THE DEPARTMENT OF
MOLECULAR BIOPHYSICS AND PHYSIOLOGY
Message from the Chairman, Bob Eisenberg

Physiology is the study of function and the mechanism of function of the various components of plants and animals. Physiological questions are asked of tissues at every level of structural complexity, ranging from questions of human behavior (how does a conscious human control the position of his joints?), to questions of the physics of ionic movement (how does an ion cross a membrane 3 nm thick?). Physiological questions have a unity because the evolutionary process that built biological structures is the same, whether the structures are enzymes or ankles. Physiological questions have a unity because the methods of describing and analyzing function are not diverse, even if the structures performing those functions are. For example, the mathematics describing the flow of current across membranes is intimately related to the mathematics describing the control system for movement: a linear differential equation is a linear differential equation, no matter what the meaning of its coefficients. The unity of physiology arises then from the questions it asks of biological systems and the unity of the mechanism that created those systems. The diversity of physiology arises from the range of systems about which those questions are asked. The excitement of physiology arises from the tension between its unity and diversity. When new systems arise, old questions are asked of them, at first. When new questions or methods arise in the new system, they are then applied to old problems. And applied to old problems, the new questions are often as revealing as when applied to the new systems, where they arose. Whether the unity of physiology is a reflection purely of reality (that is, of the unity of biology), or a reflection of the paucity of our knowledge, or even a reflection of the inherent limitations of our nervous system, is not known. Probably all three.

Graduate Studies

The program leading to the PhD provides training in quantitative areas of modern physiology and biophysics for those interested in pursuing a career in research. Programs of study are individualized, and most training occurs in the research laboratories of faculty members. The PhD program is directed by Fredric Cohen, PhD: fcohen@rush.edu.
Multicellular organisms coordinate the activity of individual cells through signals that determine metabolic changes. Inside cells, signals are often transient changes in $[\text{Ca}^{2+}]$. The Section of Cellular Signaling studies these changes and tries to understand their varied mechanisms and roles within healthy cells, as well as their alterations and failure modes in a number of diseases.

The section was designed to be an environment of collaboration, where these studies, which are necessarily carried out at various and diverse levels of complexity, from aqueous solutions all the way to full animals, could be easily integrated among these diverse levels. As a result, the interested students can choose to participate, under the same roof, in all of the following:

- Atomic-level studies of ion permeation through channels (cf. Gillespie and Eisenberg lab descriptions)
- Single-molecule-level studies of permeation, gating, or regulation of ion channels in lipid bilayers or on-cell patches (cf. Fill and Ramos-Franco descriptions)
- Sub-cellular (organellar) and cellular level studies of ion movements and their regulation, largely on skeletal and cardiac muscle (cf. Ríos, Zhou, Blatter and Shannon descriptions)
- Studies at the tissue or organ level, especially as regards whole hearts in healthy or diseased conditions, or skeletal muscle in mice with mutations that lead to conditions that model human hereditary diseases

The goal of “vertical” integration among these levels or research has been accomplished, as best exemplified by collaborative publications.

A second goal of the section is to facilitate the formal teaching of molecular biophysics, physiology and related subjects. The ongoing collaborations within the section foster interesting interdisciplinary courses, an example of which is “Fundamental Basis of Molecular Biophysics and Physiology.” This course is presented in the form of an ongoing Journal Club, in which the faculty present lectures on fundamental topics of their expertise, while the students contribute readings of publications, selected by the faculty to illustrate and reinforce the fundamentals.

Address questions regarding the Section of Cellular Signaling to its section director, Eduardo Ríos: erios@rush.edu.
As stated before, Ca\(^{2+}\) signaling is ubiquitous and crucial. The laboratory of Eduardo Rios, PhD, is interested in understanding “Ca signaling.”

