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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

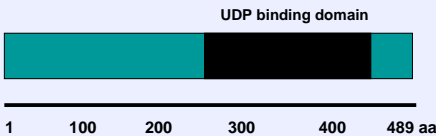
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Abstract

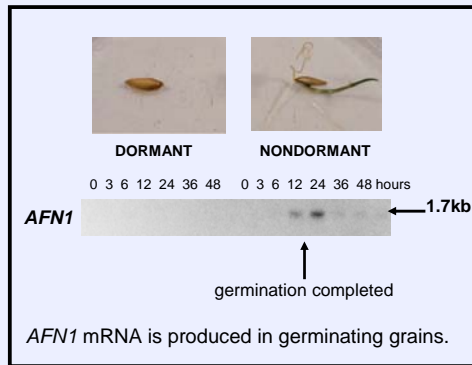
The *AFN1* gene is transiently expressed in germinating oat grains. As *AFN1* is not expressed in dormant oat grains during imbibition, we hypothesize that *AFN1* may be involved in stimulating the germination process. Sequence analysis of an *AFN1* cDNA clone indicates that the *AFN1* polypeptide is 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.



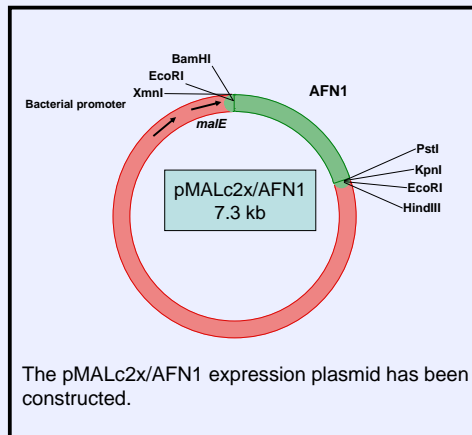
The *AFN1* cDNA encodes a 489 amino acid polypeptide.

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.

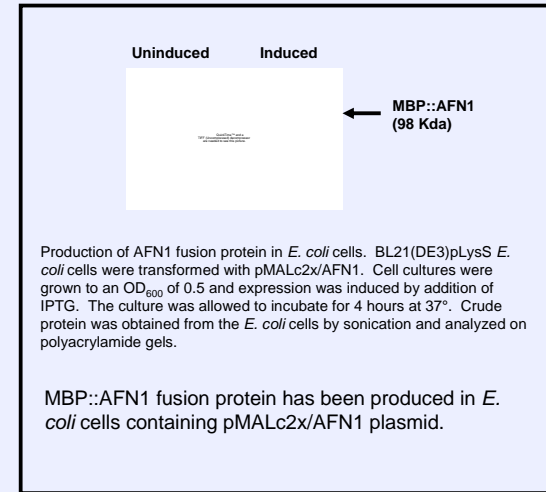
Results



AFN1 mRNA is produced in germinating grains.

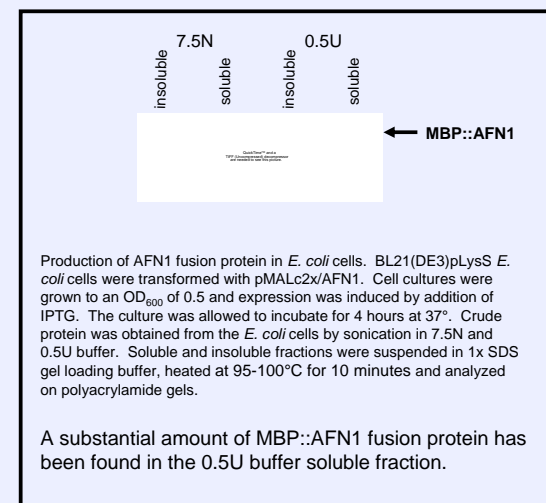


The pMALc2x/*AFN1* expression plasmid has been constructed.



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 OD₆₀₀ 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 OD₆₀₀ 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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