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Rapid reversal of translational silencing: Emerging role of microRNA degradation pathways in neuronal plasticity

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Abstract

As microRNAs silence translation, rapid reversal of this process has emerged as an attractive mechanism for driving *de novo* protein synthesis mediating neuronal plasticity. Herein, we summarize recent studies identifying neuronal stimuli that trigger rapid decreases in microRNA levels and reverse translational silencing of plasticity transcripts. Although these findings indicate that neuronal stimulation elicits rapid degradation of selected microRNAs, we are only beginning to decipher the molecular pathways involved. Accordingly, we present an overview of several molecular pathways implicated in mediating microRNA degradation: Lin-28, translin/trax, and MCPIP1. As these degradation pathways target distinct subsets of microRNAs, they enable neurons to reverse silencing rapidly, yet selectively.

Introduction

Compelling evidence showing that long-term memory and enduring forms of synaptic plasticity require *de novo* protein synthesis has focused attention on the signaling pathways linking neuronal stimulation to protein translation (Mayford et al., 2012). Furthermore, evidence that defects in these pathways contribute to the pathophysiology of cognitive disorders has stimulated interest in deciphering how they operate (Kindler and Krienkamp, 2012; Richter et al., 2015). As the microRNA system plays a prominent role in silencing protein translation, the possibility that neuronal stimulation triggers translation by reversing silencing has emerged as an attractive mechanism. In this review, we summarize recent studies demonstrating that neuronal stimulation can trigger rapid decreases in microRNA levels that lead to rapid reversal of translational silencing. Furthermore, as these studies indicate that rapid degradation of microRNAs plays a key role in reversing silencing, we present an overview of several molecular pathways implicated in this process.

Viewing microRNAs as second messengers

Unlike other second messenger systems, such as the cyclic AMP and phosphoinositide systems, which were originally identified as mediators of extracellular signals on cellular

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function, the discovery of the microRNA system emerged from studies of *C. elegans* development (Ruvkun, 2008) and RNA-mediated silencing (Fire et al., 1998; Bartel, 2004). This pivotal advance revealed that cells have a sophisticated system for delaying translation of transcripts until needed.

To understand the operation of this complex and novel system, investigators initially focused on deciphering how microRNAs are made and how they silence translation. Accordingly, we now have a reasonably clear idea of microRNA biosynthesis (Figure 1) and have identified key components of the silencing process (Bartel, 2009; Eacker et al., 2013). However, we are only beginning to discern how this system responds to extracellular signals, i.e. functions as a second messenger system that enables neuronal stimulation to trigger translation by reversing silencing. This area is particularly timely in light of recent studies, summarized below, indicating that reversal of silencing plays a key role in driving translation of plasticity transcripts.

Neuronal stimulation triggers rapid reduction in microRNA levels

A signature feature of second messenger systems is their ability to respond quickly to extracellular signals. As microRNAs were initially thought to have rather long half-lives, on the order of hours to days, they did not appear to fit into a second messenger scheme. However, this perspective changed as a result of seminal studies demonstrating that neuronal stimulation can trigger reversal of translational silencing and cause a rapid reduction in microRNA levels *in vitro* (Table 1).

Schratt et al. (2006) examined the impact of a brain-enriched microRNA, miR-134, on dendritic morphology. They found that overexpression of this microRNA, which is localized to dendrites, decreases the size of dendritic spines. Furthermore, they showed that this effect is mediated by silencing expression of *Limk1*, a kinase involved in regulating spine development. In addition, these investigators demonstrated that brain derived neurotrophic factor (BDNF) can drive translation of *Limk1* by overriding its silencing by miR-134. Interestingly, these seminal studies did not address whether BDNF might act by decreasing levels of miR-134. However, these findings yielded a major breakthrough by demonstrating that extracellular signals, such as BDNF, can inactivate microRNA-mediated silencing.

