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Small regulatory RNAs in neurodevelopmental disorders

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Increasingly complex networks of small RNAs act through RNA interference pathway to regulate gene expression. Recent evidence suggests that both development and proper function of central nervous system require intricate spatiotemporal expression of a wide repertoire of small regulatory RNAs. Misregulation of these small regulatory RNAs could contribute to the abnormalities in brain development that are associated with neurodevelopmental disorders. Here, we will review recent progress made toward understanding roles of small regulatory RNAs in neurodevelopmental disorders and discuss the potential involvement of newly discovered classes of small RNAs in these disorders.

INTRODUCTION

RNAs are an integral component of chromosomes and contribute to their structural organization (1). RNAs can regulate gene expression at many levels and via an array of mechanisms. Genome projects have shown that at least 93% of human genome nucleotides analyzed are transcribed in different cells, with similar findings for the mouse and other eukaryotes, indicating that there may be a vast reservoir of biologically meaningful RNAs that could far exceed the ~1.2% of encoding proteins (1–3). Uncovering the functions of these non-coding RNAs could significantly improve our understanding and treatment of human diseases. Recently, small non-coding RNAs were found in such abundance that they were dubbed the 'dark matter' of the cell (4); small non-coding RNA guides, including microRNAs (miRNAs), Piwi-interacting RNAs (piRNAs) and endogenous small interfering RNAs (esiRNAs), are 18–30 nt in length and can shape diverse cellular pathways, from chromosome architecture, development and growth control to apoptosis and stem cell maintenance (5–8).

The common trait linking the large group of neurodevelopmental disorders is that disease onset occurs during periods of ongoing maturation and development (9). These disorders are often associated with complex neuropsychiatric problems, including intellectual disability, autism, attention deficit hyperactivity disorder (ADHD) and epilepsy, among others. Neurodevelopmental disorders are caused by a wide range of genetic mutations and epigenetic and environmental factors, which lead to the changes in development, possibly via the same alterations in neurogenesis, cell migration and neuronal connectivity that are responsible for cognitive deficits in adults (9). Small regulatory RNAs, particularly miRNAs, are known to be dynamically regulated in neurogenesis and brain development (10,11). Some recent studies have suggested that the alterations in small regulatory RNAs could contribute to the pathogenesis of several neurodevelopmental disorders. In this review, we will focus on the role(s) of small regulatory RNAs in several well-defined genetic disorders, although the basic information presented here is more broadly relevant and therefore applicable to neurodevelopmental disorders in general.

BIOGENESIS OF SMALL RNAs

Given the pivotal roles of endogenous small RNAs in diverse biological pathways and the broad application of RNA interference (RNAi), understanding the mechanism of the small RNA pathway is of great importance (12). In recent years, extensive research has revealed distinct classes of small RNAs and the key protein components involved in the biogenesis of each class of small RNAs.

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MicroRNAs

miRNAs are 18–25 nt, small non-coding regulatory RNAs that are known to regulate translation of target messenger RNA (mRNA) molecules in a sequence-specific manner. In mammals, the majority of endogenous miRNA genes are transcribed initially as primary transcripts (pri-miRNAs) that range from hundreds to thousands of nucleotides in length and contain one or more extended hairpin structures (13). The nuclear RNase III enzyme Drosha, working with DGCR8, cleaves both strands near the base of the primary stem-loop and yields the precursor miRNA (pre-miRNA) (Fig. 1). After being exported to the cytoplasm by exportin-5/RanGTP, pre-miRNAs are further cleaved by the RNase III Dicer, along with a double-stranded RNA (dsRNA)-binding protein, and TAR RNA-binding protein (TRBP) (13). The Dicer–TRBP complex is also required for the processing of short hairpin RNA (shRNA) into small interference RNA (siRNA) of ~21 bp. After cleavage by Dicer and unwinding by RNA helicase, one strand of the miRNA/miRNA* or siRNA duplex (the antisense, or guide strand) is then preferentially incorporated into the RNA-induced silencing complex (RISC), whereas the other strand (the sense, or passenger strand) is degraded (Fig. 1). The RISC is a large and heterogeneous multi-protein complex. Components of the RISC that have been identified include Dicer, TRBP and Argonaute 2 protein (AGO2) (13).

