ESSAY

Mentors: Ron Kaback

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There is no question that H. Ronald Kaback's research has been extraordinarily innovative, creative, and of high impact. But in fact, his impact as a mentor is as legendary as his scientific contributions. The number of scientific children, grandchildren, and great-grandchildren that Ron has all over the world is a testament to this. I am fortunate enough to have experienced the nurturing and supportive environment that he created, having completed my postdoctoral training with Ron at the Roche Institute of Molecular Biology in Nutley, New Jersey.

Ron started to do research, and pretty much charted his own course, while a medical student at Albert Einstein College of Medicine. He had the idea that one could prepare sealed membrane vesicles from bacteria to study transport processes in a far more controlled experimental system than was possible before—a concept that profoundly challenged the conventional wisdom in the field at the time. He succeeded brilliantly in preparing the vesicles, but had to contend with a tremendous amount of skepticism toward his breakthrough. It took him years to get his vesicle paper published because people simply couldn't bring themselves to accept his data (1). Membrane vesicles are osmotically sealed sacs with a defined orientation but without any cytoplasm. These vesicles mediate active transport in the way that intact cells do, but they do not metabolize the accumulated substrates. Using this experimental system, Ron demonstrated that an electrochemical proton gradient ($\Delta\mu\tilde{P}_{H^+}$) is the driving force for the accumulation of many different substrates (2, 3). Peter Mitchell considered these results the first piece of conclusive evidence for his chemiosmotic hypothesis.

The cell or plasma membrane separates the interior of the cell from the external milieu. One of the quintessential characteristics of biological membranes is their selective permeability. Membrane transport proteins are responsible for this selective permeability and many mediate the translocation of solutes across a membrane or epithelium against a concentration gradient—i.e., they actively transport solutes. In primary active transport, energy derived from light, respiration, or ATP hydrolysis is used directly to drive the transport of either protons or sodium, thereby generating an electrochemical gradient of the cation ($\Delta\mu\tilde{P}_{H^+}$ or $\Delta\mu\tilde{P}_{Na^+}$). Thus, the cell membrane behaves like a battery. This battery provides the driving force for secondary active transport by cotransporters or permeases—ubiquitous polytopic membrane proteins that transduce the free energy stored in $\Delta\mu\tilde{P}_{H^+}$ or $\Delta\mu\tilde{P}_{Na^+}$ into substrate concentration gradients across cell membranes.

Members of Ron Kaback's laboratory in the 1980s, just before (upper panel) and after (lower panel) the laboratory's foray into molecular biology.

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Many such transport proteins belong to the Major Facilitator Superfamily (MFS), whose members mediate the transport of ions, carbohydrates, amino acids, peptides, vitamins, neurotransmitters, nucleobases, nucleosides, nucleotides, drugs, and many other substrates (4, 5). Over the last six decades, Ron has pushed the field of membrane transporters from the phenomenological level to the biochemical and even to the atomic level. He has concentrated mainly on the lactose (lac) permease of *Escherichia coli*, an MFS family member that uses a proton gradient to cotransport lactose into cells. By combining detailed biochemical and biophysical studies, he has gained an unparalleled mechanistic understanding of all the different reactions that the lac permease carries out: active transport, facilitated diffusion, efflux, exchange, and counterflow.

I joined Ron's laboratory at a particularly exciting time. The *lacY* gene had recently become the first gene coding for a membrane transport protein to be cloned and sequenced—a feat accomplished by Müller-Hill and colleagues (6) that made it possible to overexpress its product, the lac permease. Shortly thereafter, the permease was solubilized with detergent, purified to homogeneity in the presence of *E. coli* phospholipids, reconstituted into proteoliposomes, and shown to be fully functional; all of this was accomplished by Ron and his colleagues (7–10). In addition, his group elucidated the topology of the lac permease and generated monoclonal antibodies that uncouple proton movements from lactose transport (11–13), and Ron's laboratory set out on a new adventure into the world of molecular biology to begin investigating the roles of specific amino acid residues using site-directed mutagenesis. This was the beginning of the journey to identify key residues involved in substrate binding and proton translocation, which led to the realization a few years later, after Ron moved to UCLA, that only six residues are essential to the activity of the protein.

