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Influences: Childhood, boyhood, and youth

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Peering into the fog of time through memory's astigmatic lens, I can cubbyhole my scientific embryogenesis into three stages: childhood in graduate school, boyhood in postdoc research, and youth in my first few years at Brandeis. As an undergraduate physics major who had avoided biology classes even in high school, I signed up in my senior year for a course called something like "Biophysics for Biological Dummies." That class would change my life. Each week we'd read papers by a scientist in the Philadelphia area and then visit that scientist to discuss the papers we'd studied. I became enchanted with our visit to Gilbert Ling, whose contrarian schtick dismissed the "membrane theory"—the idea that cells maintain their ion gradients by energy-consuming ion pumps. Instead, he asserted, cell membranes don't exist, and the gradients reflect ion binding to protein groups and low ion solubility in a "special" water with anomalous physical properties dictated by the crowded cellular milieu.

Ling lavished quality time on us during our five-hour visit to his laboratory, arguing eloquently from his experiments that membrane pumps egregiously violate the laws of thermodynamics—and that did it for me, a bio-ignoramus arrogantly imagining myself a physicist fox set among the biologist chickens (a phenotype still recognizable in some physicists transitioning to biological applications). Ling's heresy also tickled the lèse-majesté impulses of a student in the turbulent '60s, channeling them away from politics into a harmless corner of physiology. After that visit, I dreamed of going to grad school to help Ling overthrow the "membrane establishment." In 1969, after a year of teaching high school math, I joined Ling's laboratory as a PhD student at Penn. (I was rejected from most of the graduate programs I applied to, and think now how different my career would have been had I accepted my only other offer, Yale's, into Fred Richards' new hardcore structural biology program with its then-young faculty including Peter Moore, Tom and Joan Steitz, and Don Engelman.)

Childhood: University of Pennsylvania, 1969–1974

My graduate education was a slow process of picking my way out of Ling's intricate system of thought. Early on, designing what I imagined would be a suite of crucial experiments to decide between the membrane theory and Ling's ideas, I plunged into several years of thesis work on sugar transport in mouse muscle. (I once brought home for dinner some mouse livers from my day's dissections, to my wife's disgust and my dog's delight.) As so often in research, collaboration was essential; I survived Ling's no-membrane nonsense thanks to two other students in the laboratory, Jeff Freedman and Larry Palmer, also physics-trained Ling acolytes, ignorant of matters biological. We rescued a discarded blackboard off the streets of West Philly and met weekly in our apartments for subversive nighttime seminars to read the literature (something our adviser had advised us against, as it would only confuse us). Slowly, slowly, we emerged from Ling's worldview to see that, despite its self-consistency, it just didn't mesh with the facts outside our laboratory bubble.

Gilbert Ling, circa 1965. Photo from Wikimedia.

Ling refused to sign my thesis, which claimed to refute his ideas, and he boycotted my public defense, precipitating a hilarious last-minute slapstick scene in which my depleted committee frantically commandeered a hapless passerby to "just sit there" to make up a quorum so they could get rid of me, PhD in hand. I hasten to add that, despite the personal pain Ling suffered at my "disloyalty," he never used his power to kick me out of his laboratory (as was his right; academic science retains the very best

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Efraim Racker. Photo courtesy of the author.

elements of feudalism). I remain grateful to him for tolerating what must have been emotionally taxing: the daily presence in his laboratory of a traitor.

Ling had a tremendous, abiding influence on me. He was a broad intellectual who wove music, literature, and Chinese cooking into the laboratory's buzz, and who taught us how to critically dismember research papers (of his opponents). He was a kind man with a fine sense of humor, high integrity, and an infectious passion for research, and he was a skilled experimentalist who set a lasting example by working in the laboratory side by side with his students. But he was a tragic figure, his wealth of professional virtues nullified by rigid attachment to theory, a violation of the first commandment of science: when Nature speaks, you'd better listen. He became the scientific analogue of a religious fanatic and continues today in his mid-'90s, a self-proclaimed revolutionary (http://www.gilbertling.org). I reckon that his greatest influence on me was to instill, along with a bizarre fascination with small inorganic ions, a profound aversion to becoming emotionally attached to my own ideas.