The RISC uses the guide RNA to find complementary mRNA sequences via Watson–Crick base pairing, which leads to post-transcriptional gene silencing through inhibition of either translation initiation or elongation (13,14). miRNA could also negatively regulate protein expression through targeting of mRNA coding regions (15). Furthermore, miRNAs are found to upregulate the translation of target mRNAs in a cell cycle-dependent manner, switching between translational suppression in proliferating cells to translational activation in quiescent cells (16–18). Hence, a single miRNA may simultaneously regulate the expression of multiple mRNA targets and thereby act as a rheostat to fine-tune protein expression (19).

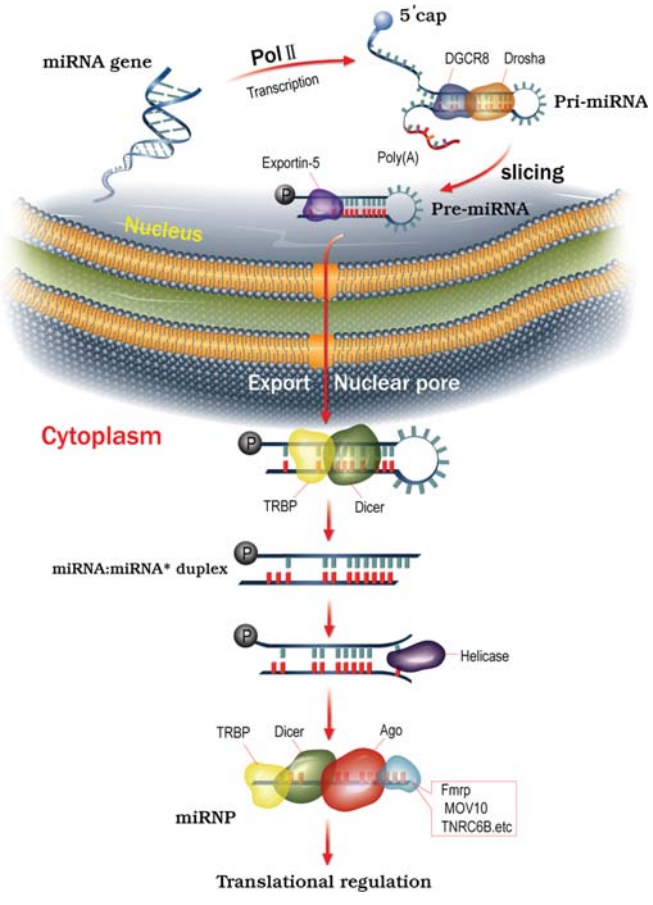

Figure 1. miRNA biogenesis. Genes encoding miRNAs are initially transcribed by RNA polymerase II or III to generate the pri-miRNA transcripts within the nucleus. The stem-loop structure of the pri-miRNA is recognized and cleaved on both strands by a microprocessor complex, which consists of the nuclear RNase III enzyme Drosha and an RNA-binding protein, DGCR8, to yield a pre-miRNA 60–70 nt in length. The pre-miRNA is then exported from the nucleus through a nuclear pore by exportin-5 in a Ran-GTP-dependent manner and processed in the cytoplasm by the RNase III Dicer–TRBP. Sliced RNA strands are further unwound by an RNA helicase. One strand of the miRNA/miRNA* or siRNA duplex (the antisense, or guide strand) is then preferentially incorporated into the RISC (or miRNP for miRNAs) and will guide the miRNP to a target mRNA in a sequence-specific manner. Once directed to a target mRNA, the RISC can mediate translational regulation by inhibiting the initiation or elongation step or through destabilization of the target mRNA. Alternatively, miRNAs may also upregulate translation of target mRNAs under certain conditions.

