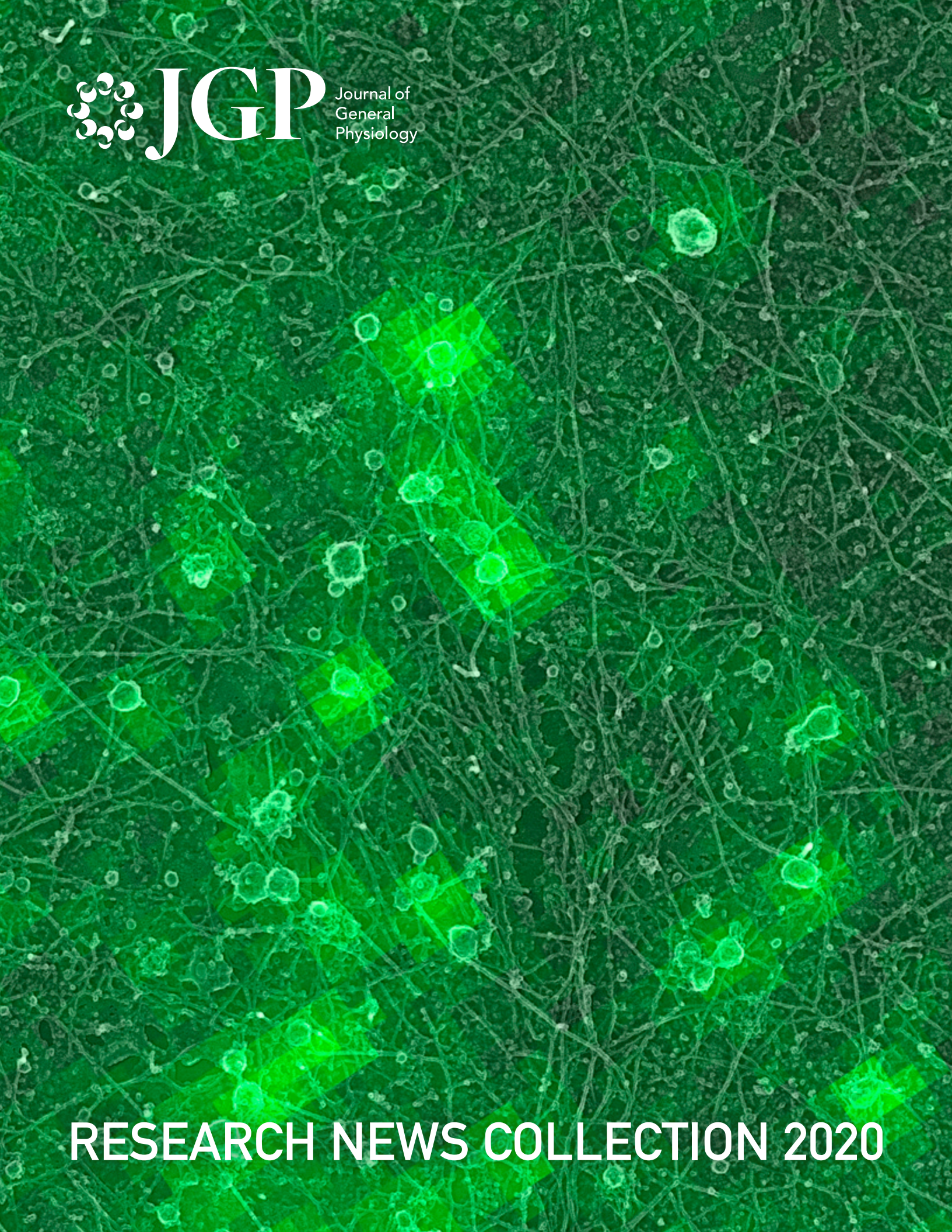
JGP
Journal of
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JGP Journal of General Physiology

RESEARCH NEWS COLLECTION 2020

The *Journal of General Physiology* (*JGP*) is pleased to present this collection of recently published articles that elucidate new advances in mechanistic and quantitative cellular and molecular physiological research. If you enjoy this collection, please visit jgp.org to sign up for email alerts and receive the latest articles in your inbox.

Brochure articles by Caitlin Sedwick, PhD, and Ben Short, PhD; Editorial by *JGP* Editor-in-Chief David Eisner

Design by Christine Candia

On the cover: Secretory vesicles containing MMP9-GFP near the plasma membrane of MCF-7 breast cancer cells. Overlay of TIRF MMP9-GFP image (green) and platinum replica TEM image (gray) of a MCF-7 cell plasma membrane sheet. Image © 2019 Stephens et al. Read "Determining the dynamics of cancer cell secretion."

CLIMBING ABOARD

AN EDITORIAL BY *JGP* EDITOR-IN-CHIEF DAVID EISNER

The beginning of the year is traditionally a time for resolutions, and beginning a term as a new Editor-in-Chief is an added stimulus to take stock. I assume the position of Editor-in-Chief with considerable pleasure and pride but also a certain amount of trepidation. It seems only yesterday that I was an undergraduate studying physiology and reading papers published in the *Journal of General Physiology.* It is also no easy task to succeed Sharona Gordon, who has done an outstanding job.

In the 100 years since *JGP* was founded, its development has paralleled that of the subject of cell and membrane physiology (Hille, 2018). In his fascinating history of the journal, Olaf Andersen, a previous Editor-in-Chief, reminded us that the term "general physiology" was first coined by Claude Bernard in 1885 (Andersen, 2005). The first paper published in *JGP* (Osterhout and Haas, 1918) studied photosynthesis in algae and used an optical indicator (phenolphthalein) to measure rates. This paper set a theme for *JGP* by comparing experimental observations with the predictions of mathematical models (Jennings, 2018). 100 years later, optical methods are very much still part of the exciting and cutting edge techniques used by many biologists and championed in articles published in *JGP.* The 100th anniversary of *JGP* was marked by the publication of a collection of historical and retrospective articles. If you have not read them already, I strongly recommend them. There are too many riches to mention all and so I only refer to those that particularly appeal to my personal interests.

Some areas of science underpin so much of modern physiology that it is important to be reminded of their development, and I note a few areas where articles in *JGP* have been drivers of the development of the fields with Milestones that summarize *JGP*'s contributions: regulation of cell volume (Kay and Blaustein, 2019); the constant field equation and its link to the membrane potential (Alvarez and Latorre, 2017); the physiology of Na and K channels (Bezanilla, 2018a); calcium and muscle contraction (Ríos 2018); and photoreception (Pugh, 2018). Another theme that runs through *JGP* is the link between structure and function (Franzini-Armstrong, 2018). Today, this is not only important at the level of the cell but also at the level of the molecule (Robertson, 2018).

