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Gene Expression in Developing *Zea mays* Embryos: Regulation by Abscisic Acid of a Highly Phosphorylated 23- to 25-kD Group of Proteins[1]

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ABSTRACT

We have earlier identified a set of proteins of 23 to 25 kilodaltons (kD), covering an isoelectric point (pI) range of 6.2 to 8.2, which accumulate gradually during normal embryogenesis of *Zea mays* and disappear in early germination. These polypeptides can be induced prematurely in immature embryos by abscisic acid (ABA) treatment. We report here that the more acidic protein forms are due to post-translational phosphorylation of at least two polypeptides of 23 kD, pI 8.2 and 25 kD, pI 8.0. A polyclonal antiserum was obtained which recognizes all forms of both the 23-kD and 25-kD polypeptides. Recovery of cDNA clones corresponding to these proteins was accomplished by hybridization with cDNA made from size-selected mRNA enriched for these sequences. Hybrid selection experiments demonstrate that clone MA12 specifically hybridizes with mRNAs encoding the 23-kD and 25-kD protein set which are recognized by the antiserum. By Northern hybridization analysis, the RNA encoded by clone MA12 is shown to accumulate in mature embryos and to be induced in young embryos upon ABA incubation.

The analysis of proteins synthesized in developing embryos from different plant species shows that it is possible to distinguish groups of polypeptides specific to the different developmental stages. One of these groups included polypeptides that become abundant during the maturation period of cotton (7), wheat (19), or maize (21) embryos, and rapidly disappear during early germination. The synthesis of some of these polypeptides can be induced prematurely in excised young embryos when incubated in the presence of the plant growth regulator ABA.

ABA inhibits germination in isolated embryos of many species. Immature embryos kept in culture in the presence of ABA continue to undergo elaborate tissue and molecular differentiation characteristic of later stages of embryonic development (19). In the absence of ABA, immature embryos can bypass the maturation process and embryos in culture precociously germinate. However, seedlings produced by precocious germination of immature embryos may be abnormal (4).

The endogenous level of ABA in embryos of monocots has been found to increase during development, from the cell division phase to the maturation stage (25). This increase is thought to be correlated with prevention of the viviparous germination of the embryo, since ABA deficient or insensitive mutants, in maize or *Arabidopsis*, precociously germinate (13, 20).

Complementary DNA (cDNA) clones have been obtained for some ABA-inducible genes and their regulation at the mRNA level has been studied in embryos of cotton (9), wheat, (26) rapeseed (5), and soybean (2); some of them have been sequenced (14). Unfortunately, a clear identification of similarly induced genes or products in any of these species has not yet emerged. It is striking that many of the ABA-enhanced mRNAs encode storage proteins (2, 5), while in cotton none of the storage protein mRNAs appears to be regulated by ABA (9).

All of these data suggest that ABA may elicit an alteration in the expression of particular genes which initiates a complex sequence of changes in the pattern of mRNA accumulation and which leads to a number of physiological and biochemical events that precede the dormancy period. Other agents, such as high osmotic potential of the media, mimic some of the ABA induction effects (6) and make it difficult to ascertain the primary role of the hormone.

We have previously described (21) a set of specific polypeptides (23–25 kD) which, by means of two-dimensional electrophoretic analysis from *in vivo* labeled or *in vitro* translated products, were rapidly induced by ABA in young embryos of maize upon hormone treatment. These polypeptides also appear during normal embryogenesis when the development of the embryo is progressing to the maturation stage, coinciding with the period where the endogenous level of ABA attains the maximum peak (12). After accumulating in mature embryos they disappear during the first hours of germination.

While all these polypeptides are detected in *in vivo* experiments by incubating excised embryos in the presence of the amino acid precursor [^{35}S]methionine, only five of them are detected in the corresponding *in vitro* experiments. Here we report that those proteins which are not detectable in the *in vitro* polypeptide pattern correspond to phosphorylated modifications of at least two major proteins of 23 kD, pI[3] 8.2 and 25 kD, pI 8.0. These proteins and their phosphorylated forms are recognized by an antiserum by means of immunoprecipitation of the *in vitro* translation products of poly(A)$^+$ RNA of mature embryos and

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[3] Abbreviations: pI, isoelectric point; mAP, messenger affinity paper.

immunoblot of total *in vivo* synthesized proteins. The isolation and preliminary characterization of cDNA clones complementary to these 23- to 25-kD protein mRNAs, and the regulation of these mRNAs in young embryos by ABA, are also presented.

MATERIALS AND METHODS

Plant Material. Plants of the *Zea mays* L. inbred line W64A were used. Immature seeds were used immediately after collection and both mature and immature embryos were manually dissected. They are referred in the text as E60 for the mature embryo, as E20 for the immature embryo, and as End 20 for endosperm 20 and 60 referring to days after pollination.