Further evidence that neuronal stimulation can regulate silencing emerged from studies evaluating the role of the microRNA system in synaptic plasticity in *Aplysia*. To this end, Rajasethupathy et al. (2009) focused on long-term facilitation elicited by repeated application of serotonin, as it is dependent on *de novo* protein synthesis. Using this paradigm, they noted that serotonin application triggers a rapid and long-lasting decrease in levels of miR-124. As they identified CREB1 as a target of miR-124, they went on to show that reversal of silencing of this transcription factor mediates long-term facilitation induced by serotonin application. *A priori*, serotonin could trigger rapid reduction of miR-124 levels by increasing degradation or blocking production of this microRNA. In this paradigm, serotonin triggers degradation of this microRNA via activation of MAP kinase, rather than by abrupt cessation of its transcription.

Subsequent studies by Krol et al. (2010) provided critical support for the idea that neuronal stimulation can trigger rapid degradation of microRNAs. Using primary neuronal cultures, these investigators identified multiple microRNAs that are degraded rapidly in neurons and found that treatment with tetrodotoxin (TTX) stabilized their levels. Thus, these findings show that endogenous neuronal activity is sufficient to drive rapid turnover of these microRNAs. Further characterization of this response demonstrated that, in the presence of TTX, NMDA receptor activation triggers rapid degradation of several microRNAs (Table 1). Accordingly, these findings stimulated interest in the proposal that reversal of silencing triggers translation that is critical for long-lasting forms of plasticity **and in identifying the targets of these microRNAs**.

As metabotropic glutamate receptors (mGluRs) have been implicated in driving dendritic translation that mediates protein synthesis-dependent long-term depression (LTD) (Huber et al., 2000), investigators have asked whether activation of these receptors might also be able to reverse silencing. Two studies have confirmed this hypothesis. In one, Muddashetty et al. (2011) demonstrated that mGluR activation stimulates PSD-95 translation by decreasing levels of miR-125a, a microRNA localized in dendrites. In the other, Chen and Shen (2013) examined how mGluR activation drives translation of MAP1B, a protein that has been implicated in mediating LTD, since it stabilizes internalized AMPA receptors. They found that mGluR stimulation decreases levels of miR-146a, blocking its ability to silence MAP1B.

The presence of multiple microRNA targeting sites in the 3'UTR segment of a transcript renders it subject to silencing by any of their cognate microRNAs. MAP1B provides an excellent illustration of this principle (Figure 2). In addition to its regulation by mGluR via its effects on miR-146a, described above, silencing of this transcript is also triggered by BDNF via its effects on a different microRNA. In the course of studying how BDNF stimulates axonal growth, Dajas-Bailador et al. (2012) found that brief stimulation with BDNF triggers increased expression of MAP1B in axons by decreasing levels of miR-9, which targets MAP1B. Of note, the effect of BDNF on miR-9 levels is restricted to axons demonstrating that microRNA degradation can display spatial selectivity. Thus, distinct microRNA target sites in the 3'UTR of MAP1B confer sensitivity to both mGluR and BDNF stimulation. Accordingly, it will also be interesting to understand how these stimuli trigger selective degradation of microRNAs.

While these studies provided clear evidence that neuronal stimulation can trigger translation by reversing silencing, it remained unclear whether this mechanism is employed in isolated instances or plays a major role in mediating translational responses to neuronal stimulation. A more comprehensive investigation of the effects of BDNF on the microRNA system in primary hippocampal cultures revealed that reversal of silencing plays a prominent role in driving translation of plasticity transcripts (Huang et al., 2012). Furthermore, these findings also help explain how BDNF uses the microRNA system to prioritize translation of a privileged set of plasticity transcripts and suppress constitutive expression of housekeeping transcripts (Figure 3). In brief, by stimulating expression of Dicer, BDNF enhances production of mature microRNAs globally which leads to widespread silencing of transcripts. However, at the same time, it activates the Lin-28 pathway to degrade the let-7

family of microRNAs, thereby allowing selective, coordinated expression of a set of plasticity transcripts that contain let-7 sites in their 3'UTRs. Thus, analysis of the impact of BDNF on the microRNA system has yielded valuable insights into how neuronal stimulation can differentially regulate microRNA generation and degradation to induce selective translation of a cadre of plasticity transcripts.

Is reversal of silencing involved in plasticity *in vivo*?

