Protein components of the microRNA pathway and human diseases

Marjorie P. Perron and Patrick Provost*

Centre de Recherche en Rhumatologie et Immunologie, CHUL Research Center, 2705 Blvd Laurier, Quebec, QC, G1V 4G2, Canada, and Faculty of Medicine, Université Laval, Quebec, QC, G1K 7P4, Canada

Summary

MicroRNAs (miRNAs) are key regulators of messenger RNA (mRNA) translation known to be involved in a wide variety of cellular processes. In fact, their individual importance is reflected in the diseases that may arise upon the loss, mutation or dysfunction of specific miRNAs. It has been appreciated only recently that diseases may also develop when the protein components of the miRNA machinery itself are affected. The core enzymes of the major protein complexes involved in miRNA biogenesis and function, such as the ribonucleases III (RNases III) Drosha and Dicer as well as Argonaute 2 (Ago2), appear to be essential. However, the accessory proteins of the miRNA pathway, such as the DiGeorge syndrome critical region gene 8 (DGCR8) protein, Exportin-5 (Exp-5), TAR RNA binding protein (TRBP) and Fragile X mental retardation protein (FMRP), are each related, in various ways, to specific genetic diseases.

Keywords

RNA silencing; microRNA; gene expression; diseases

1. Role of the MicroRNA-Guided RNA Silencing Pathway

The microRNA (miRNA)-guided RNA silencing pathway is a gene regulatory process present in most eukaryotic cells which is involved in the repressive control of messenger RNA (mRNA) translation. MiRNAs are encoded in the genome and the regulation of their expression is crucial for the maintenance of cellular homeostasis. Although estimated to represent ~2% of the genome, miRNA gene products have been proposed to regulate as many as 92% of the genes in human (1)! Several characteristics of miRNAs and their recognition of binding sites through which they regulate specific mRNAs are conserved throughout evolution. MiRNAs are a well-defined family of non-coding, ~21 to 23-nucleotides (nt) RNA species that can bind their target mRNA through imperfect complementarity usually in the 3' non-translated region (NTR), causing specific translational repression. MiRNA-based gene regulation has been shown to play an important

*Corresponding author: Patrick Provost, Centre de Recherche en Rhumatologie et Immunologie, 2705 Blvd Laurier, Local T1-49, Quebec, QC, G1V 4G2 Canada, Phone: 1 418 656 4141 (ext. 48842), Fax: 1 418 654 2765, patrick.provost@crchul.ulaval.ca.

role in development, cell growth, apoptosis and other cellular processes (for a review, see Ouellet et al. (2)).

A single miRNA can regulate several mRNAs through recognition of specific binding site that is believed to require the perfect pairing of its miRNA seed region, referred to as nt 2 to 8 from the 5' end (3). The 3' region of the miRNA can exhibit imperfect complementarity with the mRNA target, but may compensate for a weaker binding of the miRNA seed region. Conversely, more than one miRNA can recognize the same mRNA target and strengthen its translational repression. Elucidation of the architecture of the well-studied interaction between let-7 and lin-41 in *C. elegans* have allowed a better understanding of the pairing determinants of a miRNA with its mRNA target (3,4). The let-7 miRNA forms an imperfect duplex with six complementary sites present in the lin-41 3' NTR, and the occupancy of only two of the sites, separated by a 27-nt sequence, are sufficient to induce silencing of lin-41. The 27-nt sequence between the two binding sites for let-7 also seems to be important, as deletional mutagenesis or nucleotide substitutions in the sequence prevent lin-41 silencing (3). However, the characteristics of an experimentally validated miRNA:target pair are not sufficient by themselves to establish the rules governing mRNA recognition by all existing miRNAs, which may require elaborated studies of several different miRNA:mRNA combinations.

In fundamental research, the properties of this unique gene silencing machinery can be exploited by synthetic, miRNA mimics small interfering RNA (siRNA) to specifically downregulate the expression of our gene of interest to study its function. Having the ability to structurally mimic miRNAs and to hijack the endogenous pathway of the cell in which they are introduced, siRNAs represent a powerful research tool with a promising future in human therapy, with the possibility to downregulate a protein found to be overexpressed in specific diseases, such as cancer.

