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TM20, a Gene Coding for a New Class of Transmembrane Proteins Expressed in the Meristematic Tissues of Maize*

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Virginia Stiefel, Eduardo López Becerra‡, Ramon Roca, Miriam Bastida§, Torben Jahrmann, Enrique Graziano§, and Pere Puigdomènech¶

From the Departament de Genètica Molecular, Institut de Biologia Molecular de Barcelona, Centre d'Investigació i Desenvolupament, Consejo Superior de Investigaciones Científicas, Jordi Girona, 18, 08034 Barcelona, Spain

In the course of the analysis of *lachrima*, a recessive, defective kernel, embryo-lethal mutation in *Zea mays* that alters embryo and endosperm development, a gene coding for a new class of transmembrane proteins was isolated. The mutant was produced by Ac transposon tagging, and a gene located in the insertion region of the transposon was isolated as well as the corresponding cDNA. The predicted protein contains twenty hydrophobic segments that can be grouped in five repeats formed by four segments that fulfill the criteria for membrane spanning domains, and for this reason the gene has been named *TM20*. The sequences of the domains in each position of each group can be aligned, indicating that TM20 is formed by a four-domain structure duplicated five times. During embryogenesis in wild-type embryos and in the growing plant, *TM20* gene expression is associated with meristems.

Embryo development in flowering plants begins with a zygote and results in the formation of a bipolar embryo, containing the shoot and root meristems, that gives rise to the adult plant during post-embryonic development (1). The process of embryogenesis involves three main phases: pattern formation (early embryogenesis), embryo enlargement and storage product synthesis (mid embryogenesis), and desiccation, dormancy and preparation for germination (late embryogenesis).

Whereas knowledge about the regulation of pattern formation and embryogenesis in animals has made rapid advances, the mechanisms that regulate these processes in plants are just beginning to be defined. The most extensive studies in this field have dealt with *Arabidopsis thaliana* and maize, which are the best analyzed examples in the two major groups of flowering plants, monocots and dicots. The general strategy in genetic analysis is to use mutants as tools for the identification of essential genes. In Arabidopsis, many embryo mutants have been isolated and characterized. Recently, some mutations altering embryo development have been characterized at a molecular level (2–5), and in some cases the protein encoded by the gene is involved in the formation of the cell wall, an essential step in plant morphogenesis.

Besides its worldwide economic importance, maize has several intrinsic advantages which facilitate the study of embryogenesis. The size of the embryo allows isolation from the endosperm at early stages of development. Moreover, the developmental stages of the maize embryo, from one-cell zygote to mature embryo, are well characterized at the morphological level (Refs. 6–8; see the schematic drawing in Fig. 3). The maize zygote divides asymmetrically to generate a small, lenticular terminal cell and a larger basal cell. This two-cell embryo undergoes irregular cell divisions, both in orientation and in sequence, so it is not possible to trace the future organs of the embryo as it has been done in some other plants. The result of these divisions is the formation of a club-shaped embryo (proembryo stage), consisting of two regions, a small celled embryo proper, which lies above a large celled suspensor. The first evidence of differentiation within the embryo proper occurs at the transition stage with the delimitation of the protoderm and the appearance of a wedge-shaped meristematic region within the embryo. At this point, the first evidences of bilateral symmetry appear. Embryo development can be blocked at this stage either by specific mutations (like the one analyzed here) or by placing the embryos in culture conditions that block polar transport of hormones (9). Recently, new types of transmembrane proteins have been described in Arabidopsis identified from mutants blocking processes such as meristem development or gravitropism that appear to be involved in the hormone efflux processes (10–13). Until now none of these genes has been related to embryogenesis.

In maize, embryo and endosperm mutants have been identified and characterized (14–19). Two main types of embryo mutants are recognized in maize: the defective kernel (*dek*) mutants, in which both embryo and endosperm are defective, and the embryo-specific (*emb*) mutants, in which development of the embryo is profoundly altered without disrupting the morphogenesis of the endosperm. The availability of these mutants, together with the collections of maize transposon-tagged stocks, greatly facilitates the study of embryogenesis in maize. Here we describe the characterization of a gene linked to a *dek* mutation in maize and encoding a new class of transmembrane proteins.

EXPERIMENTAL PROCEDURES

Mutant Lines, Growing Conditions, and Genetic Analysis—Mutant lines were produced by Dr. S. Dellaporta (Yale University) as previously described (20). Wild-type and mutant plants were grown in the greenhouse of the Departamento de Genética Molecular (CID-CSIC, Barcelona).

RNA Blot and Southern Blot Analysis—Tissue from different parts of wild-type (W64) and *lachrima* plants was harvested and immediately frozen in liquid nitrogen. RNA was isolated, denatured, and fraction-

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) X97570.

