



this system that defines the tissue context of the hypoxic program and provides a method for tuning the responses of transcription factors under the wide range of conditions that contribute to the genesis of hypoxic stress. The paper by Ghosh and colleagues (7) adds yet another dimension to this response by demonstrating the HIF-independent hypoxic induction of a hypoxamir unique to the endothelium, which prolongs HIF expression and thereby promotes the hypoxic induction of the angiogenic response required to restore tissue perfusion and attenuate tissue hypoxia (Figure 1). It is an elegant system of extraordinary complexity that is essential for homeostatic control under variable oxygen tensions.

Acknowledgments

This work was supported in part by NIH grants HL61795, HL81587, HL70819, and HL48743. The author wishes to thank Stephanie Tribuna for expert technical assistance.

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The magic and mystery of miR-21

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MicroRNAs (miRNAs) are potent regulators of mRNA stability and thereby protein expression. As such, miRNAs have become of interest as possible therapeutics and/or therapeutic targets. In this context, small complementary miRNA sequences known as antagomirs could be used to inhibit miRNA activity, while miRNA mimics could confer gain-of-function activity. However, a note of caution is sounded by Patrick et al. in this issue of the JCI, as they show that although recent reports have suggested that an miR-21 antagomir might be therapeutically useful in preventing heart failure in mice, genetic deletion of miR-21 does not confer a similar phenotype, suggesting possible confounding factors that are only now beginning to be revealed in the techniques used to study miRNA biology.

Conflict of interest: The author has declared that no conflict of interest exists.

Citation for this article: *J Clin Invest*. 2010; 120(11):3817-3819. doi:10.1172/JCI44596.

MicroRNAs (miRNAs) are small, noncoding RNAs transcribed within the introns of other genes or encoded separately as uniquely regulated RNA gene prod-

ucts. The approximately 70-bp precursor miRNA product is processed by the enzymes Drosha and Dicer to generate a mature 22-bp product that binds mRNAs in a unique manner using Watson-Crick base pairing through a conserved 6- to 8-bp "seed" sequence as well as additional contacts (Figure 1). Binding of miRNAs to their target mRNAs inhibits protein translation and reduces mRNA stability.

miRNAs were first identified in the model organism *Caenorhabditis elegans* but soon after were shown to play potent roles in regulating mRNA stability, and thereby protein expression, in higher eukaryotes. Studies using genetic deletion or inhibi-

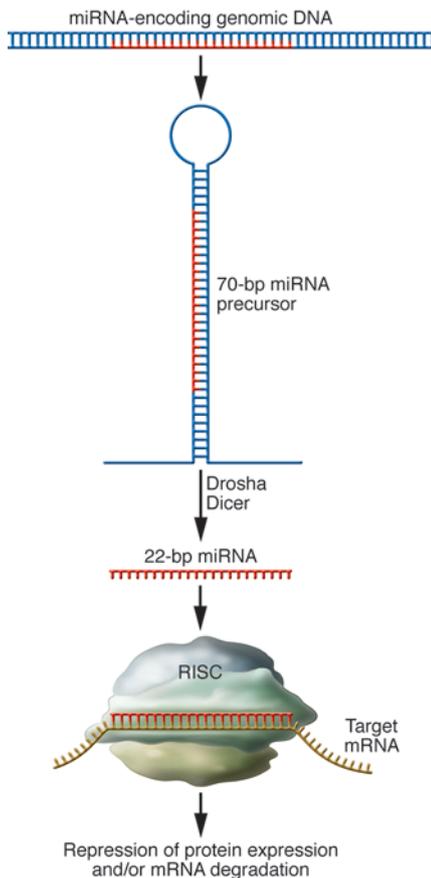


Figure 1 Biogenesis of miRNAs. miRNAs are transcribed as 70-bp precursor products that are processed into the mature 22-bp products by the Drosha and Dicer RNA-processing enzymes. The mature products interact with the multiprotein RNA-induced silencing complex (RISC) and bind to complementary sequences found in target mRNAs.

nology, several groups have shown that specific miRNAs are important for early cardiac development, skin development, and, more recently, heart failure in rodents and humans (6–8). Despite these important and intriguing findings, there have been few miRNAs that have been studied using traditional genetic inactivation techniques, including homologous recombination in ES cells.

miRNAs in muscle biology

Many early studies of miRNA function focused on the role of this pathway in muscle development (9–11). A seminal study showed that miR-208, which is expressed in an intron of the α -myosin heavy chain gene, regulates the stress response in the adult heart in part by regulating expression of the embryonic myosin heavy chain isoform, β -myosin heavy chain (10). Importantly, this study used traditional gene targeting in mice to elucidate the function of miR-208. miR-1 and miR-133a have also been shown to play important roles in cardiac development and the stress response in the heart (8, 12). Genetic inactivation of miR-1 results in disruption of cardiac morphogenesis accompanied by alterations in cardiomyocyte proliferation (8). miR-133a is also important for cardiac development, with approximately 50% of the embryos and neonates in which the miRNA is genetically inactivated dying due to ventricular septal defects. In addition

to these roles for miR-1 and miR-133a that were elucidated using genetic deletion studies in mice, studies using gain-of-function techniques have shown that these two miRNAs promote cardiac mesoderm development in differentiating ES cells (13).

