking. This is consistent with previous reports demonstrating a role for AMPAR internalisation in homeostatic downscaling (Wang et al., 2012a). While known AMPAR trafficking proteins are absent from the list of miR-134 targets, *Pum2* was shown to be downregulated by picrotoxin treatment in a miR-134-dependent manner. Furthermore, a miR-134-dependent loss of Pum2 was shown to be essential for synaptic downscaling (Fiore et al., 2014). Pum2 is involved in translational repression via a non-miRNA mechanism (Goldstrohm et al., 2018). Among the mRNA targets

of Pum2 is *Plk2*, which encodes polo-like kinase, a known mediator of GluA2 internalisation in homeostatic synaptic down-scaling. Plk2 binds to the GluA2 interacting protein NSF, thus inhibiting the function of NSF in maintaining GluA2 surface expression (Evers et al., 2010). Pum2 has been shown to bind and regulate many hundreds of mRNAs (Goldstrohm et al., 2018), so it will be interesting to investigate additional genes that may be regulated by Pum2 in the control of homeostatic synaptic plasticity. Interestingly, picrotoxin treatment did not affect translation via the *Limk1* 3' UTR, which is another target of miR-134, and encodes the actin-regulatory protein Limk1, which would be an attractive candidate for mediating dendritic spine retraction during homeostatic downscaling (Fiore et al., 2014; Schratt et al., 2006). This specific effect of miR-134 indicates that the underlying mechanism is not simply transcriptional upregulation of miR-134, but suggests an additional regulatory mechanism is at play.

- Regulation of AMPAR subunit synthesis in LTD and LTP by miR-137, miR-501-3p and miR-181a,

LTD can be induced by stimulation of NMDA receptors or of metabotropic glutamate receptors (mGluRs) (Collingridge et al., 2010). A role for the translational control of protein synthesis in mGluR-dependent LTD is well-established (Waung and Huber, 2009), however, to date only one miRNA has been identified as a critical regulator of AMPAR subunit expression in this form of plasticity. MiR-137 is rapidly and transiently upregulated in response to mGluR5 stimulation with DHPG and targets *Gria1* 3' UTR to reduce synthesis of GluA1 and hence reduce synaptic strength (Olde Loohuis et al., 2015). A critical role for this mechanism in mGluR-LTD was demonstrated by inhibition of miR-137 function with specific antagomirs, which caused a significant reduction in mGluR-LTD in hippocampal CA1. Furthermore, experimental up- or down-regulation of miR-137 showed corresponding changes in GluA1 expression and mEPSC frequency under basal conditions, indicating a direct and fundamental role for this miRNA in regulating synaptic transmission. Mature levels of miR-137 were shown to increase significantly after just 15 min DHPG treatment, with a concomitant reduction in the

levels of immature pre-miR-137 (Olde Loohuis et al., 2015). This suggests that the relevant mechanism is not enhanced transcription, but rather involves enhanced processing of pre-miR-137 by Dicer. Moreover, levels of mature miR-137 returned to baseline at 45 min, suggesting a role in reducing GluA1 levels in the early stages of LTD expression, rather than in maintaining the reduction in synaptic transmission (Olde Loohuis et al., 2015).

A different *Gria1*-targeting miRNA has been suggested to play a role in NMDAR-dependent LTD. MiR-501-3p is important in regulating GluA1 levels through development; its expression is developmentally regulated with high levels at postnatal day (P)1, reducing to ~15% of the P1 level at P56 (Hu et al., 2015). This low basal level of miR-501-3p in mature neurons was shown to be dramatically upregulated by a 5 min application of NMDA to hippocampal neurons (chemical-LTD induction, cLTD); both mature and pri-miR-501-3p levels were significantly increased at 60 min, but not at 30 min after the stimulus. The timing is a critical issue, since NMDAR-dependent LTD expression is a rapid process, with reduced synaptic transmission seen just a few minutes after the induction protocol. In contrast to miR-137 (Olde Loohuis et al., 2015); discussed above), it therefore seems unlikely that miR-501-3p activity is involved in the early stages of LTD expression, but perhaps is important for maintaining reduced GluA1 expression levels in the later stages of LTD. Indeed, a role for miR-501-3p in hippocampal LTD measured electrophysiologically was not addressed in this study (Hu et al., 2015). However, it was shown that dendritic spine shrinkage in response to NMDAR stimulation was dependent on miR-501-3p activity at later time points after stimulation (Hu et al., 2015). While it is possible that miR-501-3p targets other mRNAs encoding proteins involved in dendritic spine morphogenesis, a role for AMPAR subunit expression in modulating spine size has been suggested in other reports (Kopec et al., 2007).