Taken together, the studies summarized above have yielded compelling evidence that neuronal stimulation can induce translation of plasticity transcripts by reversing silencing and have sparked interest in assessing the role of this mechanism in mediating translational responses underlying plasticity *in vivo*. However, as those studies focused on developing neurons grown in culture, one must be cautious in extrapolating those insights to synaptic plasticity *in vivo*. Furthermore, given the increased complexity encountered in studying this process *in vivo*, it is not terribly surprising that initial attempts to assess whether plasticity stimuli trigger reversal of silencing *in vivo* have met with limited success.

Studies examining the impact of long-term potentiation (LTP) stimulation on the microRNA system *in vivo* have focused on the perforant path input to the dentate gyrus. Initial microarray profiling studies identified ten microRNAs that were elevated in the dentate gyrus following tetanic stimulation, and a comparable number that were decreased (Wibbrand et al., 2010). Follow up studies focused on miR-132 and miR-212, which displayed increases, and miR-219, which was decreased. Of note, earlier *in vitro* studies had demonstrated that miR-132 is upregulated by activity in neurons (Vo et al., 2005). As it well established that LTP induction in this pathway is NMDA receptor dependent, these investigators checked if these changes in microRNA levels were blocked by systemic injection of CPP, a competitive NMDA receptor antagonist. Surprisingly, administration of high-frequency stimulation in the presence of CPP had the opposite effect, increasing levels of all three microRNAs relative to the levels found after high frequency stimulation (HFS) alone. Thus, these unexpected findings indicated that HFS regulates microRNAs by both NMDA and non-NMDA receptor mechanisms, with NMDA receptor activation driving suppression of these microRNAs. Subsequent studies demonstrated that blockade of mGluR receptors during HFS abolishes the observed increases in miR-132 and miR-212. Thus, these studies uncovered opposing actions of NMDA and mGluR receptor activation on microRNA regulation in this paradigm.

As Arc protein is robustly induced in dentate granule cells by HFS stimulation of the perforant path, Wibbrand et al. (2012) examined the possibility that reversal of silencing might mediate expression of Arc in this plasticity paradigm. Although they confirmed that translation of Arc transcripts in cultured cells is subject to silencing by a variety of microRNAs, they did not detect alterations in the levels of any of these microRNAs *in vivo* following LTP stimulation. Thus, this negative result indicates that reversal of silencing is not involved in regulating Arc translation following HFS of this input. Perhaps, silencing is reserved for situations in which there is a substantial delay in translation following transcription and trafficking of transcripts. In contrast, in this paradigm, HFS produces rapid

and robust induction of Arc mRNA followed immediately by translation and may, therefore, not be needed.

More recent studies have also performed microRNA profiling in dentate gyrus following tetanic stimulation of the perforant path (Joilin et al., 2014). In contrast to previous studies that employed anesthetized animals, these investigators used un-anesthetized, freely moving rats. They identified two microRNAs, miR-34a and miR-132-3p, that are down regulated at 20 min following LTP stimulation. Of note, the corresponding primary microRNAs were elevated or unchanged suggesting that the decrease observed in these mature microRNAs is mediated by increased degradation, or decreased pre-microRNA processing, rather than by decreased transcription. Although these microRNAs are predicted to target plasticity transcripts, further studies are needed to assess their role in regulating translation following LTP.

An alternative approach to assessing the role of silencing reversal in plasticity and learning has been to examine its role in long-term memory using a robust auditory fear conditioning paradigm (Griggs et al., 2013). In this paradigm, pairing a shock and tone elicits enhanced freezing when tested at 90 minutes or 24 hours post-training. As this behavioral response is dependent on the lateral amygdala, Griggs et al., (2013) examined the impact of training on microRNA levels in this region. They identified 32 microRNAs that were decreased following paired training. Of these, they chose to focus on miR-182 as it showed more than a 50% reduction compared to naïve controls and is predicted to target several proteins that regulate the actin cytoskeleton which has been implicated in mediating fear conditioning in the amygdala. They confirmed that overexpression of miR-182 suppresses expression of several actin-regulating proteins and also blocks expression of fear memory as monitored by freezing in response to tone presentation at 24 hours, but not at 90 minutes post-training. Accordingly, these findings suggest that training-induced decreases in miR-182 lead to enhanced translation of actin-regulating proteins critical for synaptic changes mediating long-term memory.

