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MINIREVIEW

miRNAs and regulation of cell signaling

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MicroRNAs (miRNAs) regulate gene expression post-transcriptionally by binding to target mRNAs in a sequence-specific manner. A large number of genes appear to be the target of miRNAs, and an essential role for miRNAs in the regulation of various conserved cell signaling cascades, such as mitogen-activated protein kinase, Notch and Hedgehog, is emerging. Extensive studies have also revealed the spatial and temporal regulation of miRNA expression by various cell signaling cascades. The insights gained in such studies support the idea that miRNAs are involved in the highly complex network of cell signaling pathways. In this minireview, we present an overview of these complex networks by providing examples of recent findings.

Introduction

In higher organisms, the regulation of the transcriptome is extremely complicated. Traditionally, regulation of the transcriptome referred mainly to the activation or repression of gene expression by transcription factors. However, gene expression in higher organisms is now known to be controlled by a multilayered regulatory network that includes epigenetic modification of the genome and post-translational modification of gene products. The discovery of microRNAs (miRNAs), which regulate gene expression post-transcriptionally, has added to the complexity of transcriptional regulation. At present, the expression of miRNAs can be profiled using various available platforms, which are based on microarrays, high-throughput sequencing or quantitative real-time PCR. Many studies have reported that miRNAs show specific spatiotemporal patterns of expression. Expression profiling studies have identified miRNAs that are specific to particular organs or cell lines and have revealed an inverse correlation between the expression of a miRNA and that of its target mRNAs [1]. Several previous studies have revealed that miRNAs play an important role in various cellular processes, including proliferation, differentiation, apoptosis and development [2]. The negative regulation of gene expression by miRNAs has been reported to contribute to the fine regulation of important physiological and pathological responses, such as oligodendrocyte cell differentiation [3], epigenetic modification [4] and DNA damage response [5], as well as embryonic stem cell function and fate [6]. Further studies have demonstrated that a large number of miRNAs are under the control of various important signal transduction cascades. These miRNAs appear to contribute to the regulation of different signaling pathways via the

Abbreviations
AP-1, activation protein 1; EcR, ecdysone receptor; EMT, epithelial–mesenchymal transition; ERα, estrogen receptor-α; ERK, extracellular signal-regulated kinase; GPC, granule cell progenitor; Hh, Hedgehog; MAPK, mitogen-activated protein kinase; MB, medulloblastoma; miRNA, microRNA; NF-κB, nuclear factor kappa B; R-smad, receptor-regulated SMAD; TGF, transforming growth factor.

repression of their target genes, which results in the regulation and modulation of signal transduction [7]. However, the precise mechanisms that regulate miRNA expression remain unclear.

In this minireview, we describe the role of miRNAs with respect to the complicated regulation of the transcriptome and signal transduction. Although miRNAs and well-established cell signaling pathways have been the subject of recent reviews [7–10], few have focused upon the role of miRNAs in regulatory network of various cell signaling pathways. We summarize the current knowledge of the interdependence of miRNA and cell signaling pathways, which results in highly complicated networks for the regulation of the transcriptome. Current findings on the role of miRNAs in cardiac diseases [11] and recent discoveries involving the miRNA–epigenetics regulatory network [12] are reviewed in the accompanying minireviews.

miRNAs are involved in various signal cascades

First, we focus on the roles of miRNAs in various conserved signaling pathways. Many miRNAs are induced by the action of conserved signaling pathways but, in turn, the induced miRNAs regulate these pathways by repressing the expression of components of the signaling pathways and, in some cases, components of other signaling pathways, thus forming a complex regulatory network (Fig. 1).

