zones. Further analysis confirmed that α2A-ARs and HCN channels are found together on spine membranes, often within tens of nanometers of each other.

Individually, each one of these results is subject to possible alternative interpretations, but collectively they make an extremely strong case for the biochemical cascade proposed by the authors. Furthermore, this cascade has immediate implications for the design of effective clinical therapies, because an abnormal capacity for working memory is thought to contribute to cognitive deficits, such as attention deficit hyperactivity disorder (ADHD) (Scahill et al., 2001).

It is not often that changes in behavior can be traced to specific molecular interactions in the brain, and it is even more rare to see a complex cognitive function, such as working memory, dissected in this way. Indeed, the work by Wang et al. (2007) is a remarkable feat that sets a high standard for future research in neuroscience.

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miR-1-2 Gets to the Heart of the Matter

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Although many microRNAs (miRNAs) and their targets have been identified, the importance of miRNAs in vivo is still unclear. In this issue, Zhao et al. (2007) generate mice deficient in a cardiac-specific miRNA, miR-1-2, and reveal that this microRNA plays a crucial role in heart development and physiology.

Organogenesis is a complex biological process that requires precise spatial and temporal control of gene expression. Recently, miRNAs have emerged as central post-transcriptional repressors of gene expression that interact with the 3′ untranslated region (UTR) of specific target mRNAs (Kloosterman and Plasterk, 2006). In this issue of *Cell*, Zhao et al. (2007) investigate the role of miRNAs during cardiac development in mice. Using elegant genetics, these authors uncover important roles for the miRNA, miR-1-2, during heart morphogenesis in the mouse embryo and for the regulation of cardiomyocyte proliferation and electrophysiology in the adult heart (Zhao et al., 2007).

To understand the role of miRNAs in the developing heart, the authors engineered mice that lacked the microRNA processing enzyme Dicer in heart tissue alone. These embryos showed cardiac failure due to a variety of developmental defects, including pericardial edema and underdevelopment of the ventricular myocardium. These phenotypes are consistent with the defects during heart development observed in zebrafish embryos devoid of Dicer function (Giraldez et al, 2005)

To further investigate the role of miRNAs in cardiac development, the authors focused on miR-1, a highly abundant class of miRNAs in the mammalian heart. Two members of the miR-1 class of RNAs—miR-1-1 and miR-1-2—are specifically expressed in cardiac tissue and skeletal muscle. miR-1-1 and miR-1-2 are encoded separately but appear to target the same mRNAs. To determine the particular role of miR-1-2, the authors using a targeted deletion strategy to eliminate the locus coding for miR-1-2 without affecting adjacent genes. Fifty percent of mice lacking miR-1-2 died during

embryonic development, displaying defects in their ventricular septum indicative of abnormal heart morphogenesis. Interestingly, 15% of the miR-1-2-deficient mice that did not die in utero suffered severe heart defects and died 2–3 months after birth. Some of the remaining embryos survived to adulthood and appeared to have normal cardiac morphology but suffered sudden death, suggesting additional functions of miR-1-2 during postembryonic development.

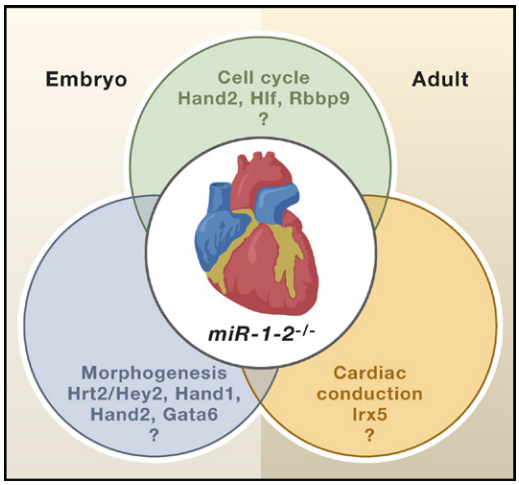

Figure 1. miR-1-2 Function in the Mouse Heart
Absence of the cardiac-specific microRNA miR-1-2 affects heart morphogenesis (potential targets shown) during embryonic development. Mice lacking miR-1-2 that survive to adulthood have defects in cardiac conductivity and cardiomyocyte proliferation. These effects are likely through the regulation of the expression of the transcription factors Irx5 and Hand2 by miR1-2. Other putative miR-1 targets involved in cell cycle/cancers include Hlf (hepatocyte leukemia factor) and Rbbp9 (retinoblastoma-binding protein 9).