Antigen Purification. Proteins from mature embryos (E60) were extracted and subjected to SDS gel electrophoresis as previously described (22). Protein bands were visualized by immersing the gel in 4 M sodium acetate and the 23-kD band was excised. Bands excised from several gels were pooled and protein was eluted by electrodialysis in SDS-gel running buffer. Purity and absence of degradation in the eluted polypeptides was assessed prior to immunization by electrophoresis in SDS gels and staining with Coomassie brillant blue and silver nitrate.

Immunization and Analysis of Antibody Specificity. New Zealand White rabbits were immunized according to the following schedule: intradermal injections of 1.0 ml containing 100 to 200 μg of the 23-kD proteins in Freund's complete adjuvant, at multiple sites on the back of the animals. Second and third injections with similar amounts of protein but in incomplete Freund's adjuvant followed at monthly intervals. Three weeks after the last set of injections, blood was drawn from an ear vein, and serum collected and stored at −20°C.

To analyze antibody specificity, samples containing total E60 protein extract and the 23-kD protein used as immunogen were subjected to both one dimensional SDS/polyacrylamide and two-dimensional electrophoresis (21, 22), transferred electrophoretically onto nitrocellulose paper (15) and processed for the detection of immune complexes using peroxidase-conjugated anti-rabbit IgG and 4-chloro-1-naphtol as substrate. Immune serum was used at a 1/200 dilution.

***In Vivo* Protein Labeling and Protein Extraction.** Mature embryos were *in vivo* labeled with (500 μCi/ml) L-[^{35}S]methionine (1000–1500 Ci/mmol, Amersham) for 2 h and proteins extracted both as previously described (21).

In the samples subjected to alkaline phosphatase treatment, the *in vivo* labeled E60 proteins were extracted with 50 mM Tris-HCl (pH 8.0). Alkaline phosphatase from calf intestine (Boehringer) was added to the solubilized proteins at 460 units/ml with incubation for 30 min a 37°C. The reaction was terminated by addition of TCA to a final concentration of 15%. For double labeling experiments, embryos were incubated simultaneously with L-[^{35}S]methionine and [^{32}P]phosphate (100 μCi/1 ml) and processed as described except that exposure of dried gels was done using two films, one in close contact with the gel and the other separated by an opaque layer in order to be exposed only by the more energetic β-emission of ^{32}P.

cDNA Synthesis, Transformation, and Differential cDNA Screening. Poly(A)$^+$ RNA obtained from E60 embryos was purified by two succesive passages through oligo (dT)-cellulose chromatography (1). Double-stranded cDNA was prepared (11, 16) and inserted into the *PstI* site of pBR322 by dC/dG tailing (24). Transformation was performed with competent *Escherichia coli* HB101 cells (18). Replicate nitrocellulose filters of the library of recombinant clones were incubated with ^{32}P-labeled single-stranded cDNA (10^6 cpm/ml) synthesized from poly(A)$^+$ RNA of both E60 and E20 embryos. Following hybridization for 16 h in a buffer containing 5 × SSC (1 × SSC = 0.15 M NaCl-0.015 M Na-citrate) and 5 × Denhard's solution, (0.5 g Ficoll, 0.5 g PVP, 0.5 g BSA, H_2O to 50 mL) filters were washed in 0.5 × SSC at 65°C, dried, and autoradiographed. Colonies showing greater hybridization with cDNA from E60 embryos were saved.

Analysis of RNA and Isolation of Poly(A)$^+$ RNA Enriched for those Encoding the 23- to 25-kD Polypeptides. RNA and poly(A)$^+$ RNA were isolated as previously described (21). Total RNA was obtained from manually dissected embryos at different stages of development (E15, E20, E60, and 24 h or germination) and from young embryos incubated in the presence of 1 μM ABA for 21 h (E20 + ABA). For Northern blot analysis, 20 μg of RNA went seperated by electrophoresis in 1.5% agarose and 2.2 M formaldehyde gels and transferred to nitrocellulose membranes. Immobilized RNA was hybridized with ^{32}P-labeled probes for 24 h at 65°C in 25 mM sodium phosphate buffer, pH 7.5, 3 × SSC, 3× Denhart solution, and 0.1% SDS (17). The filters were washed at 65°C in 0.1 × SSC and 0.1% SDS, dried, and exposed at −80°C to MAFE RPA film with intensifying screen. To obtain poly(A)$^+$ RNA enriched in those encoding the 23- to 25-kD proteins, poly(A)$^+$ RNA (12.5 μg) from E60 was electrophoresed in a 1.25% agarose gel and blotted onto mAP (27). Size markers were run in parallel and the mAP blot was cut into strips containing mRNAs of sizes predicted to code for 23- to 25-kD polypeptides. The RNA was eluted from each mAP strip, following the procedure described by the manufacturer (Orgenics) and translated *in vitro* using rabbit reticulocyte lysate. cDNA was prepared from a fraction having high template activity for the 23- to 25-kD polypeptides. It was hybridized to the pool of selected cDNA-containing colonies and those showing high signals were again saved.

