People & Ideas

Fernando Martín-Belmonte: Epithelia embrace the space

Martín-Belmonte studies epithelial morphogenesis in 3D culture and in zebrafish.

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Fernando Martín-Belmonte

For multicellular animals, the interface between "inside" and "outside" is demarcated by epithelia. Epithelial sheets form a barrier separating internal tissues from the environment, and inside the body they delineate different tissues and line the open spaces, or lumens, that riddle even the simplest organisms. In fact, lumenal spaces—the gut, blood vessels, and other tubules and chambers—are themselves generated by epithelia during organismal development through mechanisms that are only now being understood.

Fernando Martín-Belmonte was seduced by the study of epithelial biology as a graduate student (1) and has since striven to detail the mechanisms of epithelial morphogenesis (2–4) and lumen development (4, 5). Having delved deeply into these questions using in vitro models, Martín-Belmonte is now exploring how these processes occur in vivo, as we learned when we spoke with him at his office in Madrid's Centro de Biología Molecular Severo Ochoa (CSIC-UAM).

> "I was completely seduced by the work I was doing."

THE PEAKS BECKON

What interests did you have as a child?

I grew up in Madrid, but I loved mountaineering and hiking outdoors. I remember doing a lot of that when I was a kid. My father was very fond of it, and we often went to the mountains to climb and hike. I also went a lot with one of my brothers, Alberto, but unfortunately he died while climbing in the mountains when I was a teenager. After that I stopped going for a while. But now enough time has passed that I'm able to enjoy being out there again. Every year I go with a bunch of friends to the Pyrenees to summit one of the peaks.

Are you looking forward to your next mountaineering trip this summer?

Actually right now I am looking forward to getting some sleep. [Laughs] My wife and I have a new baby daughter, who arrived just a few weeks ago. We also have a two-and-a-half-year-old boy, and it is true what they say: having two kids is more than double the work. But it's very nice as well. I can't complain. I'm really happy.

What did you want to be as a kid?

Not a scientist, for sure! Maybe I wanted to be a soccer player, like probably everybody else in Spain. I had a really good teacher when I was 14 or 15, and he was an enthusiastic follower of Santiago Ramón y Cajal's work in the nineteenth century. He was always telling us that Ramón y Cajal was the "greatest genius ever." That was the first time I really thought about doing research. But even at university I was more preparing myself to do engineering. I only switched to biology very late in college when I realized that with an engineering degree I would probably end up doing something like telecommunications or informatics. I didn't think that was for me. Biology and biochemistry were much more interesting to me.

LOOKING WITHIN

Why did you choose Miguel Alonso's lab for your PhD?

I had actually started working in his lab as an undergraduate. When I had to decide where to go for my PhD, I stayed there because I was completely seduced by the work I was doing in his lab.

I was studying a protein called MAL, which is part of the machinery involved in protein trafficking. It's associated with lipid rafts, and at that time lipid rafts were a fancy concept that everyone was interested in. But it was a real challenge to work with this protein because it is actually a proteolipid—a protein with the chemical properties of a lipid. It was difficult to make antibodies to it, and we spent a lot of time trying to figure out how to analyze and characterize MAL. Then it became possible to use RNA interference to knock down proteins, and we were really excited to use RNAi to find out what the protein was doing. We found that it is involved in apical protein trafficking.

Why did you choose to do your postdoc with Keith Mostov in San Francisco?

When I finished my PhD at Universidad Autónoma de Madrid, I had already published a bunch of papers, so it was relatively easy to get into a lab and continue my work on epithelial polarity. I interviewed with a lot of labs, but I chose to go to Keith's lab at the University of California in San Francisco because I was really impressed by what he was doing with organotypic three-dimensional cell cultures.

In Keith's lab my work was influenced by Henry Bourne's lab, which was nearby. Henry's people were working on neutrophil motility and signaling and had found that phosphoinositides segregate during migration in neutrophils. Many people in Keith's lab were interested in this because we thought epithelial cell membranes might also be organized into different domains enriched with different phosphoinositides. We decided to have a look at it, and we found that PIP2 is enriched at the apical surface and PIP3 is enriched at the basolateral surface. Many proteins have phosphoinositide-specific binding domains that specifically bind to PIP2 or PIP3, so this is one of the ways that proteins can become compartmentalized in epithelia.

