# Generally Physiological

## Taking a translational turn


This month's installment of *Generally Physiological* takes a translational turn, discussing functional analyses of two proteins whose mutation is associated with disease (heart failure and cystic fibrosis, respectively) and the mechanism underlying the painful response to cold found in ciguatera.

### A tunable brake on cardiac contractility

Cardiac contraction, like that of skeletal muscle, depends on the interaction of myosin thick filaments with actin thin filaments, which slide past each other to shorten the sarcomeres that make up muscle fibers. Although mutations in cardiac myosin–binding protein C (cMyBP-C, which binds to thick filaments) have been linked to heart disease, its precise physiological role—and thus the mechanisms underlying the pathophysiological consequences of its malfunction—has been unclear (see Burghardt and Ajtai, 2012). Previs et al. (2012) developed an in vitro sarcomere model in which they visualized the movement of fluorescently labeled actin filaments along thick filaments immobilized on coverslips. Analyses of actin movement along thick filaments isolated from the hearts of wild-type mice or mice lacking cMyBP-C revealed that cMyBP-C, which localizes to a particular region of the thick filament (the C-zone), acts in this region to slow actin movement. β-adrenergic stimulation of the heart promotes cMyBP-C phosphorylation, and manipulation of the degree of phosphorylation revealed that increasing phosphorylation of cMyBP-C was associated with a graded reduction in its inhibition of actin filament velocity. Thus, the authors propose that cMyBP-C enables the fine-tuning of cardiac contraction, providing a potential mechanism to link its loss or dysfunction to aberrant cardiac contractility.

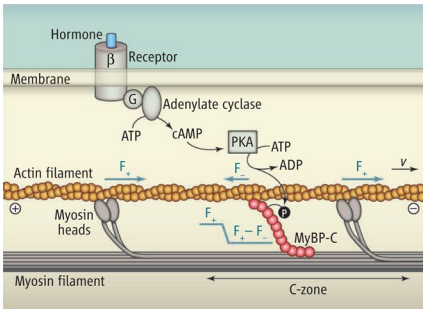


**Image 1. Model for how β-adrenergic signaling could modulate cardiac contraction through phosphorylation of MyBP-C. (From Burghardt and Ajtai. 2012. *Science*. 337: 1182–1183. Reprinted with permission from AAAS.)**

### A one-way cycle for CFTR gating

The cystic fibrosis transmembrane reporter (CFTR, mutation of which leads to cystic fibrosis) is unusual in being an ATP-gated chloride channel in a family of active transporters (the ATP-binding cassette [ABC] protein superfamily). Unlike active transporters, which couple ATP hydrolysis to substrate movement against a concentration gradient, channels mediate the passive transmembrane diffusion of ions down their concentration gradients. Thus, there is no clear requirement for the energy derived from ATP hydrolysis in CFTR function (see Tsai, 2012). Noting that CFTR retains key structural elements common to other members of the ABC family, Jih et al. (2012) exploited a mutant form of the CFTR that exhibits two different open states (characterized by distinct conductances) to explore the role of ATP hydrolysis by CFTR. They found that ATP hydrolysis promoted transition between these two open states in a preferred order, indicating that CFTR gating involves an irreversible step that requires input of energy, so that it progresses through open and closed states unidirectionally through a "transporter-esque" energy coupling mechanism.

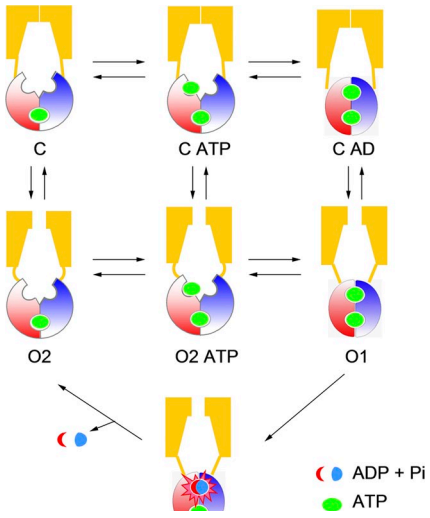


**Image 2. Model for the energetic coupling of CFTR gating; ATP hydrolysis provides a shortcut from open state O1 to open state O2. (from Jih et al., 2012).**