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§ Recipient of a Predoctoral Fellowship from CIRIT, Generalitat de Catalunya.

¶ To whom correspondence should be addressed. Tel.: 34 934006129; Fax: 34 932045904.

ated on a denaturing gel as described by Logemann *et al.* (21) and transferred to a nylon membrane (Nytran, Schleicher and Schuell) using 20× SSC as transfer buffer. Maize genomic DNA was isolated as described by Chen and Dellaporta (22), and transferred under alkaline conditions onto Nytran (Schleicher and Schuell) following the protocol of the manufacturer. Northern and Southern blots were fixed, hybridized, and washed as described by Church and Gilbert (23). Probes were labeled by the random priming method (Random Primed DNA Labeling Kit, Roche Molecular Biochemicals, Germany), following the protocols of the manufacturer.

Molecular Cloning and Sequencing—General recombinant DNA techniques were performed as described by Sambrook *et al.* (24). All the genomic and cDNA libraries were made according to the instructions of the manufacturer (Stratagene, La Jolla, CA). The partial *Eco*RI genomic library of *lachrima* mutant DNA was made in λZapII vector and screened with the central 0.9-kb[1] *Eco*RI/*Hin*dIII fragment of the Ac element. After partial Sau3A restriction of W64 genomic DNA, a genomic library was generated in λDASHII and screened with the DNA fragment flanking the Ac element. A cDNA library from 12 days after pollination (DAP) embryos was directionally constructed in the *Eco*RI 5′ to *Xho*I 3′ λZapII vector and screened with the coding region deduced from the genomic clone. The remaining sequence of the cDNA was obtained using the 5′-RACE technique (Life Technologies, Inc.). DNA sequencing was performed using automatic fluorescent sequencing (ALF, Amersham Pharmacia Biotech). Analysis of the predicted protein sequence of TM20 was carried out using the BLAST network server (25) and the GCG sequence analysis software package (Madison, Wisconsin). The hydropathy profile has been obtained using the DNAStar package (Lasergene Inc., Madison, WI) that follows the Kyte and Doolittle algorithm (29). A 19-amino acid window was used for the analysis.

In Situ Hybridization—Fixation of the embryos and kernels was done in ethanol:formaldehyde:acetic acid (80:3.5:5) for 1 h at room temperature. Once the fixative was removed, the samples could be stored in 70% ethanol at 4 °C indefinitely. Embedding, sectioning, and pretreatment of the tissues was performed as described by Langdale (26). Riboprobes, hybridization, washes, blocking, and antibody incubation and detection were done according to the instructions of the manufacturer (RNA Color Kit for nonradioactive *in situ* hybridization, Amersham Pharmacia Biotech). Digoxigenin-labeled hybrids were viewed using bright field microscopy and photographed using Kodak Ektachrome 160 film.

Scanning Electron Microscopy—Maize embryos and kernels were prepared for scanning electron microscopy analysis following the procedure of Irish and Sussex (27), with the following modifications. Fixation of the samples was performed as for *in situ* hybridization. After the dehydration series, the 100% ethanol solution was removed and replaced by isoamyl acetate, 100% ethanol, 1:2 (10 min); isoamyl acetate, 100% ethanol, 1:1 (10 min); isoamyl acetate, 100% ethanol, 2:1; and isoamyl acetate (3 × 10 min). Dehydrated material was critical point dried in liquid CO_2 and examined in a Hitachi S2300 scanning electron microscope from Serveis Científico-Tècnics (Universitat de Barcelona).

RESULTS

Isolation of a Maize dek Mutant Produced by Ac Transposition—Using the strategy of gene tagging by the *Activator* (Ac) transposon (20), a *dek* (*defective kernel*) mutation associated with an Ac transposition event was isolated. This *dek* mutation is a recessive embryo-lethal mutation. Segregation analysis of this mutant line shows a complete cosegregation between the mutant phenotype and a transposed *Ac* element as seen by Southern blot. Although several copies of sequences that hybridize to Ac probes are present in all maize lines, the *dek* phenotype segregates with a unique Ac element that is not present in the parental DNA line, appearing as a 3.2-kb *Eco*RI fragment in Southern blots (Fig. 1*I*, *panel A*). More than 100 plants were analyzed in this fashion, and Fig. 1*I*, *panel A*, is an example of the Southern blot obtained. The *dek* mutant kernels always possessed an Ac element in homozygosis, whereas heterozygote kernels had just one copy of this Ac element. The Ac

