People & Ideas

Antonio Giraldez: At the tip of the microRNA iceberg

Using zebrafish, Giraldez studies how microRNAs guide the body's development.

MicroRNAs (miRNAs) are single-stranded RNA molecules of approximately 22 nucleotides. They regulate protein expression by binding to and suppressing translation of messenger RNAs. Less than 20 years ago, they were unheard of, but the number of identified miRNAs is growing.

This burgeoning field captured Antonio Giraldez's attention while he was a PhD student studying *Drosophila* wing morphogenesis (1–3), yet he didn't start his own experiments on the subject until after he had started his postdoc. Better late than never, as Giraldez's studies produced important data showing that zebrafish zygotes unable to make their own miRNAs cannot undergo brain morphogenesis, and that one particular miRNA can rescue the defect (4, 5).

Now running his own laboratory at Yale, Giraldez has stuck with zebrafish and investigates how miRNAs shape gene expression to orchestrate embryonic development (6).

> "It always amazes me how we can modify the development of living animals using genetics."

WINE, WINGS, AND A PhD

Whereabouts in Spain did you grow up?

Jerez, which is south of Seville, where the sherry comes from. Wine is really big there, and my dad actually worked in a winery, as an administrator.

When I was little, he would bring home Petri dishes and beakers and gadgets that the scientists had given him. That was one of my first contacts with science. I had this little desk that you could close—that was my incubator. I would drive my mom crazy, spreading the Petri dishes with everything, seeing which substances would grow. You can imagine the smell in my room.

How did you get from Petri dishes in your bedroom to university in Madrid?

Actually, first I went to the University of Cádiz in the south to study chemistry. I did an undergraduate project on wine, in fact. But then I decided that I was more fascinated by the chemistry of life. So I went to Madrid to study molecular biology and biochemistry for an extra two years.

Tell me about your wine project. What were you analyzing?

In white wine, polyphenolic compounds become oxidized in a process called browning (because it turns the wine brown). The process is influenced by sunlight.

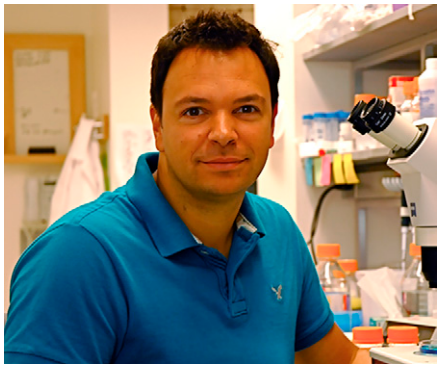
In the south, white wine is produced in dark bottles, as it was thought to stop the sun's effect. But in other markets, they use clear bottles for white wine, and dark bottles for the red wine—which actually doesn't make much sense because red wine doesn't get easily oxidized: it's protected by the tannins. The companies in the south were making a shift toward using clear bottles, because of market demands. So I put wine in different colored bottles on my sunroof, and found that even if you have your wine in a dark bottle it will still get completely messed up if you leave it in the sun. It's better to have your wine in cardboard.

Madrid was where I first had contact with really big science. I think that the Center for Molecular Biology in Madrid has some of the greatest scientists in Spain.

In my last year of university I did a dissertation project with Ginés Morata—a great developmental biologist. Working with him was a life-changing experience.

You weren't tempted to stay in his laboratory for your PhD?

Ginés introduced me to genetics, but I wanted a middle ground between molecular biology and genetics. I had heard about the European Molecular Biology Laboratory (EMBL), so I went there for a summer internship with Steve Cohen in my last year of university. It was a great experience. I decided to do a PhD with Steve after university. Actually, there was some competition between the groups of Steve and Ginés, so it was a bit awkward.



Antonio Giraldez

Tell me about your PhD project.

We did an overexpression screen, where you randomly insert an enhancer controlled by gal4 (a yeast transcription factor) into the genome of flies, and then cross these flies with gal4-expressing flies. This generates a library of fly lines that overexpress particular genes.

I looked for flies that had defects in their wing development. It was an exciting time. For the first year of my PhD we screened 20,000 lines. It was wonderful because every day you discovered something.

We found around 50 novel genes that could affect wing patterning. One of the genes that I chose to work on made a fly with no wings. Instead it would duplicate the notum— the back of the fly. It was pretty cool. It always amazes me how we can modify the development of living animals using genetics.

How did your interest in miRNAs start?

Steve's laboratory was also interested in growth control. Using the same screen, he had identified an insertion that caused a particular growth defect, but for several years no one could find the gene. It almost ruined the careers of several postdocs. When the miRNA field started emerging, Julius Brennecke, a student in the laboratory, thought to look for a miRNA, and found one—it was the first miRNA identified in the fly.

Text and Interview by Ruth Williams
ruth.williams@rockefeller.edu

There's a lesson: believe in the results rather than the hypothesis.
Absolutely. It was a mutant with a phenotype. It was worth pursuing because it had to be true.

