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MANUEL FRANCISCO MORALES
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A Biographical Memoir by

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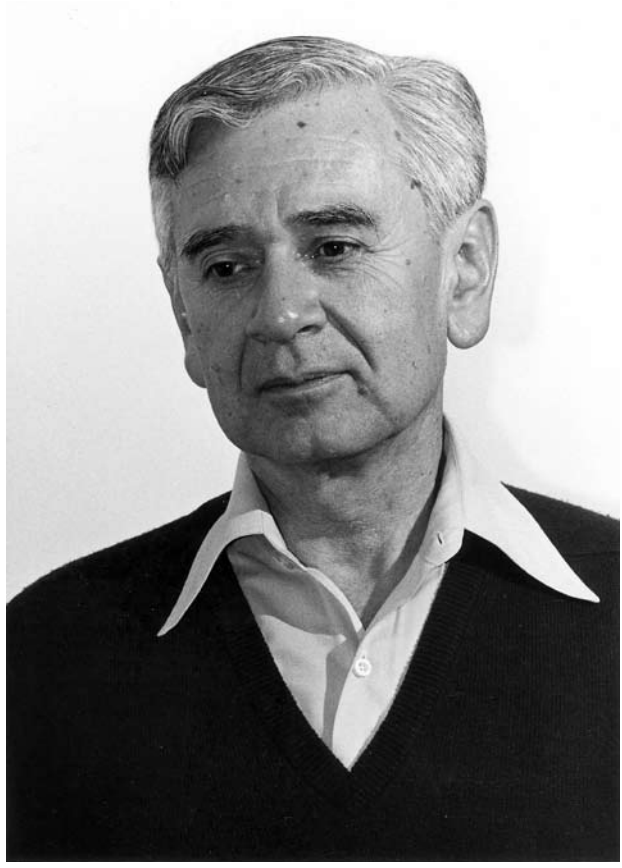
Biographical Memoir



Manuel F. Morales

MANUEL FRANCISCO MORALES

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BY ROGER COOKE AND STEFAN HIGHSMITH

MANUEL MORALES HAD A LONG and distinguished career devoted to understanding the molecular mechanism of force generation by actin and myosin in skeletal muscle. His published articles span an astounding 66 years from 1941 to 2007. Manuel brought a number of biophysical techniques into the field of muscle, including the use of fluorescence energy transfer to measure distances within proteins and fluorescence polarization and electron paramagnetic resonance to monitor both protein conformations and cross-bridge orientations in muscle fibers. He trained a large cadre of students, who populated the field of muscle and the wider field of biophysics. These included a number of students from Japan, a country where he forged connections with the laboratories studying muscle. In 1989 he was awarded the Order of the Rising Sun by the Japanese government in recognition of his contributions to Japanese science. He was president of the Biophysical Society in 1968 and was one of a very select number of individuals to receive a Career Investigator Award from the American Heart Association.

Manuel Francisco Morales was born in San Pedro Sula, Honduras, on July 23, 1919. He moved with his family as a child to San Francisco, California. After graduating from Balboa High School, he studied physiology at the Univer-

sity of California, Berkeley, graduating in 1939 with highest honors as a member of Phi Beta Kappa. He earned a master's degree in mathematics and physics at Harvard University, where he was a Whiting fellow, and returned to Berkeley to obtain a doctorate in physiology in 1942. During World War II, Morales served in the U.S. Naval Reserve as a line officer on the USS *Washington* and as a staff officer while working as an instructor in physics at Western Reserve University. He acquired U.S. citizenship in 1944 by virtue of wartime service in the U.S. Navy. After the war, he joined the faculty of the department of biophysics at the University of Chicago. During this time, he met and married Jean Botts, with whom he collaborated for most of the rest of his life. In 1949 he moved to the U.S. Naval Medical Research Institute, where he was chief of the physical biochemistry division. In 1957 he moved to Dartmouth University to chair the biochemistry department. In 1960 Morales moved to the University of California, San Francisco, where he had a position as professor and rapidly established a large research group. During that period, he married his second wife, Patricia Rainford. Required by law to retire from UCSF at age 65 (in 1984), he moved to the University of the Pacific School of Dentistry in San Francisco, where he continued working until his death on November 12, 2009.

