Plant Physiol. (1995) 108: 831–832

Plant Gene Register

Characterization and Expression of a cDNA Encoding a Seed-Specific Metallothionein in Maize[1]

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Developing embryos of many higher plants undergo a maturation process in which a variety of storage proteins and putative dessication protectants are synthesized and stored. One of the proteins found to accumulate in maturing wheat embryos is a Zn^{2+}-associated class II metallothionein called E_c (for early Cys) (Lane et al., 1987). Like many other maturation proteins, the promoter regions of wheat E_c genes have been found to contain ABA-response elements, and their expression is ABA inducible (Kawashima et al., 1992). We have characterized a cDNA from maize (*Zea mays* L.), pMEC (maize E_c), whose sequence, regulation, and expression patterns indicate that it is the equivalent of wheat E_c (Table I).

Several clones having strong homology with transcripts encoded by the wheat E_c genes were isolated from a cDNA library that was differentially screened for messages expressed in maturing wild-type embryos but absent from *vp*1 mutant embryos. These mutant embryos synthesize normal levels of ABA but are not ABA responsive (Neill et al., 1986; Robichaud et al., 1986). The product of the *Viviparous*-1 locus has been shown to act as a transcriptional activator of promoter targets carrying ABA-response elements (Hattori et al., 1992). We observed complete sequence identity among all pMEC isolates and single-band patterns on genomic Southern analysis, indicating that pMEC is probably encoded by a single gene. The approximately full-length clone, pMEC, contains a complete coding region for a polypeptide of 7.8 kD having 77% amino acid identity and 83% similarity with wheat E_c.

Maize embryos accumulate pMEC mRNA specifically in maturing seeds. On northern blots of RNA isolated from developing maize embryos, pMEC detects an mRNA species migrating at approximately 530 bases. The message level is low in immature embryos and increases to a peak as embryos enter the mid-maturation phase. It remains high throughout later development. We have not assessed the relative contributions of transcription or stability to this persistence. Significant hybridization to pMEC was not found in RNA from seedling shoot, cob, tassle, or adult leaf.

Table I. *Characteristics of a cDNA for a seed metallothionein in maize*

Organism:	
	Zea mays L.
Gene Location:	
	Single copy, nuclear gene.
Gene Product:	
	Seed class II metallothionein.
Method of Identification:	
	Sequence and predicted translation product comparison with wheat class II metallothionein, E_c (Lane et al., 1987). The predicted 7.8-kD maize protein is significantly similar to the E_c amino acid sequence (77% identical, 83% similar).
Techniques:	
	λZapII (Stratagene) cDNA library constructed with poly(A)$^+$ RNA from maturation-phase embryos. Clones were selected in a differential screen using probes from wild-type and *Vp*1 mutant embryos. Sequencing was done both manually and by automated methods (Applied Biosystems). Sequences were analyzed and checked against data base libraries using Genetics Computer Group (Madison, WI) software.
Expression and Regulation:	
	Northern blot analysis shows an RNA of approximately 530 nucleotides that is present in maturation-phase embryos but not in younger embryos or in other plant tissues. Expression is dependent on both ABA and the product of the *Vp*1 gene.

Expression of pMEc message is modulated by ABA; it is abolished in *vp*1 mutants and reduced at least 4-fold in *vp*5 mutant embryos, which do not synthesize ABA (Neill et al., 1986). To test whether ABA and/or osmotic stress could induce pMEC expression in young embryos, wild-type and *vp* embryos were cultured in the presence of either 10 μM ABA or high osmoticum (Rivin and Grudt, 1991). Exogenous ABA enhanced pMEC message accumulation in both wild-type and *vp*5 embryos. Osmotic stress elevated pMEC message levels in wild-type embryos but not in *vp*5, implying that de novo ABA synthesis was required for the osmotic stress response of this message. The treatment with high osmoticum prevented precocious germination of *vp*1 embryos in culture, but no pMEC expression was observed, confirming the requirement for a functional *Vp*1 gene product. Although we do not know whether ABA acts by stimulating transcription or by increasing message stability, we

[1] Supported by National Science Foundation grant No. DCB9007481 to C.J.R. Oregon Agricultural Experiment Station Technical Paper No. 10657.

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Abbreviation: *vp*, *viviparous*.

believe that the former is likely, since *Vp1* stimulates the transcription of maturation-associated genes in response to increased ABA levels (McCarty et al., 1991).

It has been proposed that wheat E_c acts as part of a zinc-homeostasis system supporting nucleic acid synthesis during periods of cellular proliferation (Kawishima et al., 1992). Although a similar function has been proposed for some mammalian metallothioneins, they may also be induced by physical or chemical stress (reviewed by Kagi and Schaffer, 1988). The ABA inducibility of pMEC and its accumulation after completion of embryogenesis is consistent with E_c protein either playing a role in preparing seed tissues for desiccation or serving as a metal storage system in preparation for germination processes. If the latter is true, the failure of *vp* mutants to express pMEC indicates that this function is not obligate for successful precocious germination.

One other metallothionein-like gene has been isolated from maize (de Framond, 1991). Unlike pMEC, its expression is confined to root tissue. Maize and wheat E_c-coding elements diverge from this metallothionein as well as other metallothioneins isolated from higher plants in that they do not contain a Cys-poor internal spacer (Kawashima et al., 1992, and refs. therein).

Received October 14, 1994; accepted November 21, 1994.

The GenBank accession number for the sequence reported in this article is U10696.

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