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Regulation of Gene Expression in Developing *Zea mays* Embryos

PROTEIN SYNTHESIS DURING EMBRYOGENESIS AND EARLY GERMINATION OF MAIZE

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ABSTRACT

Polypeptides synthesized in dissected embryos of *Zea mays* at different stages of embryogenesis and early germination have been characterized by their migration in two-dimensional gel electrophoresis. This analysis has been carried out with *in vivo* labeled polypeptides from excised embryos and with proteins synthesized *in vitro* in the rabbit reticulocyte system directed by poly(A⁺) RNA isolated from the different developmental stages. We have identified three main sets of expressed polypeptides: (a) embryonic set: this group of polypeptides is synthesized in young and mature embryos but not in early germination; (b) maturation set: this group of polypeptides is not present in young embryos and appears during the maturation period. Some of these polypeptides are still present in early germination while others disappear from stored mRNAs in dry embryos. One particular group from this set can be induced prematurely in young embryos by incubation with abscisic acid; and (c) germination set: this group of polypeptides is not expressed in the maturation period and appears after brief imbibition of the dry embryos.

Little information is available on the molecular events that underlie plant development. The lack of sufficient genetic information, the long life periods of the individuals, and the large size of the genome present in general in higher plants are, among other factors, the reasons that have made these developmental studies more difficult than those carried out on some animal species where embryogenesis has been studied at the molecular level. One important step in the study of the molecular mechanisms involved in plant development is the characterization of the proteins synthesized at the different stages of seed maturation and plant germination and the corresponding expression of their mRNA.

A thorough characterization of mRNA populations expressed during embryogenesis and germination has been reported for cotton seeds (11, 12), wheat (26) and radish embryos (2, 14), and one early expressed gene from dry wheat embryos has been cloned (7, 28). In this respect, research in maize seeds has been focused on the period of rehydration of quiescent embryos in an attempt to clarify the role of stored mRNA in the seed embryos (10, 24). However, no comparable data are available for maize embryogenesis and early germination.

Organogenesis in the maize embryo includes the formation of three lobes (posterior, distal, and anterior) that are evident 10 d after pollination. The development of the embryo is completed by about d 30 to 40 and maturation and drying take place during the next 10 to 20 d (23). The stages of development considered in the present study are those where the embryonic organs (root, stem, coleoptile, and scutellum) are already defined: young embryo (10–20 DAP[1]), embryo in late embryogenesis (30–40 DAP), and mature embryo (45–50 DAP), as well as dry and germinating embryo. To study the mechanisms of regulation of differentially expressed genes during the embryogenesis of *Zea mays*, we have analyzed the polypeptides synthesized in dissected dry embryos after 2 h imbibition and these polypeptides have been compared

