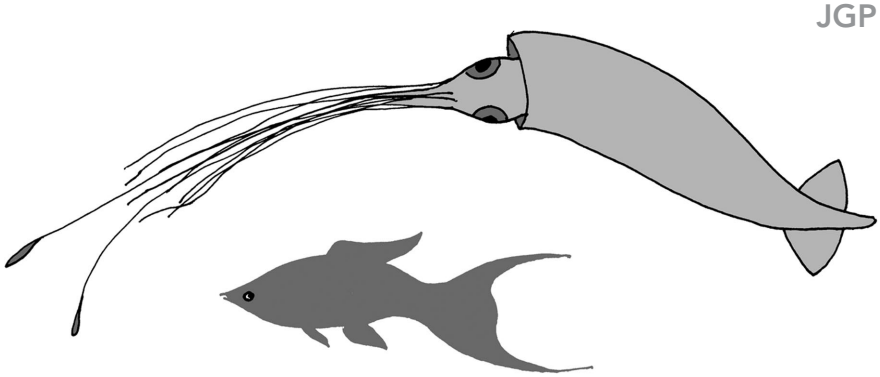
Friends of Physiology: An Interview with Clara Franzini-Armstrong and Clay Armstrong



Transcript of a conversation between Elizabeth M. Adler,[1] Clay M. Armstrong,[2] Clara Franzini-Armstrong,[3] and Sharona E. Gordon[4]

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Transcript condensed and edited by EMA

EMA: Hi, I'm Liz Adler, Executive Editor of* The Journal of General Physiology. *Incoming Editor-in-Chief Sharona Gordon and I are in Woods Hole for the 67th Annual Meeting of the Society of General Physiologists. This year's meeting sees the unveiling of the Friends of Physiology Lecture Series Honoring Clara Franzini-Armstrong and Clay Armstrong, and to mark the occasion, we're delighted to present an interview with Clay and Clara in which we discuss some of the work that identifies them as friends of physiology.

Clay and Clara, thank you so much for agreeing to the interview.

CMA: Thank you for inviting us.

CFA: Yeah.

EMA: So, where did the name "Friends of Physiology" for the lecture series come from?

CMA: Well, it's two things: One is that we've made lots and lots of excellent friends in this pursuit and we wanted to acknowledge them, and the second is that we just enjoy people whose enthusiasm is in science and in trying to find things out.

EMA: Clara, you've done many beautiful electron microscopic studies dealing with excitation–contraction coupling in skeletal and cardiac muscle. Do you have a favorite paper, or series of papers?

CFA: Well, yes, I presume the favorite is my first real entry into the field, when by total serendipity I happened to fix some fish from the tank of Mrs. Porter.

I was in Keith Porter's laboratory, and those tail muscles in the fish revealed a direct opening of transverse tubules to the extracellular space. That was something that several laboratories had been looking for and had a hard time finding because usually the peripheral segment is too tortuous and you cannot follow it in a single micrograph, whereas in the fish it went just straight out, so there was great excitement. There was actually simultaneously three laboratories got the equivalent evidence. One was Hugh Huxley, who infiltrated ferritin molecules into the muscle and found that they penetrated into the lumen of the T-tubule, and one was Makoto Endo, in Andrew Huxley's laboratory, who, using a fluorescent dye, for the first time showed the T-tubules in fluorescence microscopy. [*Editor's note*: The third was Sally G. Page.]

So, the indication that there *should* have been an opening of the T-tubule was due to the famous local stimulation experiments of Andrew Huxley, where he found that a specific site along the sarcomere was a place where a local—totally localized—depolarization would induce the resulting local contraction. And so he postulated that there had to be an element that went into the muscle fiber that carried the effect of the depolarization in a localized fashion. So, he had first postulated there was some transverse element, and then Porter and Palade demonstrated that there were triads at the site where the local stimulation effect happened, and then Anderssson-Cedergren showed that the central element of the triad—the transverse tubule—was a continuous element going into the fiber. And then, he said, well, you needed an opening—and the opening was hard to find.

EMA: Right, so then you found the opening.

CFA: So, we found the opening. And that was fun.

EMA: Yeah, that must have been fun. … What was it that attracted you to EM as a technique?

CFA: That, actually, was another serendipity. That was the fact that the Ministry of Education decided that microscopy was an uncommon technique, so he gave an electron microscope to the University of Pisa; my professor got his hands on the microscope, put it in a room, gave me a book, and said "You learn to do microscopy." … and then Keith Porter came visiting and he took pity on us,

trying to learn to do microscopy on our own, and then I came as a postdoc and really learned to do it well.

EMA: So, what are you working on these days?

CFA: Well, I actually am very excited because I just got a very nice set of experiments using zebrafish. Now, zebrafish has become my favorite, because the structure of the striated muscle in zebrafish is wonderfully organized within 48 hours after fertilization—so it has these triads, absolutely straight in the right place. And it has a component that has been postulated to be necessary to large sparks of localized calcium release, which is a type of ryanodine receptor that's associated with the one that's directly involved in EC coupling.