The mitogen-activated protein kinase (MAPK) signaling pathway is a highly conserved module that is involved in various cellular functions, including cell proliferation, differentiation and migration [13]. Recently, the mechanisms of transcription and the functional roles of miRNAs associated with MAPK signaling have been revealed. miR-21 is one of the most interesting examples of an miRNA that is associated with the MAPK signaling pathway. Thum *et al.* [14] reported that miR-21 regulates the extracellular signal-regulated kinase (ERK)/MAPK signaling pathway in cardiac fibroblasts [14]. The expression of miR-21 is increased selectively in fibroblasts of the failing heart, which augments ERK/MAPK activity through the inhibition of sprouty homolog 1, a negative regulator of MAPK [15]. Furthermore, it has been reported that miR-21 is upregulated during cardiac hypertrophic growth and represses the expression of Sprouty 2 (Spry2), which negatively regulates ERK1/2 [16]. Hence, miR-21 increases the basal activity of ERK1/2 by repressing Spry2. Recently, Huang *et al.* [17] reported that the expression of miR-21 is upregulated via the ERK1/2 pathway upon stimulation of HER2/neu signaling and that miR-21 suppresses the metastasis suppressor protein PDCD4 (programmed cell death 4) in breast cancer cells. The expression of miR-21 is also upregulated by overexpression of other ERK1/2 activators, such as RASV12 and ID-1, in HER2/neu-negative breast cancer cells. Moreover, Fujita *et al.* [18] have reported the activation of miR-21 expression by 4β-phorbol 12-myristate 13-acetate in HL60 cells [18]. The transcription factor activation protein 1 (AP-1) triggers the expression of miR-21 through binding to several AP-1 binding sites that are found in the promoter of the gene for miR-21. Taken together, these studies suggest that miR-21 acts as a positive-feedback regulator of the MAPK-ERK signaling pathway because miR-21 is both induced by the activation of ERK1/2 and enhances the activity of ERK1/2 by repressing negative regulators of the ERK/MAPK signaling pathway.

Some other miRNAs are also reported to be induced by the MAPK signaling pathway. In the human B-cell line Ramos, miR-155 is induced by signaling by the B-cell receptor through the ERK and *c*-Jun N-terminal kinase pathways but not by the p38 pathway. The induction of miR-155 depends on a conserved AP-1 site that is approximately 40 bp upstream from the site of initiation of miR-155 transcription [19]. We previously reported that simulation with nerve growth factor induced the expression of miR-221 and miR-222 in PC12 cells, and that this induction is dependent on sustained activation of the ERK1/2 pathway [20]. Furthermore, the induction of miR-34a depends on the activation of ERK1/2 in K562 cells [21,22]. We have demonstrated that the activation of MEK/ERK signaling by 4β-phorbol 12-myristate 13-acetate induces the expression of miR-34a, which then inhibits MEK1 expression, and leads to the repression of cell proliferation during megakaryocytic differentiation in K562 cells [21]. In addition, miR-34c is induced under the control of both p53 and p38-MAPK, and prevents Myc-dependent DNA replication by targeting c-Myc [23]. Kawashima *et al.* [24] reported that brain-derived neurotrophic factor upregulates miR-132 expression via the ERK-MAPK pathway, which results in the upregulation of glutamate receptors in cultured cortical neurons. These studies indicate that many miRNAs are involved in the MAPK signaling pathway and these miRNAs have important roles in various cellular functions. Because a single miRNA usually targets many genes, the influence of miRNAs on the components of different signaling pathways could be complex. Many studies in various model organisms, including *Drosophila* and *Caenorhabditis elegans*, have provided evidence to support this scenario.

