From the Archive

Cilia get arms for bending

After completing his PhD in Sweden, Björn Afzelius took a position at Johns Hopkins University (Baltimore, MD) with the idea of working on luminescent organisms. But his experiments weren't working, and "at the end of the year I was desperate," he says. "I picked up some embeddings I had brought over from Sweden."

model in cross-striated muscles proposed by Huxley [1957]."

Before he wrote those words, Afzelius had to convince himself that his intuition was consistent with what physical reality would allow. "The summer I was writing the paper I had a bamboo stick and I was pulling a rope to bend the bamboo," he says. "It was so simple and

Keith Porter's laboratory and then independently, now sought to add information about the dynamic movement of cilia.

His model—the mussel gill—was one that had been used for decades to study cilia. Each cilium in this structure beats slightly out of phase with its neighbor, thus setting up a series of so-called metachronal waves of activity.

> **Björn Afzelius identifies cilia arms and comes up with the filament sliding model of cilia movement. The sliding is visualized first by Peter Satir and more directly by Ian Gibbons.**

Afzelius had tried to improve on existing electron microscopy (EM) contrast by preparing his samples using 40% osmium tetroxide—"a nasty mixture," he says, "that I wouldn't touch now." But the chemical did its trick. Earlier EM had revealed the now familiar 9 + 2 pattern of the axoneme (the functional core of both eukaryotic flagella and cilia), with 9 doublet microtubules surrounding a central pair. With the extra contrast, Afzelius could spot arms that reached from one outer doublet toward another. The presence of the arms turned out to correlate with the ATPase activity of the cilia (Gibbons, 1963), and the structures were subsequently named dynein (Gibbons and Rowe, 1965).

But long before those discoveries, the significance of the structures was evident to Afzelius. Theoretical literature on cilia movement had focused mainly on the idea that a part of the structure would contract, thus inducing a wave-like movement. But, as Afzelius noted in his paper (Afzelius, 1959):

"The filaments must not necessarily be contractile in the ordinary sense of the word; the work done by them could also be the result of a sliding of the filaments in relation to each other, each filament retaining its original length and thickness…The underlying mechanism for such a filament sliding, if it exists, is certainly not understood, but it is probable that the arms would be active in this process. This would thus be a mechanism reminiscent of the contraction

naïve I didn't want to write about it. But it was more to convince myself that having a microtubule climbing on another would allow bending."

The excitement from the 1950s is still evident in Figure 1 of the completed paper. "Over Figure 1 there is a [white] line," says Afzelius. "I found the dynein arms in this section. I rushed to the boss of the institute and in my hurry I broke the photographic plate. I put it together as best as I could. The line is still there, reminding me of my eagerness."

Adding the time dimension

After Afzelius's paper and a detailed study from Gibbons and Grimstone (1960), the structure of the axoneme had been well picked apart. But ideas about movement mechanisms were still just that: ideas. Peter Satir, working first in

Dynein first showed up as arms between adjacent microtubule doublets; the white line is a mark of Afzelius's early enthusiasm.

"I had the idea that if you could stop the cilia in different stages of the beat, whatever was happening during the beat would vary from one cilia to another in a systematic way and you would be able to read it," he says. "I was tremendously excited. I thought it was a tremendously original idea. Of course, it turned out it had been done in the 1920s and again in the 1950s."

But those earlier studies had all been restricted to light microscopy. In his studies, Satir successfully activated the metachronal wave in isolated gills, and then captured it by fixation followed by EM (Satir, 1963). He then studied the distal ends of the cilia, first qualitatively (Satir, 1965) and then quantitatively (Satir, 1968), and found that "different microtubules stuck out, and the ones that stuck out were consistent with sliding."

Satir reasoned that, in a sliding model, "the bottom filaments [those on the inside of the curve] would be obliged to slide out past the top ones to accommodate the curvature." This is exactly what he found, with the identity of the protruding microtubule pair changing with the direction in which the particular cilium was moving.
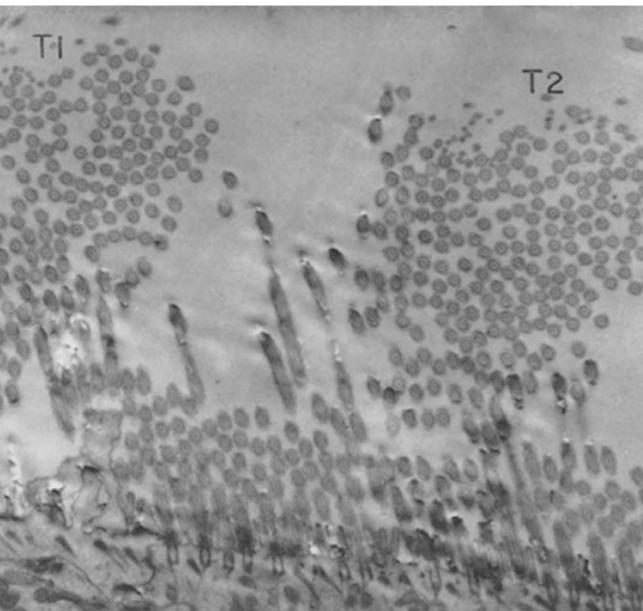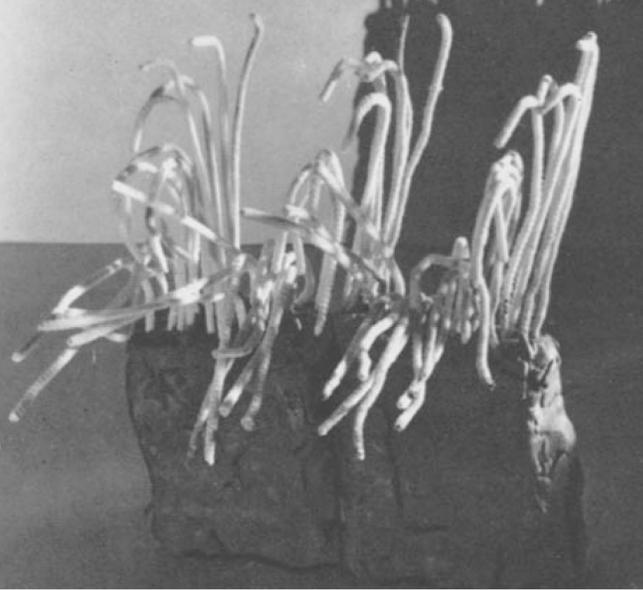
As the papers were published, "I would present the evidence…and essentially I was not believed," says Satir. "Most people were convinced by theoretical studies that contraction was the model."

