Retraction then publication

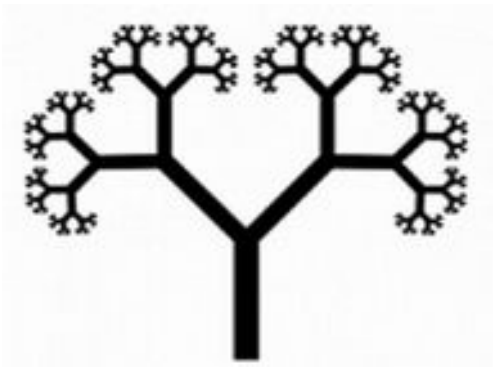
In the world of scientific research, 'retraction' is not a happy word. Retractions are what happen (or what should happen, anyway) when authors realize there is something terribly wrong with a paper they have published, and they ask the editor of the journal to publish a formal retraction in the next issue of the journal and to attach a copy fo the retraction notice to the electronic version of the archived paper as well. 'Terribly wrong' does not mean writing about a theory or explanation that turns out to be incorrect – that is the normal path of scientific progress and proposing an idea interesting enough to stimulate someone to do a clever experiment that proves it wrong is something for which the proposer can take great credit, not shame. Much of what we 'know' about the universe, we have found out by disproving every other possible explanation anyone has proposed. For retractions, 'terribly wrong' usually means a bad internal mistake, like muddling data from different experiments or - worst of all - finding out that someone in the team has been committing fraud. And under these circumstances, retractions are often forced on authors by the journal itself.

Sometimes, though, 'retraction' can have a more interesting meaning. We have just published a brief paper announcing that a process we have called 'node retraction' is a feature, quite a big feature, of kidney development that has, as far as we know, gone unnoticed. To tell the story, I need to explain a little about the internal anatomy of kidneys. Kidneys are packed full of fluid-carrying tubes that are arranged in a very precise relationship to one another. Blood vessels carry blood, and another set collectively classed as 'epithelial tubes' carry and process the fluid that will eventually emerge as urine. Much of the production and processing of urine takes place in peculiarly bent and convoluted epithelial tubes called nephrons, and we have around a million of these in each of our kidneys. Each nephron drains into a tree-like system of epithelial tubes called colecting ducts, that eventually open into a small holding area called the renal pelvis, and that in turn drains through the ureter to the bladder.

The earliest tubules to appear during kidney development are those of the collecting duct tree: the 'trunk' of he tree, which will eventually become the ureter, enters the kidney-forming area of the embryo and branches repeatedly inside it to create a simple tree. A picture of a real one, in a mouse kidney growing in a culture dish, is shown in the figure below. The shape is vaguely similar to the

purely mathematical 'fractal' trees made famous by Beniot Mandelbrot. To emphasize this point, I wrote a little computer program based on Mandelbrot's ideas, and it generates a broadly similar but not identical pattern (the difference being that the mathematical version generates outer branches much finer than biology does).

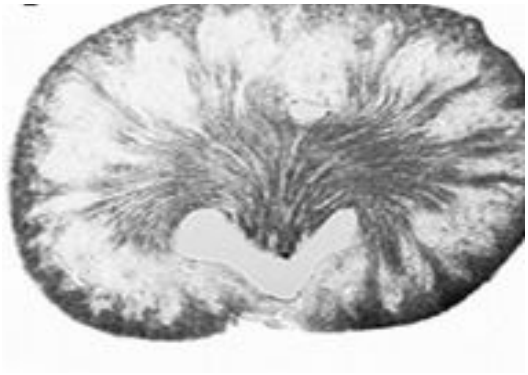
An image of the growing collecting duct system of a mouse kidney growing in culture. The kidney has been stained so that only the collecting duct tree shows up,. and other tissues remain transparent.



A fractal 'tree' generated by computer using the ideas of Beniot Mandelbrot ("The Fractal Geometry of Nature" - a brilliant book!), but with branching angle set to 90 degrees rather than 180 as in Mandelbrot's own model.

A feature of the tree – the real one as well as the computer-generated one – is that branch points (nodes) appear at intervals all the way from trunk to outside. In the mathematical model, the intervals shrink by half as one travels outwards. In the real biological version, the spacing is a bit more regular.

The branching of the early collecting duct system has been studied for many years (indeed, this lab has frequently published papers on the molecular systems that control its branching) but one problem has always been, to use a tired cliché, an 'elephant in the room'. In a mature kidney, the inner part of the collecting duct system does not look like a tree at all. Instead, once ducts leave their branching systems in the outer part of the kidney (the cortex), they travel more-or-less parallel to one another, draining into the pelvis independently so that the pelvis connects to many tubes and the hand connects to many (5) fingers.



An older mouse kidney, showing how the collecting ducts pass through the inner part fo the kidney as almost parallel bundles without the regular branching structure of the young version. Photo credit: Todd Valerius.