***In Vitro* Protein Synthesis, Immunoprecipitation, and Hybrid Selection.** Rabbit reticulocyte lysate (Amersham) was used to translate *in vitro* the poly(A)$^+$ RNA from E60 embryos as described (21). Immunoprecipitation of the ^{35}S-labeled polypeptides was done immediately after a 30 min translation period, using the antiserum raised against the 23-kD embryo protein (23). The fraction bound to protein-A Sepharose was eluted with SDS gel electrophoresis buffer.

To identify polypeptides encoded by cDNA insert of pMA12 complementary mRNA, 20 μg of *Eco*RI-cleaved plasmid DNA was fixed to a nitrocellulose filter following denaturation by heat at 70°C for 10 min poly(A)$^+$ RNA from mature embryos (40 μg) was hybridized at 50°C for 4 h with the filter in a buffer containing 65% formamide, 10 mM Pipes, and 0.4 M NaCl (18). The hybridized RNA was eluted, translated *in vitro,* and the products resolved by one- or two-dimensional electrophoresis.

RESULTS

Pattern of 23- to 25-kD Protein Phosphorylation. The two-dimensional electrophoretic analysis of proteins from mature *Z. mays* embryos (E60), synthesized *in vivo* during a 2 h incubation period with [^{35}S]methionine as protein precursor, shows the existence of a group of proteins of 23 to 25 kD with a pH range from 6 to 8.2 which have been described earlier (21). This group of polypeptides appears as highly radiolabeled spots when compared to the incorporation of other polypeptides (Fig. 1B). The more basic of the newly synthesized proteins of these groups are not accumulated in sufficient amounts to be detected by Commassie blue staining of the gel (Fig. 1A).

A diagram is shown in Figure 1F comparing the two-dimensional pattern of the 23- to 25-kD set of proteins detected following *in vitro* translation of mature embryos poly(A)$^+$ RNA (see Fig. 5) with those detected by *in vivo* labeling (21). In both systems the main radioactive spots corresponding to the more basic polypeptides (numbered 1, 2, 3, 4, and 5, Fig. 1F) are equally detected. However, the more acidic proteins (unnumbered open spots in Fig. 1F) are not synthesized in the *in vitro* translation. We hypothesized that a post-translational phosphorylation may be responsible for the differential occurrence of

