

a surface membrane Ca channel that is influenced by depletion of intracellular Ca stores. In 1986, when Putney proposed the "capacitative model . . . for receptor-regulated calcium entry," few electrophysiologists must have predicted its impact. The capacitative model was a generalization and extension of earlier proposals of van Breemen (1977), Casteels and Droogmans (1981), and Brading et al. (1980) for smooth muscle: that the sarcoplasmic reticulum (SR) forms a "superficial buffer-barrier" against the movement of extracellular calcium to regions deep within the cell, and that the filling of the SR occurs by a special access pathway to the extracellular medium. Thus, the SR and surface membrane were hypothesized to act as a coordinated system controlling Ca penetration. Putney posed that in cells that are regulated through surface membrane receptors, emptying the endoplasmic reticulum (ER) would increase Ca permeability of the surface membrane. In other words, he emphasized that the *degree of filling* of the ER was the important parameter regulating the movement of Ca into the cell through this special access pathway, hence the name "capacitative model."

Occupation of surface membrane receptors regulates intracellular Ca stores through the diffusible intracellular second messenger, D-myo-inositol 1,4,5-trisphosphate (IP₃). A salient feature of the Putney hypothesis is that IP₃ or other metabolites of the inositol lipid system do not act directly on specialized Ca channels of the surface membrane; rather, they act only indirectly by emptying the intracellular stores.

Signaling from surface membranes to intracellular membranes has been firmly established: for example, via IP₃ or direct coupling between membranes. The capacitative model requires that information be transferred in precisely the opposite direction, i.e., from intracellular to surface membranes. Evidence has recently begun to support this idea in a number of cellular systems. Notably, in T-lymphocytes (Zweifach and Lewis, 1993) and in mast cells (Hoth and Penner, 1993), a Ca current can be elicited by a number of maneu-

vers that deplete ER stores. In both cells, the Ca pathway appears to have a very low single-channel conductance. In lymphocytes, the single-channel conductance has been estimated by noise analysis to be ~24 fS in 110 mM CaCl₂, too low for detection in conventional single-channel records.

Furthermore, a diffusible messenger that increases the surface membrane permeability has been found and partly characterized in human tumor lymphocytes (Randriamampita and Tsien, 1993). The soluble mediator has a molecular weight under 500, is inactivated by alkaline phosphatase but not by heat or protease, and is released into the cytoplasm (and into the extracellular medium) upon depletion of the intracellular Ca stores.

The article by Lückhoff and Clapham describes a Ca channel in an epidermal cell line (A431) that is transiently activated by experimental conditions expected to reduce the Ca content of the ER. The slope conductance, although small (2 pS in 200 mM CaCl₂), was larger than the 24-fS channel of lymphocytes and large enough to be observed as single-channel openings. Their report represents the first single-channel records of a Ca depletion current, and it demonstrates that all Ca depletion channels cannot be identical because theirs has different conductance and selectivity properties than the 24-fS channel already described.

Now that a Ca depletion channel has been found with conductance large enough for single-channel recordings, more techniques can be applied to the problem. In addition to having the availability of single-channel analysis, one can envision a time when such channels (and perhaps accessory molecules) will be incorporated into artificial membranes, and purified chemical messengers will be added to regulate the channel openings.

A large number of unknowns remain. How many types of Ca channels are coupled to the depletion of internal stores? What are the coupling mechanisms? Might physical, as well as chemical, links be utilized? What is the nature of the chemical messenger that has already been partly identified

(Randriamampita and Tsien, 1993), and are there more than one? Finally, can this pattern of functional communication from intracellular stores to the surface membrane be generalized? Are there comparable signals between other intracellular membranes; for example, does the ER talk to the mitochondria?

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Reining in Calcium Release

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In striated muscles, skeletal or cardiac, contraction is triggered by a sudden increase in the concentration of free Ca²⁺ near the myofilaments. The relaxation that follows contraction is brought about by the return of [Ca²⁺] to resting levels. A fast contraction-relaxation cycle depends on the mechanisms that ensure a fast decay of the elevated [Ca²⁺] as much as on those that determine its rapid increase.

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Therefore, muscles have elaborate systems for rapid removal of Ca^{2+} from free solution in the myoplasm. It is increasingly clear, in addition, that the mechanisms of Ca release are also adapted to rapidly saturate the Ca binding sites on troponin and then stop. This avoids overburdening the intracellular milieu with Ca^{2+} , whose prolonged elevation has damaging consequences.