In “electric signaling” — when Na\(^{+}\) enters a cell during action potentials — the message is electric; Na\(^{+}\) is just the medium that carries it. By contrast, in Ca\(^{2+}\) signaling, Ca\(^{2+}\) is the medium and the message. While electric signals move at meters per second, Ca\(^{2+}\) signals move at the slow pace of diffusion. For this reason, Na\(^{+}\) and K\(^{+}\) channels can be sparsely distributed and gate independently; intracellular Ca\(^{2+}\) release channels instead must cluster and rely on mutual interactions to gate open together. Only then can they achieve the high local gradients necessary to effectively supply Ca\(^{2+}\) to its targets.

The basis of the mutual interaction is Ca-Induced-Ca-Release, or CICR. CICR inherently leads to unstable, all-or-none behavior; however, Ca\(^{2+}\) signals stay graded with stimuli. A powerful Ca\(^{2+}\)-dependent inactivation (CDI) is needed for signals to remain graded. CICR and CDI are the necessary yin and yang of Ca signaling.

But that is not all: There is evidence for a third mechanism, intra-store Ca\(^{2+}\) control, or ISCC, whereby release channels are activated by high loads and deactivated when load decays.

These mechanisms constitute the focus of our research, which is conducted on skeletal and cardiac muscle using a variety of molecular and cellular approaches. For example, to understand ISCC we are developing novel methods to image quantitatively [Ca\(^{2+}\)] inside cellular organelles (nuclei, SR, mitochondria). The figure illustrates work on a nuclear Ca\(^{2+}\) sensor.

Excitation-contraction (E-C) coupling, the process that translates muscle membrane depolarization to increase in intracellular [Ca\(^{2+}\)] and contraction, is the first recognized example of Ca signaling. Our group strives to understand E-C coupling at the molecular and cellular levels. These questions transcend muscle physiology; indeed, the concepts emerging from the muscle studies find general applicability, given the widespread distribution of Ca signaling in other types of muscle, nerve cells and other tissues.

Here we describe the general aims and approaches of our research. Readers interested in current issues should see our website: http://www.phys.rush.edu/ERios/physiorio.html.

Approaches are cellular and molecular. Cellular methods include recording of calcium changes “globally” (that is, averaged over the whole cell) or locally, which is done by confocal microscopic imaging. The study of local events started in cardiac muscle with the discovery of Ca\(^{2+}\) sparks (Cheng et al., Science 1993), followed in skeletal muscle with our description of sparks (Tsugorka, Rios and Blatter, Science 1995). While they are local, sparks were soon found to involve multiple intracellular Ca channels. Example of studies of calcium spark include Launikonis et al. (PNAS USA 2006) and Rios et al. (J Gen Physiol 2008). While sparks were found to be key components of the signals in muscles of amphibians, they were decidedly non-physiological in the adult mammal.

The definition of processes and mechanisms is often achieved through formal quantitative modeling. In this paper we introduced the “coupon” as the functionally relevant multi-channel unit: Stern, Pizarro and Rios: “A local control model of excitation-contraction coupling in skeletal muscle” (J Gen Physiol 1997).

More recent examples of work at the global level include the following:


Molecular approaches include modifying the endowment of native proteins — or adding foreign ones — and then exploring the functional consequences of these changes. An example: Pouvreau et al.: “Ca\(^{2+}\) sparks operated by membrane depolarization require isoform 3 ryanodine receptor channels in skeletal muscle” (PNAS USA 2007). This study demonstrated that the functional differences among species are due to the absence of a specific version of the Ca release channel (RyR3) in the muscle of mammals.
The laboratory of Dirk Gillespie, PhD, uses theories of liquids (such as density functional theory, DFT) to model ion movement. All projects have a very close relationship with experimental groups that provide data and test the models’ predictions. Of particular interest are the following:

- Ryanodine receptor (RyR) and L-type calcium channels in muscle, which are involved in excitation/contraction coupling, to understand how ions move through these pores (permeation) and what determines which ions are conducted (selectivity). The physiological consequences of these mechanisms (and their disruption in disease states) are also studied.
- The excitatory effect of Ca\(^{2+}\) release on neighboring RyRs (Ca\(^{2+}\)-induced Ca\(^{2+}\) release, CICR). The goal is to understand the mechanism of CICR, the mechanism for stopping CICR, and changes in CICR during disease states.
- Understanding and developing new nonbiological nanofluidic devices, devices that move electrolytes through nanometer-sized pores to create current. The goal is to understand their physics and to predict new unique device properties for future applications.