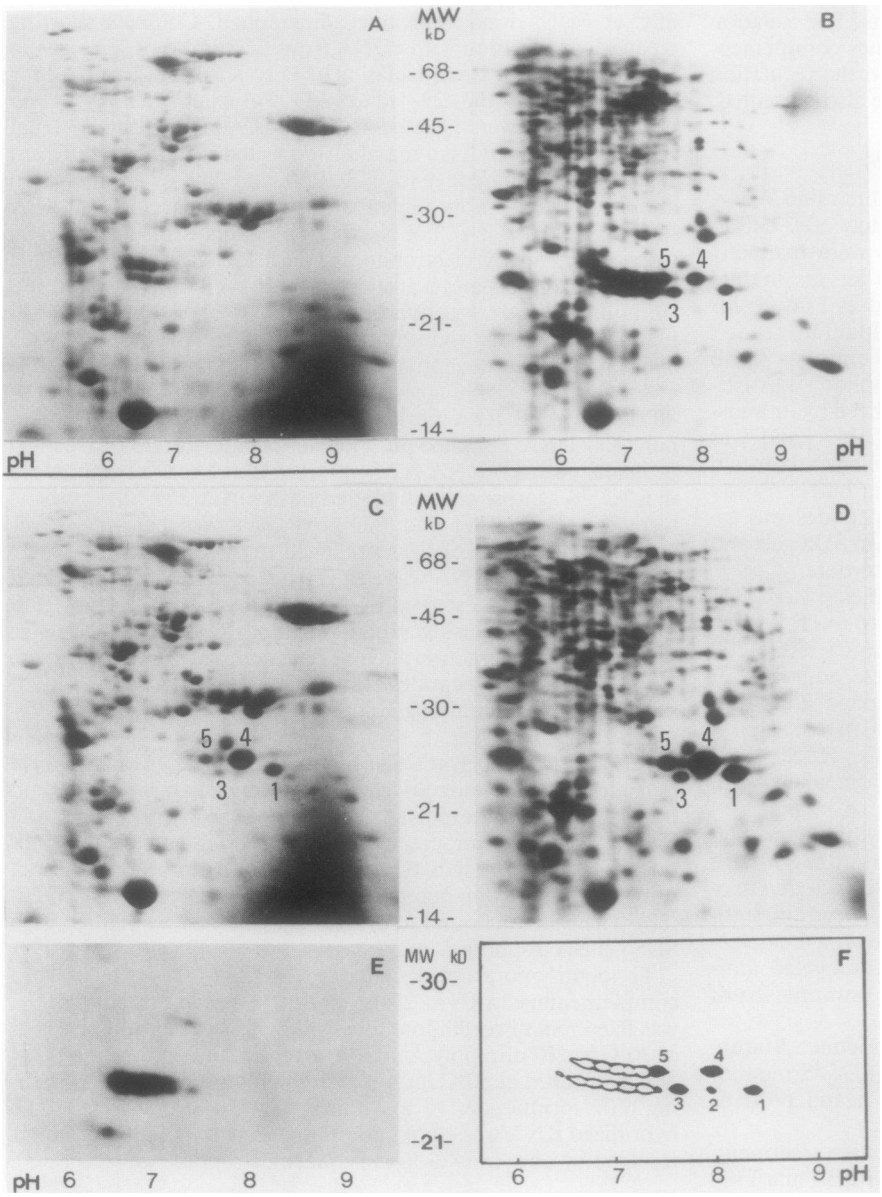

FIG. 1. Phosphorylation of the 23- to 25-kD proteins. Mature E60 embryos were incubated with [^{35}S]methionine (A–D) or [^{35}S]methionine and [^{32}P] phosphate (E) and analyzed by two-dimensional gel elcetrophoresis. A, Coomassie brillant blue-stained gel, and B, its corresponding fluorograph of ^{35}S-labeled proteins. Total protein extracts were subjected to protein dephosphorylation with alkaline phosphatase treatment and then electrophoresed. C, Coomassie stained pattern and D, fluorograph of the same gel; E, detail of the autoradiograph of ^{32}P-labeled proteins, labeled *in vivo* showing the incorporation of ^{32}P into the more acidic polypeptides in the 23- to 25-kD set; F, diagram of the 23- to 25-kD set: open unnumbered spots represent the acidic phosphorylated polypeptides which are visible with Coomassie staining of total E60 protein content (A) and synthesized *in vivo* (B and E). Numbered closed spots indicate the polypeptides of the 23- to 25-kD set that are detected only in the *in vitro* translation products (compare with Fig. 5 and 6) of E60 poly(A)$^+$ RNA and upon alkaline phosphatase treatment (C and D). They are rapidly synthesized *in vivo* (B) but do not accumulate (A).

these polypeptides in the *in vivo* and *in vitro* systems. To test this possibility, total protein extracts of E60 were subjected to treatment with alkaline phosphatase before two-dimensional electrophoresis. Both the stained gel (Fig. 1C) and its fluorograph (Fig. 1D) show a shift in the distribution of the 23- to 25-kD set of polypeptides toward the basic end of the gel, with simultaneous dissapearence of the more acidic polypeptides and a significant increase in amount of basic polypeptides. This indicates that alkaline phosphatase treatment has effectively removed phosphate groups from proteins with pI ranging from 6.3 to 7.2 (unnumbered in Fig. 1F). These are probably derived from the two principal basic proteins (numbers 1 and 4, Fig. 1F) of M_r 23 kD, pI 8.2. and 25 kD, pI 8.0.

More evidence for the existence in mature embryos of phosphoproteins in the 23- to 25-kD range was obtained from double labeling experiments. The two-dimensional electrophoretic pattern of proteins isolated after incubation of E60 embryos both with [^{35}S]methionine and [^{32}P]phosphate has allowed us to identify the proteins phosphorylated *in vivo* (Fig. 1E) as being the same phosphorylated proteins (unnumbered, Fig. 1F) which disappear after the alkaline phosphatase treatment.

Immunological Analysis of 23- to 25-kD Proteins in Embryos. One-dimensional gel electrophoresis of the total protein extracts from immature E20 and mature E60 embryos from *Z. mays* indicates, in Coomassie stained polyacrylamide gels, the presence of a broad protein band of about 23 kD in E60 and its absence in the immature embryo (Fig. 2A). The accumulation of significant amounts of this band and the absence of other nearby proteins facilitated its purification by electrophoresis and electroelution. Purity of the eluted protein was confirmed by SDS-gel electrophoresis and silver staining (Fig. 2B). It probably contains both the 23-kD to 25-kD sets observed in two dimensional electrophoresis (see below).

