and a second site that regulates the fast kinetic changes."

Although Eckert and Armstrong (1987) have shown that Ca^{2+} channels in other cell types must be phosphorylated to open, support of this hypothesis for cardiac Ca^{2+} channels has been ambiguous. Recently, though, Katsushige Ono and Harry Fozzard (1992) have provided stronger support by showing that phosphorylation restores Ca^{2+} channel activity after rundown in excised patches. Other data that the cardiac Ca^{2+} channel does not require phosphorylation to function can be reconciled if the probability that the unphosphorylated channel will open is very small, but finite. This gating mode might not have been detected in the experiments of Herzig et al. (1993) and would have appeared as blank sweeps. This idea of dual phosphorylation sites is made even more attractive by a recent paper by Ono and Fozzard (1993) showing that low concentrations of okadaic acid increased channel availability without affecting channel open time, whereas higher concentrations increased both availability and open time. They interpret these data to indicate that one phosphorylation site controls availability and is dephosphorylated by a different phosphatase than the second site that controls open time.

Although the biophysical consequences of phosphorylation are becoming more clearly defined, biochemical correlates have lagged behind (Hartzell and Duchatelle-Gourdon, 1992). Despite convincing evidence that the skeletal muscle Ca^{2+} channel α_1 subunit is phosphorylated by cAMP-dependent protein kinase (PKA), evidence that the cardiac channel is phosphorylated is limited. There have been several reports that the α_1 subunit is not a substrate for PKA (Hartzell and Duchatelle-Gourdon, 1992). Furthermore, only two of the seven consensus sequences for PKA in the skeletal muscle α_1 subunit are conserved in the cardiac channel. Perhaps more problematic is the absence of an effect of cAMP on I_{Ca} generated by the cardiac α_1 subunit expressed in frog oocytes. Steps toward solving these two problems have recently been published. Yoshida et al. (1992) have found that the gene for the cardiac α_1 subunit expressed in CHO cells produces two forms of Ca^{2+} channel: a minor 250-kDa form which is phosphorylated in response to dibutyryl cAMP and a major 200-kDa species (proteolytic fragment?) which is not phosphorylated. Dibutyryl cAMP also produced a one-fold increase in I_{Ba}. In contrast, I_{Ca} produced by the cardiac α_1 subunit expressed in frog oocytes is not increased by cAMP unless the β subunit of the channel is coexpressed (Klöckner et al., 1992). These data raise the question whether one or both of the putative phosphorylation sites that regulates Ca^{2+} channel function is on the α_1 subunit, another subunit, or another regulatory protein. The slow kinetics of the increase in I_{Ca} in response to cAMP may suggest that an intermediate protein is phosphorylated (Frace et al., 1993).

Although the dogma that the cardiac Ca^{2+} channel is regulated by cAMP-dependent phosphorylation of the channel itself is an attractive model, it is clear that there are still many gaps that remain in tying the biophysics of channel gating and availability to channel phosphorylation. If there are indeed multiple phosphorylation sites, possibly on different subunits or affected by the presence of different subunits, site directed mutagenesis of putative phosphorylation sites should be able to distinguish between sites that regulate gating and availability.

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Calcium Oscillations and Waves: Is the IP_3R Ca^{2+} Channel the Culprit?

Joel Keizer

Institute of Theoretical Dynamics and Section of Neurobiology, Physiology, and Behavior, University of California, Davis, California 95616 USA

In this issue of *Biophysical Journal* Atri et al. (1993) present evidence from modeling studies that the inositol 1,4,5-trisphosphate receptor (IP_3R) is respon-

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sible for Ca^{2+} oscillations and waves in immature *Xenopus* oocytes. The IP_3R is a tetrameric Ca^{2+} channel that is widely distributed in the endoplasmic reticulum (ER). Ca^{2+} that is normally stored in the ER can be released by stimulating the IP_3R channel using physiological amounts of inositol 1,4,5-trisphosphate (IP_3), which is known to be an important second messenger in a number of Ca^{2+}-mediated signaling processes.

Over the past 2 years experiments have implicated the IP_3R as a major player in calcium oscillations and waves in a variety of tissues. Finch and colleagues noted that Ca^{2+} ions both activate and inhibit Ca^{2+} efflux from microsomal preparations, presumably by acting on IP_3Rs. At about the same time Ehrlich's group incorporated IP_3Rs from cerebellum into planar lipid bilayers and determined that the percent open time of these Ca^{2+} channels had a bell-shaped dependence on Ca^{2+} applied to the cytoplasmic face. Since the maximum was relatively sharp with a value near that of resting cytosolic Ca^{2+} (Ca, 100 nM), it seemed possible that cytoplasmic Ca^{2+} could *activate* its release from stores in the ER when the cytoplasmic Ca^{2+} concentration was low and *inhibit* it when the cytoplasmic Ca^{2+} concentration became too high. Indeed, according to Finch, activation was a rapid process with respect to inhibition. These results dovetailed nicely with the work of Parker and Ivorra, whose elegant two-pulse experiments with caged IP_3 indicated that Ca^{2+} release from the ER inhibited further Ca^{2+} release on the time scale of 10–20 s.

