

## Dispatches

# Ciliary Ion Channels: Location, Location, Location

The elegant waveforms of motile cilia derive from temporal and spatial regulation of dynein-driven microtubule sliding. A new study reveals the surprising localization of the channel responsible for waveform switch.

Lynne Quarmby

Eukaryotic cilia (also known as flagella) beat with two distinct waveforms. One is known as the ciliary waveform and the other as the flagellar waveform.

The ciliary waveform involves an asymmetric breaststroke-like motion, with a power-stroke and a recovery stroke. In contrast, the flagellar waveform is symmetrical. In metazoans, it is common for cells to use one waveform or the other exclusively. For example, mammalian respiratory epithelia use the ciliary waveform to drive fluid flow in one direction, whereas sperm use the flagellar waveform to push the cell forward. Many protists, however, have the ability to change waveform: *Paramecium* swim forward, pulled along by numerous cilia beating with the ciliary waveform, until they bump into something. Such mechanical stimulation induces backward swimming as a result of the reorientation of the ciliary beat cycle. Similarly, *Chlamydomonas* uses its two flagella in a ciliary waveform to pull the cell forward, but bright light or mechanical stimulation induce a switch to flagellar waveform that pushes the cell backwards, a behaviour known as the 'photoshock' response (Figure 1).

In a recent issue of *Current Biology*, Fujii *et al.* [1] report the identification of CAV2 as the voltage-gated calcium channel that transduces both bright light and mechanical stimuli into a switch from ciliary to flagellar waveform in *Chlamydomonas*. One of the more interesting aspects of the report by Fujii *et al.* [1] is the localization of the channel by indirect immunofluorescence. Although it was anticipated that the channel would be localised along the length of the cilium, the observation that it is restricted to the distal regions of the cilium was not predicted by electrophysiological experiments. Knowing that the calcium transients

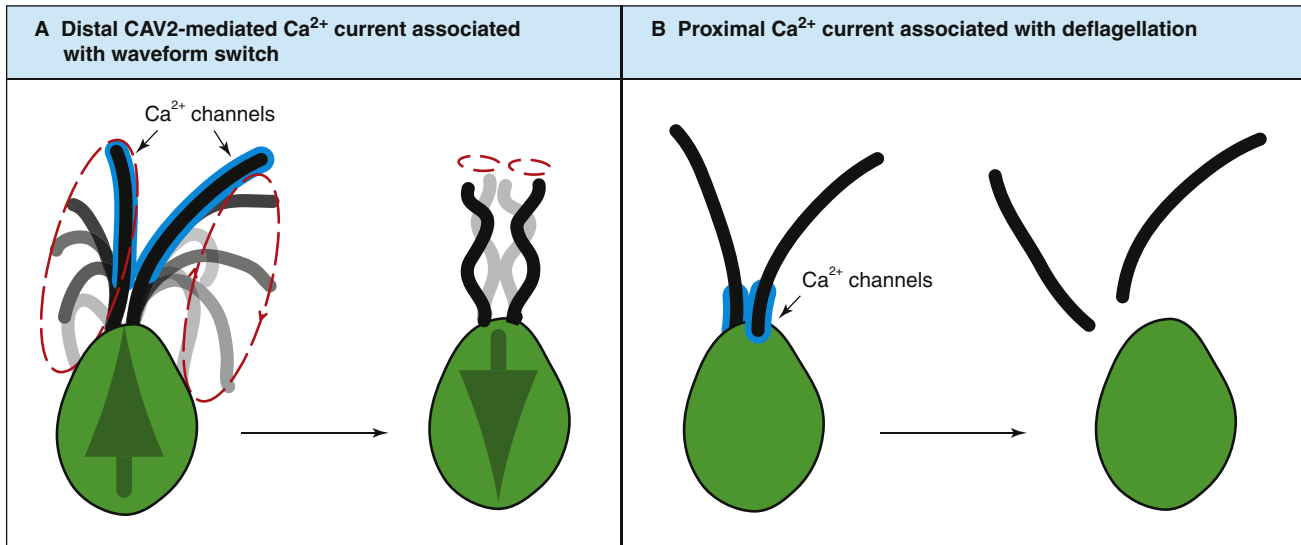
that induce waveform switch are excluded from, or minimized in, the proximal regions of the cilia will guide future experiments on the intriguing problem of waveform transition.