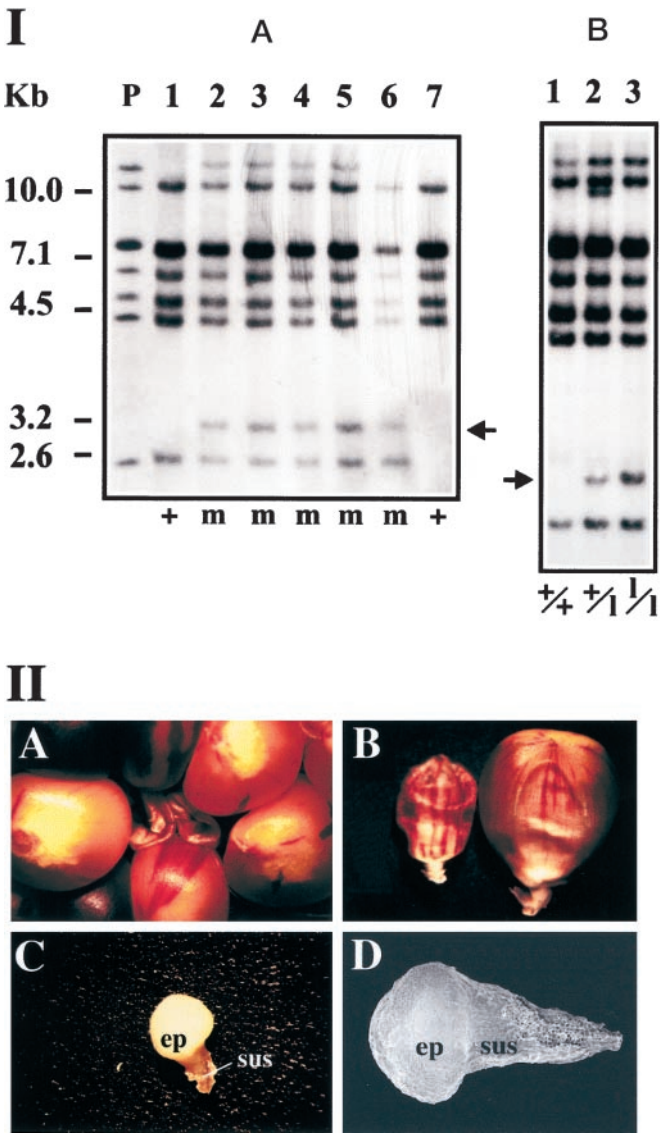


FIG. 1. **Segregation analysis of the *lachrima* mutant line and the transposed *Ac* element.** *I*, *panel A*, an example of Southern blot analysis of different genomic DNAs isolated from plants of segregating ears. The DNAs were digested with *Eco*RI and hybridized with a 0.9-kb internal fragment of Ac (34). *Lane P*, parental DNA; *lanes 1* and *7*, genomic DNA wild-type for *lachrima*; *lanes 2–6*, genomic DNA heterozygote for *lachrima*. *Arrow* points to the 3.2-kb *Eco*RI transposed Ac fragment. *I*, *panel B*, Southern blot analysis of wild-type kernels (*lane 1*), heterozygote kernels (*lane 2*), and mutant kernels (*lane 3*). The digestion and hybridization of the genomic DNAs is the same than in (*I*, *panel A*,). *II*, *panel A*, segregating ear showing the collapsed *lachrima* mutants; *II*, *panel B*, wild-type (*right*) and *lachrima* kernel (*left*) of a segregating ear; *II*, *panel C*, fresh dissection at kernel maturity of a *lachrima* mutant. *ep*, marks embryo proper; *sus*, suspensor. *II*, *panel D*, scanning electron microscopy of a *lachrima* embryo at kernel maturity. The diameter of the embryo proper is 1 mm.

was never found in homozygous wild-type sibling kernels (Fig. 1*I*, *panel B*). These results indicate that the 3.2-kb *Eco*RI fragment is associated with an Ac element linked to this *dek* mutation.

The *dek* mutant was named *lachrima* because of the teardrop shape of the embryo at seed maturity (Fig. 1*II*, *panel C*). Mutant kernels are first distinguishable because of their smaller size at about 10 DAP for our summer material and about 12 DAP for our winter greenhouse material (Fig. 1*II*, *panel A*). They remain retarded in kernel growth and in both their embryo and endosperm development until kernel maturity, when compared with normal embryos from the same ear.

[1] The abbreviations used are: kb, kilobase(s); RACE, rapid amplification of cDNA ends; DAP, days after pollination.

