Onward from the cradle

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ABSTRACT **This essay records a voyage of discovery from the "cradle of cell biology" to the present, focused on the biology of the oldest known cell organelle, the cilium. In the "romper room" of cilia and microtubule (MT) biology, the sliding MT hypothesis of ciliary motility was born. From the "summer of love," students and colleagues joined the journey to test switch-point mechanisms of motility. In the new century, interest in nonmotile (primary) cilia, never lost from the cradle, was rekindled, leading to discoveries relating ciliogenesis to autophagy and hypotheses of how molecules cross ciliary necklace barriers for cell signaling.**

How lucky to be there at the beginning! The Rockefeller Institute for Medical Research began its graduate program in 1955, and I was accepted into the program in 1956, when Keith Porter and George Palade, just promoted to members (i.e., professors), were first accepting students into what Palade later referred to as the "cradle" of cell biology (Moberg, 2012). Every day was an adventure into the new fine structure of the cell revealed by the transmission electron microscope (TEM), when everyone in the laboratory gathered at teatime to see the newest images hot off the drier and to try to decipher what they meant in terms of organelle structure and function. In 1960, the American Society for Cell Biology was born. As a student completing my Ph.D. with Porter, I was encouraged by him to join the society, subscribe to the *Journal of Biophysical Biochemical Cytology*, soon to be the *Journal of Cell Biology*—the journal of the ASCB before *Molecular Biology of the Cell*—and to consider presenting an abstract at the first meeting. I took his advice.

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The Rockefeller Institute graduate program had a special feature: to illustrate the international nature of the scientific endeavor, students were encouraged to spend a year abroad. In 1958–1959, I chose to work in the laboratory of Eric Zeuthen, one of the first cell biologists, in Denmark—a choice that was to influence my life profoundly, since that is where and when I met Birgit Hegner, my partner in life. When I returned to New York, I began my thesis work in earnest. I wanted to learn how cilia, the oldest known cell organelle, moved. Porter had done pioneering work on the TEM of 9+2 motile and modified nonmotile 9+0 cilia. I've told the story of my thesis discovery—fixation of the metachronal wave of mussel gill cilia—and some of the consequences of that discovery elsewhere (Satir, 2010; Moberg, 2012).

By the autumn of 1961, impatient to start my own laboratory, I had left the cradle to become an instructor in biology and zoology at the University of Chicago. I chose that position, in part, because Frank Child, one of the first people to seriously work on the molecular biology of cilia, was also a young faculty member in the department. In the following years, the University of Chicago did indeed become, if not the cradle, certainly the "romper room" of cilia and microtubule (MT) biology. In addition to Frank, Birgit, and me, the following years saw Sid Tamm, Gary Borisy, Joel Rosenbaum, David Phillips, and eventually Fred Warner studying cilia, while across the street in biophysics, our colleague Ed Taylor and his group began working on the structure of MTs.

By 1967, when I had finally figured out that the fixed metachronal wave showed cilia whose tip patterns varied with beat stage, and I was beginning to study serial sections to show that the patterns were consistent with a sliding MT hypothesis of ciliary motion, I was recruited to the Department of Physiology–Anatomy at the University of California–Berkeley. Birgit and I with our two young children

DOI:10.1091/mbc.E14-05-1014

Peter Satir is corecipient of the 2014 E. B. Wilson Medal from the American Society for Cell Biology.

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Abbreviations used: CLEM, correlated light and electron microscopy; GAS, growth arrest–specific; MT, microtubule; TEM, transmission electron microscope.

