Andrea Ladd: Getting to the heart of alternative splicing control

Ladd identifies alternative splicing regulators and examines how they shape the heart during development.

One of the surprises that arose from the Human Genome Project is that there are far fewer human genes than anyone had guessed. The complexity and diversity of the human proteome is generated in part by alternative splicing—once thought to be a relatively rare event, but now believed to affect approximately 75% of our genes. Ladd is one of a growing breed of investigators that examines how alternative splicing events shape the developing embryo.

Factors that control alternative splicing, such as the CELF proteins that Ladd helped to identify (1, 2), are starting to be thought about in the same way as transcription factors—as key regulators of gene expression programs. Researchers are thus investigating how the factors themselves are developmentally regulated, how they work, and what transcripts they target for splicing. Ladd made great headway in tackling these questions during her postdoc studies at the Baylor College of Medicine in Houston, Texas (1–5). Now, Ladd continues to ask and answer the questions of alternative splicing regulation in her own laboratory at the Cleveland Clinic in Ohio.

"I came home and announced to my entire family that I was going to be a research biologist!"

A SURE START

As a kid, what did you want to be when you grew up?

I pretty much always wanted to be a biologist. I was a nerdy little kid, very interested in science. I loved animals and plants and going to the zoo and the science museum; those were my favorite places. I think the first time I really thought about what I wanted to do when I grew up was when I was in seventh grade.

It was the first year we studied biology. And I had a really fantastic biology teacher named Mr. Keyes. The second week of school I came home and announced to my entire family that I was going to be a research biologist!

And that was that! Where did you go to university?

I did my undergrad at the University of Wisconsin in Madison and studied molecular biology.

At what stage did you know that you definitely wanted to do a PhD?

Actually, I had it in my mind right at the beginning.

In seventh grade?

[Laughs] Well, maybe not from seventh grade, but right from the beginning of college. I got a job working in a laboratory when I was an undergrad and worked there for several years, and I really loved it. I loved interacting with the graduate students and postdocs, and I thought, "This thing's really cool, I want to do that."

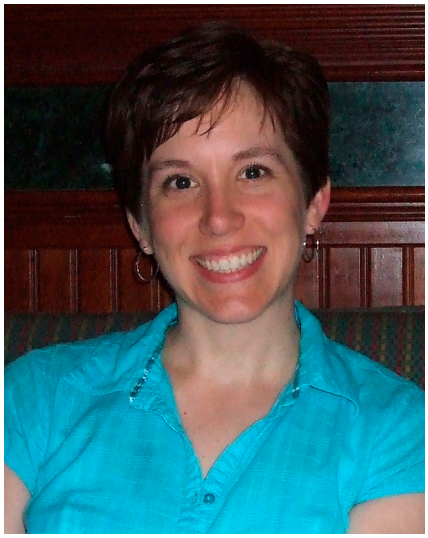
What made you choose the University of Arizona for your PhD?

It was more of a personal decision than a professional one. I had never been anywhere outside the Midwest, and I thought this could be a big adventure, to move someplace so far away and so different from Wisconsin.

They were very smart; they brought me down for my interview in January. It was the coldest winter on record back home, it was 50 below at the airport when I left, and when I arrived in Tucson, it was 72 degrees. It was clever, because then I moved to Tucson in August, and it was 116 degrees! A little less enjoyable.

Your PhD was with Parker Antin. What was it about developmental biology, and specifically heart development that captured you?

The heart is the first organ that forms in the embryo, and the idea that an embryo that looks like almost nothing at the time—just a few thousand cells—can create a functioning heart was fascinating. The heart is particularly interesting because as it's forming and developing, it already has to be working. It's constantly pumping, and yet undergoing morphogenesis and differentiation at the same time.



Andrea Ladd

APPEAL OF THE ALTERNATIVE

How did you get into studying splicing?

When I was thinking about a postdoc, I was looking at other developmental biology laboratories but felt that I would just be looking at the same kind of problems in another tissue. I wanted instead to go into a different field so that I could then come back to developmental biology from a new angle.

Splicing was chosen by accident. I was at a Gordon Conference on muscle development with Parker, and at the meeting we hung out with Tom Cooper, who had done his postdoc in the same laboratory as Parker. I had a lot of great conversations with Tom, and then after the meeting Parker sent an email to Tom and said, "You know, Andrea's looking for a postdoc." So Tom contacted me, and we started talking. I don't think I would've thought of looking at alternative splicing if it hadn't been for that.

