MicroRNAs: A new class of regulatory genes affecting metabolism

Minireview

Jan Krützfeldt[1] and Markus Stoffel[1,*]

[1] Laboratory of Metabolic Diseases, The Rockefeller University, 1230 York Avenue, New York, New York 10021
*Correspondence: stoffel@rockefeller.edu

MicroRNAs (miRNAs) are short noncoding RNAs that regulate gene expression by binding to target mRNAs, which leads to reduced protein synthesis and sometimes decreased steady-state mRNA levels. Although hundreds of miRNAs have been identified, much less is known about their biological function. Several studies have provided evidence that miRNAs affect pathways that are fundamental for metabolic control in higher organisms such as adipocyte and skeletal muscle differentiation. Furthermore, some miRNAs have been implicated in lipid, amino acid, and glucose homeostasis. These studies open the possibility that miRNAs may contribute to common metabolic diseases and point to novel therapeutic opportunities based on targeting of miRNAs.

MicroRNAs (miRNAs) are 19–22 nucleotide RNAs that regulate gene expression posttranscriptionally by base pairing with complementary sequences in the 3′ untranslated regions (3′UTRs) of protein-coding transcripts. This interaction leads to translational repression and, in many cases, to decreased mRNA levels (Valencia-Sanchez et al., 2006). More than 300 human miRNAs, many of them evolutionarily conserved, are currently listed in the miRNA Registry version 7.1 (Griffiths-Jones, 2004). The total number of miRNAs encoded in the human genome is currently unclear. Computer-based predictions estimate that microRNAs constitute as many as 2%–3% of all genes in the genome (Berezikov et al., 2005). The function of most miRNAs is largely unknown, but many miRNAs in invertebrates and vertebrates are clearly involved in important cellular processes such as differentiation and development (Alvarez-Garcia and Miska, 2005). It is estimated that up to one-third of all human genes may be miRNA targets (Lewis et al., 2005).

Like conventional mRNAs, miRNAs are transcribed by RNA polymerase II as long primary transcripts that are capped, polyadenylated, and spliced (Kim, 2005). The genomic localization of many miRNAs overlaps with known protein coding or noncoding genes (Rodriguez et al., 2004). These miRNAs share expression patterns with their host-gene mRNA, thus indicating that they are coordinately expressed. Furthermore, approximately 40% of miRNAs are located in clusters that are <3.0 kb apart (Altuvia et al., 2005). MicroRNAs are processed into 19–22 nt duplexes by a two-step process involving nuclear and cytosolic RNase III-type endonucleases, known as Drosha and Dicer, to yield the ''mature'' miRNA. In a final step, this RNA duplex is loaded into the RNA-induced silencing complex (RISC), one of the strands is eliminated, and the remaining strand engages in imperfect base pairing with specific sequences in their target mRNAs.

The ability to control the rates of metabolic processes in response to changes in the internal or external environment is indispensable for all living cells. Mechanisms that are essential for metabolic control and maintenance of homeostasis are complex and involve transcriptional, translational, posttranslational, and allosteric regulation. MiRNAs constitute a novel class of genes that add a new level of regulation and fine tuning for gene expression that is likely to be important for a wide range of cellular functions, including metabolism.

Although the function of most miRNAs is currently unknown, several studies indicate that they may play important roles in diverse aspects of signaling and metabolic control. The earliest phylogenetic evidence for a role of miRNA in responses to extracellular stimuli comes from plants, where particular miRNAs have been shown to accumulate in response to sulfur starvation, abiotic stresses, or phytohormones (Jones-Rhoades et al., 2006). For instance, several miRNAs have been identified as influencing transcriptional regulators that are altered by auxin, a phytohormone that plays critical roles during plant growth. More recently, several intriguing studies have begun to shed light on the role of miRNAs for metabolic control in invertebrates and vertebrate animals.

