Michael T. McManus: Interrupting biology

Michael McManus wants to use small bits of RNA to end the mischief caused by genes gone bad.

The human genome encodes snippets of RNAs ranging from 21–23 nucleotides in length, called microRNAs (miRNAs). Rather than code for proteins, miRNAs instead sabotage the translation of mRNAs. miRNAs are first generated as longer, hairpin-like precursors in the nucleus. In the cytoplasm, a collection of enzymes strips down the hairpins into their tiny and unwound final structure. miRNA-bound mRNAs are either sequestered from translation or destroyed outright—processes known as RNA interference (RNAi).

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Michael McManus has been a fan of small RNAs and their manipulative power his entire career. As a graduate student, he studied how small RNAs in trypanosomes guide RNA editing, whereby incomplete mitochondrial mRNAs are filled in with uridine residues (1, 2).

During his postdoctoral stay in Nobel Laureate Phillip Sharp's laboratory at MIT, McManus's work led to one of the first reports of RNAi activity in mammalian cells (3). He showed that T cell genes could be suppressed by a class of small double-stranded RNA called short interfering RNAs (siRNA). His next discovery—that synthetic miRNA hairpin structures could be stably introduced into cells via DNA vectors—gave the field a reliable tool for studying RNAi (4).

Now heading his own lab at the University of California, San Francisco, McManus is exploring the role of miRNAs during development and disease. His team has developed a mouse model in which the gene for the miRNA-processing enzyme Dicer can be selectively switched off (5) and a technique to visualize miRNA expression patterns in situ (6). They are now creating RNAi libraries that can be used to analyze gene function in cells and in animal models. McManus is also involved in collaborations to understand how miRNA failures lead to diseases such as diabetes. He recently began a project funded by the Keck Foundation to create 100 different miRNA mouse knockouts.

FINDING THE WAY TO RNA

When did you decide that you wanted to be a scientist?

During my early high school years, I was painting a lot and creating three-dimensional art and thought of making a living as an artist. Then, in my last year in high school, I realized that my second passion, science, was a more practical route. I figured that, as an artist, it would've been hard for me to do molecular biology on the side. But as a molecular biologist, I could maybe do some art on the side.

Has it actually worked out that way?

I'm not doing as much art as I would like. But had I gone the art route, I definitely wouldn't have done as much molecular biology as I have.

How did your interest in RNA biology come about?

I initially thought I wanted to be a plant biologist and went off to agricultural school at Auburn University in Alabama, which is where I'm from. But by my second year as an undergrad, I was swapping courses like pomology (the study of pitted fruits) for plant genetics and biochemistry. I remember sitting in the dean's office trying desperately to get out of taking courses like pesticide management.

So when I went off to graduate school, I tried out neurobiology and glycobiology before going to Steve Hajduk's lab to study RNA editing. All the lab rotations were fantastic, but the RNA work thrilled me because I saw a lot of black boxes.

FROM EDITING TO INTERFERING

What was your Ph.D. project?

I studied how guide RNAs direct RNA editing—a process that's only been seen so far in trypanosomes. By the end of the project, we had a good idea of how these RNA bits got anchored to the about-to-be-edited mRNA and guided its posttranscriptional processing. I discovered the identity of the RNA ligase that stitches the edited sections of the mRNA together.

Michael McManus

How did you segue from guide RNA to microRNA?

I was fascinated by the idea that small, noncoding RNAs could actually direct and regulate gene expression. I read a paper that Victor Ambros had published in 1993 in which he described the first miRNA, *lin-4*, which regulated developmental timing in *C. elegans*. At the time, everyone thought that this was an interesting and peculiar oddity in worms.

As I was wrapping up in graduate school, it was becoming clear that biologically active small RNAs could be generated from longer RNA duplexes, and there were some ideas in the field about how these RNAs were operating in plants and worms. But of course RNAi could also be occurring in mammalian cells. So I emailed Phil Sharp at MIT, and we had an interesting discussion about the topic.

Later, when I started to explore my postdoctoral options, my wife, who is a professional ballerina by training, suggested that we move to Boston where she wanted to explore the arts scene. I remembered my discussions with Phil, who is also in Boston, and decided to go there to see whether RNAi occurred in mammalian cells.

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TRYING TO DELIVER

Apart from studying RNAi in T cells, you also had other projects in Phil's lab?

After it was shown that RNAi was not just a worm-specific phenomenon, there was a great interest in using RNAi in other models. And so there was a race to optimize the process of expressing siRNA in various cells and organisms. As the idea is to silence a gene to study its function, the constant degradation and dilution of siRNA is very much an irritation. You've got a battle against time and a small window through which to explore your question.