In general physiology, as in all other areas of science, advances depend on many factors, including conceptual breakthroughs and the development of new techniques. No less important, however, is the academic environment in which the work is done. The success of modern general physiology can be traced back to institutions as far apart as Chile (Bezanilla, 2018b), Russia (Sobolevsky, 2018), and New Haven (Aldrich, 2018), as well as my alma mater (Hodgkin, 1976). As scientists, we take it for granted that research depends on international collaboration. There have been many occasions over the last 100 years when this has been impossible and, even today, the ability of scientists to travel between certain countries is limited. I think it is essential that scientific journals and societies stand up for the

international nature of science. Another important factor is the level of support that students and junior scientists receive from their laboratory heads. Many of the most successful scientists of today have benefited greatly from committed mentoring (Clapham, 2018; Miller, 2018; Carrasco, 2019). But we all know that many junior scientists (students, fellows, and junior faculty) do not receive the mentoring they need, and I thank Sharona Gordon for her initiative to establish the Junior Faculty Networking Cohort system (Gordon, 2017), which has made *JGP* an active participant in the development of the next generation of general physiologists. This is a clear example of how a journal can add value, which, together with Sharona's unstinting efforts

to further increase the scientific stature of *JGP,* sets the path for moving forward.

Colleagues ask me what I want to achieve in the next three years with *JGP.* One obvious answer is, "It ain't broke, so don't fix it." That said, I would like to expand the influence of *JGP* beyond its current core community. While *JGP* is appreciated by those whose primary interest is in the fundamental properties of channels, transporters, and other properties of membranes, it is less recognized by others who are primarily concerned with the effects of these proteins on organ function. This is despite the fact that recent issues of *JGP* contain many articles with direct relevance to these broad communities; we need to be better at highlighting *JGP*'s contributions and our wish to publish papers that make important contributions to a wide range of problems. To paraphrase from our Aims and Scope, if your work elucidates mechanisms of broad physiological significance, then come to us!

Finally, please feel free to contact me with suggestions for *JGP.* Let me know what you like and, equally important, what you don't.

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◊ Alvarez, O., and R. Latorre. 2017. *J. Gen. Physiol.* https://doi.org/10.1085/jgp.201711839
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HOW A MUTATION UNDERMINES CARDIAC FUNCTION

Researchers explore how a mutated troponin T causes cardiac hypertrophy

Hypertrophic cardiomyopathy (HCM), a common disease of the heart that can cause sudden death, is often linked to mutations in the proteins that make up the contractile unit of cardiac muscle, the sarcomere. In their paper in *JGP,* Piroddi et al. explore how a mutated version of the regulatory protein troponin T alters heart muscle function (1).

"We're trying to understand the impact of mutations leading to congenital heart disease, and we do that by investigating sarcomere function from human surgical samples," says Corrado Poggesi, a Professor of Physiology at the University of Florence in Italy.

In cardiac muscle sarcomeres, the motor protein myosin produces contractile force by forming cross-bridges with and pulling against actin-based thin filaments. Myosin's access to its actin binding sites is controlled by thin-filament proteins such as tropomyosin, and the troponin subunits C, I, and T. In the absence of calcium, tropomyosin blocks myosin from binding actin. But when calcium floods the cytoplasm after the arrival of an electrochemical action potential at the cell membrane, it binds to troponin C, which undergoes a conformational change that's communicated to tropomyosin by troponin T. This shifts tropomyosin out of the way, allowing myosin to do its work.

Prior studies by Poggesi's laboratory have demonstrated that some mutations in myosin can affect the energetic efficiency of cardiac contraction by changing how the protein binds to actin (2). But recently, as part of the European "Big Heart" research consortium, Poggesi's group had the opportunity to study the heart tissue of a young man with severe HCM caused by a mutation in the C-terminal region of troponin T (3).

Nicoletta Piroddi, an Assistant Professor working in Poggesi's lab, headed a multi-center effort to understand how this mutation affects cardiac muscle function.

A study by Nicoletta Pirrodi (left), Corrado Poggesi (center), and colleagues (not shown) show that the K280N mutation in troponin T increases the energetic cost of cardiac muscle contraction (see graph) by accelerating cross-bridge kinetics (not shown). Photos courtesy of the authors.

"The impact of mutations associated with HCM on sarcomere function is often difficult to identify in cardiac preparations from humans," notes Poggesi. One reason for this is that most patients are heterozygous for sarcomere protein mutations, so the mutant proteins can be variably expressed throughout the heart. But Piroddi et al. found that this patient was homozygous for mutated troponin T, so they began their studies by isolating myofibrils from the patient's tissue and comparing their mechanical properties with myofibrils from normal donors, from patients with aortic stenosis, and from patients who had HCM but no mutations in sarcomere proteins. "Because we were comparing a single patient with many different controls, it was a challenging statistical problem to determine what the meaningful differences were between the patient and the controls," says Poggesi.

Cardiac tissue can undergo molecular remodeling to try to compensate for impairments in heart function, which can obscure the effect of the mutated protein (4). Piroddi et al. addressed this problem by conducting troponin replacement experiments (5). They studied the mechanics of the patient's myofibrils in which the mutant troponin was replaced with normal protein, and of normal myofibrils where the protein was replaced with the mutant version.

"In the presence of the mutant protein, there was a significant change in cross-bridge kinetics and in the energetic cost of tension generation," says Poggesi. The mutant troponin accelerated both formation and detachment of cross-bridges; the latter resulted in higher energy consumption for muscle contraction. This was accompanied by higher tension in resting myofibrils, suggesting a cellular basis for the diastolic dysfunction observed in HCM disease.

Interestingly, the functional effects of the mutated troponin strongly resemble what has been observed in some myosin mutants, but the underlying molecular mechanism is unknown. This needs more investigation, observes Poggesi.

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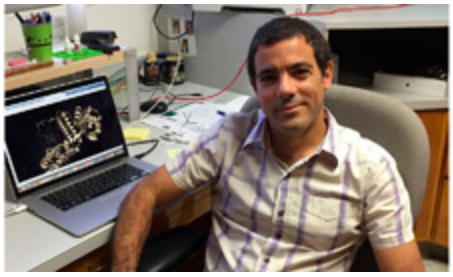
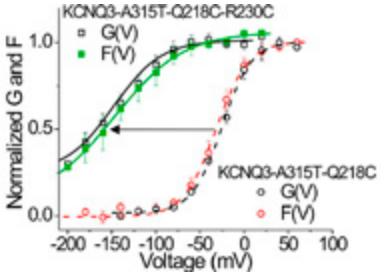
INVESTIGATING AN EPILEPTOGENIC MUTATION

Study examines how a mutation in KCNQ3 affects channel behavior

There are more than 100 naturally occurring mutations that cause neuronal hyperexcitability, which results in a variety of disorders. For example, mutations in KCNQ voltage-gated potassium channels are commonly associated with epilepsy. One such mutation, the substitution of a cysteine in place of an arginine at residue 230 in the KCNQ3 subunit, is linked with a particularly severe form epilepsy called epileptic encephalopathy. A *JGP* paper by Rene Barro-Soria explores how this mutation affects the channel's function (1).





Rene Barro-Soria shows that the R230C mutation in KCNQ3 (solid lines in graph) shifts channel closing to extreme negative membrane potentials. Photo courtesy of the author.

In the nervous system, KCNQ3 assembles into an ion channel with another KCNQ subunit, KCNQ2. Shifts in membrane voltage prompt a conformational change in the channel's voltage-sensing domain that controls the opening of the transmembrane pore. The channel is normally closed at the resting neuronal membrane potential (about −70 mV), but prior studies (2, 3) have established that the R230C mutation, found in the voltage-sensing region of KCNQ3, causes the channel to remain open and act as if it were constitutively active. Although this might be expected to inhibit neuronal excitability by driving membrane hyperpolarization, the R230C mutation instead generates hyperactivity in the brain, possibly by impairing the inhibitory interneurons responsible for tamping down the excitability of other neuronal types (3). But this is just one of the mysteries regarding this mutation.

