ABA signalling: **A messenger's FIERY fate**

Lee Hunt and Julie E. Gray

There is considerable circumstantial evidence that the Ca^{2+}-mobilizing second messenger IP_3 is involved in plant responses to the drought hormone abscisic acid. More direct evidence for this has now come from studies in which endogenous IP_3 levels have been manipulated in plants.

Address: Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield S10 2TN, UK.
E-mail: J.E.Gray@sheffield.ac.uk

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The plant hormone abscisic acid (ABA) regulates seed germination and is thought to be involved in plant responses to various stresses, including drought, salinity and cold shock [1]. So far, five genes involved in ABA signalling pathways have been isolated from the model plant *Arabidopsis*. These include genes for protein phosphatases (*ABI1* and *ABI2*) and for putative transcription factors (*ABI3–5*) [1]. One of the best understood ABA signalling pathways is the closure of the stomatal pore in response to ABA. ABA application is known to cause elevations in guard cell cytosolic Ca^{2+} ion levels [2], and oscillations in the cytosolic [Ca^{2+}] are necessary for stomatal closure [3]. There is some evidence that the Ca^{2+}-mobilizing intracellular second messenger inositol 1,4,5-trisphosphate (IP_3), generated through the action of phospholipase C (PLC), is involved in this response. Application of ABA generates increases in IP_3 in guard cells [4] and release of IP_3 leads to elevations in cytosolic [Ca^{2+}] [5] and stomatal closure [6]. An inhibitor of PLC, the enzyme responsible for releasing IP_3 from the membrane, inhibits ABA-induced cytosolic [Ca^{2+}] oscillations and reduces stomatal closure [7], and several genes in the phosphoinositide signalling pathway have been shown to be upregulated by ABA (Table 1).

Elevations in cytosolic [Ca^{2+}] also occur in whole seedlings following stresses such as cold-shock [8], and this has led to the identification of several genes as ABA or cold responsive, and these gene have been used to study the ABA/stress response. It is the induction of one of these ABA/stress related genes, *RD29A*, which has formed the basis for recent research that has provided convincing genetic evidence that IP_3 does indeed play a general role in plant responses to ABA, drought, cold and salinity stresses.

In their recent study, Xiong *et al.* [9] successfully used a firefly luciferase luminescent screen to isolate components of the ABA and/or stress-related signal transduction chain(s) in plants. The luciferase reporter gene (*LUC*) was expressed in *Arabidopsis* plants, under the control of the ABA and stress-regulated *RD29A* promoter. When these plants are exposed to stress or ABA, there is an increase in promoter activity as shown by increased luciferase activity.

To identify further components of the ABA signalling pathway, *RD29A–LUC* plants were subjected to EMS mutagenesis and progeny plants were examined for altered luminescence patterns. One of the mutagenised plants identified by Xiong *et al.* [9] gave over four-fold enhanced levels of luminescence following stress treatments — such as cold, salt or ABA application — and was consequently named *fiery1*. As well as having high levels of *RD29A* expression, *fiery1* plants exhibit increased expression of a number of other stress or ABA induced genes, such as *HSP70*, following ABA or stress induction, indicating a general role for *FIERY1* in activating gene transcription in response to stress or ABA.

Perhaps most importantly, the *fiery1* plants exhibit a clear stress and ABA related phenotype, which confirms the role of the *FIERY1* gene in ABA/stress signalling. For example, treatment with 200 mM NaCl kills almost all *fiery1* seedlings, whilst 90% of wild-type seedlings survive. Mutant *fiery1* plants also exhibit increased damage on exposure to drought simulation, are unable to adapt normally to cold, and show increased sensitivity to ABA — seed germination and seedling development are impaired by concentrations of ABA too low to affect wild-type plants significantly. These results indicate that *FIERY1* affects the signal transduction pathway(s) that link ABA or stress stimuli to altered gene expression patterns, leading subsequently to altered stress tolerance in plants.

Table 1

Effect of ABA on the expression level of characterised *Arabidopsis* phosphoinositide signalling genes.

Gene name	Enzyme encoded	Effect of ABA
ATPLC1	Phospholipase C	Upregulated [10]
ATPLC2	Phospholipase C	No effect [20]
PIP4K	Phosphatidylinositol 4-kinase	No effect [21]
PIP5K	Phosphatidylinositol-4-phosphate 5-kinase	Upregulated [22]
ATIP5PII	Inositol(1,4,5)P3 5-phosphatase	No effect [10]
FIERY1	Inositol phosphate polyphosphatase	No effect [9]

Map-based cloning, coupled with an extensive T-DNA mutagenesis programme, led to isolation of the *FIERY1* gene, which was found to encode a bifunctional enzyme with 3′(2′),5′-bisphosphate nucleotidase and inositol polyphosphate 1-phosphatase activities. FIERY1-like proteins are thought to act predominantly as inositol phosphatases in multicellular eukaryotes, and are believed to dephosphorylate catabolites of IP_3 and terminate the IP_3 signalling process. It is thus possible that *fiery 1* plants are unable to catabolise IP_3 efficiently and so have enhanced levels of this second messenger following ABA/stress induction. This is exactly what Xiong *et al.* [9] found: they observed that *fiery1* plants have approximately ten times the level of IP_3 found in wild-type plants after ABA induction, indicating that the level of IP_3 is important for ABA and stress related signalling pathways [9].