So there are type 1 and type 3 ryanodine receptors; type 1 is directly involved in the mechanical interaction with calcium channels of the T-tubules, as mostly shown by the work of Kurt Beam (postulated by Schneider and Chandler first), but then a second type of channel is there, and if that is present, then you have a large unitary calcium release event—the calcium spark. So we found that in the zebrafish—and the zebrafish can be manipulated—so we decided that we would silence the type 3 ryanodine receptor and see what effect it would have on the sparks and on the structure. And we did a really quick set of experiments with my last postdoc—Stefano Perni—we showed that, yes, indeed, with silencing the type 3 ryanodine receptor the structure changes appropriately and, with Steve Baylor and Steve Hollingworth, we showed that the spark disappears.

CMA: And how does the standard transmission work in a muscle?

CFA: So the standard transmission is specifically limited to an interaction between the calcium channels in the transverse tubules, which are, you know, a slowly activating Ca_v1 voltage-activated calcium channel, and the ryanodine receptor of the sarcoplasmic reticulum. [*Editor's note*: The ryanodine receptor involved in "standard transmission," which occurs in skeletal muscle, is type 1 (RyR1).]

What I've shown over the years is that the ryanodine receptors are located at the site where the sarcoplasmic reticulum associates with the transverse tubule very specifically. So then they are ready to get a signal and the Ca_v channels are located in the transverse tubule and associate themselves very specifically so that a group of four calcium channels are linked to the four subunits of the ryanodine receptor in a very specific manner. And that forms the link that allows the functional connection to happen.

EMA: Right.

CFA: We've shown in lots of collaborations—mostly with Paul Allen and Kurt Beam and Isaac Pessah—that this specific interaction is always there when you have the functional interaction, and if by swapping components you block the interaction, then the structural component also is disarranged.

SEG: Let me ask you what has been your biggest disappointment in your scientific career.

CFA: Mostly I used to say that I had to get down to the pit of desperation before I got a result. You know, microscopy is not as easy as you would think; you don't just throw something in a fixative, put it in a microscope, and get beautiful images. And so often I had to fight against something that was not working and I had to get really unhappy, and then suddenly it came out and worked. But this last experiment I didn't have to go to the pit of desperation; I was very happy; it was really unusual.

But mostly I've been quite happy with what I've done. I have enjoyed it because the structures are *so* beautiful.

EMA: Yes.

CFA: And in addition to what I have described as functionally relevant, I have looked at almost the entire animal kingdom. I have images of dragonflies—muscle from dragonflies from Woods Hole—scorpions, spiders, all sorts of things—earthworms—in search for what is the common structure. And the common structure is that you always have an element of the sarcoplasmic reticulum—there is the ryanodine receptor associated with it—which interacts either with the surface membrane or the transverse tubules. And that's absolutely always the case. And then the interaction between cardiac and skeletal muscle and muscle from invertebrates can happen slightly differently. But the components are there. In all of them.

EMA: Clay, your work has focused on ion channel permeability mechanisms and gating processes. How did you get interested in that area?

CMA: Well, at the beginning, I went to NIH from Washington University, where I was a postdoc. And so I got an appointment at NIH. So there, there was this … everyone was in shock. All of the neurophysiological community, as best I could tell, was in enormous shock because of the Hodgkin and Huxley papers. This was 10 years later, but still, people were shaking their heads. I mean, you know, the established professors would basically have to go back to school in order to understand what these men were talking about. Either that or it was wrong—so let's just say that it's wrong. There was great desire to add something to change—or perhaps overthrow—the Hodgkin and Huxley formulation. So …

SEG: Did people think it was too complicated or too simple?

CMA: Oh, I don't think most people really understood it at all, you know. I mean it is *very* complicated, with several factors governing each permeability. You have to know about electricity; you have to know about thermodynamics, within limits; you have to know the ion distributions across membranes in the resting state. All of those things had been put together by Hodgkin and Huxley and by Katz, but had, I think, not really permeated largely to the neurophysiological community, which was used to looking at brain waves.

Which I was doing for a while. Which I liked.

You know the final blessing on the bilipid layer was not until about 1952.

So, KC [*Editor's note*: Kenneth Cole] was making new formulations and trying to explain, for example, why the potassium conductance had a sigmoid time course. And I think, as late as 1965, that his favorite explanation was that it involved the Nernst–Planck equations and that the potassium ions were held at the inner end of the membrane at the resting potential and migrated slowly across the membrane during the rise of the potassium conductance.

SEG: At that point they still didn't know that the conductances were mediated by proteins.

CMA: Oh, no. In fact, I would claim some of the first clear evidence of that based on our experiments with pronase. Although Bert Hille, of course, who was a lively presence, always had had the ideas of specific receptors for tetrodotoxin, for example.

In 1965, Goldman said in one of his papers, I think in the *JGP*, that the ions moved through the membranes—through the lipids—and that the proteins are insulation.

EMA: Are insulation? Huh.

CMA: Basically the ions go through with a Nernst–Planck sort of mechanism. Go through the lipids.

EMA: Right.

