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.

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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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Ca^{2+} gradients are not small, Ca^{2+} obeys a nondiffusive transport equation. The transport equation comes in two pieces. The first term is the usual type of diffusion term but with a diffusion coefficient dependent on Ca^{2+}. This is the same expression that was derived by Irving et al. However, the second term in the transport equation is a nonlinear function of the Ca^{2+} gradient and can be significant in regions where the gradient is large.

This elegant result has a number of important consequences. Most importantly, it shows that the effects of buffers cannot, in general, be modeled by a reduction in the diffusion coefficient of Ca^{2+}. A small amount of mobile buffer can have a disproportionately large effect on the transport equation. A number of ways in which this result affects the interpretation of experimental data spring to mind. For instance, there has been some controversy in the literature as to the identity of the diffusing messenger that propagates the Ca^{2+} waves observed in *Xenopus*, and calculation of the effective diffusion coefficient of Ca^{2+} has played a central role in these arguments. However, such effective diffusion coefficients cannot always be defined and, therefore, such arguments are at best unreliable. Other examples are discussed by Wagner and Keizer.

Although the work of Wagner and Keizer has advanced our understanding of the effects of buffers, many questions remain unanswered. How will buffers affect the existence of waves? Can mobile buffers cause the breakdown of wave propagation, or will they have little effect? How will the wave speed be affected? Intuitively, one expects that mobile buffers will have a tremendous influence on the speed of propagating waves, but this remains to be quantified. What effect will buffers have on wave profiles? It has already been shown that the relationship between the space constant of the wave front, the speed of the wave, and the diffusion coefficient of Ca^{2+} is profoundly affected by buffers (Sneyd and Kalachev, 1994), but can one make more explicit predictions? The new transport equation will play a pivotal role in the study of such theoretical questions. The incorporation of buffering terms into a single transport equation will, one hopes, simplify the analysis of wave propagation in buffered systems. Instead of having to deal with multiple diffusion equations, theoreticians, instead, can study the behavior of a single equation, with all the simplifications this implies.

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Resonance Raman Microspectroscopy in Biology

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The development of resonance Raman spectroscopy as a spectroscopic and analytical tool has occurred rapidly over the past twenty years. Progress in implementing this technique has been driven by the molecular level insight it provides, by the experimental versatility with which it can be implemented, and by advances in laser, detector, and spectrograph technology. The article in this issue of *Biophysical Journal* by Salmaso et al. demonstrates many of these advances in a Raman microspectroscopic characterization of mammalian peroxidases. This class of enzyme has attracted considerable recent interest because of their critical roles in the antimicrobial defense systems in higher animals.

The insight into molecular process available from resonance Raman spectroscopy derives from the fact that it provides vibrational data under conditions of resonance with optical (electronic) transitions. Thus, it ties the inherently high information content of vibrational spectroscopy to the dissective capabilities of selective optical excitation and is ideally suited to modern high resolution laser technology. The underlying principles of both its vibrational and electronic aspects are well understood. This sound theoretical basis, coupled with the fact that it can be carried out over a broad temperature range with both pulsed and continuous wave lasers, extends its range beyond static structural characterization to kinetic and dynamic applications (e.g., Riordan and Vallee, 1993).

Laser excitation is bright and easy to manipulate optically, which provides considerable flexibility in sample geometry and physical state. All that is necessary is to bring laser light to a focus on a sample positioned at the focus of the spectrometer collection optics. This situation minimizes the requirements for sample volume, which can be considerably less than 1 μl, and allows solid, liquid, and gaseous samples to be used. The sustained progress in laser technology over the past three decades has provided continuously tunable excitation frequencies from the infrared to the vacuum ultraviolet region; moreover, with modern mode-locking methods, temporal resolution to the subpicosecond regime is routinely available. Only uncertainty broadening, which becomes appreciable in the femtosecond region, now limits the time resolution of a Raman measurement.

These developments in laser technology have been matched by improvements in detector technology,

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