Direct miRNA-dependent regulation of AMPAR subunit expression in LTP has been suggested for miR-181a, whose expression was down-regulated by chemical-LTP (cLTP) induction in cultured neurons and also by the learning phase of an object location memory

task *in vivo* (Rodriguez-Ortiz et al., 2020). Other studies have demonstrated that miR-181a targets the 3'UTR of *Gria2* mRNA, causes a decrease in GluA2 protein levels in cultured hippocampal neurons and a corresponding decrease in mEPSC frequency, indicating a fundamental role in regulating synaptic function ((Saba et al., 2012); see below). Hence, a down-regulation of miR-181a expression in response to cLTP induction results in an increase in GluA2 protein synthesis and an increase in AMPAR expression. Interestingly, several plasticity genes appear to be regulated by miR-181a, including cFos and CamKII, all of which were upregulated by chemical LTP induction in a miR-181a dependent manner (Rodriguez-Ortiz et al., 2020). A mechanism for the reduction in miR-181a levels was shown to involve an increase in the expression of Translin, observed 1 h after cLTP induction, a protein that is involved in regulating miRNA degradation (Rodriguez-Ortiz et al., 2020). However, it is unclear precisely how LTP regulates Translin expression. The same study also investigated a role of miR-181a in a model for Alzheimer's disease (AD), which is discussed below.

Synaptic plasticity in specific brain regions mediate distinct forms of memory or behavioural modifications. A specific form of plasticity in the nucleus accumbens (NAc) is a central process in the formation of behaviours associated with drug addiction (Chen et al., 2010). In the search for miRNAs involved in this process, miR-181a was identified in a screen for miRNAs enriched at NAc synapses (Saba et al., 2012). Importantly, mice exposed to amphetamine for 5 days showed a significant increase in miR-181a levels in the ventral midbrain, the sublimbic forebrain (containing the NAc) and the hippocampus, which would cause a reduction in GluA2 protein synthesis. In cultured neurons, dopamine receptor stimulation also caused an increase in miR-181a expression, suggesting a likely mechanism. Since the expression of GluA2-lacking AMPARs has been shown to be involved in the plasticity associated with drugs of abuse (Wolf and Tseng, 2012), up-regulation of miR-181a and consequential repression of *Gria2* translation might be a contributing factor to the process of addiction.

- Regulation of AMPAR trafficking by miR-135, miR-9-3p, miR-146a-5p.

As well as targeting AMPAR subunit expression directly, miRNAs can regulate synaptic function and plasticity by modulating the translation of proteins involved in AMPAR trafficking or synaptic localisation.

Increases in synaptic strength associated with LTP require the insertion of additional AMPARs into the neuronal plasma membrane by SNARE complex-mediated exocytosis. Complexin-1 and -2 associate with SNARE complexes and are a necessary regulatory factor for AMPAR exocytosis during LTP expression (Ahmad et al., 2012). While much less studied compared to the role of exocytosis in LTP, it has been suggested that a reduction in AMPAR exocytosis is involved in LTD, to synergise with the internalisation of surface AMPARs needed to bring about a decrease in synaptic strength (Hu et al., 2014). MiR-135 has been shown to target complexin-1 and -2 in response to NMDAR stimulation (cLTD), and hence to reduce exocytosis of GluA1 in hippocampal neurons (Hu et al., 2014). MiR-135 levels were increased by NMDA application with a very similar time course as miR-501-3p, showing a significant increase in mature miR-135 and pri-miR-135 at 60 min, but not 30 min after a 5 min exposure to NMDA. Inhibition of miR-135 blocked the NMDAR-dependent exocytosis of recombinant GluA1 in hippocampal neurons in culture, and of LTD expression in hippocampal slices within a few minutes after low frequency stimulation. In a similar manner as miR-501-3p, discussed above (Hu et al., 2015), miR-135 not only regulates AMPAR trafficking, but also dendritic spine shrinkage in response to NMDAR stimulation (Hu et al., 2014). It will be important to investigate the mechanism underlying how miR-135 regulates the early stages of LTD expression, since the increase in its expression is not seen until 60 min after stimulation. These experiments also suggest that complexins are actively involved in constitutive AMPAR trafficking under basal conditions, whereas other reports have suggested that constitutive AMPAR trafficking is unaffected by complexin knockdown (Ahmad et al., 2012).

