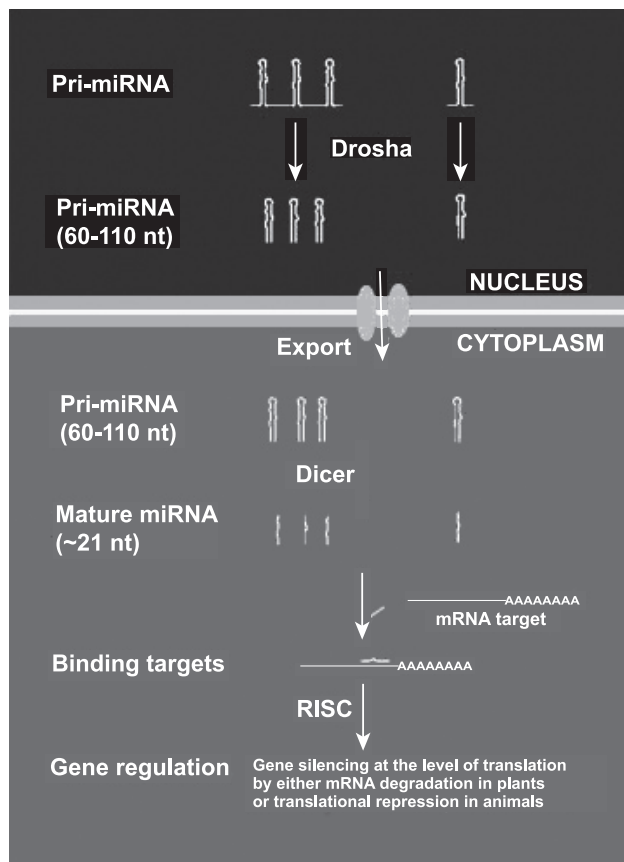


From Precursor to Mature MicroRNA

Synthesis of a microRNA begins in the nucleus as a pri-miRNA is transcribed. The pri-miRNA is typically more than 100 nucleotides long. It base-pairs within itself to form an elongated secondary structure that is open, with 2 strands at 1 end, and forms a small hairpin at the other end, with base-pairing in a neck region that might not be perfect, leaving a small bump (Figure 1.1).

Figure 1.1. Model for MicroRNA Biogenesis



Source: GenoSensor Corporation

2.2. Inhibiting MicroRNAs

The classic approach of the geneticist is to delete a gene or block its activity and then to observe the effect on the phenotype to infer wild-type function. Investigating microRNA function uses modified oligonucleotides that are antisense (complementary) to all or part of a specific microRNA. The oligos both resist degradation by nucleases and bind with great affinity to their complementary microRNAs. Following are descriptions of several such approaches.

Locked Nucleic Acid Modified Antisense Oligonucleotides

An LNA, as described previously, can basically immobilize the microRNA to which it binds. The first demonstration of the LNA technique inhibited a specific microRNA in *Drosophila melanogaster* cells in culture and tracked the increase of the protein, whose gene the microRNA usually holds in check (Ørom et al., 2006). LNA technology has been used in conjunction with in situ hybridization in a loss-of-function assay for microRNAs (Naguibneva et al., 2006).

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Morpholinos

In chemistry, a morpholine is a 6-membered ring functional group that includes an oxygen and a nitrogen. In molecular genetics, a morpholino is a specific type of subunit that includes any of the 4 nitrogenous bases found in DNA, but with a morpholine ring in place of the deoxyribose and a phosphorodiamidate linkage in place of the phosphate group.

Morpholino antisense oligos are generally 25 morpholino subunits long. They bind to RNAs with specificity and great affinity, blocking the RNA bases so that transcripts cannot be translated. In addition to knocking down the activity of mature microRNAs, morpholinos can block the processing of the pri-miRNA or the pre-miRNA. Morpholino antisense oligos were invented by James Summerton, PhD, in 1985 (Summerton, 1989) and are marketed for research purposes by GeneTools (Philomath, OR).

oncogenes and tumor suppressors, such as *TGFBR2* and *RB1*. The fact that the same microRNAs are implicated in different cancers suggests that they may be targeting common regulatory pathways.