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

But once we started talking, I thought, "You know, this is really fascinating. I had no idea alternative splicing was so prevalent or so important." I thought it was something I could go after, because I didn't think the developmental biology community had really looked at this.

What was your project in Tom's laboratory?
When I joined the laboratory, they had just identified a new family of splicing factors called the CELF proteins, which stands for CUG-BP and ETR-3–like factors—those were the first two members of the family to be described.

I started out doing some Western blots and saw that the expression patterns of CELF proteins were very dynamic during development. A lot of researchers in the alternative splicing community looked at how a gene was spliced one way in muscle cells, and another way in neurons. But there hadn't been many studies into what happens within the same cell type over time. I found evidence to suggest that CELF protein expression changes drive the developmental transition between fetal and adult patterns of splicing in the developing heart.

How do CELF proteins direct the splicing machinery to control alternative splicing?
That's a great question. Actually, very little is known about that. We know they bind to the RNA, and that they have to bind to the RNA to affect splicing. But how precisely they're communicating with the basal splicing machinery is not well understood.

What else are you looking at?
We're starting to look at another family of splicing regulators called the muscleblind-like proteins, which act on many of the same subset of targets as the CELF proteins but, at least in some cases, have been shown to be antagonistic.

We have preliminary data that both the CELF and the muscleblind-like protein family are very dynamic during embryonic heart development. And so we think that they're involved in developmental stage-specific alternative splicing control.

My guess is that probably most, if not all the different splicing factor families are developmentally regulated in different tissues, in much the same way that transcription factors are.

And presumably they each have a distinct subset of targets.
Right. Each protein or each family of splicing regulators would affect different subsets of targets, and some of those subsets may overlap, and some may be very distinct.

You've identified six of these CELF proteins. Do you know all of their targets?
That's actually one of the big questions that we have now. Only a very small number of targets have been identified, and we think there are probably many more. We're now trying to identify the others.

Are you working on any other projects?
I think the big, overriding question that we really want to answer is, what are the consequences of these splicing programs for development? Not just how is splicing regulated, but why is it important?

So we're using a variety of whole embryo, primary cell, and embryonic explant assays to go in and manipulate the splicing factors and see what effects we're having on morphogenetic processes.

THE RIGHT FIT

What made you choose Ohio?
It was a combination of things. On a professional level, the Cleveland Clinic is really a top-notch place. It's got great resources, it's got great people. And scientifically, it was a great fit. Our work is sort of a combination of cardiovascular, developmental, and RNA biology, and that's an unusual combination. But here in Cleveland, between the Cleveland Clinic and Case Western Reserve University, there's actually a huge RNA community. And at the Cleveland Clinic there's a huge cardiovascular biology community.

Then on a personal level, I just really liked Ohio. I really wanted to move north after living in Arizona and Texas. I discovered the southern climate doesn't really agree with me, and I wanted to go back to a place that had a change of seasons and had a real winter, and snowed on Christmas. I really love the midwestern culture.

Are you enjoying life as a Principle Investigator?
It's fun. It's kind of stressful. When I left Tom's laboratory, I remember he told me that being a PI is like being a parent. It's one of those things that, if you had any idea going in how much work it would be or how hard it would be or how stressful it would be, you might not want to do it. But once you're there and you're doing it, it's so rewarding, you're glad you did. I don't know about being a parent, but as a PI, I'd say that's completely true. JCB

"The big, overriding question that we really want to answer is, what are the consequences of these splicing programs for development?"

1. Ladd A.N., et al. 2001. *Mol. Cell. Biol.* 21: 1285–1296.
2. Ladd A.N., et al. 2004. *J. Biol. Chem.* 279: 17756–17764.
3. Ladd A.N., and T.A. Cooper. 2004. *J. Cell Sci.* 117:3519–3529.
4. Ladd A.N., et al. 2005. *Dev. Dyn.* 233: 783–793.
5. Ladd A.N., et al. 2005. *Mol. Cell. Biol.* 25:6267–6278.

One of the CELF family of alternative splicing regulators (purple), expressed in a chick embryo's heart.