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REVIEW

miRNA: local guardians of presynaptic function in plasticity and disease

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ABSTRACT

Environmental fitness is an essential component of animal survival. Fitness is achieved through responsive physiological plasticity of tissues across the entire body, and particularly in the nervous system. At the molecular level, neural plasticity is mediated via gene-environmental interactions whereby developmental cues and experience dependent input adapt neuronal function to ever changing demands. To this end, neuronal gene regulation must be coupled to changes in neural activity. Seminal discoveries of the 20th century demonstrated neural activity modifies gene expression through calcium-dependent gene transcription. Building on this model, recent work over the last two decades shows that mRNA products of transcriptional programming continue to be regulated in the neuron through the activity-dependent post-transcriptional action of microRNAs (miRNAs). miRNAs are special post-transcriptional regulators that can tune gene expression within the spatial and temporal requirements of synaptic compartments. This mode of gene regulation has proven to be essential for synaptic function and plasticity as miRNA loss of function is highly associated with neural disease. In this review we will discuss current perspective on the link between presynaptic plasticity and miRNA biogenesis in the neuron.

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Introduction

In principal, a mature nervous system is comprised of a collection of synaptic connections with varying strengths and functional capabilities, which remain adaptable to outside environmental pressures. Developmental maturation and environmental fitness are achieved through the creation of immense synaptic heterogeneity through a process we call neural plasticity. At the molecular level, this process is mediated through gene-environmental interactions which lead to functional and morphogenic changes to the nervous system. During development, new neurons must perform a variety of morphological transformations, which include cellular polarization, articulation of axonal and dendritic structures, and synaptic formation; once initial connectivity is established during development, the morphogenic journey of neurons continues with synaptic pruning and experience-dependent refinement of individual synapses (Fig. 1). Downstream of neural activity, neural circuits are modified through the morphological transformation of synaptic terminals. The synaptic compartment is dynamically suspended between states of stability and reorganization. As spatial dimensions of the compartment are tuned, active zones and postsynaptic densities are continually reorganized inside this dynamic framework. In this way, the morphology of individual neurons defines the functional character of the neuron and the functional character of the circuit to which it belongs. During an adaptive process like learning, changes in behaviour occur through functional remodelling of synaptic terminals within underlying circuitry. Understanding how synaptic function is modified downstream of neural input remains an exciting frontier. Moreover, understanding how the genetic-environmental intersection mediates synaptic morphogenesis is critical to our fundamental understanding of neuroscience [1–3].

Responsive gene regulation: the basis for neural plasticity

Nature has devised the neuron with a suite of genetic tools to respond to the changing environment. At the molecular level, the responsive capacity of the neuron is defined by regulatory control of gene expression. The central dogma of molecular biology tells us that gene expression occurs through the transcription and translation of nucleic acids. In this model, transcription and translation not only propagate the expression of genes but also serve as points of regulation so that gene expression may be coupled to an adaptive response to changes in the environment. During the 20th century, the discovery that behavioural learning was mediated through experience dependent gene expression established a precedent for genetic regulatory principles of neuroscience. In this model, experience-dependent neural activity drives gene expression to create structural changes in the synaptic compartment thus modifying synaptic strength and subsequent circuit performance. This process is mediated by a molecular cascade where neural activity increases intracellular calcium

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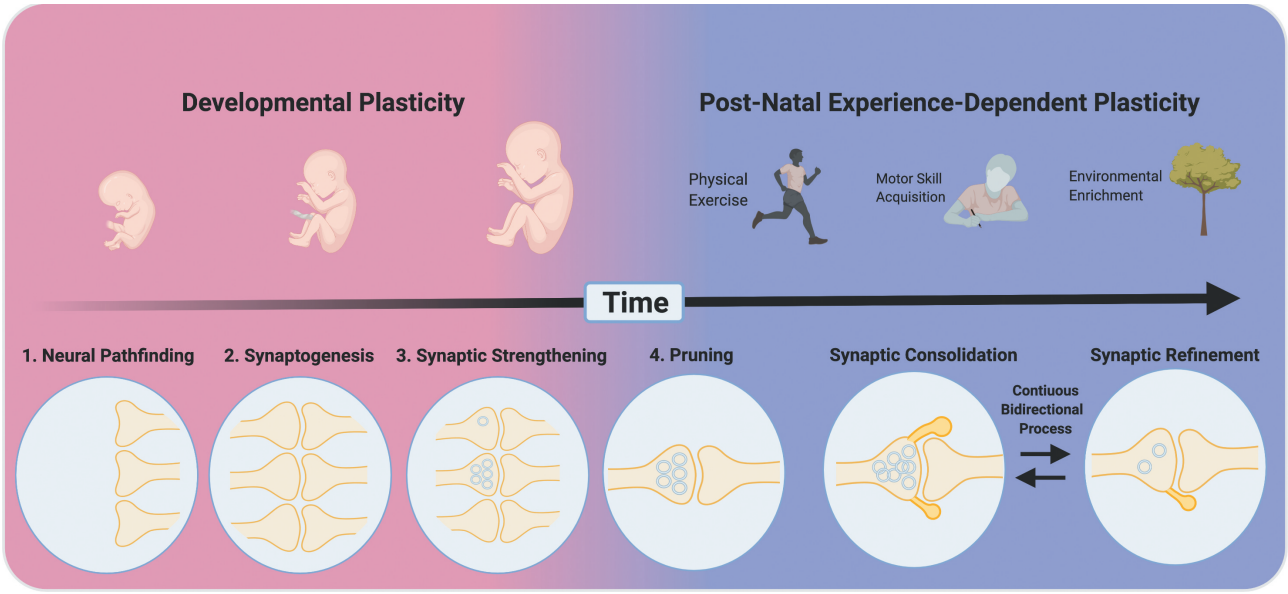

Figure 1. Developmental and experience-dependent neural plasticity.