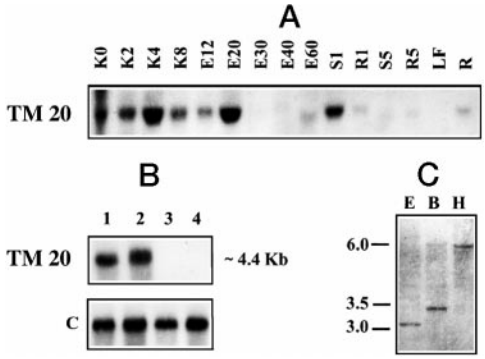


FIG. 2. **RNA and Southern blot analysis of the *TM20* gene.** *Panel A*, RNA blot analysis of *TM20* mRNA levels. RNA was isolated from different parts of wild-type plants and probed with *TM20* cDNA sequences that hybridized to a 4.4-kb band. *Lanes* are labeled as follows: 0-DAP kernel (*K0*); 2-DAP kernel (*K2*); 4-DAP kernel (*K4*); 8-DAP kernel (*K8*); 12-DAP embryo (*E12*); 20-DAP embryo (*E20*); 30-DAP embryo (*E30*); 40-DAP embryo (*E40*); 60-DAP embryo (*E60*); root, 1 day after germination (*DAG*) (*R1*); shoot, 1 DAG (*S1*); root, 5 DAG (*R5*); shoot, 5 DAG (S5); adult leaf (*LF*); adult root (*R*). *Panel B*, expression of *TM20* in 10-DAP wild-type kernels (*lane 1*), 10-DAP heterozygote kernels (*lane 2*), 10-DAP *lachrima* mutant kernels (*lane 3*), and 20-DAP mutant kernels (*lane 4*). The control (*panel C*) is an 18 S RNA probe. *Panel C*, Southern blot of wild-type DNA (W64) probed with a *TM20* cDNA fragment. Genomic DNAs were digested with *Eco*RI (*E*), *Bam*HI (*B*), and *Hin*dIII (*H*).

The mutant embryos have a symmetrical appearance, indicating that they have been uniformly blocked at the mid-transition stage of embryogenesis. After reaching this stage, the mutant embryos undergo little morphological change, slightly increasing in size while maintaining a uniform and healthy appearance until late in kernel development (Fig. 1*II*, *panels B* and *C*). No evidence of bilateral symmetry may be observed, but the basis of the embryo proper enlarges as well as the suspensor giving rise to the characteristic tear-drop phenotype. Full description of the developmental pattern of the mutation will be described elsewhere.

Molecular Cloning of TM20 Gene—A partial library was constructed from genomic DNA from a plant line heterozygous for *lachrima*. The library was screened using an Ac probe, and one clone was recovered that contained a single 3.2-kb *Eco*RI fragment which hybridized with the Ac probe and comigrated with the *Eco*RI fragment in *lachrima* genomic DNA (data not shown). The DNA sequence flanking the Ac insertion was sequenced and determined to be single copy by Southern blot. It was then used as a probe to screen for wild-type (W64A) genomic and cDNA clones. A phage containing the wild-type genomic DNA was isolated, and 10 kb of the insert were sequenced. Different fragments of the wild-type genomic clone were used as probes in RNA blots (not shown), and only a region adjacent to the transposon insertion site hybridized with embryo RNA. This region is the only one within the 10 kb sequenced having a long open-reading frame and with a GC content typical of maize coding regions. For the reasons described below, this gene was called *TM20*.

A cDNA library constructed from poly(A)$^+$ RNA obtained from immature maize embryos 12 days after pollination was also screened. Several cDNA clones were detected, and the one carrying the longest insert (1.7 kb) was analyzed. Southern blot analysis at high stringency using this cDNA as a probe showed that there was a single copy of the *TM20* sequence in the maize genome (Fig. 2, *panel C*). RNA blot analysis with the same cDNA probe shows that the cDNA hybridizes to a 4.4-kb band, indicating that the cDNA isolated was not the complete transcript (Fig. 2, *panel B*). The analysis of hybridization of the genomic sequence with the cDNA indicates that a 5-kb genomic fragment contains the entire coding sequence of the *TM20* gene. The information from the genomic sequence was used to clone the rest of the *TM20* cDNA. Two fragments that constituted the remainder of the *TM20* cDNA were obtained by using RACE. The complete cDNA is 4461 nucleotides long (see Fig. 4). This size is consistent with RNA blot analysis that shows an mRNA band of 4.4 kb.

Pattern of TM20 mRNA Accumulation—The expression of the *TM20* gene in wild-type kernels during development was analyzed. RNA blots containing RNA from wild-type maize embryos, kernels, seedling tissues, and adult tissues were hybridized with a probe covering the *TM20* coding sequence. On the blots, this probe detects a single band of about 4.4 kb. In the RNA blot presented in Fig. 2, *panel A*, *TM20* mRNA is shown to be present in all the tissues examined but to vary greatly in abundance in the different tissues examined. *TM20* mRNA is most abundant during early embryo development, whereas lower levels of mRNA accumulation are detectable in mid and late embryogenesis and in more adult tissues (Fig. 2, *panel A*). We also determined the expression of *TM20* gene in mutant and heterozygote *lachrima* kernels. *TM20* gene appears to be expressed at similar levels in both wild-type and heterozygotes, whereas no transcripts are detected in *lachrima* mutants (Fig. 2, *panel B*).