Following rabbit immunization, the specificity of the antiserum was determined by immunoblotting of total E20, E60, and endosperm (End 20) protein extracts, and the immunogen (Fig. 2C). There is a clear affinity of the polyclonal antibody for the 23-kD band of the mature embryo, whereas no reaction could be detected with the proteins extracted from immature embryos or endosperm. A mild but persistent cross-reactivity toward 45-kD and 21-kD polypeptide bands in the total E60

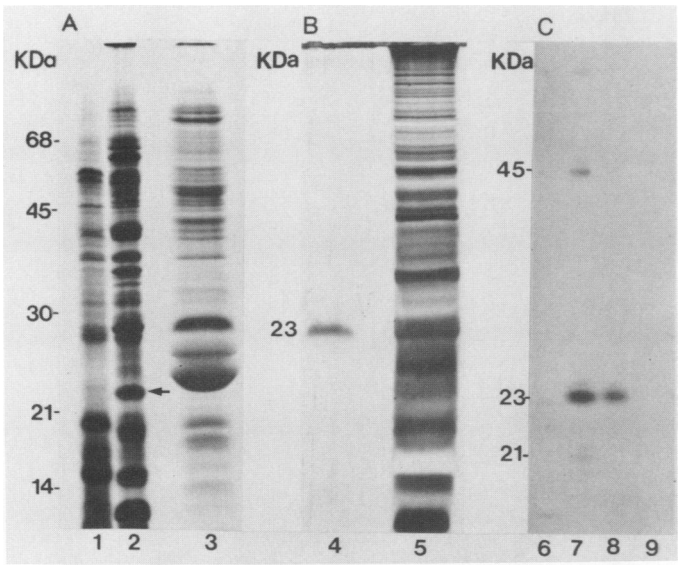

FIG. 2. Accumulation and purification of the 23-kD proteins and antibody specificity. A, Comparison of SDS electrophoretic patterns of total protein extracts from immature embryos (E20, lane 1) mature embryos (E60, lane 2) and endosperm (End 20, lane 3). The accumulation of the 23-kD band (arrow) exclusively in E60 embryos is clearly shown by Coomassie blue staining. B, Silver staining of the reelectrophoresis of the purified 23 kD band (lane 4) compared with the total E60 pattern (lane 5). C, Analysis of antiserum specificity by immunoblotting. Immune complexes were detected with peroxidase-conjugated anti-rabbit immunoglobulins and 4-chloro-1-naphthol as substrate. No reaction could be detected with the proteins of E20 (lane 6) or endosperm (lane 9), whereas specific recognition of the 23-kD band both in the purified form (lane 8) or in total E60 extracts (lane 7) was evident. Mild cross-reactivity toward 45-kD and 21-kD bands in E60 proteins is also seen.

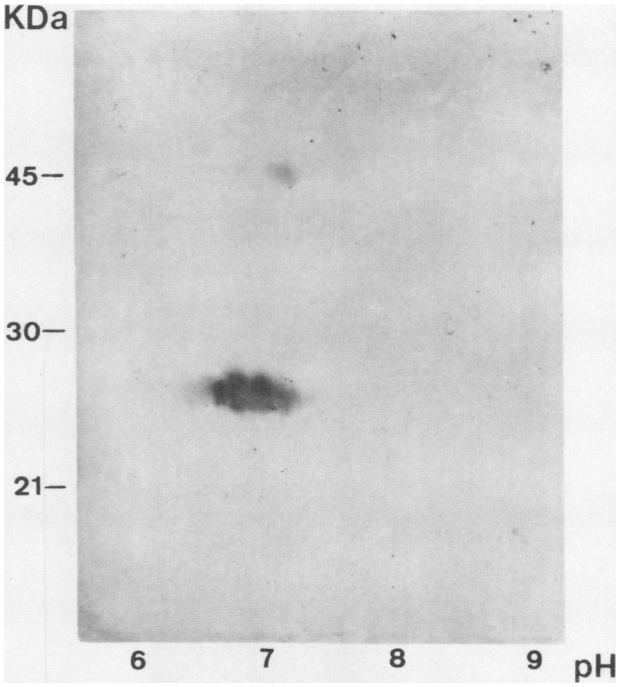

FIG. 3. Immunoblot of E60 total protein extracts resolved by two-dimensional electrophoresis. The antiserum recognizes both the 23-kD and 25-kD phosphorylated set of polypeptides localized in the acidic pI range which are not resolved by one-dimensional electrophoresis (Fig. 2, lane 7).

extract was also found and its significance will be discussed below.