Recent advances have led to the identification of miRNA targets, some of which are related to specific diseases. Among the different scenarios that may be envisioned, overexpression of a given miRNA may accentuate the translational repression of its target mRNAs, whereas the downregulation of another miRNA may lead to an enhanced translation of its target mRNAs. For instance, deregulated expression of specific miRNAs has been linked to certain cases of cancer. The miR-17-92 cluster, a region that encodes for seven miRNAs, is overexpressed in human lung cancer cell lines (5). On the opposite, frequent deletions and downregulation of miR-15 and miR-16 genes at 13q14 occur in chronic lymphocytic leukemia (6). These two miRNAs have been shown to negatively regulate the antiapoptotic Bcl2 protein at the posttranscriptional level (7). These two examples illustrate the importance of a highly regulated miRNA expression in the maintenance of normal cell function and the development of specific diseases that can result from an imbalanced gene regulation due to miRNA dysfunction.

Involved in the biogenesis and action of miRNAs, the protein components of the miRNA-guided RNA silencing machinery may also be susceptible to defects, the occurrence of which could lead to important genetic disorders and viral infection. Indeed, some protein components of the miRNA pathway have now been linked to specific diseases.

2. Major Protein Components of the MicroRNA-Guided RNA Silencing Pathway

Involving only a few proteins, miRNA biogenesis and function are well-orchestrated processes relying on numerous types of protein-protein, protein-RNA and RNA-RNA interactions that are acting in coordination for the generation of thousands of different miRNAs and regulation of tens of thousands of different mRNAs. The most notable feature of this machinery remains its ability to recognize and process specific RNA structures independently of their sequences in miRNA biogenesis, allowing any miRNAs to be synthesized and any mRNA to be regulated by miRNAs. For a recent review, see Perron and Provost (8).

MiRNA genes are transcribed by RNA polymerase II into a long non-coding RNA known as the primary miRNA (pri-miRNA) (9,10). Harboring a 5' methylated cap and a 3' poly(A) tail, this primary transcript folds on itself to form hairpin-loop structures that can be recognized and cleaved by the nuclear Microprocessor complex, composed of Drosha and the DiGeorge syndrome critical region gene 8 (DGCR8) protein, into a miRNA precursor (pre-miRNA) (11–16). Following its export from the nucleus mediated by Exportin-5 (Exp-5) (17), the pre-miRNA is cleaved by the pre-miRNA processing complex, formed by Dicer and TAR RNA binding protein (TRBP), into a miRNA:miRNA* duplex (18–20). This complex then assembles with Argonaute 2 (Ago2) protein to form a miRNA-containing ribonucleoprotein (miRNP) complex (21), after which the mature miRNA strand of ~21- to 23-nt is selected. Depending on whether the complementarity of the miRNA to its target is perfect or not, the miRNP complex can lead either to mRNA cleavage and degradation or to initial translational inhibition (22). In this latter case, the repressed mRNA is translocated to the P-bodies, after which the mRNA can either be destroyed or relocalized to the translational machinery for expression upon a specific cellular signal (see figure 1) (23,24).

2.1 Drosha

The initial step in miRNA biogenesis is the recognition of the pri-miRNA structure by the Microprocessor complex. Drosha is the core enzyme of this nuclear complex. This ribonuclease III (RNase III) enzyme binds to pri-miRNAs independently of their sequences and excises ~70-nt pre-miRNAs. Drosha was initially reported to have a role in ribosomal RNA processing (25). We anticipate Drosha to be essential for cell viability, since its downregulation by RNA interference (RNAi) in embryonic stem (ES) cells correlated with a proportional decrease in mature miRNA production, thereby indicating its absolute requirement for miRNA biogenesis (11).

2.2 Dicer

The second major protein complex of the miRNA-guided RNA silencing pathway is the pre-miRNA processing complex containing the RNase III Dicer. This enzyme is responsible for the cleavage of the pre-miRNA into a miRNA:miRNA* duplex with extremities harboring 2-nt 3' overhangs, the signature characteristic of members of the RNases III family of enzymes (26). In mammals, the presence of Dicer is essential, as Dicer-deficient mice die at the embryonic stage, suggesting that Dicer is required for mammalian development (27,28).