To define precisely the spatial and temporal pattern of *TM20* gene expression during embryo development, we localized the *TM20* mRNA by *in situ* hybridization to different embryo sections representing embryos from proembryo to stage 3 of development (Fig. 3*III*, *panels A–F*). In Fig. 3*I*, a schematic representation of the different stages and sections is shown. *TM20* gene is expressed very early in kernel development. Approximately 40 h after pollination, the zygote undergoes its first division, while the endosperm has around 20 free nuclei. At this stage, *TM20* transcripts are localized in the embryo cells, at the placental region of the kernel, and around the endosperm nuclei (Fig. 3*III*, *panel A*). About 4 DAP, wall formation begins in the endosperm, and the proembryo commonly has about a dozen cells. Subsequently, the *TM20* gene is still expressed in the placental region and in the embryo and peripheral endosperm cells (Fig. 3*III*, *panels B* and *C*). Later in embryo development, around 10 DAP, *TM20* gene expression begins to be restricted to the embryo. From 12 to 20 DAP, *TM20* RNA accumulation is detected in meristematic centers of the embryo (Fig. 3*III*, *panels D* and *E*). At the shoot region of the embryo axis, *TM20* RNA is localized in the leaf primordia and around the provascular cells of the coleoptile procambium (Fig. 3*III, panel D*). At the node region, *TM20* mRNA is also localized in the meristematic and provascular cells of the embryo axis. In the root primordia, *TM20* gene expression is detected in actively proliferating cells, which occur in the pericycle and in the provascular elements of the primordial root (Fig. 3*III, panel E*). In the scutellum, *TM20* RNA is detected in the provascular strands, and in the subepidermal layers, forming a short gradient toward the embryo axis. These results indicate that *TM20* gene expression is associated with tissues undergoing proliferation.

The TM20 Gene Encodes a Protein with 20 Hydrophobic Domains—The complete genomic and cDNA clones were sequenced (GenBank™/EBI accession number X97570, Fig. 4). Full agreement was found between the two sequences, indicating that there are no introns in the *TM20* sequence. The genomic sequence also matches the one obtained in the mutant strain which has the Ac transposon insertion 5′ to the starting point of the cDNA (see schematic drawing in Fig. 5, *panel A*). The cDNA and the deduced protein sequence are shown on Fig. 4. In the 3′ untranslated sequence, several putative poly-