Underlying the waveform of cilia is the regulation of the dynein-based sliding of the nine outer doublet microtubules of the axoneme, one against the next. The generation of a bend in the cilium requires active dynein on one side of the axoneme and inactive dynein on the opposite side of the axoneme. The generation of a wave that is propagated along the length of the axoneme requires precise regulation of dyneins. The motors must be turned on and off around the circumference and along the length in a highly coordinated manner. The switch between ciliary and flagellar waveforms adds a further level of complexity. The elegant work of several labs has contributed substantially to our understanding of the regulation of ciliary dynein and the numerous roles that calcium plays in this process [2–7]. In addition to regulation by calcium and by phosphorylation, it is likely that mechanical loads contribute to the precise spatial and temporal regulation of dynein required to generate a smooth wave [8]. It is striking that these beautiful waves can rapidly switch from one form to another. We know little about how this is accomplished, although we do know that calcium is involved [9,10].

Pioneering work in *Paramecium* indicated the roles of voltage-dependent channels in the switch in the direction of ciliary beat, and electrophysiological measurements during ciliary regeneration suggested that the relevant calcium channel was localized to cilia [11]. Using a suction pipette approach, Harz and Hegemann [12] characterized an eyespot current and a flagellar current that was

responsible for waveform switch. Subsequent identification and cloning of the eyespot channel, chlmyrhodopsin (now known as channelrhodopsin) provided a light-sensitive channel that has become a valuable tool for neuroscientists [13,14]. In *Chlamydomonas*, the eyespot current on the cell body triggers a flagellar current, presumably by a depolarization that stimulates the opening of a flagellar voltage-gated calcium-permeant channel. By way of a *Chlamydomonas* mutant strain that is unable to generate this flagellar current [15], CAV2 has now been identified as the photophobic response channel [1].

Fujii *et al.* [1] report that CAV2 localizes to the distal regions of the flagella. That the channel would be localised to cilia was expected [11,12] but exclusion from the basal region will be a surprise to many researchers in this field. An early idea was that ciliary calcium conductance was mediated by the 'ciliary necklace', an array of particles at the base of the cilia revealed by freeze-fracture electron microscopy [16]. This idea was an appealing explanation of waveform reversal in part because the flagellar waveform is propagated from the base of the flagellum. More recently, Beck and Uhl [17] studied the reappearance of the conductance during flagellar regeneration and predicted that this channel would be uniformly distributed along the length of the flagella. Based on these and many other studies, Tamm [18] proposed that, whereas calcium conductances involved in activation or enhancement of ciliary beat would be found at the base of the cilium, conductances involved in 'reprogramming' of the waveform (reversal of the ciliary beat in ciliates and a switch to the flagellar waveform in *Chlamydomonas*) would be localized along the length of cilia. There may be more commonality between the activation/hyperactivation of mammalian cilia and flagella and the waveform changes observed in protists: recent studies of the CatSper channel, which localizes to a central



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Figure 1. The ciliary ion channel, CAV2, is excluded from proximal regions of flagella.

*Chlamydomonas* is normally pulled forward by the ciliary beating of its two apical flagella. In response to bright light or mechanical stimulation, the flagella undergo a waveform switch to a flagellar beat and the cell is propelled backwards. Waveform switch is mediated by high levels of intraflagellar calcium, achieved through opening of the voltage-gated CAV2 channel. Localization of CAV2 to distal regions of the flagella allows waveform switch (A) without inducing deflagellation, a stress response that is triggered by similar levels of calcium at the base of the flagella via an as yet unknown mechanism (B). (Artwork courtesy of John Glover.)

region of mammalian sperm flagella, showed that it plays a role in hyperactivation of flagellar beat via the formation of a more prominent bend [19].

The exclusion of CAV2 from the proximal region of the flagellum is, however, predictable from studies of the effects of calcium on the axoneme. Using isolated flagella, Bessen *et al.* [10] found that concentrations of free calcium above 1  $\mu\text{M}$  were required in order to elicit the flagellar waveform. Below this concentration, isolated flagella beat with a ciliary waveform. The conundrum arose when later studies revealed that flagella would be shed at 1  $\mu\text{M}$  calcium. Deflagellation is a stress-induced shedding of flagella that involves a precise severing of the nine outer doublet microtubules at the base of the flagella. *In vitro* studies revealed that 1  $\mu\text{M}$  free calcium is sufficient to induce severing of the axonemal microtubules [20]. How can bright light or mechanical stimulation induce the flagella of *Chlamydomonas* to switch from ciliary to flagellar waveform without causing deflagellation? The localization of CAV2, the transducer of both bright light and mechanical stimulation, provides a tidy answer. By excluding CAV2 from the basal region, the high concentrations of calcium required for

waveform switch can be achieved along most of the length of the flagella, while protecting the flagella from deflagellation. The calcium conductance pathway of the deflagellation pathway has yet to be identified, but is predicted to lie at the base of the cilium (Figure 1). While providing a neat answer to the question of how a waveform switch can be achieved without triggering deflagellation, the new work by Fujii *et al.* [1] raises intriguing questions about how the CAV2 channels are excluded from the proximal region of the ciliary membrane. The mechanism of channel placement and the impact of this localisation on waveform switch provide new avenues of exploration in this fascinating area of biology.

