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MicroRNAs: All Gone and Then What?

MicroRNAs are abundant gene regulatory factors whose function in animal development and homeostasis is poorly understood. A new study reports the genetic elimination of miRNA function on a full genomic scale and identifies a subfamily of miRNAs involved in brain morphogenesis.

Oliver Hobert

One of the perhaps biggest surprises in molecular biology in the past few years has been the discovery of a large number of previously completely overlooked regulatory molecules, termed miRNAs. miRNAs, ~22 nt long RNA molecules, regulate the expression of target genes by binding to their 3'UTR [1] (Figure 1). They were first identified more than 10 years ago in the nematode *Caenorhabditis elegans* [2], yet thought to be a worm specific oddity for the longest time. It was only the cloning of the second miRNA, *let-7*, that led the Ruvkun laboratory to note the conservation of miRNAs across phylogeny [3]. This in turn spurred intensive, genome-wide searches for miRNAs and current estimates of miRNA gene number range in the several hundreds for vertebrate genomes [4,5].

But what is it exactly that miRNAs do and how do they do it? The only clear theme that has emerged over the past few years is that they generally appear to repress gene expression [1,4]. But what cellular processes do miRNAs control? To date, there are still only four miRNAs — *lin-4*, *let-7*, *bantam* and *lisy-6* — whose physiological function has been elucidated *in vivo* and whose targets are known [2,3,6,7]. But speculation about the breadth of cellular processes in which animal miRNAs are involved have flourished over the past two years, mainly based on computational

miRNA target prediction [8–10]. Yet, in contrast to plants, the usefulness of computational target prediction approaches has so far been limited in animals, which is illustrated by the striking lack of concordance of different target prediction algorithms. Nevertheless a common theme of all target predictions is that a large fraction of the genes in a given genome may be regulated by miRNAs. But how pervasive is miRNA function in reality?

This is where a new study by Alex Schier's lab [11] has provided fundamentally important new insights. Rather than eliminating a single miRNA, Giraldez *et al.* [11] eliminated *all* miRNAs by genetically removing the zebrafish gene coding for Dicer, an RNase required for miRNA processing [12] (Figure 1). A zebrafish Dicer mutant is not new *per se*; Plasterk and colleagues [13] had already reported the postembryonic lethality of Dicer knockout fish. However, their study was confounded by the fact that maternally supplied Dicer mRNA and/or protein from the heterozygous mothers of homozygous mutant embryos apparently allowed the generation of mature miRNAs during embryogenesis. This problem was now elegantly circumvented by using the germline replacement technique [14], which allows the study of homozygous mutant embryos devoid of both maternal and zygotic Dicer function. The observations of Giraldez *et al.* [11]

on such maternal-zygotic Dicer mutant embryos are dramatic — not only because of the type of defect they observe, but also because of the type of defect they do not observe.

Given the vast abundance of predicted miRNA target genes, including genes involved in signaling and transcriptional control, maternal-zygotic Dicer null mutants displayed surprisingly normal axis and pattern formation [9]. Individual organs and multiple cell types were present and all anterior–posterior and dorsal–ventral patterning events examined do apparently not require miRNA function. Many of these initial patterning events are known to be under control of key signaling systems, such as Nodal, Hedgehog, Wnt, Notch, FGF, BMP and Retinoic acid [15]. As many of these pathways were predicted by *in silico* approaches to be targeted by miRNAs, the absence of any defects in these systems upon global removal of miRNAs is striking. Given the negative nature of this result, the authors showed that miRNA processing is indeed globally defective in Dicer mutants using a representative sample of many miRNA species. However, it can formally not be excluded that trace amounts of miRNAs are still being produced, for example by an unknown RNase other than Dicer.

