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Characterization of AFN1, a Gene Associated with Cereal Grain Germination

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Characterization of *AFN1*, a gene associated with cereal grain germination

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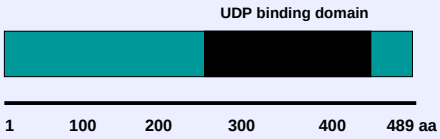
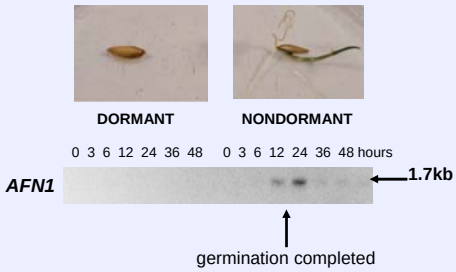
Abstract

The *AFN1* gene is transiently expressed in germinating oat grains. As *AFN1* is not expressed in dormant oat grains during imbibition, we hypothesize that *AFN1* may be involved in stimulating the germination process. Sequence analysis of an *AFN1* cDNA clone indicates that the AFN1 polypeptide is similar to a previously identified abscisic acid (ABA) glucosyl transferase. This suggests that AFN1 may be acting to glucosylate ABA, thereby inactivating it. As the hormone ABA is known to inhibit germination, ABA glucosylation/inactivation could lead to germination in grains expressing AFN1. To test this hypothesis, we have constructed an expression plasmid that encodes an MBP::AFN1 (maltose binding protein) fusion protein. *E. coli* cells carrying the expression plasmid were found to produce the MBP::AFN1 fusion protein as a substantial fraction of total protein. We are currently in the process of purifying the MBP::AFN1 fusion protein by affinity chromatography, so that it can be assayed for ABA glucosyl transferase activity. We also wish to test the effect of *AFN1* gene expression during grain imbibition on the germination behavior of the grains. To this end, we have constructed plasmids for the overexpression and RNAi-based suppression of AFN1 in transgenic plants. These plasmids have been introduced into oat cells by particle bombardment and we are in the process of regenerating transgenic plants for study.

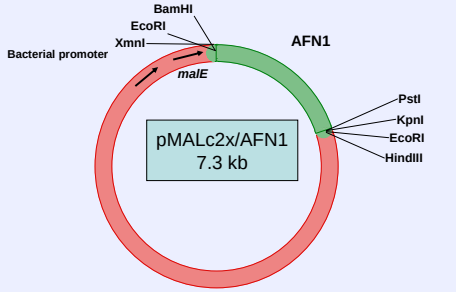


The *AFN1* cDNA encodes a 489 amino acid polypeptide.

The AFN1 polypeptide contains UDP binding domain which is also present in (ABA) glucosyl transferases. This suggests that AFN1 may act to glucosylate ABA, thereby inactivating it.

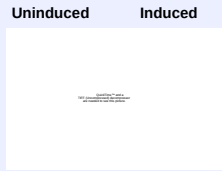


AFN1 mRNA is produced in germinating grains.



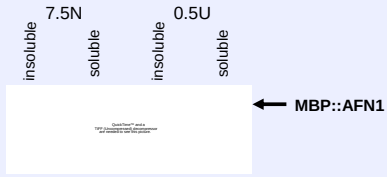
The pMALc2x/AFN1 expression plasmid has been constructed.

Results



Production of AFN1 fusion protein in *E. coli* cells. BL21(DE3)pLysS *E. coli* cells were transformed with pMALc2x/AFN1. Cell cultures were grown to an OD_{600} of 0.5 and expression was induced by addition of IPTG. The culture was allowed to incubate for 4 hours at 37°. Crude protein was obtained from the *E. coli* cells by sonication and analyzed on polyacrylamide gels.

MBP::AFN1 fusion protein has been produced in *E. coli* cells containing pMALc2x/AFN1 plasmid.



Production of AFN1 fusion protein in *E. coli* cells. BL21(DE3)pLysS *E. coli* cells were transformed with pMALc2x/AFN1. Cell cultures were grown to an OD_{600} of 0.5 and expression was induced by addition of IPTG. The culture was allowed to incubate for 4 hours at 37°. Crude protein was obtained from the *E. coli* cells by sonication in 7.5N and 0.5U buffer. Soluble and insoluble fractions were suspended in 1x SDS gel loading buffer, heated at 95-100°C for 10 minutes and analyzed on polyacrylamide gels.

A substantial amount of MBP::AFN1 fusion protein has been found in the 0.5U buffer soluble fraction.

Acknowledgments

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