

miRNAs Play a Tune

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Two new studies describe functionally relevant interactions between microRNAs (miRNAs) and their targets in the immune system and the brain (Xiao et al., 2007; Karres et al., 2007). Furthermore, these studies illustrate the involvement of miRNAs in tuning the expression of target genes to physiologically relevant levels.

MicroRNAs (miRNAs) are regulatory RNAs that repress the expression of target genes by binding to complementary sites in the mRNA of target genes (Lee et al., 1993; Wightman et al., 1993; Carthew, 2007). Given that miRNAs appear to constitute one of the largest classes of gene regulatory molecules in animals, understanding their mode of action and their physiological roles is essential. Several genetic studies in plants and invertebrates have provided initial insights into the importance of miRNAs in controlling the development and function of various cell types (Carthew, 2007). However, their importance in vertebrate development has only recently begun to emerge and is bolstered by two papers in this issue (Xiao et al., 2007; Karres et al., 2007).

In their new work, Rajewsky and colleagues (Xiao et al., 2007) provide an impressive example of the physiological importance of the regulatory relationship of an miRNA, miR-150, and a target gene, *c-myb*, in the mouse immune system. Loss of *c-myb* is known to result in a defect in early B cell development—that is, progression of pro- to pre-B cells—as well as a loss of a specific mature B cell subpopulation termed B1 cells. Xiao et al. (2007) deleted the miR-150 locus and observed an expansion of the B1 cell population. This phenotype mimics that expected from upregulation of *c-Myb*—the presumptive target of miR-150 and a regulator of B1 cell formation. Conversely, aberrant, premature expression of miR-150 in transgenic mice mimics the loss of *c-Myb*, causing a block of the pro- to

pre-B cell transition. The authors also show that miR-150 targets *c-Myb* expression directly in vitro in a manner dependent on the presence of miR-150-complementary sites in the *c-myb* 3' untranslated region (UTR). This careful study emphasizes the point that to assess the physiological effect of removal of an miRNA, one needs to be able to precisely probe the development of the cell type that normally expresses the miRNA. Therefore, as is the case for any gene, lack of an apparent miRNA-knockout phenotype should not be taken as evidence for overly subtle roles of miRNAs, but, at least initially, rather as an indication of not having looked at the right phenotype. The Xiao et al. (2007) report also underscores the importance of generating animals that lack a predicted miRNA target, which is the case of mice lacking *c-myb* that display a phenotype precisely complementary to the miRNA mutant phenotype. This is notable because miR-150 is, like most other miRNAs, predicted to regulate the expression of a substantial number of target genes. Although additional targets of miR-150 may be relevant in other cell types, the function of miR-150 in B cells appears to be largely if not exclusively mediated by a single target, *c-Myb*. Such a presumptive one miRNA-one target relationship is highly reminiscent of the genetically identified miRNAs in *C. elegans* (*lin-4*, *let-7*, *Isy-6*), whose activity in specific cellular processes can also be largely explained by their regulation of single target genes (Carthew, 2007). Similarly, genetic suppression analysis,

among other approaches, has shown that the activity of some transcription factors—the other major gene regulatory class in animals—can also be explained through their regulation of single or very few target genes. Phenotypic analyses of miRNA and knockouts of target genes such as those described by Xiao et al. (2007) will be necessary to probe how many of the large number of predicted miRNA/target interactions are indeed physiologically relevant.

The Xiao et al. (2007) study ventures into the arena of gene-dosage effects from their observation that removing just one copy of the *c-myb* gene results in developmental defects in the B cell lineage. Genetics has taught us that biology is replete with instances in which the amount of a specific gene product—modulated by mechanisms such as transcriptional regulation, transcript stability, and protein turnover—needs to be adjusted to a precise level. The Xiao et al. (2007) paper—as well as a second paper in this issue from Karres et al. (2007) that specifically addresses the notion of gene dosage—now provide evidence that miRNAs join the crowd of regulatory mechanisms that tune the expression of genes to physiologically relevant levels. Tuning the expression of a gene to a precise level at which the gene will execute a specific function is of course different from completely switching off a gene, which was the function assigned to the first miRNAs genetically identified in *C. elegans* (Carthew, 2007). Yet experimental examples in which an miRNA is involved in reducing expression of