During development, new neurons must perform a variety of morphological transformations, which include cellular polarization, (1) articulation of axonal and dendritic structures (neural pathfinding). Once this occurs, synaptic connectivity is then established through (2) synaptogenesis, functional connections are (3) strengthened, and remaining connections are refined through (4) synaptic pruning. After development synaptic connectivity is further adapted downstream of sensory and experience dependent input from the ever-changing environment. Several models of experience-dependent plasticity, including physical exercise, motor skill acquisition, and environmental enrichment, show that experience dependent neural input modulates synaptic function through continual reorganization of transmission machinery and synaptic architecture over time. Increased neural input can be consolidated by expansion of synaptic architecture, whereas decreased neural input results in a reduction of synaptic architecture. Synaptic plasticity occurs through this continual and dynamic process, which allows synaptic function to remain adaptable to the changing environment.

(Ca [2]+) levels, which ultimately induces changes in gene transcription through the activation of Ca [2]+ binding kinases such as the Ca [2]+/calmodulin-dependent protein family of kinases (CaMK) [4] and downstream transcription factors like cAMP response element binding (CREB), calcium responsive trans-activator (CREST), and c-fos [4–6]. While this seminal work demonstrated a fundamental role of gene transcription in neural plasticity, we now realize that this is only the first part of a much longer story. Recent work has enlightened us to many post-transcriptional gene regulation mechanisms and their indispensable roles in the neuron.

Post-transcriptional regulation: gene deployment in time and space

After transcriptional programmes are initiated, RNA transcripts are processed and the translation of these transcripts are finely tuned in response to neural activity. These post-transcriptional steps of gene expression support transcriptionally independent regulatory checkpoints at which protein synthesis can be adjusted, allowing gene expression to be acutely adapted to rapid changes in neural activity. Post-transcriptional regulation is particularly advantageous when cellular processes need to be modulated within a short time scale that would not be possible to accommodate through gene transcription. This temporal advantage is indispensable to spatially elaborate neurons such as motor neurons, where soma and nuclei are millimetres to metres away from the presynaptic terminal. The large spatial dimensionality of many neuronal cell-types poses a regulatory time conundrum that can be resolved by local gene regulation. In the synaptic compartment, protein turnover is highly dynamic and influenced by neural activity [7]. The half-lives of synaptic proteins are extremely diverse ranging from the order of minutes to weeks [8]; and may be selectively accelerated by selective protein degradation mechanisms [9]. Protein synthesis is coordinated in relation to the half-lives of synaptic proteins and is also coupled to the changing demands of the synapse. Both structural and functional properties for an individual synapse vary within the same neuron as well as within the same synapse at different points in time [10]. However, both spatial and temporal synaptic heterogeneity can be achieved through local protein synthesis in the synaptic compartment [11]. In this way, plasticity can occur through the coupling of protein synthesis to the changes in neural activity. How this coupling is coordinated to achieve diverse synaptic heterogeneity in both time and space remains an intriguing frontier.

miRNA control of local protein synthesis: a lever for synaptic heterogeneity

miRNAs are versatile post transcriptional regulators in the neuron that tune gene expression in a way that can accommodate the unique spatial and temporal requirements of different synaptic compartments. In contrast to generic set points of protein synthesis like ribosome activity, due to their dependence on seed sequence matching, miRNAs regulate specific target mRNAs within individual synaptic compartments inside a morphologically elaborate neuron. This kind of regulatory precision allows the neuron to adjust gene

expression in response to neural activity, locally within fine cytoplasmic spaces. Thus, in the context of neural plasticity, miRNAs can serve as regulatory links that couple local protein synthesis with activity-dependent remodelling of individual synaptic terminals. Ultimately, locally distinct protein synthesis can establish unique functionalities of individual synapses and their performance downstream of developmental cues and experience-dependent neural activity. While some mechanisms such as transcriptional activation set baseline programming for morphogenesis across the entire neuron, miRNAs can also serve as local regulatory guardians that can limit protein synthesis to match the unique specifications of individual synapses [11].

The significance of miRNA in synaptic biology is substantiated by recent findings demonstrating a broad range of pathologies that are associated with miRNA misfunction, many of which have a presynaptic aetiology. While many studies have illustrated the role of miRNA in post-synaptic biology, far less has been reported on miRNA regulation in presynaptic function and disease. Here, we will (1) introduce current perspective on long-term presynaptic plasticity, (2) discuss how miRNA biogenesis and expression are regulated in the neuron, and (3) discuss recent findings that demonstrate how miRNA regulate active zone proteins in the presynaptic terminal in the context of synaptic homoeostasis and disease.

Presynaptic plasticity: a set point for synaptic homoeostasis

Downstream of neural activity, the presynaptic active zone is dynamically reorganized to alter neurotransmitter (NT) release. Presynaptic long-term potentiation has been shown to occur in mossy fibre synapses of the hippocampus. In this model, neural activity drives increase of NT release through an PKA-RAB3-RIM1 dependent reorganization of Ca [2]+ channel-vesicle coupling at the active zone [12,13]. Active zone reorganization is key to homoeostatic control of synaptic transmission. In this context, presynaptic plasticity can help to maintain constant neural transmission in response to changes in post-synaptic function. This is particularly important in neural injury and resulting synaptic pathology. For example, in traumatic brain injury and cerebellar lesions, postsynaptic degeneration is compensated by presynaptic plasticity. In the cerebellum, neural degeneration reduces post-synaptic surface area that is compensated by increased presynaptic contact area and increased vesicle density within the presynaptic compartment [14]. Additionally, during neuromuscular degeneration, post-synaptic sensitivity is decreased, which is compensated by increased presynaptic NT release [15,16]. Furthermore, dynamic reorganization and increased expression of presynaptic active zone proteins have also been shown to underpin exercise amelioration of age-related neuro degeneration [17].

