… an ugly fact

The great tragedy of Science — the slaying of a beautiful hypothesis by an ugly fact..
TH Huxley, Collected Essays 8, 229.

Part of the business of science is the creation of hypotheses that might explain some observed behaviour. Hypothesizing happens at all scales; at an everyday scale, a researcher frustrated by the failure of a technique to work might hypothesize, for example, that a critical enzyme is life-expired, and test that hypothesis in a simpler way before spending more time trying the whole experiment again. At a grander scale, very long periods of careful contemplation of a complex set of facts might result in a hypothesis that, if correct, can change the course of science. The hypothesis of evolution by natural selection, published by a one-time medical student at this university, Charles Darwin, is an example. When a hypothesis is proposed, it is subjected to experimental testing, ideally through being challenged by experiments that, if the results came out one way, could prove the hypothesis wrong. When hypotheses survive many aggressive tests, we place more confidence in them and may one day call them 'theories'. Some hypotheses – evolution is one – have a kind of beauty to them: a sparse simplicity from which aspects of the richness of the natural world can arise. The more beautiful a hypothesis is, the more we tend to want it to be true and the sadder it can seem when the data say 'no': that is what Thomas Huxley meant when he uttered the phrase at the top of this blog post.

A few years ago, I was most impressed by a simple hypothesis that was proposed by Celeste Nelson to explain a feature of cell behaviour that is critical to human development. The anatomy of many of our internal organs is arranged around an internal tree of branching tubes. The airways of the lung, the urine collecting ducts of the kidney and the excretory ducts of salivary and mammary glands are well-known examples. In each case, the tree grows, much like an actual tree, from an initially unbranched trunk, the tip of which divides to make two tips, which then grow out before each divides again and so on. Some years ago, Sanjay Nigam's group developed culture systems in which single cells could be placed in gels that mimic extracellular proteins. These cells would multiply to make hollow spheres and, if these spheres were treated with the right signalling molecules, they would spontaneously produce little branched trees. This raised an interesting question: all the cells were the same (they were all clones of the original), and the signalling

molecule was everywhere; why, then, did some cells become tips to lead new branches while others were content just to follow and become stalks?

The phenomenon is an example of spontaneous symmetry-breaking, which is a general term for what happens when a system that is the same everywhere ('symmetrical') suddenly starts to show differences without those differences being imposed from outside. Usually, spontaneous symmetry breaking involves feedback, so that differences tend to get amplified, and this can happen at a vast range of scales and does not need biology. Matter, for example, attracts other matter through gravity so that random clumps that form will tend to grow, getting larger by pulling in neighbouring matter and at the same time denuding the space between the random clumps: start with a random spread of hydrogen and helium and end up with galaxies separated by empty space and, within those galaxies, end up with concentrations of matter in stars and planets.

If symmetry-breaking usually relies on feedback, what feedback is operating when symmetrical spheres turn into branched trees? Celeste Nelson proposed an elegant idea. The cells, she said, may be excreting a molecule that discourages them to advance. Any cell that happens to advance slightly ahead of is neighbours, just by random chance, as the sphere grows will enter a region a little less polluted by the inhibitory molecule and will be able to advance a bit more, taking it further away from the inhibitor and allowing it to advance still faster and so on. The neighbours left behind now find themselves in concave 'caves', which because they are semi-surrounded by cells, accumulate inhibitor and really, really inhibit advance. Thus the initially tiny difference between tips and non-tips becomes more and more exaggerated. When new branch tip has grown out far enough, the same sort of thing can happen again along its side to make new generations of branches. As well as proposing this model, Nelson and colleagues set up a series of tests of it in culture, growing breast cancer cell lines in wells in a gel. In a circular well, the cells would try to grow out in any direction. If two wells were put close to one another, outgrowth was inhibited in the vicinity of the other well, as if inhibitor was accumulating there and, similarly, convex well edged allowed much more outgrowth than concave. The group identified the inhibitor involved as the molecule TGF-beta. I loved this paper when I read it, and with a PhD student in the lab published a small summary review of it at the invitation of a journal editor.

Here, we work on kidney rather than mammary tissue. Kidneys also have branching trees and their cells have similar symmetry-breaking properties and we felt it would be helpful to find out what

molecule was involved in kidney. Huabing Yin, a colleague and friend in the engineering department in nearby Glasgow, had already developed an elegant printing technique that could create precisely shaped islands of cell-friendly surface separated by a 'sea' of a surface too slippery for cells to grip. She offered to make thousands of islands with edges that had a complex but reproducible sequence of straight, convex and concave lines so that we could grow cells on them, and monitor their attempts to advance. This work was begun by MSc and undergraduate project students but most of it was done by Kim Martin, a PhD student (now a PhD graduate) who took the work on from preliminary data to publication.

The first results were most encouraging. Cells on straight edges tried to advance somewhat. Those in concave edges really did not want to advance very much at all while those on convex curves were very enthusiastic about trying to extend. This is just what the Nelson hypothesis would predict. The stage was set for a series of experiments that would identify the molecule involved but, before starting them, we did a simple test to confirm the whole idea. Kim placed the whole culture system in a flow cell – a piece of apparatus that allows culture medium to flow over the cells – connected up a pump, and turned on the flow. If the hypothesis of the secreted inhibitor were correct, we would expect the flow to sweep the inhibitor away and we would expect to see all areas showing much more attempts to advance, with (if the washing away was complete) all going at the same fast rate. The actual result could not have been more different: the flow made no difference at all!

Confused, we performed all sorts of 'control' experiments to verify that the flow really was reaching the cells and that it was capable of clearing away fluorescently labelled test proteins. It was, and quickly. This was perplexing. Even if our flow system was not quite good enough, and only reduced rather than eliminated concentrations of inhibitor, one would expect to see some change of behaviour but there was none (and poor Kim studied the cells on literally thousands of these islands so that we could be sure that we had made enough measurements that even a small effect would have shown up).

There was only one conclusion: at least in kidney, the control of cell motility by curvature is not mediated by a secreted inhibitor. Another beautiful hypothesis has been slain by an ugly fact. Kim's paper describing this work is in the 'links' section below. It may of course be that symmetry-breaking in mammary cancers and normal kidneys are controlled by completely different mechanisms, and obviously we intend to repeat our experiments using the mammary tumour line to

find out. But for the kidney, we are back to square one. Curvature does matter, certainly, and thanks to Kim we have some hints about how, but more work needs to be done before we publish those (and I make it a rule not to describe experiments in this blog that have not already gone through peer-review).

I greatly admire Kim's patience in her painstaking analysis, and I like her data… but some little part of me still grieves for the beautiful hypothesis on whose grave those data lie.

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Links:

- Celeste Nelson's paper: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2933179/
- Our review of Nelson's paper: http://onlinelibrary.wiley.com/doi/10.1002/bies.20541/abstract
- Kim's paper: Martin KC, Yuan X, Stimac G, Bannerman K, Anderson J, Roy C, Glykofrydis F, Yin H, Davies JA. (2017) Symmetry-breaking in branching epithelia: cells on micro-patterns under flow challenge the hypothesis of positive feedback by a secreted autocrine inhibitor of motility. J Anat. 2017 Mar 29. doi: 10.1111/joa.12599. [Epub ahead of print] https://www.ncbi.nlm.nih.gov/pubmed/28369863