Taken together, studies examining the microRNA system in plasticity and learning *in vivo* illustrate the difficult challenges encountered (Ryan et al., 2015). Therefore, advances in understanding the signaling pathways mediating reversal of silencing are likely to be helpful in deciphering the role of the microRNA system in mediating synaptic plasticity and learning. For example, the preponderance of evidence indicates that reversal of silencing triggered by neuronal stimulation is associated with degradation of microRNAs. Thus, recent advances in delineating the signaling pathways mediating degradation of microRNAs, summarized below, may make it easier to monitor the activation of these pathways by plasticity stimuli *in vivo*.

Pathways mediating degradation of microRNAs

A priori, it seems reasonable to postulate that multiple microRNA degradation pathways might be needed by the cell in order to regulate the activity of this system. As Dicer mediates production of nearly all microRNAs, regulation of Dicer activity would serve to increase or decrease production of mature microRNAs globally. In contrast, recent studies

have identified multiple microRNA degradation pathways that display differing substrate specificities. Thus, selective activation or inactivation of one of these would provide a mechanism for regulating silencing mediated by a subpopulation of microRNAs. To date, three pathways mediating degradation of pre-microRNAs have been identified: Lin-28, translin/trax and MCPIP1.

Lin-28

Lin-28 is a conserved RNA-binding protein present in higher eukaryotes that plays important roles in development, tumorigenesis, glucose metabolism, differentiation and pluripotency (Huang, 2012; Mayr et al., 2013). It was originally identified as a heterochronic gene that controls *C. elegans* developmental timing (Ambros et al., 1984). In mammalian organisms, two paralogs Lin-28a and Lin-28b encode basic 23- or 28- KDa proteins and both contain two RNA-binding motifs, cold-shock domain and CCHC zinc finger (Moss et al., 2003; Darr et al., 2009; Mayr et al., 2013). The observation that Lin-28 and let-7 microRNAs show complementary expression patterns during larval development of *C. elegans* provided a critical clue to identifying Lin-28's role in degrading let-7 microRNAs (Moss et al., 1997; Moss et al., 2003; Pasquinelli et al., 2000). At an early developmental stage, Lin-28 is readily detected, but mature let-7 microRNA is not, despite clear expression of its precursor transcript, pri-let-7, suggesting that Lin-28 may block formation of mature let-7 (Van Wynsberghe et al., 2011). As development proceeds, Lin-28 is down regulated and the expression level of mature let-7 increases quickly, consistent with this view of Lin-28 action. The complementary expression pattern of Lin-28 and let-7 microRNAs is also reported in mammals (Rybak et al., 2008; Viswanathan et al., 2008). Lin-28 is mainly expressed in undifferentiated cells, while mature let-7 is absent until differentiation (Rybak et al., 2008; Viswanathan et al., 2008). Taken together, these studies indicated that Lin-28 serves as a negative regulator of the maturation of let-7 microRNAs.

A more detailed understanding of how Lin-28 acts to suppress expression of let-7 microRNAs emerged from studies by Heo et al. (2008) demonstrating that Lin-28b binds directly to pre-let-7. Furthermore, knockdown and overexpression of Lin-28 only affected pre-let-7a expression levels but not that of the primary transcript, pri-let-7a. In addition, overexpression of Lin-28 induced an unexpected fuzzy band that migrates above pre-let-7a. This band was identified as a modified form of pre-let-7 containing a 3' tail of approximately 14 U residues. As uridylated pre-let-7 is rapidly degraded and resistant to Dicer cleavage, these findings indicate that Lin-28 overexpression promotes uridylation of pre-let-7 and, thereby, blocks its maturation. However, the inability of Lin-28 to catalyze uridylation of pre-let-7 prompted a search for the enzyme responsible for this modification.