"There is a really big need to understand how these channels work, but surprisingly little is known about how they open and close," explains Barro-Soria, an assistant professor at the University of Miami in Florida. "The mutated channel remains open at physiological voltages. One hypothesis to explain this is that the voltage sensor may be locked in its activated position so that the gate is always open. Alternatively, the mutation may let the voltage sensor move up and down but uncouple it from the gate's opening, or it could shift the membrane voltage at which the channel closes."

To explore these hypotheses, Barro-Soria expressed KCNQ3 as a homomer in frog oocytes and used voltage clamp fluorometry (4) to simultaneously monitor the channel's electrophysiological properties and movement of its voltage sensor. These experiments demonstrated that R230C KCNQ3 is not locked open. It is capable of closing, but only does so at membrane voltages lower than −100 mV. At these nonphysiological voltages, however, movement of the channel's voltage sensor was still tightly coupled to opening of its gate.

"This implies that compounds that shift the voltage dependency to more positive voltages would actually promote gate closure, and hence may have therapeutic potential," notes Barro-Soria.

For insight into the physical mechanism that drives this behavior, Barro-Soria examined the structural features of the mutation. It is expected that having a positively charged amino acid, like arginine, at position 230 may be important because charged residues can both sense electric fields and form electrostatic interactions with other moieties within the molecule. To confirm this, Barro-Soria substituted R230 with lysine, another positively charged amino acid. Surprisingly, lysine only partially restored the normal gating behavior of the channel. Barro-Soria hypothesized this could be because lysine, though charged, is physically smaller than arginine. To test this idea, he substituted in an artificial amino acid called citrulline that is structurally similar to arginine but lacks a positive charge (5). Citrulline also shifted channel opening back toward less-negative voltages (albeit much less than lysine did), suggesting that both the charge and physical size of the arginine at position 230 is important for channel gating.

Determining whether the R230C mutation affects channel gating by destabilizing its closed state or by stabilizing its open state will require additional work. Having just started his own laboratory, Barro-Soria is also interested in identifying compounds that could help treat epilepsy. "I'm looking for students!" he says.

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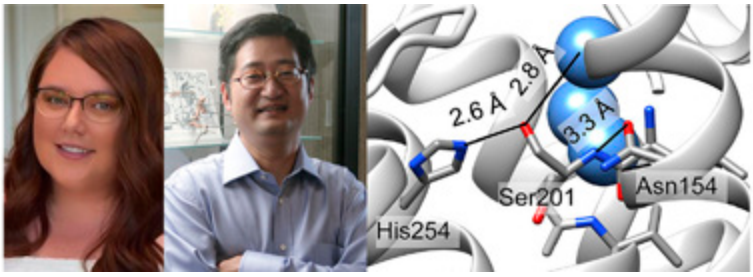
RHOMBOIDS MAKE DO WITH A WEAK HYDROGEN BOND

Researchers explore the strength of the hydrogen bond network at the active site of GlpG

Rhomboids are proteases that specifically cleave membrane proteins, a process that can result in release of various growth factors and transcription factors, and even activation of other enzymes (1). Although rhomboids are related to the larger class of serine proteases, their rate of catalysis is unusually slow (2). In their article article published in *JGP*, Gaffney and Hong investigate the bacterial rhomboid protease GlpG, providing important insight into how this class of protease functions (3).



Kristin Gaffney (left) and Heedeok Hong (center) studied the hydrogen bond between serine and histidine at the active site of the bacterial rhomboid protease GlpG (ribbon diagram at right; water molecules depicted in blue). Photos courtesy of the authors.

Serine proteases are so named because the region that performs catalysis, the active site, contains the amino acid serine. This amino acid residue carries out a nucleophilic attack that severs the peptide bond making up the backbone of target proteins. Simply possessing serine does not make a protein into an enzyme, though; serine needs help to become capable of nucleophilic attack.

"The first step in proteolysis is the serine residue is activated by certain nearby residues, most often a histidine residue. The histidine's nitrogen atom pulls the hydrogen in serine's hydroxy group strongly so that the oxygen in the serine residue becomes a better nucleophile and can better attack a peptide bond," explains Dr. Heedeok Hong, an associate professor in chemistry at Michigan State University.

The hydrogen bond formed between serine and histidine is therefore crucial for catalysis. Many serine proteases possess a third amino acid in their active site, an aspartate residue that assists with catalysis and participates in forming a tight hydrogen bond network. However, rhomboids only contain a serine–histidine dyad (1, 4). The strength of the hydrogen bond between these residues, and how proteolysis occurs, is unknown.

Hong's laboratory is primarily interested in the process by which membrane proteins fold into their final, functional shape. However, Hong and graduate student Kristen Gaffney realized that tools developed to study protein folding could also be used to probe a rhomboid protease's active site.

The authors used a method called steric trapping (5) in which a protein is labeled with a pair of small biotin tags that bind bulky streptavidin probes. The tags are placed so that simultaneous binding of two probes only occurs when the protein has become unfolded. This technique allows analysis of protein folding under native conditions and without use of chemical denaturants.

Gaffney and Hong combined this with a mutational approach called double mutant cycle analysis, in which the individual effects of two single mutations are compared with that of having both residues mutated simultaneously. The authors investigated the strength of interaction between GlpG's active site hydrogen bond residues by mutating them to alanine (which cannot participate in hydrogen bonding) and assessing their effect on protein stability via steric trapping. These experiments showed that GlpG's active site hydrogen bond is surprisingly weak: around 1 kilocalorie per mole, compared with 5–7 kilocalories per mole for other serine proteases. "That may be partly responsible for the slow proteolysis reaction mediated by rhomboid proteases," theorizes Hong.

Gaffney and Hong were also curious about how GlpG's hydrogen bond affects the protein's structure overall. To assess this, they positioned steric trapping affinity tags at different positions within GlpG, then investigated whether mutations in the active site hydrogen propagate to distant sites. The results suggest that mutations at the active site are strongly propagated throughout the protein.

"We conclude that the hydrogen bonding network is not just important for function. Those highly conserved interactions might have evolved for communication between different parts of the protein," says Hong.

Although Gaffney and Hong studied GlpG folding in detergent, Hong thinks steric trapping may enable studies of protein folding within lipid bilayers—a technically challenging problem that few groups have attempted. "We are currently working on that," he says.

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SMALL CALCIUM LEAKS, BIG MUSCLE ADAPTATIONS

Mild calcium leak is associated with improved resistance to muscle fatigue

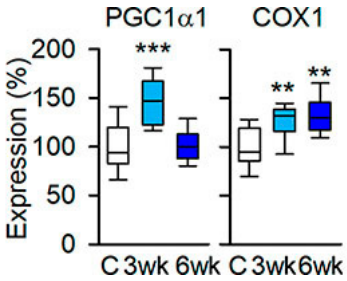
Muscle training results in muscle adaptations that increase both performance and resistance to fatigue, whereas overtraining can result in muscle weakness. Muscle weakness also appears alongside various diseases such as muscular dystrophies and rheumatoid arthritis. A better understanding of the pathways affecting muscle adaptation could therefore lead to major improvements in both disease settings and athletic performance. A *JGP* paper by Ivarsson et al. uncovers a pathway that increases muscle fatigue resistance (1).

