Once regarded as a biological anomaly, microRNAs (miRNAs) have since been recognized as a prevalent RNA species that regulates a wide array of biological processes, from fat storage and insulin secretion, to apoptosis and cell growth. Recent studies show that miRNAs are expressed at precise times and locations in embryonic development. Moreover, disruption of miRNA processing triggers widespread developmental defects. These findings bolster the idea that miRNAs also regulate multiple aspects of embryonic development. This primer focuses on the emerging roles of miRNAs in development. The basics of miRNA biogenesis and miRNA and mRNA target identification are covered, with an emphasis on miRNA function in development. The primer also features a dialog about current topics in the field. Developmental Dynamics 235:846 – 853, 2006. c 2006 Wiley-Liss, Inc.

**Key words:** microRNA; dicer; heterochronic; lin-4; lsy-6; miR-1

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**THE JOURNEY FROM miRNA TRANSCRIPT TO GENE REPRESSION**

Despite its small size, the ~22-nt mature miRNA is a potent inhibitor of posttranscriptional gene expression. The journey from miRNA transcript to target recognition is complex, involving a number of enzymatic steps summarized in Figure 1 (Zamore and Haley, 2005; Hammond, 2005). First, the Ribonuclease (RNAse) III enzyme Drosha excises a ~70-nt stem-loop, or hairpin precursor (pre-miRNA) from the primary transcript (pri-miRNA). Next, a second RNAse III enzyme, Dicer, liberates the mature ~22 nt miRNA, comprised of half of the hairpin, from the pre-miRNA. These processing events require the dsRNA-binding domain cofactors (dsRBD) Pasha, Loquacious, and R2D2. The mature miRNA is then incorporated into the RNA-induced silencing complex (RISC), which includes the RNA binding protein Fragile X mental retardation protein (FMRP) and the mRNA “slicer” Argonaute. Finally, the miRNA guides RISC to its complementary sequence, usually in the 3’UTR of its target mRNA.

The degree of base pairing between a miRNA and its target dictates the mechanism by which the target gene is silenced (reviewed in Zamore and Haley, 2005). Nearly perfect base pairing, a phenomenon that is rare in animals but common in plants, triggers mRNA destruction via the RISC “slicer” enzyme, Argonaute (Ago-2 in humans) (Liu et al., 2004; Song et al., 2004). Imperfect complementarity between the miRNA-mRNA target instructs inhibition of protein synthesis by an unknown mechanism. Imperfect sites fall into one of two categories: 5’ dominant sites and 3’ compensatory sites (Brennecke et al., 2005). The most common, the 5’ dominant site, frequently base pairs with a “seed sequence,” 7–8 nt near the 5’ end of the miRNA. 5’ dominant sites base pair at both the 5’ and 3’ ends of the miRNA, often leaving an unpaired bulge in the middle. It is not known if one mode of repression offers a distinct biological advantage over another.

**miRNAs REGULATE MULTIPLE ASPECTS OF DEVELOPMENT**

Many studies demonstrate that miRNAs function in development. Large-scale miRNA expression profiles show that many miRNAs are expressed transiently during specific stages of embryonic development (Aravin et al., 2003; Miska et al., 2004; Wienholds et al., 2005). In addition, dozens of miRNAs are exclusively expressed in single tissues including brain, lung, spleen, liver, heart, skeletal muscle,
kidney, and ES cells (Miska et al., 2004; Sempere et al., 2004; Suh et al., 2004; Houbaviy et al., 2005). These data imply that miRNAs are poised to regulate a myriad of developmental events.

Recently, Schier and colleagues tested the requirement for overall miRNA function during development by creating zebrafish lacking both maternal and zygotic dicer (MZ dicer), rendering animals incapable of producing mature miRNAs (Giraldez et al., 2005). MZdicer embryos unexpectedly generate all major cell types, and exhibit normal embryonic patterning. Despite a good start, things quickly go awry. Late embryos exhibit defects in the retina, spinal cord, heart, somites, and, most noticeably, in the brain. These findings corroborate expression profile experiments showing that miRNAs regulate multiple developmental events. Unexpectedly, the findings also suggest that miRNAs play a limited role in early development, and instead mainly regulate later morphological and differentiation events.