Two groups independently identified TUT4/Zcchc11 functions as the uridylyl transferase involved (Hagan et al., 2009; Heo et al., 2009). Hagan and colleagues (2009) demonstrated that in the presence of Lin28, TUT4/Zcchc11 catalyzes addition of a poly U tail to pre-let-7. Conversely, depletion of TUT4/Zcchc11 selectively increases the expression levels of mature let-7 microRNAs and represses expression of its target genes (Hagan et al., 2009). Heo et al. (2009) also confirmed that uridylation of pre-let-7 by TUT4/Zcchc11 is Lin-28 dependent, and only TUT4/Zcchc11 of the known human TUTases can uridylate pre-let-7 (Heo et al.,

2009). Furthermore, their microarray results showed that in cells depleted of both TUT4/Zcchc11 and Lin-28, only let-7 family members are upregulated, while most of the other microRNAs are unchanged, indicating that Lin-28 and TUT4/Zcchc11 selectively target pre-let-7.

The basis for this remarkable selectivity emerged from studies aimed at analyzing the interaction of Lin-28 with pre-let-7 family members. These studies revealed that the CCHC zinc finger motif in Lin-28 binds to a GGAG motif present in the loop of pre-let-7 family members. Mutations in this motif confirmed that it is essential for the interaction of Lin28 with pre-let-7 and the subsequent recruitment of TUT4/Zcchc11 to this complex (Heo et al., 2009). After uridylation, pre-let-7 is rapidly degraded by the Perlman syndrome exonuclease Dis312 in mouse embryonic stem cells (Change et al., 2013). Change and colleagues used biochemical reconstitution assays to show that 3' oligouridylation stimulate the RNase activity of Dis312 *in vitro* and depletion of Dis312 in mouse ESCs leads to an accumulation of uridylated pre-let-7, confirming that Dis312 is responsible for the degradation of uridylated pre-let-7 (Change et al., 2013). Taken together, these studies delineate the sequence of events that mediate Lin-28 directed degradation of let-7 pre-microRNAs: Lin28 binds to the GGAG motif present in the terminal loop of pre-let-7 microRNAs (Figure 4). Then, the Lin28/pre-microRNA complex recruits TUT4/Zcchc11 which proceeds to uridylate the 3' terminal of these pre-microRNAs. The uridylated pre-microRNAs are then degraded by Dis312.

Translin/trax

The translin/trax complex has recently been identified as an RNase that targets pre-miRNAs (Asada et al., 2014). Initially, translin was isolated based on its ability to bind to single-stranded DNA or RNA (Aoki et al., 1995). Subsequent studies demonstrated that translin can form a heteromeric complex with a closely related protein, trax, and that this heteromeric complex possesses RNase activity (Aoki et al., 1997; Liu et al., 2009). In the course of identifying key components of the *Drosophila* RNAi system, Liu et al. (2009) found that C3PO, the *Drosophila* orthologue of the translin/trax complex, enhances silencing activity by hastening loading of the guide strand onto the RNA-induced silencing complex (RISC). It does so by degrading the complementary or passenger strand of the siRNA after it has been nicked by Ago. Structural studies indicate that the translin/trax complex is an octamer composed of two translin homodimers and two translin/trax heterodimers. Furthermore, the RNase activity of the complex is dependent on trax, as a homomeric octamer composed entirely of translin is devoid of RNase activity. In addition, point mutations of key catalytic residues in trax abolish the RNase activity of the translin/trax complex (Ye et al., 2011; Tian et al., 2011).

Unexpectedly, the translin/trax complex was also identified in a biochemical screen looking for enzymes that degrade pre-microRNA (Asada et al., 2014). Since translin/trax is not able to cleave double-stranded RNA, it was assumed that the complex cleaves the loop present in pre-microRNA. However, analysis of the initial cleavage fragments generated by brief incubations of pre-microRNA with translin/trax indicated, unexpectedly, that this complex targets mismatches located in the stem (Figure 4). Eliminating these mismatches blocks

cleavage by translin/trax confirming that it targets bulges located in the stem. Thus, these findings suggest that translin/trax may have opposite effects on silencing mediated by microRNAs and siRNAs. The ability of translin/trax to degrade pre-microRNAs *in vitro* predicts that it acts to decrease microRNA-mediated silencing, in contrast to its ability to enhance siRNA-mediated silencing in *Drosophila*.