The laboratory is best suited for those with a background in physics and math who are interested in using, developing or implementing new modeling techniques, within an environment of collaboration with both theorists and experimentalists.

**SELECTED PUBLICATIONS**


Contact: Dirk_Gillespie@rush.edu
Amyotrophic lateral sclerosis (ALS) is a fatal neuromuscular disorder characterized by degeneration of motor neurons and atrophy of skeletal muscle. The laboratory of Jingsong Zhou, PhD, discovered hyperactive Ca release events in skeletal muscle of a transgenic mouse model of ALS. Structurally and functionally altered mitochondria (see figure) were also identified and are likely responsible for the uncontrolled Ca signaling. Muscle cells use Ca as a messenger to control their contraction, while excessive elevation of Ca inside the cell can cause cell death. Two major organelles tightly control the intracellular Ca level, the sarcoplasmic reticulum (SR) and mitochondria — a “power house” that can also produce detrimental oxidative stress. The SR and mitochondria are intimately associated in muscle cells. Combining gene targeting, transgenic animal models and electrophysiology with confocal imaging, this laboratory aims at a comprehensive understanding of the cellular mechanism that contributes to the progressive decline in mitochondria-SR coupling that leads to the compromised Ca signaling and muscle atrophy in ALS.

SELECTED PUBLICATIONS


Contact: Jingsong_Zhou@rush.edu
The laboratory of Josefina Ramos-Franco tries to understand intracellular Ca\(^{2+}\) signals arising from opening of inositol trisphosphate receptor (IP\(_3\)R) channels, which are present in intracellular organelles (ER to nuclei).

The lab is currently delineating IP\(_3\)R local control mechanism(s) in both cardiac myocytes and endothelial cells. Ongoing work seeks to define the control and physiological role(s) of IP\(_3\)R Ca\(^{2+}\) signals in heart. This includes determining how these signals can be effective in the presence of the ryanodine receptor-mediated “Ca\(^{2+}\) noise” of normal cardiac excitation-contraction coupling.

A developing interest is the IP\(_3\)R regulation by intra-organelle Ca\(^{2+}\) levels. This line of research includes site-directed mutagenesis to identify key residues of the Ca\(^{2+}\) sensing site in the IP\(_3\)R molecule.

The lab applies a multidisciplinary approach at different levels: Global intracellular Ca\(^{2+}\) and electrical signals are recorded in whole hearts. Local Ca\(^{2+}\) signals, called puffs, are imaged in isolated cells. Single IP\(_3\)R channel Ca\(^{2+}\) currents are measured in artificial bilayers. Individual IP\(_3\)R amino acids are manipulated in vitro and mutant channels expressed in cell lines. The goal is to define how IP\(_3\)R are controlled in cells in order to understand both how IP\(_3\)Rs contribute to malfunction in disease and hopefully determine how their function can be manipulated to therapeutic advantage.

**SELECTED PUBLICATIONS**


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Research in the laboratory of Wayne Chen, PhD, is directed towards understanding the structure/function relationships and physiological roles of the calcium release channel (ryanodine receptor) (RyR). Current research in the laboratory focuses on the following:

- The molecular and cellular basis of cardiac arrhythmia and sudden death
- The molecular actions of pro- and anti-arrhythmic drugs and the development of novel anti-arrhythmic therapies
- The molecular mechanisms of RyR activation by cytoplasmic and luminal calcium
- Regulation of RyR by phosphorylation, calmodulin and other protein regulators
- Three-dimensional structure of RyR

Techniques used in our studies include site-directed mutagenesis, single channel recordings in planar lipid bilayers, fluorescence Ca\(^{2+}\) imaging, purification of recombinant RyR proteins for biochemical and structural studies, cryo-electron microscopy, and gene targeting in mice.