To test the possibility that the rapid activation and slow inhibition of the IP_3R by cytosolic Ca^{2+} might be responsible for Ca^{2+} oscillations, De Young and Keizer developed a kinetic model of the IP_3R. Treating the tetrameric receptor as consisting of identical, independent subunits they were able to reproduce the experimental data of Parker and Ivorra (1991) and others. In combination with a slow Ca^{2+} leak from the ER and reuptake into the ER via a SERCA-type Ca^{2+} ATPase, they demonstrated that fast Ca^{2+} activation and slow Ca^{2+} inhibition of the IP_3R produced Ca^{2+} oscillations remarkably like those observed experimentally in a number of cell types. Later they also showed that the kinetic scheme gave rise to Ca^{2+} waves when diffusion of Ca^{2+} and IP_3 were added to these calculations.

In this issue of *Biophysical Journal*, Atri et al. (1993) take that work one step further. Like Li et al. (1993) they have identified rapid activation and slower inhibition of the IP_3R by Ca^{2+} as the essential feedback mechanisms acting on the receptor. In this way the kinetics of the IP_3R channel can be modeled after the familiar Hodgkin-Huxley treatment of ion channels in the squid giant axon. In this case, however, Ca^{2+} replaces the voltage as the variable controlling the conductance of the channel. Using these ideas the open probability of the channel is the product of an activation term due to IP_3 binding, an instantaneous cytosolic Ca^{2+} activation term, and a time-dependent term that inactivates exponentially to a Ca^{2+}-dependent value. The main virtues of this model of the IP_3R are its simplicity and its lack of detailed assumptions regarding molecular mechanism.

Using plausible values for kinetic parameters, Atri et al. (1993) show that their simplified model gives rise to a modified bell-shaped open probability that is compatible with experiments on IP_3-induced Ca^{2+} release from *Xenopus* oocytes. This is important because oocytes have become the preparation of choice for testing ideas about Ca^{2+} waves. Indeed, Miyazaki et al. (1992) have shown that monoclonal antibodies to the IP_3R eliminate the fertilization wave in hamster oocytes. Compelling experiments by Clapham and coworkers have shown that immature *Xenopus* oocytes also support Ca^{2+} waves, including an intriguing assortment of spiral waves.

With their model Atri et al. (1993) have carried out realistic calculations in two spatial dimensions that include the effect of diffusion of Ca^{2+} and IP_3. It is now known that IP_3 has an effective diffusion constant in cytoplasm that is several orders of magnitude larger than Ca^{2+}. This is due to the fact that only one of approximately every one hundred Ca^{2+} ions is free to diffuse in the cytosol, with the remainder sequestered by immobile or slowly diffusing endogenous buffers. Their calculations demonstrate that the Ca^{2+} wave speeds of 7–15 $\mu m \cdot s^{-1}$ observed experimentally can be explained primarily by diffusion of Ca^{2+} alone. They also explain the recent observation that increasing Ca^{2+} influx from the external medium increases the wave speed.

The mechanism of Ca^{2+} oscillations based on this type of mechanism is simple: IP_3 primes the receptors by increasing their open probability. Once the open probability reaches a significant level, more Ca^{2+} is released than can be pumped back into the ER by the Ca^{2+} ATPase and the cytosolic Ca^{2+} concentration starts to increase. This, in turn, activates the IP_3R, releasing even more Ca^{2+} until activation gives way to Ca^{2+}-induced inhibition, which turns off the release. This permits the Ca^{2+} ATPase to pump Ca^{2+} back into the ER, thus lowering the cytosolic Ca^{2+} and reactivating release. In this mechanism, Ca^{2+} waves are nothing more than a manifestation of localized Ca^{2+} oscillations propagating via diffusion—a typical result for excitable media.

So to what extent does this model *explain* Ca^{2+} oscillations and waves? Certainly it does not do so in cells where the IP_3R is absent and where Ca^{2+} release via the ryanodine receptor/Ca^{2+} channel predominates. Nonetheless, recent work by Györke and Fill (1993) suggests that related mechanisms may control Ca^{2+}-induced Ca^{2+} release through the ryanodine receptor. On the other hand it seems likely that in addition to oocytes these ideas apply to certain excitable cells, such as the pancreatic β cell (Keizer and De Young, 1993) and pituitary gonadotrophs (Li et al., 1993). Despite the fact that the bell-shaped activation and inhibition of the IP_3R is best documented in cerebellar Purkinje cells, their ER contains both IP_3Rs and ryanodine receptors. The differential compartmentalization of the two receptor types in those cells suggest that understanding Ca^{2+} kinetics in the cerebellum and other tissues will require considerably more work.

