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New and Notable

Phospholamban, Phosphorylation, and Phosphorescence

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The Ca^{2+} pump from skeletal muscle sarcoplasmic reticulum (SR) has been studied for many years as a prototype for understanding structure/function relationships of membrane transporters. The calcium pump from heart SR is of special interest in that its pumping is modulated by phospholamban, an amphipathic basic polypeptide of approximately 6000 molecular weight that appears to exist in the membrane as a pentamer (Tada et al., 1988). In the submicromolar Ca^{2+} concentration range, phospholamban attenuates the calcium pumping rate. Phosphorylation of phospholamban by protein kinases relieves the inhibition of the calcium pump so that more Ca^{2+} is pumped into the SR (Inui et al., 1986). This regulation of the calcium pump in heart SR explains, at least in part, the basis for the stronger heart beat (inotropy) by the action of catecholamines on the heart. Thus, an understanding of the molecular and physical basis for the regulation of the Ca^{2+} pump by phospholamban has great physiological and clinical import. A report in this issue by Voss et al. provides evidence that the regulation of the Ca^{2+} pump (Ca-ATPase) of cardiac sarcoplasmic reticulum (SR) is related to critical changes in protein dynamics and protein-protein interactions.

Time-resolved phosphorescence anisotropy (TPA) is a powerful spectroscopic technique that has been used to detect microsecond rotational motions of the Ca-ATPase and, thus, to provide insight into the distribution of oligomers in the membrane. Using this technique, Birmachu et al. (1990) showed that the dependence of SR Ca-ATPase activity on temperature correlates with inhibitory self-association of the Ca^{2+} pump. Voss et al. (1991) used TPA to show that aggregation-based inhibition could also be induced by melittin, a peptide from bee venom that has been shown to inhibit a variety of membrane ion pumps. This result was intriguing for investigators of cardiac SR, because melittin shares phospholamban's basic amphipathic character. Recent research in this field has focused on the proposal that pump inhibition and activation depend on a physical interaction between phospholamban and the Ca^{2+} pump (James et al., 1989).

In the report in the present issue, Voss et al. have taken an important step toward elucidating the physical basis of this regulatory system. They labeled the Ca^{2+} pump with a phosphorescent dye, then used laser-pulsed TPA to detect the microsecond rotational dynamics of the labeled protein as a function of $[Ca^{2+}]$ and phospholamban phosphorylation. A distribution of oligomeric complexes of the Ca^{2+} pump protein was detected, ranging from rapidly rotating monomers to slower oligomers and immobile large aggregates. The enzyme was much less mobile in cardiac SR than in skeletal SR. The most striking difference was a substantial population of large, immobile aggregates in the cardiac sample. They proposed that phospholamban inhibits the Ca^{2+} pump by aggregating it, and phosphorylation of phospholamban activates by reducing this inhibitory aggregation. This model was supported by the $[Ca^{2+}]$ dependence of protein disaggregation, which correlated well with that of Ca^{2+}-uptake activity. The effect of phosphorylation was maximal at submicromolar $[Ca^{2+}]$ and negligible at micromolar $[Ca^{2+}]$, where calcium activates and disaggregates the pump by itself. Thus, it appears that either phospholamban phosphorylation or micromolar calcium releases the pump from a kinetically unfavorable associated state. At submicromolar $[Ca^{2+}]$, the positive charge on unphosphorylated phospholamban encourages the association with negatively charged regions of adjacent Ca^{2+} pump proteins, thus restricting the ability of the pump to undergo required conformational changes. The binding of Ca^{2+} to the pump proteins or the addition of the negatively charged phosphate to phospholamban provides an electrostatic switch that dissociates the complex and frees the pump to go through its catalytic cycle.

These results help provide a unified physical explanation for a number of recent biochemical observations in cardiac SR. The direct physical interaction of phospholamban with the Ca^{2+} pump has been demonstrated (James et al., 1989), and this interaction is dependent on phospholamban's positive charge (Xu and Kirchberger, 1989). Kinetic evidence suggests that phospholamban inhibits by stabilizing the E2 conformational state of the enzyme, which has been correlated with self-association of the Ca^{2+} pump.

Further testing and refinement of this model will require more direct probing of the structure and dynamics of phospholamban itself and its effects on structural changes within the pump protein. An essential component of these studies will be site-directed spectroscopic probes of both phospholamban and the Ca^{2+} pump, which are being made possible in part by rapid advances in site-directed mutagenesis and expression of these proteins (Toyofuku et al., 1994). These results could have broader implications. Electrostatically controlled protein-protein interaction is an attractive general mechanism for the regulation of membrane proteins. Phospholamban-like peptides (i.e., amphipathic, basic membrane-binding peptides that are kinase substrates) and phospholamban-like protein domains are associated with a wide range of membrane enzymes and pumps. More generally, lateral association of membrane proteins has been implicated in a wide range of signal transductions, from growth factor binding, to

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G-protein action, to heat shock. The development of the TPA technology for detecting and quantitating protein association will be a powerful technique in probing physical mechanisms in these processes.

REFERENCES

Birmachu W., and D. D. Thomas. 1990. Rotational dynamics of the Ca-ATPase in sarcoplasmic reticulum studied by time-resolved phosphorescence anisotropy. *Biochemistry.* 29:3904–3914.