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The research program led by Lothar A. Blatter, MD, PhD, focuses on the role of calcium ions in the regulation of cellular functions in the cardiovascular system. On the one hand, we investigate, at the cellular and subcellular levels, the pathways and mechanisms through which calcium itself is regulated in cellular compartments such as the cytosol, the sarcoplasmic reticulum and mitochondria. On the other hand, we study how specific changes in cellular calcium concentrations control functions of cardiac myocytes and vascular endothelial cells. Of particular interests in cardiac cells are the regulation of calcium during excitation-contraction coupling (i.e., the rhythmic elevations of calcium that lead to contraction with every heartbeat) and in excitation-transcription coupling, where we investigate the sources and specific role of calcium for the activation of transcriptions factors (such as NFAT) that are involved in pathological cardiac remodeling. We are further interested in studying the specific changes in calcium signaling that occur in the diseased heart — e.g., in cardiac hypertrophy, heart failure and arrhythmias. In vascular endothelial cells, we are interested in the interplay between calcium signaling and the generation of nitric oxide, an important endothelium-derived relaxing factor through which the vascular endothelium contributes to the control of blood flow and blood pressure. For the study of these calcium-signaling processes, we employ a wide palette of methodological approaches, including high-resolution confocal imaging using a wide spectrum of fluorescent probes, electrophysiology (patch clamp and lipid bilayer single channel recordings), photolysis of caged compounds, molecular biology and biochemical approaches, and transgenic animals.

An important area of research (led by Elena N. Dedkova, PhD, and Dr. Blatter) centers around the role of mitochondria in cardiovascular function. This research investigates the contribution of mitochondria to the regulation of cytosolic calcium concentration through its capacity to store and release calcium ions. This research also investigates the role of calcium ions for the regulation of mitochondrial functions, including energy metabolism and ATP production, regulation of mitochondrial ion channels such as the mitochondrial permeability transition pore, and control of the cellular redox state and protection against oxidative stress.

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The laboratory of Bob Eisenberg, PhD, is focused on using physics, chemistry and mathematics to understand how ion channels and enzymes work.

Selectivity of calcium and sodium channels can be explained nearly quantitatively with the same physical model, in an enormous range of solutions and conditions, and in both types of channels. Recent efforts are to understand and extend this finding.

For example, a new mathematical method called energetic variational analysis is being used to make predictions of current voltage relations from the equilibrium-binding analysis of previous papers.

Another example: A new experimental channel has been developed to allow the reproducible and easy study of currents from single channels in a wide range of conditions. This channel, NanC, has a known 3D structure, and mutants can be built with standard methods that collaborators are eager to crystallize and measure. Theory and simulations can be focused then on a complete system, in which everything can be done and tested with relative ease.

Recently, we have started looking at enzymes in this light. A scan of some 150 enzymes of known structure shows that they have enormous densities of charged side chains, as do calcium and sodium channels. The mean is around 20 molar. For comparison, solid NaCl is 37 molar. Biology must use this very special physical system of highly concentrated charges for a reason, even though we do not yet know what that reason is.

Dr. Eisenberg’s group will try to exploit these advances using whatever it takes to get answers, whether designing and building amplifiers, picovalves, single channel measurements, stochastic or variational theories, etc.

SELECTED PUBLICATIONS


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Cell membranes are not homogeneous; particular proteins and lipids assemble within microdomains. Proteins that participate in a wide range of cellular functions, such as intracellular signaling, are thought to be enriched within the same domains, allowing them to interact quickly with each other. To determine the protein and lipid compositions of membrane domains, they must first be isolated. Unlike all other labs that work at 4°C, we utilized a number of biophysical principles to develop a means to isolate domains from cells at physiological temperature, 37°C.