Piwi-interacting RNAs

piRNAs represent a distinct class of small RNAs that interact with Piwi proteins in both mammals and *Drosophila* (6–8,20). piRNAs interact with the Piwi proteins, but not Argonaute 2, the key protein in the RNAi pathway (21). Piwi protein is required for piRNA biogenesis and stability (22). piRNAs are 24–31 nt long, which differs from both siRNAs and miRNAs (21). High-throughput sequencing has revealed that the number of distinct piRNAs is much higher (more than 50,000) than miRNAs (several hundreds) (22). Most piRNAs match to the genome in clusters of 20–90 kb in a strand-specific manner, with each cluster likely representing a long single-stranded RNA precursor or, more often, two non-overlapping and divergently transcribed precursors (22). In contrast, siRNAs and miRNAs are derived from dsRNA and shRNA precursors, respectively.

Unlike siRNAs or miRNAs, the biogenesis of piRNAs is Dicer-independent (21). piRNAs are likely produced from long single-stranded precursors by yet-to-be-identified endonucleases. In *Drosophila*, a 'ping-pong' model is proposed to be involved in the generation of some transposon-derived piRNAs, although the detailed biogenesis of piRNAs in both mammals and *Drosophila* remains to be determined (23,24). Most piRNAs map to unique sites in the genome, including intergenic, intronic and exonic sequences. For example, only 17–20% of mammalian piRNAs map to annotated repeats, including transposons and retrotransposons (25). Thus, piRNA could have diverse functions, from epigenetic programming and repressing transposition to post-transcriptional regulation (21).

Endogenous small interfering RNAs

More recently, several groups described a rich diversity of esiRNAs in mice and *Drosophila* (6,8,26–28). Most of these

esiRNA classes seem to be analogous between species and include those derived from transposable elements, from complementary annealed transcripts and from lone 'fold-back' transcripts called hairpin RNAs (29). esiRNAs in particular could be generated from mammalian pseudogene-gene pairs (26). Studies in *Drosophila* suggest that esiRNA biogenesis requires components involved in the siRNA/miRNA pathway; however, the mechanism of esiRNA biogenesis remains a mystery (29), as does the specific biological function(s) of esiRNAs.

miRNAs IN NEURODEVELOPMENTAL DISORDERS

Fragile X syndrome

Fragile X syndrome (FXS), one of the most common forms of inherited mental impairment, was the first genetic disorder linked to the miRNA pathway (30–32). Clinical presentations of FXS include learning disabilities and more severe cognitive or intellectual disabilities. Fragile X patients have characteristic physical and behavioral features and experience delays in speech and language development (33).

In 1991, positional cloning of the fragile X mental retardation-1 (*FMR1*) gene revealed the molecular basis of FXS; the syndrome is associated with a massive unstable CGG trinucleotide repeat expansion within the gene's 5′ untranslated region (5′-UTR) (34–36). The functional *FMR1* gene product, fragile X mental retardation protein (FMRP), belongs to a small and highly conserved RNA-binding protein family that has been implicated in translational control (37–41). FMRP functions as a suppressor of target mRNA translation via binding of non-coding RNA structures, including G-quartets and 'kissing complexes' (also known as loop-loop-pseudoknots), within the UTRs of target mRNAs (37,39–45).