Invertebrates

The first evidence for participation of miRNAs in metabolism came from a forward genetic screen in the fruit fly *Drosophila melanogaster*. Xu et al. (2003) found that loss of miR-14 doubled the amount of total body triacylglycerides. Conversely, additional copies of miR-14 in the fly genome decreased triacylglyceride levels. The mechanism for this effect is unknown. Using a similar screening method, Teleman and Cohen (2006) more recently identified a role for miR-278 in energy homeostasis of *Drosophila*. MiR-278 is prominently expressed in the fat body of flies, and homozygous mutations for miR-278 have a smaller fat body and reduced ratio of total body triglycerides to total protein. This phenotype could be rescued by miR-278 expression. Mutant flies also exhibited hyperglycemia in spite of elevated insulin-like peptide levels. The authors conclude that miR-278 regulates insulin sensitivity through its target, the membrane-associated protein *expanded*. These two studies point to an important role for miRNAs in fat tissue of *Drosophila*. Work by Sokol and Ambros (2005) demonstrated that miR-1, one of the most highly conserved miRNAs in the animal kingdom, is also essential for proper skeletal muscle function in the fruit fly. Loss-of-function of muscle-specific miR-1 in *Drosophila* results in small, immobilized larvae with severely deformed musculature. The lethality of these mutants can be rescued when

a *miR-1* transgene is expressed specifically in the mesoderm and muscle. Interestingly, feeding triggers *DmiR-1KO*-associated paralysis and death in *DmiR-1KO* flies, a phenotype that can be prevented when *DmiR-1KO* larvae are starved. These results indicate that *DmiR-1* is not required for the formation or physiological function of the larval musculature but is essential for postmitotic growth of larval muscle. The target genes of miR-1 that mediate this effect are unknown. The high evolutionary conservation of miR-1 suggests that miR-1 could also be important for adaptation of vertebrate skeletal muscle to environmental challenges.

In another invertebrate organism, *Caenorhabditis elegans*, the miRNA lin-4 has been found to be critical in development. Recently, it was shown that lin-4 also regulates life span in this organism (Boehm and Slack, 2005). Animals with loss-of-function mutation in lin-4 displayed a life span that was significantly shorter than that of the wild-type. In contrast, overexpression of lin-4 led to a lengthened life span. Lin-4 binds with imperfect complementarity to the 3′UTR of its target, lin-14, to prevent its translation. Consistent with this data, mutations in lin-14 exhibited longevity. Interestingly, life-span extension conferred by a reduction in lin-14 was dependent on the forkhead transcription factor daf16, which is known to regulate life span through insulin-PI3 kinase-Akt signaling. It is intriguing that the closest daf-16 homologs in mammals are the transcription factors Foxo1 and Foxa2, both of which are regulated by insulin signaling and play important roles in glucose and lipid homeostasis (Puigserver et al., 2003; Wolfrum et al., 2004). While the exact mechanism of the extended-life-span phenotype remains to be determined, the data provide an example of gene-regulation crosstalk of miRNAs and insulin signaling. Thus, the above summarized studies provide persuasive evidence that connects miRNA function and metabolism in invertebrates. However, it is worth noticing that, with the exception of miR-1, the analyzed miRNAs are not conserved in vertebrates. Thus, these results cannot directly be related to mammalian and human tissues. However, it is still possible that miR-14 and miR-278 regulate the expression of evolutionarily conserved target genes that may shed light on novel pathways affecting insulin signaling and energy homeostasis.

Vertebrates

The first evidence for a role of miRNAs in hormone secretion in vertebrates came from an unbiased cloning approach of small RNAs from the pancreatic β cell line MIN6 (Poy et al., 2004). This approach identified 11 novel miRNA sequences, among them two pancreatic-islet-enriched miRNAs, miR-375 and miR-376. Overexpression of miR-375 decreased insulin secretion, while inhibiting miR-375 function increased insulin release from pancreatic β cells. Although the precise mechanism for the negative regulation of insulin secretion by miR-375 is not understood, this miRNA seems to inhibit the exocytosis process of insulin-secretory granules, possibly through SNARE proteins (e.g., Vti-1a) and myotrophin, two predicted and experimentally validated target genes of miR-375. Future studies of mutant mice with genetic miR-375 deletions will be able to address whether this miRNA also affects pancreatic β cell proliferation, differentiation, and survival.