Another protein involved in the forward traffic of AMPARs to the synaptic plasma membrane in LTP is SAP97, a scaffold protein that binds GluA1 C-terminus via a PDZ-domain interaction (Leonard et al., 1998). *Dlg1* mRNA encodes SAP97 and is a target for miR-9-3p, which is highly expressed in the brain and is involved in various aspects of neuronal development. A specific role for miR-9-3p in LTP was discovered by demonstrating that miR-9-3p inhibitors block hippocampal LTP, with no effect on basal synaptic transmission or LTD (Sim et al., 2016). This study also investigated a role for miR-9-3p in hippocampal memory processes, and demonstrated that animals expressing the miR-9-3p inhibitor showed deficits in spatial memory (water maze) and object in place memory. Interestingly, contextual fear conditioning, which is also a form of hippocampal memory, was unaffected by miR-9-3p inhibition, indicating that these different forms of memory have distinct mechanisms with respect to miRNA-dependent control of translation. Seven target genes for miR-9-3p are involved in LTP, including *Dlg1*, *Cacng2* (stargazin) and *Dmd* (dystrophin), all of which were translationally repressed by miR-9-3p. Since this report indicated that miR-9-3p activity is required for LTP, these data suggest that down-regulation of protein synthesis from these genes is necessary for LTP. This is perhaps surprising, since SAP97 and stargazin have been suggested to play positive roles in AMPAR trafficking towards the synapse, and hence in LTP expression (Nakagawa et al., 2004; Opazo and Choquet, 2011).

Microtubule-associated protein MAP1B has been identified as an important component in the internalisation of GluA2-containing AMPARs in both NMDAR- and mGluR-dependent LTD (Benoist et al., 2013; Davidkova and Carroll, 2007). The precise role for MAP1b in regulating AMPAR endocytosis in spines is unclear, but involves activation of the small GTPase Rac by the Rac GEF Tiam1 (Benoist et al., 2013). *Map1b* is a target for miR-146a-5p, whose expression was shown to increase through development, and decrease as a result of group 1 mGluR signalling following application of the agonist DHPG in mature neurons (Chen and Shen, 2013). DHPG caused a corresponding increase in MAP1b protein expression, which was blocked by overexpression of miR-146a-5p. DHPG-induced AMPAR internalisation was

also blocked by miR-146a-5p overexpression. Taken together, these observations suggest a mechanism whereby miR-146a-5p associates with *Map1b* 3'UTR to repress basal levels of MAP1B translation. Group 1 mGluR signalling relieves miR-146a-5p-dependent inhibition of translation, increasing MAP1B expression, resulting in enhanced AMPAR internalization and LTD. Important unanswered questions include the mechanism for down-regulation of miR-146a-5p activity in response to DHPG. Levels of pri-miR-146a-5p are unaffected by DHPG, indicating that regulation is not at the level of transcription (Chen and Shen, 2013). It has been demonstrated that group 1 mGluR signalling causes an increase in phosphorylation of the RNA-binding protein FMRP, which in turn relieves translational repression of *PSD95* via miR-125a (Muddashetty et al., 2011). Perhaps a similar mechanism is at play with miR-146a-5p and *Map1b*. While there is no direct evidence for such a mechanism, it has been demonstrated that FMRP binds *Map1b* mRNA, and it has been suggested that FMRP phosphorylation regulates this interaction (Coffee et al., 2012).

