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Spikes and Waves: Calcium-Mediated Signaling in Tip-Growing Cells

Plants have evolved remarkable strategies to coordinate their growth and development in response to internal and external signals in varied and changing environments. Although plant responses to these physical and chemical signals have been known for some time, the mechanisms that couple signal perception to the response have taken longer to identify. By the mid-1980s, calcium was recognized as a potential intracellular messenger in plants, modulating responses through changes in its cellular concentration (Hepler and Wayne, 1985).

Much of the basis for postulating this role for calcium came from parallels with signal transduction in animals and a few critical measurements of intracellular calcium levels in lower plants (Saunders and Hepler, 1981; Williamson and Ashley, 1982). The role of calcium in animal signal transduction had been demonstrated with experimental evidence that met three criteria outlined by Jaffe (1980): (1) the responses were preceded or accompanied by an increase in intracellular calcium; (2) blockage of natural calcium increase inhibited the responses; and (3) experimental generation of an increase in intracellular calcium stimulated the responses. Although preliminary evidence pointed to a comparable role for calcium in plant signaling, it was clear that convincing support for this hypothesis would require information about calcium levels before and after perception of a stimulus, identification of mechanisms that regulate intracellular calcium levels, and links between changes in cellular calcium levels and specific physiological and developmental responses.

In the past few years, significant progress toward these goals has been made. For example, calcium transport proteins (channels, pumps, and carriers) on cellu-

lar membranes have been identified and their biochemical and electrophysiological properties determined (reviewed in Bush, 1995), indicating that mechanisms capable of regulating intracellular calcium levels are present in plant cells. More recently, two technological advances have made it possible to link perception of stimuli with changes in intracellular calcium levels, and changes in calcium levels with specific physiological responses.

The first advance has been the ability to introduce indicator dyes into living cells, which permits the visualization of changes in cytoplasmic calcium levels. As a result, calcium concentrations have been shown to change during a plant's response to a variety of signals including wind and cold (Knight et al., 1991, 1992), as well as pistil S proteins (Franklin-Tong et al., 1993, 1995) and nodulation factors (Ehrhardt et al., 1996). The second advance has been the ability to introduce molecules into cells that alter calcium levels directly (e.g., UV-releasable caged calcium and calcium chelators) or indirectly (i.e., molecules that promote or block the release of calcium from intracellular stores). From studies using these approaches, there is mounting evidence that changes in calcium levels affect processes including stomatal closure in guard cells (Gilroy et al., 1991), transcription of phytochrome-regulated genes (Bowler et al., 1994), and pollen tube growth during a self-incompatible interaction (Franklin-Tong et al., 1996).

On pages 2015–2031 of this issue, Taylor and coworkers begin to link the mechanisms regulating intracellular calcium with changes in calcium levels during the response of the intertidal alga, *Fucus*, to changing osmotic conditions. Zygotes of the order *Fucales* have been used widely to study the mechanisms in-

involved in the generation of asymmetry during plant development. In response to a number of stimuli, these spherical zygotes establish and fix an axis of polarity that defines the subsequent differentiation of the organism into the thallus and rhizoid. From the data reported in their paper, Taylor et al. provide evidence that *Fucus* may also be a useful model in which to develop an understanding of how organisms that experience fluctuations in their ionic environment regulate the osmotic balance in their cells.

To do this, Taylor and colleagues have characterized mechanosensitive plasma membrane channels in the apex of the rhizoid pole of the developing zygote. These calcium-permeable channels may provide the mechanism for the polar calcium influx (measured as a transient increase in calcium at the rhizoid apex) seen when cells perceive a hypoosmotic shock (i.e., when they are shifted to a lower osmotic pressure). Using successive shocks, the authors provide evidence for a link between changes in cytoplasmic calcium levels and the ability of the plant to osmoregulate by showing that a reduction in the ability to generate the increase in intracellular calcium leads to increased osmotic sensitivity.

Although studies such as the one described above have shown that calcium levels change in response to the perception of a signal, perhaps not surprisingly, the picture is more complicated than just the simple presence or absence of calcium. Varied spatial and temporal patterns of calcium distribution have been identified in numerous studies. For example, Taylor et al. also show that the increase in intracellular calcium that occurs in the response of *Fucus* to osmotic shock is initiated at the rhizoid apex and expands to the base, lasting approximately 100 sec.

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Conversely, in the response of alfalfa root hairs to compatible *Rhizobium* nodulation factors, Ehrhardt and coworkers (1996) have shown that an asymmetric calcium oscillation (60 sec mean duration) begins approximately 9 min after perception of the elicitor signal and can last more than 1 hr. This increase originates near the nucleus and subsequently radiates outward into the cytoplasm.

In a third study, published in the August issue of THE PLANT CELL, Franklin-Tong and coworkers used both calcium imaging and the introduction of molecules that alter intracellular calcium levels to study the signaling mechanisms underlying self-incompatibility (Franklin-Tong et al., 1996). This group showed that cessation of pollen tube growth in an incompatible response in *Papaver rhoeas* likely involves a "wave" or "flood" that is due to slow increases in calcium initiated behind the tip of the tube which expand to the tip within 200 sec. By introducing molecules that promote or block the release of calcium from intracellular stores, Franklin-Tong et al. provided evidence that once initial increases in calcium take place, the flood is propagated via the release of calcium from these stores.

It is clear that in each of the above-mentioned responses, calcium is involved in the growth modifications; however, the patterns of calcium changes in each organism are quite different. This raises a number of interesting and important questions that will hopefully be the focus of future studies. For example, in addition to questions concerning where and how the signals are perceived, it will be critical to identify, for each of these patterns of calcium change, both the source of calcium and the transport mechanisms that mediate the initial increases in calcium levels.

Furthermore, if additional calcium is subsequently required to sustain and

propagate the transient increases or oscillations, what is its source? Does the vacuole usually hold the major intracellular calcium store, or does the calcium source vary among organisms, responses, or within a given response? What is the mechanism of calcium release? Are the transient increases or oscillations of calcium necessary and sufficient to activate the responses that develop? How do the waveforms and distribution (spatial and temporal patterns) of calcium direct the different outcomes of cellular differentiation?

Answers to questions like these will lead into studies aimed at identifying the downstream components of signal transduction pathways, and will ultimately provide the information necessary to link the perception of stimuli to physiological responses in plants.

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