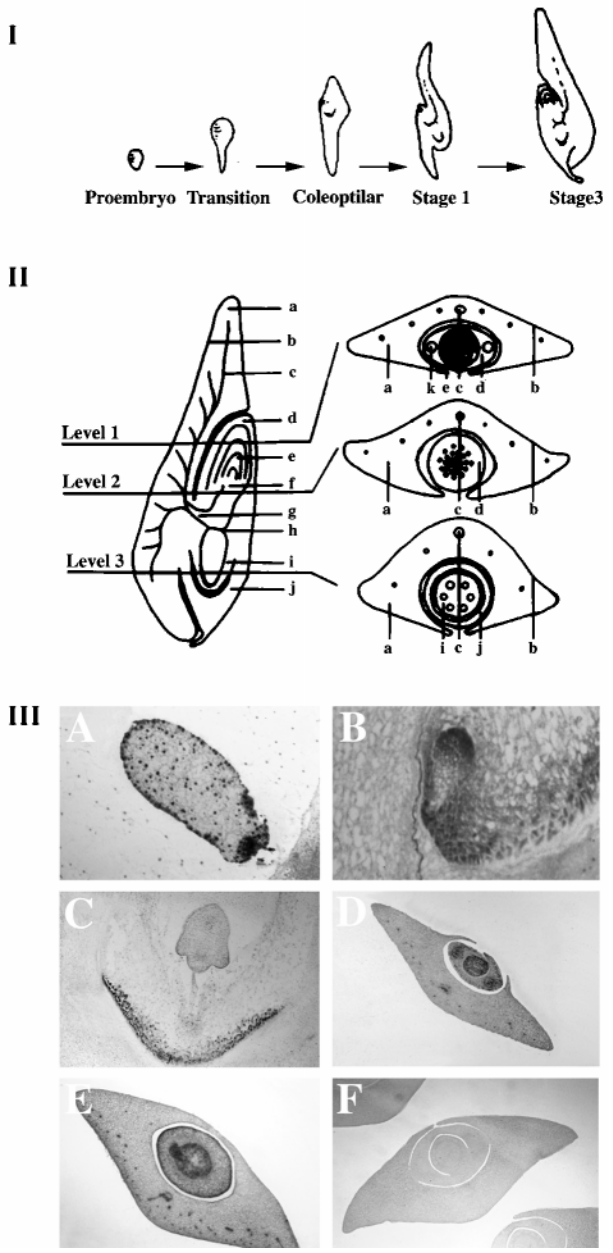


FIG. 3. *I*, schematic representation of the development of normal maize embryos from the proembryo to stage 3 according to Abbe and Stein (8). For each stage, the drawing shows a longitudinal radial section with the face of the embryo at *left*. *II*, schematic representation of a maize 20-DAP embryo. Longitudinal (*left*) and transverse (*right*) sections show the arrangement of organs and tissues at the shoot (1), node (2), and root (3) levels. *a*, scutellum; *b*, glandular layer of the scutellum; *c*, scutellar procambium; *d*, coleoptile; *e*, plumule; *f*, first internode; *g*, lateral seminal root; *h*, scutellar node; *i*, primary root; *j*, coleorhiza; *k*, coleoptilar procambium. *III*, *in situ* localization of *TM20* RNA in wild-type kernels. Sections were hybridized with a 3′ end riboprobe of the *TM20* cDNA. Longitudinal sections of a 4-DAP (*panel A*), 8-DAP (*panel B*), and 10-DAP kernel (*panel C*). Transversal sections of a 20-DAP embryo at the shoot (*panel D*) and root (*panel E*) level. A control with the sense probe (*panel F*) is also shown. *Scale bar* is 300 μm in (*panel A*) and 200 μm in (*panels B–F*).

adenylation signals were found, as is normally the case in plant genes. The single open reading frame in the sequence has 1389 amino acids, encoding a putative 177-kDa protein. Neither the DNA nor the protein sequences showed any significant similarity to others found in the EBI or GenBank™ data bases. Analysis of the deduced protein sequence indicates that it contains a number of hydrophobic domains (indicated in Fig. 4), most of them exhibiting the properties expected for membrane spanning segments (28). These domains are better seen in a hydropathy plot of the sequence (29) as shown in Fig. 5, *panel B*.

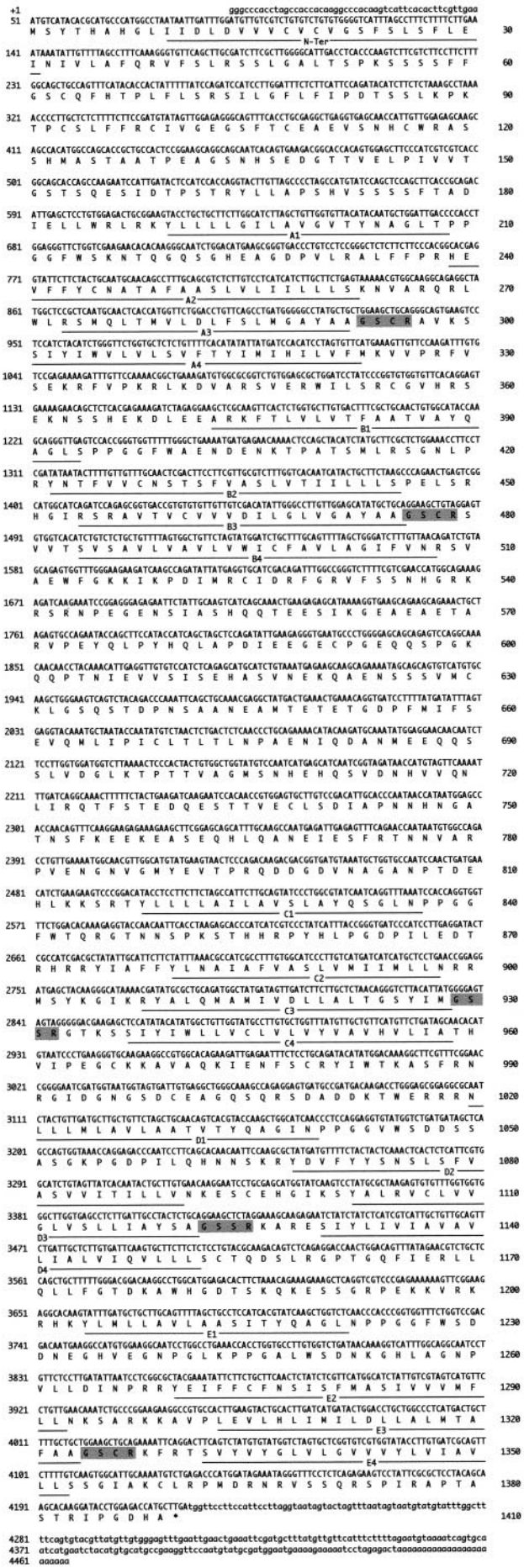
The twenty hydrophobic domains in TM20 protein sequence can be grouped in five homologous classes of four domains, and therefore they are termed A1 to A4, B1 to B4, etc. This structure can be observed in the hydropathy plot, and it is confirmed from the similarity existing between the different domains. Indeed, the protein sequences of domains A1, B1, C1, D1, and E1 can be aligned, and the same is true for most of the other sequences in the respective positions (not shown). This result may allow proposing that the basic unit of the protein is a four-domain transmembrane element that is tandemly repeated five times. A hydrophobic domain is also present in the N terminus of the protein as well as in the middle of a hydrophilic domain present in the central part of the protein. Five regions containing consensus sequences for phosphorylation by protein kinase C are found after each one of the hydrophobic domains placed in third position (see Fig. 4), and twelve putative *N*-glycosylation sequences are also present, distributed throughout the sequence. A possible model for the structure of the protein is shown in Fig. 5, *panel C*, where the putative transmembrane and hydrophilic domains and the glycosylation sites are shown.