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## Visual Neuroscience: A Hat-Trick for Modularity

**A new study using transcranial magnetic stimulation of the brain shows that each of three neighboring areas of visual cortex plays a specific and causal role in perceiving faces, bodies and other kinds of objects.**

**Paul E. Downing**

Vision provides the human brain with its main source of information about the surrounding world. Accordingly, the visual cortex is large and complex — considerable machinery is required quickly and accurately to decode the wealth of information that is latent in the retinal input. How is visual cortex organised? Much of it can be partitioned into multiple maps of space that analyse the input for primitive features such as edges. But a large part of the visual brain plays a more complex role in comprehending what we see — for example, identifying objects across changing perspectives or lighting — and this has been a fertile ground for debate.

A lightning rod for this debate is the issue of whether and to what degree the organization of visual areas is modular. Are there focal brain areas that specialise in the perception of certain classes of things? This idea takes support from neurological patients who can perceive everyday objects without difficulty but fall down on faces (and others who show the reverse dissociation), although such ‘pure’ deficits are rare [1]. Observations like these have inspired neuroimaging studies that identified small areas of visual cortex that respond highly selectively — to faces, for example [2]. When neuroimaging data are analysed with sophisticated pattern recognition methods, however, it appears that broad swathes of visual cortex contain diffuse, distributed information about many visual kinds, contrary to the modularity hypothesis [3].

Hence a key question in the field is whether brain areas that respond strongly to a single category are uniquely and causally involved in perceiving items of that kind. Neuroimaging methods can only provide correlational evidence, so transcranial magnetic stimulation (TMS) steps in to cross the gap. TMS works by driving a strong electrical current through a coil placed over the scalp. The resulting magnetic field induces an electrical current into the neurons that lie beneath the coil — effectively adding noise to neuronal activity and hence interfering with normal processing. The effects of TMS are temporary, working on a time scale of milliseconds to tens of minutes (depending on the protocol), making it the ideal tool for reversibly impairing the function of a brain area in order to probe its workings.

Conveniently, several apparently category-selective regions of visual cortex lie on the lateral surface of the brain, where they are in reach of TMS (Figure 1). These include the occipital face area [4] and the extrastriate body area [5] — selective for human faces and bodies, respectively — and the lateral-occipital complex [6], selective for general object form but not especially for bodies and faces. Some recent studies [7–9] of neurological patients indicate, for each of these regions, a causal role in perceiving a particular category. Similarly, in each case, initial TMS studies support the same view. For one example, TMS over the extrastriate body area, but not over primary visual cortex or prefrontal cortex, impairs tasks requiring subtle judgments of the form of the human

body [10]. Similar tasks on other object types were unaffected. In all of these TMS studies to date, the category-selective area of interest has been compared to sites elsewhere in the brain — typically not even in visual cortex — leaving open the question whether this method can distinguish among them with precision.

As they report in this issue of *Current Biology*, Pitcher *et al.* [11] were bold enough to seek a triple-dissociation among the occipital face area, the extrastriate body area, and the lateral occipital complex. Each region was localised individually in each participant with an fMRI scan, to provide a target for TMS. Participants performed carefully balanced tasks that involved making subtle discriminations about images of faces, bodies, or novel objects. The results showed that in each test, TMS over a given area (such as the occipital face area) impaired accuracy only for that area’s ‘preferred’ stimulus (for example, faces). Performance on the other categories was just as good as when no TMS was applied. This spatial precision is remarkable given the close proximity of these regions (Figure 1) and is good news for researchers hoping to use TMS as a tool to investigate visual cortex.

Thus, brain areas that respond selectively in fMRI appear to be uniquely involved in analysing their preferred categories, strongly favouring a modular view in which at least some visual kinds are analysed in focal brain areas. Caveats apply: in principle there could be minute but undetected effects of TMS on the other categories, and there could still be some other, untested category that will activate these areas equally well, although many have been tested [12]. But on the whole this triple-dissociation pattern is very hard to accommodate with a model in which the information about object form (or at least bodies and faces) is spread diffusely across visual cortex.