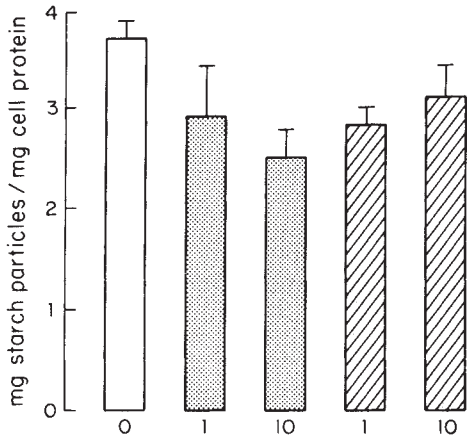

Fig. 4 Preservation of phagocytic function after deactivation of macrophages by TGF-β1 or TGF-β2. Macrophages (8.5×10^5) were plated on 13-mm glass coverslips and nonadherent cells removed after 2 h. Test media were added for 2 days as indicated: complete medium alone (open bar), or complete medium containing TGF-β1 (stippled bars) or TGF-β2 (hatched bars) at the concentrations indicated in ng ml^{-1}. After 2 days, macrophages were washed and incubated in complete medium for 2 h with 2 mg per coverslip of ^{14}C-acetylated starch granules isolated from seeds of *Amaranthus caudatus*. These conditions optimize the quantification of maximal phagocytic capacity by macrophages[11]. The coverslips were washed and the monolayers solubilized to determine the mg particles phagocytized per mg cell protein. Means ±s.e.m. of triplicates are shown.

induces chemotaxis[20,21] (EO_{50}, 0.004 pM)[20], release of fibroblast growth factors and accumulation of IL-1 mRNA (EC_{50}, ~40 pM)[20], and release of angiogenic factors[21]. On the other hand, exposure of macrophages to TGF-β1 is associated with ~50% suppression of TNFα release and ~35% suppression of Ia antigen expression (EC_{50}, 40–400 pM) (ref. 21 and personal communication, C. Czarniecki). Above, we have described virtually complete suppression of macrophage respiratory burst capacity by TGF-β1 (EC_{50}, 0.6 pM) and TGF-β2 (EC_{50}, 4.8 pM). These effects may reflect a coordinated response in wound healing[23], in which macrophages are recruited to scavenge debris and foster the growth of fibroblasts and endothelial cells, while being suppressed in their capacity for a respiratory burst that could be inimical to these cells.

The ability of TGF-β1 and TGF-β2 to ablate the respiratory burst of macrophages raises the possibility that these agents might have a role in the treatment of inflammatory disorders involving excessive macrophage activation.

We thank Moses Chao, Joshua Adler, Yuko Arita, Genentech, Inc., Amgen, Inc. and Immunex, Corp. for providing some of the cytokines. This work was supported by the National Cancer Institute.

Received 6 February; accepted 19 May 1988.

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A gene induced by the plant hormone abscisic acid in response to water stress encodes a glycine-rich protein

Jordi Gómez, Demetrio Sánchez-Martínez*, Virginia Stiefel, Joan Rigau, Pere Puigdomènech & Montserrat Pagès†

Departamento de Genética Molecular, CID (CSIC), Jorge Girona Salgado 18–26, 08034 Barcelona, Spain

Plant hormones such as abscisic acid (ABA) appear to modulate the responses of plants under adverse conditions[1,2] ABA has a poorly-understood role in embryogenesis, accumulating in the stages before dessication[3,4], and altering the rate of transcription of a specific set of genes[5,6]. The functions of the proteins encoded by these genes, however, are unknown, and their messenger RNAs decrease again during early germination[7–9]. No correlation has been established between ABA levels and the induction of particular genes in non-embryonic organs. The level of ABA increases substantially in leaf tissues subjected to water stress[10] and thus it has been proposed that ABA mediates plant–water relations[1,10]. Here we describe the isolation of complementary DNA and genomic clones of a gene that is ABA-inducible in the maize embryo, and whose messenger RNA accumulates in epidermial cells, which is also induced by water stress and wounding in leaves. The deduced protein is rich in glycine. Identification of this gene will contribute to our understanding of the role of ABA.