Expression of miRNAs is not limited to embryonic stages, and most miRNAs are expressed continuously during the life of a tissue or organ (Wienholds et al., 2005). Nevertheless, current research has focused on their roles during development and has tended to overlook miRNA function during adulthood (Kloosterman and Plasterk, 2006). Zhao et al. (2007) examined the mice lacking miR-1-2 that survived to adulthood to determine the function of this miRNA in the adult heart. They observed physiological defects in the adult heart in the absence of miR-1-2, particularly an increase in cardiomyocyte proliferation and electrophysiological defects including reduced heart rate and prolonged ventricular depolarization. These phenotypes suggest that miR-1-2 has distinct functions in the embryonic and the adult heart, where it modulates the machinery that establishes and maintains cardiac rhythm (Figure 1). Although these cardiac conductivity defects appear in miR-1-2-deficient heart tissue that appears morphologically normal, such defects could be reminiscent of subtle cardiac embryonic phenotypes. Thus, future experiments will need to investigate the postembryonic roles of miR-1 in the adult heart by analyzing conditional knockouts that eliminate miRNA function after birth.

microRNAs are known to reduce target mRNA translation and accelerate mRNA decay (Lim et al., 2005; Giraldez et al., 2006). Based on these observations, microarray analyses have contributed to the identification of miRNA targets based on gain- and loss-of-function experiments (Lim et al, 2005; Giraldez et al., 2006). To investigate the molecular targets responsible for the cardiac phenotypes in miR-1-2-deficient mice, Zhao et al. (2007) used microarray analysis to identify mRNAs upregulated in the absence of miR-1-2. One might predict that the phenotypes observed would be caused by misregulation of a small number of key effector genes. Zhao et al. (2007) identified a dozen putative direct targets and propose that misregulation of the genes encoding the transcription factors Hand2 and Irx5 is responsible for the phenotypes of cardiac proliferation (Zhao et al., 2005) and repolarization, respectively. But the whole picture is likely to be far more complex given that miRNAs can target hundreds of genes (Lim et al. 2005; Giraldez et al., 2005). For instance, loss of miR-1-2 might result in dysregulation of multiple target genes that encode proteins with synergistic effects during cardiac function. Additional experiments are necessary to conclusively show that the potential targets highlighted by Zhao et al. (2007) are indeed responsible for the observed phenotypes. In this regard, reduction of expression of Hand2 or Irx5 in miR-1-2-deficient mice should attenuate the observed cardiac phenotype. Interestingly, previous studies have suggested that miRNAs might regulate genes that are not essential for function of a particular tissue. For example, a muscle miRNA might eliminate noisy expression of neuronal mRNAs (Lim et al., 2005; Stark et al., 2005). In contrast to these observations but as predicted in the rheostat model (Bartel and Chen, 2004), the Zhao et al. (2007) study suggests that miR-1-2 indeed tunes the levels of a set of targets that are essential rather than extraneous for heart function.