Clinical Applications of MicroRNAs in Cancer

Clinical applications of microRNAs are and will in the future be based on the fact that these RNAs are expressed quite differently in cancer cells than in the normal cell types from which the aberrant cells descend, and that the particular patterns are reproducible from patient to patient. MicroRNAs will become important in diagnosis and staging, prognosis, selecting treatment, and monitoring response to treatment (Jay et al., 2007). MicroRNAs that actually help protect against cancer, such as by dampening metastases, may suggest new treatments (Tavazoie et al., 2008).

Diagnosis

In the diagnostic arena, microRNA expression profiles correlate extremely well with traditional histological classification of tumor types. One study of 334 samples from leukemias and solid tumors found that microRNA profiles define the same subgroups as diagnoses based on cell lineage or state of differentiation (Lu et al., 2005). The Lu study, in fact, found that microRNA expression profiles are better classifiers than mRNA profiles.

MicroRNA profiles as diagnostics may prove especially attractive, and with large markets, for the more common cancers that currently have unpleasant, invasive, costly, or otherwise less-than-ideal diagnostic workups. This is the case for lung cancer. Current diagnostic tests include helical computed tomographic (CT) scans, sputum testing, and x-rays. Screening is not yet routine. Rosetta Genomics is developing a diagnostic test, performed on sputum and blood, to detect a panel of 20 microRNAs with altered expression in lung cancer cells. A microRNA-based diagnostic is also in development at Rosetta for colorectal cancer, for which current diagnostics include Hemocult testing and colonoscopy.

The specificity of microRNA expression profiles in distinguishing cancer subtypes is especially valuable for “cancers of unknown primary” (CUP), in which the first evidence of disease is metastasis, with the tumor of origin unknown. According to Dalia Cohen, “In 3% to 5% of all cancer patients, clinicians cannot identify the origin of a patient’s tumor, information that is crucial for determining treatment type.

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Table 4.1. MicroRNAs Implicated in Development and/or Differentiation

Process	MicroRNA
Adipocyte differentiation	<i>miR-143</i>
B-cell progenitor determination	<i>miR-181</i>
Myeloid differentiation	<i>miR-196</i>
T- + B-cell differentiation	<i>miR-155, 150</i>
Muscle cell differentiation	<i>miR-1, 133, 206, 208</i>
Cardiovascular system development	<i>miR-1-2, 133</i>

Source: *Insight Pharma Reports*

MicroRNA Control in Embryos

MicroRNAs control even the earliest embryos. These actions can shed light on decades-old observations. This is the case for Spemann's organizer, the group of cells in the equatorial region of amphibian blastulae that intercepts signals that set up the fundamental 3-layered organization of the embryo. One set of signals, entailing ligand binding to transforming growth factor-beta, sets the developmental course toward forming the 3 primordial germ layers. The other set of signals, operating through the *Wnt* gene, sets up dorsal-ventral distinctions. Stefano Piccolo, PhD, and coworkers at the University of Padua (Italy) recently found that *miR-15* and *miR-16* control the size of Spemann's organizer in *Xenopus laevis* embryos by targeting a Nodal receptor (Martello et al., 2007).

In the zebrafish embryo, microRNAs orchestrate later developmental events, such as segmentation (Wienholds et al., 2005). Ronald Plasterk, PhD, and his group at the Centre for Biomedical Genetics (Utrecht, The Netherlands) examined 115 conserved microRNAs in zebrafish embryos and used locked nucleic acid (LNA)-modified oligonucleotide probes to track their expression during segmentation (Kloosterman et al., 2006). They also identified mutations in microRNA genes that arrest development. The group concluded that the microRNAs they investigated function in maintaining the differentiated state, rather than the earlier determination of cell fate shown for the amphibian embryos.

Researchers and industry watchers cite several reasons why the microRNA field is so promising:

- MicroRNAs as a whole have broad effects, so they can target a variety of clinical areas.
- MicroRNAs may provide treatments for common conditions that have been very challenging to treat and for which targeting conventional gene products—proteins—has not been successful.
- Small noncoding RNAs as diagnostic tools and therapeutic agents may fill niches left by lags in nurturing other classes of small molecules toward the clinic.