NEW YORK

Why New York? Why Alexander Schier's laboratory?
I knew I wanted to experience science in the US. And I had made up my mind that I wanted to change to a vertebrate system. I just had to decide which laboratory to go to.

When I met Alex for the first time, I had this gut feeling that joining his laboratory would be a good decision. We had a great connection. It struck me that he was a '70s version of Steve Cohen—Steve with an Austin Powers dress sense.

> "miRNAs really function as the sculptors of gene expression that carve away mRNA and protein to control development."

Tell me about the project.
I started off studying morphogens—one of Alex's preferred subjects— but it didn't feel like I was at the tip of the iceberg (although we still don't understand how morphogen gradients function). Researchers were already moving down the morphogen iceberg. In a moment of reflection in the laboratory, I thought, "This is not what I want to do for life." The miRNA field, however, was up and coming. And I thought there was a good chance that miRNAs would be important in development.

Ronald Plasterk had produced a zygotic mutant that lacked Dicer, the enzyme that makes miRNAs, but its phenotype was not as dramatic as we thought it would be. So we made a mutant zygote that also lacked any Dicer contributed by the mother (in the egg). Despite having no miRNAs, these mutants could still make many cell types—neurons, muscle cells, cardiomyocytes, pigment cells—but the cells could not organize into proper functional organs.

At the time, David Bartel had cloned some miRNAs expressed in early zebrafish development. I contacted him and said, "We have to talk." He had found this miRNA, called miR-430, that was abundant in the zygote. The dream experiment was to put this miRNA in our Dicer-less embryos and see if it could rescue them. I told David, "This experiment will never work, but we have to try."

Did it work?
The first time I did the experiment, I thought that I had mistakenly taken a wild-type clutch. That experiment was a dream come true for a postdoc.

We found that miR-430 targets and degrades up to 40% of the maternal transcripts present in the zygote. For more than 20 years we have known that degradation of the maternal mRNAs occurs at this time in the zygote. But we had no idea what factor was responsible for this degradation. miR-430 fits the puzzle perfectly.

Hasn't miR-430 also been linked to cancer and embryonic stem cells?
Yes. So, discovering its target transcripts in the adult would be extremely important. Absolutely. In the Dicer-lacking embryo, miR-430 restores morphogenesis of organs, a process that requires cell motility. If miR-430 can also make cancer cells go from immotile to motile, you've got metastasis.

THE MYRIAD miRNAs

Are you looking at other miRNAs and their targets?
Yes. We continue to study the effects of miRNAs in neural development, but have also moved to study the muscle. The embryonic muscle is a more homogeneous tissue, and so it should be easier for looking at gene expression changes. We're comparing wild-type and Dicer mutant cells, and also cells where individual miRNAs have been removed. The beauty is that we can easily manipulate the embryo and ask what happens when we do so. This approach has allowed us to show that miRNAs really function as the sculptors of gene expression that carve away mRNA and protein to precisely control embryonic development.

Why did you choose Yale?
Several reasons. My wife, Valentina Greco, who is also a scientist, was working in New York with Elaine Fuchs. So, I wanted to stay in the US.

But also, I was struck by the amazing scientific caliber of my colleagues and my chair, Richard Lifton. He's a great scientist. And he has given me everything I need to succeed. So now the ball is in my court.

I also got an offer from Cold Spring Harbor, which is an amazing place. I had no idea which would be the best choice. It's an experiment with no control. It was the toughest decision I've ever had to make, but for a good reason: I was choosing between great and great.

Are you enjoying Yale so far?
Yes. The first year was the toughest of my scientific career. Also, we had a baby three months after my laboratory started, so that made it a little more interesting.

But now the laboratory is running, and we're focused on the question of how miRNAs regulate development. We're searching for their targets and trying to understand the physiological functions of individual miRNA–target interactions. This is just the tip of the iceberg. miRNA targets are still relatively hard to identify, and many novel noncoding RNAs are still to come. It's going to be extremely exciting in the future. It is already.

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3. Giraldez, A.J., and S.M. Cohen. 2003. *Development.* 130:6533–6543.
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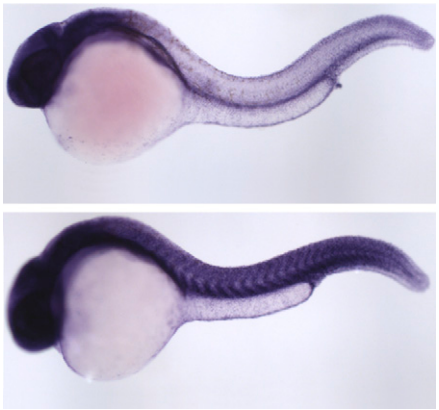

A ubiquitously transcribed transgene mRNA gets degraded by a miRNA in muscle (top). When the miRNA is inhibited (bottom) the message is restored.