Intraflagellar transport—the "new motility" 20 years later

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ABSTRACT **Intraflagellar transport is the rapid, bidirectional movement of protein complexes along the length of most eukaryotic cilia and flagella. Discovery of this intracellular process in *Chlamydomonas reinhardtii* 20 years ago led to a rapid discovery of cellular mechanisms that underlie a large number of human ciliopathies. Described herein are the events that led to this discovery.**

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As a graduate student, I thought how strange it must be to look at your own research 20 years later. In my hands was a review titled "Two Decades since the Naming of Tubulin" (Mohri and Hosoya, 1988). I certainly did not imagine at the time that I would ever make a similar retrospective. Yet, here I am, reflecting upon my discovery of intraflagellar transport (IFT) 20 years ago (Kozminski *et al.*, 1993b), in January 1992, when I was a doctoral student with Joel Rosenbaum in Yale's Department of Biology.

The Mohri and Hosoya review interested me because my dissertation research initially focused on the functional significance of a tubulin posttranslational modification, acetylation. Studying the biflagellate, unicellular, green alga *Chlamydomonas reinhardtii*, I asked whether alterations in the amount of acetylated α-tubulin affected the stability or function of the flagellar axoneme, the microtubule core of the flagellum. I found no mutant phenotype, making for a quick but publishable end to my nascent project (Kozminski *et al.*, 1993a). As I learned, a quick end can be a good thing.

The swan song of my tubulin project, and the first step toward the discovery of IFT, was the December 1991 American Society for Cell Biology meeting in Boston. The night before the meeting, my dissertation advisor Joel Rosenbaum aptly deemed the micrographs on my poster substandard. What ensued, learning that it is never too early to start a poster, was an all-nighter with my coauthor Dennis Diener, reprinting micrographs from photo negatives. With the sun rising, I had just enough time to catch an early morning train to Boston. As the Amtrak train swayed out of New Haven station I heard *the* voice barking, "Kozminski, Wake up!" It was no dream. Rosenbaum was in the aisle, talking about tubulin acetylation and my need for dissertation plan B. In serious outside voice, he said, "You need to look at Bloodgood's Balls." I concurred. Heads turned. There was certainly no better conversation on which to eavesdrop that morning.

Chlamydomonas flagella are an outstanding model for substrate–cell surface interactions. This became clear in 1977, when Robert Bloodgood, then a postdoc in the Rosenbaum lab, discovered a novel flagellar motility that is independent of flagellar beating. He found that polystyrene balls attached to the surface of a flagellum move in a rapid, bidirectional, saltatory manner (Bloodgood, 1977). This ball movement is thought to be a manifestation of whole-cell gliding motility, which occurs when *Chlamydomonas* cells move along a substrate via their flagella, in a manner completely independent of flagellar beating. To this day, the mechanism of whole-cell gliding is not fully known and remains a very ripe area for research in cell signaling at cell surface–substrate interfaces.

My question that morning on Amtrak was, "What are we looking for?" Rosenbaum wanted me to find the mechanism driving ball movement on the flagellar surface by using a permeabilized cell model. He made the pitch, telling me about earlier studies on dynein reactivation. I countered, saying we should look for kinesins within the flagellum, because ball movement is bidirectional and dyneins, which seemed well studied at the time, may only be applicable to motility in one direction. I recall that my favoritism of kinesin over dynein was only because kinesin was a relatively new discovery and hence "cool." Rosenbaum liked my kinesin idea and launched into a 60-mile explanation of how cilia/flagella are the same as neurons; that is, if kinesin was found in axoplasm, it will be found in a flagellum. Sixty miles on Amtrak is a long time. That was it—after the winter holidays, I was to search for the flagellar

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Abbreviations used: DIC, differential interference contrast; IFT, intraflagellar transport.
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The new year brought a new discussion. On returning from the holidays, Rosenbaum and Mark Mooseker, also on my dissertation committee, pushed me hard to work on radial spoke assembly. Spokes are the protein complexes that extend from the central pair of microtubules of the axoneme toward the outer doublet microtubules. In Petrine style, I refused three times. I said, "Spokes are *boring*," relative to molecular motors. In the end, I prevailed, and Rosenbaum permitted me to explore, at least for a short time, flagellar surface motility, as we had discussed on the train to Boston. I was extremely fortunate to have an advisor who allowed explorative forays. It made science extra fun. And, so the record is clear, Rosenbaum was not wrong in his push for radial spoke research. Spokes have emerged at the center of some very interesting cellular and evolutionary biology (Pigino *et al.*, 2011; Barber *et al.*, 2012).

