# miRNA Role in Development and Disease

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Regulation by microRNAs (miRNA) appears to be the most abundant mode of post-transcriptional regulation, affecting most genes. With some 450 miRNA genes in mammals, occupying ~2% of the transcribed genome, miRNAs are a big family of regulators, in the order of magnitude of bHLH transcription factors or the family of genes encoding for kinases. Each miRNA has a few dozen downstream targets that can be reliably predicted by sequence complementation. The combination of hundreds of known miRNAs, together with their set of downstream mRNA targets, suggests that practically all cellular and organismal processes, whether in health or in disease states, are regulated by miRNAs. The aim of the projects in our lab is to understand the logics governing miRNA-based regulation of gene expression and the specific involvement of miRNAs in a variety of developmental systems.

## miRNAs confer robustness to development

One role for miRNAs is in preventing inappropriate ('leaky') expression of genes in domains where ill-controlled expression may affect the phenotypic outcome. For example, we have found that in the developing hindlimb miR-196 works as a safeguard against leaky expression of Hox genes such as Hoxb8 (Hornstein et al., 2005). Interestingly, the miR-196 gene is embedded within the Hox genomic cluster, which it regulates. miR-196 is likely just the first miRNA to be functionally described in this cluster. Using high throughput methods such as tiling arrays, we seek (together with the Segal lab) novel miRNA genes, transcribed from the Hox clusters that may regulate Hox gene expression and thereby affect developmental processes.

The idea that miRNAs confer robustness to biological programs (reviewed in Hornstein and Shomron 2006) is, of course, not limited to the Hox cluster and implies that de-regulation of miRNAs may contribute to a large set of human diseases. Thus, we explore miRNA involvement in models of human disease including metabolic/endocrine and bone diseases.

## miRNA in pancreas development and Diabetes Mellitus

Pancreatic beta cells are responsible for regulation of blood glucose levels by secretion of the hormone insulin. We have used a specialized expression array to create pancreas specific miRNA profiles from different developmental stages and physiological conditions. Promising miRNA candidates are now being investigated using *in vivo* gain- and loss- of function approaches.

In a complementary study we have inactivated the miRNA pathway through a beta-cell specific deletion of Dicer, the enzyme necessary for biosynthesis of functionally-mature miRNAs (Figure 1). We have recently uncovered that mice harboring a deletion of Dicer in beta cells exhibit hallmarks of Diabetes

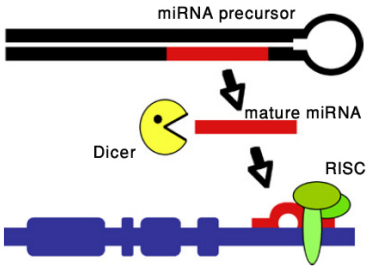


***Fig. 1*** *miRNAs (red) are processed from a precursor form (black) that folds back on itself to form a hairpin. Dicer (yellow) digests the double stranded hairpin to generate a single strand miRNA. This mature miRNA recruits an RNA induced posttranscriptional silencing complex (RISC, greens) that binds to the 3' untranslated region of messenger RNA (blue).*

Mellitus, such as impaired glucose tolerance and elevated fasting plasma glucose levels (Figure 2). We now study, in collaboration with Yuval Dor (Hebrew University), the molecular mechanisms by which miRNAs regulate glucose-insulin homeostasis (Melkman et al., submitted).

## miRNA in the development of skeletal tissues

Hereditary disorders such as dwarfism, cleft palate and craniosynostosis are diseases frequently observed in humans. We study miRNA contribution to these devastating conditions. For example, we have recently noticed striking features such as complete loss of the skull vault and the jaws upon deletion of Dicer in neural crest cells (figure 3). We have profiled the relevant miRNA populations using a miRNA-specific microarray and are now studying the intertwined network of miRNAs and transcription factors in skeletogenesis with the aim of understanding the basis for the human disorders.

Another key component in bone structure and physiology is the osteoclast, a matrix digesting cell. We study the role of miRNAs in differentiation and function of osteoclasts using high throughput screening techniques (A joint project with the Geiger lab). Together these projects will increase our understanding of bone development and homeostasis and provide insight into the involvement of miRNAs in differentiation decisions and tissue function.


***Fig. 2*** *Adult mice wherein the miRNA pathway was inactivated in a beta cell specific manner (RIP-Cre;DICER$^{cond/cond}$) fail to normalize their plasma glucose levels 120 minutes after being challenged with a standard glucose tolerance test.*

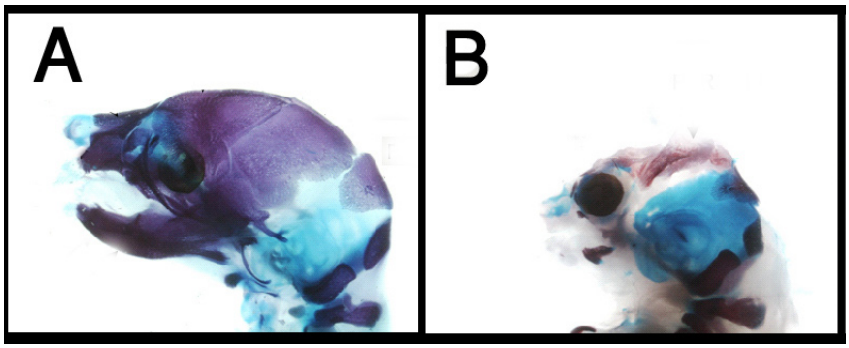


***Fig. 3*** *Dicer null mutant embryos (Wnt1-Cre;DICER$^{cond/cond}$) are missing the lower and upper jaws, the nasal bone and the frontal bone. A, side view of a wild type E17.5 embryo stained for bone (red) and cartilage (blue). B mutant littermate.*

**Modeling the genetic network underlying cell fate determination**

miRNAs and transcription factors orchestrate cell fate determination in multicellular organisms. However, the complexity of the genetic network often hampers our ability to uncover the rationale underlying its design. In collaboration with Namma Barkai we take a modeling approach to reveal how transcription factors and miRNAs work in establishing cell fates. We then use Drosophila to experimentally test predictions from our computed model. Drosophila provides a good model system as it is highly amenable to genetic perturbations and has a significantly simpler genetic network. We also use Drosophila to ask the question at the population level, understanding how miRNA-conferred genetic robustness controls the generation of reproducible phenotypic outcome.

In summary, posttranscriptional regulation by miRNA is pivotal for cellular function. Our research uncovers miRNA role in development and in disease.

**Selected publications**

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