"With endurance exercise, the muscle adapts so you can perform a task better," says Johanna Lanner, an Assistant Professor at the Karolinska Institutet in Stockholm, Sweden. Exercised muscle contains more mitochondria, demonstrating increased mitochondrial mass and the expression of genes involved in mitochondrial biogenesis, such as PGC1α1 (2). "We wondered, 'How can a contracting muscle lead to more mitochondria?'"

During each muscle contraction, calcium ions (Ca^{2+}) are released from the sarcoplasmic reticulum into the cytoplasm. There, Ca^{2+} enables interaction between myosin motors and actin thin filaments to produce shortening of muscle filaments, and then it is returned to the sarcoplasmic reticulum by the SERCA Ca^{2+} pump. Ca^{2+} release from the sarcoplasmic reticulum is controlled by the ryanodine receptor (RyR1), a giant protein whose stability is regulated by a subunit called FKBP12 (3).

"If FKBP12 is not there, you get an unstable receptor that turns leaky," explains Lanner. "Such leak has always been considered something bad that contributes to muscle weakness."

Extreme RyR1 leakiness causing prolonged elevations in cytoplasmic Ca^{2+} is observed both in disease settings (4) and during muscle overtraining (5). However, recent work hints that lower levels of RyR1 Ca^{2+} leak might actually be beneficial (6). Because



First author Niklas Ivarsson (left), co-senior authors Johanna Lanner (center) and Håkan Westerblad (not shown), and colleagues found that mild Ca^{2+} leak through ryanodine receptors correlates with increased expression of markers for mitochondrial biogenesis (graph, right) and improved muscle function. Sadly, Ivarsson passed away on October 10, 2018. Photos courtesy of the authors.

Ca^{2+} is a potent second messenger, Ivarsson and colleagues hypothesized that exercise training may provoke changes in baseline cytoplasmic Ca^{2+} levels, thereby changing gene expression or other processes. To probe this, the researchers gave mice access to running wheels and examined how exercise affects Ca^{2+} leak, baseline cytoplasmic Ca^{2+}, and muscle mitochondria.

After three weeks of voluntary exercise, mouse skeletal muscle displayed higher levels of FKBP12 dissociation from RyR1 and 25% higher baseline Ca^{2+} concentrations. This was accompanied by a marked increase in expression of PGC1α1 and other genes involved in mitochondrial biosynthesis. By six weeks of exercise, however, the period of muscle adaptation had ended; mitochondrial content was higher, PGC1α1 gene expression and FKBP12 dissociation had returned to normal, expression of SERCA protein had increased, and baseline Ca^{2+} concentration was back to its original level.

The strong correlation between RyR1 leakiness and expression of mitochondrial biogenesis markers prompted Ivarsson et al. to investigate whether inducing a mild Ca^{2+} leak with pharmacological agents might prompt muscle adaptation. They found that muscle injected periodically over three weeks with low levels of the drug rapamycin, which binds FKBP12 and forces its dissociation from RyR1, exhibited similar levels of baseline Ca^{2+} and mitochondrial biosynthesis as exercised muscle. Rapamycin-treated muscle also showed functional improvements compared with muscle from rested mice.

"It appears that a small RyR1 Ca^{2+} leak can be a trigger for signaling pathways that improve fatigue resistance in muscle. We want to investigate the molecular mechanisms involved," says Lanner. And, she adds, if it works the same in humans as in mice, a pharmacological pathway providing a shortcut to improved muscle function could help patients suffering muscle weakness due to disease, or athletes training their muscles. Although it could be dangerous to use rapamycin for this purpose because the drug can cause severe immunosuppression and cardiac arrest, future work might produce a safer route.

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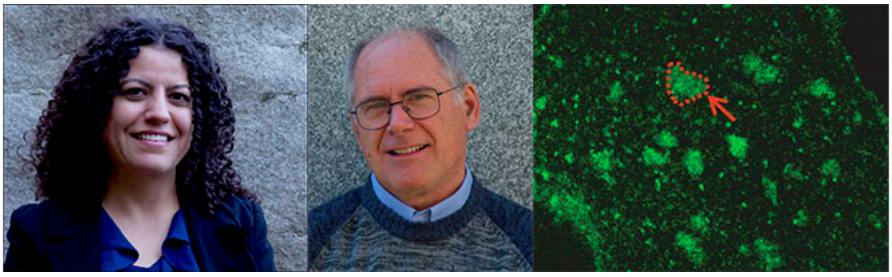
CFTR GETS TOGETHER

Pro-secretory agonists prompt CFTR to assemble into large lipid platforms

Cystic fibrosis is a heritable disease caused by mutations in CFTR, a protein expressed on the epithelial cells that line mucosal tissues throughout the body. Although its incidence varies according to ethnicity, the disease is fairly common, appearing in ~1 in 3,500 people of northern European descent. Despite decades of research, fundamental questions about CFTR and the disease process remain. A *JGP* paper by Abu-Arish et al. details some unexpected findings regarding CFTR distribution and dynamics in the plasma membrane (1).



First author Asmahan Abu-Arish (left), senior author John Hanrahan (right), and colleagues show that agonists that stimulate secretion cause CFTR to assemble into large, ceramide-rich platforms on the surface of airway epithelial cells (outlined area in micrograph).

"CFTR is an ABC transporter family member that is unique in that it functions as an anion channel," explains John Hanrahan, a Professor of Physiology at McGill University in Montreal, Canada. "Disease-associated mutations cause a reduction in CFTR channel activity, which then leads to reduced chloride currents and fluid secretion, so mucus becomes viscous and isn't cleared properly from the airways."

For CFTR to function, it must be present at the cell surface. Little is known about how CFTR is distributed there, or how its distribution might change under different conditions. Prior work has shown that CFTR is located in specialized membrane domains at the cell surface called lipid rafts (2, 3). These dynamic structures are assembled from clustered lipids—principally, cholesterol and sphingomyelin—that form a spatially and biochemically distinct region to segregates certain membrane proteins within the plasma membrane.

As a postdoc in Hanrahan's lab, Asmahan Abu-Arish investigated CFTR's distribution in the cell membrane. Using a microscopy technique called k-space Image Correlation Spectroscopy (kICS), developed by Paul Wiseman's group at McGill, the group saw that CFTR resides in tiny lipid raft microdomains on the surface of resting epithelial cells (4). They became curious whether CFTR distribution changes when the channel is activated by exposure to agonists that stimulate secretion from airway epithelial cells, such as the neuropeptide vasoactive intestinal peptide (VIP).

"We were interested to understand what controls where CFTR goes on the cell surface and also the role of lipids in that process," says Hanrahan.

"We wanted to work with live cells because we wanted to see that behavior in real time and in a relevant living system," adds Abu-Arish, now a research associate working with Hanrahan.

To address this, Abu-Arish et al. again used kICS to observe CFTR molecules in live primary airway epithelial cells collected from human donors. They found that stimulation with VIP and other pro-secretory agonists prompted CFTR lipid raft microdomains to coalesce into large, ceramide-rich platforms. CFTR surface expression is known to increase after VIP stimulation (5), and Hanrahan speculates that CFTR inclusion in these lipid platforms may promote cell surface retention by preventing internalization.