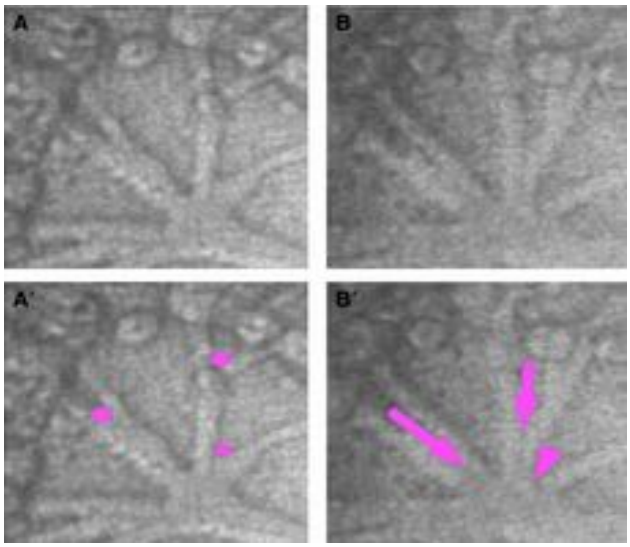
How can the early form turn into the later form? Other researchers have identified strong growth of the inner ducts to make them long as a possible mechanism but that clearly cannot work: if you start with a tree and grow some of its branches to be very long, then you get a long, spindly tree but it is still a tree: you cannot transform a 2-lead-into-one trunk branching system into a many-lead-into-one trunk (renal pelvis) system in this way. It's mathematically impossible. When I read these papers, I knew they had to be wrong (not in their report of growth, which was correct, but in their assertion that it solved the whole problem), but I had not intended to work on the problem myself.

My attention to the problem was provoked by watching a movie in a lab meeting. The movie was of a kidney developing in a new culture system that allowed the little organs to grow and thrive for much longer than they did in older systems. The movie was made and was being shown by C-Hong Chang, then a PhD student working with me who is now doing a post-doctoral fellowship at the University of Cambridge. The point of the movie was really about the development of a part of the nephron called the loop of Henle and had nothing to do with the collecting duct tree. I have, however, worked on that tree, off-and-on, for more than twenty years and if one is visible I cannot help being distracted by it, usually to the irritation of my colleagues interested in other things. After we had watched the thing a few times to discuss the loops of Henle, something was nagging at me, and I asked to see it again. Watching, I had the distinct impression that the nodes of the collecting duct system were moving!

Curious, I asked C-Hong to put all of his movies in to a USB stick. I also put on the stick another set of movies prepared for another quite different purpose by Nils Lindström; I have known Nils since he was an unnervingly bright undergraduate: he came to my lab to do a PhD and, after a spell with Martin Collinson in Aberdeen, returned to Edinburgh for a post-doctoral fellowship with my long-term collaborator, Peter Hohenstein. Nils is now about to leave for a position in the University

of California. His movies, like C-Hong's, are of unusual clarity.

Sitting at home in front of an annoyingly smoky coal fire, and armed with a really large mug of strong Assam tea, I inspected the movies frame-by-frame. The nodes did indeed move! What is more, some moved so much that they obliterated the node, going from Y to V and then to II (the two branches now joining the trunk/developing renal pelvis independently.



This figure shows (top row) branch points before and after node retraction, with the pink marks in the bottom row drawing attention to the movement.

Detailed measurements and mathematical analysis showed that while this node retraction, as we call it, is a feature of tree maturation it dfoes not take place at the same time for all of the branches, so cannot be a response to a global (eg hormonal) signal. What is more, as far as we could see the cells themselves did not stream back as quickly as the branches, suggesting that movement of nodes is done by rearrangement of cells not bu bulk movement (our data on this were not quite strong enough to make a big deal of in the paper: we will follow the story up).

So, it looks as if we had an explanation, at least at the descriptive level, of how a tree formed of two-from-one branches can transform into a many-from-one arrangement of renal pelvis to many collecting ducts!

Of course, as often happens in science, gaining an 'answer' really just generates new questions. How does this happen at a cellular level? What controls it? Do any of the congenital diseases that include a messed-up renal medulla stem from a fault in this process? Answering these questions will be

slow. An application for funding to do it quickly, with a dedicated member of staff, was rejected on the understandable grouds that basic work like this cannot take priority over competing applications that have a tight focus on a known kidney disease. We can, though, perhaps make these questions the core of an MSc or PhD research project, for which having an interesting question that will provide strong scientific training in a range of methods, wet-lab and computer-modelling, is more important than direct clinical applicability. If any prospective PhD students happen to be reading this, please do get in touch.

Jamie Davies,
Edinburgh,
January 2015

Links:

The publication itself: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4299504/