

microRNAs role in Development

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We study the roles of microRNAs (miRNAs) in development, focusing on vertebrate organogenesis.

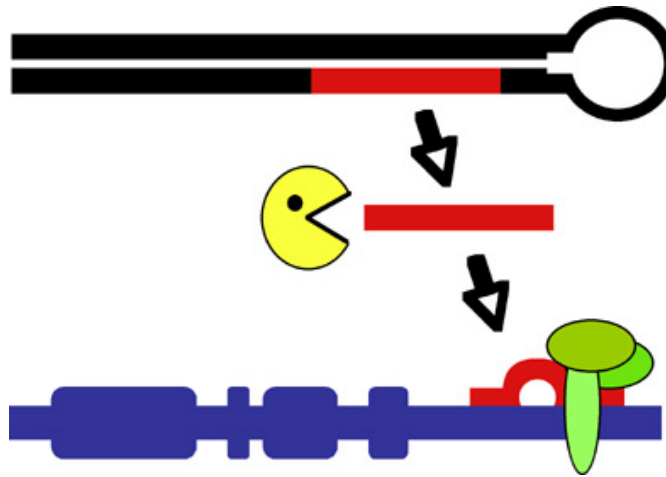


Fig. 1 miRNAs (red) are processed from a pre-miRNA precursor (black) that folds back on itself to form a hairpin. Dicer (yellow) digests the double stranded hairpin to generate a single strand miRNA. This mature miRNA recruits a protein complex (RISC, greens) that binds to the 3' untranslated region of messenger RNA (blue) and target it either to cleavage or translational repression.

We seek to gain insight into the role of miRNAs in key developmental events including differentiation, patterning and tissue size control and in miRNA contribution to the evolution of developmental pathways.

The genomes of animals contain hundreds of miRNA genes, which encode for short regulatory RNA molecules. miRNAs repress the expression of protein-coding mRNA ('targets'), providing a previously unappreciated regulatory mechanism for gene expression. Upon binding of an individual miRNA, or a combination of several miRNAs to the 3' untranslated region of a target mRNA, either translation repression or mRNA cleavage is induced through activation of the RNA-Induced Silencing Complex (RISC). Phylogenetic analysis on multiple vertebrate genomes indicates that miRNAs can potentially repress more than a third of all genes and therefore may be relevant to the vast majority of cellular processes. Nonetheless, these predictions are supported experimentally only in a handful of cases and the true impact provided by the network of miRNA:Target interactions is unknown.

One way to get a handle on miRNA role in vertebrate development, is provided by Dicer knockout alleles. Dicer is necessary for biosynthesis of functionally-mature miRNAs and thus its genetic deletion results in inactivation of hundreds of miRNAs and in expected de-regulation of thousands of targets.

Surprisingly, most vertebrate tissues differentiate and pattern properly in the absence of miRNA activity. The discrepancy between the minimal phenotypic changes observed in zebrafish knockout and in mouse-conditional Dicer alleles on the one hand, and the thousands of predicted targets on the other hand, is intriguing. One possible interpretation is that miRNAs may mainly play accessory role, being involved in enhancing the fidelity of primary gene expression programs.

miR-196 acts upstream of Hoxb8 and Sonic Hedgehog in limb development

The concept that miRNAs confer robustness to developmental programs gained support from our recent study (Hornstein et al., 2005), showing that a main role for miRNAs may be to prevent inappropriate ('leaky') expression of genes in domains where they are predominantly regulated transcriptionally. Specifically, we have found that a previously described, yet uncharacterized, hindlimb inhibitory activity can be explained by the presence of a miRNA. Thus, retinoic acid activates Hoxb-8 transcription in the