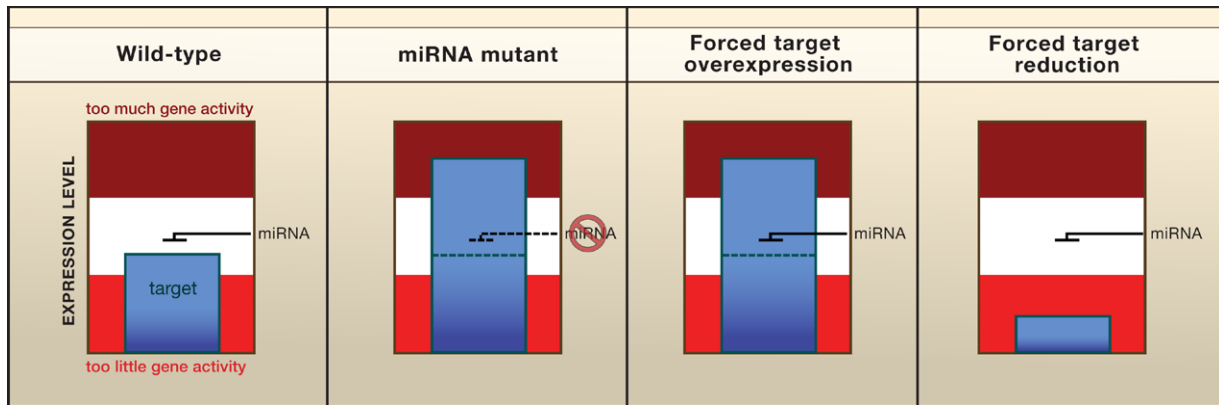


Figure 1. Tuning Gene Activity with microRNAs

Too high or too low levels of gene activity often produce aberrant phenotypes in a given biological context. For an miRNA to be involved in setting the correct level of gene activity, loss of the miRNA should cause an aberrant phenotype due to elevated target gene expression, as should forced overexpression of the target. Further reduction of target gene expression below the level set by the miRNA should also cause a mutant phenotype. For a more extensive discussion of tuning versus switching modes of miRNA action, see Bartel and Chen (2004).

a target gene to a level at which the target gene still plays a vital role had not yet been discovered.

The experimental criteria that need to be fulfilled to support a tuning relationship between an miRNA and its target are at least threefold (Figure 1). First, the miRNA and the protein product of the target gene must both be detected in a cell. Second, the absence of the miRNA and the resulting upregulation of the target must be detrimental to a cellular process. Third, forced downregulation of the target below the levels normally set by the miRNA must also be somehow detrimental to a functional process in the same cell. Karres et al. (2007) indeed describe a case in which all these criteria appear to be fulfilled. They show that the broadly expressed *Drosophila* miR-8 gene dampens expression of the even more broadly expressed atrophin protein, a phylogenetically conserved transcriptional regulator. The miRNA-target relationship is convincingly shown by genetic interaction tests—i.e., reduced expression of the target gene suppressed the loss of the miRNA—and by the analysis of 3'UTR reporter genes. Both overexpression of and reduction of the atrophin target in a wild-type background below the levels normally set by miR-8 are detrimental to an as yet uncharacterized aspect of nervous system function in these animals, thereby fulfilling the

major tenets of the “tuning model.” It remains to be examined, though, whether the phenotype resulting from reduction of atrophin levels is really caused in the same cells as those in which miR-8 normally tunes atrophin levels. This is important because it is unclear whether miR-8 tunes atrophin expression in all cells in which miR-8 and atrophin are coexpressed. A more precise definition of the cellular processes that miR-8 and atrophin are involved in is required to resolve this issue.

The regulatory relationship between miR-150 and *c-myb* as described in the Xiao et al. (2007) paper may also provide an example for a tuning relationship. The authors find that removal of one copy of the *c-myb* gene (in *c-myb* heterozygous mice) leads to a partial reduction of the B1 cell numbers, as compared to a complete loss of these cells upon complete loss of *c-Myb* in the B cell lineage. This observation argues that in wild type animals miR-150 downregulates *c-myb* to a level at which it promotes the generation of a precise number of B1 cells. Further reducing *c-myb* levels eliminated B1 cells, and higher *c-myb* levels lead to an increase in the number of B1 cells.

To further illustrate the difference between these examples of a tuning relationship to that of a switch relationship, let us consider the example of the *Isy-6* miRNA and its target *cog-1* (Hobert, 2007). *Isy-6* switches

off *cog-1* in a neuron termed ASEL. Aberrant expression of *cog-1* in ASEL (either through ectopic expression or in an *Isy-6* mutant) causes ASEL to be converted to ASER. In a *cog-1* null mutant, however, the ASEL fate is unaffected; that is, *cog-1* has no role in the cell in which it is normally repressed by *Isy-6*. In contrast, in those cells in which *mir-8* and *mir-150* downregulate their targets, the respective target genes may still fulfill a vital function.

Extensive further genetic analysis is required to determine whether the tuning or switch relationship are each widespread modes of miRNA/target regulation or whether one of the two mechanisms is a predominating theme of miRNA function. In any case, the wide spectrum of effects that miRNAs exert on their target genes should perhaps not be too surprising. Several conceptual similarities in the action of transcription factors and miRNAs have already been summarized before (Hobert, 2004). The Xiao et al. (2007) and Karres et al. (2007) studies drive these analogies further as the tuning of the precise level of expression of their target genes is a feature shared with the action of many transcription factors (Davidson, 2001), let alone signaling pathways that ultimately result in transcriptional regulation. Advantages of tuning gene-expression levels by miRNAs rather than

transcriptional or other forms of posttranslational mechanisms may lie in some specific and unique features of miRNA action, such as the potential for rapid reversibility of the regulatory relationship. Alternatively, the relatively limited effort with which a regulatory relationship between an miRNA and its target can evolve may have merely been the fastest way for nature to acquire a trait of selective advantage.

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