"We believe the major mechanism for platform formation is diffusion," says Abu-Arish. "CFTR clusters can diffuse in the membrane, and when rafts containing ceramide meet, the ceramide molecules form strong bonds, and the rafts coalesce."

CFTR-containing platforms were located near cilia, and their formation depended on an increase in membrane ceramide content driven by the enzyme acid sphingomyelinase (ASMase). Most cellular ASMase is found in lysosomes. There, it can be activated by cell stress and then trafficked to the cell surface. However, Abu-Arish et al. found no evidence for such trafficking in VIP-stimulated epithelial cells.

"We think that there's probably a redox signaling mechanism that activates the acid sphingomyelinase on the outer surface of the cell," says Hanrahan. His laboratory is already working to identify this pathway. Because cell membrane lipid imbalances are a common feature of cystic fibrosis, they're also investigating whether common CFTR mutations affect the channel's distribution and dynamic behavior at the cell surface.

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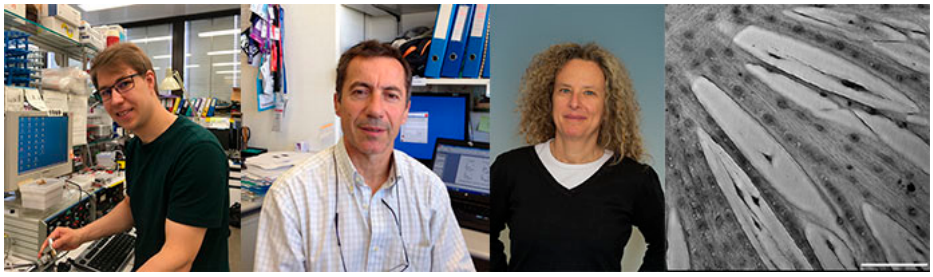
IT'S TIME TO LOOK MORE CLOSELY AT RYR3

Ryanodine receptor 3 is important for extraocular muscle function

Ryanodine receptors (RYRs) are calcium (Ca^{2+}) channels that reside on the endoplasmic reticulum (ER). Type 1 and type 2 RYRs (in skeletal and cardiac muscle, respectively) release Ca^{2+} from the ER in response to excitation of muscle membranes to promote muscle contraction. Type 3 RYR (RYR3) is ubiquitously expressed but is less well studied. A *JGP* paper by Eckhardt et al. sheds more light on this mysterious protein (1).



Left to right: Jan Eckhardt, Francesco Zorzato, Susan Treves, and colleagues (not pictured) demonstrate that RYR3 is involved in force production and calcium handling in extraocular muscle (see micrograph).

"Nobody really knows what RYR3 does," explains Dr. Susan Treves, senior author on the paper. "It seems to increase in expression in developing muscle cells, called myotubes, but in mature skeletal muscle its level of expression is very low."

Accordingly, RYR3 knockout mice don't show any strong phenotype changes in mature muscle (2, 3). RYR3-deficient mice are slower than wild-type mice at completing a behavioral task called the water maze, but this was attributed to a neuronal deficit because RYR3 is strongly expressed in neurons (4). For this reason, neither Treves nor co-senior author Francesco Zorzato was initially focused on RYR3. That is, until Maya Sekulic-Jablanovic and Jan Eckhardt, PhD students in Treves and Zozato's laboratory in Basel, Switzerland, began studying how some congenital myopathies affect the muscles that surround the eye (called extraocular muscles, or EOM). They observed strong RYR3 expression in human and murine EOM (5).

"RYR3 is such a large protein that if you don't need it you wouldn't usually be expressing it, so we thought it might be important in eye muscles," says Treves. Graduate student Jan Eckhardt headed the group's efforts to test this hypothesis.

EOM control the ability of the eye to focus, so functional defects in these muscles may result in poor vision. The authors therefore investigated the vision of RYR3-deficient mice using two behavioral tests: the water maze and the optokinetic reflex test. In both tests, the mice behaved as if they had trouble tracking their surroundings, but their problems could stem from either poor EOM function or from neuronal or retinal defects. To determine whether RYR3-deficient mice have impaired EOM function, the researchers needed to examine the extraocular muscle fibers themselves—a challenging task because the eye muscles are complex and tiny.

After perfecting methods to isolate EOM fibers, Eckhardt et al. found that RYR3-deficient EOM produce much less force than their wild-type counterparts, and do so at a slower velocity. These flaws were not due to muscle atrophy or large-scale morphological changes.

"We also excluded a change in expression of excitation–contraction coupling proteins," says Treves. "There could have been downregulation of RYR1 in EOM, for example, but all the coupling proteins except for parvalbumin were the same in control and knockout mice."

RYR3-deficient muscle fibers did, however, show differences in how they handle Ca^{2+}.

"The peak Ca^{2+} level during muscle contraction was the same in the wild type and the knockout, but the knockout's half-relaxation time was much slower," notes Treves.

Prior studies in amphibians and birds showed that RYR3 is involved in Ca^{2+} sparks—rapid, tiny releases of Ca^{2+} from the ER that help regulate cytoplasmic Ca^{2+} levels. Surprisingly, Eckhardt et al. found no evidence for this in single isolated fibers from mouse EOM. Instead, they observed that RYR3 was needed for rapid Ca^{2+} oscillations that take place in EOM-derived myotubes. That's interesting because EOM are under constant use, and the abundant myotubes are constantly fusing into adult extraocular muscles.

"Our conclusion was that RYR3 is involved in something like Ca^{2+}-induced Ca^{2+} release to amplify the Ca^{2+} signal in myotubes of EOM," explains Treves. She suspects this may also be the case in adult EOM fibers. "It just might be so fast that we don't have the systems to see it, and we're missing it."

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HOW DENDRITIC SPINES SHAPE CALCIUM DYNAMICS

Mathematical model that describes how calcium signaling could be influenced by spine geometry and ultrastructure

Dendritic spines are small, actin-rich structures that protrude from neuronal dendrites and receive inputs from neurotransmitters released by neighboring axons. Calcium ions flood into the spine immediately after neurotransmitter binding, triggering a wide variety of signaling pathways that are crucial for synaptic remodeling and plasticity. A *JGP* study by Bell et al. describes how the spatio-temporal dynamics of dendritic calcium are affected by both the size and shape of individual spines and by a specialized organelle within spines called the spine apparatus (1).

Miriam Bell (left), Padmini Rangamani (right), and colleagues develop a 3-D multicompartment reaction–diffusion model of calcium dynamics in dendritic spines. The model shows that altering the shape of the spine head or the specialized ER known as the spine apparatus has complex, nonlinear effects on calcium's spatial distribution 5 ms after stimulation.

Calcium dynamics in dendritic spines have been extensively studied (2, 3), and researchers have identified numerous proteins that influence these dynamics and/or respond to them. In recent years, however, high-resolution imaging has shown that individual spines can adopt a variety of sizes and shapes that could have important impacts on their function (4). "We were interested in the physical aspects of this," explains Padmini Rangamani from the University of California, San Diego. "How are calcium dynamics affected by spine geometry?"