FMRP interacts biochemically and genetically with known components of the miRNA pathway. Experiments in *Drosophila* revealed specific biochemical interactions between *dFmrp* and functional RISC proteins, including *dAGO1*, *dAGO2* and *Dicer* (30,31,46). *dFmr1* displays strong genetic interaction with *dAGO1*, and *dAGO1* dominantly interacts with *dFmr1* in both *dFmr1* overexpression and loss-of-function models (32). Furthermore, *dFmr1* also interacts genetically with *AGO2*, as exemplified by their ability of co-regulating ppk1 mRNA levels (46). Additional studies provide further evidence supporting the involvement of FMRP in miRNA-containing RISC and P body-like granules in *Drosophila* neurons (47). Recombinant human FMRP is able to act as an acceptor for Dicer-derived miRNAs, and importantly, endogenous miRNAs themselves are associated with FMRP in both flies and mammals (30–32). In adult mouse brain, *Dicer* and *eIF2c2* (the mouse homolog of *AGO1*) interact with FMRP at postsynaptic densities (48). Presumably, this interaction works to regulate translation of target mRNAs in an activity-dependent manner. Based on these findings, it has been proposed that the RISC proteins, including Argonaute and Dicer, could interact with FMRP and use the loaded guide miRNA(s) to interact with target sequences within the 3′-UTR of mRNA bound to FMRP, and suppress translation (32,49). In this model, FMRP facilitates the interaction between miRNAs and their target mRNA sequences, ensuring proper targeting of guide miRNA-RISC within the 3′-UTRs and proper translational suppression (Fig. 2).

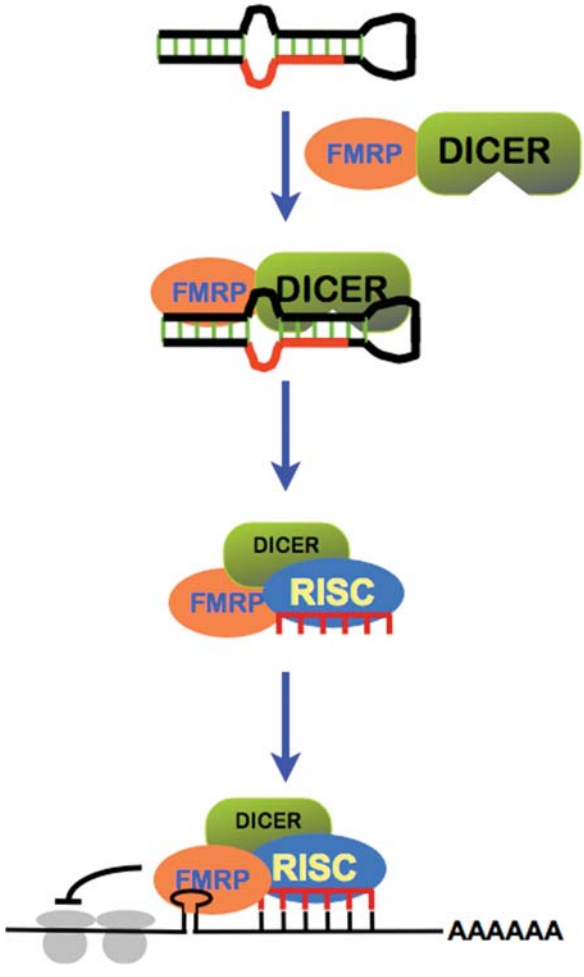

Figure 2. miRNA pathway in FMRP-mediated translational control. FMRP interacts with Dicer and RISC, and could participate the processing of miRNA precursors into mature miRNAs (i.e. miR-124a in *Drosophila*). FMRP could bind to mRNA through either G-quartet/stem structure or 'kissing complexes'. Once FMRP binds to its mRNA ligands, it could recruit RISC along with specific miRNAs (i.e. bantam in *Drosophila*) to its mRNA ligands and facilitate the recognition between miRNAs and mRNAs, which could modulate the translation of the bound mRNA ligands.