Throughout this time, I had grown fascinated with ion channels from reading papers on the single-molecule stochastic behavior observed with certain bacterial peptides added to "planar bilayer" membranes (1, 2), whose teraohm leak resistance made such measurements feasible. Paul Mueller, a master of electronics tinkering, and whose nearby laboratory I'd also visited while in that undergraduate biophysics class, had invented planar bilayers in the early '60s (3). During my last few months at Penn, I asked Paul to teach me the technique, and he let me tinker along with him. There, I fell in love with those "artificial" membranes whose existence nobody, not even Ling, could deny. That summer was a golden time; I'd ride my motorbike up to Paul's laboratory to play with bilayers in the morning and then return by noon to watch, spellbound, Sam Ervin's Watergate hearings on TV, and in the evening write up thesis chapters and manuscripts (single author, because they argued against Ling's theory [4, 5]).

Boyhood: Cornell, 1974–1976

As summer became winter and spring, I wrote a postdoctoral grant proposal to work with Efraim Racker at Cornell. In a brilliant experimental flash of Gordian knot cutting (6), Ef had engineered reconstituted membranes to disprove the reigning idea of chemical coupling in mitochondrial ATP synthesis, thereby ensuring Peter Mitchell's Nobel Prize for his heresy that proton gradients thermodynamically drive oxidative phosphorylation. My postdoc interview had been unpromising; after I'd explained my odd situation as a born-again membrane researcher, Ef probed me with an arcane question about nucleotide metabolism, a subject about which I understood little. Downhearted, I confessed that I had no clue what his question even meant. For what seemed like minutes, Ef silently contemplated the carpet in his office with his characteristic frown, and then looked up and said: "Well, if I don't accept you, you are lost forever." To this day, I am sure he was right about that.

Though substantively idiotic in retrospect, my postdoctoral grant proposal to reconstitute the Ca^{2+} ATPase of SR into planar bilayers, where I'd measure its electrical properties, was funded. I was elated to move to Cornell (and not at all unhappy at the rise in yearly stipend from $2,400 to $12,000, a $6,000 check appearing biannually in the mail). Ef was an amazing adviser who, while doing his own benchwork, somehow kept himself deeply informed of the diverse membrane reconstitution projects of his 15 postdocs. My close companion was Baruch Kanner, whose work there led to his later breakthroughs identifying neuronal glutamate and GABA transporters. I spent two exceedingly happy years learning to handle defined proteins in defined membranes, entirely free of ideologies associated with scientific orthodoxy or heresy, which were daily fare in Ling's laboratory. Here I was hypothesis free, just exploring dark territory with liposomes and planar bilayers, doing a completely different kind of research: discovery rather than epistemology.

I stumbled on something unexpected: an ion channel. It was known that SR membranes are chock full of Ca^{2+} pumps and that they must also harbor some sort of Ca^{2+} release channel to trigger muscle contraction. But in fusing SR membranes into planar bilayers, I recorded only an unknown voltage-dependent, K^+-selective channel (7). As soon as I saw its single-channel fluctuations—a "real protein" rather than a bacterial peptide—I lost all interest in Ca^{2+} pumps. Of the scores of job applications I sent out toward the end of my postdoc, I scored just one interview, at Brandeis's Biochemistry Department. (In those days, when unsuccessful paper applicants would sometimes receive form letters of rejection, I received the same rejection letter from the same university on four successive Fridays: a case of either a copier gone psychotic, or a department that really, really didn't want me on their faculty.)