Rangamani and her graduate student Miriam Bell are computational biologists rather than neuroscientists, but thanks to a Multidisciplinary University Research Initiative grant from the United States Air Force, they were able to collaborate with Tom Bartol and Terrence Sejnowski to build a 3-D, multicompartment model of how spine calcium dynamics respond to stimulation.

"There's a wealth of information out there, so the most challenging part for me was deciding what proteins to include in the model," says Bell. In the end, the researchers incorporated numerous proteins, including NMDA receptors and voltage-sensitive calcium channels that transport calcium into spines, membrane pumps that remove the metal, and calcium-binding proteins that buffer cytosolic calcium. The model also incorporated the spine apparatus (5), a specialized type of endoplasmic reticulum that can mop up calcium from the cytosol and is present in ~14% of spines, particularly mature ones.

When Bell and colleagues adjusted the size of the spine head in their mathematical model, they found that smaller spines generate higher peak calcium concentrations, but the total amount of calcium they see over time is reduced. "So there's an interesting trade off that could have important functional implications," explains Bell. "Larger spines tend to be more stable, and even though they have lower calcium peaks, they are exposed to more total calcium, which corresponds to them seeing more information overall."

The effect of spine shape was far more subtle, however. "We thought that adjusting the spine head from a spheroid to an ellipsoid would have a much more dramatic effect," Rangamani admits. Instead, dendritic spine shape showed a complex, nonlinear relationship with calcium dynamics. This is because, while altering spine shape changes its surface area to volume ratio, thereby affecting calcium flux across the cell membrane, it also changes the distance between the postsynaptic density (a major location of calcium influx) and the spine apparatus (a major calcium sink that removes calcium from the cytosol).

Accordingly, changing the size or shape of the spine apparatus altered its capacity to act as a calcium sink and had similar nonlinear effects on calcium dynamics. Bell et al. speculate that spines may dynamically regulate their size and shape and control the presence or absence of the spine apparatus to fine tune their calcium dynamics for optimal synaptic function.

The researchers' model also reveals that calcium dynamics are affected by the spatial distribution of membrane fluxes and calcium buffers. Rangamani stresses that all of these factors should be taken into account when modeling the signals received by individual spines. The next step, the researchers say, is to model how all of these individual signals are integrated by the dendrite as a whole.

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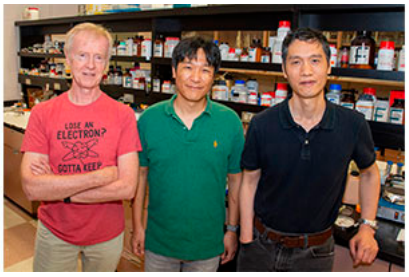
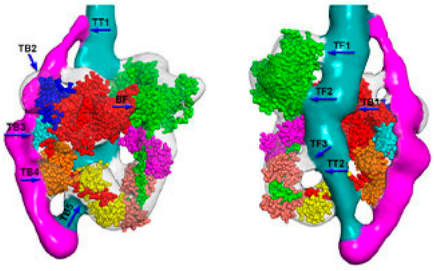
HOW MYOSIN II ACHIEVES TOTAL SHUTDOWN

Study describes 3-D structure of the 10S form of myosin II, identifying key interactions between the head and tail domains that keep the motor protein switched off

The myosin II motor protein drives a wide variety of processes, from muscle contraction to cell migration, by binding to F-actin and hydrolyzing ATP. Composed of two heavy chains, two essential light chains, and two regulatory light chains (RLCs), myosin II molecules perform their functions by polymerizing into extended filaments. To conserve energy and facilitate their delivery to sites of filament assembly, however, myosin II monomers are maintained in a compact, inactive conformation named after its sedimentation coefficient of 10S. Yang et al. provide new insights into the structure of 10S myosin II that help explain how its motor activity is completely shut off (1).





Left to right: Roger Craig, Kyoung Hwan Lee, Shixin Yang, and colleagues generate a 3-D reconstruction of the 10S conformation of myosin II that explains how interactions between the tail and head domains help to completely switch off the protein's motor activity. Key interactions include TB3, which inhibits the ATPase activity of the "blocked" head, and TF1, which masks the actin-binding domain of the "free" head.

10S myosin is remarkably inactive, hydrolyzing ATP at a rate of just one molecule per hour (2). 3-D reconstructions of the 10S structure suggest that the two head domains of myosin II—comprising the light chains and the N-terminal halves of the heavy chains—inhibit each other in an asymmetric manner (3, 4). The actin-binding domain of one head is "blocked" by the other head. This second head, in contrast, may be "free" to bind actin, but its ATPase activity is inhibited because its converter domain—which helps release the products of ATP hydrolysis—is immobilized by its interaction with the blocked head.

The tail domain formed by the C-terminal halves of the heavy chains is also thought to contribute to the inhibition of 10S myosin, but this region of the molecule is largely missing from the 3-D reconstructions. 2-D electron microscopy has shown that the tail wraps around the head domains of 10S myosin II (5), but these head–tail interactions have not been observed in 3-D. "We wanted to fill in this gap," explains Roger Craig from the University of Massachusetts Medical School.

Craig and colleagues, including co-first authors Shixin Yang and Kyoung Hwan Lee, therefore generated a 3-D reconstruction of 10S myosin using negative staining and single particle analysis of the full-length molecule. Due to their high flexibility, some parts of the tail are missing from the reconstruction, but the structure includes all of the tail regions closely associated with the heads.

The researchers were able to identify numerous interactions between the head and tail domains, many of which are likely to stabilize the asymmetric interaction of the blocked and free heads as well as the overall conformation of the 10S molecule. But several interactions appear to play a more direct role in inhibiting 10S myosin activity.

One interaction between the tail and the converter domain of the blocked head likely inhibits this head's ATPase activity, while another interaction between the tail and the actin-binding domain of the free head probably prevents this head from attaching to actin filaments. "So actin-binding and ATPase activity are switched off in both heads," Craig says. "Everything is totally shut down." In addition, interactions between different segments of the tail allow it to fold up so that it is unable to mediate myosin polymerization and filament formation.

10S myosin is activated when myosin light chain kinase phosphorylates a serine residue on each of the two RLCs. Yang et al.'s reconstruction reveals that the serine residue in the free head RLC is completely accessible. But the equivalent serine in the blocked head is masked by an interaction with the tail. The researchers suggest that phosphorylation of the first serine may slightly destabilize the 10S conformation, whereas phosphorylation of the second serine is likely to trigger the molecule's complete unfolding and activation.

"We'd now like to get a higher resolution structure using cryo-EM so that we can see exactly which residues are involved in all these interactions," Craig says.

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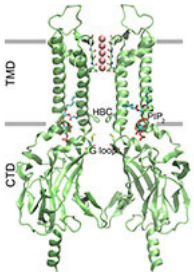
MODELING GIRK CHANNEL CONDUCTANCE

Researchers use MD simulations to investigate the gating and conductance of the inwardly rectifying potassium channel GIRK2



Anna Stary-Weinzinger (left), Harald Bernsteiner (right), and colleagues use MD simulations to provide new details about the gating and conductance of the inwardly rectifying K^+ channel GIRK2. The crystal structure of the channel bound to its activator, PIP2, shows the position of the HBC and G-loop gates, as well as five K^+ ions (pink spheres) passing through the selectivity filter via what the simulations suggest is a direct knock-on mechanism.