To test this prediction, Asada et al. (2014) assessed whether knockdown of translin or trax enhanced the ability of overexpressed pre-microRNAs to silence a microRNA sensor. Surprisingly, this manipulation had little impact on silencing suggesting that Dicer's access to these pre-microRNA substrates is not regulated by the translin/trax complex under control conditions. However, treatments that impair Dicer activity might enable translin/trax to compete more effectively with Dicer for pre-microRNA substrates. Testing the impact of knocking down translin or trax in cells expressing a hypomorphic variant of Dicer, that decreases Dicer activity by about 50%, confirmed this prediction. As expected, impaired Dicer activity decreased microRNA levels and silencing activity. However, knockdown of translin or trax reversed both these parameters, demonstrating that depletion of microRNAs observed with decreased Dicer activity reflects both decreased generation of microRNAs as well as their increased degradation by translin/trax. Of note, in the context of reduced Dicer activity, translin/trax knockdown elevates about half of microRNAs monitored in a high-throughput screen, suggesting it may only be able to cleave a subset of pre-microRNAs.

As recent studies indicate that Dicer expression in neurons is regulated by MAPK activity (Huang et al., 2012), as well as the GSK-3/beta-catenin pathway (Dias et al., 2015), it is conceivable that inhibition of translin/trax may help restore normal silencing function in the face of reduced Dicer activity found in pathological conditions. In fact decreased Dicer activity has been implicated in mediating neuro-degeneration and deleterious effects of stress (Gascon and Gao, 2012; Dias et al., 2015). As the expression of translin and trax is enriched in brain where it is predominantly expressed in neurons (Finkenshtadt et al., 2000), further studies are warranted to assess the role of this complex in regulating microRNA mediated silencing in neurons and whether inhibition of this complex may have therapeutic potential.

MCPIP1

MCPIP1 (monocyte chemoattractant protein [MCP]-1 induced protein 1, also known as Zc3h12a [Zinc finger CCCH-type containing 12A]) was identified as another RNase that targets pre-microRNAs in a screen looking for regulators of the microRNA pathway (Suzuki et al., 2011). Overexpression of MCPIP1 blocks the ability of pri-microRNAs to silence a luciferase reporter containing complementary target sites in its 3'UTR. Initial characterization of this response demonstrated that MCPIP1 did not affect the levels of ectopically expressed pri-microRNAs, but did suppress levels of the corresponding pre-microRNAs and mature microRNAs. Of note, other MCPIP family members or other proteins containing CCCH-type zinc finger domains do not share MCPIP1's ability to robustly suppress microRNA-mediated silencing, indicating that MCPIP1 has diverged from other family members to acquire this activity. Analysis of its mode of action revealed that MCPIP1 targets the terminal loops of pre-microRNAs (Figure 4). Consistent with that

finding, co-expression with Lin-28b, which binds to the terminal loop of let-7 microRNAs, blocks the ability of MCPIP1 to cleave a let-7 family member, pre-let-7g (Suzuki et al., 2011).

MCPIP1 first attracted attention because its transcript is robustly induced in immune cells by pro-inflammatory molecules, such as TNF α , MCP-1, IL-1 β and lipopolysaccharide (LPS) (Zhou et al., 2006; Bidzhekov et al., 2006). Subsequent experiments revealed that it served as a negative regulator of macrophage-mediated inflammatory responses (Liang et al., 2008). Furthermore, Matsushita et al. (2009) found that mice lacking this gene display excessive inflammatory responses and develop autoimmune phenotypes. Tracking down the molecular mechanism underlying the exaggerated response to LPS revealed that levels of IL-6 mRNA, which is normally induced by LPS, reach much higher levels in the absence of MCPIP1. This observation led to the discovery that MCPIP1 degrades IL-6 transcripts by targeting the loop formed by a hairpin element located in its 3'UTR. Thus, MCPIP1 is able to cleave hairpin loops present in either 3'UTR of selected transcripts, such as IL-6, or located in pre-microRNAs.