We are utilizing these procedures to follow movement of proteins within plasma membranes that results from trans-activation of the epidermal growth factor receptor (EGFR) and have shown how the three key membrane-signaling molecules of trans-activation — EGFR, TACE, and TGF-a — come into close contact. By following the movement of additional membrane proteins and translocation of proteins between the plasma membrane and cytosol, we are characterizing the mechanisms that underlie the cellular changes that occur upon stimulation by growth factors.

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Current members of the laboratory of Thomas E. DeCoursey, PhD, include Vladimir Cherny, Deri Morgan and Boris Musset. Our main interest over the past decade has been in two molecules that reside in the membranes of white blood cells. These are proton channels and NADPH oxidase. Both play vital roles in white blood cells when these cells kill bacteria and other microbial invaders. When NADPH oxidase does not work, white cells cannot kill many types of bacteria. Patients afflicted with hereditary chronic granulomatous disease (CGD) lack this enzyme, and if not treated often die in childhood of recurrent infections.

We have shown that inhibiting proton channels prevents NADPH oxidase from working (DeCoursey et al., 2003, Nature 422:531-34) and that this results from the effects of proton channels on membrane potential and pH (Morgan et al., 2009, Proc. Natl. Acad. Sci., USA 106:18022-27). We found that in human basophils, inhibiting proton channels prevents histamine release (Musset et al., 2008, Proc. Natl. Acad. Sci., USA 105:11020-25). We continue to investigate roles played by proton channels in a variety of cells, such as B-lymphocytes (Capasso et al., 2010, Nature Immunol. 11:265-72). We recently discovered a new proton channel gene in a dinoflagellate, which we believe may trigger the bioluminescent flash produced by these creatures when seawater is disturbed at night.

Another focus of our current research is understanding how the proton channel works on a molecular scale. We design mutations to the protein, express the mutant channels in cultured cells, and then record from the cells to determine how the mutation affected the function of the molecule. For example, we found that the regulation of channel activity by phosphorylation occurs at one specific threonine residue in the intracellular part of the channel (Musset et al., 2010, J. Biol. Chem. 285:5117-121). The figure shows how we believe the proton channel dimer is assembled (Musset et al., 2010, J. Physiol. 588:1435-49). Because the proton channel gene was identified only in 2006, much work remains to be done.

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Michael Fill, PhD, defines mechanisms that control intracellular Ca^{2+} signaling in excitable cells — specifically, those attributed to the ryanodine receptor (RyR) Ca^{2+} release channel found on the surface of intracellular Ca^{2+} storage organelles. These channels control a wide array of cellular phenomena, from contraction to secretion, in many types of cells. Thus, the fundamental RyR control mechanisms represent broadly applicable points of potential pathological failure and therapeutic intervention. The lab’s most recent efforts have focused on cardiac muscle because it is exceptionally RyR rich and has robust intracellular Ca^{2+} signaling. As in many tissues, neighboring RyRs work in concert to generate localized Ca^{2+} signals, called sparks, and abnormal RyR function can lead to heart failure and arrhythmias. To explore cardiac RyR control, the lab isolates single channels from cells and examines their function in artificial membranes using a wide array of experiment tools (genes to lasers). The information gained is interpreted using parallel spark measurements in cells. The goal is to define and understand the local RyR control mechanisms that govern the spark and its role in certain cardiac pathologies. Exemplifying other collaborative efforts within the Section of Cellular Signaling, the Fill laboratory has published with Eduardo Ríos, PhD, and coworkers (e.g., Kettlun et al. 2003); with Dirk Gillespie, PhD, and coworkers; and with Lothar Blatter, MD, PhD, and colleagues.

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