PHOTOSYNTHESIS

Coming out of the dark

Seeds are tough cookies — they can withstand hostile surroundings until conditions favour germination. Emergent seedlings, though, are very vulnerable — particularly when first exposed to sunlight. Seedlings accumulate a small amount of protochlorophyllide, the precursor of chlorophyll, before they reach the surface so that, on sensing the first rays of sun, they are ready to photosynthesize. But too much protochlorophyllide can induce oxidative damage (in the form of bleaching), so chlorophyll biosynthesis must be carefully controlled. The identification, by Huq *et al.*, of PHYTOCHROME-INTERACTING FACTOR-1 (PIF1), provides a mechanism by which this can be achieved.

Phytochrome (phy) receptors perceive light signals, which are thought to be transduced through PIFs to impinge on gene expression. The authors' investigations focused on PIF1, a basic helix–loop–helix (bHLH) transcription factor. Whereas *pif1*-insertion-mutant-seedlings showed no adverse effects when grown in the light from germination, those that were germinated and grown in the dark before being transferred to the light became bleached. This was reminiscent of a mutant phenotype that is caused by excess protochlorophyllide and, sure enough, protochlorophyllide levels were higher in the *pif1* mutants that grew in the dark after germination. And the longer the time spent in darkness before transfer to light, the more severe the bleaching phenotype. This hinted that PIF1 might prevent the build-up of excess protochlorophyllide in the dark. Huq *et al.* indeed found that PIF1 negatively regulated the chlorophyll biosynthetic pathway — if *pif1*-mutant seedlings were grown in the dark for only a short period and then exposed to light before they had accumulated lethal levels of protochlorophyllide, they subsequently accumulated chlorophyll much faster than wild-type seedlings did in response to light.

The authors next established that PIF1 could bind to the so-called G-box DNA-sequence motif that is present in the promoters of many light-regulated genes. So what was the effect of PIF1 on transcription? It induced a marked increase in the activity of a luciferase reporter gene

TECHNIQUE

A natural view

Signalling proteins are under tight control both temporally and spatially to ensure that they carry out their roles correctly. For example, the small GTPase Cdc42 functions in processes such as cell motility, proliferation and apoptosis, so its activation at specific subcellular locations needs to be strictly regulated. It has been difficult to visualize Cdc42 activation dynamics *in vivo*, because of the limitations of present approaches. But, in *Science*, Hahn and colleagues now report the development of a biosensor that allows unlabelled, endogenous Cdc42 activation to be visualized in living cells.

They covalently labelled a domain from Wiskott–Aldrich syndrome protein (WASP) — a Cdc42 effector protein — with a dye that specifically reports protein interactions and protein conformational changes in living cells. The dye did not significantly perturb the Cdc42–WASP interaction, and it showed a threefold increase in fluorescence intensity on binding to Cdc42–GTPγS (GTPγS is a non-hydrolysable analogue of GTP). No increase was observed in the presence of Cdc42–GDP. The biosensor could also distinguish between Cdc42 and the related RhoA and Rac GTPases.

Although this biosensor could be used to measure Cdc42 activation in cell lysates, Hahn and co-workers developed a ratiometric imaging approach that allowed Cdc42 activation to be visualized in living cells. The final biosensor was named Mero-CBD — a merocyanine dye plus a Cdc42-binding domain.

The authors used this biosensor to monitor Cdc42 activation during cell adhesion and spreading. They found that Cdc42 is activated at the cell periphery, which extends filopodia, but not in actual filopodia. In addition, using specific inhibitors, they showed that Cdc42 activation at the cell periphery is microtubule dependent. They also found that Cdc42 is activated at the *trans*-Golgi apparatus: this indicates that it regulates the directional sorting/trafficking of polarity signals or that microtubules mediate the transport of activated Cdc42 to the cell periphery. Finally, they showed that increases and decreases in Cdc42 activity are precisely coordinated spatially and temporally with cell extension and retraction.

So, Hahn and colleagues have developed a biosensor that gives us a natural view of Cdc42 dynamics — it allows the activation of endogenous protein to be detected at physiological concentrations in living cells, and does not require Cdc42 to be modified with a fluorescent label. This sensitive methodology could therefore be extended to "…proteins that cannot be derivatized or overexpressed for live cell studies", and could allow us to carry out a "…detailed kinetic analysis of rapid cellular processes".

Rachel Smallridge

References and links

ORIGINAL RESEARCH PAPER Nalbant, P. *et al.* Activation of endogenous Cdc42 visualized in living cells. *Science* **305**, 1615–1619 (2004)

FURTHER READING Erickson, J. W. & Cerione, R. A. Multiple roles for Cdc42 in cell regulation. *Curr. Opin. Cell Biol.* **13**, 153–157 (2001)

WEB SITE

Klaus Hahn's laboratory: http://www.med.unc.edu/wrkunits/2depts/pharm/faculty/hahn.htm

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