Activation of degradation pathways

Although these findings indicate that degradation of pre-microRNAs plays a prominent role in regulating silencing, relatively little is known about how neuronal stimulation activates this process. However, the identification of three mechanisms mediating degradation of pre-microRNAs sets the stage for deciphering the signaling pathways involved. Initial studies have identified clues to how each of these three pathways may be regulated. Lin-28 expression is robustly regulated by BDNF via a post-transcriptional mechanism (Huang et al., 2012). As MCPIP1 mRNA is robustly induced by a variety of extracellular signals in immune cells, it will be interesting to check if it is also highly responsive to changes in neuronal activity. Little is known about the regulation of translin/trax activity. However, it is able to translocate between the nucleus and cytoplasm and displays increased translocation into hippocampal dendrites in response to increased neuronal activity (Wu et al., 2011). Accordingly, studies aimed at elucidating how these degradation pathways are regulated in neurons are needed to decipher their role in reversal of silencing underlying neuronal plasticity (Figure 5).

As these degradation pathways target pre-microRNAs, an interesting question is whether pre-microRNAs are the sole target of degradation activated by neuronal stimulation or if mature microRNAs might be targeted as well. As direct monitoring of pre-microRNA levels is more technically demanding than measuring mature microRNA levels, most studies have not distinguished between these possibilities. In theory, degradation of mature microRNAs rather than pre-microRNAs might provide a more direct and rapid way of reversing silencing. However, evidence that Dicer is intimately associated with the RISC complex suggests that mature microRNAs may be protected from degradation as they are directly loaded onto the RISC complex (Chendrimada et al., 2005; Haase et al., 2005).

Is reduction in microRNA levels sufficient to reverse silencing?

Although numerous studies cited above indicate that reductions in microRNA levels elicit reversal of silencing, it is unclear how that alone could reverse the interaction of the guide-strand bound to the RISC complex with its target site in the 3' UTR of a silenced transcript. Certainly, selective reduction in a specific microRNA would be expected to block formation of the guide-strand/RISC complex. However, *a priori* one would not expect that reductions in microRNA levels would be able to trigger dissociation of the guide strand/RISC complex with its target site in order to enable the silenced transcript to undergo translation. Accordingly, this question has focused attention on whether additional signaling pathways may be involved in liberating the silenced transcript from the RISC complex. Although this is still an active area of investigation, detailed analysis of the mechanisms mediating reversal of PSD-95 by mGluR1 activation have yielded key insights into this issue (Muddashetty et al., 2011). In particular, these findings indicate that mGluR1 activation leads to de-phosphorylation of FMRP which leads to dissociation of Ago2 from PSD-95 mRNA. Thus, mGluR1 activation triggers both reduction of miR-125a and de-phosphorylation of FMRP, both of which appear to play key roles in suppressing silencing of PSD-95 mRNA. De-phosphorylated FMRP is then rapidly ubiquitinated and degraded (Nalavadi et al., 2012). However, since FMRP is estimated to bind to only ~ 4% of brain mRNAs, it is likely that we have only begun to understand the molecular mechanisms involved in driving dissociation of the Ago2-guide strand complex from silenced transcripts.

Furthermore, even for those transcripts that bind FMRP, its role in regulating silencing appears to vary widely across transcripts (Kenny et al., 2014). The RNA helicase, MOV10, has also been implicated in regulating microRNA-mediated silencing (Banerjee et al., 2009). MOV10 is degraded by the proteasome in response to NMDA receptor activation, which leads to reversal of microRNA-mediated silencing. Furthermore, MOV10 has recently been shown to associate with FMRP. In general, MOV10 appears to facilitate microRNA-mediated silencing by helping to expose microRNA target sites via its helicase activity. Furthermore, FMRP bound to transcripts helps to recruit MOV10 to these transcripts, which fosters their silencing. However, if both FMRP and MOV10 bind in close proximity to a microRNA target site, they prevent the Ago2-guide strand complex from binding to this site, thereby blocking silencing. Thus, these findings indicate that the role of FMRP in regulating silencing is likely quite complex and that FMRP de-phosphorylation may not represent a general mechanism utilized to reverse silencing. Accordingly, further studies aimed at understanding the mechanisms mediating dissociation of the active silencing complex regulated by FMRP and other RNA binding proteins are clearly warranted.