Having stood my ground with Rosenbaum and still able to draw breath, I, at his direction, started working with Paul Forscher, a new assistant professor on our floor. Using high-end optics, Forscher studied polystyrene ball movement on the surface of *Aplysia* growth cones—inductopodia formation. The collaboration made sense on many levels. I had a chance to learn high-resolution video microscopy and image analysis; cilia equal neurons; and inductopodia are studied in perfusion chambers. Reactivation studies required good perfusion chambers. So, in January 1992, I started my optical training with Forscher. It was not an auspicious start. On my first day, I dropped Forscher's never-used, just-out-of-the-box Nuvicon video camera on the floor. As the camera rested after a second bounce, Forscher turned beet red. Forscher's patience with me that day was key to my success. I was extremely fortunate to have in Paul Forscher a teacher willing to give me a second chance. After optical training and setting up a high-resolution, video-enhanced, differential interference contrast (DIC) microscope, I was ready to look at *Chlamydomonas*.

January 24, 1992, brought the first observation of IFT. Paul Forscher and I were working with *pf1* cells, a paralyzed flagella mutant of *Chlamydomonas*. Immotility has obvious advantages for microscopic observations. With these immotile cells, we first saw, based on my recollection, membrane blebbing (snoodling) from the tips of flagella. We did not pay much attention to this phenomenon, though retrospectively one might wonder whether these packets of membrane, released from the flagellar tip, function like exosomes. We also saw, based on my notes, flagella that were bulbous at one point along their length. This in itself was not a discovery, because it was known that the flagellar membrane swells locally prior to stress-induced excision, for example, when cells are placed under intense light such as the light we were using. The unexpected observation was the occasional visualization of lumps (birefringent pulses or particles), smaller than the aforementioned membrane bulge, moving rapidly along the length of the flagellum to its tip. Forscher asked me to identify the rapidly moving lumps. I said, "We are the first people to ever see them." I do not have videotape of that first cell, but I logged the following in my notebook that day:

> N.B. Cells in M_I medium looked great. Observed movement <u>within</u> flagellum. Birefringent pulses travelling from cell body end to distal end. ~0.5–0.25 µm big. rate ~10 µm/s. Several in rapid succession. Like intracellular transport, perhaps it could be membrane rippling.

I found Rosenbaum and told him I saw things moving in the flagellum. He gave me an indescribable look and then said, "Optical artifact." That was not an unreasonable response at all because decades of electron microscopy had not revealed (and has not yet revealed) vesicles within flagella. Thus the idea of an intracellular transport system akin to molecular motor–driven vesicle movements seemed far-fetched. In the end, Rosenbaum gave me the benefit of the doubt and took a look for himself. He saw the movement immediately and brought in colleagues to look as well. Once again, cutting-edge microscopy techniques opened a new window into the cell. Previous light microscopy observations of flagella showed them as featureless, high-contrast rods. Here, with video-enhanced DIC, a fiber-optic scrambler to provide full and even illumination of the condenser aperture, and flagella oriented parallel to the DIC shear axis to reduce contrast, we were seeing much more (Figure 1), though without a clear idea of what we were viewing. Because of either scheduling or computer problems, we did not take another look until January 31, 1992. On that day, my notebook entry took a less dispassionate tone:

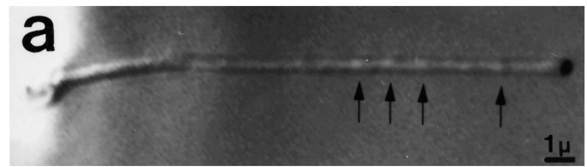

FIGURE 1: Digital, video-enhanced DIC image of IFT in a *Chlamydomonas moewusii* flagellum. Although IFT was first discovered in the flagella of *C. reinhardtii*, *C. moewusii* was favored for microscopic observations, because it has longer flagella than *C. reinhardtii*. The increased distance from the highly refractive cell body (white area on left) allowed for an easier visualization of IFT particles along the length of a flagellum (arrows). Reprinted with permission from Kozminski *et al.*, 1993b. ©1993 National Academy of Sciences, U.S.A.

> In Paul's lab, looked at pf1 63X oil lens … used fiber optic scrambler, green light. Saw movement in the flagellum as noted earlier, anterograde. However, this time also saw retrograde transport!!! <u>Wow</u>.

