FORUM Cell biology

Calcium contradictions in cilia

Organelles called primary cilia that protrude from cells have been thought to sense the surrounding environment through calcium-channel proteins that respond to force. Two scientists discuss the implications for developmental biology and kidney disease of a study that challenges this hypothesis. SEE LETTER P.656

THE PAPER IN BRIEF

- The primary cilium is a hair-like projection that is often regarded as the cell's signalling antenna.
- It harbours a calcium-channel protein that was thought to open when the cilium was bent by the force of fluid flow, allowing calcium ions (Ca^{2+}) to enter the cell (Fig. 1a).
- On page 656 of this issue, Delling *et al.*[1] report that they have engineered mice to express a sensor protein that fluoresces in response to Ca^{2+} influx into primary cilia, and measured Ca^{2+} signals after applying a mechanical force.
- They find no evidence of force-driven Ca^{2+} influx (Fig. 1b), and therefore conclude that these structures are not involved in calcium-based mechanosensation.

Asymmetric implications

DOMINIC P. NORRIS

Although human bodies show left–right symmetry externally, internal organs are asymmetrically located — our heart and stomach, for example, are on the left side of the body. This asymmetry is established at the time when the embryo comprises a flat disc of cells, in which a small pit called the node transiently forms. Each node cell has a primary cilium that rotates clockwise, somehow driving extracellular fluid from right to left to establish asymmetry[2]. Mounting data[3–5] have supported the idea that cilia on the left of the node directly sense and respond to fluid flow through PKD2 Ca^{2+}-channel proteins, which open to produce a left-biased ciliary Ca^{2+} signal. Delling and colleagues' evidence that this is not the case could have a profound impact on our understanding of left–right patterning.

Debate about how cells in the embryo perceive flow goes back almost 20 years, and several explanatory models have been developed[6]. One posits that a secreted signalling molecule called a morphogen becomes enriched on the left side of the node in response to flow. Another argues that vesicles containing morphogens travel leftward, delivering their cargo unilaterally. A third, the mechanosensation model, suggests that the leftward force itself is perceived by cilia in crown cells that surround the node. Although no model has been proved or disproved, over time, the mechanosensation hypothesis gained the most traction.

The authors' cilium-specific Ca^{2+} sensor allowed them to test this hypothesis directly. Validating the sensor was challenging, but, luckily, the authors found that it was expressed not only in primary cilia, but also in cilium-like structures on hair cells in the inner ear, which are known to respond to force through Ca^{2+}-based mechanosensation[7]. Delling and colleagues applied a flow of fluid onto the hair cells and found that the sensor reliably reported Ca^{2+} signals in response to physiological levels of force.

Next, the researchers isolated mouse embryos at the ages at which left–right asymmetry is established, and applied fluid flows to either the left or right side of the node for up to 10 seconds. This consistently failed to elicit a ciliary Ca^{2+} response. Similarly, the authors failed to detect Ca^{2+} signals in the cilia of bone cells.

Delling *et al.* endeavour to explain how their study contradicts so much previous work. First, they propose that previous studies might have misinterpreted imaging artefacts that arose when flow moved the cilium out of the plane of focus, causing changes in fluorescence. Second, they argue that imaging speed is key, because the cilium can become rapidly infused with Ca^{2+} that flows in from the cell body, and this might be mistaken as originating in the cilium.

These results seem to discount the mechanism by which mechanosensation has been proposed to act during the establishment of left–right asymmetry. However, the authors are careful to state that they have tested only whether the nodal primary cilium is a Ca^{2+}-responsive mechanosensor, not whether force detection plays any part in asymmetry. Mechanosensation could, for instance, occur through the cell body or through cilia in a calcium-independent manner. Morphogen-dependent mechanisms also remain possible. Whatever the mechanism, it must explain why PKD2 is essential for crown-cell cilia to detect flow — a fact that is now unexplained.

As with all potentially revolutionary findings, possible caveats are likely to be tested in depth by other laboratories. The authors openly lay out some of the limits of their data. But, importantly, left–right patterning of an embryo occurs over hours rather than seconds, and inside the mother rather than in a Petri dish. The extent to which the real-life situation has been modelled in the current study is likely to be questioned.

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Signals straightened out

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The hypothesis[8] that forces generated by urinary flow elicit ciliary mechanosensation in the kidney dates to 2001, and defects in this process have been proposed to cause adult polycystic kidney disease. A technical tour de force has allowed Delling *et al.* to question this long-standing model. The strength of their argument lies in their use of recently developed Ca^{2+}-signalling reporters (described above) and their ability to rapidly visualize Ca^{2+} signalling while controlling for ciliary movement.

The past few years have seen a renaissance in the study of primary cilia, thanks to genetic studies[9] in model organisms. Another rich source of information comes from the many human-gene mutations that cause ciliary dysfunction, which have allowed researchers to identify structural and trafficking components of cilia, and ciliary signalling mechanisms[10].

