

## Evangelia Kranias The Mother of Phospholamban

Susan Ince

After a sheltered childhood, Evangelia (Litsa) Kranias defied her parents' wishes and boarded a ship from Athens to the United States, having been accepted at the University of Chicago and cautioned that how she performed would determine whether other foreign undergraduates would be admitted.

"When I think about it, I am amazed at the courage and determination of that 18-year-old Greek girl!" says Kranias. "My mother wrote to me every other day and I did the same. She often said, 'If we were to lay down our letters, they would form a bridge between Athens and New York.'"

Kranias has kept that bridge intact throughout her career, directing the molecular cardiology group of the Biomedical Research Foundation of the Academy of Athens while maintaining multiple positions at the University of Cincinnati—Director of Cardiovascular Biology in the Department of Pharmacology and Cell Biophysics, codirector of the Cardiovascular Center of Excellence, and Hanna Professor of Cardiology.

Shortly after joining the faculty in Cincinnati, Kranias recognized the importance of the recently discovered PLN (phospholamban) molecule in the regulation of calcium cycling through the sarcoplasmic reticulum and in the overall regulation of cardiac function. Kranias and John Solaro soon provided the first evidence that PLN is phosphorylated in the intact heart in "fight or flight" situations.<sup>1</sup> After generating a series of genetically altered mouse models, her laboratory revealed that, rather than stimulating contractility, PLN inhibits the calcium pump in the sarcoplasmic reticulum and this inhibition is relieved when the molecule is phosphorylated under  $\beta$ -agonist stimulation.<sup>2</sup> Surprisingly, PLN-deficient mice exhibited enhanced myocardial performance without changes in heart rate, raising the possibility of PLN as a target to improve calcium cycling and improve function in patients with heart failure.

In the clinical arena, Kranias' laboratory showed that heart failure is associated with an increase in the relative ratio of PLN to the calcium-transporting ATPase SERCA2a (sarcoplasmic/endoplasmic reticulum  $\text{Ca}^{2+}$ -ATPase 2) and a higher degree of dephosphorylated PLN. In 2006, Kranias identified a mutation in the PLN gene (deletion of arginine 14) that resulted in dilated cardiomyopathy

and death by middle age in heterozygotes in a large family—similar to the heart abnormalities and premature death in transgenic mice overexpressing the mutant gene.<sup>3</sup> Later, a group of mutant gene carriers created the PLN Genetic Heart Disease Foundation in the Netherlands, which has the largest patient population of this life-threatening condition. The foundation, which calls Kranias the "mother" of phospholamban, sponsors international collaborative research on the deadly condition.

Over the years, Kranias' work on PLN has expanded to include additional regulators of sarcoplasmic reticulum calcium cycling and apoptosis, including the small heat-shock protein Hsp20<sup>4</sup> and the antiapoptotic protein HAX-1 (hematopoietic cell-specific Lyn substrate 1-associated protein X-1), a modulator of PLN activity.<sup>5</sup>

In 2009, Kranias was designated as a distinguished scientist by the American Heart Association. Her sustained achievements in understanding the cellular mechanisms regulating the heart's pumping action were recognized by the International Society for Heart Research with the Peter Harris Research Achievement Award in 2014

and by the American Heart Association Basic Cardiovascular Sciences Council by selecting her to deliver the George E. Brown Memorial Lecture in 2016. Throughout her career, Kranias has mentored a large number of graduate students and postdoctoral fellows, providing daily interaction to refine their research, draft manuscripts, and prepare them for success as independent researchers, earning her the 2016 award for excellence in mentoring of doctoral students at the University of Cincinnati.

### Where Were You Born?

In Thessaloniki, Greece. It is the second largest city in Greece and was named after the sister of Alexander the Great.

### How Would You Describe Your Childhood?

I grew up in a suburb of Thessaloniki and it was a fun time. We did not worry about locking our doors and people used to drop in any time to say hello and have tea with us. As children, we played outdoors every day with our friends and we walked to school, even when it was far away.



Evangelia Kranias

### **What Were the Earliest Indications of Your Interest in Science?**

I was fascinated by a biology course in high school. We had an inspiring teacher and I wanted to read more and more and learn “how the body works.”

### **What Was Your High School Like?**

I went to a private “American” high school, Anatolia College, with a full scholarship. We were required to speak English among ourselves during recess, at lunch time, and any time we were outside the classroom. We also had an English class every day and a few other classes conducted in English, such as home economics, biology, and sociology. My class size was very small: only 16 girls.