GIRK proteins are a family of G protein–regulated inwardly rectifying potassium channels that, in response to dopamine and other neurotransmitters that stimulate G protein–coupled receptors, generate small, hyperpolarizing outward K^+ currents that inhibit neuronal activity. Like all other inwardly rectifying potassium channels, however, GIRK activity is also regulated by the phospholipid PIP_2, and the precise details of how these channels are gated and how they conduct K^+ ions remain uncertain. Bernsteiner et al. use MD simulations to uncover new details about the mechanisms controlling K^+ flux through GIRK2, a GIRK family member also known as Kir3.2 (1).

Experiments on GIRK2 embedded in planar lipid bilayers suggested that channel opening requires both PIP_2 and $G_{\beta\gamma}$ (2), whereas studies on GIRK2 incorporated into liposomes indicated that PIP_2 is sufficient to activate the channel (3). Crystal structures have been obtained for wild-type GIRK2 in the presence and absence of both PIP_2 and $G_{\beta\gamma}$ (4, 5). "However, none of these structures appear to be in an open state," explains Anna Stary-Weinzinger, an assistant professor at the University of Vienna.

To learn more about the channel's gating, Stary-Weinzinger and colleagues, including first author Harald Bernsteiner, performed MD simulations based on the crystal structure of GIRK2 bound to PIP_2 but not $G_{\beta\gamma}$,in which the channel's helix bundle crossing (HBC) and G-loop gates both appear to be closed. "In our simulations, however, we were quite surprised to see that, when embedded in a membrane, the channel spontaneously opens and allows K^+ ions to pass through the gates," Stary-Weinzinger says.

This spontaneous opening was facilitated by the wetting of the HBC and G-loop gates by water molecules diffusing into the channel, opening them wide enough to permit the passage of K^+ ions. The gates remained closed when the researchers removed PIP_2 from the simulations, indicating that the phospholipid is sufficient to activate GIRK2. One possibility is that, in vivo, $G_{\beta\gamma}$ promotes channel opening by acting as an allosteric modulator, increasing GIRK2's affinity for PIP_2.

The researchers then applied an electric field to their simulations to mimic membrane potential and, in a series of microsecond-long simulations, saw that, after diffusing through the G-loop and HBC gates, K^+ ions move through the channel's selectivity filter via a "direct knock-on" mechanism. Such a mechanism, in which K^+ ions entering the selectivity filter directly push the ions ahead of them through to the other side, has recently been demonstrated in voltage-gated K^+ channels. But the subject remains controversial, with many studies supporting a "soft knock-on" mechanism in which the permeating K^+ ions are separated by intervening water molecules. "Further studies are needed, but maybe all K^+ channels can conduct in both ways," Stary-Weinzinger suggests.

Finally, free energy profiling revealed that, rather than the HBC or G-loop gates, the selectivity filter represents the main barrier to K^+ ion movement through GIRK2. In voltage-gated K^+ channels, a phenomenon known as C-type inactivation can restrict ion movement by narrowing the selectivity filter. Surprisingly, however, Bernsteiner et al. found that the selectivity filter of GIRK2 was dilated in MD simulations showing little or no conductance.

"If the filter gets as little as 1 Å wider, it appears to stop conductance," Stary-Weinzinger says. "In contrast, we did not find any correlation between conductance and the diameters of the HBC or G-loop gates."

Mutations in the selectivity filter of GIRK channels have been linked to the rare developmental disorder Keppen-Lubinsky syndrome as well as cases of aldosterone-producing adenomas. "We now want to investigate the effects of these disease-causing mutations in our MD simulations," Stary-Weinzinger says..

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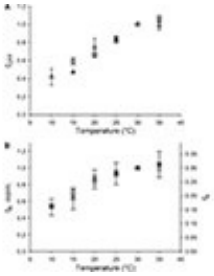
COLD TEMPERATURES PUT A FREEZE ON MYOSIN ACTIVATION

Study reveals that low temperatures reduce force production in mammalian skeletal muscle by trapping myosin motors in a refractory state unable to bind actin

Contraction of striated muscle is triggered by an influx of calcium into muscle fibers that induces structural changes in the thin filament proteins troponin and tropomyosin, making actin available to the myosin motors present in the thick filaments. In recent years, however, it has become clear that structural changes in the thick filament are also crucial for contraction; in relaxed muscle, myosin is organized into helical tracks on the filament surface that render the motors incapable of binding to actin and hydrolyzing ATP. Caremani et al. reveal that low temperatures convert this ordered OFF state into a disordered state that is nevertheless unavailable to bind actin (1). This refractory state explains why mammalian muscles generate less force at lower temperatures and may help hibernating animals conserve energy.



Left to right: Marco Caremani, Massimo Reconditi, Vincenzo Lombardi, Elisabetta Brunello, and colleagues discovered that cold temperatures convert myosin motors in mouse skeletal muscle at rest to a disordered refractory state unable to bind actin upon muscle activation. This refractory population is revealed by x-ray diffraction measurements showing that, in resting muscle, low temperatures reduce the number of myosin motors in the ordered OFF state (top graph) whereas, in contracting muscle, low temperatures cause a parallel reduction in the number of actin-attached motors (bottom graph).

The ordered OFF state of myosin in relaxed muscle was originally defined by electron microscopy (2). In 2015, Vincenzo Lombardi and colleagues from the University of Florence and from King's College London used the x-ray diffraction patterns of frog muscle cells (fibers) to show that this organization is disrupted upon muscle stimulation by stress in the thick filament, increasing the number of myosin molecules in a disordered ON state capable of attaching to actin when the muscle works against a high load (3).

In frog muscles, at least, this mechanoregulation of thick filaments is not affected by temperature; the number of actin-attached motors recruited during an isometric contraction is identical at both 0°C and 20°C (4). The reduced amount of force generated by frog muscles at lower temperatures is explained by the reduced structural change responsible for force generation in the motor domain of individual myosin molecules (5). "However, temperature has a much greater effect on mouse muscles," Lombardi says. "The amount of force generated is threefold less at 10°C than it is at physiological temperatures."

Lombardi and colleagues, including Marco Caremani, Elisabetta Brunello, and Massimo Reconditi, therefore suspected that low temperatures might have a more dramatic effect on the control of myosin in mouse thick filaments. To investigate this possibility, the researchers collected x-ray diffraction patterns from intact mouse skeletal muscle at temperatures ranging from 10–35°C.

The x-ray signals revealed that lowering the temperature progressively disrupts the helical arrangement of myosin in the muscle at rest, such that the number of motors in the OFF state at 10°C is half of that at 35°C. But these disordered motors appear to have lost the ability to be switched into an ON state capable of binding actin; in response to stimulation, the number of actin-attached motors at 10°C is also half of that at 35°C. This suggests that cold temperatures push myosin motors into a disordered state that is refractory to activation, and only those motors that remain in the ordered OFF state are capable of being activated and attaching to actin upon muscle stimulation.

"The challenge now is to define this refractory population either biochemically or pharmacologically," Lombardi says. The researchers propose that cold temperatures may disrupt the intra- and intermolecular interactions that stabilize the helical arrangement of myosin motors on the surface of thick filaments, while maintaining the interactions that inhibit the motors' actin-binding and ATPase activity.

