

pressure. Not much evidence on any of these questions is yet available, but it neatly sketches exciting research agendas stimulated by this study. Last but not least, and as mentioned above, *C. bombi* belongs to a group of important disease agents. Might it be that some compounds of nectar could do the same trick in *Leishmania* or *Trypanosoma*? The methodical and conceptual advance provided by the study of Koch *et al.* now provides us with the right tools and ideas to study such questions. And, maybe, one day the humble bee — which we now call the bumblebee — will prove to be an instructive stepping stone towards finding ways for protecting humans and their resources from parasite attack.

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## Animal Evolution: The Hidden Biology of the Ctenophore Cilium

Sally P. Leys<sup>1,2</sup>

<sup>1</sup>Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada

<sup>2</sup>Twitter: @SallyLeys

Correspondence: [sleys@ualberta.ca](mailto:sleys@ualberta.ca)

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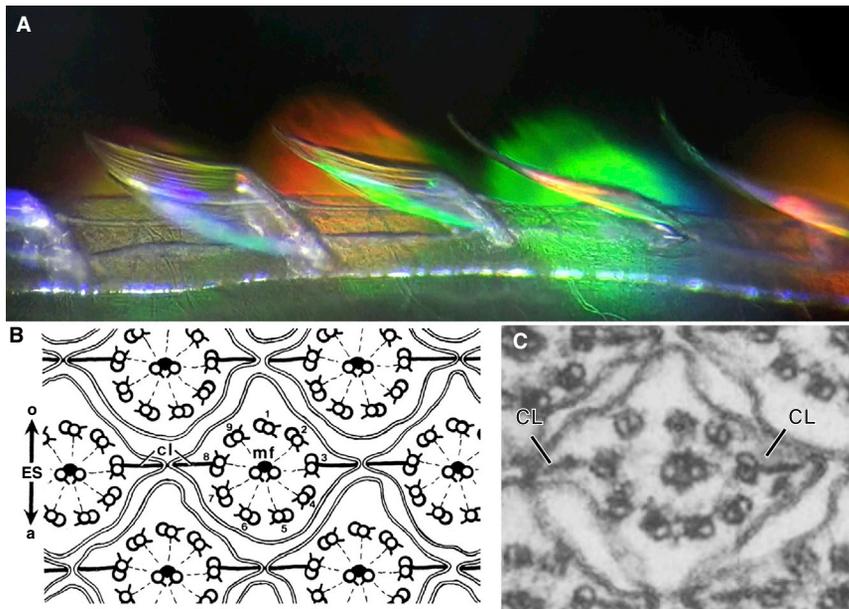
**A new study uncovers the function of a completely novel protein in the eye-catching iridescent cilia of the ctenophore comb plate.**

Consider the feather. It's light yet strong and incredibly effective as a paddle. Yet unlike man-made paddles, it is made of hundreds of thousands of filaments. In fact, most biological structures that function as paddles, moving either air or water, are made of highly aligned filaments. Cilia are essentially tiny filaments that project from the surface of cells. They arise singly or in groups from a

single cell and when in groups, the distance between the cilia is what makes them function as a paddle or a rake [1]. Keeping the filaments together correctly is key to the ability of biological paddles like cilia to function well hydrodynamically. To be effective in moving fluids, however, they must also beat in synchrony. How do cilia stay synchronized? What we know about how

cilia become oriented to beat in the correct direction comes largely from studies on the development of cilia in vertebrate epithelia. There, cilia are mostly positioned by developmental signals and then correct orientation of the ciliary beat comes with feedback from flow generated by other cilia [2]. If any animal should have sorted out how to organize cilia properly it's the ctenophore.





**Figure 1. The cilia of ctenophores.**

(A) Multiciliated comb rows from the ctenophore *Bolinopsis mikado* from Video S2 in [4]. (B) Diagram of the ciliary array in a comb row of the ctenophore *Pleurobrachia pileus* [11]. (C) Cross section through a cilium in *Pleurobrachia pileus* showing the organization of microtubules in the axoneme and the compartmenting lamella [11]. ((B) and (C) republished with permission of Rockefeller University Press from [11] © 1981).

Ctenophores are grape-sized gelatinous marine animals that are most easily identified by the presence of 8 rows of iridescent giant 1 mm-long cilia (Figure 1A) which they use to row themselves around. How are so many long cilia held together to beat synchronously? A new paper in this issue of *Current Biology* by Kei Jokura, Kazuo Inaba and colleagues [3] has now revealed the molecular basis of the protein that keeps the ciliary arrays in ctenophore comb plates ordered.

Cilia themselves are typically well-organized structures: each cilium consists of a ring of nine microtubule pairs surrounding a central pair (together called the axoneme), all enclosed by the cell's membrane. In the ctenophore, thousands of cilia are arranged hexagonally in a structure called the comb plate, and the entire comb plate beats as one (Figure 1B). A range of meticulous experiments has shown that, like normal sized cilia, ctenophore comb plates are entrained to beat synchronously by hydrodynamic coupling [4,5]. Hydrodynamic (or mechanical) coupling means a nervous stimulus is not required to propagate sequential beating of the cilia. Experiments have shown that separating the comb cilia mechanically

(like parting the filaments of a feather) does not prevent the whole comb from beating. Adjacent cilia in a comb plate must therefore be mechanically coupled to one other [4].