The abbreviated timeline of Manuel's career shows a rapid ascent followed by a long career. His first 20 or so publications were in various areas where biophysics could be applied to questions in physiology. Like many fellow scientists during the war years, he used his background in physiology and physics to explore topics relating to very practical problems associated with the war effort. Papers published during this period included titles such as "Influences of Weather, Combat Status and Overcrowding on the Incidence of Disease and Accidents aboard Naval Vessels," and "Studies on the Effect

of Air-Cooling on Personnel aboard the USS Washington." Following his stint in the navy, Manuel returned to academic life.

His initial investigations after the war were concerned with the properties of enzymatic reactions, concentrating on the hydrolysis of ATP. In an era when the mistaken concept of a high-energy enzyme-phosphate bond was employed in bioenergetics, Manuel was first to show that transfer to and from ATP is a property of a reaction, not of any special bond. It was also he and his collaborators who first deduced correctly the standard free energy and enthalpy of ATP hydrolysis. He helped to develop several mathematical formulations that are now commonplace in enzyme kinetics, such as temporal periodicity, description of substrate-modifier systems, and the exact treatment of the time-dependent enzyme, enzyme•substrate, and enzyme + products case. He and his colleagues developed the very useful scheme for separating substrate from enzyme•substrate by using acrylamide quenching of the fluorescent analog, ε-ATP. However, he increasingly focused on the molecular mechanism of chemomechanical energy transduction by striated muscle.

Manuel's main contributions to science are in elucidating how muscle works. His first publication on muscle contraction was in 1946, and he stayed narrowly focused on that subject for the rest of his career. Manuel contributed to the characterization of the myosin system in these earlier years by measuring its colligative properties, for example, by establishing the molecular weight of myosin by light scattering, sedimentation, and osmotic pressure. He was first to provide a kinetic formulation of the myosin ATPase system. His work helped establish the concept that the rate limiting step in the ATPase cycle was the release of products, and that factors that destabilized the enzyme-products complex, removal of magnesium, modification of sulfhydryls, increased

the ATPase rate. These investigations also included interesting results on the influence of hydrostatic pressure on head-head interaction. He investigated the systematic effect of various ions in various catalyzed systems, an investigation that was useful in calling the attention of biophysicists and biochemists to the importance of the Hofmeister series and the work of von Hippel. This led, for example, to the replacement of chloride ions by carboxylate ions in studies of the contractile proteins. From the beginning Manuel thought the key to understanding force generation required identifying the forces created in the ATP site of myosin during hydrolysis, the pathway through myosin that increased its affinity for actin, and the moving part of myosin that accounted for the power stroke that had been measured by A. F. Huxley. The forces in the active site had to be large, and he started by measuring the repulsive electrostatic forces generated by ATP hydrolysis due to the negative charges of ADP and Pi. As the biological sciences became increasing molecular and atomic he drew on developments in other areas, mainly the more physical sciences, and applied them to understanding the mechanism of force production from ATP hydrolysis.

There were three optical spectroscopic methods that Manuel and his associates were first to exploit in the contractile system. One was the analysis of the frequency spectrum of noise from polarized fluorescence fluctuations in the active muscle. Another was time-resolved fluorescence anisotropy decay, where the sudden imposition of a direction in a random distribution of absorbers is followed by tracking (with fluorescence polarization) the effect of how rotational diffusion disperses the distribution in time. The third was fluorescence resonance energy transfer, where after placing an isotropic emitter at point A, examining whether a receiver at point B has its orientation sufficiently randomized so that the description of it as a dipole is reduced to one unknown

(the A-B distance). To apply these methods they devised the means of placing the emitters and receivers on their system (this came from their studies of cysteine modification in actin and myosin), and of interpreting their results in terms of the geometry of their system. It was by these means that they (1) first demonstrated the existence of repetitive cycling of cross-bridges, (2) observed the segmental flexibility in myosin and measured acto-myosin affinity, and (3) obtained proximity maps of S-1. In addition to the optical techniques, Manuel introduced electron paramagnetic resonance into the study of the contractile system. The results and insights obtained by these various techniques are summarized in more detail below.