Inui, M., B. K. Chamberlain, A. Saito, and S. Fleischer. 1986. The nature of the modulation of Ca^{2+} transport as studied by reconstitution of cardiac sarcoplasmic reticulum. *J. Biol. Chem.* 261:1794–1800.

James, P., M. Inui, M. Tada, M. Chlesi, and E. Carafoli. 1989. Nature and site of phospholamban regulation of the Ca^{2+} pump of sarcoplasmic reticulum. *Nature.* 342:90–92.

Tada, M., M. Kadoma, M. Inui, and J. Fujii. 1988. Regulation of Ca^{2+}-pump from cardiac sarcoplasmic reticulum. *Methods Enzymol.* 157:107–154.

Toyofuku, T., K. Kurzydlowski, M. Tada, and D. H. MacLennan. 1994. Amino acids glu2 to ile 18 in the cytoplasmic domain of PLB are essential for functional association with the Ca^{2+}-ATPase of sarcoplasmic reticulum. *J. Biol. Chem.* 269:3088–3094.

Voss, J., D. Hussey, W. Birmachu, and D. D. Thomas. 1991. Effects of melittin on molecular dynamics and Ca-ATPase activity in sarcoplasmic reticulum membranes: time-resolved optical anisotropy. *Biochemistry.* 30: 7498–7506.

Voss, J., L. R. Jones, and D. D. Thomas. 1994. The physical mechanism of calcium pump regulation in the heart. *Biophys. J.* 67:190–196.

Xu, Z., and M. A. Kirchberger. 1989. Modulation by polyelectrolytes of canine cardiac microsomal calcium uptake and the possible relationship to phospholamban. *J. Biol. Chem.* 264: 16644–16651.

Calcium Buffering and Diffusion: On the Resolution of an Outstanding Problem

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In recent years, there has been an explosion of interest in the dynamic behavior of intracellular Ca^{2+}, as it has been discovered that its behavior is considerably more complex than had been previously realized. Not only does the concentration of intracellular free Ca^{2+} show complex oscillatory patterns ranging from baseline spiking to sinusoidal oscillations, but the spatiotemporal properties of Ca^{2+} wave propagation, within a single cell or between cells, are equally complex. For instance, in large cells such as *Xenopus* oocytes, propagating waves of Ca^{2+} can form target patterns, periodic plane waves, and even multiple spiral patterns (Lechleiter and Clapham, 1992), whereas in glial cell cultures, intercellular Ca^{2+} waves can coexist with asynchronous intracellular oscillations (Charles et al., 1991). There is general agreement that Ca^{2+} oscillations serve to regulate many aspects of cellular function, whereas waves are important for the communication of such regulation over greater distances, and the coordination of whole-cell and multicellular activity. This complex dynamical behavior has also attracted the attention of theoreticians. Because of the nonintuitive nature of the underlying control mechanisms, theoretical work has a vital role to play in the study of these phenomena. A system that is capable of spiral wave formation, frequency regulation, and repetitive wave activity simply cannot be understood in detail without theoretical models. To date, modeling has made some significant contributions toward the understanding of the possible mechanisms underlying Ca^{2+} oscillations and wave propagation. Nevertheless, despite the importance of theoretical work, there is one aspect of Ca^{2+} control that has so far been comparatively neglected, and that is the question of Ca^{2+} buffering.

For convenience, many models (where they did not ignore the question completely) have assumed that Ca^{2+} buffering is fast and nonsaturable. This has the advantage of merely introducing a scale factor for the Ca^{2+} dynamics, but suffers from the disadvantage of not being terribly accurate in many circumstances. Another approach has been to perform detailed numerical studies of models that incorporate different types of buffers with many kinetic parameters. The paper by Backx et al. (1989) is an excellent example of this kind of work. However, these studies do not usually give insight into the ways in which buffers can change the qualitative behavior of a system. Results for specific kinetic parameters can be obtained, but the behavior of the model for different kinetic parameters remains unknown until a specific simulation is carried out. Because of the large number of parameters in such models, a comprehensive study of the effects of buffers would require an inordinate amount of computer time, and in any event, would be extremely difficult to interpret. One would wish to have a more general theory of the effects of buffers on oscillatory and wave activity, a general theory that could be used to understand specific results in terms of a broader framework.

One of the most important theoretical questions for which a general theory would be desirable is that of effective diffusion coefficients. That is, when Ca^{2+} is buffered, does it still move according to the diffusion equation, but with a lower diffusion coefficient, or does Ca^{2+} obey a fundamentally different transport equation? Although it has been known for some time that fast nonsaturating buffers merely reduce the diffusion coefficient of Ca^{2+} by a constant factor, the more general case of saturating, mobile buffers was not well understood at all. An important advance was made by Irving et al. (1990), who derived the expression for the effective diffusion coefficient of Ca^{2+} in the presence of multiple mobile buffers. According to Irving et al., Ca^{2+} does obey a diffusion equation in this case, but the diffusion coefficient will depend on the concentration of Ca^{2+}, as well as the diffusion coefficients and kinetic parameters of the various buffers. However, their analysis was restricted to the case where the Ca^{2+} gradients are small. The question of effective diffusion coefficients (or lack thereof) has been finally resolved by Wagner and Keizer (1994) in this volume. They derive a transport equation for Ca^{2+} in the presence of multiple buffers, mobile and immobile, and show that, in the general case when

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