

Commentary

How plants learn

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The identification of signal-transduction components in plant cells is proceeding on an almost daily basis. In this issue, Kudla *et al.* (1) continue this important process by describing the isolation and characterization of regulatory subunits of calcineurin, a Ca^{2+} /calmodulin-dependent protein phosphatase. Since the pioneering work of Cohen (2) and collaborators, biologists have classified protein phosphatases into four types: PP1, PP2A, PP2B, and PP2C. Although this classification was sensibly designed to bring order out of chaos, it had one major drawback. Division into just four types implied a limited diversity and perhaps a lack of specificity in the mechanism of protein dephosphorylation. Consequently, investigators have tended to concentrate attention on protein kinases where, it was surmised, all the action was to be found. Indeed, several hundred protein kinases have now been cloned or purified from plant tissues (3). Estimates suggest there will be nearly a thousand identified when the *Arabidopsis* genome sequence is finally published. However, the observations of Kudla *et al.* (1) suggest that the regulation of dephosphorylation by calcineurin (classified as PP2B) might be more complex than is currently appreciated.

Calcineurin is a heterodimeric phosphatase. A conserved “A” type catalytic subunit combines with a class of variable and regulatory “B” type subunits. It is the latter subunit class that is the subject of the paper by Kudla *et al.* (1). Originally, calmodulin was thought to be the only B class subunit. Most early studies in plant cells suggested calmodulin to be of very limited diversity—one or two sequences at the most. It was assumed, therefore, that specificity in dephosphorylation would have to reside in some unique characteristics of individual calcium signals. Indeed, a multiplicity of hormonal, physical, chemical, and stress signals induces transient elevations in cytosolic Ca^{2+} in plant cells, and each signal induces unique kinetics in the generated Ca^{2+} transient (4). However, unlike animal cells, plants now are known to contain many isoforms of calmodulin, with the current record being 13 in wheat (5). These isoforms have cell and tissue specificity in expression, distribution, and cell location (6), and they provide the potential for at least 13 specific calcineurin activities. However, the description by Kudla *et al.* (1) of a distinct class of calcineurin B subunits increases the potential number of specific calcineurin activities yet again. These B subunits can be used interchangeably with calmodulin to regulate calcineurin. Some reassessment of the emphasis placed on protein-kinase control of plant-cell phosphorylation is probably long overdue.

The identification of this class of calcineurin regulatory subunits in *Arabidopsis* is secure. Kudla *et al.* (1) have used the now familiar complementation of yeast mutants (in this case lacking calcineurin) and dihybrid technologies (with rat A calcineurin subunits) to characterize subunit function and binding properties. This gene family contains six members. Sequencing identifies the presence of four EF hands or calcium-binding regions in each subunit. So these *Arabidopsis* calcineurins retain their dependence on signal-initiated changes in cytosolic Ca^{2+} . However, strikingly, the B subunit

sequence is most similar to frequenin, a neuronal cytosol Ca^{2+} sensor involved with vesicle fusion and neurotransmitter-release functions (7). Of the three B subunits investigated in detail, the expression of one subunit increased greatly when plants were signaled by cold, wounding, or drought, as determined by Northern analysis.

What function can be deduced for this class of subunits? Specifying a cellular location is one likely possibility. It has been apparent from the earliest days of phosphorylation research that the spatial location of kinases and phosphatases is important in defining substrate specificity (8). For example, in the absence of spatial localization, many proteins, not usually endogenous substrates for protein kinase, can be illegitimately phosphorylated and potentially activated; similarly for dephosphorylation. The simplest way for a signal to navigate its way through the cellular forest of kinases and phosphatases safely and induce specific responses is by a clearly defined path. This perspective sees the specificity in signal transduction as the result of a kind of hard wiring of the cell: a defined, spatially structured path involving the cytoskeleton, membranes, or wall. The function of transduction switches, like cytosolic Ca^{2+} , is to connect and then direct information flow through one or another well structured path; much as identically constructed switches are used to connect and direct electrical current separately to the lights, the television, or the computer.

