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small polarons, there is a barrier to their formation. Electrons with finite parallel momentum can help to overcome this barrier. On the other hand, fast-moving electrons will be more difficult to trap at specific sites. From these classical arguments, one intuitively expects an optimum parallel momentum to facilitate localization. The experiment suggests that this effect is present but that there is an additional strong quantum contribution from resonant tunneling into intramolecular vibrational modes that are involved in the formation of the polaron state. The determination of the time scales and energetics as well as the identification of the relevant phonon modes surely provide an excellent basis for further in-depth theoretical studies of the localization dynamics for this system.

Such detailed experimental information is hardly accessible with transient absorption or other purely optical techniques that have been used to time-resolve electron dynamics in bulk materials (*11*, *12*). It is one of the great virtues of time-resolved photoelectron spectroscopy from two-dimensional systems that by measuring the kinetic energy, the emission angle and possibly also the spin of the emitted photoelectron, one is able to completely determine the time evolution of its quantum state before ejection. On the picosecond time scale, these capabilities have been applied most successfully at semiconductor surfaces (*13*, *14*). Recent progress toward a time resolution of 10 fs and the investigation of coherence phenomena have opened up many possibilities (*10*, *15*, *16*). The work by Ge *et al.* might very well mark the point where this powerful probe for ultrafast electron dynamics is applied to areas of research that are not directly connected to the physics and chemistry of surfaces.

In chemistry and biology, for example, the interplay between localization and delocalization of weakly bound electrons is crucial for electron transfer reactions in large molecules such as photosynthetic reaction centers. Furthermore, self-trapping phenomena are important in the dynamics of solvated electrons (that is, excess electrons in liquids that are used as models for studying the chemical physics of solvation). And, quite generally, there is a close relation between the theory of polarons and the electron transfer theory of Marcus (*17*). In terms of technological applications, small polarons are believed to play an essential role in recently discovered materials, such as high-temperature superconductors and manganates with giant magnetoresistance, as well as in conductive polymers, organic light-emitting diodes, and other devices in the rapidly expanding field of molecular electronics.

Certainly, the preparation and characterization of surface layers of some of these materials will not always be simple or even possible. There are obvious differences in the physics of electron localization in two dimensions and in three dimensions. The presence of a conducting substrate, which is highly desirable for photoemission experiments, may lead to undesired coupling effects. However, over the years, surface scientists have learned how to master many of the preparative difficulties. Coupling effects, for instance, can easily be eliminated or controlled by using oxidized surfaces or spacer layers (*18*, *19*). To this end, the fact that solids have well-defined surfaces might very well prove to be a blessing for future investigations of electron dynamics in many areas of science.

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SIGNAL TRANSDUCTION

Calcium Signaling: Up, Down, Up, Down.... What's the Point?

James W. Putney Jr.

The simple, ionized form of the element calcium belies its value as a key carrier of information in cells. Just over a decade ago, this messenger was first seen: Calcium-sensitive photoproteins and fluorescent dyes allowed scientists to track calcium concentrations in the cytoplasm of single, living cells in real time and as they responded to outside cues (*1*). In neurons and other excitable cells, where calcium channels are opened by membrane depolarization, it was not surprising that intracellular calcium concentrations rose and fell along with the cyclical depolarizations associated with action potentials. However, it came as something of a shock that, even in nonexcitable cells, hormone stimulation triggered a series of pulses of calcium inside cells, superimposed on a baseline level (*2*).

Two fundamental questions remain: How do these oscillations arise? And what is their function (*3*)? From years of research, something is known about the answer to the first question, but the answer to the second—what the oscillations are actually doing—has remained a mystery. Now, an elegant and creative experimental approach to understanding how molecules decode intracellular calcium oscillations is described in a report by De Koninck and Schulman on page 227 of this issue (*4*).