Dicer-deficient mouse ES cells are defective in differentiation and centromeric silencing. The role of miRNAs in ES cell differentiation has been recently studied by generating an inducible Dicer knockout model (29). Analysis of Dicer-null ES cells revealed an impairment in miRNA biogenesis and a severe defect in differentiation both in vivo and in vitro. Epigenetic silencing of centromeric repeat sequences and concomitant expression of homologous small double-stranded RNAs (dsRNAs) were also markedly reduced. Noticeably, the phenotype was rescued by the re-expression of Dicer in these cells (29). These results suggest that Dicer participates in multiple, fundamental biological processes in mammals, ranging from stem cell differentiation to the maintenance of centromeric heterochromatin structure and centromeric silencing (29).

Deregulation of Dicer expression has also been observed in cases of cancer. A reduced expression of Dicer in non-small cell lung cancer (30) or an overexpression of Dicer in prostate adenocarcinoma and in precursor lesions of lung adenocarcinoma (31,32) have been reported, indicating that an adequate level of Dicer expression may be required for maintaining normal cell functions.

2.3 Argonaute 2

The Ago2 protein is the major component of the miRNP effector complex. Ago2 is a member of the PAZ and PIWI domain (PPD) protein family (33). A remarkable feature of the miRNP complex remains its versatility in inducing either mRNA cleavage or translational repression on the sole basis of base pairing between the miRNA and its mRNA target. This notable aspect resides in the structure of the Ago2 protein, as determined from the structure of the *D. melanogaster* Ago1 PAZ domain solved by nuclear magnetic resonance (34,35). The PAZ domain specifically recognizes siRNAs via their characteristic 2-nt 3' overhangs (34–37). The 3' end of the guide siRNA strand is anchored in the PAZ domain, whereas the PIWI domain, acting in concert with the PAZ, cleaves the mRNA strand at the phosphodiester bond facing that present between nt 10 and 11 of the siRNA. An active RNA-induced silencing complex (RISC) can then be regenerated and initiate, armed with the same siRNA, a new round of mRNA cleavage (38). When a miRNA perfectly complementary to its mRNA target is loaded into a miRNP, the cleavage occurs at precisely the same site as that seen for siRNA-guided cleavage (39). After cleavage of the mRNA, the miRNA remains intact and can guide the recognition and destruction of additional mRNAs (39).

Translational repression induced by miRNAs implicates that the target mRNA is not cleaved by Ago2. In fact, structural studies have shown that the imperfect complementarity occurring between a miRNA and its mRNA target prevents mRNA cleavage by Ago2 by moving away from the catalytic site the portion of the mRNA facing nt 10 and 11 of the miRNA. The mechanism underlying translational inhibition likely involves multiple binding sites for the same or different miRNAs in the mRNA 3' NTR and cooperation among the different miRNPs attached to these binding sites on the targeted messages. These miRNPs may prevent initiation of translation or block active translation through combinatorial control and steric hindrance of the ribosomal machinery (40,41). This scenario is supported by the

relatively high degree of conservation among the different miRNAs recognizing the same target throughout evolution.

Ago2 is essential for mouse development, and cells lacking this protein are unable to mount an experimental response to siRNAs (42). Recently, another strategy of disruption of Ago2 in mice indicates that the absence of this protein leads to embryonic lethality early in development, i.e. after the implantation stage (43). It was also shown that Ago2 deficiency impairs miRNA biogenesis from pre-miRNA followed by a reduction in miRNA expression levels (44). Using hematopoiesis in mice as a model system to study the physiological function of Ago2 in vivo, it was found that Ago2 controls early development of lymphoid and erythroid cells (44). Thus, there is no doubt that Ago2 is essential for the miRNA-guided RNA silencing pathway and there is no homologue in the cell capable of compensating the loss of Ago2. These results suggest that among the Ago family members, only Ago2 seems to have mRNA cleavage activity (43).

