Six drugs, Rac and Rho

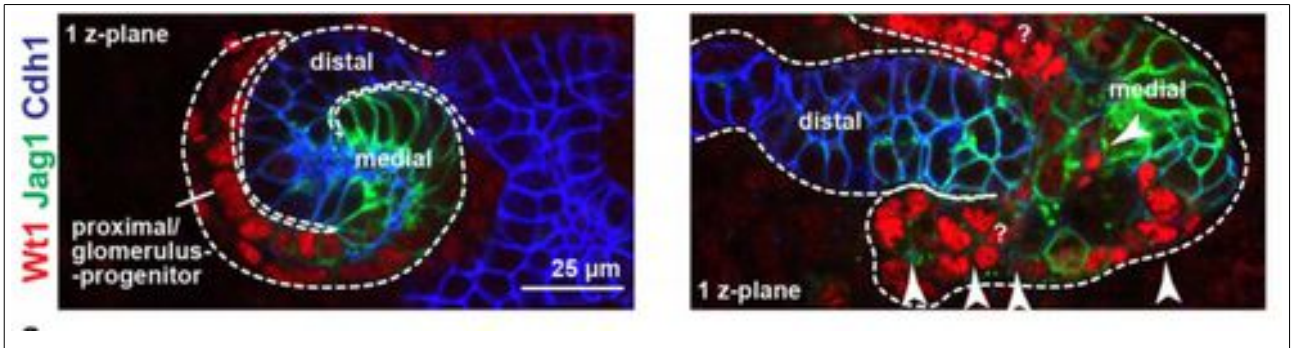
One of the conventions of scientific writing is that one structures a paper so that it presents the evidence for, and relevance of, a new discovery in the most logical and coherent way possible. The finished article is therefore a very poor reflection on what actually went on in the lab, in terms of why things were done, and who thought of what, and when. Papers are very useful for other scientists trying to follow the work (‘to follow’ as either ‘to understand’ or ‘to build on’) but they are pretty useless for historians because they do not reflect the real motives for things nor the real process of discovery. A recent paper by Nils Lindström, arising from his doctoral studies in my lab, provides a concrete example.

Nils, whom I have known since he was a (scarily bright) undergraduate student of developmental biology, came to my lab to do his PhD in the field kidney development. To a first approximation, kidneys consist of a large and organized mass of tubes of various types. Many years work on the development of these tubes, when the kidney first appears in foetal life, has allowed this lab and others to identify a number of critical biochemical signals that pass between cells and organize the tubes’ formation, growth, and acquisition of specific shapes. What we know very little about is how the signals get translated into actual three-dimensional shapes of tubes.

Tubes are made from cells, and it is a reasonable hypothesis (‘guess’!) that the shape of the tube is controlled at least in part by the shapes of its constituent cells. The shape of a cell is determined mainly by its internal skeleton, which consists of protein filaments such as microfilaments and microtubules. The behaviour of the microfilament cytoskeleton, in particular, is controlled by biochemical pathways inside the cell that modulate the activity of enzymes such as Rac and Rho. Rho activity organizes the microfilaments into strong, contractile cables that pull (indirectly) against the cables of adjacent cells. Rac activity organizes microfilaments in a very different way that makes them push parts of the cell outwards: this outpushing is the basis of most animal cell movement. Fortunately, drugs are available to control the activities of Rac and Rho, and of microfilament proteins, and we had access to six of these. Nils’ first experiment was therefore a very simple one: take mouse embryonic kidneys, grow them in culture, apply the six drugs separately and at different times, and see if any of the drugs cause a reproducible change in the shape of any of the types of tube in the kidney (for example, making them grow straight when they

should bend). If we found any such change, that might be a good target for closer study. Simple, naive exploratory experiments like this are a very common beginning to doctoral training because they will probably turn up something interesting, and they allow the student to start making early decisions about the direction of the project.

Nils did the experiments, and found that drugs that inhibit Rho's action (actually, by inhibiting an effector of Rho called Rho-kinase) cause the excretory tubules of the kidney to acquire very weird shapes indeed. If the drug was applied early, the cyst-like balls of cells that are the first signs of these tubules formed properly but they failed to elongate into tubes. If the drug was applied later, tubes formed but they were odd shapes and made unusual connections, sometimes joining to one another. The odd shapes were associated with bizarre directions of cell division. Normally, as these excretory tubules mature, they develop specialized domains (eg proximal, medial and distal). Nils realized that if he was going to make sense of the weird tubules formed in the presence of the drugs, he would need to be certain about what domain of the tubule he was looking at. He therefore stained his normal and his drug-treated cultured organ rudiments for a range of domain-specific markers. This revealed a surprise: in normal kidneys the domains are distinct and the markers do not overlap but, in the drug-treated kidneys, a significant amount of overlap was seen, as if the cells were either confused about their identities or are living in mixed groups when they should be segregated. Both seemed to be true; cells expressing a pure identity were in mixed groups and some individual cells showed markers of two different domains at the same time.



The left-hand figure shows a developing tubule in a normal kidney, with proximal (red), medial (green) and distal domains well segregated. The right-hand figure shows the same staining technique applied to a drug-treated kidney: the domains overlap and the cells are muddled up. The overall shape is also very odd.

A lot of detailed analysis of the cells' behaviour and identity (the analyses can be seen in the manuscript – see Links below) led to a simple overall conclusion: with Rho-kinase inhibited, cells

had lost their sense of direction and position. Rho-kinase is known to be important in cells' internal sense of direction in other parts of the body in simpler organisms such as flies and zebra fish. Finding this in kidney may therefore add to the growing general story about how important this pathway is to a cells' internal compass.

When all of this was turned into a paper, the focus was naturally on the importance of Rho-kinase in giving kidney tubule cells a sense of location and direction. The formal abstract to the paper is as follows:

Epithelial tubules must have the right length and pattern for proper function. In the nephron, planar cell polarity controls elongation along the proximal-distal axis. As the tubule lengthens, specialized segments (proximal, distal etc.) begin to differentiate along it. Other epithelia need Rho-kinase for planar cell polarity but it is not known whether Rho-kinase is involved in this way in the nephron. We show that Rho-kinase is essential for the morphogenesis of nephrons, specifically for correct cell orientation and volume. We use fluorescent reporter-models and progenitor-specific markers to demonstrate that inhibition of Rho-kinase prevents proper proximal-distal axis formation, causes segments to develop abnormally, and progenitor-cell segregation to fail. Our data demonstrate the importance of Rho-kinase in normal nephron tubulogenesis and patterning.

It will be apparent that nowhere in the abstract, or in the paper itself, is there anything much about the original question – whether the shaping of tubules is controlled by the shaping of cells, and whether the shaping of cells is controlled mainly by the cytoskeleton. The results had moved the research on and had replaced the original question. Trying to tell the real story of the work would have been pointless and confusing, so what emerged was a paper that is internally coherent and that gives a clear message about Nils' discovery, but that has little to do with why the key experiments were actually done. Just like almost every other paper that is published.

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Links:

Nils' paper: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3776198/