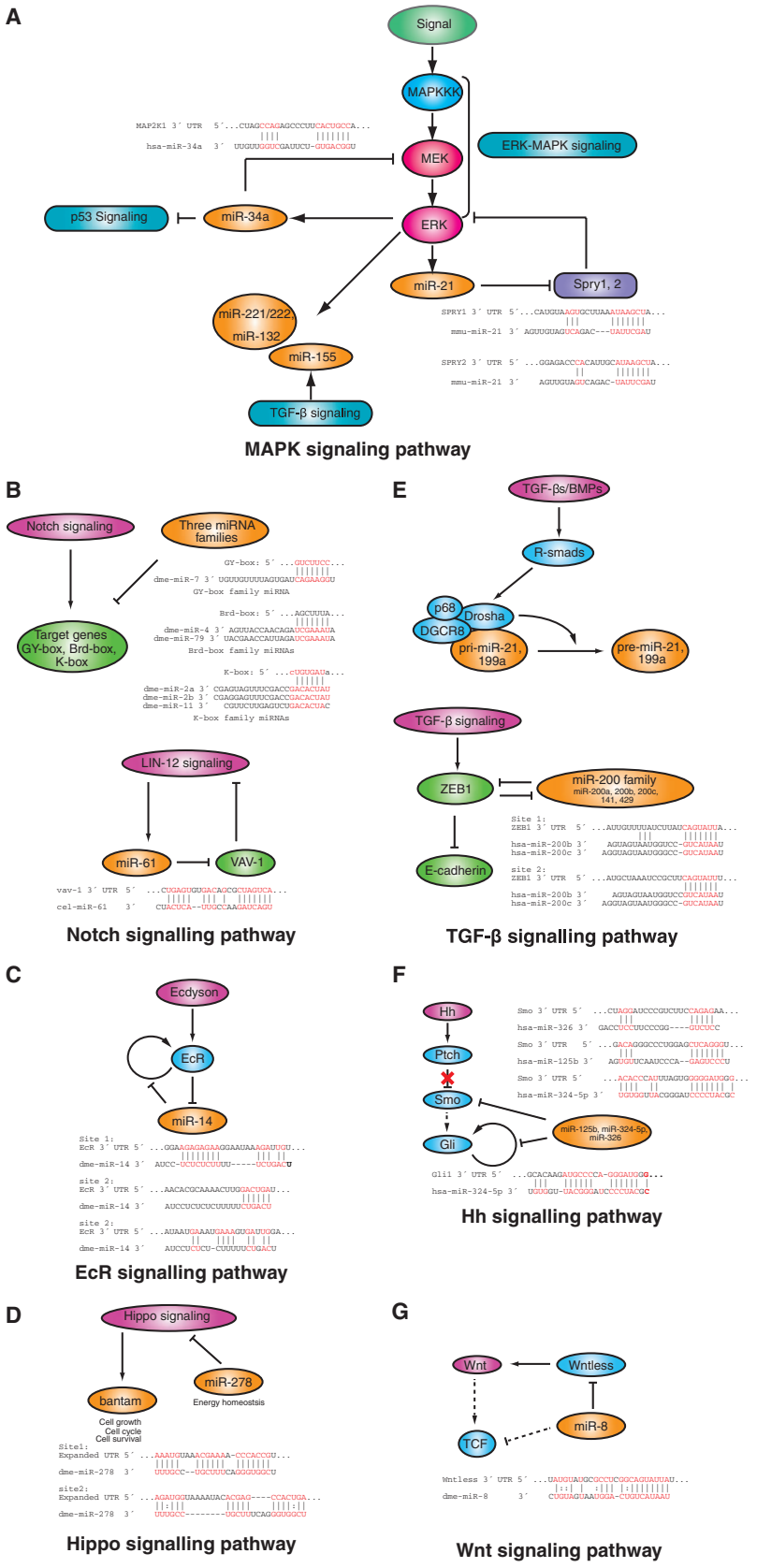

Fig. 1. Involvement of miRNAs in various signaling cascades. Many miRNAs are under the control of various conserved signaling pathways and in turn regulate components of these pathways, which results in the formation of complex regulatory networks. Model of regulatory networks in the (A) MAPK signaling pathway, (B) Notch signaling pathway, (C) EcR signaling pathway, (D) Hippo signaling pathway, (E) TGF-β signaling pathway, (F) Hh signaling pathway, and (G) Wnt signaling pathway.

The Notch signaling pathway plays an essential role in a variety of biological processes in multicellular organisms. In *Drosophila*, two large families of Notch target genes are clustered at two genomic locations. These families are named the bearded and enhancer of split complexes. These Notch target genes contain conserved motifs, which are named the GY-box, Brd-box and K-box, in their 3′ UTR. The members of three different families of miRNAs (miR-2, miR-4, miR-7, miR-11 and miR-79) have been shown to regulate the Notch target genes, negatively, by binding to these motifs. This negative regulation prevents the aberrant activation of Notch signaling [25]. In *C. elegans*, miR-61 is a direct transcriptional target of lin-12/Notch. In addition, miR-61 targets Vav-1, which is a negative regulator of LIN-12, and hence functions in a positive-feedback manner [26].

A steroid receptor signaling pathway in flies is also reported to be regulated by an miRNA. Ecdysone receptor (EcR) signaling constitutes an autoregulatory loop, in which the activation of EcR induces the expression of EcR itself. miR-14 targets EcR mRNA and modulates this loop. Interestingly, EcR signaling reciprocally regulates transcription of the genes for miR-14 and EcR. This prevents activation of the loop by transient transcriptional noise [27].

