Victor Ambros: The broad scope of microRNAs

Ambros studies how microRNAs impact development.

PHOTO COURTESY OF ROSALIND LEE

Victor Ambros

Once, we thought we understood all there was to know about how gene expression is regulated: A cell can tinker with the expression level of a given protein's messenger RNA by modifying the activity, abundance, and type of transcription factors in the nucleus or with the RNA's stability once it is made. But then came a surprising story about a short RNA in *C. elegans* called *lin-4*, which didn't encode a protein but prevented expression of the protein encoded by another gene, *lin-14*, through antisense binding to *lin-14* mRNA (1, 2). Today, we know that *lin-4* was just the first example of a large number of small RNAs, called microRNAs, which regulate the expression of various other proteins in a similar way.

Victor Ambros, whose lab published that first story about *lin-4*, has been studying microRNAs (3, 4) and their regulation (5, 6) ever since, pushing forward our understanding of this powerful mechanism. We called him at his office at the University of Massachusetts Medical School to get some perspective on microRNAs and his career and to learn about some of the latest developments in his lab.

> "That shared discovery is one of the most precious moments in my career."

FROM FARM TO LAB TABLE

How did you end up doing a PhD with David Baltimore?

I was the first scientist in my family. My dad was an immigrant from Poland. He came to the States just after World War II and met my mom. They got married, moved to a farm in Vermont, and started farming. My siblings and I grew up amongst the cows and pigs and helped with the haying and cutting corn, stuff like that.

When I was about nine, I got interested in science, and after that I always wanted to be a scientist. I was an amateur astronomer; I built a telescope and started to imagine that I could actually do astronomy or physics as an occupation. But I quickly changed my mind when I reached college, in part because I realized that my math skills weren't really up to the task of being a physicist and also because I discovered molecular biology and genetics and just fell in love with both subjects. David taught one of the advanced biology classes I took as an undergraduate at MIT, and that probably had some influence on my decision to work with him.

After college, I worked as a technician in David's lab for a year. I liked it a lot and stayed on in his lab when I entered graduate school at MIT. I was lucky because I had gotten a little bit of traction on a project and continued on that as a grad student, so I ended up finishing grad school fairly efficiently.

What did you work on?

I was working on poliovirus. David had a nice group of people working on this little virus at the time, and there were two terrific senior postdocs that I learned a ton from—Ralph Patterson and Bert Flanegan. They were really, really expert in what they did and very enthusiastic about it. I discovered that I didn't necessarily have to be a genius to make progress, if I was lucky enough to find myself amongst smart, accomplished, and enthusiastic people. There have been many people like that in my career, including my wife, Rosalind Lee, who works in my lab and has contributed an enormous amount to my success. She and a postdoc in my lab, Rhonda Feinbaum, were the ones who cloned and characterized the first microRNA, *lin-4*, while I was at Dartmouth.

EARLY WORM DAYS

Why did you switch fields as a postdoc?

I was trying to finish up my graduate work when Bob Horvitz arrived to start his lab at MIT. He gave a series of talks about his research, and I really got excited about the opportunity to work on *C. elegans,* a new model organism that had a lot of interesting attributes. I imagined that it was simpler than *Drosophila* and was interested in it because it followed a very clear developmental program. You could learn a lot from genetics studies in this organism, before having to resort to the time-consuming and difficult process of cloning gene products.

Also, this may sound silly, but I was really turned on by the idea that you could freeze the worms. You could neglect them for months and then come back and revive your cultures by thawing out the worms. There wasn't the burden of having to maintain your fly strains. So, I could imagine that it was a forgiving enough animal that it would allow me to do science without tripping myself up too badly. [Laughs]

I got excited by some mutants that Bob had in the lab at the time, which we later called heterochronic mutants. One of these mutants was a *lin-4* loss of function, which repeated early developmental stages in many lineages. But Bob also had a *lin-14* loss-of-function mutant that had the opposite phenotype, skipping the early stages and going right to the later stages. So Bob brought these mutants to me and suggested I work on them. They were immediately captivating.

In the end, I took *lin-4* with me to study in my own lab, and Gary Ruvkun, who was a postdoc in Bob's lab at the same time as me, took *lin-14* to his lab. Because they were so obviously intertwined, he and I kept in close contact about our work.