The fact that FMRP is associated with Dicer, miRNAs and specific mRNA targets raised the question of whether FMRP is associated with specific miRNAs and modulates their processing. To address this question, the expression and processing of miRNAs were examined in *Drosophila dfmr1* mutants. In fly brain, *dFmrp* was found to be specifically associated with miR-124a, a nervous-system-specific miRNA (50). The proper processing of pre-miR-124a requires *dFmrp*, whereas the loss of *dFmr1* leads to a reduced level of mature miR-124a and an increased level of pre-miR-124a. These results suggest a modulatory role for *dFmrp* to maintain proper levels of miRNAs during neuronal development (50). In our own studies, we have shown that *dFmr1* plays a role in the proper maintenance of germline stem cells in

Drosophila ovary, potentially through the miRNA pathway (51). To further test this hypothesis we used an immunoprecipitation assay and revealed that specific miRNAs, particularly the bantam miRNA (*bantam*), are physically associated with *dFmrp* in ovary (52). We found that like *dFmr1*, *bantam* is not only required for repressing primordial germ cell differentiation, but also functions as an extrinsic factor for germline stem cell maintenance (52). Furthermore, we showed that *bantam* genetically interacts with *dFmr1* to regulate the fate of germline stem cells (52). Collectively, our results support the notion that the FMRP-mediated translational pathway functions through specific miRNAs to control stem cell regulation; however, we saw no effect of *dFmrp* on the biogenesis of the bantam miRNA. Whether FMRP is associated with specific miRNAs in mammalian cells remains to be determined.

Intriguingly, another member of the fragile X-related (FXR) protein family, FXR1, has also been implicated in miRNA-mediated translational upregulation through an association with AGO2 on AU-rich 3′-UTRs in quiescent cells (16–18). Nonetheless, the relevance of these observations to FMRP-mediated translational regulation requires further exploration.

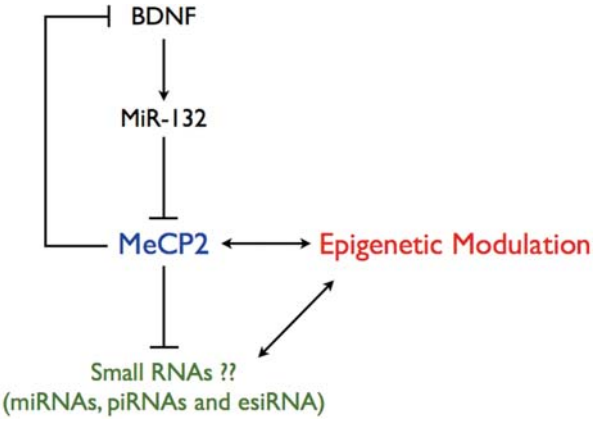

Figure 3. Small RNAs in MeCP2-mediated epigenetic modulation. Homeostatic regulation of MeCP2 expression by miR-132 is mediated by BDNF-activated CREB. Besides directly influencing the expression of mRNA protein-coding transcripts, MeCP2 may also regulate the transcription of non-coding RNA elements, such as miRNAs, piRNAs and esiRNAs. The altered expression of small regulatory RNAs could impact epigenetic modulation as well.

Rett syndrome

De novo mutations in *MECP2* are known to cause the X-linked dominant neurodevelopmental disorder Rett syndrome (RTT) (53). *MECP2* encodes the DNA methyl-CpG-binding protein, MeCP2 (54). The general association of methyl CpG dinucleotides with heterochromatic or transcriptionally silent regions of the genome led to the hypothesis that MeCP2 normally functions as a component of transcriptional repressor complexes (55,56). More recently, MeCP2 has also been shown to function as a transcriptional activator at certain loci (57). MeCP2 null and MeCP2 transgenic mouse models, which, respectively, mimic loss-of-function *MECP2* mutations and *MECP2* gene duplications, also display RTT-like phenotypes (55). Furthermore, recent clinical observations correlated duplications of *MECP2* with Rett-like phenotypes, although overall such duplications result in clinically distinct phenotypes (55). Together, these observations are consistent with a dose-dependent mechanism for MeCP2-mediated regulation of target transcripts whose misexpression during development is pathogenic. To date, most concerted efforts to identify MeCP2 target transcripts have focused on protein-coding mRNA transcripts. These approaches have revealed a number of direct MeCP2 target genes in specific cell and tissue types; however, there are several recent observations surrounding the involvement of small regulatory RNAs in MeCP2 function.