Youth: Brandeis, 1976–1980

Arriving at Brandeis as a 29-year-old assistant professor of biochemistry, I excitedly set up my own laboratory to continue working on SR K^+ channels in planar bilayers. I knew nothing about mechanistic enzymology, my department's widely regarded strength. Two giants of that field—Bill Jencks, a deep scholar, and Bob Abeles, a true genius—had laboratories just upstairs from me. Sergei Timasheff, a highly respected physical biochemist, and Bob Schlief, a young, creative geneticist in the early days of DNA manipulation, were also close by. Al Redfield, a brilliant pioneer

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in nuclear magnetic resonance relaxation theory (and arguably the worst undergraduate teacher ever), was just down the hall. Andrew Szent-Györgyi shared floor space with David DeRosier, Don Caspar, and Carolyn Cohen, structural biology gurus, while Michael Rosbash, a brash assistant professor working on something called RNA, and John Lisman, a biophysically minded neuro geek, lived in the adjacent Biology Department. This small university was literally crawling with terrific scientists. Our department was small, so we collided in the halls often and discussed each other's research in monthly informal lunch presentations. They hired me, I surmised, as the "membrane guy," thinking that membrane proteins should be brought into the biochemical fold. They knew I would be incompetent at teaching standard biochemistry classes, so I was asked to design a course on biochemical thermodynamics, which in one form or another I've been teaching for over 40 years.

For a lowly assistant professor, this was a heady time, in part because our undergrad research students were so talented—my first was a shy transfer student from UMass Boston named Rod MacKinnon—and in part because of a complete absence of departmental hierarchy. These jaw-droppingly eminent scientists treated me like a peer, seeming to want to learn from me about ion channels and the power of single-molecule kinetics, subjects with which I was comfortable by that point. In my second year at Brandeis, I stumbled upon yet another channel that wasn't supposed to be there, a strange Cl^- channel in an electric fish, but I've told that story already (8). Suffice it to say that Brandeis biochemistry provided an almost effortless, learning-by-osmosis immersion in a foreign subject that deeply informed the "enzymological" approach to channels championed in the '70s by Bertil Hille, one that I applied experimentally to ion channels in chemically defined membranes.

By the early '80s, thanks also to my friendship with Ramon Latorre, at Harvard on what Boston's vibrant Chilean expat community called a "Pinochet Fellowship," I had learned enough about and produced enough work on ion channels to have been noticed, to my amazement, by my electrophysiological heroes, Clay Armstrong, Chuck Stevens, Knox Chandler, Alan Finkelstein, and Peter Läuger, as well as by colleagues just out of the postdoctoral hatchery: Rick Aldrich, David Clapham, David Corey, and Fred Sigworth. These young stars made me realize that my own scientific youth was over, that I was now an adult embedded in an effervescent, blooming field—an unusual one where your uncompromising competitors are also generous collaborators and helpers, to the untainted benefit of the collective progress of our science.

Lesley C. Anson served as editor.

References

1. Bean, R.C., W.C. Shepherd, H. Chan, and J. Eichner. 1969. *J. Gen. Physiol.* 53:741–757. https://doi.org/10.1085/jgp.53.6.741
2. Hladky, S.B., and D.A. Haydon. 1972. *Biochim. Biophys. Acta.* 274:294–312. https://doi.org/10.1016/0005-2736(72)90178-2
3. Mueller, P., D.O. Rudin, H.T. Tien, and W.C. Wescott. 1962. *Nature.* 194:979–980. https://doi.org/10.1038/194979a0
4. Miller, C. 1974. *Biochim. Biophys. Acta.* 339:71–84. https://doi.org/10.1016/0005-2736(74)90333-2
5. Miller, C. 1974. *Biochim. Biophys. Acta.* 339:85–91. https://doi.org/10.1016/0005-2736(74)90334-4
6. Racker, E., and W. Stoeckenius. 1974. *J. Biol. Chem.* 249:662–663.
7. Miller, C., and E. Racker. 1976. *J. Membr. Biol.* 30:283–300. https://doi.org/10.1007/BF01869673
8. Miller, C. 2015. *J. Physiol.* 593:4085–4090. https://doi.org/10.1113/jphysiol.2014.286260