The Hippo signaling pathway, which is involved in the control of tissue growth, has been studied extensively in *Drosophila* and recently emerged as an important contributor to turmorigenesis in vertebrates. The *Drosophila* miRNA bantam is a direct transcriptional target of the Hippo signaling pathway, and it has been shown to promote growth and inhibit apoptosis [28,29]. The *Drosophila* miR-278 plays a role in the control of energy homeostasis. This miRNA is also known to target and regulate a component of the Hippo signaling pathway [30,31]. However, no homologs of bantam or miR-278 are found in vertebrates and no functionally equivalent miRNAs have been found to date. In humans, miR-372 and miR-373, which have been implicated as oncogenes in tumors of testicular germ cells, have been reported to target and regulate LATS2, which is a homolog of a component of the Hippo signaling pathway [32].

An interesting finding concerning the biogenesis of miRNAs has been reported with respect to signaling by members of the transforming growth factor β (TGF-β) family [33]. Receptor-regulated SMADs (R-smads) are involved in the processing of pri-miRNAs. Stimulation by an appropriate ligand causes the recruitment of R-smads to specific pri-miRNAs that are bound to the Drosha–DiGeorge syndrome critical region gene 8 complex and RNA helicase p68. The recruitment of the R-smads stimulates the production of these miRNAs and thus represses the expression of their target genes. TGF-β signaling is known to be involved in the epithelial–mesenchymal transition (EMT). The transcription factors ZEB1 and ZEB2 are downstream mediators of TGF-β signaling and negatively regulate the expression of E-cadherin. The miR-200 family is reported to target ZEB1 and ZEB2, which results in the inhibition of EMT in vertebrate cell lines [34–36]. The miR-200 family is markedly decreased in cells that have undergone EMT as a result of stimulation with TGF-β [35]. Interestingly, ZEB1 reciprocally represses the expression of the miR-200 cluster and hence promotes EMT in a feed-forward manner [37].

The Hedgehog (Hh) signaling pathway has a pivotal role in animal development and functions as a master regulator of cerebellar granule cell progenitors (GPCs). Medulloblastoma (MB) is the most common pediatric brain malignancy and is caused by the disruption of Hh signaling. Microarray analysis of human MBs with high levels of Hh signaling identified miRNAs that had been downregulated. Some of these miRNAs (miR-125b, miR-326 and miR-324-5p) target activator components of the Hh signaling pathway and suppress Hh signaling, which suggests that these miRNAs are involved in MB. miR-324-5p also targets a downstream transcriptional regulator of Hh signaling and, interestingly, is located in a genomic region whose deletion is associated with MB. Moreover, the above-mentioned miRNAs are upregulated during GPC differentiation, which suggests that they might function *in vivo* by inhibiting Hh activity during the differentiation of GPCs [38].

With respect to the Wnt signaling pathway, a screening assay has identified miRNAs that modulate Wnt signaling [39]. In *Drosophila*, miR-8 negatively regulates Wnt signaling at multiple levels, targeting the downstream component T cell factor and two upstream positive components, including Wntless, which is required for the secretion of Wnt. Mammalian homologs of miR-8 were also shown to inhibit Wnt signaling in a cell culture model [39].

Taken together, the results show that the transcriptional hierarchy downstream of various important signal cascades appears to include multiple miRNAs. miRNAs may mediate cross-talk between various signaling pathways via the repression of their target genes. Indeed, several examples of feedback regulation that involve miRNAs have been reported. Below, we attempt to summarize our recent understanding of feedback regulation of signal cascades that involve miRNAs.

