Plants use calcium to resolve salt stress

Long ago plants ventured forth from the relatively stable world of the oceans to the land, where mineral nutrients and water were often scarce commodities. In their quest to cope with the changing conditions of life on land, many plant species appear to have lost much of their ability to deal with higher sodium concentrations. With the exception of the halophytes, which occupy the sodium-rich terrestrial habitats of estuaries, marshes and other niche environments, land plants have evolved a requirement for fresh water. However, in recent years, we have learned that the cells of most higher plants are capable of adjusting to high levels of NaCl. Indeed, if exposed in a gradual manner, plants can grow and reproduce during exposure to very high concentrations of sodium. It is this ability to adjust that has led many to believe that most of the cellular machinery for dealing with excessive sodium still exists in the majority of plant species. What distinguishes many salt tolerant species is the ability to engage that machinery when needed. By understanding the signaling system that allows a plant to sense excess sodium in the environment and to make appropriate adjustments, plant biologists hope to be able to influence the growth behavior of crop plants in arid and inhospitable conditions.

When plants are challenged with salinity stress, an increase in the concentration of Ca$^{2+}$ often can ameliorate the inhibitory effects on growth. Although the underlying mechanism has remained largely unexplained, prevailing models for Ca$^{2+}$ function include both membrane stabilization and signaling roles. Numerous studies indicate that a variety of stress conditions, including salinity, induce cytosolic Ca$^{2+}$ accumulation. The role of Ca$^{2+}$ as a second messenger in many biological systems, coupled with these observations, indicates that plants are able to adjust to high salt environments by activating a signal transduction system involving Ca$^{2+}$.

Recent advances

Insight into the role of Ca$^{2+}$ in salt adaptation has been provided by the recent reports from the laboratory of Jian-Kang Zhu. Zhu’s group have used a molecular genetic approach, focusing on the isolation of loss-of-function mutants, to identify the determinants of salt tolerance. In Arabidopsis, this approach has led to the identification of a genetic locus that is necessary for salt tolerance. A mutation in this locus, sos1, results in hypersensitivity to NaCl and LiCl. Increased Ca$^{2+}$ abrogated this hypersensitivity, and a transgenic plant expressing a dominant negative form of the SOS3 gene suppressed the mutant phenotype. The sequence of the SOS3 allele was recently determined. The gene codes for a protein with regions of homology to EF-hand Ca$^{2+}$-binding domains and has highest sequence homology with the yeast calcineurin B subunit and a neuronal calcium sensor, both of which are activated by Ca$^{2+}$.

Involvement of calcineurin

Previous work had already implicated calcineurin, a Ca$^{2+}$- and calmodulin-dependent PP-2B protein phosphatase, in the regulation of ion transport in plants. Pharmacological and genetic evidence indicates that the activity of calcineurin is regulated by the cytoplasmic concentration of Ca$^{2+}$. This concentration is controlled, in part, by the action of the calcium-sensing neuronal Ca$^{2+}$-sensor, both of which are involved in neuronal signaling. The Ca$^{2+}$-dependence of calcineurin activity has been demonstrated in vitro and in vivo.

According to Zhu and colleagues, the SOS3 gene encodes a protein that is similar to the yeast calcineurin B subunit and is likely to be a calcium sensor or a target for Ca$^{2+}$-dependent calcium sensors.

