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

GENE EXPRESSION

Clocks and cycles: unveiling the link



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-1,4,5trisphosphate — 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 receptormediated 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 Ca²⁺ 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 Ca²⁺ with low affinity — rather than high-affinity Ca²⁺-binding sites of calmodulin and other Ca²⁺ sensors. Binding studies subsequently showed there to be low-affinity/high-capacity Ca²⁺-binding sites in the amino terminus of CAS.

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 12hour 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 cellcycle 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 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.

Katrin Bussell

References and links
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receptor mediates extracellular Ca²⁺ sensing in guard cells.
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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 bluelight 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

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