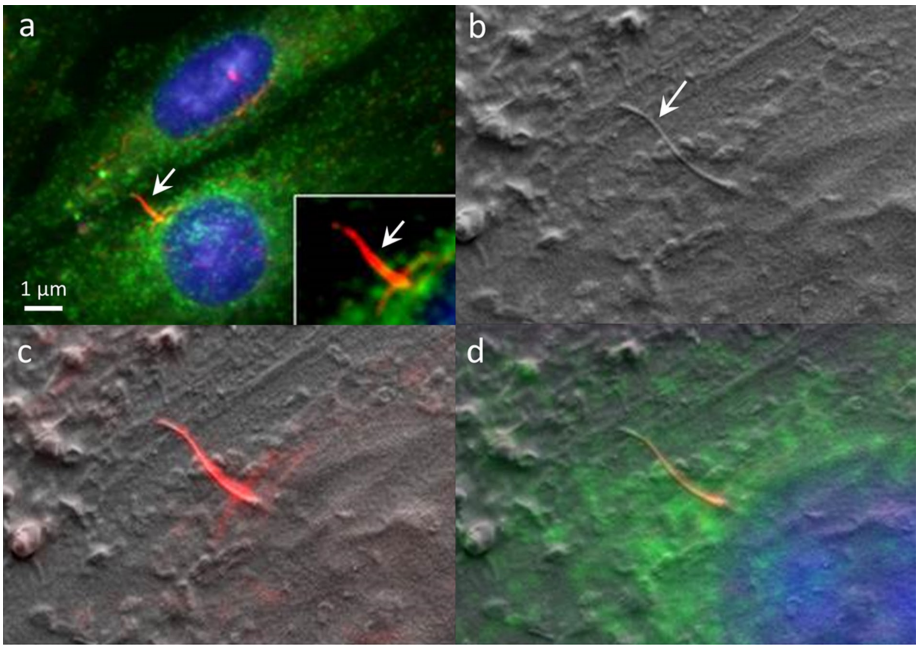

FIGURE 1: (a) An MEF primary cilium (arrows, inset) labeled in IFM for localization of acetylated α-tubulin (red), 4′,6-diamidino-2-phenylindole (blue), and clathrin (green). (b) SEM and (c and d) CLEM of the same specimen. At the base of the cilium, a ciliary pocket is surrounded by clathrin-coated vesicles. (From J. Kolstrup, Thesis, University of Copenhagen [2012], with permission.)

welcomed the move. It was the "summer of love" and the times they were a-changin! Again lucky, we were the first couple to break the nepotism rule at UC–Berkeley and were allowed to work independently in the same department.

I began to attract graduate and postdoctoral students. My first postdoctoral student was Fred Warner, who rejoined the lab after completing his Ph.D. in Chicago. The first graduate student to complete a degree with me was Norton B. (Bernie) Gilula. While with me, dodging gas canisters from helicopters and National Guardsmen with fixed bayonets (signs of the protest against the Vietnam War that shook the campus), these people became extraordinary electron microscopists whose images, some of which we published together (Gilula and Satir, 1971, 1972; Warner and Satir, 1973, 1974), remain classic.

At Berkeley, freeze fracture, a new technique to study cell membranes, was being used by Dan Branton in the botany department. A description of the structure of mussel gill membrane junctions with the new technique would provide a thesis for Bernie Gilula. So I asked Dan to teach Bernie the technique. With freeze fracture, Bernie discovered that the mussel gill cells had true gap junctions (Gilula and Satir, 1971) and at the base of the ciliary membrane, Bernie and I (Gilula and Satir, 1972) described the ciliary necklace in both motile and primary cilia, a structure that has come back into fashion 30-odd years later.

Meanwhile, the sliding MT hypothesis was receiving definitive proof (Summers and Gibbons, 1971). The next graduate student caught in the ciliary web was Win Sale; I asked him to do the near impossible, to take the Summers and Gibbons results to TEM resolution, which might demonstrate how the dynein arms worked, The images from Sale and Satir (1977) show that axonemal dynein functions as a minus-end motor, in that active arms on one doublet (N) push the adjacent doublet (N+1) tipward during active sliding. For the first time, we could visualize the arms along the doublets in negative stain. These findings led to two new ideas: 1) that we could study the mechanochemistry of dynein by looking at changes in dynein arm structure in different activity states (Satir *et al.*, 1981) and 2) that all arms couldn't be active at once during a ciliary beat. As seen in the fixed metachronal wave, arms were switched off across about half of the axoneme, where doublet N+1 was found basal to doublet N at the ciliary tip, which later led to the switch-point hypothesis (Satir 1985). Differential sliding activity of the doublet MTs was demonstrated in different beat stages, corresponding to "hands up" and "hands down" cilia (Satir and Matsuoka, 1989), wherein switching depends in some part on a central pair projection of hydin (Lechtrack and Witman, 2007).