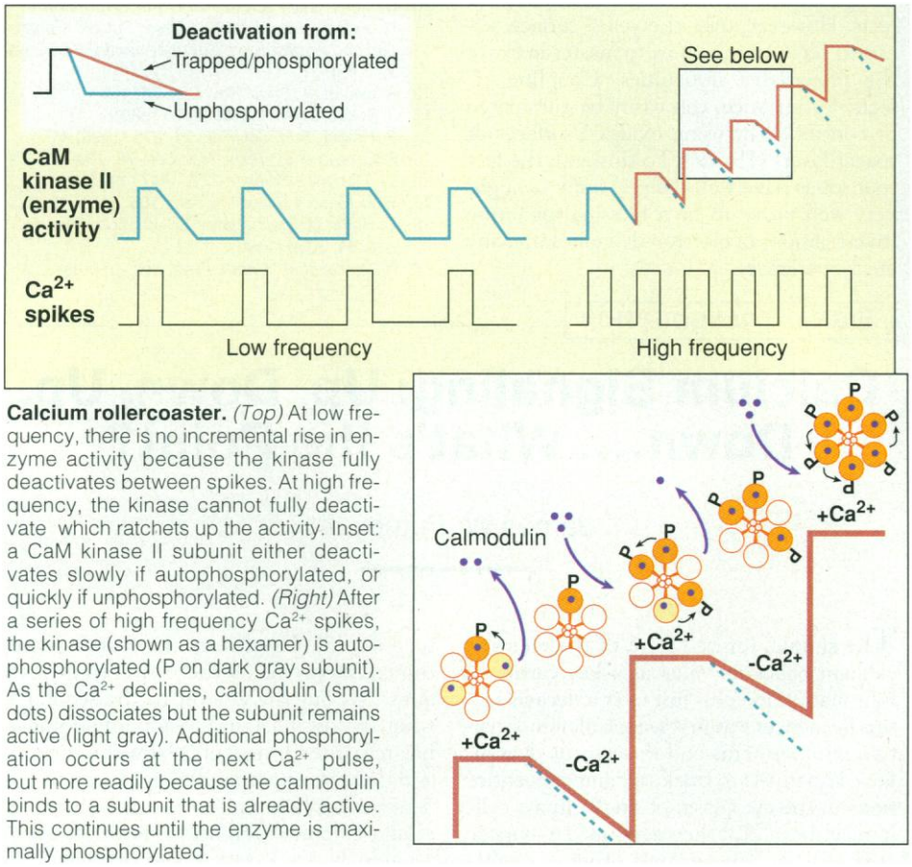
Calmodulin-dependent protein kinase II (CaM kinase II) is a ubiquitous enzyme target of calcium signaling pathways. It is not directly activated by calcium, but rather responds to another ubiquitous molecule, calmodulin, but only when in its calcium-bound form (calcium-calmodulin). The kinetics of this interaction are complex. In addition to acute activation of the enzyme resulting in phosphorylation of appropriate protein substrates, association of calcium-calmodulin also catalyzes the autophosphorylation of CaM kinase II (*5*), with the result that the enzyme "traps" calmodulin and continues to be active even after calcium levels decline (*6*). In this state, the enzyme becomes au-

The author is at the Laboratory of Signal Transduction, National Institute of Environmental Health Sciences–National Institutes of Health, Research Triangle Park, NC 27709, USA. E-mail: putney@niehs.nih.gov

tonomous and can be said to have a short-term molecular "memory" that could sustain its activity between repetitive oscillations in intracellular calcium levels. This property could thus impart the complex, nonlinear behavior of the enzyme in response to the digital and cyclical activation associated with intracellular calcium oscillations. The potential for such behavior has been demonstrated in computer simulations (*7*). Until the current study, however, this nonlinear behavior has not been demonstrated experimentally.