The importance of these brakes on Ca release transcends the simple protection from unwanted Ca^{2+} in the myoplasm. The chief mechanism of activation of Ca release from the sarcoplasmic reticulum of cardiac muscle is calcium-induced calcium release (CICR). The trigger Ca^{2+} is supplied by the sarcolemmal Ca current (I_{Ca}), which crosses L-type membrane Ca channels in response to depolarization. The ghost of instability lurks near the models that physiologists make: because both the trigger for release (I_{Ca}) and release itself result in increased $[\text{Ca}^{2+}]$, the process should be self-perpetuating and all-or-none.

Somehow, both increases are not hopelessly intertwined. In voltage clamp experiments, Ca^{2+} release remains a graded, monotonic function of the amount of Ca^{2+} supplied by I_{Ca} . More dramatically, it is possible to stop Ca release instantly by stopping I_{Ca} with a large increase in transmembrane potential (Cleeman and Morad, 1991). The stability of such a system necessarily relies on the presence of self-limiting mechanisms in Ca release (Fabiato, 1985; Stern, 1992).

Which are these mechanisms? In skeletal muscle, it appears that the release channels are closed by Ca^{2+} on the cytoplasmic side, an effective means of self-limiting Ca^{2+} flux. Recent measurements revealed that release also decays rapidly after an early peak (Wier et al., 1994). When studied in planar bilayers, however, the cardiac Ca^{2+} release channels differ from their skeletal muscle counterparts in not showing inactivation at high $[\text{Ca}^{2+}]$ (Rousseau et al., 1990). Because the sarcolemmal channels that pass I_{Ca} are endowed with their own inactivation mechanism, it was long thought that cardiac Ca release did not need to inactivate inde-

pendently, and its decay was tied to the inactivation of I_{Ca} . Yasui et al., in this issue of *Biophysical Journal*, uncover another mechanism that terminates Ca release. Using a drug, they were able to slow inactivation of I_{Ca} in cardiac myocytes; release flux, simply inferred from the time derivative of the Ca^{2+} transient, was still found to inactivate rapidly in these cells. A more quantitative derivation of Ca release (Weir et al., 1994) will be necessary to clearly establish the role of I_{Ca} inactivation, but the rapid decay of release inferred by Yasui et al. could not be caused by a decay in I_{Ca} and must be a manifestation of an intrinsic inactivation of release.

Slowing inactivation of I_{Ca} had an additional payoff. After a depolarization that elicits I_{Ca} and a large Ca release of a duration sufficient to allow for inactivation of Ca release but not I_{Ca} , returning the membrane potential to resting level resulted in a large "tail" of membrane current. The increased intracellular $[\text{Ca}^{2+}]$ caused by this tail elicited additional release. The ability of apparently inactivated channels to reopen upon exposure to higher $[\text{Ca}^{2+}]$ was previously demonstrated for cardiac Ca release channels in bilayers, and the phenomenon termed "adaptive inactivation" (Gyorke and Fill, 1993). In that work, adaptive inactivation was seen as a spontaneous decay of channel activity induced by a step increase in $[\text{Ca}^{2+}]$, but this decay seemed too slow to play a significant functional role. The new results in this issue indicate that adaptive inactivation proceeds much more rapidly in living cells, and is probably a chief mechanism limiting Ca release.

The functional relevance of this adaptive response to Ca^{2+} gives more meaning to the concept of a superfamily of intracellular Ca^{2+} channels (Marks and Brillantes, 1993). The IP₃ receptor, a major Ca^{2+} release channel of smooth muscle and nonmuscle cells, also features the ability to respond adaptively to increases in IP₃ (Muallem et al., 1989). These parallel discoveries of adaptive properties in the two main classes of intracellular Ca channels pose two clear challenges; to clarify the molecular mechanism of this flexible response, and to understand the func-

tional rewards that justify the existence of such mechanisms.

The demonstration of an intrinsic inactivation property brings the cardiac Ca release channels closer to their skeletal muscle counterparts. Is there more to be learned here for skeletal muscle physiology? There is increasing evidence of participation of CICR in skeletal muscle, but the system remains always under control by the membrane potential. Thus, the problems of stability in the presence of positive feedback are as mystifying in skeletal as in cardiac muscle. The detailed mechanisms, however, are likely to be different. Unlike cardiac release channels, the skeletal muscle channels are inactivated by elevated Ca^{2+} in bilayers. When confronted with a step increase of $[\text{Ca}^{2+}]$ the skeletal muscle channels display adaptive inactivation, (Gyorke et al., 1994), but its rate in bilayers is several-fold slower than for cardiac channels, to the point that one must wonder whether it has any physiological significance.

In both types of muscle, local increases in $[\text{Ca}^{2+}]$, associated with steep spatial gradients largely determined by the details of supramolecular structure and association of channels, will be a necessary ingredient of our view of mechanisms (Stern, 1992; Weir et al., 1994; Gyorke and Palade, 1993). Classical macroscopic, or single-pool models of activation will yield to local models, in which three-dimensional structural features and the open-close kinetics of individual channels will be important. One great challenge is to produce such a picture with a minimum of parameters. The recent findings suggest that this picture will have many common features in muscle and non-muscle cells.