The identification of the *FIERY1* gene and the characterisation of the *fiery1* plant phenotype strongly indicate that a transient increase in IP_3 level, presumably brought about by PLC-mediated cleavage of phosphatidylinositol 4,5 bisphosphate (PIP_2), is an important component of the ABA and stress induced signalling pathways. In the mutant plants where the FIERY1 inositol phosphatase is inactive, the IP_3 messenger is not degraded as efficiently as normal and continues to activate the pathway(s), leading to high levels of ABA/stress induced gene activation. These high levels of ABA/stress related gene expression lead to a less ABA/stress tolerant phenotype in *fiery1* plants, suggesting that attenuation of the IP_3 signal is necessary for ABA/stress tolerance.

Further support for the role of IP_3 action in ABA signalling comes from recent experiments using an antisense approach to reduce the level of expression of the PLC gene *AtPLC1* in *Arabidopsis* [10]. These plants have much reduced levels of IP_3 following an ABA stimulus and also show reduced levels of expression of stress-inducible genes, such as *RD29A*. We might therefore expect that these low PLC plants would have the opposite phenotype to *fiery1* plants — which have increased levels both of IP_3 and *RD29A* mRNA following ABA induction — and this indeed appears to be the case. Antisense *AtPLC1* seedlings show no inhibition of germination or growth in the presence of concentrations of ABA that are normally inhibitory to germination, whereas the germination of *fiery1* seedlings is hypersensitive to ABA, as described above.

The conclusion that IP_3 acts as a second messenger during the induction of stress related gene expression is supported by other recent findings in the field. Work on cultured *Arabidopsis* cells [11] has shown that IP_3 levels rise rapidly and transiently following a hyperosmotic shock. Pharmacological inhibition of PLC activity, which as mentioned above is responsible for the release of IP_3, inhibits both the increase in IP_3 levels and the expression of stress-inducible genes such as *RD29A*. Although Takahashi *et al.* [11] found evidence for IP_3 acting as a signal in response to hyperosmotic stress, in contrast to Xiong *et al.* [8] they could not find evidence for its involvement in the ABA inducible gene expression pathway. It was thought that, in these experiments, the IP_3 levels rose too slowly following ABA induction to be responsible for inducing gene expression, raising the question of whether or not IP_3 is necessary for the ABA inducible gene expression pathway.

The three recent reports [9–11] all agree that an elevation in IP_3 levels is important in mediating the response to stress signals, but they are divided on the question of whether IP_3 is important in the ABA signalling pathway. It is possible that each group is correct, and that ABA induction of gene expression may occur in either an IP_3-dependent or IP_3-independent manner. This illustrates how complex plant cell signalling pathways may be. An emerging view is that multiple signalling pathways can bring about the same cellular response, and that perhaps factors such as the previous experiences, conditioning or plant cell type determine which pathway is used [12]. In guard cells, for example, where an ABA stimulus results in a reduction of the stomatal pore, at least three other Ca^{2+}-mobilising signals have been identified: cyclic ADP ribose [13,14], sphingosine-1-phosphate [15] and inositol hexakisphospahate (IP_6) [16], in addition to IP_3, have each been shown to act via a Ca^{2+}-dependant pathway in the guard cell ABA response. To further illustrate the plasticity of signalling pathways in guard cells, *fiery1* plants have no apparent decrease in transpirational water loss, suggesting that stomatal regulation is not affected by the increase in IP_3 level in these plants (or that perhaps a different inositol phosphatase isoform is active in guard cells).

Of course, the analysis of signalling pathways is never clear-cut, especially it appears, in plant cells. The reduced activity of inositol polyphosphate 1-phosphatase in *fiery1* will inevitably affect the levels of other phosphoinositide pathway intermediates, as well as IP_3. Some potentially affected phosphoinositides, such as PIP_2 and IP_6, also play active signalling roles in plant cells. For example, PIP_2 can regulate the activity of a phospholipase D isoform in *Arabidopsis* [17] and modulates the plant cytoskeleton [18,19]. So, although we can conclude from the results discussed here, that IP_3 is important in mediating plant responses to stress and, at least under some circumstances, responses to ABA, there is still much work to do before we can fully understand the importance of IP_3 in transducing these signals. Improving our understanding of how plant stress response pathways act is nevertheless an important step in approaching the production of crops with enhanced stress tolerance for future agriculture.

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