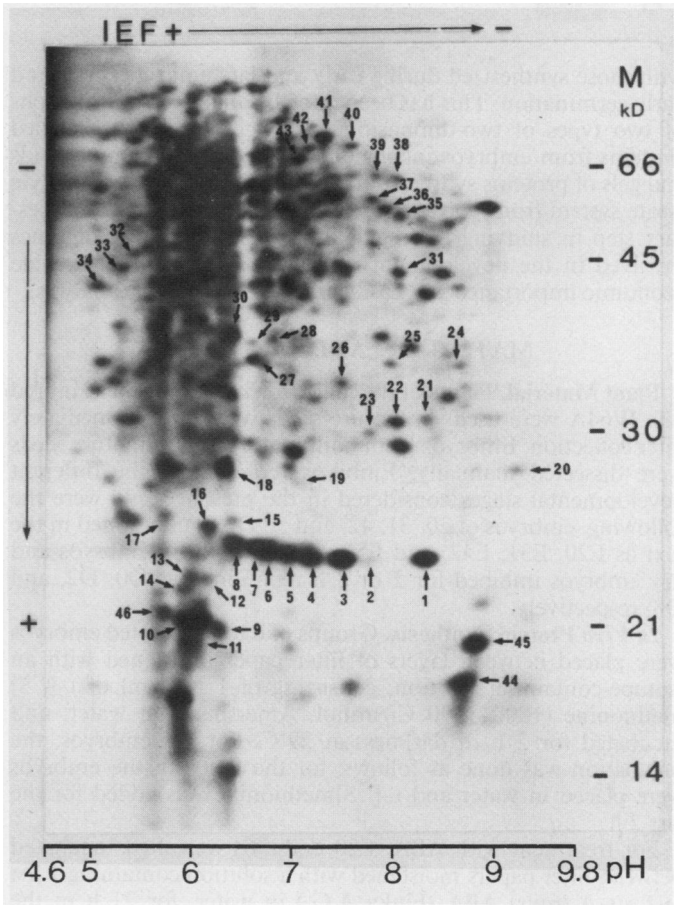

FIG. 1. *In vivo* protein synthesis in embryos imbibed for 2 h. Analysis by two-dimensional electrophoresis. Numbered black arrows show 46 radioactive polypeptides synthesized *in vivo* and relevant to this stage. Isoelectric focusing was run in the first dimension as indicated (pH scale is shown below) and electrophoresis in SDS in the second dimension is top to bottom. The mol wt of markers are indicated on the right. BSA 66 kD, ovoalbumin 45 kD, carbonic anhydrase 30 kD, soybean trypsin inhibitor 21 kD, lysozyme 14 kD).

[1] Abbreviation. DAP, days after pollination.

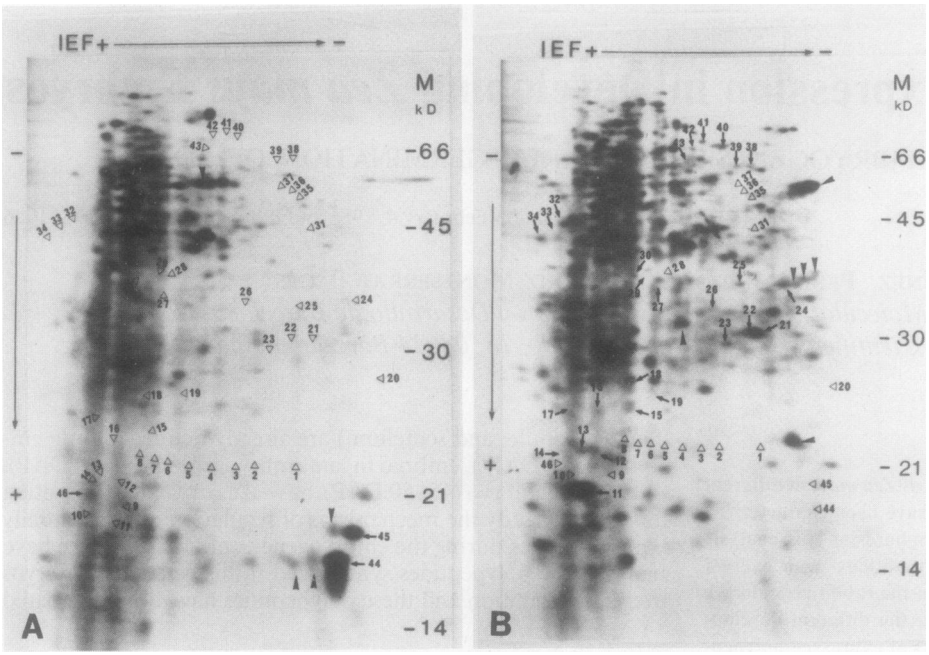

FIG. 2. Protein synthesis in embryos 20 DAP (A) and dry embryos after 8 h imbibition (B). Two-dimensional electrophoretic patterns of polypeptides synthesized *in vivo*. Numbered black arrows show polypeptides present, and white arrows polypeptides lacking in comparison with the gel shown in Figure 1. Unnumbered black arrows indicate stage-specific polypeptides.

with those synthesized during early and late embryogenesis and early germination. This has been done by analyzing fluorographs of two types of two-dimensional gels: gels of *in vivo* labeled proteins from embryos incubated with radioactive amino acids and gels of proteins synthesized *in vitro* in the rabbit reticulocyte lysate system from poly(A^+) RNA. This type of work is a necessary step in studying the regulation of the expression of genes involved in the development of maize, a cereal of worldwide economic importance and easily amenable to genetic analysis.

