Profile of Martin Chalfie

Despite having a bad reputation, cholesterol is an essential component of the plasma membranes of animal cells, where it is thought to modulate the properties of the lipid bilayer. Cholesterol can also bind directly to proteins in the membrane. In his Inaugural Article published in 2006, Martin Chalfie, the William R. Kenan, Jr., Professor of Biological Sciences at Columbia University (New York), in collaboration with Thomas Benzing (University of Cologne, Germany), identified a new class of cholesterol-binding proteins among the prohibitin (PHB)-domain protein family (1). PHB-domain proteins appear to regulate a variety of membrane functions, from cell signaling to mechanosensation. Studying two members of the family, MEC-2 and Podocin, Chalfie, Benzing, and their colleagues found that cholesterol is crucial for the activity of two different classes of channel proteins to which the PHB-domain proteins bind. They suggest that the binding of cholesterol by the PHB-proteins alters the local lipid environment of associated membrane proteins and changes their activity.

Martin Chalfie

Questioning Research

Chalfie, born in 1947 and elected to the National Academy of Sciences in 2004, grew up in Chicago. As a child, all elements of science interested him, but he feels his early activities were somewhat mundane. "Unfortunately, I did not have that real indicator of a career in science that many of my friends have. I did not make explosives and almost destroy my home," he says. As a child, he cut out newspaper comics about nature for a scrapbook and, in high school, participated in a weekly science club after school. "I was fairly good at science in school," he recalls. "That was the positive reinforcement to keep me going." Chalfie entered Harvard University (Cambridge, MA) in 1965 and thought that he would major in math. He soon switched gears. "I was attracted to biochemistry because I could do a little bit of everything: chemistry, math, and biology," he explains. "The subject also seemed new and exciting."

During his junior year, Chalfie took a cell physiology class with Woody Hastings, but Chalfie could not register for the laboratory portion and wrote a paper instead. The subject, the role of cyclic AMP (cAMP) in sodium transport in the toad bladder, would later spark an idea that led to Chalfie's first published research article. In the meantime, Hastings provided Chalfie with one of his fondest memories of Harvard. "I never seemed to be able to get to the biology library when it was open, so I asked Woody for permission to get a library key for late night reading," he recalls. "Most of my professors seemed very distant, so I was amazed when he got up from his desk, walked down four flights of stairs to the library office, and said, 'Give this boy a key.' I've since learned that this kindness was characteristic of him."

Chalfie spent the summer after his junior year working in the laboratory of Klaus Weber at Harvard. Chalfie set out to study the active site of aspartate transcarbamylase, but "although I kept trying to do the experiments, I failed miserably all summer," Chalfie says. "I decided I shouldn't be in science." So for his senior year, Chalfie took the last required course for the biochemistry major and then other courses that interested him, including law, theater, and Russian literature.

After graduating in 1969 and still unconvinced about a career in research, Chalfie took a series of short-term jobs, including a stint selling dresses for his parents' dress manufacturing business in Chicago. In 1970 he began teaching high school at Hamden Hall Country Day School (Hamden, CT).

Chalfie took the advice of a fellow teacher and applied to work in the laboratory of Jose Zadunaisky at Yale University (New Haven, CT) during the summer of 1971. During the initial interview, Zadunaisky told Chalfie about his work measuring chloride transport in the frog cornea by using an Ussing chamber. "I thought about the paper I had written for Woody Hastings, and forgetting that that research involved sodium, not chloride, transport, toad and not frog, and bladder and not cornea—although it did measure transport with a Ussing chamber—I tried to impress Jose by asking if cAMP was involved," Chalfie recalls. "He liked the

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question but assigned me a completely different project. Jose then left to do research for the summer in France." Chalfie recalls, "Enamored with my own idea and left on my own, I didn't do the assigned project but tried to see if increasing cAMP affected the current. Fortunately, it did so dramatically, and Jose was very supportive when he found out what I had done at the end of the summer." This research would garner Chalfie his first publication (2) and give him the confidence to consider a career in biology.