Activity-dependent changes to the presynaptic active zone not only support homoeostasis, but also can drive post-synaptic reorganization and plasticity. This has been demonstrated at the *Drosophila* larval neuromuscular junction (NMJ), where increased activity-dependent accumulation of the active zone protein Bruchpilot (Brp) in the presynaptic terminal has been shown to influence assembly and maturation of multimeric postsynaptic ionotropic glutamate receptors (iGluRs) [18]. In this way, synaptic homoeostasis and plasticity require the core active zone machinery to be adaptable in both activity and expression downstream of neural activity. Recent years have brought to light the role of miRNAs in regulating active zone assembly and dynamics. Insights from the *Drosophila* NMJ indicate that presynaptic miRNA action is critical for the regulation of synaptic formation, homoeostasis and presynaptic neurotransmitter release [19]. Similar to the mammalian examples referenced above, in the *Drosophia* NMJ system, reduction of postsynaptic glutamate receptors is compensated by expansion of presynaptic active zone content that results in augmented neurotransmitter release [19]. These insights underscore the important role of presynaptic miRNA action during synaptic formation and homoeostasis. In the following section we will review recent literature illustrating the function of miRNA in presynaptic biology, and moreover how the miRNA regulatory apparatus is employed in the context of activity-dependent plasticity. To begin, we will first explore how miRNA expression is controlled and directed by neural activity.

A regulatory sensor: responsive miRNA expression

Biogenesis: miRNA biogenesis commences with transcription to produce the primary transcript. miRNAs are typically transcribed by polymerase II into longer transcripts known as primary (pri-) miRNA [20,21]. Next, the RNAse type III nuclease Drosha, in conjunction with an RNA binding protein DGCR8 (named Pasha in flies and nematodes), process the primary miRNA transcript to yield the pre-miRNA transcript [22]. The pre-miRNA transcript can then be transported out of the nucleus and into the cytoplasm by exportin-5 [23]. Once in the cytoplasm, the miRNA-selective endonuclease Dicer processes the pre-miRNA into a transient miRNA Duplex – whereby one of the strands is degraded, and the other retained to function as the mature miRNA [24]. The mature miRNA transcript is essentially a guide RNA that is loaded into the RNA Induced Silencing Complex (RISC), and targets RISC for the silencing of specific mRNA targets bearing the miRNA Response Element (MRE). Subsequent silencing of mRNA expression can occur through RNA degradation and/or inhibiting the mRNA from being fully accessed by ribosomes [24]. Each step in the biogenesis pipeline, from primary transcription through processing and loading, represents a potential regulatory node that can be employed to modulate miRNA function in response to neural activity (Figs. 2 & figure 3).

miRNA Transcription: To serve as a regulatory link between the environment and neuronal gene expression, miRNA expression needs to be competent to respond to changes in neural activity and to changes in the cellular microenvironment (e.g. proximal to the synapse). In response to neural activity, many studies reveal miRNA expression and activity can be regulated at many steps in the miRNA biogenesis pipeline. At the transcriptional level, miRNA expression is coupled to neural activity through calcium dependent