Text and Interview by Caitlin Sedwick
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Had you any idea at the time what the nature of the* lin-4 *mutant was?

The assumption was that it was a protein product. I mean, nobody ever thought that there would be any other kind of regulator. There really wasn't any reason to imagine that there were any other kinds of molecules necessary, other than proteins, to carry out everything that's done in a cell—especially with regard to the regulation of gene expression. The complexity of gene regulation by proteins alone was so enormous that I never imagined—and nobody I knew imagined—that we needed to look for new kinds of regulatory molecules.

The realization that *lin-4* was antisense to the 3′-untranslated region of *lin-14* was totally the result of communication between Gary and me. That shared discovery is one of the most precious moments in my career. But at the time I didn't realize that this might be the first example of a general mechanism for regulating gene expression because I was prone to thinking that whatever I was studying in the worm was not generally applicable. It wasn't until genome sequences were made available that the prevalence of this mechanism became clear.

THE RIGHT CONTEXT

You've moved to studying processes that modulate microRNA function…

One protein we've studied is called Nhl-2. It's an example of an emerging class of proteins that can modulate, positively or negatively, the RNA-induced silencing complex (RISC) that inhibits mRNAs targeted by microRNAs. This class of genes may have either general effects on RISC activity or, in some cases, more specific effects. One area of interest in the lab right now is trying to understand the specific outcomes for the regulation of particular microRNAs. Do they always interact with all their targets, or is their activity on some targets promoted or inhibited at the expense of other targets? Can their interaction with certain targets be modified depending on context? We're using genetic and genomic approaches to identify new modulatory cofactors.

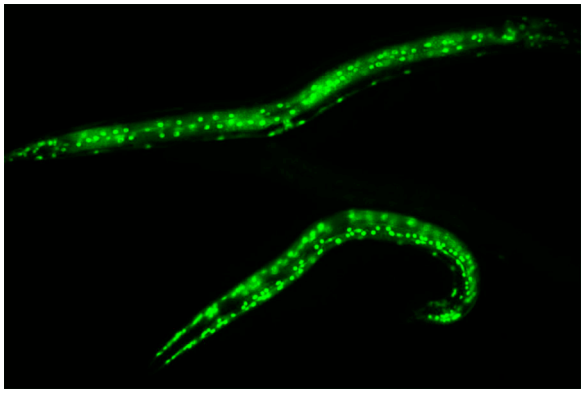

IMAGE COURTESY OF VICTOR AMBROS

Expression of col-19::GFP (green) is regulated by the *lin-4* and *let-7* microRNAs.

Do defects in microRNA regulation have consequences as dramatic as* lin-4 *mutations?

Nhl-2 is an example of a protein whose null mutation has a very weak phenotype. It's a conserved protein, and there are other family members that are similar to it, but if you eliminate those at the same time you don't really aggravate its phenotype. So what is this protein doing? We found that if you compromise microRNA activity by mutating additional microRNA genes that themselves don't cause a very strong phenotype, now the combination with Nhl-2 mutations has a very strong phenotype. There's a kind of genetic synergy. This could be because they interact with one another or because they each contribute in parallel to similar outcomes, so the challenge is to figure out the basis for their synergy.

> "The interface between developmental regulation of gene expression and stress responses is really interesting to us."

We're also trying to understand the activity of genes, particularly microRNA genes, by exploring what kind of conditions—genetic backgrounds, environmental stresses, diets, and so forth—synergistically affect the phenotype of mutants. We're particularly interested in how microRNAs may be involved in mechanisms that buffer development against these kinds of stresses. The interface between developmental regulation of gene expression and stress responses is really interesting to us. This is important no matter what kind of animal you are, and I'm excited that the worm offers a particularly appropriate system to explore these questions. We think it'll be fruitful.

Any other projects you can talk about?

Right now, Rosalind is working on a collaboration with some colleagues here at UMass who are interested in using microRNAs as biomarkers. MicroRNAs are easily assayable in bodily fluids, and, because they're regulators of gene expression, they have a lot of implicit functional import. So, we're helping our colleagues profile microRNAs in samples of body fluids from patients with a variety of indications. It's a bit of a departure for my lab but an interesting field for us to get into.

PHOTO COURTESY OF GREG AMBROS

Victor Ambros works closely with his wife, Rosalind Lee.

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