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Lipid Molecular Shape and High Curvature Structures

Sek Wen Hui

Biophysics Department, Roswell Park Cancer Institute, Buffalo, New York 14263 USA

It has been a long standing puzzle why cells go to such length to meticulously maintain distinct lipid composition profiles for each of their organelle membranes. This question is especially puzzling when there is still a prevailing group of membranologists regarding membrane lipids to be merely a passive matrix serving no more than a structural frame for functioning protein components. A closer look into the lipid profiles of biomembranes reveals an interesting correlation, that those membranes having higher protein-to-lipid ratios contain more lipids that do not self-assemble into a planar bilayer form, which is expected for membrane lipids (Hui and Sen, 1986). These membranes include the energetically active mitochondrial inner membranes, and membranes of thylakoid and of photoreceptor discs in the retina rod outer segment. On the other hand, inactive membranes such as myelin, which has a low protein to lipid ratio, contains mostly bilayer-forming lipids. It seems that lipids which do not remain in plane bilayer form by themselves at physiological conditions are necessary for certain membrane functions. The article by Lee et al. (1993) adds a simple but intriguing aspect to many recent studies of the molecular packing of these lipids.

What distinguishes lipids that self-assemble into planar bilayers from those that do not? In spite of a wealth of experimental data on the classification of membrane phospholipids according to their polymorphic phase preference (see review by Seddon, 1990), a quantitative criterion is still not available. Isrealachvili et al. (1977) first defined a packing factor $f = v/al$ for amphiphilic molecules, where v and l are the unconstrained volume and length of the hydrophobic moiety of the lipid, and a is the optimal (unconstrained) hydrophilic surface area. Molecules with $f = 1$ tend to form planar bilayers, whereas those with $f < 1$ or $f > 1$ tend to form positive or negative curvature structures, respectively. In other words, if the cross sections of the hydrophobic and hydrophilic moieties of the lipid molecule are unequal, it is energetically unfavorable to pack these lipid molecules into a planar bilayer form.

The next problem is to measure the parameters v, a, and l experimentally. Intuitively, one would expect these geometric parameters, especially v and l, to vary with temperature. Increasing temperature presumably would increase v and reduce l due to increasing thermal motion of the hydrocarbon chains, and indeed increasing temperature favors the formation of negative curvature structures from lamellar structure (Seddon, 1990). Yet to obtain the absolute values of v, l, and a is not trivial. Hui and Sen (1989) approximated the values of l by equivalent chain lengths deduced from chromatographic retention times, and the values of v by the equivalent specific volumes of corresponding hydrocarbons. Determining the values of a required more assumptions and approximations. The conformation of many headgroups are still unknown. Furthermore, there is the question: should any water molecules be included in the headgroup volume estimation, according to the polarity of the headgroup? How to account for possible hydrogen bonding or electrostatic repulsion between headgroups? Even if all this information is in hand, reducing them to a representative value of a still involves some modelling assumptions.

Despite the considered complications in the determination of a, Lee et al. (1993) showed that the sum of covalent volumes of headgroup atoms of a series of homologous phospholipids inversely correlated with their potential to destabilize the bilayer. In other words, when v and l of these lipids are equal, the tendency to form negative curvature structures decrease linearly with $a^{3/2}$ (hence $1/f^{3/2}$) of equivalent covalent spheres of the headgroup. This simple correlation is very impressive, considering the years of qualitative argument of the shape hypothesis without reliable ways to measure the optimal shape of molecules. Although it is not a direct determination of the f values, it gives some confidence in using covalent volume of headgroups to deduce f.

The monotonic relation of a and T_H reported by Lee et al. (1993) is not surprising even that the polarity and charge of the headgroups were deliberately overlooked. The electrostatic repulsion between headgroups of charged lipids is negligible in mixtures containing only dilute charged lipids. The dipole field that orders water molecules on the bilayer surface is not a simple sum of those of isolated headgroups, therefore accounting water molecules is imprecise. These factors may not be of sufficient significance to disrupt the reported monotonic relationship. However, an intriguing point of this paper is the linear relationship. The lowering of T_H is not simply related to the free energy ΔG due to curvature frustration. The value of ΔG, when a spontaneously curved surface is constrained to a flat bilayer, is proportional to the bending modulus k and the square of the spontaneous curvature c_0 (Winterhalter and Helfrich, 1992):

$$\Delta G = (\tfrac{1}{2})kc_0^2.$$

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