Commercializing microRNAs will present challenges as well as promises. Gene expression studies will be required to predict off-target effects that are inherent in microRNA function. For example, silencing a microRNA would upregulate the target that it normally represses, but may also downregulate other genes. Silencing an oncogene via altering microRNA function could be counterproductive if it introduces a secondary problem. It is hard to know what we have yet to learn. “To a large extent, we still don’t know their biological functions. For most miRNAs, there has been no established role,” said Scott Hammond, assistant professor of cell and developmental biology at the University of North Carolina in Chapel Hill.

Commercializing microRNAs will present challenges as well as promises.

5.2. Commercialization

The companies associated with microRNAs came to the field in a few general ways. These include:

- New companies devoted exclusively to microRNAs
- RNAi-focused companies that are exploring microRNAs on a small scale or are acquiring companies with microRNA expertise
- Spinouts from 1 or more companies
- Suppliers of reagents, software, devices, and other tools essential to microRNA research

have 1 or 2 people investigating them. Some companies are toying with this on a small scale. Big Pharma is getting involved. Merck bought Sirna, and they had a microRNA program. They are paying attention, perhaps more through acquisitions than developing in-house groups.

CHI: *What is your group working on now?*

Dr. Slack: We are pursuing the roles of microRNAs in development, cancer, and aging. We are still using *C. elegans* to help identify additional important microRNAs. We anticipate that the story will not end with the few known microRNAs; there will be many, many more stories in the future.

Phillip D. Zamore, PhD

Gretchen Stone Cook Professor of Biomedical Sciences, Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, MA

CHI: *Can you trace the beginnings of the microRNA field?*

Phillip Zamore: It started in 1993, with Victor Ambros' work, when he was at Harvard. The second paper came in 2000, and then a whole bunch of papers were published in 2001.

CHI: *Why the time lag?*

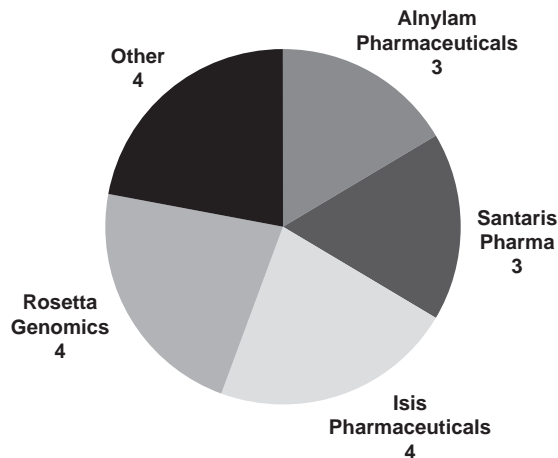
Dr. Zamore: I think it really came down to the idea of people being ready to discover things. When Victor made his original discovery, no one doubted that he'd discovered something important. I was a young post-doc then. The work wasn't ignored, but it was unusual and difficult to understand. Not until relatively recently was there a mechanistic framework within which to fit his discovery. On the one hand, microRNAs could have been discovered earlier, but I'm not sure what we would have done with them.

Understanding RNAi and how it works made it easier to figure out what kinds of questions to ask about microRNAs. Smart questions. Are these microRNAs really just siRNAs? No. Then we had to figure out how they differ. They have a lot in common, but they also have differences.

In response to the question concerning their involvement with companies conducting drug target screening based on microRNA, respondents were almost evenly split among Isis Pharmaceuticals and Rosetta Genomics at 4 each (27%), and Alnylam Pharmaceuticals and Santaris Pharma at 3 each (20%), with 4 citing others.

Figure 19A. Companies Involved in Drug Target Screening Using MicroRNA

Do you work with any of the following companies that are conducting drug target screening based on microRNA?



N = 52

Source: *Insight Pharma Reports MicroRNA Survey—December 2007*

When asked to estimate the time it will take for the first microRNA product to be used in the clinic, 51 individuals responded; 13 (25%) projected within 2 to 3 years, 10 (20%) went for 4 to 5 years and 6 to 8 years, 6 (12%) projected 8 to 10 years, and 12 (24%) were unsure.