DISCUSSION

Different mutations arresting embryo or kernel development at defined stages have been described (16, 19) in maize, but no molecular data are available about genes involved in these mutations. We report here the characterization of a gene coding for a new transmembrane protein, TM20, that is tightly linked to and its expression inhibited by a new defective kernel (*lachrima*) mutation that appears necessary for the passage through the transition stage of embryogenesis. The uniform blockage of the embryos at the proembryo/transition stage border of embryogenesis indicates that the function of the *lachrima* gene is required for processes leading to the acquisition of an asymmetric embryo and to the formation of the shoot apex and the coleoptilar primordium. The function of *lachrima* is also important in the formation of a normal endosperm because the mutation produces an altered phenotype in both embryo and endosperm development.

The mutation here analyzed is produced by the insertion of an Ac transposon in the maize genome, and this fact has allowed us to clone a gene coding for a new type of transmembrane protein named TM20. Although maize is an excellent system for genetics studies, only a limited number of embryo mutations are available and less so alleles of a given mutation. This fact does not allow us to conclude beyond the complete genetic linkage between the *lachrima* and *TM20*, although the mutation inhibits the expression of the *TM20* gene. This fact allows us in any case to conclude on the involvement of the TM20 protein in the developmental processes blocked by *lachrima*.

Higher plants develop with little change in cell shape and without cell migration. Therefore, the precise molecular mechanisms responsible for plant cell differentiation have to be coordinated with a precisely controlled pattern of cell division and depend on cell-cell communication in a position-dependent manner (30). The spatial and temporal pattern of *TM20* gene expression during embryo development is restricted to a population of cells active in cell division in the meristematic regions. *TM20* transcripts are detected from the very first cell divisions that occur in the endosperm and in the embryo. During the next embryo developmental stages, *TM20* expression remains associated with the newly appearing embryo struc-