In striking contrast to the lack of early patterning defects, Dicer mutants display severe defects in the morphogenesis of several distinct organ types [11]. In the nervous system, neurulation was severely affected, brain ventricles did not form properly, subregions of the brains were not appropriately demarcated and neuron position and axon projections were disrupted. Gross defects were also observed in cell arrangements during gastrulation, cardiovascular morphogenesis

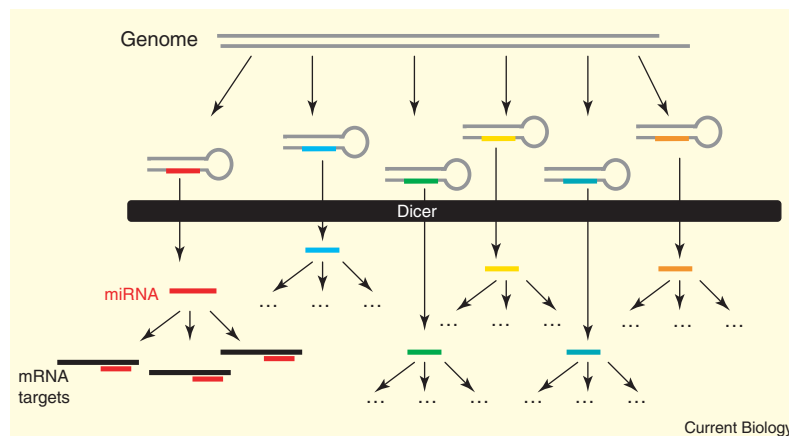


Figure 1. Dicer globally affects miRNA processing.

Genomes are likely to contain many hundreds of miRNA encoding genes, which are expressed as hairpins and processed by various enzymes (only Dicer is shown here) to produce a ~22 nt miRNA species. Each of the mature miRNAs may regulate the expression of scores of target genes to which miRNAs bind in a sequence specific manner. The role of *Dicer* in regulating the production of other types of RNA species is not shown here. For more details see [12].

and function as well as during somitogenesis.

The third surprise of the paper came through an experiment that one would not have expected to work at all. Giraldez *et al.* [11] re-supplied fish embryos with synthetic, mature miRNAs, which are beyond the step of requiring Dicer processing. Focusing on a set of miRNAs expressed at the relevant stages of development, the authors report that injections of a single subfamily (miR-430-a/b/c) of miRNAs can rescue the brain morphogenesis defects of *Dicer* mutants. However, some non-neuronal defects were either only partially rescued or not rescued at all, which hints at a tissue-specificity of miRNA function. Several aspects of these rescue experiments are notable. First, from a technical point of view they conclusively demonstrate that the observed effects of loss of Dicer are indeed caused by the lack of miRNA processing and not by other functions of Dicer, such as siRNA-dependent DNA methylation, histone modification and centromeric silencing [16].

Second, it is as surprising as it is satisfying to see that many, very different aspects of brain morphogenesis are controlled by a single miRNA subfamily. This also makes miR-430-a/b/c the first vertebrate miRNAs with a

firmly established role in development. It will be very intriguing to find the target genes of these miRNAs, particularly as some of the morphogenetic defects in *Dicer* null mutants, such as ventricle formation, are only poorly understood on a molecular level.

Lastly, there is a notable oddity of the miR-430 subfamily — its three members come in a cluster of 90 copies. Clustered miRNA families have been known before but the sheer number of copies of miR-430 is unique. The functional relevance of this clustering — if there is one — remains mysterious.

Naturally, we are now left with many new questions. Why are miRNAs abundantly employed in cell fate diversification rather than in early patterning? A general role of miRNAs in cell fate diversification can be extrapolated from several previous studies on individual miRNAs [6,17,18]. But why are they not employed in early patterning? Unfortunately, we currently understand too little about mechanisms of miRNA action to engage in anything but wild speculations. Perhaps there is something intrinsic about miRNA regulation that predestines miRNAs to fulfill a function in determining 'stable' gene expression programs in differentiating cell types. In one of

the best characterized examples, the *C. elegans* miRNA *Isy-6* is required to diversify and then lock two terminal differentiation programs into their stable end state [6]. In contrast, early patterning events are characterized by dynamic changes in gene expression. These regulatory dynamics require the activity of gene regulatory factors to be transient, plastic and/or reversible. Mechanistic features of miRNAs, such as their long half-life, may not allow them to participate in such dynamic processes.