The notion that miRNAs predominantly function in differentiation contradicts previously published results. For example, Dicer1-deficient mice die before axis formation and fail to express the mesodermal specification gene T brachyury (Bernstein et al., 2003). In addition, hundreds of miRNAs were isolated from early stage Drosophila embryos (Aravin et al., 2003). Finally, miRNAs regulate many aspects of stem cell development (Houbaviy et al., 2003; Suh et al., 2004; Hatfield et al., 2005; Kanellopoulou et al., 2005). These data suggest that miRNAs also take part in early developmental events. Why there are discrepancies between the two sets of studies remains to be determined. Nevertheless, miRNAs perform vital roles in embryonic development.

**METHODS FOR UNDERSTANDING miRNA FUNCTION**

The first discovered miRNA, lin-4, was isolated in a *C. elegans* screen looking for genes that control timing of postembryonic developmental events (see “miRNAs in Development: From Target to Function” Section). Although genetic screens have since proven to be among the most successful methods of isolating miRNAs that regulate development, it is a labor-intensive and identification of miRNAs is serendipitous. Outlined below are alternative means for isolating and verifying miRNAs and their targets, and discovering their biological roles.

**miRNA Prediction and Validation**

miRNAs are most commonly isolated by size-fractionating, amplifying, and cloning the small RNA population.
from cells. Only clones shown by bioinformatics to fit the following criteria make it to the next round. They should have high-sequence conservation between related species, located in intergenic sequence, flanked by sequence that has the potential to form a stem-loop precursor, and mature miRNA should be expressed as a ~22-nt transcript (Lee and Ambros, 2001; Berezikov et al., 2005).

Candidate miRNAs are not considered bona fide until they are experimentally validated either by expression or functional analysis. Expression is verified by Northern analysis or even against miRNA microarrays from other species (Lee et al., 1993; Krichevsky et al., 2003; Wienholds et al., 2005). Sites of miRNA activity are revealed through miRNA sensors. miRNA sensors are comprised of a reporter under the control of a heterologous promoter, and have 3’UTR sequence that is perfectly complementary to the miRNA (Brennecke et al., 2003). In transgenic animals bearing sensors, cells with miRNA activity show reduced reporter expression. An important caveat to keep in mind is that because mismatches are tolerated between miRNA and its target, each of the above methods tolerates a few mismatches, meaning that they may detect more than one miRNA species (Miska et al., 2004; Suh et al., 2004). The problem of mRNA-miRNA binding specificity is largely circumvented by a more recent approach. Incorporation of high-affinity RNA analogues, locked nucleic acids (LNA), into oligonucleotide probes vastly increase the sensitivity and specificity of oligo:RNA hybridization. LNA modified oligos have improved resolution of Northern analysis, and have made in situ hybridization against the small miRNAs possible (Valoczi et al., 2004; Wienholds et al., 2005).

Aside from mutant analysis, overexpressing miRNAs (Zhao et al., 2005) or inactivating miRNAs via anti-sense oligonucleotide injections (Leaman et al., 2005) can provide insights into miRNA function. While different methods have collectively validated hundreds of miRNAs, identifying and verifying their targets has proven to be more challenging.

**Target Prediction and Validation**

miRNA targets have been difficult to identify due in large part to the lack of strict base pairing between miRNA and mRNA target sequences. Computational target prediction programs rely mainly on four criteria: base pairing rules between mRNA and miRNA target sequences. Computational target prediction programs rely mainly on four criteria: base pairing rules between mRNA and miRNA target sequences. Computational target prediction programs rely mainly on four criteria: base pairing rules between mRNA and miRNA target sequences. Computational target prediction programs rely mainly on four criteria: base pairing rules between mRNA and miRNA target sequences. Computational target prediction programs rely mainly on four criteria: base pairing rules between mRNA and miRNA target sequences. Computational target prediction programs rely mainly on four criteria: base pairing rules between mRNA and miRNA target sequences.