Summary

Reversal of silencing in response to neuronal stimulation has emerged as an attractive mechanism for mediating rapid changes in protein synthesis that underlie neuronal plasticity. In fact, the ability of neuronal stimulation to elicit rapid reductions in microRNA levels suggests that this family of molecules be viewed as second messengers capable of eliciting coordinated changes in translation. To date, three mechanisms mediating microRNA degradation have been identified. The translin/trax complex and MCPIP1 encode RNases

that target pre-microRNAs, while Lin-28 does not. Instead, its binding to the let-7 family of pre-microRNAs leads to their polyuridylation by TUT4 followed by degradation by Dis3l2. Of note, each of these degradation mechanisms targets different parts of the pre-microRNA structure and may target a subpopulation of pre-microRNAs. Since nearly all mature microRNAs are generated by Dicer, regulation of this central enzyme would not be expected to affect subpopulations of microRNAs. In contrast, the presence of multiple mechanisms of pre-microRNA degradation would enable neurons to reverse silencing in a more selective fashion. Elucidation of the signaling pathways linking neuronal stimulation to microRNA degradation and reversal of silencing may help define their role of silencing reversal in neuronal plasticity and the pathophysiology of psychiatric disorders.

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Highlights

- 1.** Neuronal stimulation triggers rapid degradation of selected microRNAs
- 2.** Rapid reversal of silencing drives translation required for plasticity
- 3.** Lin28, translin/trax and MCPIP1 mediate degradation of distinct subsets of microRNAs
- 4.** Differential activation of these pathways may elicit selective reversal of silencing

Figure 1.

Canonical pathway generating microRNAs that mediate silencing. Formation of microRNAs involves sequential processing of the primary transcripts (pri-microRNA) by Drosha and Dicer enzyme complexes. Cleavage of the pri-microRNAs in the nucleus yields hairpin structures, pre-microRNAs, which are shuttled to the cytoplasm where they are processed into short (21–23 nt) double stranded fragments, called mature microRNAs, by the Dicer complex. One of these strands, the guide strand, is loaded onto the RNA-induced silencing complex (RISC) which mediates its binding to a complementary target site in the 3'UTR of the silenced transcript.

Figure 2.

Neuronal stimulation regulates silencing of MAP1B via multiple microRNAs. The MAP1B transcript illustrates the concept that a single transcript can be targeted by multiple microRNAs to produce silencing. mGluR activation mediates degradation of miR-146a and reverses silencing of MAP1B in dendrites; in axons, BDNF elicits degradation of miR-9 to reverse silencing of this transcript.

Figure 3.

BDNF reverses silencing of plasticity transcripts. BDNF selectively triggers translation of plasticity transcripts by activating Lin-28 to reverse silencing by let-7 family members. At the same time, BDNF enhances Dicer activity to increase silencing of constitutively expressed transcripts.

Figure 4.

Multiple pathways of pre-microRNA degradation. Three pathways mediating degradation of pre-microRNAs target different features of these hairpin structures. Lin-28 binds to a GAGG motif present in let-7 family members that triggers their uridylation and degradation by TUT4 and Dis312, respectively. MCPIP1 also binds to the terminal loop. In contrast, the translin/trax complex targets mismatches in the stem, a common feature of microRNAs.

Figure 5.

Neuronal stimulation reverses silencing by activation of pre-microRNA degradation pathways. Neuronal stimulation elicits selective, rather than global, reductions in subpopulations of microRNAs. Based on BDNF's ability to activate degradation of let-7 family members by Lin-28, this model posits that neuronal stimulation also activates other pre-microRNA degradation pathways to reverse silencing in a selective fashion.

Table 1Neuronal stimuli that trigger a rapid decrease in microRNA levels *in vitro*

Extracellular signals	miRNAs	Targets	References
BDNF	miR-134	LimK1	Schratt et al., 2006
	miR-9	MAP1B	Dajas-Bailador et al., 2012
	let-7	plasticity transcripts	Huang et al., 2012
5-HT	miR-124	CREB1	Rajasethupathy et al., 2009
mGluR	miR-125a	PSD-95	Muddashetty et al., 2011
	miR-146a	MAP1B	Chen et al., 2013
NMDA	miR-124	?	Krol et al., 2010
	miR-128		
	miR-134		
	miR-138		