After a series of differential screenings of a cDNA library constructed from maize dry embryo using cDNA synthesized from immature embryo poly(A)$^+$ RNA, with or without ABA treatment, six non cross-hybridizing clones were selected. These were shown to correspond to mRNAs present in dry embryos by Northern analysis, and their level increased precociously after ABA treatment of immature maize embryos. One of them (clone pMAH9) had an insert of 732 base pairs (bp) and hybridized to an RNA band of approximately the same size (Fig. 3). This clone was chosen for further study, and was used to screen a genomic library. The identity of a hybridizing genomic clone was confirmed by restriction mapping.

The sequences of the cDNA and genomic clones are shown in Fig. 1. They show perfect identity except for an insertion of 146 bp in the genomic sequence with the sequence features of an intron. There is a TATA box at the 5′-end. Only one plausible open reading frame was identified in the cDNA, and the sequence of the putative protein is shown in Fig. 1. The encoded protein is 157 amino acids long with a predicted relative molecular mass of 15,427 and an isoelectric point of 5.7. These values correspond closely with polypeptides detected in hybrid-released experiments using the pMAH9 plasmid (results not shown).

The protein sequence has a well-defined domain structure, clearly seen in hydrophilicity plots (Fig. 2). The first half of the sequence (residues 1–88) is composed of alternating α and short

* Present address: Herpes Branch, Centers for Disease Control, Atlanta, Georgia 30333, USA
† To whom correspondence should be addressed


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cgccgttgtttgcgcggtcgccgcccgttccgttcttgtcggcgcccgtcacgtccgtcgcgc  -124

tataaatctacgggggtaggccttcctcactccctcgtgctctctcaggggtgggctggcttct  - 61

cctcccccctcttaccaagtcctcctcgggttagggttccttagggtttttgtgggggggcgATG    3
                                                              Met    1

GCG GCG GCT GAT GTG GAG TAC CGT TGC TTC GTC GGC GGG CTT GCC TGG     51
Ala Ala Ala Asp Val Glu Tyr Arg Cys Phe Val Gly Gly Leu Ala Trp    17

GCC ACC AGC AAC GAG TCG CTG GAG AAT GCC TTC GCC TCC TAC GGC GAG     99
Ala Thr Ser Asn Glu Ser Leu Glu Asn Ala Phe Ala Ser Tyr Gly Glu    33

ATC CTC GAC TCC AAGgtttgcttcttcctcgctaggtttcattccgcggtctgtttgtg   158
Ile Leu Asp Ser Lys                                               38

ccgttgggggctagatccgggtcgtggttcaacagatctgcttcgtttttggtacagatctgcgt  221

tcgctcgaatcgagcatgacgttttcatgtgattatgcagGTC ATC ACC GAC CGG GAG   279
                                        Val Ile Thr Asp Arg Glu    44

ACG GGG AGG TCT CGC GGC TTC GGC TTC GTT ACC TTC TCC TCC GAG AAC   327
Thr Gly Arg Ser Arg Gly Phe Gly Phe Val Thr Phe Ser Ser Glu Asn    60

TCC ATG CTC GAC GCT ATC GAG AAC ATG AAC GGC AAG GAG CTC GAC GGC   375
Ser Met Leu Asp Ala Ile Glu Asn Met Asn Gly Lys Glu Leu Asp Gly    76

CGT AAC ATC ACC GTT AAC CAA GCC CAG TCC CGT GGC GGT GGC GGT GGC   423
Arg Asn Ile Thr Val Asn Gln Ala Gln Ser Arg Gly Gly Gly Gly Gly    92

GGC GGT GGC TAC GGC GGC GGT CGC GGC GGC GGC GGC TAT GGT GGC GGG   471
Gly Gly Gly Tyr Gly Gly Gly Arg Gly Gly Gly Gly Tyr Gly Gly Gly   108

CGC CGT GAC GGC GGT TAT GGC GGC GGT GGC GGC TAC GGC GGT CGG CGC   519
Arg Arg Asp Gly Gly Tyr Gly Gly Gly Gly Gly Tyr Gly Gly Arg Arg   124

GAG GGT GGT GGC GGC GGC TAC GGA GGC GGT GGC GGC TAC GGC GGT CGG   567
Glu Gly Gly Gly Gly Gly Tyr Gly Gly Gly Gly Gly Tyr Gly Gly Arg   140