2.4 The P-bodies

The P-bodies are specific cytoplasmic foci of aggregated mRNA-containing RNP (mRNP) complexes associated with the translation repression and mRNA decay machineries (45,46). P-bodies are referred to as the GW182-containing bodies, because they contain the GW182 RNA-binding protein as well as other proteins implicated in protein degradation, such as the decapping enzyme Dcp1 (47,48). Recently, a link between these bodies and the miRNA-guided RNA silencing pathway has been established. In fact, Ago proteins have been localized to these protein complexes (45) and found to interact directly with GW182 (23). Indeed, silencing of GW182 delocalizes resident P-body proteins and impairs silencing of a miRNA reporter system. The importance of P-bodies in mRNA repression is supported by the findings that mutations that prevent Ago proteins from localizing to P-bodies also prevent translational repression of mRNAs (23).

MiRNAs are also present in P-bodies, as the formation of this structure seems to be a consequence, rather than the cause, of miRNA biogenesis (49). The authors reported that endogenous let-7 miRNA co-precipitates with a GW182 protein complex. In addition, knockdown of two proteins, Drosha and DGCR8, which are essential for the generation of mature miRNAs, results in a loss of the P-bodies (49). P-bodies thus represent the cellular site where repressed mRNAs accumulate and are ultimately either degraded or rescued and redirected to the translational machinery.

MiRNA-guided mRNA repression appears to be a reversible process. Recently, it was observed that upon a specific stress, a repressed mRNA can be released and translated back into proteins (24). The authors showed that cationic amino acid transporter 1 (CAT-1) mRNA and reporter genes bearing its 3' NTR can be relieved from miR-122-induced inhibition in human hepatocarcinoma cells subjected to amino acid deprivation. The derepression of CAT-1 mRNA is accompanied by its release from cytoplasmic P-bodies and its recruitment to polysomes. mRNA derepression seems to require binding of HuR, an AU-rich element (ARE) binding protein, to the 3' NTR of CAT-1 mRNA. The authors proposed that proteins interacting with the 3' NTR of mRNAs will generally act as modifiers, altering the potential of miRNAs to repress gene expression (24).

Interestingly, autoantibodies directed against protein components of the miRNA pathway have been detected in human patients with immune diseases. First, anti-GW182 antibodies were found in patients harboring the Sjögren's syndrome, which is the most common clinical diagnosis, followed by mixed motor/sensory neuropathy and systemic lupus erythematosus (50). Recently, it was reported that autoantibodies from patients with rheumatic diseases as well as from a mouse model of autoimmunity recognize Ago2, a component of the P-bodies (51). Indirect immunofluorescence studies demonstrated that these autoantibodies target the P-bodies. These autoantibodies were also capable of immunoprecipitating additional components of the miRNA pathway, including Dicer (51).

3. The Accessory Proteins of the MicroRNA Pathway and Human Diseases

Assisting the major enzymes in their function, different accessory proteins play an important role in miRNA biogenesis and action. Of these accessory proteins, some have been shown to be implicated in genetic disorders or viral infection.

3.1 The DiGeorge Syndrome Critical Region Gene 8 Protein and the DiGeorge Syndrome

The DGCR8 gene is present in a common monoallelic deleted genomic region containing ~30 genes located in the q11.2 region of the human chromosome 22 (52). There are two types of deletion, a 3.0-Mb deletion and a 1.5-Mb deletion. Although most patients have in common the 1.5-Mb deletion, some patients harbor chromosomal deletions that have either no overlap with those two types of deletions (53,54) or no detectable deletion in the q11 region of chromosome 22 (55–57). Heterozygous deletion of this locus leads to the most common human genetic deletion and patients presenting this deletion display clinical phenotypes defined as the DiGeorge syndrome, Conotruncal anomaly face syndrome and Velocardiofacial syndrome (52). The patients carrying this deletion demonstrate various conditions, ranging from congenital heart defect and characteristic facial appearance to immunodeficiency and behavioral problems (58). Many efforts have been made to identify the genes located in this region that could be correlated with these phenotypes.

The DGCR8 protein interacts directly with the RNase III Drosha (14) within the Microprocessor complex. DGCR8 has been proposed to guide Drosha by functioning as a molecular anchor that determines the position where Drosha cleave its pri-miRNA substrate, since Drosha lacks processing specificity in the absence of DGCR8 (14,15). Knockdown of DGCR8 by RNAi, like its partner Drosha, decreased the level of mature miRNAs, indicating that both proteins are required for miRNA biogenesis (14). These findings were confirmed in DGCR8 knockout ES mouse cell (59).

