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Calcium dynamics in single plant cells

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Kath Carr
Alistair Hetherington

Abstract

A new noninvasive technique has been developed that successfully measures calcium dynamics in single *Arabidopsis* guard cells.

Significance and context

This successful application of a new recombinant technique for measuring cytosolic calcium ($[Ca^{2+}]_{cyt}$), when combined with *Arabidopsis* genomics, will open up plant calcium signaling pathways to genetic analysis. The small size of most *Arabidopsis* cells makes microinjection of fluorescent calcium indicators into cells very difficult, and recombinant techniques that incorporate the gene for the luminescent calcium indicator aequorin into the genome have not been routinely successful at the level of the single cell. Allen *et al.* have used an ingenious recombinant construct made up of fluorescent proteins and the calcium-binding protein calmodulin - a 'cameleon' - as a calcium sensor in *Arabidopsis* guard cells. This first use of cameleons as calcium sensors in plants is an exciting and important advance that will enable researchers to tackle previously intractable questions in topics such as gravitropism, fertilization and cell differentiation at the level of the single plant cell.

Key results

In epidermal peels taken from plants transgenic for the calcium indicator yellow cameleon 2.1 (YC2.1), the cameleon protein was localized to the guard cell cytosol and no signal was reported from chloroplasts, suggesting that YC2.1 was not present in this organelle. The presence of YC2.1 in guard cells did not interfere with guard-cell closure of the stomatal pore induced by the plant signaling molecule abscisic acid (ABA). From the guard-cell response to extracellular calcium and ABA, the authors conclude that the calcium responses measured with YC2.1 compare well with those detected using microinjection of fluorescent calcium indicators. Although Allen *et al*. encountered some problems with chloroplast autofluorescence, they were able to investigate cytosolic calcium dynamics on a semi-quantitative basis. The cameleon technology enabled $[Ca^{2+}]_{cyt}$ to be measured in both guard cells of a single stoma at the same time. This produced an unexpected result, clearly showing that stimulus-induced increases in $[Ca^{2+}]_{cyt}$ were not synchronized in the two halves of the stomatal

apparatus (although some stomata did show similar patterns). The authors also report cameleon localization in root tips and root hairs.

Methodological innovations

Cameleons are an ingenious application of fluorescence resonance energy transfer (FRET) to detect changes in calcium concentration. The cameleon is composed of two 'green fluorescent proteins' (GFPs) which are bound together via calmodulin and a calmodulin-binding peptide, M13, to form a Ca^{2+} sensor, as described in detail in Figure 1.

Figure 1 Fluorescence resonance energy transfer (FRET) is an analytical technique that is useful for investigating processes that involve a change in molecular proximity, such as the assembly of protein complexes, immunoassays and the distribution and transport of lipids.FRET relies on the distance-dependent interaction between two chromophores within the system under investigation. Specifically, this interaction involves the transfer of energy from an electronically excited donor molecule to an acceptor molecule without the emission of a photon. Usually, the donor and acceptor are different entities. A key aspect of FRET is the Förster radius, which relates interchromophore distance and spectroscopic properties for any given donor-acceptor pair. It describes the separation (in å) at which a 50% transfer of excitation energy occurs. It is important to select a donor-acceptor pair that possesses a Förster radius comparable to the spatial arrangements in the biological system being studied. For FRET to occur, there are a number of spatial and spectral criteria which must be fulfilled. The donor-acceptor pair must be in close proximity (typically 10-100 å) and their transition dipole orientations must be approximately parallel. The emission spectrum of the donor and the absorption spectrum of the acceptor must overlap to some extent. With different donor and acceptor chromophores, FRET can be detected in two ways: probably the more common method involves the measurement of donor quenching, but the appearance or enhancement of acceptor fluorescence is also useful. The latter can sometimes be complicated by the fact that many acceptors absorb some of the incident light used to excite the donor and hence exhibit a degree of fluorescence even before energy transfer occurs. In cases where the donor and acceptor are the same, FRET is monitored by fluorescence depolarization. For some time, donor and acceptor moieties have been introduced into biological systems using organic chemical techniques such as covalent bond formation with synthetic fluorescent dyes. A typical donor-acceptor pair of this type is fluorescein (donor) and tetramethylrhodamine (acceptor). The natural incidence of donors and acceptors is somewhat limited but recent years have seen the introduction of a family of molecules called green fluorescent proteins (GFPs). In the approach described by Allen *et al*., two GFPs - enhanced cyan fluorescent protein, ECFP, the donor (D) and enhanced yellow fluorescent protein, EYFP, the acceptor (A) - have been used, bound together via calmodulin and a calmodulin-binding peptide, M13, to form a Ca^{2+} sensor referred to as a 'cameleon'. The figure illustrates how the binding of calcium to the calmodulin domain causes conformational changes that serve to bring the ECFP donor into closer proximity to the EYFP acceptor, facilitating fluorescence resonance energy transfer. This can be monitored by the detection of enhanced emission at 535 nm. The process has the added advantage of being fully reversible, allowing ratiometric measurement of cytoplasmic free Ca^{2+}.

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Links

Julian Schroeder's laboratory website, Marc Knight's laboratory homepage and Michael Whitaker's laboratory website provide information on the detection of calcium signaling in plants and other organisms.

Conclusions

The authors identified seven separate advantages of using the cameleon technology over existing procedures. Probably the main advantage in *Arabidopsis* is the ability to study calcium homeostasis at the single-cell level. This will enable plant physiologists studying calcium signaling in plant cells to exploit the opportunities presented by advances in *Arabidopsis* genomics.

Reporter's comments

This first use of cameleons as calcium sensors in plants shows their potential for studying calcium homeostasis in individual cells. It is likely that the problems with chloroplast autofluorescence will be ironed out; however, even as it stands, the approach is a major advance. Although Allen *et al.* used isolated epidermal peels, it seems likely that with confocal microscopy the approach could be made totally noninvasive and applied to cells and tissues not previously amenable to experimentation. In addition, as the authors were careful to engineer multiple cloning sites upstream and downstream of the YC2.1 coding site in their expression vector, it will be possible to target YC2.1 to organelles and, through fusion to specific signaling proteins, to analyze highly localized calcium dynamics.

Table of links

Plant Journal

Julian Schroeder's laboratory

Marc Knight's laboratory

References

1. Allen CG, Kwak JM, Chu SP, Llopis J, Tsien RY, Harper JF, Schroeder JI: Cameleon calcium indicator reports cytoplasmic calcium dynamics in *Arabidopsis* guard cells. Plant J. 1999, 19: 735-747. 0960-7412

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