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developing vertebrate forelimb, whereas Hoxb-8 expression in the hindlimb is apparently unwanted. Loss of miRNA function in Dicer-deficient hindlimbs relieves this blockade, thereby enabling Hoxb-8 induction by retinoic acid. To look for a hindlimb-specific candidate miRNA, downstream of Dicer, we have screened a miRNA expression array with probes from hind- and fore- limb tissues. We uncovered this way the hindlimb-specific expression of miR-196, a known inhibitor of Hoxb-8. We next established that in the unmanipulated vertebrate hindlimb, miR-196 indeed directs the cleavage of 'leaky' Hoxb-8 transcripts. Further, miR-196 over-expression in chick embryo forelimbs attenuated the levels of endogenously expressed Hoxb-8 and was able to titrate out retinoic-acid induced Hoxb-8 mRNA. Finally, the expression of Sonic hedgehog (Shh), a key regulator of limb development downstream of retinoic acid and Hoxb-8, was also damped. The discovery that miR-196 inhibits Hoxb-8 expression in a hindlimb-specific fashion, provides one of the earliest examples for a miRNA playing a role in vertebrate development and supports the idea that miRNAs may function in fail-safe mechanisms, assuring fidelity of expression domains.

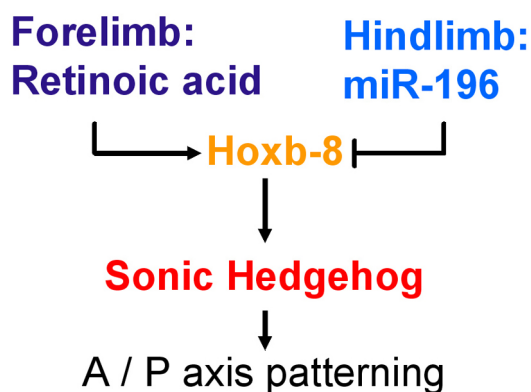


Fig. 2 Retinoic acid induces Hoxb8 transcription, upstream of Sonic hedgehog in the developing vertebrate forelimb. The presence of Hoxb8 is unwanted in the hindlimb and its transcription is shut off. However minute levels of mRNA are still transcribed and the hindlimb specific miR-196 directs cleavage of the unwanted Hoxb8 transcript.

miRNA function in skull development

The vertebrate skull is a complex structure composed of a number of dermal (intramembranous) bones that function in physical protection of the brain, mastication and hearing. Most of the dermal bones ossify directly from the cranial neural crest-derived mesenchyme. To reveal a potential role for miRNAs in craniofacial development we used a conditional allele of Dicer. We have removed Dicer in cells originating from the dorsal neural tube, including cranial neural crest cells. The resulting striking phenotype includes skull bone agenesis and aberrant cell fate of some other cranial structures. We are now profiling the miRNA populations of the cranial structures using our miRNA-specific microarray. We will try to establish the in vivo role of a few candidate miRNAs from our screen in skull development using the rapid chick misexpression system and mouse genetics. We believe that this study, will enable us to uncover some of the roles played by miRNAs in skull bone formation. More broadly, this setting would provide insight into the involvement of miRNAs in differentiation decisions to create diverse fates from a homogenous population of progenitors.

miRNA global effects

While extensive in-silico studies predict the genome-wide interactions of miRNAs and their mRNA targets, no systematic attempts were yet taken to analyze the global downstream effects of the miRNA network on gene expression. To this end we have generated a cell line in which Dicer can be knocked-out at will, in culture. In collaboration with Chris Burge's lab (MIT), which provides the strong computational power needed for this analysis, we correlate expression levels of specific miRNA species to the magnitude of their effect on target mRNAs. We provide global experimental evidence supporting the rationale of current bioinformatic predictions that miRNA broadly affect the transcriptome. Further we uncover that miRNA loss of function can be depicted at targets mRNA level, reflecting very broad miRNA-directed mRNA cleavage.

Selected publications

- Hornstein E., Mansfield JH., Yekta S., Hu Kuang-Hsien J., Harfe BD., McManus MT., Bskerville S., Bartel DP., Tabin CJ. (2005) The microRNAs, miR-196, acts upstream of Hoxb-8 and Shh in limb development. *Nature*, 438, 671-4.
- Harfe BD., McManus MT., Mansfield JH., Hornstein E., Tabin CJ. (2005) The RNaseIII enzyme Dicer is required for morphogenesis but not patterning of the vertebrate limb. *Proc Natl Acad Sci U S A.* 102, 10898-903.
- Hornstein E., (2006) Hox cluster-embedded miRNAs *Cell Cycle*, in press.
- Hornstein E., Shomron N., (2006) Canalization of Development by miRNAs *Nature Genetics*, in press

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