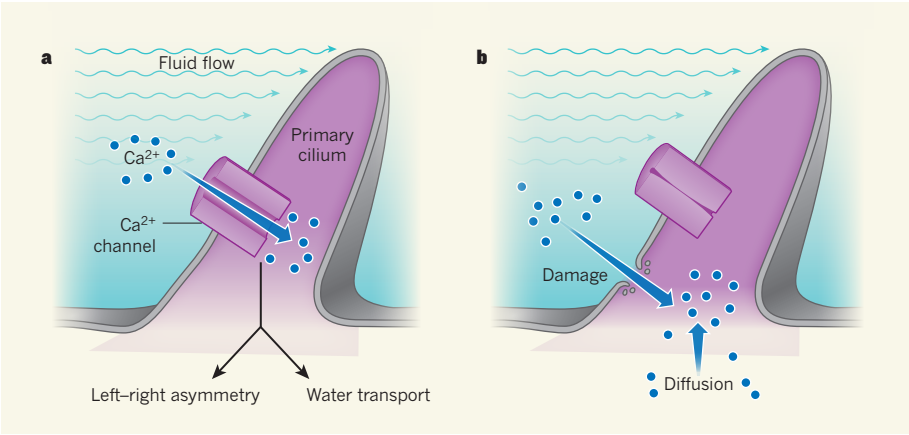

Figure 1 | The mechanosensation hypothesis. **a**, Much research suggested that cells' primary cilia respond to force through mechanosensation. In this original model, fluid flow pushes the cilium, which triggers the opening of calcium-sensitive channel proteins and so allows calcium ions (Ca^{2+}) to enter the cilium. Intracellular signalling cascades are activated by the Ca^{2+} influx, leading to altered gene expression on the left side of the embryo, or promoting water transport in the kidneys. **b**, Delling *et al.*[1] find that, contrary to this hypothesis, ciliary bending in response to force does not open Ca^{2+} channels. Instead, the authors propose that the Ca^{2+} influx observed in previous experiments might have been due to diffusion from the cell body, or to damage to cilia in response to extreme levels of force.

Several analyses support the model that cilia are involved in specialized Ca^{2+} signalling. First, work[11] by the group that performed the current study indicates that Ca^{2+} signals in primary cilia are as strong as Ca^{2+} influxes that trigger signalling cascades in other cell types. Second, cilia contain many Ca^{2+}-binding proteins, including adenylate cyclase III — an enzyme that is located only in cilia, is vital for several cell-regulatory roles (including limiting cystic growth in kidneys) and is Ca^{2+}-dependent. Third, the childhood kidney cystic disease nephronophthisis can be caused by mutations in genes that encode calcium-dependent ciliary proteins; similarly, adult polycystic kidney disease has been linked[10] to mutations in the *PKD1* gene and in the gene that encodes its binding partner, the PKD2 Ca^{2+} channel. However, another study[12] by the current group indicates that the strong Ca^{2+} signals observed in cilia do not require PKD1 or PKD2, but rather the related proteins PKD1L1 and PKD2L1, underscoring the fact that we understand neither the breadth of ciliary Ca^{2+} signalling nor the fundamental pathways in which channel proteins such as PKD2 function.

Cells of the proximal collecting duct make up the kidney tubules involved in antidiuretic (urination-reducing), hormone-stimulated water transport. Malfunction of cilia in these cells is thought to be involved in water-transport-driven swelling of the collecting ducts and cysts in cystic kidney diseases. Delling *et al.* examined collecting-duct cilia using a flow chamber to approximate urinary flow rates and to cause varying degrees of ciliary bending, but observed no effects on Ca^{2+} signalling. Occasionally, Ca^{2+} signals were generated by damaging the cilia, and this would explain the discrepancy between these data and older work. Indeed, without modern sensors and control markers, measurement of bona fide ciliary signals in past work would have been difficult.

For the Ca^{2+}-mechanosensation model to make sense physiologically, the location and timing of Ca^{2+} influx would connect an observed signal to the physiological effect. For example, observations of fluid flow, ciliary bending, Ca^{2+} influx and water transport in the collecting duct would have to be temporally and spatially linked. Delling and colleagues find no evidence of either the mechanical changes or the appropriate Ca^{2+} signals in this setting, thus disputing this model. Although it is hard to categorically disprove a signalling hypothesis, the quality of the authors' work sets a high bar for any future study that supports ciliary mechanosensation.

Researchers must now find another explanation for how collecting-duct cilia function. An attractive alternative is that urinary flow provides a flux of molecules, possibly metabolites or toxins, that signal through other ciliary receptor proteins to control water retention or to direct toxin clearing. G-protein-coupled receptors are a particularly attractive class of potential receptor, but channels such as PKD2 and PKD2L1 are also candidates, because little is known about potential ligands that activate them. The key regulatory output of ciliary signalling in collecting ducts — calcium-dependent or otherwise — and how this signal impinges on physiology and cystic diseases, remain to be defined. Further study of cilia could help to find a broader explanation of how kidneys regulate water balance without retaining toxic metabolites. ■

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CANCER IMMUNOTHERAPY

Killers on sterols

Increasing cholesterol levels in the cell membranes of killer T cells boosts the cells' ability to mount an immune response against tumour cells in mice. Such a strategy might be valuable in anticancer immunotherapies. SEE LETTER P.651

MICHAEL L. DUSTIN

The dream of stimulating the body's immune response to fight cancer has become a reality in the past decade. Several potent drugs have been clinically approved that block inhibitory checkpoints in the immune system, boosting the ability of the system's T cells to mount responses against a range of cancers[1]. Furthermore, patients' own T cells have been successfully genetically engineered to attack blood-cancer cells[2]. Although these studies have established the immune system as a powerful ally in cancer therapy, there are still many challenges to overcome, and further advances would increase the number of people who stand to benefit from immunotherapy. In this issue, Yang and colleagues[3] (page 651) propose a way to boost the function of antitumour T cells, using a metabolic trick