A newer algorithm also takes into account miRNA binding site accessibility based on secondary structure (Zhe et al., 2005). A searchable predicted target and miRNA database, miRBase, can be accessed at http://microrna.sanger.ac.uk/ (Enright et al., 2003; Griffiths-Jones, 2004).

Presently, there are distinct disadvantages to using algorithms for miRNA target prediction. First, because miRNA-target pairing is based on computational information, certain biological tools, such as miRNA mutants, are often not available to verify an in vivo role. Second, existing experimental data, such as expression data, are frequently used to narrow a list of potential miRNA targets. This approach categorically excludes identification of novel genes as targets. Third, each target prediction algorithm incorporates slightly different criteria. This has resulted in few overlapping targets between predicted target datasets, prompting the researcher to wondering, “Which dataset should I use?” As more miRNA-target pairs are validated, undoubtedly prediction programs will evolve to become more accurate.

After target prediction, candidates must be verified as miRNA targets. One method is to incorporate the target’s 3’UTR sequence into a miRNA sensor and monitor cells where the native miRNA is expressed, or where it is experimentally overexpressed, for downregulation of the sensor (Hipfner et al., 2002; Chang et al., 2004; Mansfield et al., 2004). Another method is to examine cells in which an miRNA has been overexpressed for stable target mRNA expression and reduced protein expression (Zhao et al., 2005). Below is a published example using many of the methods for target prediction and validation described above.

**Targeting Hox Clusters**

Bioinformatics successfully identified targets of the miRNA subfamily, miR-196 (Yekta et al., 2004). miR-196 consists of several highly related miRNAs that are embedded throughout Hox clusters. Based on this observation, the authors hypothesized that the miR-196 family regulates Hox genes. Using bioinformatics, evolutionarily conserved miR-196 complementary sequence was found in the 3’UTRs of HoxB8, HoxC8, HoxD8, and HoxA7 (Lim et al., 2003). Four lines of evidence suggest that at least HoxB8 is a bona fide target of miR-196. First, expression of HoxB8 in mouse embryos is opposite to miR-196 sensor activity, implying that miR-196 downregulates HoxB8 mRNA (Mansfield et al., 2004). Second, miR-196 and complementary sites in HoxB8 show nearly perfect base pairing. Consistent with this finding, a modified form of 5’ RACE detected HoxB8 RNA products that were cleaved at the putative miR-196 binding site in mouse embryonic tissue. Finally, ectopic miR-196 downregulates sensors bearing target HoxD8, HoxC8, and HoxA7 sequence in cell culture. Although Hox genes are likely targets of miR-196, the developmental consequence of losing miR-196 activity has not been determined.

**miRNAs IN DEVELOPMENT: FROM TARGET TO FUNCTION**

To date, only a handful of miRNA-driven developmental processes have been worked out from miRNA to target to function. Three examples that exploited different methods in their discoveries are described below.

**Gatekeepers of Developmental Timing**

The first identified miRNA was isolated through a C. elegans genetic
screen for heterochronic mutants, or mutants that experience a change in relative timing of developmental events. Among genes isolated from the screen were lin-4 and lin-14. During the first larval stage (L1), lin-14 mutants precociously develop cell fates typical of the L2 stage. lin-4 mutants exhibit the opposite phenotype: L1 fates are reiterated during subsequent stages (Ambros and Horvitz, 1984, 1987). Cloning of lin-4 revealed that it is a short RNA transcript with no coding sequence, what we now know is a miRNA (Wightman et al., 1984, 1987). Cloning of lin-4 directly downregulates LIN-14 expression via its 3’UTR during the L2 stage. The back-to-back Cell papers published by the Ruvkun and Ambros groups reflected a novel and, at the time, improbable finding from which an entire research field was born. Since then, it has been discovered that a number of heterochronic genes are regulated by miRNAs (Moss et al., 1997; Reinhart et al., 2000; Abrahante et al., 2003; Abbott et al., 2005; Li et al., 2005).