MiRNA-dependent regulation of AMPAR subunit synthesis in neurological disorders

In addition to physiological roles of miRNAs, there has been much research focus in recent years to define the function of miRNA systems in neurological disorders, largely in the hope that they will emerge as novel therapeutic targets. Some miRNAs that are involved in physiological forms of plasticity as discussed above, have also been shown to be involved in pathological forms of plasticity. This might be expected since many neurological disorders (AD, ASD, ALS, etc) are classified as synaptopathies, i.e., they involve synaptic dysfunction, and neurological disorders that involve cognitive deficits can often be attributed to dysfunctional synaptic plasticity. On the other hand, some miRNAs that have been found to play an important role in synaptopathies have yet to be assigned a role in physiological forms of plasticity.

- MiR-181a, mir-30b and miR-124 in Alzheimer's disease

As discussed above, a downregulation of miR-181a is involved in the expression of LTP by releasing translational repression of *Gria2* (Rodriguez-Ortiz et al., 2020). MiR-181a has also been implicated in the cognitive impairments associated with AD, since increased levels have been observed in AD patients compared to controls (Ansari et al., 2019). It is therefore a good example of a miRNA involved in physiological plasticity and in the deficits of plasticity associated with disease. Exogenous application of Aβ oligomers to cultured neurons is widely used to model the effects of amyloidopathy on neuronal cell biology. While Aβ application had no effect on basal levels of miR-181a, it blocked the cLTP-dependent decrease in miR-181a levels in hippocampal neurons (Rodriguez-Ortiz et al., 2020). Furthermore, Aβ oligomers inhibited the cLTP-dependent increase in GluA2, CamKII, cFos and other miR-181a targets, an effect that was blocked by miR-181a inhibitors. MiR-181a is upregulated in the 3xTg mouse model for AD, which carries familial AD mutations in Amyloid Precursor Protein (APP), tau and presenilin. 3xTg animals express a lower level of GluA2 compared to WT controls, and miR-181a inhibitors increased GluA2 expression towards WT levels (Rodriguez-Ortiz et al., 2020). Importantly, miR-181a inhibitors rescued deficits in object recognition memory in 3xTg mice, defining a role for miR-181a in the memory deficits observed in these animals. Hence, this study suggested a role for miR-181a in physiological synaptic plasticity and in AD pathology (Rodriguez-Ortiz et al., 2020). Taken together, these reports indicate that miR-181a inhibits *Gria2* expression under basal conditions, which is released by LTP induction, resulting in an increase in AMPAR subunit expression to enhance synaptic strength. In AD, increased levels of miR-181a cause a reduction in basal GluA2 and inhibit the increase in GluA2 protein synthesis needed for LTP and consequent memory formation.

Another miRNA that targets *Gria2* and is upregulated in AD is miR-30b (Song et al., 2019). MiR-30b was found at increased levels in AD patients and also in the 5xFAD mouse model, which carries three mutations in APP and two in presenilin1, resulting in increased levels of

Aβ. 5xFAD mice show reduced expression of GluA2 protein as well as other proteins involved in synaptic structure and function (Song et al., 2019). Expression of a miR-30b inhibitor in the hippocampi of 5xFAD mice increased AMPAR subunit expression (in this case, data for GluA1 was shown, rather than for the miR-30b target GluA2), and rescued deficits in hippocampal LTP, in spatial memory analysed by performance in the Morris water maze, and in object recognition memory (Song et al., 2019). To strengthen the importance of miR-30b in synaptic dysfunction and cognitive deficits, it was shown that miR-30b overexpression in WT mice resulted in disruption of LTP and behavioural phenotypes that were quantitatively indistinguishable from those of 5xFAD mice. This study also suggests a mechanism for pathological increases in miR-30b that required the transcription factor NF-κB, which is strongly up-regulated in AD patients and 5xFAD mice compared to controls. NF-κB expression was increased in cultured neurons as a result of addition of pro-inflammatory cytokines or of Aβ, both of which are central to AD pathology (Song et al., 2019).

Although not well characterised, some reports suggest that AD involves disruption to homeostatic synaptic plasticity (Jang and Chung, 2016), which provides another example of a physiological form of plasticity regulated by miRNA-dependent regulation of AMPAR subunit translation that is dysregulated by Aβ. As described above, miR-124 represses *Gria2* translation as a critical component of synaptic up-scaling after TTX treatment (Hou et al., 2015). Following a similar mechanism as the response to TTX-induced activity blockade, Aβ application to cultured neurons caused a small increase in miR-124 expression via EV11-HDAC interactions, and an increase in synaptic up-scaling via CP-AMPAR expression caused by repression of *Gria2* translation (Gilbert et al., 2016).