Lombardi and colleagues further speculate that this cold-induced refractory state may be beneficial to hibernating animals when their body temperatures fall to ambient temperatures. "Muscles account for ~40% of body mass, so reducing the fraction of muscle that can be switched on by residual neuronal activity would greatly reduce metabolic demand," Lombardi suggests..

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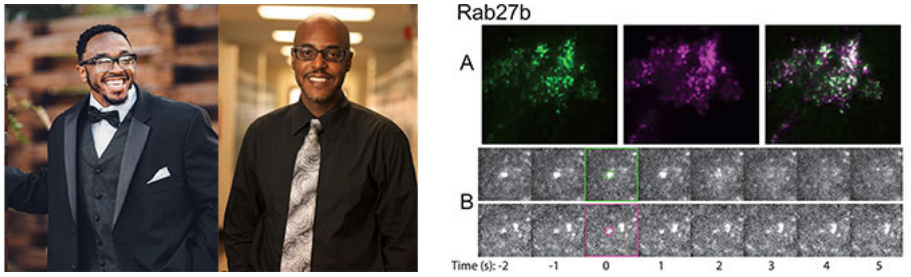
DETERMINING THE DYNAMICS OF CANCER CELL SECRETION

Study describes the spatiotemporal dynamics of proteins and lipids involved in the exocytosis of the matrix metalloproteinase MMP-9 from breast cancer cells

The metastasis, or spread, of cancer cells to other parts of the body begins with the breakdown and remodeling of the extracellular matrix surrounding the primary tumor. This is achieved, in part, by matrix metalloproteinases (MMPs), a family of matrix-degrading enzymes that are secreted from cancer cells via a highly regulated process involving dozens of protein and lipid factors. Stephens et al. reveal the complex dynamics of many of these factors at individual sites of MMP exocytosis in breast cancer cells (1).



Dominique Stephens (left), Dinari Harris (center), and colleagues used TIRF microscopy to identify factors associated with secretory vesicles containing the enzyme MMP-9 in breast cancer cells and monitored their dynamics over the course of exocytosis. In the example shown, Rab27b (magenta) colocalizes with MMP-9 (green) at steady state (top images). Both proteins rapidly disperse from exocytic sites following membrane fusion (bottom images).

Increased expression of two closely related MMPs, MMP-9 and MMP-2, is found in a wide variety of cancers and is associated with poor prognosis. Secretory vesicles containing these enzymes are trafficked to the plasma membrane of cancer cells, where they can fuse and release their cargo into the extracellular space. This process, and the numerous factors regulating it, have been extensively studied using traditional biochemical methods. "But what's been lacking in the field is an understanding of the real-time dynamics of these factors at the sites of exocytosis," says Dinari Harris, an assistant professor at Howard University.

This type of information is more readily available for the factors that regulate secretion from neuronal cells (2,3). These factors, or "organizational elements," can be broadly classified into three distinct groups: scaffolding proteins, such as Rab GTPases and SNARE proteins, that link secretory vesicles to the plasma membrane; lipid molecules within the membranes themselves; and components of the actin cytoskeleton.

Harris and colleagues, including first author Dominique Stephens, used two-color TIRF microscopy to assess the colocalization of ~150 different proteins with MMP-9-containing secretory vesicles and saw that a similar set of organizational elements are involved in the secretion of MMP-9 from breast cancer cells.

Multiple Rab GTPases, for example, were found to be associated with MMP-9-positive vesicles, including two closely related isoforms, Rab27a and Rab27b, that have previously been linked to both exocytosis and tumor cell metastasis (4,5). Despite their similarity to each other, Stephens et al. found evidence to suggest that Rab27a and Rab27b may have distinct roles in MMP-9 secretion. By analyzing the kinetics of individual exocytic events, the researchers discovered that some MMP-9-containing vesicles fuse with the plasma membrane as soon as they reach the cell cortex, while others dock at the membrane for a short period before undergoing fusion. Overexpressing Rab27a appears to promote the former type of fusion, whereas overexpressing Rab27b promotes the latter type. Harris plans to investigate this potential specificity using CRISPR/Cas9 to knockout each of the two isoforms.

Stephens et al. found that Rab GTPases, along with many other scaffolding proteins, assemble on MMP-9-containing vesicles docked at the plasma membrane and then quickly dissipate as soon as the vesicle undergoes fusion. In contrast, amphiphysin1 and syndapin1—two BAR domain–containing proteins commonly associated with endocytosis—were transiently recruited to exocytic sites at the moment of fusion. The researchers also saw a similarly transient accumulation of the regulatory phospholipid PIP2. "That suggests that there is some reason for PIP2 recruitment at exocytic sites," Harris explains.

Harris and colleagues are now investigating the localization and dynamics of other lipids, as well as the behavior of the actin cytoskeleton, the third class of organizational element known to regulate exocytosis. "The bigger question then is how these different elements interact and communicate to cause vesicle fusion and the release of MMP-9 from breast cancer cells," Harris says.

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ORIGINAL PAPER

Stephens, D.C., N. Osunsanmi, K.A. Sochacki, T.W. Powell, J.W. Taraska, and D.A. Harris. 2019. Spatiotemporal organization and protein dynamics involved in regulated exocytosis of MMP-9 in breast cancer cells. *J. Gen. Physiol.* 151:1386–1403. doi: https://doi.org/10.1085/jgp.201812299

JGP Journal of General Physiology

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Print ISSN 0022-1295
Online ISSN 1540-7748

Rockefeller University Press

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Journal of General Physiology (ISSN 0022-1295) is published monthly by Rockefeller University Press, 950 Third Avenue, New York, NY 10022. Periodical postage paid at New York, NY and additional mailing offices.

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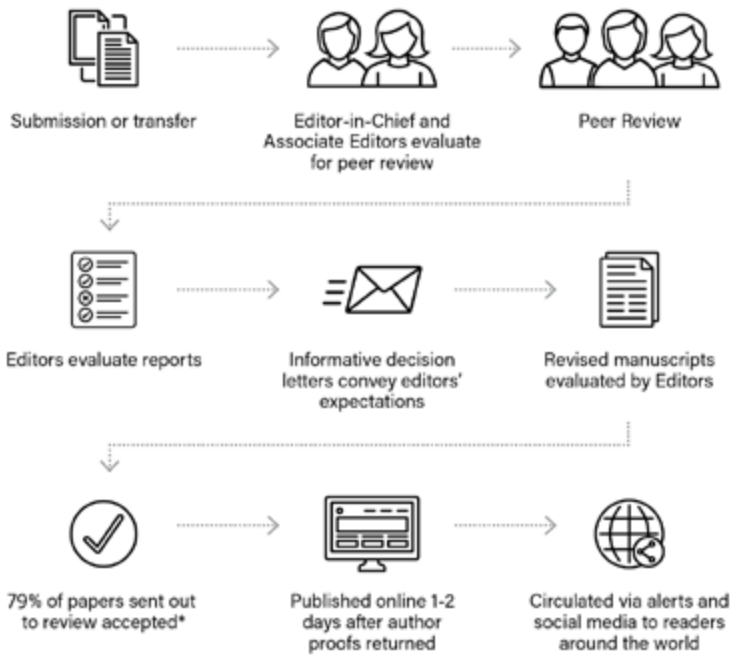

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