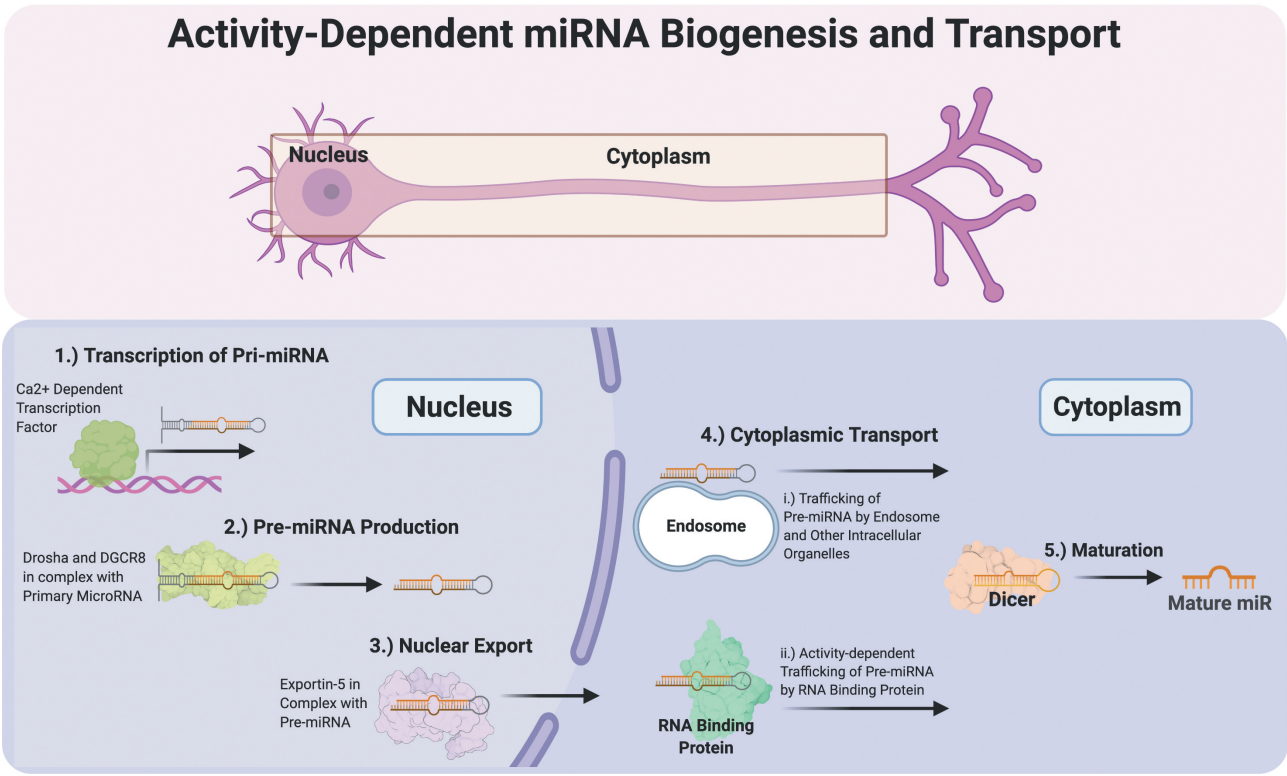

Figure 2. Activity-dependent miRNA biogenesis and transport.

miRNAs function as regulatory sensors that respond to changes in neural activity. Importantly, miRNA expression is directed by neural activity at many steps in the biogenesis and targeting pipeline. (1) This first begins at the transcriptional level where Ca2+ dependent transcription factors regulate primary miRNA expression, which is followed by (2) processing and pre-miRNA production by Drosha and RNA binding protein DGCR8, then followed by (3) nuclear export by the transport protein Exportin-5. Once in the cytoplasm, pre-miRNA are (4) transported in RNA binding protein granules downstream of neural activity. Recent work has also shown non-canonical forms of (4) transport where endosomes taxi miRNA down the axonal shaft. (5) miRNA maturation also occurs in the cytoplasm, and can persist in an activity-dependent manner

transcription factors. The first example of this was discovered when miR-132 was identified as a target of the transcription factor CREB. Further investigation showed miR-132 regulates synaptic plasticity by targeting the GTPase-activating protein, p250GAP, and this processes could be controlled in an activity dependent manner through CREB, thus linking miRNA to neural plasticity [25]. The connection between neural activity and miRNA action was further elucidated when activity-dependent action of the transcription factor MEF2 was shown to target the miR-379-410 cluster and miR-134 cluster, which are necessary for synaptic growth [26]. In further exploration of activity-dependent control of miRNA expression, other studies have identified additional ways in which miRNA biogenesis is influenced by neural activity.

Cytoplasmic Transport: RNA species are transported throughout the neuron in higher order ensembles of RNA-protein complexes called RNA granules. mRNA transcripts can be targeted for delivery using 'zip-code' sequences in the 3′UTR that interact with RNA binding proteins that aid in the directed transported of mRNA within the cytoplasmic space [27]. Similarly, miRNA biogenesis and transport can be regulated by sequence specific RNA binding proteins that act on the terminal loop structure of pri- and pre-miRNA [28,29]. miRNA confers transport specificity through a five central nucleotide sequence within the terminal loop and is sufficient to direct miRNA into the synaptic compartment [30]. Recent work shows that activity-dependent dendritic trafficking of pre-miR-134 occurs via interaction with the RNA binding protein DHX36. DHX36 binds the five nucleotide sequence in the terminal loop of pre-miR-134 and shuttles pre-miR-134 to the dendritic shaft in response to the activity-dependent signalling factor, Brain-Derived Neurotrophic Factor (BDNF) [31]. Interestingly, mobilization of pre-miRNA can deviate from canonical RNA-protein modalities of transport; recent findings show that non-coding RNA can be transported by subcellular organelles. Axonal transport of pre-miR-181a has been shown to occur through a 'hitchhiking' mechanism, where subcellular endosomes taxi pre-miR181a along the axonal shaft [32,33]. Whether this can occur in an activity-dependent context remains unexplored. Similarly, a recent study shows that miR-338 is present within the axonal shaft and associates closely with mitochondria within the axonal compartment, whether the mir-338 is transported by mitochondria directly remains unclear [33]. Additionally, mature miRNA can also be transported within the cytosol. This will be discussed more thoroughly in the section below on miRNA targeting.

Regulation of miRNA Processing and Maturation: In the 2000s, components of the RNA-induced silencing complex including Dicer, were found to accumulate within axons and developing growth cones, opening the possibility for local maturation of miRNA transcripts [34]. Moreover, such