Direct visualization

Ian Gibbons had stated in 1960 and 1963, respectively, that current evidence either

Text by William A. wells

wellsw@rockefeller.edu

Pipe cleaners (top) were the best material for modeling the wave of cilia on mussel gills (bottom).

field microscopy, so Gibbons resorted to the reflections from dark-field microscopy. "I was working late one evening, because our microscope was not set up well for dark field, so I had to wait for it to be dark," says Gibbons. "I used a little 6V lamp bulb, low concentrations of ATP, and, I think, 30 second time exposures."

"Once people saw that, then the sliding model was accepted," says Satir. "That made the sliding model a reality for most people. Mine was a true demonstration of sliding but it was an argument that was difficult to follow and people were not convinced." Sliding was seen even more directly by Brokaw (1989), who attached individual gold particles to flagellar microtubules and then tracked their oscillations in moving sperm.

Progress after 1971 has included the identification of many proteins, but the mechanistic advances have been more difficult to come by. "There's still a mystery of how you get part of the axoneme to do one thing and the other part to do something else," says Satir. In some species a rotating central pair may act as a regulator for distributing activation to dyneins in different parts of a cilium. Other researchers argue that tension exerted by dynein motors in one region may turn off opposing motors in another region by simply stalling them out. Testing of such ideas may have to await the use of inhibitors that can be targeted to an axoneme with high spatial and temporal precision. **JCB**

Afzelius, B. 1959. *J. Biophys. Biochem. Cytol.* 5:269–278.
Brokaw, C.J. 1989. *Science.* 243:1593–1596.
Gibbons, I.R., and A.V. Grimstone. 1960. *J. Biophys. Biochem. Cytol.* 7:697–715.
Gibbons, I.R. 1963. *Proc. Natl. Acad. Sci. USA.* 50:1002–1010.
Gibbons, I.R., and A.J. Rowe. 1965. *Science.* 149:424–426.
Huxley, H.E. 1957. *J. Biophys. Biochem. Cytol.* 3:631–648.
Satir, P. 1963. *J. Cell Biol.* 18:345–365.
Satir, P. 1965. *J. Cell Biol.* 26:805–834.
Satir, P. 1968. *J. Cell Biol.* 39:77–94.
Satir, P. 1974. *J. Cell Biol.* 63:35–63.
Summers, K.E., and I.R. Gibbons. 1971. *Proc. Natl. Acad. Sci. USA.* 68:3092–3096.

"favors" or is "consistent" with a contraction rather than a sliding mechanism. But it was he who finally saw sliding occurring in real time.

The visualization came after a chance difference in the in vitro behavior of *Tetrahymena* and sea urchin flagella led Summers and Gibbons (1971) to add trypsin to weaken the sea urchin structure. They succeeded in cleaving the nexin links between adjacent microtubule doublets, so that when ATP was added "it was obvious what was happening—[the microtubules] were telescoping." Freed of their constraints, individual microtubule doublets motored over one another and protruded out of the flagella.

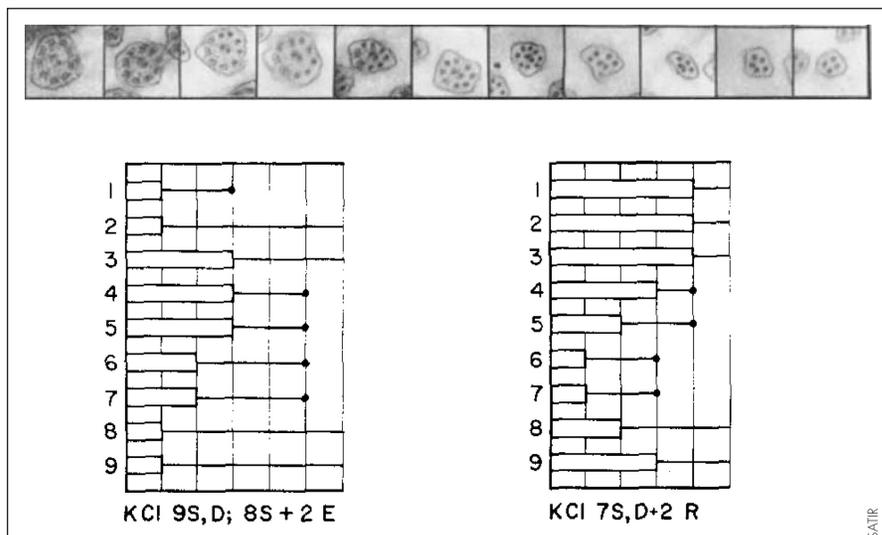The protruding microtubules were too small to be seen by simple bright-

Serial sections (top) show which doublets end first; the identity of these doublets changes depending on the orientation of the cilium (bottom, left: 1 ends first and 5 later; bottom, right: 1 ends later and 5 earlier).