Generating Neuronal Diversity

In a series of thorough studies, Hobert and colleagues showed that a miRNA-driven feedback loop governs the decision between two neuronal cell fates in C. elegans. ASER and ASEL (asymmetric chemosensory neuron right and left) are chemosensory neurons that undergo a shared precursor state (ASE), but differentiate as two functionally distinct neurons. The miRNA lsy-6 was uncovered in a screen for genes that control the ASER/ASEL cell fate decision (Johnston and Hobert, 2003). Lsy-6 mutants exhibit a two ASER phenotype as a result of ASEL transforming to ASER. How does lsy-6 control ASER cell fate? Lsy-6 is expressed in ASER but not ASEL, and represses cog-1, an Nkx-type homeobox gene with lsy-6 binding sites in its 3’UTR. Cog-1, in turn represses the homeobox gene lim-6, which represses the ASER fate (Chang et al., 2004) (Fig. 2). Therefore, lsy-6 promotes the ASEL cell fate by indirectly activating lim-6, a repressor of the ASER fate.

The authors went on to show that another double ASER mutant, die-1, functions upstream of lsy-6. They reasoned that like the ASE laterality gene cog-1, die-1 might also be regulated by a miRNA. This suspicion was confirmed when they found that die-1 3’UTR-bearing GFP sensors are downregulated in ASER. Moreover, bioinformatics identified die-1 3’UTR sites that are conserved with C. briggsae and have complementarity to the miRNA mir-273. Validating a role in ASE laterality, mir-273 is preferentially expressed in ASER, and forced expression of mir-273 in ASEL represses die-1 (Chang et al., 2004). Adding another twist to the story, genetic epistasis experiments revealed that lsy-6 and cog-1 are required for the asymmetric expression of mir-273 (Johnston et al., 2005) (Fig. 2). Because ASER and ASEL develop from an equivalent precursor state, the miRNA-driven feedback loop may serve to reinforce the terminal cell fate.

Regulating Heart Development

Bioinformatics were instrumental in identifying a target for the heart-enriched miRNA subfamily, miR-1 (Zhao et al., 2005). The two highly related miRNAs, miR-1-1 and miR-1-2, are separate genes that are both expressed in the mouse heart. Consistent with a role in heart development, heart-directed overexpression of miR-1 results in proliferation defects. In an attempt to flesh out the miR-1 pathway, the authors identified 13 candidate target genes using bioinformatics. Based on previously published gene expression patterns, the candidate Hand2, which is expressed in heart, was tested further. Hand2, a transcription factor that regulates ventricular cardiomyocyte expansion, was experimentally verified as a target gene when Hand2 protein, but not mRNA, was downregulated in hearts bearing overexpressed miR-1. Interestingly, Srivastava and colleagues also discovered that direct binding of serum response factor (SRF) to miR-1-1 and miR-1-2 enhancers is required for their heart expression (Zhao et al., 2005). This finding suggests that SRF may work through miR-1 to exert its known role in controlling the balance between proliferation and differentiation during cardiogenesis.

AN INTERVIEW WITH THE EXPERTS

What has miRNA research taught us so far? Where is the field heading? These questions and more are answered by two experts in miRNA research: Oliver Hobert, Ph.D., HHMI Investigator and Associate Professor, Columbia University, and Michael McManus, Ph.D., Assistant Professor, University of California at San Francisco (Fig. 3).

Developmental Dynamics: What initially interested you in microRNA research?

Oliver Hobert: I find it fascinating to think of the whole non-coding RNA world, populated by miRNAs, siRNAs, tncRNA, or antisense RNA, as a parallel universe out there, more or less completely uncharted. As such, I was extremely happy that my own lab stumbled upon miRNAs and their targets by accident, meaning we got mutant alleles of miRNAs and their targets from a screen for neuronal cell fate mutants.