In the approach used by De Koninck and Schulman, the CaM kinase II enzyme is immobilized on the inner surface of a section of polyvinyl chloride tubing, so that calcium-calmodulin–containing or calcium-calmodulin-free solutions can be alternatively perfused, producing rapid and controlled changes in the levels of the activator complex. Perfusion with calcium-calmodulin–containing solutions induces autonomous activation of the enzyme, measured as kinase activity that persists even in the absence of the activating principles.

With this system, De Koninck and Schulman make a number of intriguing observations. Regardless of the calcium-calmodulin pulse duration, autonomous activation of CaM kinase II increased steeply as a function of frequency. As one might expect, shorter pulse durations required greater frequencies for activation, but once the threshold was achieved, the steepness of the frequency activation curve was much greater. In other words, it is possible to prime the system with calcium bursts of a given frequency and subsequently to maintain the response level with signals of substantially lower frequency. An important feature of these results is that the pulse durations and oscillation frequencies to which CaM kinase II can respond span a broad range, from rapid, action potential–dependent spiking associated with synaptic transmission in the brain to the slower but much broader waves and oscillations of nonexcitable cells.



Calcium rollercoaster. *(Top)* At low frequency, there is no incremental rise in enzyme activity because the kinase fully deactivates between spikes. At high frequency, the kinase cannot fully deactivate which ratchets up the activity. Inset: a CaM kinase II subunit either deactivates slowly if autophosphorylated, or quickly if unphosphorylated. *(Right)* After a series of high frequency Ca^{2+} spikes, the kinase (shown as a hexamer) is autophosphorylated (P on dark gray subunit). As the Ca^{2+} declines, calmodulin (small dots) dissociates but the subunit remains active (light gray). Additional phosphorylation occurs at the next Ca^{2+} pulse, but more readily because the calmodulin binds to a subunit that is already active. This continues until the enzyme is maximally phosphorylated.

Why does the cell use these calcium pulses to control downstream targets instead of seemingly simpler steady-state calcium levels? Speculation has focused on the advantages of a digitally encoded signal (all or none pulses) for favorable "signal-to-noise" ratios (*3*). By relying on large, discrete digital events (intracellular calcium spikes), cells can readily distinguish an "intentional" calcium signal from potentially spurious wanderings of the steady-state, cytoplasmic calcium concentration. Indeed, in the brain, bursts of electrical activity are more readily perceived as signals than are action potentials that arrive singly (*8*). In addition, a much broader range of signal strengths can potentially be distinguished with a digitally encoded system, because the baseline value is essentially zero. For example, agonists can sometimes activate gene expression in response to extremely low concentrations of peptide hormones that are known to act through the phospholipase C–calcium pathway (*9*). In such cases, however, the extremely low-frequency oscillations one might expect would be unlikely to result in autonomous activation of CaM kinase II, but pulsatile activation of the enzyme could produce integrated phosphorylation of other proteins whose rates of dephosphorylation are very slow. Thus, the kinetic properties of CaM kinase II are well suited for detecting and, more important, qualitatively distinguishing acute, moderate- to high-frequency oscillations from chronic, lower frequency signals.

CaM kinase II can immediately integrate or decode frequency-encoded intracellular calcium signals. Oscillating calcium signals may also be decoded by other means. For example, mitochondria can apparently selectively sense and integrate intracellular bursts of calcium release because of their intimate spatial association with the calcium release sites in the endoplasmic reticulum and their ability to retain accumulated calcium for prolonged periods (*10*). And in some instances, the final cellular output or response may retain the oscillatory behavior of the initial calcium signal; that is, no integration may occur. For example, secretory rates in single pituitary cells appear to track hormone-induced intracellular calcium oscillations (*11*).

The breadth of functions controlled by calcium is sizable, ranging from subsecond secretory and contractile events, to the initiation of cell division or cellular apoptosis requiring several hours. Thus, it should not be surprising that the kinetics of intracellular calcium signaling similarly exhibit significant variation in patterns and mechanisms of recognition.

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