There is also emerging evidence that miRNAs might play a role in differentiation of insulin-sensitive organs such as the adipocyte and muscle. Esau et al. (2004) inhibited the activity of 86 miRNAs in primary human preadipocytes with 2′-O-methoxyethyl (2′-O-MOE) phosphorothioate-modified antisense RNA oligonucleotides (ASO) by transfection prior to induction of differentiation. They found that two ASOs targeting miR-9* and miR-143 inhibited the differentiation process as assessed by a reduction in triglyceride accumulation and decreased expression of adipocyte-specific genes. Furthermore, miR-143 expression levels were found to be higher in adipocytes than in preadipocytes. In muscle, miRNAs have been implicated in myoblast differentiation. MiR-1 and miR-133 are transcribed from a common polycistronic gene in a tissue-specific manner during development (Chen et al., 2006). However, they have opposite roles in modulating skeletal muscle proliferation and differentiation in cultured myoblasts in vitro and in *Xenopus laevis* embryos. MiR-1 promotes myogenesis by targeting histone deacetylase 4 (HDAC4), a transcriptional repressor of muscle gene expression. In contrast, miR-133 enhances myoblast proliferation by repressing the serum response factor (SRF). These data demonstrate that miRNAs can carry out distinct biological functions even when they derive from a common precursor. The skeletal muscle phenotype is controlled not only by miR-1 and miR-133 but also by miR-181. Mir-181 is strongly upregulated during differentiation of embryonic stem cells and myoblast cell lines and is required for this process, partly through inhibition of its target Hox-A11, an inhibitor of MyoD (Naguibneva et al., 2006). Interestingly, SRF and MyoD itself are transcriptional regulators of miR-1 (Zhao et al., 2005). Together, these findings add to the complexity of coordinated gene expression and transcriptional circuits that control skeletal muscle gene expression during development and possibly regeneration (Figure 1).

Several lines of evidence indicate that miRNAs may also regulate pathways in amino acid metabolism. Mersey et al. (2005) showed that the vertebrate miR-29b controls the amount of the branched-chain α-ketoacid dehydrogenase (BCKD) complex in mammalian cells, which catalyzes the first irreversible step in branched-chain amino acid (BCAA) catabolism. The BCAAs leucine, isoleucine, and valine are amino acid components in almost all proteins and in addition play important roles in nitrogen metabolism, e.g., the synthesis of glutamine and alanine. Furthermore, leucine can stimulate protein synthesis and act as a stimulus for insulin secretion. MiR-29b targets a component of the BCKD complex in HEK293 cells and thus inhibits its activity. Cellular BCKD activity is also tightly regulated by allosteric and covalent mechanisms. The work of Mersey et al. is an intriguing example of how a miRNA can add another level of regulation for a metabolically significant enzyme. An involvement of miRNAs in the regulation of amino acid metabolism has also been suggested in flies, where bioinformatic approaches have predicted several enzymes to be targets of miR-277 (Stark et al., 2003).

Recent technical advances to silence miRNAs in mice using RNA analogs have generated further evidence for a role of miRNAs in metabolism. The predominant miRNA in the liver, miR-122, has been shown to regulate cholesterol and lipid homeostasis in two independent studies. This miRNA is abundantly expressed in human and rodent liver tissue, with estimates ranging from 50,000 to 80,000 copies per cell. Using a new class of miRNA inhibitors, termed antagomirs, Krutzfeldt et al. (2005) studied the effect of miR-122 on glucose and lipid metabolism in mice. Antagomir-122 induced efficient