The antiserum was further characterized by immunoblotting of total protein extracts from E60, E20, and endosperm resolved by two-dimensional electrophoresis. No positive reaction could be detected in E20 or endosperm immunoblots (data not shown), confirming the specificity of the antiserum obtained. In the E60 two-dimensional immunoblots (Fig. 3), the immunoreactive proteins can be easily identified by their pI and M_r as the same phosphorylated forms of both 23 and 25 kD, which are detected in Coomassie stained gels (Fig. 1A). Furthermore, the IgG fraction specifically immunoprecipitates polypeptides 1, 3, 4, and 5 of the 23- to 25-kD sets from *in vitro* translation products of mature embryo RNA (see Fig. 6).

Isolation of cDNA Clones for the 23- to 25-kD Polypeptides. A cDNA library from mature embryos (E60) was plated on filters in duplicate and each replicate was hybridized with radioactive single-stranded cDNA made from young embryo (E20) or mature embryo (E60) poly(A)$^+$ RNA. Forty clones were detected which had mRNA complement sequences abundant in mature embryo poly(A)$^+$ RNA and not abundant in the poly(A)$^+$ RNA of young embryos. In order to identify which colonies had cDNA inserts encoding the 23- to 25-kD polypeptides, a cDNA was made from mRNAs enriched for those 23- to 25-kD polypeptides. Poly(A)$^+$ RNA from E60 embryos was first electrophoresed and transferred to mAP filter which was cut according to RNA size, as described in "Materials and Methods." RNA was eluted from four consecutive fractions, in which the RNA length diminishes stepwise in 150 nucleotides per fraction, translated *in vitro* and products analyzed by one-dimensional electrophoresis (Fig. 4). Only the RNA eluted from the paper strips containing RNAs of 700 to 1000 nucleotide lengths (lanes 2 and 3), effectively codes for the 23- to 25-kD polypeptides. A cDNA probe was made from the 850–1000 nucleotide fraction and hybridized to the 40 colonies which displayed differential expression in embryos. Six positive clones were obtained. These clones are included in a larger set (10) detected as ABA inducible when young embryos are treated with the hormone (J Gomez, M Pages, unpublished observations).

To identify whether these six clones contained the same or very similar sequence, the cDNA inserts contained in the selected plasmids were cross-hybridized, clearly indicating differences in sequence homology (results not shown). From the clones with the indentical hybridization behavior the plamid MA12 containing the longest insert (670 nucleotides) was chosen as representative for additional study.

Hybrid Selection and Northern Analysis. To determine whether clone MA12 coded for the 23- to 25-kD proteins E60 mRNAs hybridizing to MA12 were obtained through the hybrid-selection method and were translated *in vitro*. The products were subjected to one (not shown) and two-dimensional electrophoresis (Fig. 5A) clone MA12 recognized mRNAs encoding polypeptides 1, 3, 4, and 5. These results are compared with those obtained using total E60 poly(A)$^+$ RNA in which are displayed the entire products (Fig. 5B), or those immunoprecipitated using the serum raised against the 23-kD eluted polypeptides (Fig. 6). The isoelectric focusing:SDS electrophoretic pattern in both experiments (Figs. 5 and 6) demonstrates that the same polypeptides are identified using two different probes (the specific clone and the antibody).

The relative abundance of pMA12-complementary mRNA was examined at different developmental stages and in young embryos treated with ABA. Insert purified from pMA12 was labeled by nick-translation and hybridized to electrophoretically separated total RNA isolated from young embryo (E20), mature

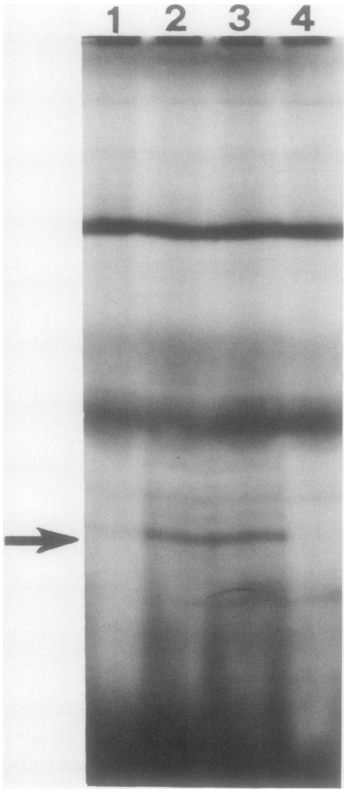

FIG. 4. Identification of the eluted mRNA fraction encoding the 23- to 25-kD proteins. Poly(A)$^+$ RNA eluted from mAP strips was translated *in vitro* in the rabbit reticulocyte system and products resolved by SDS electrophoresis. The nucleotide sizes of each mRNA fraction were: 1000–1150 (lane 1), 850–1000 (lane 2), 700–850 (lane 3), and 550–700 (lane 4). Arrow indicates the 23-kD protein band.

