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 abil gene encodes a protein phosphatase 2C (PP2C). The mutant allele abil-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 abil-1 over-expression on ABA signal transduction in P. patens. We introduced Em-GUS with or without a CaMV 35S promoter-driven abil-1 into P. patens protonemal tissue via particle bombardment. Over-expression of abil-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.