CGC GAG GGT GGT GGT GGC GGC TAC GGC GGC GGC GGC GGC TGG AGG     615
Arg Glu Gly Gly Gly Gly Gly Tyr Gly Gly Gly Gly Gly Trp Arg     156

GAC TGA tgtgtgggcccatcctggcttcggccgagttatcttatcatctatagtatcgtgt  676
Asp End                                                           157

taccgttcgcttctgtcaccgtgttagtgtccgttctacctttggattaggtgttggtacccc  739

tgttgttcccctttgtttgctcccgctatgaaaacgagacgagagaagaatgagcaaggttttg  802

ttcgcagctatttttgtccaatgatattgatatgttgttcccctgtctgttgctggattgttg  865

tgtggctatacaaatatcaacaaactgttactccgaaagccctacaaatatcaacttccgaga  928
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Fig. 1 Restriction endonuclease map and sequence of the insert of cDNA clone pMAH9 and the corresponding genomic clone. The sequenced zone is represented by a rectangle including the cDNA (hatched) and the main restriction sites. The nucleotide sequence includes a TATA box (underlined) and the limits of cDNA (shown by an asterisk). The open reading frame present in the sequence is shown (the repetitive Gly-Gly-Tyr-Gly-Gly motif is underlined with arrows). Clone pMH9 was selected by differential screening of a dry embryo cDNA library in pBR322 using single-stranded labelled cDNA probes synthesized from poly(A)$^+$ RNA extracted from immature embryos (20 days after pollination) before and after treatment with 1 μM ABA for 21 hours. The positive clones accounted for 2% of the cDNA library. The genomic clone was detected in a library constructed in λ charon35 after partial restriction of endosperm DNA with *Sau*IIIA[16]. DNA sequence was carried out by chemical degradation[17]. cDNA and genomic sequences were identical except for the insert in the genomic fragment, containing the consensus sequences of an intron.

β structures, whereas the second half (residues 89–157) has an unusual amino-acid composition (Gly 69.4%, Arg 12.5%, Tyr, 9.7%, Asp 2.8%, Glu 2.8% and Trp 1.4%). The sequence of the second domain is highly repetitive with two main motifs, Gly-Gly-Tyr-Gly-Gly and Arg-Arg-Glu. The most abundant motif (Gly-Gly-Tyr-Gly-Gly) is different from that found in a putative cell-wall protein, rich in glycine, isolated from petunia[11].

Northern analysis and *in situ* hybridization were used to check the relative abundance of RNAs hybridizing with pMAH9. Hybridization of the cDNA insert with RNAs extracted from embryos and plants at different stages of development (Fig. 3) shows that a basal level of this mRNA exists in young roots

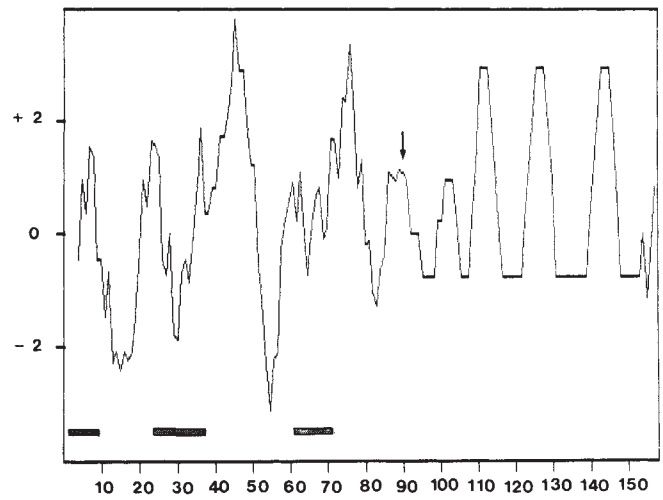

Fig. 2 Hydrophilicity plot of the predicted amino-acid sequence of the glycine-rich protein[18]. Hydrophilicity is plotted as a function of the residue number. The arrow indicates amino acid number 88, separating the two domain structures of the protein. The bars show the segments where α-structure is predicted[19].

and leaves, and in immature embryos 20 and 30 days after pollination. This starts to increase 33–35 days after pollination (not shown) and the highest level is detected at 40 days, when embryo desiccation starts. The mRNA is also present in dry embryo but is barely detectable after 24 hours of germination. When young embryos are incubated in the presence of ABA a 25-fold increase in the level of this mRNA is seen.