MATERIALS AND METHODS

Plant Material. Plants of the maize (*Zea mays* L.) pure inbred line W64A were used. Immature seeds were used immediately after collection. Embryos from both mature and immature seeds were dissected manually. Embryos representing the different developmental stages considered in the present study were the following: embryos of 20, 31, 42, and 50 DAP, mentioned in the text as E20, E31, E42, and E50 respectively; dry embryos and dry embryos imbibed for 2 or 8 h mentioned as D0, D2, and D8, respectively.

***In Vivo* Protein Synthesis.** Groups of 6 to 8 dissected embryos were placed between layers of filter paper moistened with an isotope-containing solution, consisting of 1 mCi/ml of L-[^{35}S] methionine (1000–1500 Ci/mmol, Amersham) in water, and incubated for 2 h in darkness at 29°C. For D8 embryos, the incubation was done as follows: for the first 6 h the embryos were placed in water and L-[^{35}S]methionine was added for the last 2 h.

For treatment with ABA, E20 embryos were first incubated between filter papers moistened with a solution containing 1 μM (±2 *cis*–4 *trans*) ABA (Fluka A.G.) in water, for 21 h in the darkness at 29°C and then transferred to filter paper wetted with the isotope-containing solution and incubated for 2 h at the same temperature. After incubation, the embryos were rinsed with water (to remove the unincorporated radioactivity), wiped with filter paper and quickly frozen at −80°C.

To extract proteins, frozen embryos were ground in the presence of liquid N_2 using a mortar and a pestle. The resultant powder was resuspended in lysis buffer containing 9.5 M urea, 2% (w/v) Nonidet P-40, 0.8% (w/v) ampholines (LKB, 3.5–10 pH range), 100 mM DTT, and 1 mM phenylmethanesulfonyl fluoride, homogenized for 1 h in an ultrasonic bath with cold water (4°C) and centrifuged for 5 min at 10,000*g* in a bench centrifuge. The supernatant was used to measure the incorporated label and loaded on the electrofocusing gels.

***In Vitro* Protein Synthesis.** Rabbit reticulocyte lysate (Amersham) was used to translate *in vitro* the poly(A^+) RNA from E20, D0, and D2 embryos. The translation reaction mixture contained (in a final volume of 20 μl): 14 μl lysate, 10 to 20 μCi L-[^{35}S] methionine (1000–1500 Ci/mmol), 0.5 to 1 μg of poly (A^+) RNA. The incubation was for 30 min at 30°C. The samples were precipitated with 20 volumes of cold acetone and kept in ice for 1 h, then centrifuged for 5 min at 10,000*g* and the pellet, after drying in a vacuum-speed, was dissolved in lysis buffer as described above for two-dimensional electrophoretic analysis.

The poly(A^+) RNA was prepared as follows. Batches of 100 embryos were ground under liquid N_2 in a mortar and a pestle and the resultant powder was resuspended in a buffer (6 ml of buffer per g of tissue) containing 0.2 M Tris-HCl (pH 8.0), 0.35 M sucrose, 50 mM KCl, 10 mM $MgCl_2$, 1.3% (w/v) Triton X-100, and 1% (w/v) iodoacetic acid. The suspension was shaken for 5 min in ice and centrifuged at 2,000*g* for 5 min at 4°C (to remove nuclei).

To extract the RNA, EDTA was added to the supernatant to a final concentration of 50 mM, NaCl to 0.15 M and SDS to 1% (w/v). The homogenate was extracted with a phenol:chloroform: isoamyl alcohol (50%:48%:2%, v/v/v) mixture, and the aqueous phase precipitated overnight at 4°C, with the same volume of LiCl:urea (4M:8M) (3). The pellet was centrifuged at 10,000*g* for 20 min at 4°C, dissolved in a buffer containing 50 mM Tris-HCl (pH 8.0), 0.5% (w/v) SDS, and 10 mM EDTA (pH 8.0), then extracted three times with the phenol:chloroform:isoamyl alcohol mixture, once with chloroform:isoamyl alcohol (96%:4%, v/v), and once with ethyl ether saturated with water.