Insensitive Worms

For his doctoral work, Chalfie entered Harvard University's physiology department in 1972, where he chose Robert Perlman, "who still remains a terrific friend," as his thesis advisor. "Bob was very patient and willing to listen to all of my unformed and misinformed ideas. He was also a clear and deep thinker. He was soft-spoken, but what he said was important and thoroughly considered," recalls Chalfie.

For his thesis, Chalfie used cell suspensions from rat pheochromocytomas (adrenal tumors) to look at the biosynthesis and release of catecholamines (3). Chalfie initially looked for postdoctoral positions in the same field for his graduate work but soon decided that he wanted to do something different. A visit from high school friend Bob Horvitz, who was doing a postdoc with Sydney Brenner at the MRC Laboratory of Molecular Biology (Cambridge, U.K.), prompted Chalfie to apply to Brenner's laboratory.

In 1977, Chalfie traveled to England to work with Brenner. Although intending to study neurotransmitters in *Caenorhabditis elegans*, Chalfie instead took up a project started by John Sulston, then a staff scientist in the laboratory, on the genes involved in mechanosensation.

Sulston had earlier found that some *C. elegans* mutants are unable to respond to touch (4). Working with Sulston, Chalfie began studying these mutants and the cells that sensed touch; together they identified the first collection of genes needed for touch sensitivity (5). "These genes have been keeping me busy ever since," Chalfie says. One discovery, however, came unexpectedly. "The first real skill that I used in my postdoc was my ability to count," he remembers. The touch-sensing cells were easily identified in electron micrograph because their processes were filled with prominent microtubules. One day, Chalfie decided to study the cells in the serial sections of the worm that were being used to reconstruct the animal's neural anatomy. He felt he should look at "his cells" as part of his research but was not sure what exactly to watch. At a loss, he decided to count the number of microtubules in each section. Instead of decreasing the further from the cell body (as would be expected if microtubules started in the cell body), the count "fluctuated all over the place." Nichol Thomson helped Chalfie acquire more sections, and Chalfie soon showed that, contrary to prevailing theory, the microtubules did not span the entire length of the nerve process but started and stopped along the way (6).

Chalfie relished his time at the laboratory. "My time as a postdoc made me the scientist I am today," he says. "The complete freedom, terrific colleagues, and extensive material support that were characteristic of the LMB were very stimulating. Everything was provided; so much so that you had no excuse for not being able to work except your own limitations." After five years in England, Chalfie left the laboratory in 1982. He joined the faculty of Columbia University in the department of biological sciences and continued to study *C. elegans* touch mutants. He found that some mutants were insensitive to touch because the cells were missing or incompletely differentiated, a result that led to the study of how cell type is determined (7–9). Other touch-insensitive mutants have fully formed but nonfunctional cells. These mutants led to investigations into the molecular basis of mechanosensory transduction (e.g., refs. 10–14).

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During the 1980s and 1990s, Chalfie and his laboratory studied the molecular biology of the touch genes. Convincing evidence that some of these genes encoded components of a transduction complex, however, did not arise until 2005 (14). At that time, one of his graduate students, Bob O'Hagan, working with Miriam Goodman, a former postdoc who had established her own laboratory at Stanford University (Palo Alto, CA), recorded electrical responses from the touch-sensing cells of wild-type and mutant *C. elegans*. The loss of some touch genes abolished the electrical response to touch, confirming that the genes were necessary for the touch response. Even more exciting to Chalfie, particular missense mutations in channel genes altered the nature of the electrical response (14). "The absence of the mechanosensory current could have occurred for many reasons, but the alteration of the properties of the current suggests that the channel proteins were directly involved in transduction," he explains. Chalfie is not done with his studies of mechanosensation. "Now that we have good evidence that we have identified the transduction complex, the biggest problem lies ahead: How does this complex translate touch into an electrical response?"