The DGCR8 gene is present in the most common 1.5-Mb deletion (52). The deletion encompasses ~30 genes, thus it is difficult to conclude which gene(s) is(are) involved in the disease. It is probably due to a combinational effect between all or some of these genes. Since the deletion occurs in one allele, the cell still possesses one copy of the genes, such as that encoding for DGCR8. Whether this single DGCR8 gene copy is sufficient for an efficient miRNA-guided RNA silencing pathway remains to be elucidated.

3.2 Exportin-5 and Adenovirus Infection

First discovered as a new family member of the nuclear karyopherin β transporter family, Exp-5 was found to mediate nuclear export of dsRNA binding proteins (60). The initial model stipulates that Exp-5 and Ran-GTP associate with a dsRBD-containing protein in the nucleus and that this exporting complex translocates through the nuclear pore complex (NPC) to the cytoplasm (60). Then, Ran-GTP hydrolysis releases in the cytoplasm the dsRBD-containing protein that will allow it to interact with regulatory elements of its mRNA target. Further release or degradation of the mRNA target would permit import of the dsRBD-containing protein back to the nucleus (60). More recently, Exp-5 was found to interact directly with pre-miRNAs and to mediate their transport from the nucleus to the cytoplasm in a Ran-GTP hydrolysis dependent manner (17,61).

Downregulation of Exp-5 by RNAi showed that this transporter is required for efficient inhibition of gene expression induced by a pre-miRNA or a pre-miRNA mimetic short hairpin RNA (shRNA), but not by a siRNA, in dual luciferase reporter gene assay in 293T cells (17), suggesting that the pre-miRNA or shRNA export precedes their processing. Entering in the RNAi pathway downstream of Exp-5, siRNAs do not required Exp-5 for their activity. Moreover, miRNA biogenesis is dependent on the presence of Exp-5 in HeLa cells (61). Indeed, upon depletion of Exp-5 by RNAi for 48 to 72 hours, the levels of mature miRNAs was reduced by 40 to 60% (61). On the other hand, episomal overexpression of Exp-5 enhances the capacity for RNAi induced either by miRNAs or shRNAs, but not siRNAs (62). The expression of endogenous pre-miRNAs and mature miRNAs is also enhanced upon Exp-5 overexpression, indicating that Exp-5 is the rate-limiting component of miRNA biogenesis.

The structural VA1 RNA overexpressed by adenoviruses was found to be able to saturate the transporter Exp-5 (63). VA1 RNAs are 160-nt long and can be recognized by Exp-5 via their minihelix RNA motif (63), which are dsRNA molecules similar in structure to pre-miRNAs (64). Exp-5 directly interacts with VA1 RNA in a Ran-GTP-dependent manner (63). VA1, which is expressed at very high levels in adenovirus-infected cells, potentially inhibited RNAi induced by shRNAs or pre-miRNAs, without affecting RNAi induced by siRNA duplexes (65). Competition binding for the Exp-5 and inhibition of Dicer function via direct binding of VA1 RNA appear to be the cause of the inhibition of RNAi function in adenovirus-infected cells (65).

Later, it was found that small RNAs derived from VA1 RNAs, similar to miRNAs, can be found in adenovirus-infected cells (64). These small RNAs are efficiently bound by Ago2, and behave as functional siRNAs, in that they inhibit the expression of reporter genes with complementary sequences. Inhibition of small VA1 RNA function negatively affected adenovirus production, indicating that they are required for optimal virus replication (64). Adenoviruses thus appear to utilize the endogenous miRNA-guided RNA silencing pathway to their advantage in viral production.

3.3 TAR RNA Binding Protein and HIV-1

Initially discovered in 1991, TRBP is a cellular factor acting in synergy with the viral TAT protein in the transactivation of the long terminal repeat (LTR) of human immunodeficiency virus type 1 (HIV-1), a process that results in the transcription of viral genes (66). TRBP is also known to interact with the protein Tax encoded by the human T-cell leukemia virus type 1 (HTLV-1) and inhibits its function of activation of the transcription from the LTR via the association with host cellular factors (67). TRBP exerts other functions, including inhibition of the interferon (IFN)-induced dsRNA regulated protein kinase R (PKR) (68), as well as a growth promoting role with oncogenic potential activity (69). The tumor suppressor Merlin regulates the oncogenic activities of TRBP through a direct interaction (70).