Probably one function of this class of calcineurin B subunits will be to pin phosphatase activity either to specific regions of the cytoskeleton or to membranes. Many Ca^{2+} -binding proteins are attached to the cytoskeleton (4), and calcineurin might be among them. However, the primary difficulty with involving the cytoskeleton is its lability; the hard wiring would have to be constantly remade as the cytoskeleton changes structure.

Given our current understanding, attaching calcineurin to membranes is a much more credible function for the B subunits. Kudla *et al.* (1) indicate that their sequence contains a conserved myristoylation site. Transient attachment of calcineurin to membranes controlled by myristyl transferases can be anticipated. But even stronger evidence comes from one well characterized function of calcineurin: the regulation of the inward flux of K^+ , Na^+ , and Ca^{2+} ions through plasma membrane-located channels.

Immunophilins are receptors for immunosuppressive drugs such as cyclosporin and FK506. Binding of the drug to its immunophilin receptor generates a potent inhibitor of calcineurin that modifies the immune response by impairing T cell function. In a series of ground-breaking articles, Luan and coworkers (9–11) showed that plant cells also contained several active immunophilins. When the plant immunophilins combined with FK506, calcineurin activity was blocked. Furthermore, treatment of leaf guard cells with FK506 antagonized the inhibitory effect of Ca^{2+} on inward K^+ flux. The implication is that inward K^+ channels are active when phos-

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changes in the expression of some other genes induced by these signals are regulated by cytosolic calcium (4, 13). However, the elevation of calcineurin-subunit expression after signaling is becoming part of a much more general pattern in plant cells.

Calmodulin, phospholipase C, Ca^{2+} -ATPase, Ca^{2+} -dependent protein kinase, and other protein kinases are all critical proteins concerned with the transduction of signals through a cytosolic Ca^{2+} pathway. The expression of each of these proteins (as well as calcineurin) is increased by orders of magnitude when unstimulated plants are exposed to cold, drought, or mechanical signals. Within several hours, the cellular concentrations of these proteins rise (16–19). Similar results have emerged for second messengers that are concerned with Ca^{2+} signaling. In unstimulated plant cells, the Ca^{2+} -mobilizing signal, IP_3 , the IP_3 precursor PIP_2 , and the Ca^{2+} -mobilizing cyclic ADP-ribose are present at virtually undetectable levels. A signaling episode greatly increases the resting levels of these second messengers for many hours, but elevation itself takes hours to accomplish (20, 21). Exposing unstimulated plants to one or more signals elevates critical elements of the transduction apparatus involving Ca^{2+} by an order of magnitude.

The rate of information flow, the flux, through a signal-transduction pathway will be constrained by the concentration of signal-transduction constituents. In an unstimulated plant, information flow from a signal through Ca^{2+} -dependent pathways will be slow; in a stimulated plant, information flow from the self-same signal will be enormously faster. However these data are viewed, they represent a form of cellular learning. No doubt, anticipation of further signals is the reason for cellular learning. In any case, some intriguing questions have been raised. Does enhanced expression of the Ca^{2+} apparatus occur in all cells? Or is the elevation limited to the recruitment of new cells into a full blown signaling mode? On current understanding, the latter possibility is more likely.

Signal-transduction networks share properties with neural networks, and the learning parallels can be drawn easily (22). Neural networks learn by increasing the numbers of connections (and the strength of the connections) between the neurons representing the chosen path to connect signal and response. The result of learning (reinforcement) is to accelerate the information flux rates between the signal and the response. Elevating Ca^{2+} transduction constituents is analogous to increasing the numbers of connections between neurons in a neural network. The increased information flow that results represents a kind of cellular learning. This cellular learning, coupled with the memory built into signal-transduction systems (22), suggests an unexpected form of cellular intelligence (23).

Some important questions remain to be answered. What are the identities and locations of proteins that bind B subunits? Are there other proteins that regulate calcineurin? How does calcium regulate B subunit expression? Does the elevation of B subunits involve uniform increases in all cells or are there simply more cells responding? Could tissue Ca^{2+} waves specify the spatial pattern of response of B subunit accumulation? How can we test the learning capacity of the plant-cell signal-transduction network? The important observations made by Kudla *et al.* (1) generate important questions. As always, important advances generate more questions than answers.

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