PLANT CELL BIOLOGY

Keep your balance!

Like animals, plant cells are exposed to fluctuating extracellular levels of Ca^{2+} (Ca_o^{2+}), but somehow manage to keep their internal Ca^{2+} levels on a relatively even keel. This implies that there is a mechanism for maintaining Ca^{2+} homeostasis, but until now, what this was and how it operated was elusive. But a large clue has come from the work of Shengcheng Han *et al.*, which is reported in *Nature*.

Ca^{2+} has pleiotropic roles in plants, but Ca_o^{2+} in particular can increase the concentration of cytosolic Ca^{2+} ($[Ca^{2+}]_i$) in guard cells (which surround stomatal pores), thereby promoting closure of the stomata. The authors reasoned that this so-called Ca_o^{2+}-induced $[Ca^{2+}]_i$ increase (CICI) in guard cells might correspond to a means by which Ca_o^{2+} is sensed. So, first they verified that Ca_o^{2+} did indeed increase $[Ca^{2+}]_i$ in guard cells. Next, on the basis that inositol-1,4,5-trisphosphate — through the actions of phospholipase C (PLC) — can often mediate receptor-induced Ca^{2+} release, they inhibited PLC. This inhibited guard-cell CICI and stomatal closure, implicating receptor-mediated Ca^{2+} sensing in CICI.

So the authors screened complementary DNA libraries from *Arabidopsis*, measuring $[Ca^{2+}]_i$ increases using a mammalian cell line, and identified a 1.4-kb cDNA that they named CAS (for Ca^{2+} sensing). Homologues of the 387-amino-acid CAS protein were identified in other plants, but not in animals. The amino terminus of CAS contains many acidic amino acids — which can bind Ca^{2+} with low affinity — rather than high-affinity Ca^{2+}-binding sites of calmodulin and other Ca^{2+} sensors. Binding studies subsequently showed there to be low-affinity/high-capacity Ca^{2+}-binding sites in the amino terminus of CAS.

Han *et al.* then showed that CAS is mainly expressed in leaf shoots — including guard cells — and used an antisense approach to study the requirement of CAS in guard-cell Ca_o^{2+} signalling. CICI and Ca_o^{2+}-induced stomatal closure were impaired following the introduction of an antisense transgene. Furthermore, and in line with the known role of Ca^{2+} in several developmental processes, CAS-antisense-transgenic plants were severely defective in bolting — the rapid upward growth at the transition to seed production — although they did eventually bolt at reduced Ca^{2+} concentrations.

The pathway by which CAS mobilizes Ca^{2+} has yet to be determined, but being able to manipulate CAS should greatly help in clarifying the mechanism of $[Ca^{2+}]_i$ control, and in assessing the molecular function of Ca_o^{2+} in plants.

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References and links

ORIGINAL RESEARCH PAPER Han, S. *et al.* A cell surface receptor mediates extracellular Ca^{2+} sensing in guard cells. *Nature* **425**, 196–200 (2003)

GENE EXPRESSION

Clocks and cycles: unveiling the link

Daily oscillations in many biological processes that are controlled through an endogenous circadian clock occur during a 24-hour period. This clock measures changes in light and mediates photoperiodic responses. Previous research indicated that circadian rhythms could control the activity of the cell cycle, but only now has work by Hitoshi Okamura and colleagues indicated that the underlying mechanism involves direct transcriptional regulation of the cell-cycle gene *Wee1* by Clock — a master control switch of circadian gene expression.

Using a mouse model with a partial hepatectomy (PH), Okamura and colleagues analysed the relationship between the circadian clock and the cell cycle. Liver cells from wild-type mice that have undergone a PH rapidly re-enter the cell cycle and repopulate the liver in a few days. The rate of liver regrowth was studied in mice that were maintained in a 12-hour light/dark cycle, with a PH on the liver performed at the start of the 12-hour light period (ZT0) or 8 hours later (ZT8). The results of bromodeoxyuridine (BrdU) incorporation, which marks cell proliferation, indicated that although S-phase kinetics were similar for both ZT0 and ZT8, there was a delay in cells entering mitosis if the PH was performed at ZT0. This indicates that the timing of the hepatectomy affects cell-cycle progression in the regenerating cells. Peaks of the cell-cycle protein kinase Cdc2 and messenger RNA levels of other cell-cycle regulators, including *Wee1* — which is a known Cdc2 regulator — mimicked the BrdU incorporation peaks, which pointed to the involvement of cell-cycle regulators in this process.

Performing a PH on mice that are mutant for known clock regulators, such as the blue-light sensitive photoreceptor cryptochromes (*cry*), prevented normal liver regeneration and inhibited the peak of Cdc2 activity. Levels of Wee1 were increased in *cry*-mutant mice, but decreased in *clock*-mutant mice. Clock regulates gene expression by binding E-box motifs and three of these were identified in the 5′ UTR of *Wee1*. When these regions are mutated, transcription of *Wee1* by Clock/Bmal1 is decreased, presumably because Clock no longer binds upstream of *Wee1* to regulate its transcription. This indicates a direct but unidirectional regulatory mechanism (the authors show that there is no feedback) between the circadian clock and cell-cycle regulation.

As the molecular mechanisms that underlie the action of the circadian clock are still poorly understood, further genetic analysis of the links between cell-cycle regulators and clock components should provide important clues to the control of this regulatory mechanism.

Sarah Greaves, Nature Publishing Group

References and links

ORIGINAL RESEARCH PAPER Matsuo, T. *et al*. Control mechanism of the circadian clock for timing of cell division *in vivo*. *Science* 23 Aug 2003 (doi:10.1126/science.1086271)

FURTHER READING Panda, S. *et al*. Circadian rhythms from flies to humans. *Nature* **417**, 329–335 (2002)