Sounds Physiological

Fleeing complexity: An interview with Christopher Miller

Transcript of a conversation between Elizabeth M. Adler and Christopher Miller[1]

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Transcript condensed and edited by EMA

EMA: Hi, this is Liz Adler, Executive Editor of *The Journal of General Physiology*. I'm in South Hadley, Massachusetts, at Mount Holyoke College, at the Gordon Research Conference on Ion Channels, speaking with Chris Miller from Brandeis. Chris, thanks for taking the time to speak with me.

CM: It is an unalloyed delight, Liz.

EMA: As I understand it, you've devoted much of your career to studying proteins that move ions across membranes. You've studied ion channels and ion transporters and so forth. But I also understand that you started out your research career, trying to prove that the distribution of ions between cells and their environments has nothing to do with *anything* involving a cell membrane. Can you tell me about that?

CM: Well, yes, that's very old history. I was a physics undergraduate student. And in my senior year, I took a class on biophysics. What we did was, we read papers of biophysics-type scientists in the Philadelphia area, where I was going to college. We would spend the week reading a couple of papers, and then at the end of the week, we would go visit those scientists in their labs.

One of the scientists we visited made an argument that all the ion pumps, like the sodium pump, violate the First and Second Laws of Thermodynamics. So to me, you know, well, if that's the case, they *can't* be right.

Anyway, he was a very inspiring person, very articulate, a very appealing guy. So as a result of that week, I read his book and I became a convert. So I decided I would be his graduate student, so I joined his lab. Anyway, I set up my experiments essentially to try to make a *crucial* test of his theory. During the course of my thesis, it was an experience of sort of picking my way out of an intricate, self-consistent theory that simply, as I learned slowly, didn't correspond to the outside world.

It was a very important experience for me, because it really showed me the *danger* of being religiously attached to one's own ideas. Because that's what I came to realize. This was a man who was a *brilliant* scientist, a *really* good experimentalist who, as a senior scientist, was still doing experiments in the lab. He was a *kind* person, he was a *fair* person, but he was attached to a theory, and he could not *shake* it.

And that was a pretty good experience to have early on in a scientific career.

Well, of course, it also gave me my basic interest that has prevailed through my entire career, of questions like, "Gee, what's the difference between sodium and potassium that they can be handled *so* differently by cellular proteins?"

So the upshot is that I ended up doing a postdoc in a *totally* different kind of scientific environment, which is, I went to Ef Racker's lab, and he was doing work on transport proteins but in exactly the opposite way. Racker said, "Let's break it down, purify the individual components, put them back together into membranes." Well, that's what biochemists do. But he was a biochemist of membrane transport. He was one of the early ones.

So, that was so much fun to be there, and learn how to do that, and to be freed from the immense complexity of the cell. It felt like a real liberation for me. So my two years in Racker's lab was really wonderful, and it set me on my path to be working on reconstituted systems and purified proteins, and staying away from the complexity of the cell.

And of course, that means I'm not dealing with biology per se in my work.

EMA: Right.

CM: I've always run away from biology. It's always been too overwhelming for me.

EMA: So once you got settled on an approach and a way of doing things, did you have a favorite series of experiments or problem that you worked on? Or is it just the one that you're currently on?

CM: I guess it was always the one I was on. Because the only thing that ever drew me to a problem was self-indulgent aesthetics. It's what I wanted to do. It rarely had anything to do with the importance of the system or the biology of the system.

You know, at the beginning, I was working out a technique—a technique to put ion channels into artificial membranes, and be able to do the biochemist analog of patch recording.

And it was really exciting to be able to actually see single channels in those days. Just seeing them. It almost didn't matter what they were; you know, I saw my first single channels in 1975, when I was still a postdoc. That was my first system, and I just thought, "Well, what is it?" We didn't know what the channel *was*, and it was from sarcoplasmic reticulum membranes. It wasn't expected to be there; it was a potassium channel, and I just said, "Well, this is an interesting model system, maybe, where we can look up-close in a defined system."