Examination of an imprinted locus on mouse chromosome 9, in which genes are known to be imprinted and expressed specifically in brain, revealed that MeCP2 binds upstream and regulates the paternal expression of an miRNA (miR-184) located within 55 kb of the imprinted locus. Moreover, the induction of miR-184 expression in depolarized cultured neurons is concomitant with a loss of MeCP2-binding upstream of the miR-184 locus. These data suggest that the regulation of miR-184 expression by MeCP2 is activity-dependent; however, the expression of miR-184 was not significantly changed in whole brain tissue derived from *MeCP2*-deficient mice (58).

The cAMP response element-binding (CREB) protein is known to be a critical transcription factor regulating neuronal plasticity and activity-dependent refinement of dendritic branching, both of which are defective processes in RTT patients. Initial identification of CREB protein targets revealed an miRNA (miR-132) that was predicted to post-transcriptionally regulate MeCP2. In postnatally cultured rat neurons, miR-132 did in fact directly repress the expression of MeCP2. However, by blocking miR-132-mediated regulation of MeCP2 and thereby increasing MeCP2 levels, the expression of brain-derived neurotrophic factor (BDNF) was found to be increased (59). Since BDNF is both a known target of MeCP2 and an activator of CREB, these findings together led to the hypothesis that miR-132 functions within a feedback loop involving homeostatic regulation of MeCP2 expression via BDNF-activated CREB (Fig. 3). Homeostatic regulation of MeCP2 by miR-132 may indicate a mechanism by which MeCP2 levels are normally maintained within the narrow range required for proper neuronal development and synaptic maturation in the postnatal brain, highlighting the importance of miRNA in these processes (60).

Regulation of miRNA expression provides an alternative means by which MeCP2-mediated epigenetic regulation could ultimately influence protein expression and phenotype. Rather than directly influencing the expression of mRNA protein-coding transcripts, MeCP2 may also regulate the transcription of non-coding RNA elements, such as miRNA. Thus, in the absence of MeCP2, some miRNAs might display increased expression, which may result in a negative effect on the translation of mRNAs targeted by that particular miRNA (Fig. 3). So it is important now to determine whether MeCP2 can directly regulate the expression of miRNA genes and the role of miRNA(s) in the pathogenesis of RTT.

DiGeorge syndrome

DiGeorge syndrome (DGS) is a rare congenital disease that is inherited in an autosomal dominant manner. Symptoms vary greatly among individuals, but commonly include a history of recurrent infection, heart defects and characteristic facial features. Individuals with DGS have behavioral and cognitive deficits that lead to childhood pathologies, including ADHD, obsessive-compulsive disorder and autism spectrum disorder (61,62). These manifestations are the result of a common chromosomal abnormality, a large 3 Mb hemizygous deletion on chromosome 22 (22q11.2) that is produced by an error in recombination at meiosis (63). This region (called the DiGeorge critical region) comprises more than 25 genes, making this syndrome a classic contiguous gene syndrome. Despite numerous human and mouse studies implicating a small subset of these genes (e.g. *Tbx1*, *Comt*, *Prodh* and *Gngl1*) as contributors to the morphological or behavioral phenotypes of this syndrome (64–67), the genetic basis of the cognitive impairments has gone largely unexplained. Recently, the heterozygous disruption of a single gene found in the DiGeorge critical region, *Dgcr8*, was found to result in cognitive delay, specifically in spatial working and memory-based learning (68). DGCR8 forms a microprocessor complex along with Drosha to process the pri-miRNAs (Fig. 1) (5). Mature miRNAs were reduced in the brains of mice containing either the *Dgcr8* disruption or the syntenic hemizygous deletion of the DiGeorge critical region (68). Together, these data argue that the heterozygous loss of DGCR8 causes abnormal miRNA biogenesis and leads to a deficit in cognitive performance; however, whether specific miRNAs are responsible for cognitive deficits associated with these mutants remains to be determined. The identification of the downstream targets that are misregulated in these miRNA-deficient mutants would also provide further insight into the pathogenesis of DGS, as well as a better understanding of learning and cognition more generally.