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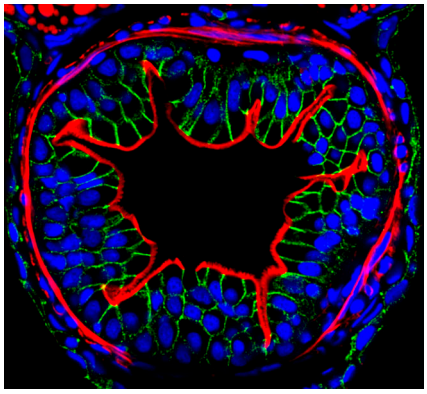

IMAGE COURTESY OF ALEJO RODRIGUEZ

Cross section of a larval zebrafish gut showing actin (red), E-cadherin (green), and nuclei (blue).

You returned to Spain after your postdoc…

That was a decision I made together with my wife, who is also from Spain. We had done our PhDs together in Madrid, and she had come to San Francisco for her own postdoc. We decided to return to Spain when my wife got an offer for a position with a big consulting company in Madrid.

It was also partly an emotional decision to return here. My country had made such a big investment in me: my college, PhD, and two years of my postdoc were paid for by the Spanish government. I felt an obligation to return and try to contribute toward science development in this country, which is something that is badly needed.

When I returned to Spain I wanted to continue my work with organotypic cultures. But I'd been working with cell models for most of my career, and I really wanted to start working with animals because, while cell models are interesting and can be very informative, they're not the real thing. What is going on in animals will be different in important ways. So although we've done a lot of good work with our organotypic culture models, half the people in my lab are now working with zebrafish and even doing a little work with mice.

EMBRACE THE SPACE

You investigated lumen formation using the 3D culture model…

A few years ago we showed that epithelial cells growing on printed micropatterns overlaid with Matrigel will form cysts with internal lumens if the micropatterns are small enough (or spatially constrained). This is completely different from what is observed when epithelia are grown in a monolayer because the cells' interactions with the extracellular matrix help them organize into little balls with a lumen at the center. We've since found that, if the micropattern is a line instead of a round dot, the cells will form tubes instead of cysts. Ideally we'd like to get tubes with microfluidic flows inside so we can actually model how epithelia really form tubes and how materials are transported through the lumen.

You've investigated the molecular requirements for lumen formation…

We knew that cells behave completely differently in 2D compared to 3D culture, and we used this fact to conduct a screen and analyze what genes are differentially induced when cells are grown in 3D culture. One of the proteins that came out of this screen was synaptotagmin-like 2 (Slp2a). We decided to study Slp2a further because there was information from many cell types that these proteins have a role in protein trafficking, particularly in membrane fusion.

> "While cell models are interesting and can be very informative, they're not the real thing."

The beautiful thing was that Slp2a was induced very early, about 12 hours after we put the cells in 3D culture, and Slp4 was induced 12 hours later. This reflects the role of these proteins in the process. We found that Slp2a is involved in the sorting and targeting of transcytosing vesicles to the PIP2-enriched apical domain. Slp4 is induced later because it is involved in regulating the fusion of these vesicles with the plasma membrane through its interactions with the SNARE syntaxin 3.

The secretory apparatus is oriented toward the site of lumen formation…

We found that the microtubule-organizing center (MTOC) is found on the side of the nucleus closest to where the lumen will develop and that this orientation is driven by actomyosin contractility. The MTOC is often associated with the secretory apparatus, and it is commonly thought that MTOC orientation may affect the direction of secretion. In the case of epithelial lumen development, MTOC orientation is important for lumen formation, but we have not yet formally proven that this is because it regulates protein trafficking.

What kinds of questions are you working on in zebrafish?

Our initial idea was to find out whether the genes that we found in our culture model do something similar in vivo. We're looking at lumen formation and general epithelial morphogenesis by characterizing two different epithelial tubes in fish: the gut and kidney pronephros. For the most part we are finding similar patterns of gene expression in zebrafish as in 3D culture, and we're actually currently preparing a couple of papers on the roles of two proteins developmentally induced in morphogenesis: Plasmolipin (PLLP), which is essential for the developmental regulation of epithelial absorptive cells in the zebrafish gut; and Slp4 in the formation of zebrafish kidneys.

1. Martín-Belmonte, F., et al. 2003. *J. Cell Biol.* 163:155–164.
2. Martín-Belmonte, F., et al. 2007. *Cell.* 128:383–397.
3. Gálvez-Santisteban, M., et al. 2012. *Nat. Cell Biol.* 14:838–849.
4. Bañón-Rodríguez, I., et al. 2014. *EMBO J.* 33:129–145.
5. Rodríguez-Fraticelli, A.E., et al. 2012. *J. Cell Biol.* 198:1011–1023.

PHOTO COURTESY OF LUIS GOMEZ

Martín-Belmonte and friends on Brecha de Roland, Valle de Oredesa, in the Spanish Pyrenees.