The aqueous phase was made 0.1 M NaCl and the RNA was precipitated with 2.5 volumes of absolute ethanol, overnight at −20°C (16). The poly(A^+) RNA was obtained using oligothymidylic acid-cellulose (Pharmacia PL Biochemicals) chromatography (4). Fractions containing poly(A^+) RNA were made 0.1 M NaCl and precipitated with 2.5 volumes of absolute ethanol, overnight at −20°C, and the resultant pellet was washed twice with 70% ethanol, air dried and stored at −80°C.

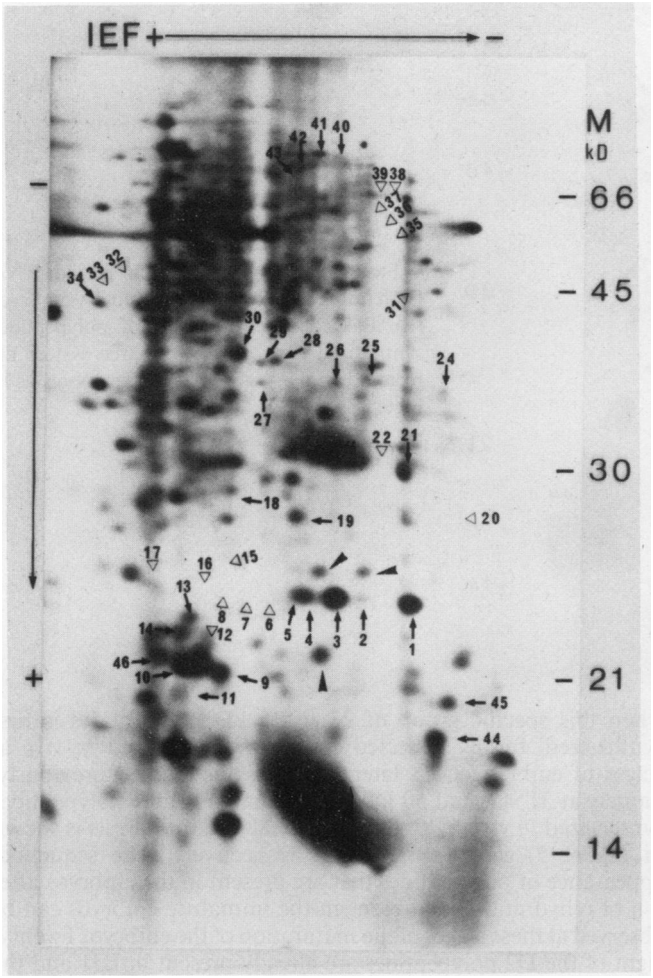

FIG. 3. *In vitro* protein synthesis with poly(A$^+$) RNA from dry embryos imbibed for 2 h. Electrophoretic pattern of polypeptides synthesized *in vitro* in a cell free system directed by poly(A$^+$) RNA. The polypeptides numbered in the *in vivo* labeling of Figure 1 are identified in this figure. Numbered black arrows show polypeptides present and white arrows lacking. Unnumbered black arrows indicate polypeptides observed only in the *in vitro* pattern.

Two-Dimensional Protein Electrophoresis. The procedure followed essentially the method of O'Farrell (19) modified by Meyer and Chartier (17). The corresponding volumes of supernatant (containing labeled polypeptides with 200,000–250,000 cpm) were loaded onto cylindrical electrofocusing gels (length 10 cm, internal diameter 2.5 mm) containing 3.7% (w/v) acrylamide, 0.21% (w/v) bis-acrylamide, 9.15 M urea, 2% (w/v) Nonidet P-40, and 0.8% (w/v) ampholines (LKB, pH range 3.5–10). Samples were loaded on the acid end of the gel (20); covered with 20 μl of overlay solution (8 M urea, 0.4% (w/v) ampholines, 5% (w/v) Nonidet P-40, and 5% β-mercaptoethanol); and electrofocused (in sets of 8 gels) for 30 min at 100 V, 45 min at 200 V, 160 min at 300 V, and 120 min at 500 V (17). After electrofocusing, gels were equilibrated in 10% (v/v) glycerol, 5% (v/v) β-mercaptoethanol, 2% (w/v) SDS, and 60 mM Tris-HCl (pH 6.8) for less than 30 min, and loaded onto SDS-polyacrylamide gels or frozen at −80°C and stored.