Down syndrome

Down syndrome (DS), which affects 1 in 700 newborns, has a variable phenotype that includes congenital heart defects, craniofacial abnormalities and cognitive impairment (69). DS is caused by triplication of all or part of human chromosome 21 and is often referred to as trisomy 21. The extra chromosomal segment results in an increase in gene dosage by as much as 50% in multiple genes, which perhaps explains the DS phenotype (70,71). Genotype and phenotype correlations of partial trisomy cases allowed for the identification of a Down syndrome critical region (DSCR); duplication of this region is associated with many of the DS phenotypes, particularly mental retardation (72,73). To date, we know of more than 30 genes overexpressed in key brain regions in DS individuals; 13 of these genes reside in the DSCR (74).

Recently, the potential contribution of miRNAs to the pathogenesis of DS has been investigated. Bioinformatic analyses revealed that chromosome 21 encodes five miRNAs (miR-99a, let-7c, miR-125b-2, miR-155 and miR-802), all of which are overexpressed in fetal brain and heart tissues from DS individuals, suggesting that they might contribute, at least in part, to the cognitive and cardiac defects seen in DS (75). Notably, none of these miRNAs are located in the DSCR; however, a role for miRNAs in DS is supported by the finding that miR-155 downregulates a human gene associated with hypertension, angiotensin II type 1 receptor (AGTR1) (76). Indeed, DS individuals do have lower blood pressure and lower AGTR1 protein levels than those without DS. Associations between an miRNA and a DS phenotype are unlikely to be rare, even for miR-155, since each miRNA has the ability to regulate a large number of protein-coding genes (77). Moreover, improved computational and experimental methods continue to reveal the location of new miRNAs, suggesting that there remain unidentified miRNAs residing on chromosome 21, and in the DSCR, which could make excellent candidates to study the molecular pathogenesis of DS further.

Other neurodevelopmental disorders linked to miRNAs

In addition to the disorders discussed above, there are several others that have a potential link to altered miRNA expression. The region associated with MRX3 and Waisman syndrome (early-onset Parkinsonism with mental retardation) harbors an miRNA, miR-175 (78). Furthermore, a microdeletion at chromosome Xp11.3 that accounts for cosegregation of retinitis pigmentosa and X-linked mental retardation in a large kindred contains two highly conserved miRNAs, miR-221 and miR-222 (79). Segmental duplications at breakpoints (BP4–BP5) of chromosome 15q13.2q13.3 result in microdeletions/duplications that are associated with a variety of neuropsychiatric abnormalities, including features of autism, ADHD, anxiety disorder, mood disorder, mental retardation, epilepsy and in some instances EEG abnormality. The ~1.5 Mb region spanning BP4–BP5 includes six reference genes and one miRNA, miR-211, although some patients were found to have a smaller ~500 kb deletion that includes only three reference genes and miR-211 (80). Furthermore, a recent study finds that altered miRNA expression is observed in postmortem cerebellar cortex from autistic patients (81). Nonetheless, it is important to note that whether the miRNAs associated with each disorder could contribute to disease pathogenesis remains to be determined.

In summary, miRNAs are abundant in the nervous system, where they are involved in neural development and are likely an important mediator of neuronal plasticity. Given that miRNAs play a role in the fine-tuning of protein production, they could contribute significantly to the molecular pathogenesis of neurodevelopmental disorders. Aside from the altered miRNA transcription and biogenesis, the dosage of miRNA genes associated with segmental duplication could contribute to the phenotypes, as well. Furthermore, there are significant numbers of single nucleotide polymorphisms in the human genome, which could potentially create or disrupt the putative miRNA target sites. Therefore, variations in the target mRNA sequences could also modulate the activity of specific miRNAs and contribute to phenotypic variation (82–84). It is likely that many of these variations will affect neuronal miRNAs.

piRNAs AND esiRNAs IN NEURODEVELOPMENTAL DISORDERS?