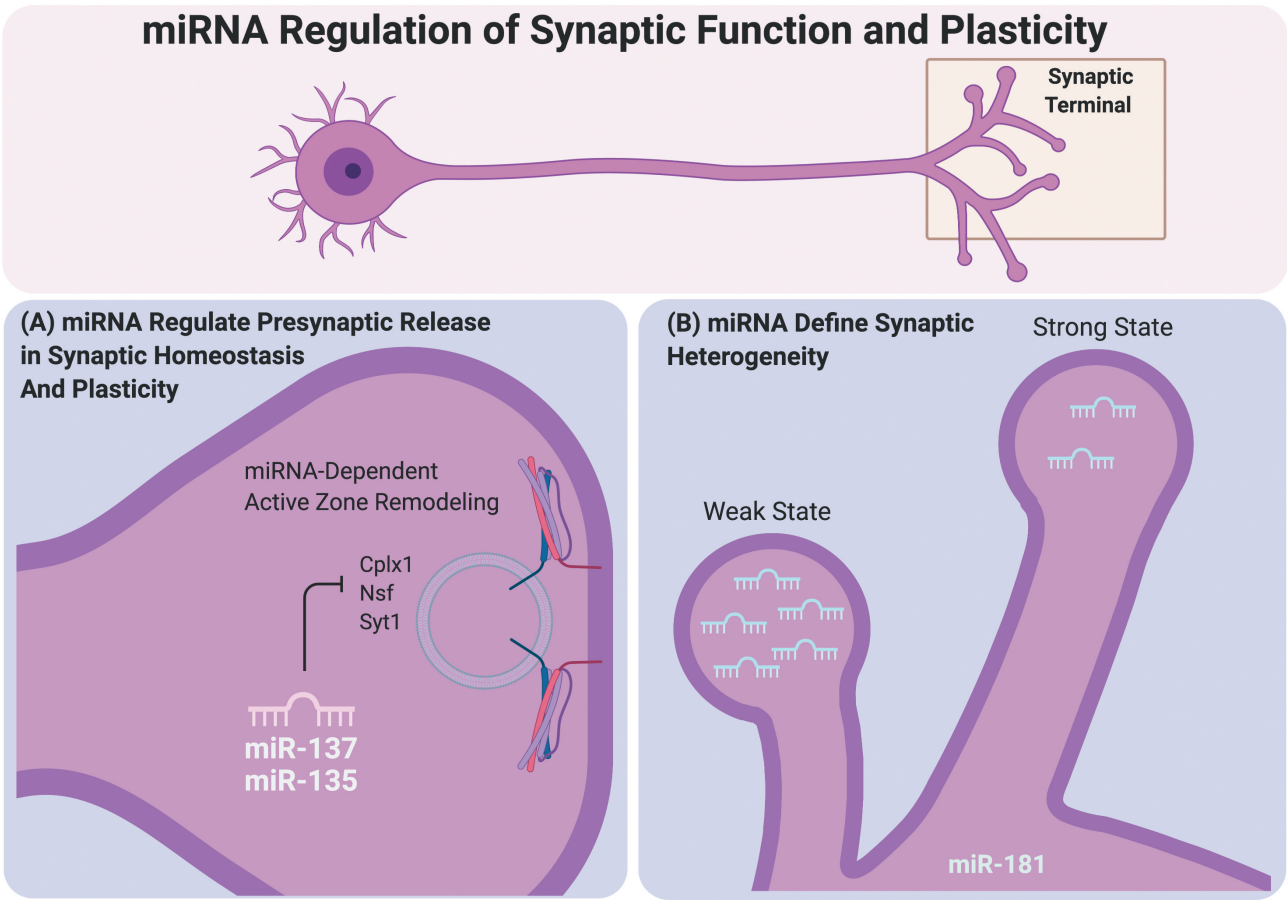

Figure 3. miRNA regulation of synaptic function and plasticity.

(A) Recent years have brought to light the role miRNA in regulating active zone dynamics to control homoeostatic control of presynaptic neurotransmitter release. miR135 and miR-137 have been shown to exert regulatory control of key active zone proteins within diverse physiological contexts (B) Other works show that the quantity of an individual miRNA, such as miR-181, can define functional states of individual synaptic compartments within the same neuron. Overall, these works demonstrate two novel and interesting ways in which miRNA regulate synaptic function and plasticity

findings also raised the question of how miRNA associated proteins like Dicer localize within distal neuronal compartments? A body of work now demonstrates roles for intracellular membranes in the transport and functionality of miRNA associated proteins Dicer, TRBP, and Argonaut (Ago) [34,35]. These studies indicate that intracellular membranes, particularly those of endosomes and rough endoplasmic reticulum, not only assist in transportation of these proteins but facilitate miRNA interactions with these proteins, thus functioning as sites of miRNA processing and maturation. More recent work shows that local assembly of the miRNA maturation complex consisting of Dicer and TRBP is regulated by neuronal activity [35]. Since then, several pre-miRNA has been shown to be locally processed within presynaptic and postsynaptic compartments downstream of neural activity [32]. For example, processing of pre-miR-16 has been shown to be down regulated downstream of neural activity in mammalian dendrites [35]. In response to neural activity, pre-miR-181a is locally processed within dendritic spines; strikingly, single-synapse stimulation is sufficient to induce local maturation of miR-181a and consequent local reduction of downstream targets [10]. Pre-miR-181a has also been shown to mature locally in the axonal growth cone in response to developmental cues [32]. Other activity-dependent mechanisms that regulate miRNA interactions include the calcium responsive protein Calpain which has been shown to enable the enzymatic activity of Dicer downstream of neural activity [36].

Regulation of RISC, miRNA Loading, and Targeting: Once miRNA is processed into mature species, they are ready to engage the RISC complex. Here we will discuss the components that comprise a functional RISC unit (miRISC), and discuss how the composition of this unit can be modified to elicit translational repression and mRNA degradation. The degradation and repressive modes of miRISC action are interconnected, where translational repression typically precedes mRNA degradation for given miRNA target [37]. While miRNA-loaded Ago is sufficiently capable of engaging downstream mRNA targets for degradation [38], the translational repressive action of miRISC can be augmented by Ago-associated proteins. Accordingly, the GW182 family of Ago-binding proteins can adapt miRISC function from degradation of mRNA targets to translational repression of mRNA targets [39,40]. GW182 recruits other translationally repressive factors like DDX6, for example, which, in concert with other components, can block mRNA accessibility from translation initiation factors [41]. It is important to note that there are

many ways in which miRNA mediated translational repression can occur, and the latter represents one example. However, an overarching factor for the mechanistic action of translational repression depends upon the addition of specific miRISC binding proteins [40]. In this way, the quality of degradation and repressive modes of miRISC activities depends upon the specific composition of miRISC [40].