Michael McManus: I’ve actually studied small RNAs my entire research career. I became interested in microRNAs as a Ph.D. student in the lab of Steve Hajduk, where I studied the biology of Trypanosome mitochondrial RNA editing, a developmentally regulated process. In Trypanosomes, this type of editing is directed by a class of small 30–50 nt RNAs known as guide RNAs—a powerful example of how small RNAs can mitigate developmentally important processes. In my Ph.D., I studied the biogenesis and activity of these small RNAs, which have real parallels to microRNAs. These studies peaked my interest, and prompted me to explore small RNA biology in the mammalian system, where I conducted such studies in the lab of Phil Sharp at MIT, a real hot-spot for many RNA/microRNA related discoveries.

Dev Dyn: What aspects of miRNA research is your lab currently focusing on?
O.H.: We are studying the function and mechanisms of a defined set of miRNAs (let-6 and the mir-273 family) within a specific cellular context in the nervous system of C. elegans. We use forward genetic approaches to delineate the pathways in which these miRNAs act in, both in terms of upstream regulators, downstream targets, and potential co-factors. We also make use of reverse genetic approaches in which we (1) attempt to validate new targets of these defined miRNAs and (2) fine-map the precise targeting requirements for known and validated miRNA/target interactions.

M.M.: My lab is focused on dissecting the functional roles of small RNAs in mammals. This includes understanding where they are expressed, when they are expressed, and how they function. We have drawn and are continuing to draw connections between the microRNA and the RNAi pathways. Feel free to get more information at my website: http://mcm anuslab.ucsf.edu

Dev Dyn: Which do you consider the most influential papers in this field?

O.H.: The top three are very clear and easy to pinpoint. The two 1993 Cell reports by Victor Ambros’ and Gary Ruvkun’s lab, that describe the cloning of the first miRNA, lin-4, and its interaction with its target, lin-14 (Lee et al., 1993; Wightman et al., 1993). And the 2000 Nature report from Gary’s lab, showed that another worm miRNA, initially like lin-4 fated to be disregarded as a worm-specific oddity, is widely conserved across phylogeny (Pasquinelli et al., 2000). That really put miRNAs on the map and prompted tons of people to join the field and explore the abundance of miRNAs. Victor and Gary will get what they deserve for their findings.

M.M.: There are many influential papers, largely because the field is growing and moving at such a feverish pace. Among the top are papers that show the biology of small RNAs: 1) The report by Fire et al. (1998) marks discovery that the experimental introduction of double-stranded RNA (ds RNA) into cells could be used to block the function of an endogenous gene, a method that is now routinely used in diverse biological systems. The discovery that dsRNA is a potent inhibitor of gene expression founded the field of RNA interference and laid groundwork for many later studies drawing the connection between the biology of RNA interference and microRNAs. 2) The work by the Baulcombe laboratory gave the first indication of the mechanism of RNAi: tiny RNAs can be potent intermediates in the RNAi pathway (Hamilton and Baulcombe, 1999). 3) The first natural regulatory RNA of 21 nt, lin-4, was discovered in the Ambros laboratory (Lee et al., 1993). This report still represents the standard as to how microRNAs can be considered functional units, and, in fact, developmentally and biologically important genes. 4) The report by Pasquinelli et al. (2000) showed that the once-thought-peculiar lin-4 gene in worms wasn’t so odd after all. I believe that many groups in the field read this paper and thought, “WOW! There must be more microRNAs!”

Dev Dyn: What is the perceived evolutionary advantage for using miRNAs as regulators of gene function?

M.M.: To be clear, it is not altogether perceived that microRNAs have an evolutionary advantage over other classes of genes. That being said, it is worth commenting that so far microRNAs appear unique to multicellular systems, and it may be speculated that microRNAs may play a role in development of a body plan.

