PROFILE

Profile of Raúl Padrón

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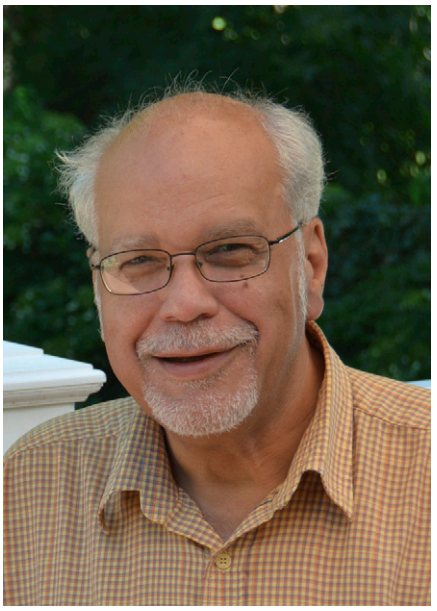
Over the past four decades, structural biologist Raúl Padrón has elucidated muscle contraction at the molecular and atomic level using a model system that he and his colleague Roger Craig developed: tarantula skeletal muscle. Padrón's research on how skeletal muscle thick filaments relax and become activated is helping to inform the molecular pathogenesis of human muscle diseases. For such advances, Padrón was elected as an international member of the National Academy of Sciences (NAS) in 2018; later, he emigrated to the United States due to his native Venezuela's ongoing political crisis. Now a professor at the University of Massachusetts Medical School, Padrón answers longstanding questions in his Inaugural Article (1) concerning striated muscle contraction that shed light on its underlying mechanisms in invertebrates and vertebrates.



Raúl Padrón. Image credit: Marie Craig (photographer).

Home Laboratory at Age 11

Padrón was born in Caracas to a concert pianist mother and pharmacologist and microbiologist father. Through his mother he gained a strong work ethic. He was also greatly influenced by his father, who maintained a home laboratory. "I still remember my surprise when he showed me paramecia swimming under a microscope," Padrón says. "Soon, I started raising yeast on chicken soup to see them under magnification."

When he was 11 years old, Padrón was permitted to have a separate laboratory, where he did chemistry and biology experiments and built electronic equipment. He attended San Ignacio High School in Caracas, where mathematician Angel Urmeneta and biologist Raphael Bredy reinforced his academic interests. In 1966, Bredy suggested that Padrón visit the Venezuelan Institute for Scientific Research (IVIC). During the visit, he met biochemist Karl Gaede, whose laboratory he joined at age 16. Padrón says, "Under Gaede's advice, I decided to study electrical engineering at the Central University of Venezuela, as he thought this would provide a good background for a career in biology."

Upon earning his undergraduate degree in 1973 under electrophysiologist Carlo Caputo, Padrón studied under molecular biologist Leonardo Mateu at IVIC for a master's degree in biology and a doctorate in physiology and biophysics, which he earned in 1979. His doctoral thesis was a study of the structure and function of the myelin sheath of toad and frog sciatic nerves and how the nerves respond to anesthetics (2).

Tarantula Model System

In 1980 Padrón began a postdoctoral fellowship at the Medical Research Council Laboratory of Molecular Biology (LMB) in Cambridge, United Kingdom. He grew increasingly interested in the work of structural biologist Hugh Huxley, a prominent researcher of muscle physiology who became his advisor. Padrón proposed studying giant barnacle muscle fibers to learn about their activation by calcium. Obtaining the animals proved challenging, so Huxley suggested that Padrón instead analyze the effect of the ATP analog AMPPNP on muscle structure. Padrón examined how this compound can arrest the cross-bridge cycle of muscular contraction (3). The cycle, previously identified by Huxley, consists of four basic stages that include the temporary attachment of the

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thick filament protein myosin with the thin filament protein actin to produce force.

At the LMB Padrón shared an office with fellow postdoctoral biologist Craig. The duo began a joint project to determine the structure of striated muscle thick filaments. Biophysicist John Wray, who had been obtaining X-ray diffraction patterns of invertebrate muscles, told them that such filaments in tarantula muscle were helically ordered and that they should view them using an electron microscope. They followed his guidance and, in collaboration with colleague Anthony Crowther, succeeded in producing the first 3D reconstruction of negatively stained tarantula thick filaments (4). The achievement, recounted in a book edited by Huxley (5), helped visualize the two heads of a myosin molecule in its native thick filament under relaxed-state conditions.

Myosin Interacting-Heads Motif

After completing their postdoctoral stints in 1983, Craig started his laboratory at the University of Massachusetts Medical School and Padrón began his at IVIC, where he also served as an associate, senior, and emeritus investigator. Padrón, who was awarded the Polar Prize in Biology by the Polar Enterprises Foundation in 1991, additionally served as a visiting professor at the University of Massachusetts, facilitating what has become a longstanding collaboration with Craig in studying the structure of muscle thick filaments.

In 1997 the Howard Hughes Medical Institute (HHMI) designated Padrón as an international research scholar, providing an initial 5-year grant that enabled him to found the Department (now Center) of Structural Biology at IVIC, equipped with a cryoelectron microscope, which permits detailed views of cellular and molecular structures. The HHMI provided two additional grants that supported Padrón's laboratory until 2011.

Using a comparable microscope at the University of Massachusetts, John Woodhead, Craig, Padrón, and colleagues produced an atomic model of a tarantula myosin filament in the relaxed state (6). The 3D model revealed that, in this state, the two heads of each myosin molecule pack together to form an asymmetric arrangement that inhibits their activity. The authors named the asymmetric head organization the myosin "interacting-heads motif" (IHM).