Besides miRNAs, numerous piRNAs and esiRNAs have been identified in genomes (6–8,20). piRNAs in particular have been linked to control of the mobilization of transposable elements in both mouse and *Drosophila* (22). Although they were initially discovered only in reproductive systems, mounting evidence from recently published work suggests that piRNAs and esiRNAs are present in both germline and somatic tissues. So the question becomes what other functions these small RNAs could play, besides modulating the activity of transposable elements.

Recent studies in *Drosophila* suggest that piRNAs could play important roles in epigenetic regulation. Piwi protein was found to colocalize with Polycomb group (PcG) proteins to cluster PcG response sequences in the genome, as well as with HP1 protein to modulate epigenetic silencing (85–87). Conversely, Piwi protein and its associated piRNA can also promote the euchromatic character of certain heterochromatin regions and their transcriptional activity (88). Interestingly, it was found that Piwi protein interacts with *dFmrp* in *Drosophila*; however, whether *dFmrp* is involved in the piRNA pathway and related epigenetic regulation remains to be determined (89). More recently, maternally deposited piRNAs were found to play a significant role in mounting an effective silencing response, and a lack of maternal piRNA inheritance was revealed to be behind hybrid dysgenesis, in which crosses between different fly strains that differ in the presence of a particular transposon could produce sterile progeny (90). Thus, maternally inherited piRNAs could contribute to epigenetic control; however, it remains to be determined whether there is a similar phenomenon in mammals. These data together suggest that piRNAs could be involved in epigenetic modulation. In humans, the belief is that epigenetic modulations may serve as an intermediate process that imprints dynamic environmental experiences on the 'fixed' genome, resulting in the stable alteration of phenotypes (91,92). Disturbance in epigenetic regulation could lead to the inappropriate expression or silencing of genes, causing an array of multisystem disorders, particularly neurodevelopmental disorders (92). Given the role of piRNAs in epigenetic modulation, it would be interesting to explore the potential role(s) of piRNAs in neurodevelopmental disorders.

In addition to the above, in recent years, segmental changes in DNA copy number have been recognized as particularly common in mammals. A substantial fraction of genomic DNA (~2–6%) is contained within segmental duplications, and copy number variation (CNV) is widespread among different humans and chimpanzees, as well as among inbred mouse strains (93–95). This active acquisition, duplication and dispersal of large gene-containing genomic segments are part of an ongoing evolutionary process and could contribute to the pathogenesis of neurodevelopmental disorders, including autism (96–99). However, what drives this genomic evolution remains unknown. In prokaryotes, genomic evolution is assisted by the integration of gene pools from phages and plasmids, or genomic islands. Hot regions for the integration of genomic islands are close to non-coding RNAs, such as tRNAs or small RNAs (100). In addition, RNA is known to be capable of guiding genome rearrangement in ciliates, a lower eukaryote (101). So it will be intriguing to test whether small non-coding RNAs, including both piRNAs and esiRNA, could be the components of the pathway modulating the dynamics of CNV in mammals.

SUMMARY

Recent discoveries of different small regulatory RNAs, including miRNAs, piRNAs and esiRNAs, have revealed a new layer of gene regulation. These 'micro' regulatory RNAs could play 'macro' roles in shaping diverse cellular pathways. Emerging data suggest that small regulatory RNAs, particularly miRNA, could contribute to the pathogenesis of neurodevelopmental disorders. We expect that these findings are just the tip of the iceberg, with different types of small RNAs possibly being involved in disease pathogenesis at different levels and via multiple distinct mechanisms. We therefore must take small regulatory RNAs into consideration when trying to identify disease-causing gene(s) and dissect the biological pathway(s) altered in neurodevelopmental disorders.

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Conflict of Interest statement. None declared.

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