### **Tell Me About Your Fulbright Scholarship, What It Was Given for and What It Allowed You to Do**

Since I went to an American high school, I was brainwashed and, as the top student in my grade, I felt I had to pursue further studies in the United States. Although I was studying hard for the entrance exam to the Greek University, I also applied for a Fulbright scholarship to attend college in the US. It was highly unusual to get a Fulbright scholarship as an undergraduate so I was very happy to receive one. The Fulbright program originally placed me at Dickinson State University in North Dakota, as they wanted to make sure that we attended relatively small colleges and got some attention and guidance as foreign students.

### **How Did You End Up at the University of Chicago? What Was That Experience Like?**

I never went to North Dakota. As I applied for a Fulbright scholarship, I also applied to other colleges under the guidance of my high school principal. I got accepted at several colleges, including Radcliffe and Berkley, but an amazing offer came from the University of Chicago. They offered me full tuition, living expenses, and additional spending money that could afford me a trip back to Greece every summer. They told me that I was going to be an “experiment” as a foreign student, and whether other foreign students were accepted depended on how I did. I was thrilled, but my parents were very much against my decision to leave Greece at the age of 18 and after being raised in a sheltered and strict environment up to that point. We had many arguments and, finally, my father was the first one to give in. He took me to Athens and saw me off, as I boarded the ship Queen Federica to come to the US. The Fulbright office offered to pay for my travel expenses but insisted that all the scholars come by boat to help us get accustomed to the idea of going abroad.

The University of Chicago was very hard and, during my freshman year, I gained 25 pounds as I stayed up for 2 to 3 nights in a row studying for exams. All my classmates were the top high school students from around the country, and we suddenly found ourselves graded on a curve, with some of us getting C and D grades for the first time in our lives. It was rather depressing for many of us and by the end of our first year, about half of our class transferred to other colleges. Things became progressively easier with time, but then classmates started to get drafted for the Vietnam war. Some tried to break their legs or arms to avoid the draft and there were lots of demonstrations on campus.

My first exposure to a lab project was at the University of Chicago during the summer following my junior year. It was a great experience.

### **How Did You Decide to Go to Northwestern University for Your Masters and PhD Work?**

After 4 years in Chicago, the city was home to me with its Greek culture and the friends that I made. Although I was accepted at several graduate programs, I decided to stay in Chicago and move to Northwestern with its beautiful Evanston campus.

### **Tell Me About Your Work With Lawrence Dumas**

I was the first graduate student of Larry Dumas, who had just gotten out of Robert Sinsheimer’s lab at Caltech. His research program was at the forefront of molecular biology at that time, working on bacteria and bacteriophage DNA. My PhD thesis was on the DNA replication of phage- $\lambda$  174. I finished my MS and PhD degrees in 4 years. I worked day and night but I absolutely loved it.

### **How Did You Make the Decision to Stay at Northwestern for Your Postdoctoral Work? Was That an Unusual Choice? Was There Any Downside to Doing That?**

I did my graduate work at the Evanston campus of Northwestern and my postdoc at the Medical Center, which is in downtown Chicago. It really feels like these are 2 different schools. By the end of graduate school, I was married and expected my first child. My husband was an intern at Northwestern University Medical School and we could rent an apartment from Northwestern right next door to the Medical Center. This was very helpful as we were starting a family, and the Biochemistry Department at the Medical School was very strong. I was lucky to work with Richard Jungmann, who gave me not only full independence but 2 technicians to help with my research projects.

### **When Did You Become Interested in Phospholamban and Its Possible Regulatory Role in Calcium Cycling?**

When I started as a faculty member at the University of Cincinnati. My postdoc was in protein phosphorylation and phospholamban had been discovered in Arnie Katz’s lab just a few years before I moved to Cincinnati.

### **Did You Ever Think That, 4 Decades Later, There Would Still Be so Much to Discover and so Many Unanswered Questions About Phospholamban?**

I could never dream of that. We thought that we had it all figured out at many points over the past 4 decades<sup>6</sup> but I think that there is still so much more to uncover!

### **Do You Remain Fascinated by It?**

Yes! I remain totally fascinated by it and the wisdom of nature: such a small molecule and so much complexity surrounding its function. I wish that I had another 4 decades to dedicate to it!