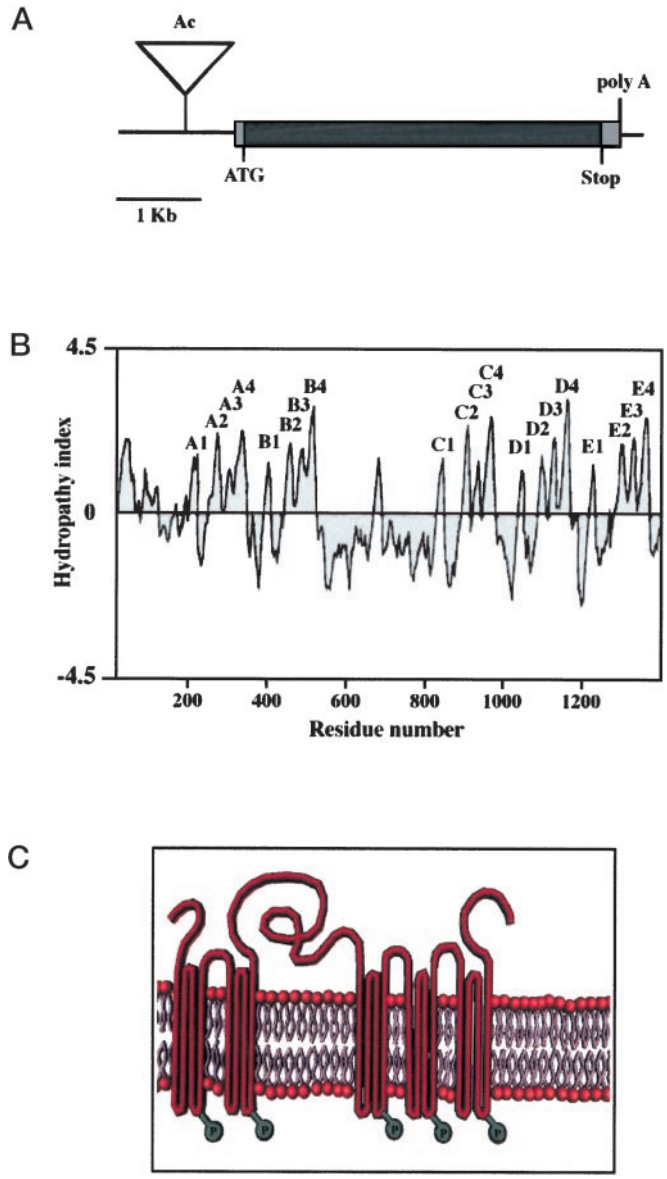


FIG. 5. **Structure of the *TM20* gene and hydrophobicity plot of the TM20 protein.** *Panel A*, schematic representation of the structure of the *TM20* gene. The location of the *Ac* transposon insertion is shown as well as the coding and noncoding sequences. No introns have been found from the comparison of genomic and cDNA sequences. *Panel B*, hydropathy plot of the TM20 sequence using standard algorithms and a 19-amino acid window. Average value is indicated by a *horizontal line*. *Panel C*, hypothetical model of the TM20 protein and its relation to the membrane.

tures. Later in embryo development, during mid and late embryogenesis, the level of *TM20* mRNA accumulation decreases considerably. Taken together, these results suggest that *TM20* may be involved in the process of differentiation during early embryo development.

Judging from the lack of similarity to known sequences, TM20 does not belong to any of the previously described classes of proteins. However, from the deduced protein sequence, 20 hydrophobic segments having the properties for transmembrane domains can be found, indicating that TM20 protein may be a complex transmembrane protein. Besides these 20 seg-

Fig. 4. **Sequence of the *TM20* cDNA and predicted amino acid sequence.** The hydrophobic domains present in the sequence are *underlined*, and the predicted phosphorylation sequences present in between hydrophobic domains are *boxed*.

ments, a hydrophobic sequence is present at the N terminus and is a possible candidate for a targeting sequence or signal peptide. A central hydrophobic domain is also found within a 310-residues-long region, having in average a hydrophilic character, features that could allow the interaction of the protein with extracellular components. The other 20 hydrophobic domains can be grouped in five groups formed by four putative transmembrane elements. The segments in an equivalent position in every group can be aligned, indicating a similar function for the five repeated groups. At the end of the third element, a sequence having the consensus for a residue phosphorylated by protein kinase C is found. Multiple (12) potential glycosylation sites are found along the sequence, indicating that the mature protein may be heavily modified. This type of protein may have relatively low sequence requirements with the exception of the central region. This fact may explain the lack of homologous sequences found using normal homology programs when searching through the data bases. A model of the TM20 protein may be proposed as shown in Fig. 5, *panel C*.

Different proteins with multiple transmembrane domains have been described in different systems, and they frequently function as channels or transporters across the cell membrane (31). A family of proteins having four transmembrane segments, the connexins, have been described, and they allow the formation of gap junctions in animal cells (32). A protein having similarity to animal connexins has been described in Arabidopsis (33). However, TM20 lacks an important feature of these proteins: a number of cysteine residues important for the establishment of the junction from one cell to another. It has also been shown that when placing wheat immature embryos in culture in the presence of either an excess of auxin or auxin transport inhibitors, the embryo is blocked at the same stage of development as the *lachrima* mutation blocks embryogenesis (9). These results indicate that an appropriate flux of hormones has to be established in the embryo to proceed from the transition stage and to allow the definition of bilateral symmetry. Recently, a number of new transmembrane proteins have been cloned and have been related to auxin transport. They are AGR, EIR1, and AtPIN2 from Arabidopsis, genes that are involved in gravitropism (11–13), and AtPIN2, which has been proposed to be a component of the auxin efflux (10). TM20 shares with these proteins the presence of a central hydrophilic domain and a number (five in Arabidopsis) of transmembrane domains before and after it. No similarity of sequence can be found between these proteins and TM20 and between rice ESTs similar to the Arabidopsis proteins and TM20, but altogether they seem to form a new class of transmembrane proteins. Whether they all form a new large family of hormone transporters in plants is a question that the availability of the *TM20* gene may allow to be addressed.

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