How is specificity encoded in calcium-based signal transduction systems? Recent evidence shows that oscillations in the cytosolic free calcium concentration of guard cells are necessary for stimulus-specific stomatal closure in plants.

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The recognition that, just as in animal cells, calcium is a ubiquitous intracellular second messenger in plants raises the question of how specificity is encoded in plant calcium-based signalling systems. One hypothesis to account for the fidelity of calcium signalling systems is based on the concept of the ‘calcium signature’, or stimulus-specific increase in the cytosolic calcium concentration, [Ca^{2+}]_{cyt}. Underlying this hypothesis is the proposition that there is information encrypted into the calcium signature that can be decoded by the cell to produce the correct response [1]. Oscillations in [Ca^{2+}]_{cyt} are an example of a class of calcium signature. Although the importance of [Ca^{2+}]_{cyt} oscillations in animal cells is well known [2,3], their precise role in plant cell signal transduction has been less clear. A recent paper from the Schroeder lab [4] reports evidence that oscillations in guard cell [Ca^{2+}]_{cyt} are necessary for stimulus-specific stomatal closure.

Before the work of Allen et al. [4] it had been established that, under appropriate conditions, treating stomata with either the plant hormone abscisic acid (ABA) or extracellular calcium, which at millimolar concentrations will induce oscillations in [Ca^{2+}]_{cyt} in the guard cell [Ca^{2+}]_{cyt}. Furthermore, it was possible to correlate the form of the oscillations with both the strength of the external stimulus and the aperture of the stomatal pore [5,6]. Some important questions remained unanswered, however; in particular, are the oscillations required to obtain a response, or are they simply a mechanism for protecting the cell against long periods of elevated [Ca^{2+}]_{cyt}?

Allen and co-workers [4] reasoned that a good way to test whether oscillations in [Ca^{2+}]_{cyt} are of primary importance in guard-cell signalling would be to interfere with the process by which they are produced and then monitor any effect on stomatal aperture. Although our understanding of how oscillations in [Ca^{2+}]_{cyt} are generated in plant cells is incomplete, there is evidence that the rising phase of the oscillation requires both the influx of Ca^{2+} across the plasma membrane and its release from internal stores. The falling phase is likely to be associated with the re-sequstration of calcium into internal stores or its expulsion from the cell across the plasma membrane [7]. Using this information, Allen et al. [4] reasoned that a plant which carried a lesion in endomembrane function might display altered [Ca^{2+}]_{cyt} oscillatory activity. Accordingly, they focussed their attention on the de-etiolated 3 (det3) mutant in Arabidopsis. This plant was likely to be a useful tool in such experiments, because it is known that calcium sequestration into internal stores is dependent on the proton gradient, and the det3 mutant exhibits reduced endomembrane energization as a result of a 60% reduction in the expression of the C subunit of the V-type H^+ ATPase [8].

In order to monitor guard-cell [Ca^{2+}]_{cyt} dynamics in wild-type and det3 mutant Arabidopsis plants, Allen et al. [4] used the ‘cameleon’ technique [9]. This technology provides a totally non-invasive way of measuring and imaging intracellular calcium dynamics. Schroeder’s group [10] have pioneered the use of this technique in plants. Given the enormous difficulties in microinjecting Arabidopsis guard cells with ratiometric [Ca^{2+}]_{cyt} indicators, it is probably true to say that these experiments would not have been possible before the advent of this technique [11].

Allen et al. [4] first investigated the effects of extracellular calcium, which at millimolar concentrations will induce stomatal closure, on guard cell [Ca^{2+}]_{cyt} in wild-type Arabidopsis. The results from these experiments confirmed previous work in Commelina communis [5] which showed that the aperture of the stomatal pore decreased, and that guard cell [Ca^{2+}]_{cyt} oscillated, when the extracellular calcium concentration was elevated. The form of the [Ca^{2+}]_{cyt} oscillations was dependent upon the concentration of the extracellular calcium. When they analysed [Ca^{2+}]_{cyt} in the det3 mutant, they found that external calcium also induced an increase in guard cell [Ca^{2+}]_{cyt}. But in marked contrast to the wild-type, this was a gradual, steady increase in guard cell [Ca^{2+}]_{cyt} and oscillations were never recorded (Figure 1).

These results suggested, as Allen et al. [4] had predicted, that the lesion in det3 did indeed affect the ability of guard cells to produce [Ca^{2+}]_{cyt} oscillations in response to extracellular calcium. What was really interesting, however, was that external calcium failed to induce any reduction in stomatal aperture. Interestingly, the authors got the same sort of result when they performed similar experiments with another closure-inducing stimulus, hydrogen peroxide. The results from these experiments strongly suggested
that oscillations in [Ca$^{2+}$]$_{cyt}$ rather than a simple elevation of [Ca$^{2+}$]$_{cyt}$ are required to induce stomatal closure.

In order to provide conclusive evidence that oscillations in guard cell [Ca$^{2+}$]$_{cyt}$ are required for extracellular-calcium-induced reductions in stomatal aperture, Allen et al. [4] carried out two additional experiments. In the first, they investigated whether artificially inducing [Ca$^{2+}$]$_{cyt}$ oscillations in det3 guard cells would result in decreases in stomatal pore diameter. Remarkably, they did. Of course the logical companion to this experiment was to investigate whether the artificial imposition of a prolonged increase in guard cell [Ca$^{2+}$]$_{cyt}$ in a wild-type plant would copy the det3 phenotype. The answer was again a resounding yes. The results of these elegant experiments establish beyond doubt the physiological significance of [Ca$^{2+}$]$_{cyt}$ oscillations in guard cell signalling. But this was not the end of the story. Allen et al. [4] also decided to investigate whether the det3 mutant was also insensitive to the closure-inducing stimuli of ABA and cold. Remarkably, these signals induced similar repetitive calcium transients in both wild-type and det3 mutant plants, and in both cases reductions in stomatal aperture were observed.

The results from these experiments are most revealing. First, they establish beyond doubt that oscillations in [Ca$^{2+}$]$_{cyt}$ have a central role to play in calcium-based signal transduction pathways in plant cells. Secondly, we learn that the production of oscillations in guard cell [Ca$^{2+}$]$_{cyt}$ does not proceed by a single common pathway. At least one component, the one that is affected by the det3 lesion, is present in the extracellular calcium and hydrogen peroxide signalling pathways, but not in the ABA or cold pathways. This is important, because it provides support for the suggestion that the key to understanding specificity might lie in understanding how individual stimuli generate their own specific calcium signatures [7]. The question of how signalling specificity is controlled is particularly intriguing, given that both opening and closing stimuli have been shown to elicit increases in guard cell [Ca$^{2+}$]$_{cyt}$ [1].
The work of Allen et al. [4] gives us an insight into the downstream processing of the calcium signal. In this work, we see that prolonged increases in [Ca\textsuperscript{2+}]\text{cyt} fail to elicit reductions in guard cell turgor. This tells us that the downstream components of cell signalling pathways must be sensitive to transient increases in [Ca\textsuperscript{2+}]\text{cyt}. In addition, the research of Allen et al. [4] points the way ahead. Given that stomatal closure proceeds rapidly, it would be interesting to determine if the period and magnitude of the first [Ca\textsuperscript{2+}]\text{cyt} oscillation is vital to dictating the outcome of the final response. Perhaps the most important general lesson from these studies however, is that Arabidopsis mutants have the potential to be enormously valuable tools for physiologists working to understand the functioning and organisation of calcium-based signalling systems in plants.

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References