- MiR-124 in frontotemporal dementia and multiple sclerosis

As discussed above, miR-124 is one of the most abundant miRNAs in the brain , and therefore dysregulation of its activity has profound effects on brain function and is a feature of a number

of neurological disorders (Lagos-Quintana et al., 2002). For example, miR-124 is downregulated in behavioural variant frontotemporal dementia (bvFTD), resulting in imbalanced levels of CP- vs CI-AMPARs, which was proposed to play a role in the behavioural phenotype (Gascon et al., 2014). The cognitive deficits associated with multiple sclerosis (MS) have also been suggested to involve miR-124, with demyelination playing a role in disrupting miR-124 expression levels (Dutta et al., 2013). Hippocampi from MS patients showing an unmyelinated phenotype expressed higher levels of miR-124 compared to myelinated hippocampi from MS patients. Experimental demyelination in mice caused an increase in miR-124, consequent reduction in AMPAR expression and deficits in performance in the water maze test for hippocampal-dependent spatial memory (Dutta et al., 2013).

- MiR-223 in ischaemia, MS, Schizophrenia

MiR-223 is also implicated in several pathologies via regulation of *Gria2* translation. However, in addition to *Gria2*, miR-223 has numerous targets involved in synaptic function in the brain, including *Grin2B*, which encodes the NMDAR subunit NR2B (Harraz et al., 2012). Hence, effects on synaptic function may be caused by changes in GluA2 or NR2B expression. Overexpression or knockout of miR-223 in the rodent brain was shown to reduce or increase GluA2 and NR2B expression respectively, with corresponding effects on basal synaptic transmission (Harraz et al., 2012). Brain ischaemia causes neuronal death via the overstimulation of excitatory synapses, a process known as excitotoxicity. Excitotoxicity has been shown previously to involve both NMDAR overexcitation as well as a shift towards GluA2-lacking CP-AMPARs (Liu and Zukin, 2007). While ischaemia was not reported to cause a change in miR-223 expression, overexpression of miR-223 resulted in neuroprotection following ischaemia (Harraz et al., 2012). Since downregulation of GluA2 might be expected to promote ischaemia-induced neuronal death by increasing levels of GluA2-lacking CP-AMPARs (Liu and Zukin, 2007), it is possible that the neuroprotective effects are caused by changes in NMDAR function following downregulation of NR2B, rather than via GluA2.

An upregulation of miR-223 has been observed in post mortem MS lesions compared to controls, and also in an experimental autoimmune encephalomyelitis (EAE) mouse model, which is often used as an animal model for MS (Morquette et al., 2019). Overexpression of miR-223 reduced neurodegeneration in response to MS-relevant pathological stimuli, which is consistent with observations indicating that glutamate receptor signalling is involved in this form of neurodegeneration. The authors of this study suggest that the upregulation of miR-223 in MS and the EAE model represents a protective response to MS pathology by reducing glutamate receptor expression (Morquette et al., 2019).

A mechanism that has recently received considerable interest is the secretion of miRNAs via exosomes, which are then taken up by recipient cells following membrane fusion events, hence modulating mRNA translation in the recipient cell. MiR-223 is known to be enriched in exosomes, and it has been demonstrated by post mortem analysis that miR-223 is upregulated in the brains of patients with Schizophrenia, also in patients with bipolar disorder at the time of death (Amoah et al., 2020). Increased levels of miR-223 correlated with reduced levels of *GRIA2* and *GRIN2B* mRNA in the same samples. Since reduced synaptic activity is suggested to be a key component of Schizophrenia pathophysiology (Coyle et al., 2020), the authors of this study suggest that the upregulation of miR-223 could contribute to chronic glutamate receptor hypofunction (Amoah et al., 2020). Interestingly, previous work demonstrated that anti-psychotic drugs reduce miR-223 expression in the mouse brain (Santarelli et al., 2013). In rodent cell culture, miR-223 expression was higher in astrocytes compared to neurons, and addition of astrocyte-derived exosomes to neurons in separate cultures caused an increase in neuronal miR-223 and downregulation of GluA2 and NR2B protein expression (Amoah et al., 2020). A critical question remaining from this work is whether exosomal transfer of miR-223 is involved in SCZ or BD pathology in humans.