Manuel introduced the application of transient fluorescence anisotropy decay (TRFAD) to rotational movement of segments of myosin and actin. Using fluorescent probes developed by Gregorio Weber, Manuel and his colleagues attached them to specific myosin amino acids to measure rotational times for specific domains. The chemistry of specific labeling, the hardware for a single-photon-counting device to measure the decay of fluorescence polarization following a brief but bright pulse of light, and the software to analyze it, all had to be developed. The apparatus was laboriously constructed by postdoctoral fellows, who had been trained in the field of physics. The software was developed by the same students. A series of papers had explored the reactivity of various amino acids in the myosin head, defining conditions that provided sufficient selectivity. Myosin is a large molecule composed of two heads attached at one end of a long coiled-coil rod. The two heads are the working end of the molecule, with sites to interact with actin and ATP. The TRFAD results demonstrated that myosin had dynamic flexibility at the motor-domain-myosin rod junction, as inferred from earlier electron micrographs. Flexibility at this loca-

tion in the molecule was a requirement of the swinging-oar theory of myosin function, and its observation provided strong evidence for the theory. Using another technique he introduced into the field—transient electric birefringence—a second dynamic flexible hinge within the myosin rod was also confirmed. Flexibility at this point in the molecule was required to enable the myosin heads to reach out from the backbone of the myosin thick filament and interact with actin. Together these conclusions provided strong support for the emerging rowing-oar model of myosin force production.

Another approach applied by Manuel to detecting the molecular geometry and movements involved in chemomechanical energy transduction was fluorescence resonance energy transfer (FRET), the molecular ruler developed by Lubert Stryer. This technique required the specific attachment of two fluorescent molecules and allowed one to measure the distance between the two. Morales and his colleagues began the mapping of distances between critical sites on the myosin motor domain. They made the first low-resolution structure of the actomyosin complex, which identified the relative locations of specific sites, such as the ATP binding site, the actin binding site, the most reactive cysteine (widely used in spectroscopic studies), and the light chains. Together TRFAD and FRET have been widely used in the field to measure changes in orientations and distances occurring during the ATP hydrolysis cycle in individual motor molecules and in muscle fibers.

When Manuel first introduced electron paramagnetic resonance (EPR) as a tool to investigate muscle contraction, the application of this technique to biological systems was very limited. Manuel recognized the utility of spin labels, developed by Hardin McConnell, as tools in the study of muscle contraction. Their application, however, was not straightforward. First, the reactivity of specific amino acids

had to be characterized. This was a daunting task in a system as large and complex as the actomyosin complex, or even much worse a muscle fiber. Manuel and his students were able to achieve sufficient specificity in the purified proteins, and amazingly in muscle fibers as well. EPR probes were applied to sorting out the conformational changes in the contractile proteins as they traversed their working cycle. However, the probes were particularly powerful as reporters of protein orientations in the organized array of the muscle fiber. The technique went on to become one of the premier techniques for revealing the details of the myosin power stroke, pioneered by Manuel and subsequently used by a number of other investigators, several of whom trained in Manuel's laboratory.

Manuel maintained an interest and expertise in mathematics throughout his career. A common experience, when working with him, was to exchange pages of handwritten mathematical schemes and proofs related to a subject at hand. He was a pioneer in the application of computer modeling to the study of muscle. He had an analog computer, a forerunner of the digital computer, that took up a considerable part of the room that housed it. A rigorous mathematical analysis was of course applied to the data, often obtained by biophysical techniques. His goal was to squeeze the maximum information from any experimental data. However, the application of mathematics went beyond data analysis to include modeling the kinetics and thermodynamics of the interactions of contractile proteins. This quantitative approach began early in Manuel's career, in collaboration with Terrell Hill, who went on to become the premier theoretician in the muscle field.