The discovery, which was commemorated on a Venezuelan postage stamp in 2007 and helped Padrón earn the 2008 National Prize of Science of Venezuela, enabled the researchers to provide a structural explanation for muscle relaxation and superrelaxation (high inhibition of ATP turnover) (7), as well as activation (8, 9). The researchers proposed that a cooperative phosphorylation activation (CPA) mechanism regulates myosin activity. The mechanism holds that the myosin heads are phosphorylated sequentially by the enzyme protein kinase C and a myosin light-chain kinase.

Motif Predating Animals

While analyzing the muscle structure of the parasite *Schistosoma mansoni*, Craig, Padrón, and their team observed an IHM identical to that identified in tarantula myosin filaments (10). They were surprised that, even though the spider and parasite are evolutionarily separate, the IHM and multiple other characteristics of these organisms' muscle structures are similar. Craig's postdoctoral scholar, Maria Zoghbi (a former student of Padrón), also showed that the IHM was present in mammalian cardiac muscle, broadening the significance of their finding in tarantulas (11).

To determine when the motif emerged, Craig, Padrón, and colleagues studied the structure of myosin molecules from sea anemones, which are the most primitive animals with muscles; sponges, which are believed to be the most primitive of all animals lacking muscle; and unicellular organisms (12). They identified the IHM, with slight modifications, in all of the studied species and in some unicellular organisms. Padrón says, "This remarkable conservation highlights the fundamental importance of the IHM as the structural basis underlying relaxation through inhibition of the heads, and is likely to underlie a critical energy-saving mechanism in cells."

Disease-Associated Mutations and IHM

In collaboration with Harvard University professors Christine and Jonathan Seidman, who study the genetic mechanisms of heart disease, Padrón and colleagues analyzed a model of human cardiac myosin (13). They discovered that mutations associated with inherited hypertrophic cardiomyopathy, which is a condition causing abnormal heart muscle thickness and cardiac dysfunction, cluster in the human cardiac IHM at sites of intramolecular interaction. The finding led to critical insights concerning the mechanistic basis for the hypercontractility, reduced diastolic relaxation, and increased energy consumption associated with the disease.

This and additional research conducted by Padrón's team and other laboratories is being applied to the development of therapeutics for cardiomyopathies. The Seidmans and biochemist James Spudich, for example, are cofounders of the California-based biotechnology company MyoKardia, which is currently conducting late-stage clinical studies of mavacamten, an investigational therapeutic being developed for the treatment of obstructive and nonobstructive forms of hypertrophic cardiomyopathy.

Challenges Posed by Venezuela Crisis

Numerous factors led to the collapse of Venezuela's economy, beginning in about 2010. Padrón says, "After my last HHMI grant finished in 2011, keeping my [laboratory] running in Venezuela became an extremely difficult task as the Venezuela crisis continued." Padrón and his dedicated team still managed to author a pair of seminal "Lessons from a tarantula" articles, reviewing their research concerning muscle thick filament and IHM structure, function, and implications for disease (14, 15).

As the socioeconomic and political crisis worsened, however, an estimated 5 million individuals left the country. Padrón and his wife, also an IVIC scientist,

became increasingly concerned. (Their four grown children had already left the country.) He says, "My election as an international member of the NAS in May 2018 opened a light at the end of the tunnel." The following month, Craig invited his longstanding colleague to join his laboratory at the University of Massachusetts. Padrón says, "It was a rescue effort that I accepted." He and his wife emigrated to the United States in November of that year. A handful of associates are currently maintaining Padrón's IVIC laboratory.

Structural Explanation

Padrón's Inaugural Article (1) represents an international effort involving researchers from his IVIC laboratory, Craig, and other colleagues in the United States, as well as scientists from Moscow State University's Institute of Mechanics. When the project was initiated in April 2018, Padrón's goal was to answer two key questions: How are myosin heads "turned on" to enable their binding to actin? And how, after tetanus, does a muscle produce a stronger twitch force than the twitch force produced before the tetanus? The latter phenomenon, discovered in 1865 by German physiologist Jonathan Ranke, is termed posttetanic potentiation.

The CPA mechanism previously proposed by Padrón helped to explain posttetanic potentiation, but he lacked direct structural evidence. In order to obtain it, Craig, Padrón, and members of their teams traveled to the Illinois-based Argonne National Laboratory Advanced Photon Source and collaborated with muscle X-ray diffraction experts Thomas Irving and Weikang Ma. He says, "We were able to place a complete live tarantula in a holder and apply high-intensity X-ray to a leg. We induced muscle contractions recording X-ray diffraction patterns every few milliseconds."

Analysis of the resulting time-resolved X-ray diffraction patterns and related data determined that the IHM is present in live tarantula muscle, and that the CPA mechanism explains the disposition of both myosin heads during tetanic and posttetanic states. The researchers observed that, after a tetanus, the released myosin heads slowly recover toward the resting, helically ordered state. During this time, they remain close to actin and can quickly rebind. Padrón says, "This enhances the force produced by posttetanic twitches, structurally explaining the posttetanic potentiation that was discovered by Ranke 155 years ago."

Craig, Padrón, and their colleagues continue to study muscle thick filament structure, function, and evolution, and their implications for human muscle diseases. They are now focusing on uncovering, by single-particle cryoelectron microscopy, the near-atomic structure of the IHM in isolated myosin molecules and thick filaments. Shixin Yang and Prince Tiwari in the Craig laboratory, together with Craig and Padrón, have recently obtained exciting new insights into the IHM, with a 4.3-Å resolution cryoelectron microscopy reconstruction (16). The structure reveals numerous new insights into myosin structure and its mechanism of inhibition, at the near-atomic level.

Despite the personal challenges that Padrón still faces, having left his homeland behind, he cannot imagine slowing down his schedule. As he says, "Structural biology is an endless story."

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