One commonality of nearly all tissue-based organisms is their dependence on microRNAs (miRNAs) for proper cell differentiation and development. These short, 16-22nt single-stranded RNA molecules facilitate post-transcriptional gene silencing across the genome in both plants and animals. However, traditional "knockout" experiments of individual (or several) miRNA-coding genes typically produce phenotypically normal outcomes, suggesting that the necessity for miRNAs in proper animal development, viability, and reproduction may be a "genome-scale" phenomenon. By studying the effects of miRNA-mediated gene regulation on generic gene regulatory networks (GRNs), we aim to better understand the basic biological laws that govern miRNA-mediated regulation, and to help explain why these regulatory units are so critical for development of plants and animals.

Challenges in traditional approaches While progress has been made in understanding the role of miRNAs in traditional "wet lab" settings, a number of intrinsic properties of miRNAs make them challenging to study in these settings. First, despite our ability to identify potentially thousands of miRNAs in the human genome, the number of truly *functional* miRNAs remains a mystery. Furthermore, the target specificity of any miRNA depends on only about seven nucleotides. As a result, there is a high level of redundancy in expressed miRNAs, and an individual miRNA may target hundreds, or even thousands of genes. Taken together, these features may help explain why animal and plant development is robust to large perturbations in the "miRNA-ome."

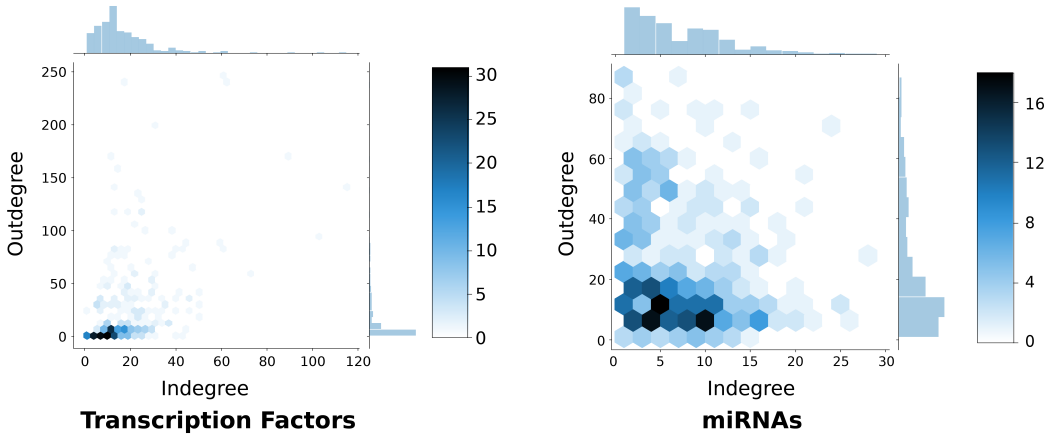

Figure 1: Density plots of *experimentally observed* regulatory connections of transcription factors (TFs) and miRNAs in the human GRN. Note that while TFs tend to have more narrowly-defined connections, miRNA connectivity is much more spread out, highlighting the deep connectivity of miRNAs in GRNs. Similar plots of *predicted* miRNA targets increase the number of connections approximately 5-10 fold.

Mathematical modeling of GRNs On the other hand, a number of generic properties of miRNAs make them quite suitable for mathematical modeling. For example, we know that miRNA-mediated regulation is required in a wide variety of plant and animal GRNs; by abstracting the network properties of these GRNs, we can apply a single model to a large set of different GRNs and look for common patterns. Additionally, *in silico* models allow us to explore more complex "knockout/knockdown" configurations than might be testable *in vitro*, allowing us to study the limits of miRNA redundancy and robustness. Finally, mathematical modeling allows us to track expression profiles continuously over time, allowing us to test the role of miRNAs in the critical early embryonic development window.

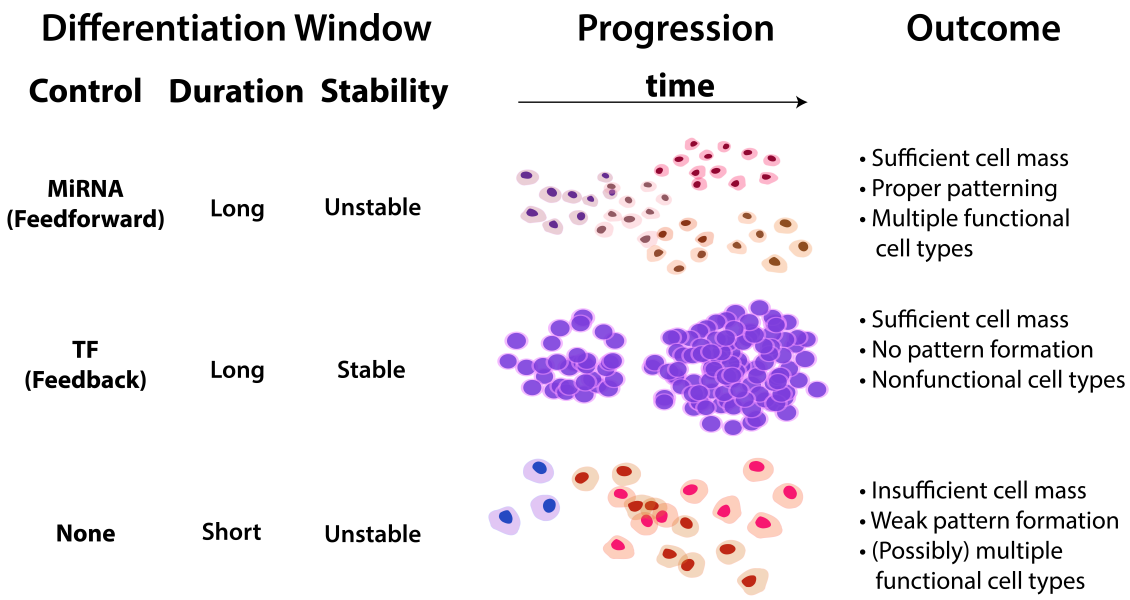

Figure 2: MiRNAs can provide a unique type of regulation which may help shape the window during which stem cells undergo differentiation. Prior to the onset of strong TF-mediated lineage-specifying feedback, miRNAs can prolong the undifferentiated state, while releasing control as differentiation proceeds. This control mechanism can allow cells time to divide and form patterns, while still guaranteeing that cells commit to a specific lineage.

A proposed role for miRNA-mediated regulation in early development By studying several different models of miRNA-mediated regulation (e.g. Boolean, continuous, and integer-valued models), we believe that miRNAs may be responsible for helping to extend the "lifetime" of undifferentiated cells prior to commitment. Unlike other regulatory mechanisms, miRNA-mediated regulation appears to be strongest when transcription factor expression is low (as in many stem cells), while also being transient. This transience is critical, as it helps to define a clear, finite "differentiation window" which can generate a large number of functional, differentiated tissues, while limiting the potential for indefinite self-renewal and hyperproliferation of non-functional cell types.