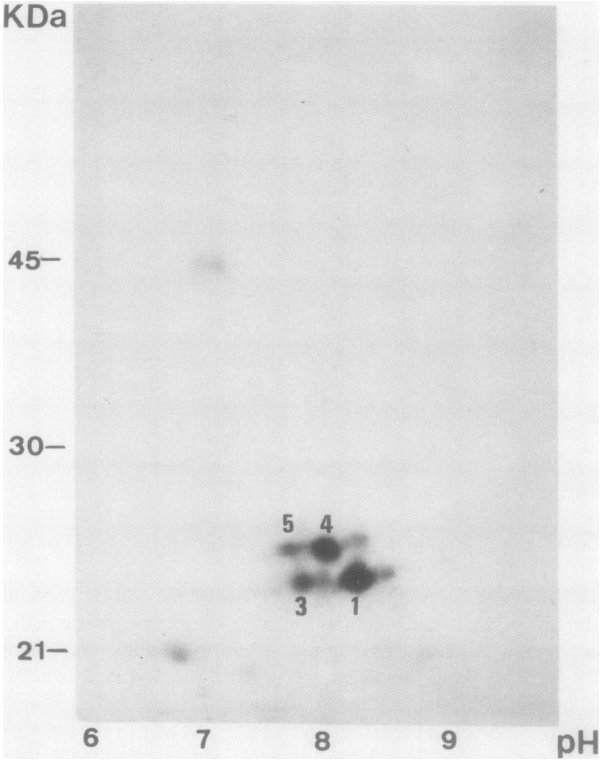

FIG. 6. Two-dimensional electrophoretic resolution of the translation products of the poly(A)$^+$ RNA from mature embryos immunoprecipitated with the rabbit antiserum raised against the 23 kD proteins. The main immunoprecipitation products, located in the 23- to 25-kD range, correspond to proteins n° 1, 3, 4, and 5 (see Fig. 1F), Two other minor polypeptides of 45 kD and 21 kD are also identified by the antiserum.

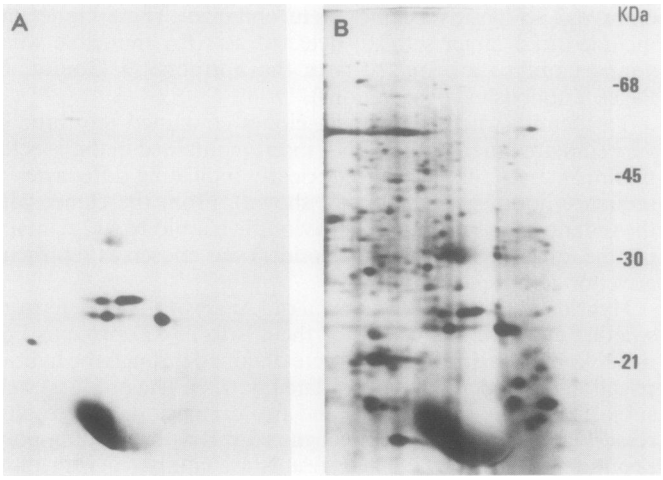

FIG. 5. Hybrid-selected translation of proteins encoded by pMA12-complementary mRNA sequences. A, Mature embryo poly(A)$^+$ RNA was hybridized with excess *Eco*RI-cleaved plasmid pMA12, eluted, translated *in vitro,* and the products resolved in two-dimensional electrophoretic gels; B, products with total poly(A)$^+$ RNA from mature embryos.

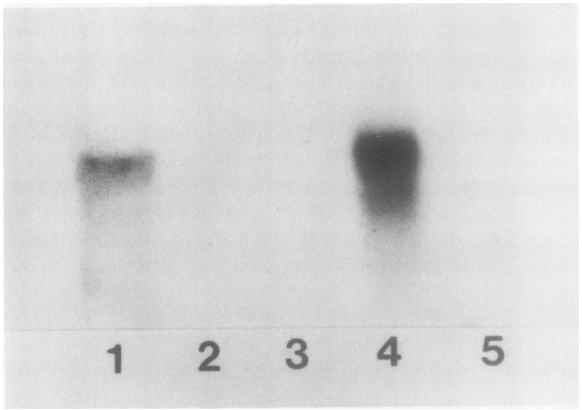

FIG. 7. Developmental regulation of pMA12-complementary mRNA in total RNA, and precocious increase in young embryos cultured in ABA. ^{32}P-labeled pMA12 insert was hybridized with total RNA from excised young embryos (E20) incubated with ABA (lane 1, young embryos (E15, lane 2, E20, lane 3), mature embryos (E60, lane 4), and 48 h germinated seedling (lane 5).