Functional modes of miRISC action also depend upon distinct localities within the cytosol. RNA species tend to aggregate together in various forms within the cytoplasm, most notable within RNA granules and Processing Bodies (P-bodies) [42]. A body work indicates that the repressive action of miRISC is concentrated within P-bodies [39]. In particular, GW182 has been shown to be instrumental in directing miRISC into P-bodies [39,43]. P-bodies function as reservoirs of miRNA-stalled mRNA that can be deployed for expression on an ad hoc basis [39,44]. Accordingly, these subcellular entities are highly enriched for miRNA biogenesis proteins, free RISC complex components (namely GW182), loaded miRISC and respective mRNA targets. Within this concentrated density, mRNA are actively degraded, and/or stalled for future use. It is postulated that stalled mRNA within p-bodies are be liberated, and translocated to polysomes for translation in response to cellular demands [39,45]. In this way, miRNA targeting can be reversable, which offers yet another regulatory node of adaptive gene expression. How this node is employed at the synapse downstream of neural activity remains a developing frontier [42]. P-bodies are also particularly mobile and can be transported by cytosolic microtubule networks [46]. Consequently, miRISC can possibly be moved across cytoplasmic domains inside P-bodies. How the mobilization of Ago-containing bodies are directed downstream of neural activity also remains an emerging field.

Recent insights from tumour biology show that phosphorylation modification to miRNA transcripts also influence miRNA loading capabilities. Notably, a pool of miR-34 has been shown to exist in an inactive dephosphorylated form, which is incapable of Ago association. Upon activation of DNA damage response pathways, miR-34 undergoes 5′ end phosphorylation which enables the modified transcript to associate with Ago [47]. Whether phosphorylation of the miRNA transcripts occurs downstream of neural activity remains to be reported.

Further work reveals that target selectivity is a highly complex process because the number of potential targets for any given miRNA vastly out numbers the number of loaded RISC molecules (for a specific miRNA) in the cell [48]. The target specificity of miRNAs relies on imperfect base pair complementarity between the seed sequence in the miRNA and miRNA recognition element (MRE) in the 3′ UTR of the mRNA. However, while many assumed that mRNA targeting was mainly dependent on its expression relative to complementary miRNAs, whether miRISC prioritizes specific MRE-bearing mRNA transcripts remains open for exploration. Recently, the selectivity of loaded RISC has been shown to be controlled through an Ago phosphorylation cycle that controls miRISC selectivity on a global level [48]. The Ago phosphorylation cycle is controlled by target engagement, where target engagement causes phosphorylation of miRISC which inhibits target engagement, which likely liberates the loaded miRISC from its target, where it is then dephosphorylated allowing it to re-engage with targets again [48]. One attractive model is this phosphorylation cycle could allow loaded miRISC to distinguish between targets with differing half-lives. mRNA transcripts with longer half-lives might require longer interaction time with Ago for degradation. In this way, the phosphorylation of Ago could potentially serve as a regulatory node available to couple miRISC target selectivity to an adaptive response in the neuron.

In the nervous system, Ago phosphorylation has proven to be a node of regulation that dictates miRNA function downstream of neural activity in the synaptic compartment. In the postsynaptic context, neural activity triggers dephosphorylation of Ago residue S387, leading to a proteasome-dependent degradation of Ago, which ultimately promotes growth of dendritic spines [49]. Conversely, in this same context, neural activity has also been shown to promote miRISC function through the NMDA receptor-dependent phosphorylation of the same S387 residue on Ago [50]. In particular, phosphorylation of Ago S387 increases recruitment of the RISC protein GW182, thus increasing the repression of synaptic genes in the synaptic compartment, namely miR-134 dependent repression of LIMK1 [51]. Another point of regulation is the RISC associated MOV10/Armitage helicase which unwinds the secondary structure of mRNA transcripts which is critical for mRNA degradation [52]. MOV10 expression is enriched in the brain and MOV10 mutants display increased anxiety and decreased dendritic arborization [53]. MOV10 degradation is controlled by NMDA activity in the dendritic compartment where MOV10 is degraded downstream of neural activity in a proteome dependent manner [54]. Activity-dependent degradation of MOV10 is associated with an increased translation of neural plasticity associated transcripts CaMKII, LIMK1, and LYPLA1.

miRNA Control of presynaptic machinery in physiology and disease

Insights from Model Organisms

Studies profiling the RNA content of neuronal axons in primary culture provided a glimpse into the complexity of miRNA content in the axonal shaft and presynaptic terminal of neurons [55], arousing further curiosity about the specific role of miRNA in presynaptic biology. While these cell culture studies have afforded great access to cellular and molecular analysis as a discovery tool for miRNA function, model organisms were ultimately essential for functional analysis within the context of intact neural tissue. As we consider the exciting study of miRNA in model organisms in the subsequent paragraphs, it is important to note that in vitro or ex vivo data does not always directly reflect the in vivo biology, just as the biology of model organisms doesn't always reflect that of human biology, as cells and processes may behave very differently in complex multicellular tissues across organisms.

Studies in Aplysia

Recent findings have defined a role for miRNA in homoeostatic control of presynaptic neurotransmitter release and plasticity. In *Aplysia*, miR-124 limits synaptic plasticity at sensory-motor synapses via serotonin-dependent downregulation of the presynaptic transcription factor CREB. Effectively, this study demonstrates how miRNAs can set transcriptional programming in response to neural activity, and how this regulatory mechanism is used for long-term plasticity and synapse maturation [56]. In this same model system, miR-22 exerts similar homoeostatic constraints during learning. miR-22 primes the presynaptic compartment for plasticity by promoting PKC expression through downregulation of PKC translational inhibitor CPEB. Downstream of sustained neural activity, miR-22 expression is quenched by Serotonin-dependent MAPK/ERK signalling, which activates CPEB downregulation of PKC and constrains presynaptic plasticity. Effectively, the miR-22:CPEB relationship is instrumental for homoeostatic control of PKC expression in the presynaptic terminal, and is vital for the stabilization of synaptic strength and memory consolidation [57]. Overall, these recent studies from *Aplysia* demonstrate that neural activity can trigger miRNA action to limit synaptic plasticity and that this function is needed to consolidate activity-dependent changes in synaptic strength during learning and memory formation.