The confusion over miR-21

In this issue of the *JCI*, Patrick et al. show that despite recent evidence of a potent role for miR-21 in regulating heart failure in rodents (6), genetic ablation of the miR-21 sequence does not result in any obvious cardiac phenotype (14). Thum et al. had previously demonstrated a striking inhibition of cardiac hypertrophy and the accompanying fibrotic response in rodents using a cholesterol-modified miR-21 antagomir (6). Much of the rationale for examining the function of miR-21 came from earlier studies by several groups that showed that miR-21 is induced at very high levels after multiple types of cardiac stress, including during the myocardial remodeling that occurs after infarction (15, 16).

Patrick et al. generated a null allele in the mouse for miR-21 using standard homologous recombination techniques in ES cells and, surprisingly, given the results of Thum et al. (6), these mice were completely normal in all assays of cardiac development and function performed (14). The authors then went on to stress the mice null for *Mir21* (miR-21-null mice) using thoracic aortic constriction, calcineurin overexpression, and angiotensin II treatment. Again, *miR-21*-null mice did not respond markedly differently from wild-type mice in any of these experimental models of cardiac stress. Patrick et al. went a step further and treated mice with a locked nucleic acid (LNA) version of a miR-21 antagomir, and even though they show data that convinc-

tion of Dicer have shown that the miRNA pathway is critical for the development of many tissues, including muscle, lung, and skin (1–3). Moreover, miRNAs play an important role in ES cell self-renewal and differentiation (4, 5). The use of antagomirs, small inhibitory sequences for individual miRNAs, has allowed researchers to relatively easily and quickly inhibit the activity of individual miRNAs in vitro and in vivo, in much the same way as antisense morpholino technology does in the zebrafish model system. Using this tech-

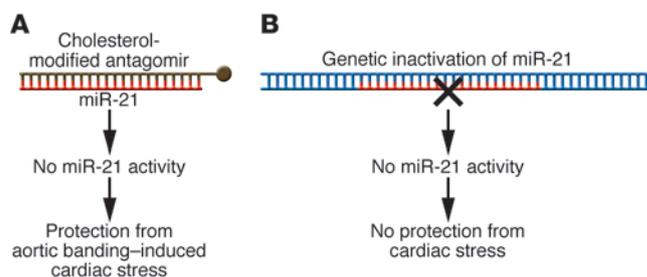


Figure 2 Two models leading to different conclusions regarding miR-21 activity. (A) Antagomir-mediated inhibition of miR-21 activity leads to protection from pressure overload stress on the heart. (B) Genetic deletion of miR-21 in mice does not alter the response to multiple types of cardiac stress, including pressure overload.



ingly demonstrate knockdown of miR-21 activity, no effect on cardiac development, homeostasis, or response to stress was observed. These data are in stark contrast to those reported by Thum et al. (6), who showed that treatment of mice with cholesterol-modified miR-21 antagomirs resulted in a blunting of the hypertrophic response of the heart after thoracic aortic banding-induced pressure overload (Figure 2A). The finding by Patrick et al. that genetic deletion of miR-21 does not alter the response of the heart to multiple stressors (14) (Figure 2B) suggests that caution is needed when interpreting studies using antagomir approaches to elucidate the function of individual miRNAs *in vivo*.

Antagomirs versus genetic deletion

The current report by Patrick et al. (14) is one of the first to compare genetic deletion of a miRNA with antagomir knockdown in the same series of experiments. These studies follow another controversial report (17) showing that miR-143/145 is critical for smooth muscle development and specification using antagomir and gain-of-function mimic techniques, while studies using genetic deletion-based inactivation of miR-143/145 in mice showed much more subtle defects in cytoskeletal dynamics and smooth muscle response to vessel wall injury (18, 19). Given these discrepancies between the results of antagomir knockdown and genetic deletion of miRNAs, it will be important to carefully analyze and interpret future data from antagomir studies.

So the question remains: why such a dramatic discrepancy between data obtained from antagomir and genetic deletion techniques? Several possibilities exist, some of which were suggested by Patrick et al., including technical variations in experimental protocols and the efficiency of LNA-based versus cholesterol-modified antagomir approaches (14). Other potentially interesting possibilities include miRNA redundancy, which could result in the suppression of multiple miRNAs that include the same or similar seed sequence as miR-21, or nonspecific effects of high levels of a cholesterol-modified antagomir on the heart. The effects of a given antagomir could result in the inac-

tivation of many additional miRNAs that share an identical or similar seed sequence, which would result in a far more robust phenotype than genetic deletion of a single miRNA species. Despite these technical limitations, carefully controlled and interpreted experiments using antagomirs and mimics will likely lead to important new insights into miRNA biology.

Where do we go from here?

The current report by Patrick et al. (14) is especially important in light of the attempts to generate novel therapies by targeting miRNAs. Antagomirs and mimics can be considered potent small molecule approaches to treat diseases as disparate as heart failure and diabetes. The possible ability of antagomirs and mimics to impact an entire class or family of miRNAs may actually be an advantage in designing future therapeutics. Alternatively, as techniques are improved and the underlying chemistry in the development of new antagomir and mimic approaches is refined, it may become easier to target individual miRNA species with high specificity. Given the relative ease with which antagomir and miRNA mimic techniques can be applied to model systems as well as cell culture, much of the functional data obtained in miRNA studies is derived from these nongenetic techniques. With proper care and further technical advancements, these techniques will not only improve, but may yet become useful therapeutic approaches to treat disease. However, until more is learned about the limits of this technology, genetic inactivation in useful model systems such as the mouse should still be the gold standard by which many experiments are measured.

Acknowledgments

The Morrissey laboratory is supported by funding from the NIH (HL100405) and the American Heart Association Jon Holden DeHaan Myogenesis Center.

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