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Q&A single-cell branching – Maria Leptin

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Maria Leptin works simultaneously in the independent fields of immunology and embryonic development. She is the director of the European Molecular Biology Organization (EMBO) and runs a laboratory in Heidelberg, as well as one in Cologne. Maria Leptin's laboratories study the development of complex cell shapes and the localisation of mRNA and proteins involved in determining the polarity of such cells. A major part of the research involves studying the terminal cells in the respiratory system (trachea) of *Drosophila* during single-cell branching.

1. What motivated you to start studying single-cell (subcellular) branching?

I would love to say 'I was so inspired by Mark Krasnow's work on tracheal development that I just had to get into that field'. But while part 1 of that sentence is definitely true, part 2 isn't. My lab started working on tracheal development for a totally different reason. In our efforts to understand the transcriptional regulation of gastrulation, we had identified a number of genes that were expressed specifically in the mesodermal primordium. One was called '4711' (which has nothing to do with Eau de Cologne, just a fun coincidence!), and we found it was also expressed in the tracheal system. We found ourselves talking to Markus Affolter about this, whose lab had just identified a deficiency with an interesting mutant phenotype in the tracheal system, and decided to see whether we might have landed on the same gene. We had, and this led to an exciting and fruitful collaboration to determine its function: an adapter acting downstream of the FGF-receptor, which we named Dof, and which turned out to be the product of a gene that had also been found by the Krasnow lab who called it *stumps*. We then continued to use both the mesoderm and the tracheal system to study the effects of FGF signalling on cell shape, which necessarily eventually led us to branching morphogenesis.

2. Why study single-cell branching in tracheal terminal cells?

Branching is obviously very interesting, with the other prime example being neurons – in fact, maybe even the most important, because neurons are among the most ancient cells in the animal kingdom. One nice thing about tracheal cells is that they (or at least their proper branching patterns) are not absolutely essential for early larval life, so one has more options for genetic analysis than for neurons. But, of course, in terms of shape, they are also among the most amazing cells of all, because in addition to being branched, they also form tubes, and more interestingly, tubes inside single cells.

3. What makes tracheal terminal cells so special? In what do they differ from other branching cells?

As mentioned above: their subcellular lumen. It is truly impressive to observe how this is produced. This happens before branching, in the embryo, but then over and over again after new branches form during larval development. The cell delivers masses of plasma membrane both to the inner tube and to the outer membrane, allowing a 10-fold expansion of embryonic terminal branches over one hour. And that membrane has to be sorted properly, too, to deliver the right amount to inner and outer plasma membrane. What a feat. And it turns out the cell uses very similar mechanisms to achieve this, as other epithelia, for example in mammals, that form tubes.

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4. Embryonic tracheal terminal cells do not branch and branching only starts at larval stages. Do you know what triggers the beginning of this single-cell branching?

We know from Mark's work that embryonic branching is genetically predetermined, and both from his and others' work that later branching is determined by the needs of the underlying tissues. So, if the embryo lays down enough cells to allow the larva to breathe and deliver enough oxygen to its tissues during the first instar, then maybe it's just not necessary to branch (this is of course a circular argument). But then as the larva grows massively during the second and third instar, cells in growing tissues are pushed further away from the pre-existing branches, and new branches will have to be made to reach them. Of course, one could have designed this differently, with tracheal cells dividing and sending daughters out to migrate to anoxic tissues. I wouldn't be surprised if some other animal hadn't invented that as a strategy. So, if you are asking 'what triggers branching', it's the anoxia of the surrounding tissue that leads to secretion of FGF which in turns triggers branching. If you're asking why does this happen only late, and why is it arranged this way, that's not a question that can be answered easily.

5. In what mechanistic part of subcellular branching is your lab currently focusing?

We have become interested in the trafficking of the plasma membrane and its correct distribution between the outer, basal membrane and the inner apical membrane that forms the lumen of the cell. This involves intricate regulation of endocytosis, proper targeting of vesicles, and directed delivery of membrane lipids. The Rab family GTPases are clear candidates for important functions in this process.

6. In tracheal terminal cells, subcellular branching is coupled to extensive membrane remodelling and concomitant luminal membrane formation. Your laboratory found that the SLP family protein Bitesize is required for the delivery of apical membrane material to the luminal plasma membrane. How do you think this is coordinated with the simultaneous growth of both outer membrane and luminal membrane?

I'm not sure I would phrase the role of Btsz exactly in this way now. The growing tracheal cell has three distinct populations of cortical actin networks: the one associated with the tube, a network at the basal membrane that is associated with integrins, and a dynamic network in the filopodia at the leading tip of the growing cell. Each of these have their own independent regulators and connectors (e.g. fascin in filopodia, and talin in the basal cortex) and I would say Btsz is needed to anchor the apical actin cortex on the tube membrane, and in the absence of this stable anchor, the tube can't form. I would not say the primary function is membrane delivery. This failure to stabilize the tube may of course also affect the rate of endocytosis and proper recycling of Crumbs, which is clearly a determinant of apical membrane morphogenesis, and which may indirectly affect plasma membrane delivery.

7. In tracheal terminal cells, developing subcellular branches to which the cell has to deliver membrane material may be located at large distances from the cell body. How do you think this can be achieved?

The cells are packed with ER, which can probably deliver membrane anywhere along the length of the cell. They are also full of microtubules which could be used to transport membrane vesicles. I think the jury is out on this question, and it is not easy to resolve. While endocytosis can be visualized by clever people doing clever microscopy, nobody has really managed to visually document delivery of vesicles to the plasma membrane in any cell type – even though It is totally obvious it must be happening all the time. So, we have to find indirect ways of figuring this out.

8. What will be the next challenges in the single-cell branching field?

I'm lousy at making predictions. I am always wrong. I look at what is fun, and follow the lead the results in the lab provide. Sometimes this takes me up the garden path, sometimes we make interesting discoveries. But they may not be about single cell branching next time!