Ron pioneered studies of helix packing using thiol cross-linking between cysteine residues engineered onto a functional transporter lacking native cysteines. He used both intact transporter molecules and split permease molecules expressed as two halves (which, strikingly, were functional; 14–16). Furthermore, Ron used a battery of other site-directed techniques—including second-site suppressor analysis coupled with chemical modification, excimer fluorescence, engineered Mn(II) binding sites, electron paramagnetic resonance, and chemical cleavage and identification of monoclonal antibody epitopes—to generate a helix-packing model at a resolution of ∼4 Å (17). The more Ron learned, the more fervently he wanted to understand the details of the mechanism by which the lac permease mediates active transport. His and his colleagues' eventual determination of the crystal structure of the protein in the inwardly facing conformation (18) only spurred him to work even harder to experimentally test the alternating access mechanism (19) and determine the structure of the outwardly facing conformation (20), and this in turn led him to ask yet more probing questions. Not satisfied with the open conformation structures, Ron recently reported an engineered occluded apo-intermediate of the protein (21). This is the kind of scientist Ron is.

Ron's discoveries have both a basic and a translational dimension to them, because the phenomenon of transport across biological membranes is at the heart of what keeps cells alive. Like ion channels, ATPases, and ABC transporters, secondary active transport proteins are highly relevant for human health and disease (depression, epilepsy, diabetes, hypothyroidism, and multidrug resistance, inter alia). Furthermore, MFS transporters, which are present in both prokaryotes and eukaryotes, fall into families whose members have known three-dimensional structures or predicted similarity to them. Unsurprisingly, the mechanisms of transport also appear to be conserved. Thus, Ron's pioneering and in-depth investigations of the bacterial lac permease have resulted in highly influential concepts and tools that have been crucial for the rapid development of the transport field. The approaches developed in Ron's laboratory have since been applied to important human transporters, including glucose transporters (GLUTs), the sodium/glucose cotransporter (SGLT1), neurotransmitter transporters (SERT, NET, DAT), and the sodium/iodide symporter (NIS), as well as to membrane receptors such as the nicotinic acetylcholine receptor and G protein–coupled receptors.

Ron is the type of scientist who will do whatever it takes to answer the scientific question he has posed. He will implement new techniques, recruit collaborators, and think about it in a thousand different ways until he solves the problem (22). There is an intensity about his approach to both science and mentorship that is hard to come by elsewhere. During the years I spent in his laboratory, there was never a boring or slow day. Seeing Ron in action and up close led me to reflect on what attributes and circumstances are needed for someone to become a great scientist. Here are some that come to mind: an abiding and in fact unquenchable curiosity; a virtually limitless passion for science; a willingness to think outside the box and challenge accepted dogma; an almost superhuman degree of persistence, even in the face of seemingly insurmountable odds. Scientists in training also need a nurturing and stimulating environment, and, of course, an excellent mentor: someone who tells you it's okay to make an experimental mistake, as long as it's not made twice; someone who is fair and generous with their time; someone who genuinely cares about you and yours, encourages you to be independent, constantly challenges you but is open to having their own ideas challenged; and someone who keeps supporting you wholeheartedly and indefinitely even after you leave their laboratory. Ron, the mentor, is exactly like this.

One telling measure of the impact of Ron's mentoring was the number of people from around the world who attended a symposium organized by Gérard LeBlanc, Shimon Schuldiner, and the late Wil Konings to celebrate Ron's sixtieth birthday in the South of France, as well as the more recent celebration of Ron's eightieth birthday at NIH, organized by José Faraldo-Gómez and Lucy Forrest. Both were memorable occasions and vivid displays of Ron's legacy, not only as a brilliant and unorthodox scientist but also as an incomparable mentor.

As one of the many beneficiaries of Ron's intense style of mentorship, I often find myself revisiting things that he told me when I was in his laboratory. He insisted, for example, that

one essential requirement for someone to become a great scientist is sheer luck. As unscientific as it sounds, this was one of the countless morsels of wisdom that I received from him. Ron's exact words were "I'd rather be lucky than smart." I remember thinking at the time that I didn't understand what he meant: it simply didn't make sense to me. My own view was that, if you wanted to succeed as a scientist, you had to be smart and do your utmost to answer key questions. It seemed to me that luck played no role in any of this. It was only much later that I finally understood what he meant: there are circumstances beyond your control that can shape your research trajectory and indeed your life. Here's one example: I myself was phenomenally lucky to be able to do my postdoctoral training in Ron's laboratory. I found in Ron not only an exceptional—in fact, unique—scientist, but also a spectacularly effective and inspiring mentor and a lifelong friend. So yes, being lucky helps. Ron was right even about that. And yet, I hasten to add, being smart helps, too. Ron has been both.

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