### **What Have Been Some of the Biggest Surprises in Your Phospholamban Findings? Anything That Made You Have to Take a Few Steps Back and Rethink Earlier Hypotheses?**

The biggest surprise was when we found out that the phospholamban knockout mouse was viable and had hyperdynamic cardiac function. This certainly changed our hypothesis regarding its role in the heart. Up until then, we thought of phospholamban as the stimulator of cardiac contractility. The mouse models revealed that it is actually an inhibitor of cardiac function and

$\beta$ -adrenergic stimulation simply relieves its inhibition. We now know that phospholamban is part of a big complex with many proteins that physically interact with each other, so the picture is more complex than we originally thought.

### How Did You End Up at the University of Cincinnati? What Factored Into Your Decision to Go There?

I just “followed my husband,” who was hired as a faculty member in ophthalmology. At that time, we had 2 small kids, 2.5 years and 3 months of age. I gave up my instructor’s position at Northwestern and thought that it would be a good idea to spend some time with the family and help us get settled in the new environment. However, I missed the lab and research so much that I could not possibly stay at home. The chair of ophthalmology who had recruited my husband was worried that we might move back to Chicago, since I was pretty unhappy being out of the lab. He introduced me to Arnie Schwartz at a Christmas party, and Arnie wrote his phone number on a cocktail napkin and asked me to call him on Monday morning. He suggested that I give a seminar to his department. Afterwards, he called a faculty meeting and 10 minutes later came out to shake my hand and offer me an instructor’s position. He asked what I would like to have, and I said just a bench to do research. There was no other discussion about salary or anything else! I was so lucky to get back to the lab within 3 months after we moved to Cincinnati.

### Did You Ever Consider Returning to Greece to Work? How Much Time Do You Spend There?

Yes. I seriously considered returning to Greece about 15 years ago. A new Research Institute was set up by the Academy of Athens, called the Biomedical Research Foundation of the Academy of Athens. I watched it being built from day 1 and it became an amazing facility with tremendous resources. I participated in the original organization and recruitment of scientists from abroad and I was offered the Directorship of Basic Sciences. Luckily, I was allowed to continue my affiliation with this Institute and direct the Molecular Cardiology group while I maintained my position at the University of Cincinnati. It has been a great blessing to have the Cincinnati and the Athens labs working so closely together and complementing each other so well. I am so fortunate!

### What Have Been the Biggest Challenges in Your Career? How Did You Overcome Them?

One of the biggest challenges was to retool my lab in the early 1990s to the new era of molecular biology/transgenesis. I was pretty comfortable with my research funding and productivity, but I knew that the only way to find out the role of PLN in the heart was to manipulate the gene in vivo. Thus, I decided to do an in-house sabbatical and learn this technology. As we started to generate the phospholamban knockout model, my chair and faculty colleagues doubted my decision to go down that path. They often asked: What makes you think that PLN is important in the heart? Why do you think that PLN ablation may have any effects in cardiac function? What if you get no phenotype and you risk losing your funding? These were difficult times and my decision to generate the mouse models could have proved to be wise or could have had major negative consequences on my career.

### How Hard Do You Work?

I work all the time. It’s funny but even after all these years, when I go to the beauty shop, my hairdresser says “OK, you can now go back to your homework” as she puts me under the hairdryer.

### What Advice Do You Have for Young Investigators?

If you love what you are doing and you are passionate about it, do not worry about success. Everything will fall in place and it will be a highly rewarding experience and a great career.

Seeking good mentors, role models, and collaborators is also very important for career development. It is so much fun to be able to discuss your ideas with colleagues and brainstorm over research challenges. Overall objectivity and challenging your own hypotheses are also important.

### What Personal Qualities of Yours Helped or Hindered You Along the Way?

My dedication, attention to detail, and punctuality helped. My continuous guilt feelings about work versus family took some of the fun away but also helped to balance my priorities.

### Can You Tell Me About Your Family?

My husband is a retinal surgeon and was born in my hometown in Greece. He was my biggest support and strength in my career development. He believed in me more than I did and encouraged me every step of the way. I was very lucky that a Greek man was so open-minded. We have 2 boys and they are both married with children. As undergraduates, both of them considered going into medicine, but they ended up with successful careers in the financial world.

### What Do You Like to Do When You Aren’t Working?

Spend time with our grandkids, travel, swim at the Greek beaches, and go shopping.

### Disclosures

None.

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