So we came up with the idea of short-hairpin RNAs (shRNAs) in which the siRNA is first stuck into the hairpin of an miRNA-like structure, which can be introduced into cells through a DNA vector. We could thus trick cells into producing specific small RNAs that target their own genes.

Are there other systems out there for RNAi delivery?

Sure. Ever since mammalian RNAi exploded into the biomedical community, a lot of people have developed their own favorite systems. RNAi delivery is a crucial issue because RNAi is more than a lab tool to study gene function. The potential of using it as therapy to turn down mutated genes has turned it into a multibillion dollar industry.

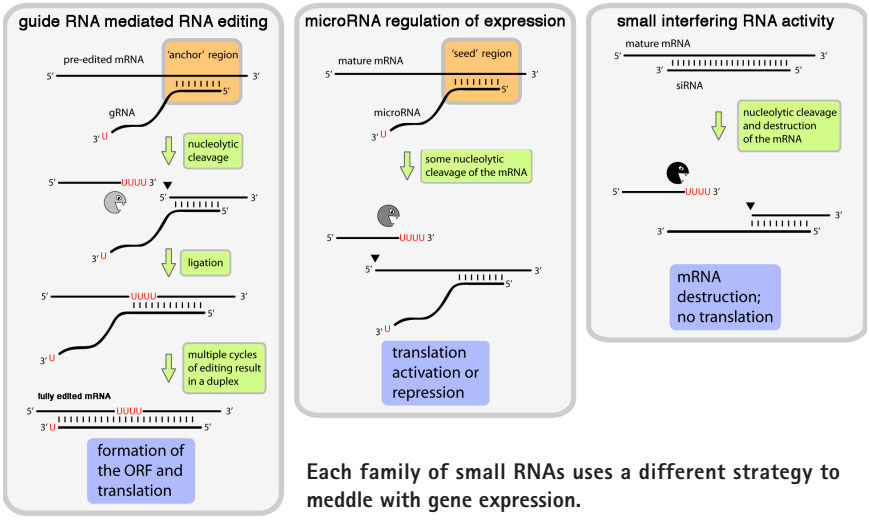
Some have tried small RNA delivery via antibodies. Another method is geared toward targeting viruses such as HIV. Lentiviruses, which can easily infect mammalian cells, can be turned into Trojan horses by including a short-hairpin RNA that targets HIV. The lentiviruses can be introduced into a person's hematopoietic stem cells, which could then be put back into the patient. The hope is that when these cells divide and differentiate, they will reconstitute the person's immune system so that all of their blood cells express the HIV-targeting shRNA. My lab at UCSF now houses a lentiviral core that provides the research community with shRNA-carrying vectors.

Others are already conducting clinical trials of this technology. It's phenomenal that we've gone from mammalian RNAi discovery in 2001 to clinical trials by 2007. But I need to be reserved in my excitement, because there's a lot of groundwork that needs to be explored regarding the safety and efficacy of RNAi.

FUTURE PLANS

We know about hundreds of miRNAs, but what do we know about their target genes?

We recently found that one of the targets—the gene for Hox8, which controls limb development—actually carries an miRNA gene within its locus. This was kind of a big deal because this locus has been studied for a very long time and people thought they knew all the genes that were in there. So the miRNA gene lurking there was a surprise. As the Hox genes are primarily regulated at the transcriptional level, the miRNA might be acting as a backup regulator posttranscriptionally.



Each family of small RNAs uses a different strategy to meddle with gene expression.

In collaboration with Mike German's group, we've also found that a specific group of miRNAs turn on the genes that are required for the development of insulin-producing β cells in the pancreas. Ultimately, this information might help in generating these cells for therapeutic purposes.

> "The potential of using [RNAi] as therapy to turn down mutated genes has turned it into a multibillion dollar industry."

We are in the process of knocking out 100 different miRNAs in the mouse and seeing if any of the knockouts will be reminiscent of any human disease. It's a very bold and ambitious project that will make the mouse models available to the community and bring together a pipeline of investigators studying RNAi in various disease contexts. This work, which is funded by the Keck Foundation, has helped me to establish a center for noncoding RNAs at UCSF.

What made you choose the UCSF Diabetes Center for starting your own lab?

I'm very much a basic scientist studying basic molecular mechanisms and gene expression. I'm not an endocrinologist or a clinician of any kind. But I have always been a very interactive scientist. So I wanted to position myself in an environment where I could rub elbows with the people who are studying various diseases, because I'd like to be able to translate some of my ideas into real-life therapeutics.

Apart from the potential for collaboration, I also wanted to move here for my wife. San Francisco really appealed to us because it's so liberal and chock full of artists. It seems like the perfect home for us.

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