Two recent studies also highlight the role of miRNAs in the heart. Yang et al. (2007) also report a function for miR-1 in heart conductivity. Interestingly, miR-1 levels were increased in humans with coronary artery disease and rats after cardiac infarction. Knockdown of miR-1 prevented heart arrhythmia, whereas miR-1 overexpression caused heart arrhythmia in normal and infarcted hearts. This effect of perturbing the levels of miR-1 prompted the authors to examine potassium channel subunits, which are important for cardiac conductance. Both the gene encoding the cardiac gap junction channel connexin43 and the gene encoding the Kir2.1 subunit of a potassium channel were targeted by miR-1. Both gain- and loss-of-function of miR-1 affect conductivity through these potassium channels. Taken together, these results strongly support a central role for miR-1 in fine tuning the regulation of cardiac electrophysiology in pathological and normal conditions. Another link between miRNAs and heart disease was reported recently by van Rooij

et al. (2007). Cardiac contractility and performance depends on the balanced expression of α- and β-myosin heavy chain (MHC). During stress, cardiomyocytes respond by hypertrophic growth and reduced contractility due to downregulation of α-MHC and upregulation of β-MHC. The authors deleted the cardiac-specific miRNA miR-208 that is encoded by an intron in the α-MHC gene. miR-208-deficient mice were viable and displayed no obvious cardiac phenotype within the first months of age. However, gene expression analysis revealed that miR-208-deficient hearts upregulate genes that encode fast skeletal muscle contractile proteins—which are absent in wild-type hearts—and cardiac stress proteins. These genes are likely secondary miR-208 targets because they lack canonical miR-208 complementary sites. Furthermore, miR-208 mutant mice failed to undergo stress-induced cardiac remodeling, hypertrophic growth, and β-MHC upregulation. Conversely, transgenic expression of miR-208 was sufficient to induce β-MHC. Given that miR-208-deficient hearts resembled hyperthyroid hearts and that thyroid hormone (T3) signaling represses ß-MHC, the authors investigated whether miR-208 was required for T3-dependent repression of β-MHC. Repression of T3 signaling in the absence of miR-208 and target validation experiments suggest that miR-208 regulates β-MHC by repressing the thyroid hormone receptor associated protein 1 (THRAP1), a cofactor of the thyroid hormone receptor (TR) and a predicted miR-208 target mRNA. Thus, miR-208 is important for cardiac growth and gene expression in response to stress and hypothyroidism. Altogether, these studies place miRNA-mediated regulation in the spotlight during heart development and adult cardiac physiology.

Finally, Zhao et al. (2007) touch on a recurrent problem in the miRNA field, the difficulty in reliably identifying the targets for any given miRNA. Current methods focus on base pairing between the miRNA "seed" comprising two to seven nucleotides and its target mRNA. Despite the presence of putative target sites, it remains unclear why some target mRNAs are strongly repressed but other targets with identical sites are only weakly repressed. In the new work and their previous study (Zhao et al., 2007; Zhao et al., 2005), the authors introduced an interesting idea for target selectivity that depends on the free energy (ΔG) surrounding the target site. They propose that a stable environment (low ΔG) might be less accessible to the miRNA silencing complex than an unstable (high ΔG) environment. Analysis of target mRNAs whose levels were altered in the absence of miR1-2 supports this hypothesis (Zhao et al., 2007). However, a genome-wide statistical analysis of the average free energy flanking artificial (shuffled) miRNA target sites is needed, together with a rigorous assessment of the efficiency of a target site in the context of a 3′UTR in which the ΔG of the 5′ and 3′ flanking regions are systematically changed. These experiments, together with experimental validation of more predicted targets, will refine current prediction methods and will consolidate the energetic considerations guiding miRNA-target mRNA interactions.

The Zhao et al. (2007) findings have several important implications. First, their work identifies miR-1-2 as a key miRNA during embryonic and adult cardiac function. Second, it defines a set of potential miR-1 target genes whose regulation is likely to play crucial roles during cardiac development and physiology. Third, it supports the hypothesis that high free energy flanking the miRNA complementary site could be used to identify functional targets of miRNAs. Finally, given that miR-1-1—which targets the same sequences as miR-1-2—is still expressed in miR-1-2-deficient hearts, the study by Zhao et al. (2007) indicates that miR-1 dosage is important during heart development. Therefore, it is tantalizing to speculate that environmental or genetic conditions that affect miR-1 expression levels during human embryonic heart development could constitute potential risk factors for cardiovascular disease and heart failure in the adult.

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