An Enjoyable Collaboration

For his Inaugural Article (1), Chalfie studied MEC-2, a protein associated with the transduction complex needed for touch sensitivity. Chalfie's laboratory had found that MEC-2, which is associated with the inner leaflet of the plasma membrane, greatly increases the current from activated touch channel proteins in frog oocytes (12, 15). MEC-2 is a PHB-domain protein; other PHB-proteins also appear to regulate membrane functions.

Chalfie recalls that in 2004, he received a call from Benzing, who was studying a similar PHB-domain protein, called Podocin, which is found in mammalian kidneys. Benzing suggested that they collaborate to study how these proteins regulate activity. Together, they found that MEC-2 and Podocin share three properties: Both proteins bind to target proteins in membranes, to themselves, and to cholesterol. Chalfie explains that multimerization and the binding to cholesterol were significant because these events could "change the lipid environment around target proteins."

The activity of the different channel proteins that bind MEC-2 and Podocin could thus be modified by the presence of these PHB-domain proteins (1).

Chalfie calls his collaboration with Benzing "one of the most enjoyable I have had with anyone." The two continue to work on questions raised in the Inaugural Article, such as whether these proteins mediate effects of steroids, which are cholesterol derivatives, at membranes. Typically, steroids are thought to act by influencing transcription, but some of their rapid effects may be independent of transcription. Benzing and Chalfie are still studying whether PHB-domain proteins mediate these nongenomic effects. Chalfie explains that anecdotal evidence, including the finding that defects in human Podocin result in steroid-resistant nephrotic disease, hint that PHB-domain proteins indeed could regulate membrane function by binding steroids.

Glow Worms

In 1994, Chalfie published what he considers his most influential paper,

which describes the first use of the now-ubiquitous green fluorescent protein (GFP) as a reporter of gene expression (16). Chalfie first heard about GFP in 1988 at a seminar given by Paul Brehm, who now is at State University of New York (SUNY)-Stony Brook. At the time, Chalfie's laboratory used lacZ fusions to localize gene activity, and he thought, "What an incredible tool this would be, especially in *C. elegans*, which, we never tire of telling people, is transparent." Chalfie found that Doug Prasher at Woods Hole Oceanographic Institute (Woods Hole, MA) was attempting to clone the GFP cDNA from the jellyfish *Aequorea victoria*. The two brainstormed about what they could do with the full-length cDNA and agreed to keep in touch. Chalfie then married Tulle Hazelrigg and joined her at the University of Utah (Salt Lake City, UT) for a sabbatical in 1989. During that time, unbeknownst to Chalfie, Prasher completed the sequence and unsuccessfully tried to contact him. Not until 1992, while searching a publications database, did Chalfie discover Prasher's publication of the GFP sequence (17). He got in touch with Prasher, and they reestablished their collaboration.

Chalfie acquired the GFP cDNA and Ghia Euschirken, a graduate student, made a construct to express the protein in *E. coli*. When he started this work, Chalfie recalls that researchers knew that the fluorescent protein formed from a modification of the polypeptide chain but had no added fluorophore.

At the time, he explains, they did not know how the modification was made; many thought that GFP might require one or more enzymes to affect the changes. Chalfie gambled on the possibility that GFP needed no other jellyfish protein, and, "when Ghia's *coli* glowed green, we knew we were right." Subsequently, they put GFP into *C. elegans*, and GFP's life as a biomarker began.

Although the paper focused on GFP as a promoter-driven construct, it mentioned that GFP could be conjugated to a protein, and Hazelrigg, by then also on the faculty at Columbia, soon showed that to be the case (16, 18).

"These papers were the beginning of the development of an ever-increasing number of applications of GFP and other fluorescent proteins, most of which I would never have imagined. I am amazed and proud at what has happened with GFP." Chalfie is also delighted with the directions his work has taken over the years, such as studies of neurodegeneration and the development of combinatorial methods to label and kill cells. Still, he is quick to credit others, "In large part, the lab's success is due to students, postdocs, and collaborators who have pushed the work along."

Tinsley Davis, *Freelance Science Writer*

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