A link has been established between HIV-1 and protein components of the miRNA-guided RNA silencing pathway. TRBP was found to interact directly with Dicer in coimmunoprecipitation experiments (19,21). TRBP possesses three dsRBD and is found in two isoforms in the cell, with TRBP2 being 21 amino acids longer than TRBP1 (68,71,72). The structure involved in the interaction with Dicer includes the third C-terminal dsRBD of TRBP, as determined by deletional analysis in the yeast two-hybrid system (19). Furthermore, pre-miRNA processing is affected when TRBP is depleted in cells (19), suggesting that its presence is required for an efficient miRNA-guided RNA silencing pathway. Although TRBP appears to be an accessory protein of Dicer, in vitro analyses demonstrated that Dicer can efficiently cleave its substrate in the absence of TRBP (21), suggesting that TRBP is not as essential to Dicer as DGCR8 is for Drosha. TRBP is also part of a ternary complex comprising Dicer and Ago2 in a sequence of events in which TRBP is required for the recruitment of Ago2 to the siRNA bound by Dicer (21), thereby coupling the initiation step of the miRNA processing and the effector steps of the miRNA pathway. TRBP may thus exert a dual role in HIV-1 pathogenesis and RNA silencing (73), but also in other important physiological processes.

3.4 PKR-Activating Protein and the PKR Signaling Pathway

Recently, a new protein has been reported to interact with a complex containing Dicer, Ago2, and TRBP: the PKR-activating protein (PACT). The direct interaction between PACT and Dicer involves the third dsRBD domain of PACT and the N-terminal region of Dicer containing the putative helicase motif (74). The accumulation of mature miRNAs in vivo and the efficiency of siRNA-induced RNAi are affected upon depletion of PACT (74). These findings were confirmed in shRNA-induced RNAi, but not when using siRNAs, suggesting that TRBP and PACT function primarily in siRNA production (75). Moreover, the presence of both TRBP and PACT increased the ability of Dicer to cleave a 566-bp long dsRNA in vitro, as compared to TRBP and PACT added individually (75).

PACT and TRBP share 44% of sequence identity and both possess three similar dsRBD domains. They bind to PKR, although exerting opposing effects, i.e. PACT activates PKR, whereas TRBP inhibits it (76–78). The third dsRBD of PACT and TRBP, which is devoid of any detectable dsRNA binding activity (78), mediates their interaction with Dicer as well as their regulation of PKR (19,21,78). The C-terminal domain of PACT can also mediate homomultimerization (79). Interestingly, a recent study demonstrated that PACT directly

interacts with TRBP and that this complex associates with Dicer to facilitate production of siRNAs (75).

PACT and TRBP may establish an interesting, but intriguing redundant link between the miRNA-guided RNA silencing pathway and the PKR signaling pathway. PKR is a dsRNA-dependent serine/threonine protein kinase that phosphorylates the translation initiation factor eIF2 to cause a general reduction of protein synthesis (80). PKR is activated in response to dsRNA of cellular, viral or synthetic origin, with a size greater than ~30 nt, but not by siRNA. PKR thus mediates a critical role in response to dsRNA, acting as a sensor of viral infections (for review see García et al. (81)). For example, VA1 is known to play a key role in blocking activation of protein PKR during the adenoviral replication cycle, presumably by viral dsRNAs. In the absence of VA1, activation of PKR induces phosphorylation of the translation factor eIF-2 α and, hence, inhibition of viral mRNA translation (81,82). It is not surprising that viruses evolved in a way that they can inhibit PKR signaling.