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Lipids Under Power Pressure

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Lipid phase transitions are very popular and interesting objects of biophysical investigation. They are, in general, easily visible by a whole host of physical techniques and, at least in chemically well defined model systems, readily open to thermodynamic analysis. X-ray diffraction on such systems is also relatively easy because of the low dimensionality of hydrated lipid specimens, the signal analysis is quite straightforward (at least in the case of multibilayer liposomes), and results in the essential parameters of bilayer and water layer thickness, from small-angle diffraction, and of hydrocarbon chain packing, from wide-angle data. As compared with single crystal structure analysis, the experimental and computational efforts are relatively small. With powerful synchrotron x-ray sources, the time resolution of modern experiments has been pushed down into the millisecond time domain, opening the field for direct approaches to the supramolecular dynamics through x-ray cinematographic observation.

The time-scale of milliseconds might look modest in the eyes of spectroscopists, at first sight, but it must not be overlooked that this is different from the usually quoted, fast time scales intrinsic to the techniques of molecular

(nuclear or electronic) spectroscopy. Although these techniques normally probe the molecular dynamics at or close to equilibrium by accumulating signals over a time period at least in the order of seconds, i.e., many times (by 5–12 orders of magnitude) larger than the motional correlation times in question, a time-resolved x-ray diffraction experiment is essentially a one-shot movie of a dynamic process.

By clever combination of time-resolved x-ray diffraction with physically well defined triggering mechanisms, lipid phase transitions can be studied in their mechanistic, structural, and thermodynamic aspects through one set of experiments. What more do we want? Indeed, there is more: by crossing the borders from equilibrium structure and thermodynamics to a non-equilibrium situation (as, e.g., in a large-amplitude jump), the linear response-regime can be left behind, and it becomes possible, and perhaps likely, that the mechanistic pathway changes and a bifurcation might occur. Knowledge in this field is still very limited for lipid systems.

Caffrey and his group have, in parallel with other groups (Gruner, 1987; Laggner and Krichbaum, 1991), pioneered this field through systematic exploitation of the possibilities of synchrotron x-ray diffraction and rapid jump-relaxation techniques. In their most recent article (Cheng et al., 1994), they report on an improved p-jump experiment, providing an important additional source of information using simultaneous small- and wide-angle detection.

Pressure as a variable has the advantage over the more commonly used temperature perturbation, because it can be varied equally rapidly in both directions through the phase transition range, and it is homogeneous throughout the system (within the limits of sound velocity). A complication, of course, is the fact that both adiabatic p- or T-jumps are always accompanied by a corresponding change in the other variable. In T-jumps, this dissipates faster (through a pressure wave), whereas with p-jumps the temperature change fades out more slowly through

heat conduction. Combining information from both types of experiments and from static, near equilibrium studies is certainly wise.

Cheng et al. found a rather complex kinetic behavior for a seemingly simple transition, the $L_{\beta'}/L_{\alpha}$ process in a hydrated phosphatidylethanolamine lipid (DHPC). Most surprising is the fact that the melting transition (depressurization from β' to α , i.e., order-to-disorder) is a two-step process, invariate in its transit time with respect to the jump amplitude, whereas in the other direction (pressurization into the ordered β' -phase) the process is by one to two orders of magnitude faster and apparently one-step. The first, fast step in the order-disorder transition appears as a lateral dilation of the ordered hydrocarbon chain packing, without change in lattice type, and the second, rate-limiting step is the chain melting and the change in inter-bilayer hydration. On a similar lipid system (1-stearoyl-2-oleoyl-*sn*-3-phosphatidylethanolamine, SOPE), laser-induced T-jump experiments with a time resolution of 1 ms have indicated a one-step, martensitic transition process that occurs within the time of energy deposition (Krichbaum et al., 1989). In the T-jump transition from the metastable ripple phase to the liquid crystalline phase of DPPC, a rapid, synchronous change of both the hydrocarbon chain packing and the interbilayer hydration was found (Rapp et al., 1983). There is no straightforward explanation for this discrepancy with the present results of Cheng et al. More, certainly, will be learned from a careful delineation of the temperature-pressure phase diagram of these systems.

Signs of transient structural intermediates upon jump-relaxation have been detected up to now in only a few lipid systems. Their nature and systematic significance for the underlying supramolecular rearrangements will have to be better defined. If these intermediates, however, prove to be bound to dynamic bifurcations (where is the threshold beyond which these occur?) between linear and nonlinear nonequilibrium situations under rapid jump conditions, a substantial expansion of