This observation of retrograde (tip-to-base) transport within flagella completed the picture, giving mechanism to the flagellar elevator hypothesis. For years, it was not known with 100% certainty whether cilia and flagella grew by the addition of axonemal subunits at their base, closest to the cell body, or from their distal tip or both, though an early experiment with *Ochromona*s by Rosenbaum and Child (1967) showed a tip growth zone. The most conclusive study came years later, when Karl Johnson, a postdoc in the Rosenbaum lab, performed a dikaryon rescue experiment with an epitope-tagged tubulin strain of *Chlamydomonas* that I made for my tubulin acetylation studies. In his classic experiment, Johnson clearly showed that tubulin assembles onto the distal end of the axoneme (Johnson and Rosenbaum, 1992). The question that remained was, "How does tubulin or any other protein reach the distal tip of the axoneme, which can be up to 10–12 µm from the cell body, the site of flagellar protein synthesis?" Diffusion was calculated to be too slow to support the known rate of axonemal assembly during flagellar outgrowth. One hypothesis, offered from earlier conversations between George Palade and Rosenbaum, was that cilia and flagella have an active transport mechanism—that is, "elevators"—to bring tubulin, radial spokes, dynein, and other axonemal precursors to the flagellar tip.

With candidate flagellar elevators in view, Rosenbaum christened the bidirectional movement of particles within the flagellum the

"new motility." I cringed. "New" is a relative term. Later, when writing our report, I proposed the name intraflagellar transport (abbreviated IFT), though at the time there was no solid evidence that this motility was transporting anything. Our model was that the IFT particles observed moving by light microscopy were the electron-dense, nonvesicular complexes Karl Johnson rediscovered, with electron microscopy, between the axoneme and flagellar membrane (Kozminski *et al.*, 1993b). Only in a later correlative light–electon microscopy experiment did Peter Beech and I show this model to be accurate (Kozminski *et al.*, 1995).

Name did not matter. *Nature* soundly trounced our manuscript because our story could not be published "in the absence of a complete definition of the movement and its function in the flagellum." A quite unfair assessment, considering at the time there were no published reports of nonmuscle motility systems in which the cargo of a specific motor protein was known. Here, in an organism with tractable genetics, we offered the ability to quantify motor activity in vivo by direct microscopic observation. Our determination that IFT is independent of ball movement on the flagellar surface was insufficient as well. One reviewer even challenged the observation with the suggestion that I was watching a video Moiré pattern. Harsh is the review that suggests psychological effect or defect. Prepublication inquiries elsewhere yielded similar declinations, as did the search for a National Academy member to communicate the paper in the Academy's *Proceedings*. Saving the day, Hewson Swift, a National Academy member and cofounder of the American Society of Cell Biology, agreed to communicate the paper to the *Proceedings of the National Academy of Sciences* upon completion of a positive and thorough peer review (Kozminski *et al.*, 1993b).

Has *Nature*'s request been fulfilled? Do we know whether IFT has broad-based biological relevance? Do we know the function of IFT, the motors driving IFT, and the cargoes carried? Yes, but the answers to these specific questions did not come soon (Kozminski *et al.*, 1995; Cole *et al.*, 1998; Pazour *et al.*, 1998; Signor *et al.*, 1999; Qin *et al.*, 2004; Pan and Snell, 2005; Lechtreck *et al.*, 2009). Although the discovery of IFT focused on flagella, the research has had, in my opinion, the greatest impact on primary cilia (Bloodgood, 2009). Building on Douglas Cole's subsequent isolation and biochemical characterization of IFT particles from *Chlamydomonas* (Cole *et al.*, 1998), studies with mammals showed that loss of specific IFT polypeptides blocks the formation and function of cilia, resulting in profound developmental defects and diseased organs (e.g., degenerating retina, polycystic kidneys; Pazour *et al.*, 2000, 2002). Thus the discovery of IFT set the cornerstone for a new appreciation of cilia as antennae that sense fluid flow, fluid pressure, or ligands that facilitate intercellular signaling. The functioning of the Hedgehog signaling pathway, for example, depends on primary cilia in most cases (Goetz and Anderson, 2010). Now in a renaissance, primary cilium research is aimed at linking specific molecular defects in this organelle to a host of human ciliopathies and cell cycle perturbations (Hildebrandt *et al.*, 2011). Clearly *Chlamydomonas* taught us a lot, and I suspect has much yet to teach us. History once again shows that model organisms—even "pond scum"—are essential for an efficient and economical understanding of the causes of human disease.

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