3.5 Fragile X Mental Retardation Protein and the Fragile X Syndrome

In human, loss of expression of the *FMR1* gene product is the etiologic factor of the fragile X syndrome, the most frequent cause of inherited mental retardation (83,84). The *FMR1* gene, which spans ~38 kb, is located in the q27.3 region located at the tip of the X chromosome long arm (85). The syndrome is transmitted as an X-linked dominant trait and it affects about 1 in 4000 males, who will develop in almost all cases moderate to severe mental retardation (IQ ~50), and about 1 in 7000 females, who present in general a milder mental handicap (85). FMRP has been detected in practically every tissue in humans and rodents, with high levels in the brain, testes, esophagus, lung, and kidney (86).

FMRP has been identified in a miRNP complex containing Dicer and Ago2 proteins in mammalian cells in vivo (87). FMRP is an RNA-binding protein (88,89) known to be involved in the regulation of mRNA translation, mRNA transfer and local modulation of synaptic mRNA translation (90–94). FMRP has been reported to behave as a negative regulator of translation, both in vitro and in vivo (90–94).

Our group recently showed that human FMRP can act as a miRNA acceptor protein for the RNase III Dicer and facilitate assembly of miRNAs on specific target RNA sequences (95). We have proposed a model in which FMRP could facilitate miRNA assembly on target mRNAs. Functioning within a duplex miRNP, FMRP may also mediate mRNA targeting through a strand exchange mechanism, in which the miRNA* of the duplex is swapped for the mRNA (96). Furthermore, FMRP may contribute to the relief of miRNA-guided mRNA repression through a reverse strand exchange reaction, possibly initiated by a specific cellular signal, that would liberate the mRNA for translation (96). Although the intracellular sites hosting these events remain to be determined, we cannot exclude the possible involvement of the P-bodies.

We hypothesized that the absence of FMRP expression may result in suboptimal miRNA assembly on, and/or disassembly from, their natural mRNA targets, leading to a perturbed protein expression profile (96). This may be expected given the requirement of FMRP for efficient small RNA-guided gene regulation (95). Elucidation of the exact role and function

of FMRP in miRNA-guided gene regulation may hold key to determining the molecular basis of the fragile X syndrome and establishing a causal link between dysfunction of the RNA-silencing machinery and a human genetic disease.

4. Conclusion

The core protein components of the miRNA-guided RNA silencing machinery are essential for cellular homeostasis. However, deregulation of some accessory proteins can be tolerated by the organism, yet resulting in a specific disease. Although attractive, hypotheses and correlations have been established between the role exerted by these components in the miRNA pathway and the phenotype related to their deregulation, these issues remain to be validated. Only then we could examine whether the clinical portrait of the patients harboring a defective protein component of the miRNA pathway could be improved upon a therapy aimed at restoring the functionality of the miRNA silencing machinery.

Acknowledgments

We are grateful to Gilles Chabot for graphic illustration. M. P. P. was supported by a doctoral studentship from Natural Sciences and Engineering Research Council of Canada (NSERC). P. P. is a New Investigator of the Canadian Institutes of Health Research and Junior 2 Scholar from the Fonds de la Recherche en Santé du Québec. This work was financially supported by a Discovery grant from NSERC (262938-03).

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Figure 1.

Protein components of the microRNA-guided RNA silencing pathway and human diseases. Encoded by the genome, microRNA (miRNA) and messenger RNA (mRNA) genes are transcribed by RNA polymerase II in the nucleus. The primary microRNA (pri-miRNA) is recognized and cleaved by the Microprocessor complex, which contains Drosha and the DiGeorge syndrome critical region gene 8 (DGCR8) protein, to form the miRNA precursor (pre-miRNA). Following its export into the cytoplasm via Exportin-5 (Exp-5), in a Ran-GTP-dependent manner through the nuclear pore complex (NPC), the pre-miRNA is cleaved by the Dicer-TAR RNA binding protein (TRBP) complex into a miRNA-miRNA* duplex. This complex is then joined by Argonaute 2 (Ago2) to form a miRNA-containing ribonucleoprotein (miRNP) complex, after which the miRNA strand is selected. Depending on the degree of complementarity between the miRNA and its mRNA target, the miRNP complex will either (1) mediate mRNA cleavage if the complementarity is perfect, or (2) initially inhibit mRNA translation if the complementarity is imperfect. In this latter case, the repressed mRNA is translocated to the P-bodies, after which the mRNA can either be destroyed or relocalized to the translational machinery for expression upon a specific cellular signal.