O.H.: Michael is making an important point in saying that miRNAs are restricted to metazoans. The challenge for every multicellular, complex organism is to be able to generate a multitude of cell-type-specific gene expression programs. The same gene regulators you have, the more options you have to evolve new programs and to diversify cell types based on novel gene expression programs. But why specifically miRNAs? I think it’s too early to answer this question because we simply understand too little about some very fundamental aspects of their mechanisms of action. Only when we understand some of these fundamental aspects will we see (or not see) conceptual advantages of their employment compared to other gene regulatory factors, such as transcription factors. For example, we need to understand whether miRNA-mediated translational repression is reversible and/or regulated. We need to understand whether miRNAs can localize to specific sites within a cell to affect gene expression. Having said all this, I would like to add that miRNAs, which perhaps a bit unfortunately became rather narrowly defined, are just one component of the small RNA world. We should not ignore that regulation of gene expression by small non-coding RNAs can be observed in bacteria and is, therefore, very ancient.

M.M.: We really think alike and I’d like to expand on our views. There are many reports suggesting that the amount of transcription in a cell may be far greater than previously thought. Why so much transcription? Does it imply that the making of a RNA is a sloppy process, and that polymerases aren’t as specific in their initiation and termination as previously thought? Certainly some of the transcription will act by modulating the activity of certain DNA binding elements, but could the data imply that there are lots of additional genes in a cell that we haven’t considered? Besides microRNAs, we do know of many additional noncoding RNAs whose functions have yet to be fully understood. microRNAs may simply be the tip of the iceberg in a set of regulatory RNA-centric biology that multicellular organisms use to sculpt themselves.

Dev Dyn: Might there be a strategy behind miRNAs repressing target mRNA via mRNA degradation vs. translational repression?

M.M.: This is a great question. One might imagine that if you could reverse the translational repression mitigated by a microRNA, you might offer a regulatable situation in a cell. For example, a timing-centric model could be postulated whereby such a de-repression could offer the cell a rapid supply of translatable mRNAs, without waiting for the gene to be transcribed. In the case of siRNA-type microRNAs, this would not necessarily be possible, since the mRNA is destroyed. Again, this could be just as important in biological processes.

O.H.: I completely agree with Michael’s assessment.

Dev Dyn: Bioinformatics have predicted a large number of miRNAs and targets, yet there are still very few pathways leading from miRNA to target to an in vivo biological process.
Why is this the case? Will it be difficult to validate novel miRNA targets?

M.M.: This is simply a matter of timing. It turns out that the bioinformatics prediction (the key word here is prediction) is the easy part, but that doesn’t mean that the predictions are always accurate. In fact, there is a strong likelihood that many targets are missed and that many targets are not correctly predicted. The hope is that investigators may use the predictions as a guide, not as a rule. It will take longer to verify and validate the biological function(s) of each microRNA. It is safe to say that the challenging part has now begun; microRNA knockouts must be made, in cells and in whole animals. What will be their phenotype, if any? It will be just as difficult (if not more difficult) to validate these computationally predicted targets. Some groups have predicted that many genes are regulated by a single microRNA. If this is the case, then the dissection of phenotypes will be challenging indeed.

O.H.: I agree with Michael’s assessment of the tentative nature of miRNA target predictions. The paucity of available miRNA knockouts is certainly the main reason why there are so few links from miRNAs to targets to biological processes. Those few examples that exist came almost all from genetic screening approaches, mainly in worms, but also in flies. But for reasons that may be trivial (such as their small size or redundancy) but also may be more complex, not many miRNAs have surfaced from genetic screens so far. Targeted knockouts are badly needed now. In regard to your second question, I don’t think it is conceptually difficult to validate predicted miRNA targets. One needs to look whether the removal of a miRNA causes an upregulation in the expression of the predicted target. Unfortunately, due to the paucity of available miRNA knockouts, most validation that is done these days is done through overexpression studies. This type of validation is, however, only tentative at best since the non-physiological concentration of the reactants may cause regulatory effects that are not existent in the natural context.