Over the course of his career Professor Morales educated several generations of scientists that are now working throughout the world. He understood that young scientists

needed to establish themselves as experts in areas that could be identified as their own, and he was helpful in achieving this important career goal. There were times when his focus on the contractile system of skeletal muscle could make it difficult for a colleague in his laboratory who was interested in pursuing a subject related but not central to the question of energy transduction. On the other hand, if one's interests were related to the mechanism of force generation, it was acceptable to pursue that interest independently. It was common in his laboratory that after an idea was developed and had led to a publication, one was free to continue with related work independently. He would decline coauthorship of manuscripts developed by members of his group unless he had made a major contribution to the study. This approach contributed to a striking feature of Manuel's career: the large number of scientists he mentored in the field of muscle biophysics. He typically had several postdoctoral students working with him. Many of these students had obtained their degrees in the physical and mathematical disciplines, and had come to Manuel's laboratory to begin their work in the biological sciences. Most of them continued to work in the field of biology and many remained in the field of muscle. Among his former students and postdocs are two distinguished members of the National Academy of Sciences and a very large number of full professors or equivalent. One of his former students started a major company producing chemicals used by researchers around the world. Manuel was particularly adept at instilling an interest in the problem of energy transduction in scientists from other fields, or who were beginning their own careers in biochemistry, biophysics, chemistry, physics, or physiology, getting them started and encouraging them to develop independence in the field. In this vein Manuel developed and maintained strong interactions with other individuals and laboratories throughout the

world working on muscle contraction and in other areas of science.

Manuel Morales was the recipient of many honors and awards: Phi Beta Kappa in 1938, Whiting fellow at Harvard University in 1940, Flemming Award in 1956 for U.S. federal service. He was American Heart Association Career Investigator VIII beginning in 1960. He was elected president of the Biophysical Society in 1968, where he initiated the National Lecturer event, now a central feature of the its annual meeting. He was a founding editor of the *Annual Review of Biophysics*. He was elected a member of the National Academy of Sciences in 1975. At the age of 80 he received a National Institutes of Health Merit Award in 1989. He was awarded the Order of the Rising Sun by the Japanese government in 1989. He was a Fogarty Scholar in Residence at the National Institutes of Health from 1991 to 1994. He was awarded an honorary doctorate from the University of the Pacific in 1992. He was elected a fellow of the Biophysical Society in 2000.

Morales was highly skilled at supporting his research program, a skill that he actively nurtured in his students. As an American Heart Association Career Investigator, his salary was independent of the institutions where he worked. This gave him a greater degree of freedom from departmental and institutional controls that are exerted on most faculty members. Other funding came from NIH and the National Science Foundation. He had many years of NIH program project support. He was frugal with grant funds, on several occasions returning unspent funds to an agency. He coached successfully many students and postdoctoral associates in their efforts to win fellowships and awards; for example, one of his postdoctoral fellows Cris dos Remedios won the first Louis Katz Award. This award is given each year by the American Heart Association for an outstanding paper in the

cardiovascular field by a young investigator. The paper that won the prize described the development of fluorescence polarization to monitor the orientations of myosin heads in a muscle fiber, work done under Manuel's supervision.

Manuel had a few interests outside of science. As an adolescent he was an enthusiastic basketball player, at about 5 feet, 8 inches in height. He liked to swim. He liked fast cars. He had a keen interest in national and international politics, using shortwave radio to tune into foreign news broadcasts. But these activities took very little of his time. His idea of a pleasant weekend afternoon was sitting in his car at the beach working out the theory of a physical method being used in the laboratory. He worked 25 years beyond what is a typical retirement age. Questions about what he might do if he retired generated a shrug; there really was not anything that approached the interest he had in his work. When he was in his seventies, one of his students, upon arriving at a conference, was asked about a rumor that Manuel was not at the meeting because he had died or retired. The student replied that he could not address the question of Manuel's death, as he had not seen him in several days, but he was absolutely positive that he had not retired.

Near the end of his life, with no grant support for his laboratory, Manuel continued to work in collaboration with Professor Hirofumi Onishi, one of his former postdoctoral students, now at the RIKEN SPring-8 Center, Kouto, Japan. His last publication, coauthored with Onishi, was in 2007; Manuel was 88. The title of this paper was "A Closer Look at Energy Transduction in Muscle." A sentence taken from the abstract shows the breadth of the approach used to combine biochemical and biophysical approaches to provide a more quantitative understanding of energy transduction: "Using results from conventional biochemistry, our own mutational studies, and diffraction images from others, we attempt, in

molecular detail, an account of a unitary interaction, i.e., what happens after a traveling myosin head, bearing an ADP-Pi, reaches the next station of an actin filament in its path."

It was a very appropriate title, and subject, which was in fact a summary of his life's work, and emblematic of Manuel's career, the paper was a collaboration with a professor in Japan.

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