In situ hybridization was carried out on cryostat sections of embryos 40 days after pollination to check whether the mRNA accumulates in a specific cell type (Fig. 4). The radioactivity accumulates in scutellar epidermal cells surrounding the embryo axis, and extends one layer of cells inwards. At higher magnification, strips of radioactivity are also seen in epidermal cells on the edges of the embryonic leaves forming the plumule (not shown). Several glycine-rich proteins[12,13] which show limited tissue-specific expression have been described as having structural and protective functions. Whether the protein encoded by the gene described here has a similar function remains to be seen.

It has been proposed that endogenous ABA levels rise as a consequence of water stress in maize leaves and return to a basal level after rewatering[14]. We therefore examined whether pMAH9 mRNA is accumulated in leaves subjected to this and to other types of stress. Northern analysis of dehydrated leaves (Fig. 3) shows a high increase in the mRNA level, decaying to a basal level after recovery. During dehydration the ABA hormone concentration increased linearly to 10-fold over a three-hour period, and to maximum correlated with the maximum mRNA abundance. After rewatering, a close correlation between ABA concentration and mRNA levels was also observed. The effect of leaf wounding was also examined. The time course of mRNA accumulation after wounding in both young (not shown) and 30-day-old leaves (Fig. 3) shows that the maximum mRNA level is achieved after two hours; mRNA is still detectable at relatively high levels after 12 hours but decreases to basal levels by 24 hours.

These results indicate that the gene corresponding to pMAH9, selected as being ABA-inducible in maize embryo, is also induced in different situations when the corresponding organ is challenged by a hydric stress. This would occur in embryos as a prelude to the dormancy period, and is one of the primary effects of wounding upon leaf cells. We suggest that the increase in ABA levels in embryos before desiccation is part of the programme ensuring the survival of embryos during dormancy. The mechanisms of protection of cereals against hydric stress conditions are not well understood. Further characterization of

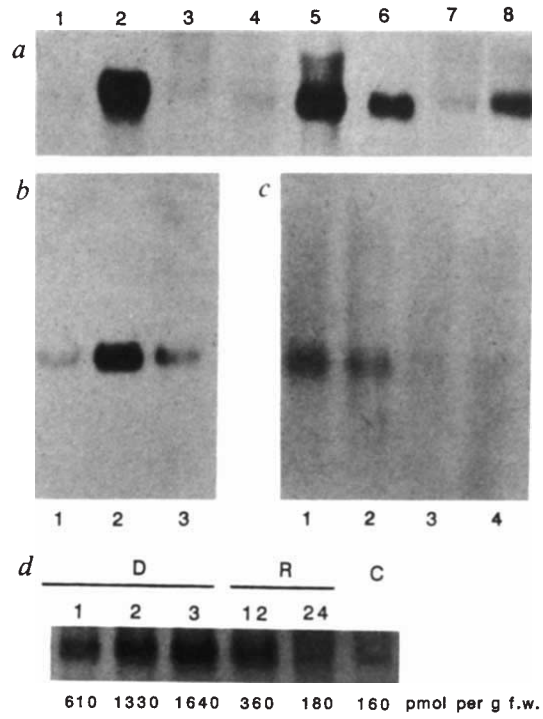

Fig. 3 Northern blot analysis of RNA hybridized with pMAH9. Total RNA was isolated[9] from: *a*, excised embryos 20 days after pollination incubated in water for 21 h (lane 1) or in the presence of 1 μm ABA (lane 2); freshly excised embryos 20 (lane 3), 30 (lane 4) and 40 (lane 5) days after pollination; dry embryos (lane 6); one-day germinated embryos (lane 7) and 7-day-old roots (lane 8). *b*, Young (7-day-old) leaves (lane 1), dehydrated leaves (lane 2) and rewatered leaves (lane 3). *c*, Wounded 30-day-old leaves 2 (lane 1), 12 (lane 2) and 24 (lane 3) hours after wounding; unwounded are shown as a control (lane 4). *d*, Dehydration of young leaves for 1, 2 and 3 h (D), and rehydration for 12 and 24 (R) control leaves (C). Numbers indicate the ABA content (pmol/g fresh weight) during and after dehydration. The blot was carried out with 10 μg of RNA as previously described[20]. The size of hybridizing mRNA is 800 nucleotides. Plants were dehydrated until they had lost 10–15% fresh weight by continuous exposure to a gentle stream of air during growing, and well watered plants were transferred to dry plates where they were dehydrated under a stream of air for 1, 2, and 3 hours. Rewatering of dehydrated plants was done during 12 and 24 hours. Leaves from 7 and 30 day-old plants were wounded by blade incision. Preparation of plant extracts for ABA measurements was done as described elsewhere[21]. The concentration of ABA was measured using a monoclonal antibody and ELISAs based on competitive binding between free and enzyme-linked ABA (Phytodetek-ABA kit from Idetek Inc., San Bruno, California).

