

T16: Molecular analysis of the ABA signaling pathway in *Physcomitrella patens*.

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Physcomitrella patens responds to abscisic acid (ABA) at the level of gene expression (Knight *et al.*, 1995). However the molecular components of the ABA signaling pathway in *P. patens* and their interaction with gene regulatory networks have not been determined. We are dissecting the ABA signaling pathway and comparing its characteristics with higher plant systems such as *Arabidopsis* and cereals. To study the ABA-response system in *P. patens* we are using the well-characterized ABA-responsive *Em* promoter from wheat, linked to GUS or GFP as a reporter.

The *Arabidopsis* *abi1* gene encodes a protein phosphatase 2C (PP2C). The mutant allele *abi1-1* strongly blocks ABA signaling in higher plants and is dominant. The moss EST database contains several ESTs that potentially encode this enzyme. We therefore evaluated the effect of *abi1-1* over-expression on ABA signal transduction in *P. patens*. We introduced *Em-GUS* with or without a CaMV 35S promoter-driven *abi1-1* into *P. patens* protonemal tissue *via* particle bombardment. Over-expression of *abi1-1* resulted in complete inhibition of ABA-induction of the *Em* promoter, suggesting the PP2Cs are involved in *P. patens* ABA signaling.

ABA-regulated transcription from the *Em* promoter is regulated by the ABI3 transcription factor in *Arabidopsis* or by the maize ortholog of ABI3, VP1 (ABI3/VP1). We searched the moss EST database for ABI3/VP1 homologues, using the highly-conserved B3 (DNA binding) domain of these proteins. One identified clone, *PpABI3*, was 3066 bp in length and encoded a 659 amino acid sequence as the putative ORF. Its B3 domain had 60% of amino acids identical to ABI3/VP1. The B1 and B2 domain, which are other highly conserved domains among ABI3/VP1 homologues, were also observed in the *PpABI3*, although the similarities were not as high as for B3.

To determine if *PpABI3* is functionally comparable to higher plant ABI3/VP1, we performed transient assays in both *P. patens* protonemal tissue and barley aleurone cells. In barley aleurone cells, the *Em* promoter requires both ABI3/VP1 and a bZIP transcription factor, ABI5, for ABA-dependent transcription. Over-expression of *PpABI3* resulted in the activation of the *Em* promoter in the absence of ABA in both cell types. However, activation of the *Em* promoter by ABA treatment was strongly enhanced by *PpABI3* only in *P. patens* cells. Interestingly, co-bombardment of *PpABI3* and barley ABI5 did not show any positive effect on the activation of *Em* promoter in either *P. patens* or barley aleurone cells. This result suggests the existence of a factor, different from ABI5, that can interact with *PpABI3* to activate the *Em* promoter in *P. patens* synergistically.

Our data clearly demonstrates that *P. patens* and higher plants share common factors in ABA signaling. However, clear differences exist among the individual molecular components. With the superb EST databases and homologous recombination technique, *P. patens* will be suitable for a comparable approach for studying the ABA signal transduction pathway in plants.

Knight, C.D., Sehgal, A., Atwal, K., Wallace, J.C., Cove, D.J., Coates, D., Quatrano, R.S., Bahadur, S., Stockley, P.G., and Cuming, A.C. 1995. Molecular Responses to Abscisic Acid and Stress Are Conserved between Moss and Cereals. *The Plant Cell* 7, 499-506.