embryo (E60), young embryos incubated for 21 h in ABA (E20 + ABA), and embryos germinated for 2 d (Fig. 7). The size of the hybridizing mRNA, calculated relative to internal rRNA standards in this particular gel system, was about 900 nucleotides. pMA12-complementary mRNA is precociously induced in excised young embryos when cultured in the presence of the hormone, it reaches the highest concentration in mature embryos, and it is not present at detectable levels in young embryos or embryos after 48 h or germination. Neither the polypeptides nor the mRNA (not shown) are induced in young embryos cultured in the absence of the hormone. These results indicate a clear correlation between the relative abundances of mRNA and the abundance of the 23- to 25-kD polypeptide group previously detected in double dimensional gel patterns (21).

DISCUSSION

Here we report the existence in mature maize embryos of phosphoproteins, originating from at least two main proteins of 23 kD (pI 8.2) and 25 kD (pI 8.0). In a previous work (21) these proteins were identified as part of a specific set of polypeptides appearing during normal embryogenesis at early maturation stages, which can be induced prematurely in young embryos by incubation with ABA. The presence of four spots (numbers 1, 3, 4, and 5) in two-dimensional gel separations of proteins after phosphatase treatment (Fig. 1 C and D), is probably not due to a partial phosphatase digestion as they are not ^{32}P-labeled (Fig. 1E) and the same four spots are present in the *in vitro* translation products (Fig. 5). Work is in progress to assess whether they are four independent translation products or represent posttranslational modifications of two polypeptides occurring both *in vivo* and *in vitro*.

The approach undertaken to further characterize this set of 23- to 25-kD ABA induced proteins, was on the one hand the isolation and preliminary characterization of cDNA clones and on the other hand the characterization of a polyclonal antiserum against the purified 23- to 25-kD polypeptides which accumulate in the mature embryo.

The antiserum raised against the 23- to 25-kD proteins did not cross-react with total protein extracts from young embryos or from endosperm, indicating that the 23- to 25-kD proteins and related antigenic forms are not present in the immature embryo nor are they zein-related polypeptides. There is a small but reproducible cross-reactivity toward a 45-kD and a 21-kD band. The recognition of a 45-kD protein by the antiserum is possibly due to protein aggregation during the electrophoretic procedure since a band of the same mol wt can also be detected by Coomassie blue staining when the purified eluted protein used as immunogen is run in high amounts in one- or two-dimensional gels (data not shown). With respect to the 21-kD polypeptide, it is recognized by the two probes, clone MA12 and antibody, suggesting that some of the observed nucleotide homology is likely to be in amino acid coding sequences. This indicates a possible structural or functional relationship between these polypeptides.

The hybrid selection experiment suggests that there are a number of different transcripts which are identified by clone MA12. These transcripts code for different polypeptides with substantial differences in their pI and mol wt. In tetraploid cotton, single cloned cDNAs arrest the translation of several different polypeptides; two of these hybrid-arrested polypeptides have been identified as putative products from two active homologous genes, each one localized in each of the two subgenomes (8). Since *Z. mays* W64A is a diploid and extensively inbred line, our results of hybrid-selection experiments could not be explained as being different products of homologous genes, or alleles of a single gene. All the hybrid-selected polypeptides were previously identified as very coordinately regulated during normal embryogenesis or after ABA induction in young embryos in culture (10). These results coupled with the differences observed in the cross-hybridization pattern of the cDNA inserts (data not shown) suggest that clone MA12 hybridizes with all species of mRNA encoding a discrete, closely related family of polypeptides. Also but less likely these related mRNAs could originate from differential post-transcriptional processing of transcripts from a single gene.

From these data we can conclude that the mRNAs coding for the 23- to 25-kD proteins, are regulated during embryogenesis, precociously induced in young embryos upon ABA treatment, and their proteins post-translationally modified by phosphorylation. Protein phosphorylation and dephosphorylation are considered important regulatory mechanisms by which the activity of key enzymes and receptor molecules is altered in response to a wide variety of external stimuli (3). The next, and more problematic, step is to determine whether the restricted pattern of expression and phosphorylation (10, 21) of this particular protein set actually reflects any developmental or functional role. Work is in progress to obtain the sequences of the cDNA clones coding for these ABA regulated proteins toward a better understanding of the relationship between ABA and embryogenesis.

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