Studies in Drosophila

Recent studies from *Drosophila* also corroborate with the action of miRNAs in presynaptic plasticity. At the NMJ a set of miRNA (namely miR-289, miR-8, and miR-958) were found to be downregulated in response to neural activity. Interestingly, these miRNAs target pro-plasticity genes, including the well-known WNT signalling factor wingless (wg) as well as Calmodulin-dependent Kinase II (CamKII) [58]. Overexpression of miR-289 was sufficient to block activity-dependent expansion of the neuromuscular terminal by inhibiting presynaptic accumulation of CamKII [59]. Taken together these findings suggest a model where miRNA supports a homoeostatic threshold that is lifted by neural activity to initiate plasticity in the presynaptic terminal.

Studies in mammalian model systems

miRNA maintenance of homoeostatic balance is also critical to proper synaptic function and plasticity. In particular, miRNA control of active zone formation and neurotransmitter release play a critical role in presynaptic function. Perturbations in miRNA action lead to both hyperactive and underactive presynaptic states which initiate a wide variety of developmental, physiological and behavioural pathologies. Studies on mammalian anxiety define an important role for miR-135a in maintaining homoeostasis of neurotransmitter release in the amygdala. Knockdown of miR-135a causes increased anxiety like behaviour in rodents which was associated with increased spontaneous postsynaptic currents. These phenomena were shown to be related to miR-135a regulation of active zone proteins Complexin 1 and Complexin 2 in the presynaptic terminal. Inhibition of miR-135a allows Complexin levels go unchecked leading to presynaptic hyperactivity in the rodent amygdala and increased anxiety like behaviour [60]. Importantly, miRNA recognition elements are not always conserved across species.

Genome-wide association studies have identified polymorphisms in the miR-137 locus to be associated with schizophrenia and other psychiatric disorders [61]. Examination of post mortem human brain tissues shows that miR-137 expression is significantly elevated in tissues with synaptic defects [61]. Exploration into the mechanistic role of miR-137 in these pathologies reveals miR-137 is an important regulator of presynaptic biology. When miR-137 is overexpressed in mouse hippocampal neurons synaptic transmission and spontaneous release is decreased by 50%. These physiological defects were also accompanied by a reduction in active zone length, vesicle density, and number of docked vesicles in the presynaptic terminal. And these cellular defects coincided with a 50% reduction in synaptic formation. In vitro and in vivo investigation shows that miR-137 targets well-known active zone proteins including, Complexin-1 (Cplx1), N-ethylmaleimide sensitive fusion protein (Nsf), and Synaptotagmin-1 (Syt1). Overexpression of miR-137 in the dentate granule cells reduces vesicle clustering adjacent to the active zone. These cells also produced lower amplitude plateaus compared to controls when subjected to sustained frequency facilitation. miR-137 overexpression in the dentate gyrus also caused a reduced context associated freezing after fear conidiation, indicating that miR-137 overexpression alters learning and behaviour. Strikingly, these in vivo phenotypes and learning defects could be reversed by miR-137 inhibition as well as Syt1 restoration. Indicating that Syt1 is a functional target of miR-137 and key to miR-137 control of active zone function in both homoeostasis and disease.

While the studies conducted in model organisms reveal the powerful regulatory capabilities of miRNAs in presynaptic function, it is also appreciated that miRNA-target pairing is under rapid evolutionary change [62], and thus, while some MRE targeting relationships are conserved from model organisms to humans, others may be species-specific. This does not preclude the possibility of more general conservation of pathway or process logic whereby a specific gene is targeted in a similar way by different miRNAs in different species, but this level of conservation can challenging to detect.

miRNAs in human presynaptic function

Analysis of various human neurological and/or neurodegenerative disorders has recently identified potential contributions of miRNAs. For example, insights from one clinical study points towards underlying contributions of miR-8485 in presynaptic homoeostasis and multiple sclerosis (MS) [63]. miR-8485 was implicated as an underlying factor in MS, when whole genome sequencing of tissue samples from 16 female patients with Relapsing Remitting Multiple sclerosis (RRMS), revealed a common mutation in miR-8485 and its downstream target Neurexin-1 (NRXN1). [63]. The authors postulate that mutations in miR-8485 lead to dysfunction of miR-8485 and consequent over-expression of NRXN1. The authors reasoned, based on studies in model organisms [64,65], that increased NRXN1 expression may lead to calcium toxicity and oxidative

stress and ultimate cell death. While these and other findings can be followed by experimentation in human cell-based model systems, they appear to corroborate known functions of miRNAs in other organisms.

Conclusion and future perspectives

The studies above illustrate the responsive nature of miRNAs as regulatory links that can couple changes in neural activity to changes in presynaptic gene expression. In this way, miRNA can be deployed downstream of a wide variety of developmental and physiological stimuli. In the neuron each step of miRNA biogenesis and targeting appears responsive to changes in neural activity. Accordingly, miRNA allow the neuron, and the local presynaptic terminal, to remain genetically malleable in the context of synaptic plasticity and homoeostasis. These studies show how miRNA can be used to adapt presynaptic function under the dynamic demands of a changing environment. While this function of miRNA has been assumed to be limited to cell autonomous effects, provocative emerging work suggests that miRNA species can be secreted into the microenvironment, and into systemic circulation, raising the possibility that miRNA may coordinate adaptive responses non-autonomously at a much larger scale. This work represents an intriguing new frontier of miRNA biology.