miRNAs act as feedback regulators of signal cascades

miR-34a is one of the most interesting examples of an miRNA that is associated with a complicated regulatory mechanism of gene expression. Initially, miR-34a was identified as a putative tumor suppressor that regulates the E2F signaling pathway and induces apoptosis in neuroblastoma cells [40]. Moreover, it was reported that the direct transactivation of miR-34a contributes to p53-mediated apoptosis in various tumors [41–44]. Subsequently, SIRT1, which is a regulator of p53 activation, was reported to be a target of miR-34a, which suggests that miR-34a participates in a double-negative-feedback loop and contributes to the fine-tuning of p53 activity [45,46]. miR-34a is also induced by a p53-independent pathway: ELK1, which is a member of the ETS family of transcription factors, mediates the induction of miR-34a during cell senescence caused by the constitutive activation of the kinase B-RAF [47]. In addition, both ourselves [21] and Navarro *et al.* [22] identified TPA-dependent transactivation of miR-34a during megakaryocytic differentiation of K562, which is a p53-null chronic myelocytic leukemia cell line. The finding that TPA-induced upregulation of miR-34a depends on the activation of the ERK signal cascade and that miR-34a downregulates MEK1, which is one of the main regulators of ERK signaling, indicates that miR-34a is involved in negative-feedback regulation of the ERK signal cascade. These studies indicate that a complicated regulatory network maintains the expression of the signaling molecules and miR-34a; at least three signalling pathways affect the expression of miR-34a and two of their components are negatively regulated by miR-34a (Fig. 2).

Some other mutual regulatory relationships between miRNAs and various signaling pathways have been reported. Xu *et al.* [48] proposed the existence of a double-negative-feedback loop controlled by miR-145 and three factors that regulate self-renewal and pluripotency: OCT4, SOX2 and KLF4. Castellano *et al.* [49] revealed that the expression of estrogen receptor-α (ERα) is autoregulated by miR-18a, -19b and -20b, which in turn are upregulated by the activation of ERα. This mechanism of regulation provides a wide range of coordinated cellular responses to estrogen [49]. In the self-renewal of neural stem cells, miR-9 acts with the nuclear receptor TLX to provide a feedback regulatory loop that controls the balance between neural stem cell proliferation and differentiation [50]. miR-9 is induced by lipopolysaccharide via the activation of the receptor TLR4 and also is involved in the feedback control of nuclear factor kappa B (NF-κB)-dependent responses by inhibiting the expression of NFKB1 in human polymorphonuclear neutrophils [51].

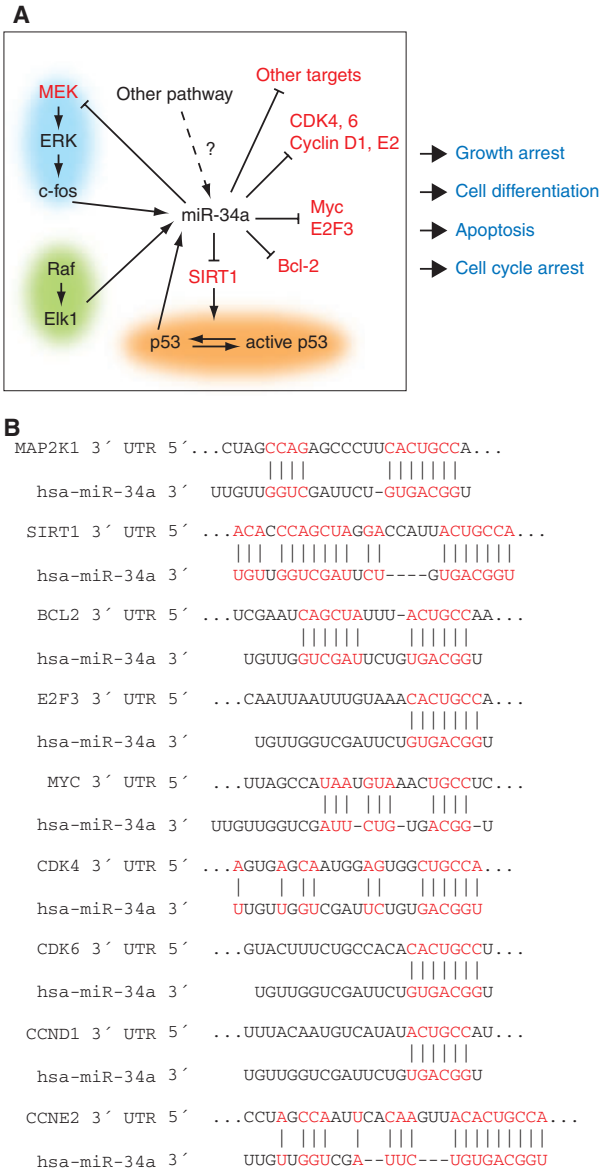

Fig. 2. miR-34a is regulated by three signaling pathways. The findings of nine studies are summarized in this model [21,22,41–47]. (A) miR-34a is regulated by at least three signaling pathways. Two components of these pathways are negatively regulated by miR-34a. miR-34a mediates several biological functions by repressing the indicated targets and presumably hundreds of other as yet unidentified targets. (B) miR-34a and the miR-34a-binding site in the 3′ UTR of genes shown in (A).