The ctenophore comb plate cilium has a propeller-like structure that extends out on either side of the axoneme and connects to the ciliary membrane. This feature is called the compartmenting lamella because it effectively divides the cilium's nine microtubule pairs in two, with three on one side and four on the other, along a plane that is parallel to the plane of the comb plate beat (Figure 1C) [6]. The lamella appears as a dark band in cross section, but in fact is a protein sheet that runs the length of the cilium. The idea that this might be what connects adjacent cilia via an extracellular protein seen between the cilia had been floated earlier [4,7], but the question remained unexplored. Now Jokura and colleagues [3] have carried out the first comprehensive molecular characterization of the protein in the compartmenting lamella and its role in ciliary beat in the ctenophore *Bolinopsis mikado*.

Jokura and colleagues [3] first dissected off the comb plates and extracted the protein with different

buffers. Eventually they were left with the bare cilium and an extract containing a very defined protein band with a molecular weight of 64 kDa, which they named CTENO64. An antibody made to that protein extract labelled half of each cilium in a distinct band proximal to the body in each comb plate. Curiously it did not label the outer portion of the cilium, despite the compartmenting lamella continuing into that region, and the point where the label ended appeared to be correlated with where the cilia became more loosely associated in the comb plate. Coupling the antibody to gold allowed the authors to use electron microscopy to confirm that the antibody recognized the compartmenting lamella and that it extended out to the ciliary membrane, which was in contact with neighboring cilia. It seemed a good guess, therefore, that the compartmenting lamella was used to hold the cilia together. But this structure could have a range of functions, including stiffening the cilia.

To determine whether CTENO64 was required for correct movement of the comb plate, the authors knocked down its expression during development using morpholinos, which they injected into fertilized eggs. They cultured the embryos until they formed larvae 30 hours later and studied the structure and motion of the cilia in the comb plates. In the CTENO64 knockdowns, cilia and comb plates appeared to develop normally, with the cilia a perfectly normal length. However, the proximal parts of the cilia no longer labelled with the CTENO64 antibody, and in nearly 16% of cilia the compartmenting lamella was missing. Moreover, in a small number the whole orientation of the ciliary axoneme was reversed by 180 degrees. To find out how these slight abnormalities affected swimming they analyzed and compared the ciliary beat of normal and morpholino injected larvae. While the comb plates beat with the same frequency in control and treated larval ctenophores, the beat was odd. In treated larvae the cilia had a much narrower angle of beat. Cilia also bent unusually at the wrong places, and about 14% of cilia beat in a different plane, which suggested that the orientation of the ciliary axoneme was different from normal comb plates. Sections through those cilia confirmed this: nearly 50% of the cilia were

misaligned, and 11% were inverted 180 degrees.

But if the antibody to CTENO64 only labelled the proximal region of the cilium, why would knockdown of the gene cause loss of the compartmenting lamella along the full length of the cilium? Cilia that lacked the compartmenting lamella had the correct orientation in the proximal region but became misaligned in the mid-distal region, causing a twist to the cilium. The authors conjecture that CTENO64 is important for holding cilia together because it maintains the alignment. They suggest that it acts as a scaffold in the proximal part to orient the cilia correctly as they form. In essence the compartmenting lamella is a stiffening rod that ensures the cilia are oriented planar to the direction beat, and at the same time it both strengthens the cilium and allows it to link to neighboring cilia in the comb plate. It has a hefty job.

If cilia are such common structures on animal cells it might make sense that this is a common solution to keeping cilia beating together in the right plane. And yet the reason the authors name the protein CTENO64 is because they only found homologs in other ctenophore genomes. This is a protein unique to ctenophores. Admittedly gene databases are still scantily populated by

invertebrates and especially poor in genes from other non-bilaterians (cnidarians, sponges, and placozoans), but it is not unusual to find that up to 40% of genes in various non-bilaterians have no known paralog in other animals [8,9]. It's not as though no other animals have the problem. Mussel gills have long frontal cilia that are held together to beat together; a number of unicellular ciliates face the same challenge. And yet the gene is not known in non-metazoans either. More studies like this on other ciliary systems might reveal an unrelated family of such proteins that are otherwise uncatalogued. As the authors suggest, CTENO64 appears to be a great example of the kind of hidden biology [10] still to be explored in non-bilaterians. Here, we have one piece beautifully dissected out to the molecular level, providing an understanding of what the gene is, what the protein is, and what role it plays in ctenophore biology.

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## Evolution: Diversification of Angiosperm Rooting Systems in the Early Cretaceous

Alexander J. Hetherington\* and Liam Dolan

Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB, UK

\*Correspondence: [sandy.hetherington@plants.ox.ac.uk](mailto:sandy.hetherington@plants.ox.ac.uk)

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**A monocot from the Early Cretaceous developed a cluster of anatomically similar roots from the base of a stocky stem. This discovery indicates that angiosperm rooting systems were more diverse than previously thought at this time.**

Most fossils of angiosperm roots from the Early Cretaceous that have been described to date are described as adventitious and developed from horizontal shoot axes called rhizomes (Figure 1) [1–8]. Rhizomes of many extant

herbaceous plants grow horizontally through the surface of soil and develop roots that supply water and nutrients as well as tethering the axis to its growth substrate [9]. Leaves, vertically growing (orthotropic) shoots and often roots

develop along rhizomes forming ramifying branched systems. The prevalence of this bauplan — root-bearing rhizomes — in the Early Cretaceous fossil record supports the hypothesis that early angiosperms developed a rhizotamous