Extracellular mobilization of miRNA has been shown to occur through distinct modalities where extracellular miRNA transport is mediated by a protein chaperone or extracellular vesicle [66]. A number of studies have shown that miRNA can be shuttled through the extracellular milieu by the RISC complex factor Ago; however, these studies are limited because it remains unclear how Ago-associated miRNA is taken up by neighbouring cells [66]. However, provokative work has recently suggested potential roles for exosome-dependent forms of extracellular miRNA secretion, transport, and uptake. These studies suggest that exosomes can be filled with individual miRNAs at a copy number high enough to downregulate mRNA targets in recipient cells [67]. Intercellular miRNA function opens the door to many intriguing possibilities of how the nervous system might employ this aspect of miRNA biology in the context of plasticity and homoeostasis.

Current studies reveal that extracellular exosome transport of miRNAs (exosome-miRNA) may be triggered by neural activity and other physiological stimuli, which represents a novel addition to the responsive capacity of miRNA action in the nervous system [55,56]. In this model, exosomal miRNA is secreted directly from cytoplasmic compartments and subsequently taken up by other recipient cells [68]. The significance of this work is underscored by recent findings that demonstrate how this pathway can be used to mediate structural plasticity under dynamic physiological conditions. Given their robust functional capabilities, neurons have demanding metabolic requirements to support their survival and operations [69]. Synaptic function is tightly coupled with available oxygen supply [69]. In a recent study, Tsai et al. suggest that peripheral glia at the *Drosophila* NMJ, sense changes in local oxygen content [70]. When oxygen content decreases, the glia relay this information coordinately to motorneurons, muscles and tracheal cells through the release of exosomal miR-274. Once taken up by neighbouring cells, miR-274 induces expansion of the neuromuscular terminal through the downregulation of Sprouty, a known regulator of synaptic and tracheal growth. This novel feature of miR-274 action to regulate the neuro-vascular unit illustrates how exosomal miRNA can be employed to elicit changes to synaptic structure and function to adapt to dynamic physiological conditions.

To this end, the underlying mechanisms of exosome-miRNA secretion, uptake, and competence remain largely undefined; furthermore, how the activities of exosomal miRNA are coordinated downstream of neural activity are open for exploration. There are studies that have begun to define how miRNA can be loaded into exosomes, which ultimately influences miRNA extracellular release. Ago has been suggested as an important player in miRNA loading into exosomes, as models of Ago deletion are associated with a decrease in extra-cellular vesicle (EV)-mediated release of highly exported miRNAs [71]. While it remains unclear as to how Ago contributes to miRNA exosome loading, it seems that Ago associates closely with EVs. Recent work indicates that Ago association with EVs is controlled though Ago phosphorylation; for example, inhibition of Ser-387 phosphorylation by MEK/ERK signalling increases Ago localization with exosomes [72]. How these activities of Ago are coordinated downstream of neural activity to mediate activity-dependent loading of miRNA into exosomes is unknown.

Multiple studies indicate that specific neuronal cell types are defined by unique miRNA profiles [66]. Interestingly, quantitative stoichiometric analysis of exosome content also suggests that distinct subpopulations of exosomes may be enriched for specific miRNA [73]. Furthermore, there is evidence suggesting that specific endosomal subpopulations can be selectively taken up by specific cell types [73]. These new findings suggest that intercellular transfer of miRNA from one cell type to another could introduce distinct exogenous miRNA in recipient cells. Thus, imported miRNA could represent a tool to tune a distinct cohort of mRNA transcripts in the recipient cell or synaptic compartment, thus providing an additional tier of neuronal gene regulation under dynamic whole-tissue physiological demands. Such a mechanism could expand the possibilities for how the nervous system communicates with other tissues to mediate plasticity and homoeostasis in the context of development and environmental fitness.

It is important to note that miRNA is but one class within a diverse milieu of non-coding RNA species. Emerging schools of thought have taken special consideration for how miRNAs might interact with other RNA entities, most notably a novel class of RNAs called circular RNAs (circRNA) that often contain sequences complementary to multiple mature miRNAs. RNA profiling studies reveal abundant expression of circRNAs in neural tissue. [74]. In both development and physiology, circRNA has been shown to act as regulators that can modulate the cellular repertoire of miRNA action. CircRNA is produced through distinct RNA backsplicing events where the 3′ and 5′ ends of a single RNA transcript are joined. The consequent product is a topologically closed loop of single stranded RNA. This molecular topology sequesters the 3′ and 5′ ends of the RNA strand from RNAse activity,

which gives circRNA enhanced stability compared to other RNA counterparts [75]. Enhanced stability might contribute to the ability of circRNA to sequester and regulate miRNA transcripts in unique ways.

While several modalities of regulation have been shown to control miRNA maturation, how mature miRNA transcripts are controlled post-maturation remains an expanding area of research. Seminal work on the human circRNA Cdrlas reveals that circRNA can exert regulatory control over the stability of mature miRNA transcripts [76]. Piwecka et al. show a novel interaction between Cdrlas and miR-7 and miR-671 in the mammalian nervous system [76]. In this context, knockout of Cdrlas caused exclusive mis-regulation of miR-7 and miR-671 expression and their respective downstream targets. Accordingly, the authors show that this circRNA:mature-miRNA interaction is essential for the normal regulation of activity-dependent gene expression and consequent synaptic plasticity and behaviour. While the regulatory roles of many circRNA have yet to be elucidated, work on Cdrlas demonstrates an important regulatory role. Like a good shepherd is to their herd, circRNA may manage cellular pools of mature miRNA to insure appropriate deployment within diverse neurological contexts. For these reasons, this new frontier is worth watching closely in the years to come.

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