Of course, I was looking for calcium [transporters], and only found potassium channels.

EMA: Huh.

CM: But that sort of gave me my first project to work on as an independent investigator at Brandeis. And that run lasted about five years. It gave me about five years of work until I got bored with it.

But by that time, there was this other system that I had developed, the CLC-0 system, the *Torpedo* chloride channel.

EMA: Oh, the first one was *Torpedo*. I didn't know.

CM: Yes, that came from the *Torpedo*. The funny thing is, this SR potassium channel . . . one of the great things that happened to me early on in my career is that nobody believed this. They thought this was some *artifact*, so I wanted to show that I could take a system where we *knew* what the channel was, and to show that the system will show that. And, obviously, the *Torpedo* electroplax was where you get your acetylcholine receptor. I wasn't purifying proteins in those days, but just purifying the membranes, and then fusing them into bilayers to just show everybody, "Oh, no, the system works! You can get

acetylcholine receptors!" The first experiments, what I got: chloride channels that nobody knew were there. And I *never* saw an acetylcholine receptor. *Never.*

But then, that led to this work on chloride channels, which continues to this day, in fits and starts, and it's very satisfying and fun.

EMA: So you started looking for ER calcium transporters, and found potassium channels, and then you looked for *Torpedo* nicotinic acetylcholine receptors, and found chloride channels . . .

CM: Right. And so it raised the question early on: If you are looking for something and you *don't* find it, and you find something else that you have no idea what it is, what should you do? There are two possibilities. You ignore the thing you don't want to see and keep trying to look for the thing that *should* be there. Or, you can study the thing and ask, What *is* that thing?

EMA: Right.

CM: And, of course, I took always the line of least resistance, which was to work on the thing that I could see. And, luckily, those all turned out to be interesting. And they were idiosyncratic enough, *weird* enough . . . in one way, every system I've ever worked on has a *weirdness* associated with it that actually is why I find it fun. The weirdness of the *Torpedo* channel showed itself pretty early on, as being this double-barreled thing that had never been seen in the channel world before, so that provided a lot of controversy to argue about, but also a lot of fun.

And, as I said, the thing that in my *earliest* days, my first five years, that was really good, is that nobody believed what I was doing was meaningful.

EMA: Really?

CM: Yeah. They'd just say, "This bilayer is not physiological; it's this or that, and it's an artifact." And that was really good for me. I knew that it was meaningful, to me at least, because it was fun to work on, and it was reproducible, and it had overtones of what real channels that we knew about did. So I knew that there was something *real* there. But it meant that, as a beginning assistant professor with, you know, one graduate student and one technician and me—six hands—we weren't going to have any competition.

And that was really good. I could kind of take things at my own pace without feeling that a lot of people might want to work on the same system.

EMA: Right.

CM: Nobody wanted to work on my system. They thought it was an artifact. So it gave me five years of freedom, and that was really great.

EMA: I guess most recently you've gotten interested in fluoride channels and transporters.

CM: That's very recent, yes. We stumbled about two years ago into this subject of fluoride transporters and channels. It was a completely unexpected thing. Before that, fluoride was considered to be a biologically irrelevant ion, except maybe . . .

EMA: Except for your teeth.

CM: . . . for your teeth.

But there wouldn't be membrane systems to handle fluoride transfer. And we now know that that's not the case. There's a whole membrane biology of fluoride transport. And it's not something that animals use. It is something that only microorganisms use to protect themselves against the direct exposure that they suffer to an environmental aqueous fluoride ion, which is an inhibitor of important basic enzymes in energy metabolism and nucleic acids.

EMA: So there's enough around to . . .

CM: There's on the order of tens of micromolar to 100 μM in groundwater, seawater, soil, everywhere. And it's been there since the beginning of evolutionary time.

EMA: Huh.

CM: It's extracted from the rocks. It's part of our environment. But it is an inhibitor of *key* enzymes in cellular metabolism. We don't have to worry about it, because we excrete it. But a unicellular organism directly exposed to it has to be able to have systems that will keep the cytoplasmic concentration low so that it doesn't go above the inhibitory levels of these enzymes.