M.M.: As you indicate above, it is true that one can look for deregulation of a predicted target by “simply” adding the microRNA into a cell, but a better experiment is to remove it and look for a change in the predicted target gene expression. But what does that mean? Does it mean that the observed changes of the given target gene are biologically pertinent? This is where the challenge is. We know that a two-fold reduction of many (most?) genes may not result in any phenotype. Furthermore, there are many studies based on siRNAs showing that even a 5–8-fold change may not produce a biological consequence. So we are left with the question about the activity of microRNAs in a cell. For a given microRNA, is there only one or two biologically pertinent targets, with many “side-effect” changes in the expression of other genes? Or is the act of a 2–8-fold change in the regulation of many genes the real activity of a given microRNA?

As the field matures, it can be expected that the bar will be set higher in future publications that “show” the target activity of a microRNA through simple Western blotting of the predicted gene. That is, it may be predicted that the future gold standard for target validation will be determining if the observed changes result in a biological consequence.

Dev. Dyn: It has been proposed that miRNAs mainly regulate differentiation rather than in cell fate specification during development. What do you think?

O.H.: Your question illustrates a current tendency to prematurely extrapolate larger themes from isolated cases. The point that you mention stems from a very elegant analysis in zebrafish in which it was shown that the elimination of a miRNA-producing enzyme causes pronounced defects in cellular differentiation but appears to have little impact on very early patterning. Similar approaches have yielded very different results in mice, and a broad analysis of fly miRNAs also is inconsistent with the idea that you are referring to. The jury is still not completely out on this one.

M.M.: I agree completely with you, Oliver. The data are just too new and incomplete. Until we have data that say otherwise, I prefer to think that microRNAs may have a diversity of functions, and will also have important roles in homeostatic pathways. In fact, there are accumulating data to support this idea.

Dev. Dyn: What are some exciting ideas that are emerging in the field?

O.H.: I am not too terribly excited about ideas that are currently floating around simply because there is too little hard data. This being said, I consider most of the existing target gene predictions as interesting ideas, not more, not less, which require experimental validation in a true, in vivo context. And that’s exactly my point. We all need to stay in the trenches and collect hard facts. It’s sobering to realize that we only have two miRNA mutants in flies and six in worms, none in any other species. As long as we don’t systematically and very carefully analyze miRNA knockouts, we (myself included) should refrain from proposing grand schemes, as tempting (and as fun) as it may be.

M.M.: It’s totally true that we need to get back to the trenches and fill in some really hard facts. But while we’re in the trenches and shoveling away, I can’t help but wonder about which of the preliminary data and recent publications from different labs will turn out to be important broad-reaching biology. Will microRNAs be involved in methylation or even heterochromatin formation? Will they act in “fortuitous” ways to prevent or assist in viral infection? Will some or many microRNAs act as “micromanagers”? And a concept that I find particularly intriguing is the question as to whether there will be insight into human disease? For example, could a microRNA(s) be the culprit in diseases where a gene has been mapped to a locus, but the candidate protein-encoding disease gene has been elusive? It is important for us to refrain from proposing grand schemes (myself included) but it will also be important to keep our minds open to the possibilities... why not revisit older mapping data and look for noncoding RNAs such as microRNAs?

Dev. Dyn: What are some important questions about the role of miRNAs in development that remain to be answered?

O.H.: The questions are simple: What do they do and how do they do it? What are their targets, and in what cellular context and what biochemical pathways do they act? These are the
basic questions that we need to address. Only once we have a more comprehensive picture can we look and see whether they really do something conceptually novel on a large scale or whether they do, just like other gene regulatory factors, lots of different things in lots of different contexts.

M.M.: One question I would postulate is whether microRNAs in general should be classified as developmentally important genes. In other words, although the few microRNAs that have been studied so far appear to have roles in development, will this hold true for the remaining hundreds or even thousands that may exist in genomes? These are exciting times for small RNA biologists since these questions are so open. I mean, sometimes it is the small things that turn out to be the most important... and sometimes not.

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