In 1977, I was invited to take the chair of the Department of Anatomy at the Albert Einstein College of Medicine. Birgit became a tenured professor. We led the department, eventually called the Department of Anatomy and Structural Biology, for 24 years. My laboratory remained small and mostly focused on cilia. A partial list of students and colleagues who worked or published with me at Einstein while I was chair includes Alastair Stuart, Ellen R. Dirksen, Michael Holwill, Tim Bradley, Marika Walter, Jeff Salisbury, John Condeelis, Tim Otter, Michael Melkonian, Michael Sanderson, Phyllis Novikoff, Allan Wolkoff, Toshikazu Hamasaki, Yuuko Wada, and Søren T. Christensen.

As cell biology transformed into molecular cell biology during this period, the complexity of ciliary structure and biochemistry was growing, new genetic and cloning techniques for studying cilia and MT molecular motors (dyneins and kinesins) were evolving. My fellow awardee, John Heuser, made a discovery crucial to dynein structure and function (Goodenough and Heuser, 1982).

In *Chlamydomonas*, a panel of swimming mutants showed that the inner dynein arms of the cilium are mainly responsible for bend amplitude and form, while the outer dynein arms control beat frequency (Brokaw and Kamiya, 1987). We (Satir *et al.*, 1993) were able to demonstrate in ciliates that cAMP phosphorylation of a small protein related to the outer arm led to faster swimming and therefore faster ciliary beat because of an increase in sliding velocity, demonstrated in vitro. It is likely that faster sliding of the inner dynein arms in vitro (Wirschell *et al.*, 2011) is the equivalent of greater bend amplitude, but the biophysics here is more complicated, and the demonstration remains incomplete.

Stepping down from the chair in 2001 was a new beginning that more or less coincided with the rediscovery of the importance of the primary cilium (Pazour *et al.*, 2000) and the growing recognition of the role of intraflagellar transport in normal ciliary growth and function (Rosenbaum and Witman, 2002). After a brief excursion into nanotechnology (e.g., Seetharum *et al.*, 2006; Bachand *et al.*, 2009), in close collaboration with Søren T. Christensen's new laboratory in Copenhagen, I began to study signaling in primary cilia.

From the work of Tucker *et al.* (1979), we knew that primary cilia grew when cultured fibroblasts were starved and went into growth arrest (G_0), so initially we examined the literature for growth

arrest–specific (GAS) genes. We discovered that PDGFRα was known to be encoded by a GAS gene (Lih *et al.*, 1996), and shortly thereafter we were able to show that PDGFRα localized to and signaled exclusively from the primary cilium (Schneider *et al.*, 2005). We were later able to show that this signal could be translated into chemotaxis (Schneider *et al.*, 2009, 2010) and cytoskeletal and membrane reorganization (Clement *et al.*, 2012). This collaboration led to an exchange of students and visits, culminating in a return sabbatical in 2012–2013 for me and Birgit at the Department of Biology, University of Copenhagen, supported by the Lundbeck Foundation. It was a time to rekindle old memories and friendships and to make new ones.

Søren and I and our laboratories made several other discoveries related to primary cilia. We showed that primary cilia with Hedgehog signaling were present on human embryonic stem cells (Kiprilov *et al.*, 2008). We also introduced a new technique, correlated light and electron microscopy (CLEM), for the study of primary cilia (Figure 1; Christensen *et al.*, 2013). Recently we have been formulating new hypotheses concerning how molecules cross the ciliary necklace barriers.

In a further development, Birgit and I noticed that starvation upregulated autophagy with about the same time course as ciliogenesis. Together with Ana Maria Cuervo and her laboratory, we showed a reciprocal relationship between the two processes—where cilia growth up-regulates autophagy, which eventually shuts down growth (Pampliega *et al.*, 2013).

When you have a good and stable childhood, you are buffered from the vicissitudes of later life. So it has been in cell biology for me: the lessons from the cradle have not been lost. But Porter and Palade knew that cell biology had a longer history, in which one of the heroes was E. B. Wilson. Together with Dan Mazia, Porter and Palade were recipients of the first E. B. Wilson award of the ASCB. I am very proud to follow in their footsteps.

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