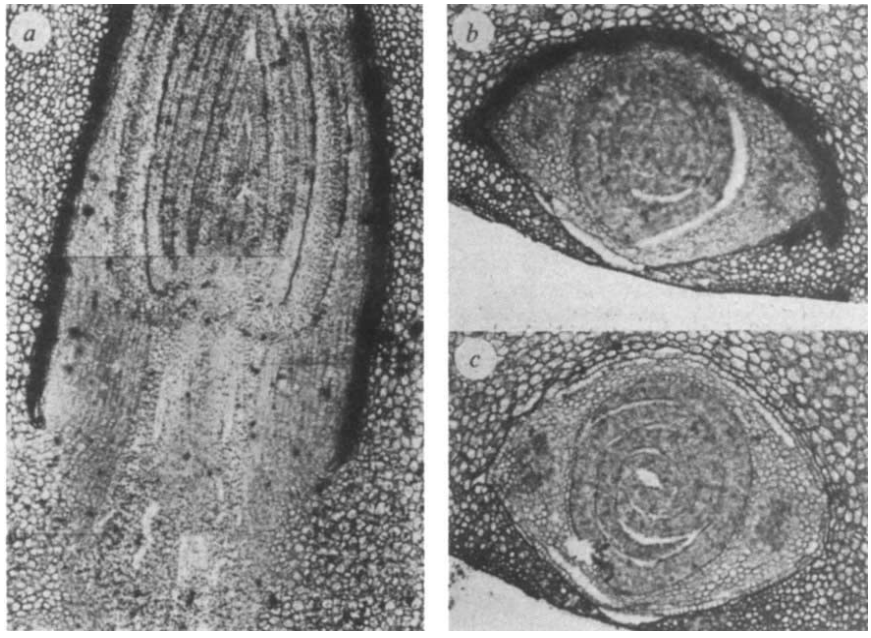

Fig. 4 Localization of mRNA complementary to pMAH9 cDNA on embryo (40 days after pollination) sections. The presence of the mRNA is observed in the scutellar epidermic cell layers surrounding the embryo axis. *a*, Longitudinal section of plumule-axis, *b*, transversal section of plumule and *c*, control transversal section of plumule hybridized with zein cDNA (clone A20[21]). Cryostat sections (8 μm) of embryos of maize (W64A) were treated as previously described[22]. The cDNA insert was digested with KpnI, SacI and HpaII and labelled with ^{35}S nucleotides by random priming (Boehringer Mannheim kit). Hybridization and washings were performed as described but DTT (10 mM) was added in all buffers. The specific activity of the probe was 1×10^8 cpm/μg and it was used at 10–30 ng per slide. Autoradiography was done with Kodak NTB-2 emulsion. Replicate sections were preincubated with ribonuclease A (100 μg/ml) and ribonuclease T1 (5 μg/ml) as a control for non-specific binding.

the gene described here should provide significant insights into the role of ABA in plant stress tolerance.

We thank Dr S. Prat for practical suggestions, Drs F. and B. Burr for providing clone A20, Ms M. Alvarez for manuscript preparation, and especially Dr M. D. Ludevid for performing the ABA determinations. This work was supported by grants from CSIC and CSIC-CDTI (Plan Proa).

Note added in proof: A recent report of Chandler *et al.*[24] confirms our results, showing the expression of ABA-inducible genes in water stressed cereal seedlings.

Received 24 March; accepted 10 June 1988.

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