Another question is how general the observed zebrafish phenotype is. And here comes another surprise. In contrast to fish, knocking out *Dicer* in mice causes early embryonic death before axis formation [19]. Moreover, embryonic stem (ES) cells derived from *Dicer* null mutant mice do not form the three germ layers that can normally be found in ES-cell derived embryoid bodies [16]. This may indicate a much broader role for miRNAs in early mouse embryos, but it is conceivable that this striking difference is 'simply' explained by one or a few specific Dicer products that control a very early process such as cellular growth or viability.

As any groundbreaking paper, the work of Giraldez *et al.* [11] raises an abundance of new and exciting questions that will lead us further in our quest for understanding microRNA function.

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Time Perception: Components of the Brain's Clock

We know the human brain contains some kind of clock, but determining its neural underpinnings and teasing apart its components have proven difficult. New work on the parietal cortex illustrates how single unit recording may be able to help.

Penelope A. Lewis¹ and Vincent Walsh²

Our brains measure time continuously. We are aware of how long we have been doing a particular thing, how long it has been since we last slept, and how long it will be until lunch or dinner. We are ready, at any moment, to make complex movements requiring muscle coordination with microsecond accuracy, or to decode temporally complex auditory signals in the form of speech or music. Our timing abilities are impressive, diverse and worthy of investigation. But they are not very well understood.

Many models of time perception have been put forward (for example, see [1–3]), collectively postulating a wide variety of different mechanisms. Regardless of their diversity, the models all agree that temporal information is processed in many ways: it is remembered, compared to other temporal information, combined with sensory information, and used in the production of motor outputs.

The holy grail of timing research is to understand the 'time-dependent process': a mechanism equivalent to a piezoelectric crystal in a man-made clock or the movement of a shadow on a sundial. This has proven an elusive goal, to the extent that ideas about how this mechanism might work remain near the level of conjecture. Researchers have had great difficulty in pinning timing-related activity in the brain to any specific type of function. This is largely because most time measurement tasks draw upon more than one process, making it difficult to tease the various components apart. In their recent study, Janssen and Shadlen [4] have shown how single unit recording can be used to partially bypass this issue.

Janssen and Shadlen [4] recorded time-sensitive responses in the lateral inferior parietal (LIP) cortex of the macaque. They trained two monkeys to perform a visual delay task: the monkeys first fixated a light, then, in response to a 'go' signal, moved their eyes to a

peripheral visual target as quickly as possible (Figure 1). The delay between target onset and 'go' signal varied according to two schedules: a bimodal schedule in which the 'go' cue could come early or late, but not between 0.75 and 1.75 seconds, and a unimodal schedule in which it came between 0.5 and 2 seconds. The schedules were presented in alternating blocks. The observed neural spike frequency in LIP correlated with the expectancy — 'hazard function' — of the 'go' cue

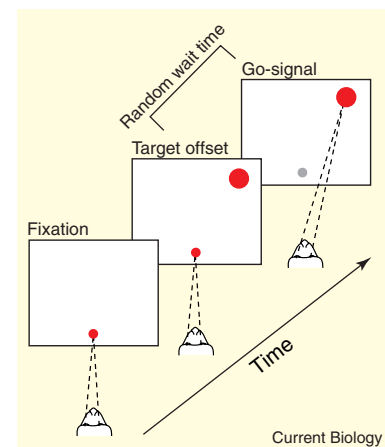


Figure 1. The task used by Janssen and Shadlen [4].

The monkey made eye movements to the red target as soon as the fixation point dimmed. Only trials in which the target appeared in the response field of the LIP neuron were reported. A bracket demarcates the random waiting time between target onset and 'go' signal. (Reproduced with permission [4].)