

## New and Notable

### A Mode of Thought in Excitation-Contraction Coupling

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Ion channels within membranes catalyze the transition of ionic species across the hydrophobic cell membrane and by opening and closing (gating) allow this transition to be controlled (Hille, 1992). The control of ion flow can be used to regulate information flow (e.g., the development of inhibitory or excitatory potentials in neurons) as well as a variety of intracellular chemical reactions to achieve cell functions, such as muscle contraction. Muscle excitation-contraction (E-C) coupling hinges on the processes that permit calcium ions to move down their electrochemical gradient from the lumen of the sarcoplasmic reticulum (SR) to the cytosol (where they regulate contractile protein interaction and metabolism). The ion channels regulating this process are ryanodine receptors (RyRs) and are one member of a super family of channels involved in calcium homeostasis (Sorrentino and Volpe, 1993; Williams et al., 2001). The intracellular location of RyRs essentially prevents analysis of their gating with *in situ* patch clamp techniques and, until now, biophysicists have generally relied on reconstitution experiments with artificial lipid bilayers or extracted SR vesicles to gain insight into their gating behavior. By combining a number of technologies Wang et al. (2002) have now gained new insight into

the gating behavior of RyRs in their native environment.

Spontaneous calcium release events (calcium sparks; Cheng et al., 1993) can be detected in isolated cells by applying fluorescent calcium indicators and confocal microscopy. By incorporating EGTA in the dialysis solution of the whole cell patch clamp pipette (to limit the spread of the dye-indicator complex) the time course of calcium release may then be measured with some fidelity. With this array of state of the art techniques, Wang et al. (2002) were in a position to examine the duration of spontaneous calcium release events at selected sites. The duration of repeated spontaneous SR calcium release events exhibited a clear mode and, since the duration of SR release should reflect the open time of the RyRs within the cell, Wang et al. (2002) concluded that the RyR open time distribution must also be modal. This result was in agreement with an earlier cardiac muscle study which showed that the amplitude of calcium sparks at given sites are modal (Bridge et al., 1999). For those interested in muscle, this observation would seem reasonable; the cell might well be expected to release calcium in quanta (sparks) that are just sufficient to activate contraction and excess calcium release would require more ATP to pump calcium back into the SR, for no obvious benefit. However, for the biophysicist this observation is puzzling. Since the spontaneous calcium release events repeated, the RyRs must have been cycling through their open states and, for a reversible reaction the open time distribution should be exponentially distributed, not modal. In fact, exponentially distributed lifetimes for ion channel states are generally observed with few exceptions. To allow steady-state behavior without micro-reversibility requires an energy source (Steinberg, 1987; Lauger, 1983). Since potential energy is stored in the cal-

cium electrochemical gradient across the SR membrane and RyR gating is controlled by calcium ions, the energy source for such non-reversible cyclical gating would seem to be readily available. To examine this possibility Wang et al. (2002) carried out experiments on isolated RyRs in planar lipid bilayers with realistic transmembrane calcium gradients. In these conditions, only exponentially distributed open times were observed showing that the energy gradient in the SR is not simply powering the modal RyR behavior observed *in situ*. In connection with this observation, it is notable that the closed time distribution (i.e., the time between spontaneous spark events) was also modal, another result which is not simply reconciled with energy being derived from the dissipation of the SR electrochemical gradient. (Modal RyR gating behavior has also been observed in skeletal muscle (Klein et al., 1999; González et al., 2000) although the possible role of the sarcolemmal voltage sensor in such behavior remains unclear.)

While it is possible that some unknown accessory protein for electrochemical gradient coupling could be lost during RyR isolation, is such extra molecular complexity really required? Wang et al. (2002) then turn to computer modeling and show that modal behavior could still be observed in a cluster of RyRs even when isolated RyRs gate with exponentially distributed open times. The open times in this model are not calcium dependent and so are insensitive to any possible coupling via calcium. In contrast, when an array of RyRs is considered the ensemble open states contain transitions which are calcium sensitive (as one or more RyRs open to join the ensemble open state). Put another way, the energy in the SR electrochemical gradient could be coupled into the RyR gating scheme by the cytosolic calcium which results from the flux of calcium via a more distant RyR. This idea is

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made even more attractive by the fact that E-C coupling occurs in a narrow region between the SR and surface membranes which restricts diffusion and thereby increases the magnitude and lengthens the time course of gradients (Soeller and Cannell, 1997). Since the purpose of ion channel gating is to control the flow of information (implying a change in energy content of the system) perhaps we should be more careful in our interpretation of equilibrium (reversible) gating in isolated channel experiments as energy sources are often modified (or removed) by the experimenter? Of course at this point we are moving into speculation, but the mathematical modeling of Wang et al. (2002) follows the best biophysical traditions. Rather than 'hand wave' the mathematical models rigorously test the validity of the hypothesis and even give insight that may be far from intuitive.

The paper of Wang et al. is "new and notable" for the elegance with which quite different state of the art methods are all brought to bear on a very difficult biophysical problem as well as showing how reintegration with computer models allows new plausible hypotheses to be created. It is likely that future progress in E-C coupling will also require similarly diverse techniques and the modern biophysicist seems to need much more than a few simple tools to open Nature's secrets—indeed, a whole box of tools would seem to be required! Finally, the paper also reminds us that insight gained from dissection of cell systems may be limited (if not completely flawed), since the macroscopic behavior of protein/chemical complexes may be quite different from their behavior in broken cell systems. After all, the cell is alive but the molecular components of the cell are not (with apologies to Albert Szent-Gyorgi).

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- Doyle et al., 1998) provided immediate qualitative answers to most questions about potassium channel selectivity. It has led to an explosion of computational papers that illustrate in great detail how the various structural features function to exclude anions, stabilize cations, select for potassium over sodium, and promote divalent block. What remains wide open is understanding their enormous diversity: they are gated in many ways and differ greatly in their conductance behavior. Selective potassium channels have five strictly conserved residues (the signature sequence) and similar inner helix sequences, motifs that form the filter and the mid-channel aqueous cavity. These features promote multi-ion stabilization, a property initially deduced by Hodgkin and Keynes (1955). Regardless of their gating mechanisms, they exhibit wide-ranging electrical properties. Maximal conductivities span a nearly 100-fold range.

## Unclogging a Pipe: Potassium Channel Pinball

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Ion channels are characterized by three main properties: their gating character-

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The crystallographic pore structure represents the channel in a closed state, the constriction on the intracellular side (the inner pore) being too narrow to permit ion (or water) passage. As has been pointed out repeatedly, structural modification of this inner pore is required for current to flow. In this issue, Chung et al. (2002) provide a reasonable and intuitive, albeit speculative, proposal for specific changes that could account for the broad spread of limiting conductances. The finding is striking: a small adjustment of the inner pore radius drastically alters channel resistivity.

In a series of papers Chung and his coworkers have exploited the advantages of Brownian dynamics (BD) to monitor ionic movement through channels and mimic experimentally observed current-voltage-concentration (I-V-c) profiles. The great virtue of BD is that, unlike molecular dynamics, large (picosecond) time steps can be used so that, with ultra-high-speed computers, multiple simulations can be