CMA: And there was—in the early part of the 1960s this—or 70s even—great interest in the behavior of lipids. Phase transitions in lipids and so how those things could be involved in this miraculous—as somebody said, almost mystical—activity called producing action potentials.

EMA: So how did you become convinced that ion channels exist?

CMA: My second set of experiments at NIH were the ones that set me on the permanent course of believing that there are channels. And that came out of a visit to London, where I talked to this wonderful student, Denis Noble.

EMA: Uh-huh.

CMA: And Denis had come up with a modification of the Hodgkin and Huxley equations, which would produce the cardiac action potential. And a part of that was the idea that there was an inward rectifier.

But, anyway, that set me to thinking about inward rectification, and so I looked at some experiments by Tasaki and Hagiwara … they had discovered that they could inject tetraethylammonium ion … they had found that it produced an action potential by, as we now know, blocking the potassium conductance. Tasaki did not believe in channels, so I think Hagiwara was quite frustrated that he couldn't explain it in good reasonable Hodgkin and Huxley terms.

But in any case, Leonard Binstock and I then began to do experiments here in Woods Hole. Over in the Lillie Building. The paper that we ultimately produced depended on one good experiment. One really thorough experiment, with a number of additional …

EMA: This was the TEA paper? Or?

CMA: Yeah, in 1965, and the phenomena were that, indeed, it produced a prolonged action potential and that if you tried to look at the currents when they were depolarized that the potassium current was very small—but if you raise the extracellular potassium concentration and then *repolarized* the axon then the current was inward and big. And it seemed clear that the TEA could get *into* the channel—it was easy to explain this in terms of a channel—with the potassium ion going through the channel and knocking the TEA out of the way at the other end of the channel.

And it was clear that the TEA could get into the channel only when the gate was open. It appeared that there was an internal gate, which protected this site where the TEA went. And so there had to be a locus inside of the membrane in the channel—I'll just call it a channel now—which was big enough to accommodate a TEA.

And so the natural thought then became that—after a while of cogitation—that there had to be a narrow part above that, which the TEA couldn't go through, but which the potassium ion could go through because it could shed its waters of hydration. TEA and a hydrated potassium are approximately the same size.

EMA: Oh, okay. So that let you know that the potassium had to lose its waters to go through.

CMA: Right. And so that was then what I worked on for many years. And then found at a certain stage that if you replaced one of the ethyl arms of the TEA with a long hydrocarbon chain that it produced spectacularly effective TEA. Clara and I were actually doing some experiments then in Chile, once upon a time.

CFA: I dissected the axons.

EMA: The* really *giant axons.

CMA: Uh-huh. And there it developed that if you used a low enough concentration of TEA you could actually see kinetics as the current would begin to turn on. You could see kinetics as the TEA blocked the channel. So those kinetics became much more visible when one used a long chain in place of the ethyl arm on the TEA. But then you could see the potassium current turn on more or less completely and then inactivate.

EMA: Huh.

CMA: So that was *very* exciting, because here you could kind of reproduce in a potassium channel, one of the Hodgkin and Huxley phenomena of the *sodium* channel. So that led to the ball and chain model, which turned out to be correct for the inactivation of the potassium channels. It's not quite correct—and we *knew* it was not quite correct when

we wrote the paper—for the sodium channel, which is more complicated.

SEG: It seems to me that one of the things that both of you have in common is thinking in terms of pictures. So that, Clay, you're thinking in terms of "ball and chain" and "foot in the door" … those are all very concrete images.

CMA: Well, that's right. No, I mean, I always thought that KC's laboratory was a little too mathematical. I was always very poor at mathematics and just didn't think in those terms.

I remember once explaining the ball and chain model at a Gordon Conference, and Chuck Stevens said, "Well, I think everybody really understood what you were saying. I think probably they were a bit scornful … (*laughs*) … I mean it can't be that simple."

SEG: What do you see as the future of physiology?

CMA: (*laughs*) Oh, I never could see the future. I mean serendipity—Clara was talking about—you just keep your eyes open.

CFA: My science has always gone from, you know, from one day to the other. You know, somebody just came up with a snake in my laboratory and found some wonderful things.

SEG: Do you want to give any advice to people who would be coming into the field now?

CFA: From my side, learn to use your hands.

CMA: Well, I think that there is still, you know, among probably most scientists in general, you know, in *medical* sciences, an ignorance of basic electrical principles. For example, knowledge of the equation relating voltage, resistance, and current. You know, those things, I think should be taught at some point. And that doesn't require much in the way of mathematics. It just requires common sense. And a little thought.

CFA: Yeah, but use your hands, I mean to say, participate directly in the experiments in your laboratory. Don't just sit in your office. That really bothers me most.

CMA: Yeah, that's one of the things that I have always found. Basically, I cannot get interested in something unless I'm working on it with my hands. And I think that probably applies not just to me but to most people.

EMA: So, is there anything else that you'd like to add?

CMA: *The Journal of General Physiology* is an institution that I hope will last forever. A specialized journal where the referees have an interest in the journal and where they have knowledge of the sorts of things that are being presented in the journal is very, very necessary, and the idea of pleasing deans by publishing in *Nature* is an abomination.