Unanswered questions and concluding remarks

While miRNA-dependent regulation of AMPAR expression is emerging as a critical determinant of various forms of plasticity and neurological disease, it must be considered together with the other important mechanisms involved, including AMPAR trafficking and in the case of homeostatic plasticity, transcriptional regulation of AMPAR subunit genes (Korb et al., 2013). Hence, miRNAs represent an important part of a multi-factorial system for regulating AMPAR synaptic expression, in both physiological and pathological forms of plasticity.

An important question is how miRNA activity is increased in response to neuronal activity, in particular NMDAR or mGluR stimulation. The signalling pathways that transduce synaptic activity into transcriptional regulation of miRNAs that regulate AMPAR subunit or associated protein expression are unclear. Some transcriptional regulators, such as EVI1 in the control of *miR-124* expression, have been identified (Hou et al., 2015), however, the mechanisms downstream of NMDAR or mGluR stimulation that regulate most of the other miRNAs discussed in this review are unknown.

Since the changes in synaptic transmission associated with NMDAR-dependent LTD and LTP occur within a few minutes after stimulation, they are unlikely to be regulated by miRNA-dependent mechanisms that require an NMDAR-dependent increase in miRNA transcription, which has been suggested to take approximately one hour after stimulation. Nevertheless, miRNA-dependent translational repression by pre-existing miRNAs is relatively rapid, hence alternative NMDAR-dependent events, such as regulation of Dicer or RISC function may be involved in such earlier stages of plasticity (Rajgor et al., 2018; Sambandan et al., 2017). The greater number of reports of miRNA-dependent control of AMPAR subunit synthesis for homeostatic synaptic scaling is to be expected, since this form of plasticity takes effect over a longer time scale.

Since miRNAs typically target numerous mRNAs, it will be important to establish whether a specific subset of targets for a specific miRNA is translationally regulated, and if so, how this is achieved. As discussed in this review, some miRNAs such as miR-134, miR-181 and miR-

223 target several genes involved in synaptic plasticity (Fiore et al., 2014; Harraz et al., 2012; Rodriguez-Ortiz et al., 2020), suggesting an elegantly orchestrated system for simultaneously modulating the synthesis of several relevant proteins. It is possible that additional regulation occurs at the level of the RISC, where phosphorylation of Argonaute and modulation of protein-protein interactions might play a role in defining which genes are preferentially repressed (Rajgor et al., 2018).

It is now well established that AMPARs form stoichiometric assemblies with so-called auxiliary subunits, including TARPs, Cornichons, Shisa and GSG1L (Greger et al., 2017; Jacobi and von Engelhardt, 2021). At the time of writing, there appear to be no published reports of miRNAs that target these essential components of AMPAR complexes to affect receptor function, although stargazin has been identified as a potential miR-9-3p target (Sim et al., 2016). Given the central role auxiliary subunits play in AMPAR gating, trafficking and desensitisation, regulation of their expression by miRNAs holds potential for an important additional level of receptor modulation.

While miRNAs can be overexpressed, knocked out or inhibited *in vivo* to investigate their role in a brain system or behavioural phenotype (Rodriguez-Ortiz et al., 2020; Sim et al., 2016; Song et al., 2019; Harraz et al., 2012; Morquette et al., 2019), there are currently technical limitations to studying the effects of miRNAs on the translation of specific genes in response to specific types of neuronal activity *in vivo*. For this level of molecular detail, the majority of papers reviewed here use reporter constructs acutely expressed in neuronal cultures. While cultured neurons are a powerful model system for elucidating molecular cell biological processes, they do not appropriately represent *in vivo* neuronal circuitry. Hence, the field awaits technical advances to allow the recording of specific miRNA-dependent gene silencing events *in vivo*, which will shed further light on the role of miRNAs in brain function.

In this review, I have focussed on miRNAs that regulate AMPAR subunit synthesis or trafficking. Numerous additional miRNAs are involved in regulating other important components of plasticity or of the pathophysiology of neurological disease, which altogether

make up a complex network of gene regulatory mechanisms, and also provide a rich potential of novel therapeutic targets.

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