**Fig. 1.** A hypothetical model of salt stress signal perception, transduction and regulation of effectors that mediate Na$^{+}$ homoeostasis based on evidence from plants and yeast. Salt stress is perceived by an unknown sensor, and initiates a Ca$^{2+}$-dependent signal transduction pathway. This cascade regulates transport proteins that control net Na$^{+}$ influx across the plasma membrane and compartmentation into the vacuole. To date, the only components of the model that are known to actually affect the phenotype of salt-tolerant plants are products of the SOS3 gene from Arabidopsis and the yeast CNA1/CIB genes. Abbreviations: AtCDPK1/1a, Ca$^{2+}$-dependent protein kinase from Arabidopsis; CAM, calmodulin; CNA/CIB, calcineurin A and B subunits (PP-2B phosphatase); PP-2C, phosphatase; SOS3, CIB-NCB-like protein.
biochemical evidence has established that the regulation of plasma membrane and tonoplast guard cell ion channels is mediated by calcineurin-like activity9,10. In addition, Ca2+-dependent signaling through protein kinases is involved in stress responses of plants. Two Ca2+-dependent protein kinases (CIPKs) function to elicit osmotic/ionic activation of a stress responsive promoter11, and mitogen-activated protein kinase (MAPK) cascades are implicated in coordinate regulation of osmotic tolerance12. It was recently reported that activated yeast calcineurin facilitated salt stress and induced expression of transgenic plants13. It has become increasingly obvious from these observations that salt stress activates a Ca2+-dependent signal transduction pathway14,15 that results in tolerance. In spite of the long-standing evidence for the involvement of Ca2+, our understanding of the process by which Na+ is perceived and a signal transduced is only now, with the identification of SOS3, beginning to take shape.

Salt tolerance in plants: following the pathway in yeast? Fortunately, Saccharomyces cerevisiae has provided us with an excellent model in which to make comparisons. The most intriguing aspect of these recent findings is the consistency of the central role for Ca2+ signaling in the salt adaptive response of both plants and yeast16-18 (Fig. 1).

Genetic and physiological evidence has demonstrated conclusively that NaCl stress perception and tolerance in yeast involves Ca2+-dependent signaling events. In yeast, calcineurin is a pivotal signaling intermediate that mediates K+ and Na+ homeostasis in saline environments19-22. Exposure to excess NaCl induces the expression of the ENA1/PMR2A gene encoding a plasma membrane Na+-ATPase that is primarily responsible for NaCl exclusion. Coordinately, there is a reduction in Na+/K+ influx mediated by a modulation of the K+ uptake system (involving TRK1 and TRK2 proteins). This results in a change from a resting state of low K+ affinity and low K+/Na+ selectivity (equivalent to system 2 of K+ uptake in plants) to a transport mode with high affinity for K+ and high K+/Na+ selectivity (analogous to system I of plants). Both responses, which function to limit net intracellular Na+ accumulation, are effected substantially by Ca2+ activation of calcineurin. Calcineurin is a ubiquitous calcium-dependent protein kinase (CPK) that functions by binding directly to the CBN regulatory subunit and through activation of calmodulin, which in turn binds to and activates the catalytic CNA subunit. Activated calcineurin interacts with the transcription factor TCN1/CRE1 of yeast, which is homologous to the mammalian CREB and other calcium-dependent genes12,13. The use of mutant forms of calmodulin and calcineurin revealed that Ca2+-calmodulin conjugates also enhance NaCl tolerance via a calcineurin-independent mechanism, which activates the ENA1/PMR2A Na+ pump post-transcriptionally13. Mutant forms of calmodulin that do not bind Ca2+ support cell growth but do not confer salt tolerance14. However, additional extra-cellular Ca2+ suppressed the NaCl sensitivity of these calmodulin mutants through an unknown mechanism. Surprisingly, this process required functional calcineurin, but was independent of Ca2+-activated calmodulin15. These findings suggest that calcineurin interacts with other Ca2+-dependent, but calmodulin-independent, components to mediate salt tolerance. At present, it is not known how yeast cells perceive ion stress and how Ca2+ signaling is subsequently initiated. External Ca2+ influx appears to be required to trigger the response because mutants deficient in CCH1 (a putative plasma membrane inward rectifying Ca2+ channel) and in MID1 (an auxiliary subunit required for CCH1 channel function) are defective in calcineurin-dependent processes, including the expression of ENA1/PMR2A and ion tolerance16.