EMA: Right.

CM: So, it's only two years into this now, but it's really fantastic. Everything you find is new.

In a way, it reminds me a little of the SR potassium channel, where it was a whole new world, and anything I would find would be interesting, because it had never been seen before.

This is also a very good lesson in how the collision of completely different areas in science—that are seen to be totally unrelated—can give rise to something new. I actually got *pushed* into this by a scientist at Yale, Ron Breaker, who is an expert in riboswitches, which are little sequences, 60–80-nucleotide sequences used by many bacteria, at the RNA level, to control gene expression.

And the way they do it is, the RNA forms a structure, which can be changed by the binding of a ligand—

EMA: Uh-huh.

CM: —which then will either turn on or turn off downstream genes. And he identified a riboswitch that was turned on by fluoride anion.

EMA: Huh.

CM: And it *turns* out that these riboswitches controlled the gene expression of not only the levels of these fluoride-inhibitable enzymes, but also an unknown transporter . . .

EMA: *Oh*, right, to get it out.

CM: . . . that, Ron then hypothesized, was an exporter for fluoride.

And he called me and he said, "Here's what I found, and a reviewer of this paper is giving us trouble because we don't have any protein level argument to say this thing's a transporter." And, as it happened, we had already been working on one of these transporters, not knowing what it was. We thought it would be a ClC. It turned out this one that started the whole thing was in a funny, distant clade of the bacterial ClC. We were trying to express lots of homologues for crystallization, and we *noticed* this clade because it had some funny sequence characteristics, and we made some of these, we

expressed some of these, and they expressed quite nicely and we could purify them—but they were inactive.

And it was just around that time that Ron gave me this call and said,

"Could you help us out, and would you be willing to do a few assays if we . . ." and I said,

"What's the system you're using?" and he said,

"Well, it's this gene from *Pseudomonas*," and I said,

"My God, we've got that in the freezer. We've already got it reconstituted, and it's inactive for chloride." So the next day, my postdoc, Randy Stockbridge, who was doing this, got it out. Put in fluoride instead of chloride. *Boom*! So that's what kicked off the whole thing and got us into this.

EMA: What an amazing story.

CM: It's the most astonishing scientific coincidence I've ever experienced. And it was really just so much fun, and it's been fun to keep in contact with Ron Breaker, who is working on these in a genetic context and is doing beautiful work. But it was an incredible coincidence, and it got us into fluoridology, which is very interesting because fluoride is such a weird, chemically weird anion.

And then, now, with this fluoride *channel* that we've been studying and that Randy has been working up very recently, that has its own *new* kind of weirdness. It's the only channel that's ever been seen that's built as an anti-parallel homodimer in the membrane. So, it's been very exciting in the last few months to begin to see the structures of these things. We've been able to crystallize them and begin to do some structural work with it.

That's what I'm working on now. Again, it's the weirdness of the system that I always find attractive, that makes it fun to work on.

EMA: Do you have anything else you'd like to say that I haven't thought to ask you or bring up?

CM: Since this is being recorded [laughs] and it's presumably there to provide some supposed wisdom from those with experience [laughs], I would just say that I still think, despite the dictates of the awful funding situation and the translational terrorism that we have coming down from the NIH, that will only get worse in the future, I still think that scientists should trust their own aesthetic pleasure in the systems they work on, and not feel they should be forced to work on something that other people think are important.

Because *that's* where most of the real novelty comes from, the out-of-the-box thinking and all of that. That people unburdened by too much knowledge can do things that the experts like me would say, "Oh, no, that will never work and don't try it."

You work on things because you like them, because they are interesting, I think there's always a good chance that something novel will come out of it that other people will also be interested in and that will start to bloom. Not to be afraid to trust your own sense of scientific aesthetics and not to be led astray by what is considered to be a hot system and an important system and all of that. I guess that's what I would like to say in ways of gratuitous advice.

EMA: Well, great. Well, thanks, Chris.

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