**Functional analyses of two proteins whose mutation is associated with disease and the mechanism underlying the painful response to cold found in ciguatera**

### Ciguatoxin-induced cold allodynia involves both $Na_v$ and TRPA1

Although fish is typically thought of as "healthy" food, fish eaters—particularly those who dine on large carnivorous reef fish in the tropics and subtropics—can run the risk of developing ciguatera. In addition to gastrointestinal symptoms characteristic of food poisoning, ciguatera is associated with a long-lasting form of cold allodynia—so that exposure to cool temperatures can elicit severe burning pain (see Voets, 2012). The ciguatoxins activate voltage-gated sodium channels ($Na_v$); however, the relationship to cold allodynia has been unclear. Vetter et al. (2012) developed a mouse model of cold allodynia and used a combination of in vivo, ex vivo, and in vitro techniques to determine that cold allodynia elicited by the Pacific Ocean ciguatoxin P-CTX-1 involves both $Na_v$ and TRPA1 (a channel that is found in nociceptors and activated in conjunction with noxious cold). P-CTX-1–induced cold allodynia was reduced in mice lacking TRPA1, and live cell imaging of cultured dorsal root ganglion (DRG) neurons combined with single-cell immunochemistry or pharmacological analysis indicated that TRPA1 mediated a P-CTX-1–dependent increase in calcium. Furthermore, P-CTX-1 sensitized previously insensitive DRG neurons to cold, an effect that depended on TRPA1. However, TRPA1 alone was insufficient to mediate the P-CTX-1 response: the DRG calcium signal was reduced by tetrodotoxin in most neurons; moreover, heterologous expression indicated that P-CTX-1–mediated calcium responses required both TRPA1 and $Na_v$. Moreover, P-CTX-1 elicited membrane potential depolarization and action potential firing in cultured DRG neurons and, as previously shown, produced a hyperpolarizing shift in $Na_v$ activation. The authors' analysis led to a model in which P-CTX-1–dependent activation of Nav—and the consequent depolarization—activates TRPA1 at higher than normal temperatures, which in combination with increased excitability promotes action potential firing and burning pain in response to typically non-noxious cold.

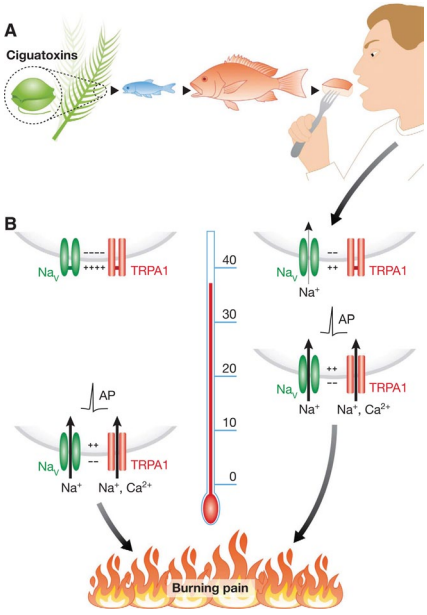


**Image 3. Model for how ciguatoxins promote cold allodynia. (Reprinted by permission from Macmillan Publishers, Ltd. *The Embo Journal.* T. Voets. TRP channel blamed for burning cold after a tropical fish meal. 31:3785–3787, copyright 2012).**


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## REFERENCES


Burghardt, T.P., and K. Ajtai. 2012. *Science.* 337:1182–1183. http://dx.doi.org/10.1126/science.1227943

Jih, K.-Y., et al. 2012. *J. Gen. Physiol.* 140:347–359. http://dx.doi.org/10.1085/jgp.201210834

Previs, M.J., et al. 2012. *Science.* 337:1215–1218. http://dx.doi.org/10.1126/science.1223602

Tsai, M.-F. 2012. *J. Gen. Physiol.* 140:343–345. http://dx.doi.org/10.1085/jgp.201210882

Vetter, I., et al. 2012. *EMBO J.* 31:3795–3808. http://dx.doi.org/10.1038/emboj.2012.207

Voets, T. 2012. *EMBO J.* 31:3785–3787. http://dx.doi.org/10.1038/emboj.2012.255