Feedback regulation by miRNAs in the context of cancer has also been reported. Aguda *et al.* [52]

Table 1. miRNAs involved in the feedback regulation of signal cascades.

miRNA(s)	Gene targets	Related signal cascade(s) and/or transcription factors	Reference
miR-34a	MYC, SIRT1, MEK1, CDK4, CDK6	p53, ELK1, ERK-MAPK	[21,22,40–47]
miR-145	Oct4, SOX2, KLF4	Oct4	[48]
miR-18a, 19b, 20b	ERα	ERα	[49]
miR-9	TLX, NFKB1	TLX, TLR4-NF-kappaB	[50,51]
miR-17-92	E2F, Myc	E2F, Myc	[52]
miR-200a, 200b, 429	ZEB1/deltaEF1, SIP1/ZEB2	ZEB1-SIP1	[53]
miR-17-5p/20a	Cyclin D1	cyclin D1	[54]
miR-206	ERα	ERα	[55]
miR-15a	c-Myb	c-Myb	[61]
let-7	Dicer	miRNA processing cascade	[62,63]
miR-21	Spry1, Spry2, PDCD4, NFIB	MAPK, AP-1, NFIB, RASV12, ID-1	[18,64]
miR-132	MeCP2	MeCP2	[65]
miR-61	VAV1	LIN-12/Notch	[26]

reported that members of a cluster of miRNAs, called miR-17-92, form a negative-feedback loop that is involved in cancer. The expression of miR-17-92 is induced by the transcription factors E2F and Myc but, in turn, miR-17-92 downregulates the expression of E2F and Myc [52]. In tumor progression, the transcription repressors ZEB1 and SIP1 and the miR-200 family of miRNAs provide a double-negative-feedback loop that regulates the phenotype of cells [53]. Furthermore, in human breast tumors and cell lines, miR-17-5p and miR-20a are induced in a manner that depends on cyclin D1 and repress the expression of cyclin D1. Hence, miR-17-5p/20a and cyclin D1 form a feedback loop and have a regulatory role in oncogenesis [54]. miR-206 and ERα repress the expression of each other reciprocally in the human breast cancer cell line MCF-7 in a double-negative-feedback loop [55].

Various other examples of feedback regulation that involve miRNAs have been reported for several important biological processes. The miRNAs that are known to be involved in feedback regulation, their target genes and the signal cascades affected are summarized in Table 1. Such studies demonstrate the highly complex regulation of signal cascades and the physiological and pathological roles of miRNAs. Hence, further investigations aiming to elucidate the mechanisms and signal cascades that regulate the expression of miRNAs should reveal complicated and multilayered cell signaling networks.

Conclusions

Considering the broad range of miRNA targets, it is possible that regulatory networks for the control of gene transcription will become much more complex as additional research is carried out [56,57]. Yu *et al.* [58] investigated the cross-talk between miRNAs and transcription factors using mathematical modeling and revealed the existence of two classes of miRNAs with distinct network topological properties. Although this analysis demonstrated extensive interaction between miRNAs and transcription factors, biological validation of mathematical models is very challenging. However, recent advances with respect to high-throughput sequencing technologies have enabled, in combination with chromatin immunoprecipitation, the cost-effective functional genome-wide investigation of transcription factor binding sites [59]. Argonaute high-throughput sequencing of RNAs from *in vivo* cross-linking and immunoprecipitation also provides genome-wide interaction maps for miRNAs and mRNAs, which enables comprehensive identification of miRNA targets [60]. By integrating mRNA and miRNA sequence and expression data with these comparative genomic data, we will be able to gain global, and yet specific, insights into the function and evolution of a broad layer of post-transcriptional control. These comprehensive analyses will yield many additional examples of functionally relevant regulatory roles of miRNAs in cell signaling pathways. The elucidation of these examples will clarify novel functions and biological roles of miRNAs.

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