The second dimension (13) was prepared as follows: the separating gel was 1.5 mm thick and contained 15% (w/v) acrylamide, 0.4% (w/v) bis-acrylamide, 0.38 M Tris-HCl (pH 8.8), and 0.1% (w/v) SDS. The upper stacking gel (in which the electrofocusing gel was included) was 1% (w/v) agarose, 60 mM Tris-HCl (pH 6.8), 2% (w/v) SDS, 5% (w/v) β-mercaptoethanol, 10% (v/v) glycerol, and 0.002% (w/v) bromophenol blue. The lower stacking gel was placed between the other two and contained 3% (w/v) acrylamide, 0.08% bis-acrylamide, 125 mM Tris-HCl (pH 6.8), and 0.1% (w/v) SDS. The gel was run at 23 mamp (constant current) for 15 h.

After electrophoresis, the gels were fixed for 30 min in a methanol:acetic acid:water solution (30%:7%:63%, v/v/v), immersed for 1 h in Enhance (New England Nuclear), rehydrated for 30 min in double distilled H_2O and dried under vacuum in a gel drier. Fluorographs were obtained by exposing the dry gels, at −70°C, for 25 to 35 d to preflashed MAFE RP-X7 film. The identification of spots in different experiments was done by comparing the mobility of mol wt markers, the pH range and the position of chosen spots present in all the samples. At least three independent observations were made for every stage studied. The pattern of spots in two-dimensional electrophoresis appeared to be highly reproducible as expected for a homozygous line, W64A, and indicating the absence of a widespread proteolysis.

RESULTS

Polypeptides Synthesized *in Vivo* in Maize Embryos. The *in vivo* synthesis of polypeptides in maize embryos during embryogenesis and at early stages of germination was studied by placing the embryos dissected from the endosperm in contact with a solution containing L-[^{35}S]methionine as a protein precursor. The incubation period in the presence of the labeled compound was in all cases 2 h. Embryo proteins were extracted in a denaturing medium and analyzed by two-dimensional gel electrophoresis using electrofocusing (real pH range in the gel 4.6–9.8) in the first dimension and SDS electrophoresis in the second. The method of analysis was chosen in order to allow a rapid separation of a wide range of polypeptides. When the embryos from dry seeds were studied in this way, corresponding to 2 h of rehydration (D2), the pattern of synthesized polypeptides shown in Figure 1 was observed. The majority of polypeptides synthesized at this stage are also found in previous stages of seed maturation or at early stages of germination. Nevertheless, among the spots that can be resolved here, 46 were chosen to study in the other stages (see below). These spots are marked with numbered black arrows in the figure.

The pattern of polypeptides synthesized at early stages of rehydration (D2) can be compared with those at later periods of imbibition (D8) or in the immature embryo (E20). Figure 2 shows fluorographs of the two-dimensional electrophoresis of total labeled polypeptides produced by a 2-h incubation with L-[^{35}S]methionine of embryos from seeds 20 DAP (E20, gel A) and from seeds allowed to germinate for 6 h and subsequently incubated 2 h with the precursor (D8, gel B). Four spots not present either in D2 or in D8 can be observed in the immature embryo, they are marked with unnumbered black arrows in Figure 2A. There are 43 polypeptides synthesized in D2 that cannot be observed in the young embryo (they are not detected even in overexposed gels). The absence of these polypeptides is indicated by white arrows in Figure 2A. At later stages of imbibition (D8, Fig. 2B) 19 of the D2 polypeptides including three (numbers 44, 45, and 46) already expressed in E20 embryos disappear (marked with white arrows in Fig. 2B). Consequently at 8 h imbibition only 27 of the D2 polypeptides are identifiable. Six new spots (marked with unnumbered black arrows) are only observed during this period. In the group of polypeptides observed in D2 embryos but not at E20 or at later stages of development, the most conspicuous are a set of 8 spots running at 23 to 25 kD; these are observed after only a 1-h incubation of the dry seed (result not shown).

***In Vitro* Protein Synthesis.** The characteristic pattern of polypeptides observed in the *in vivo* experiments in the different

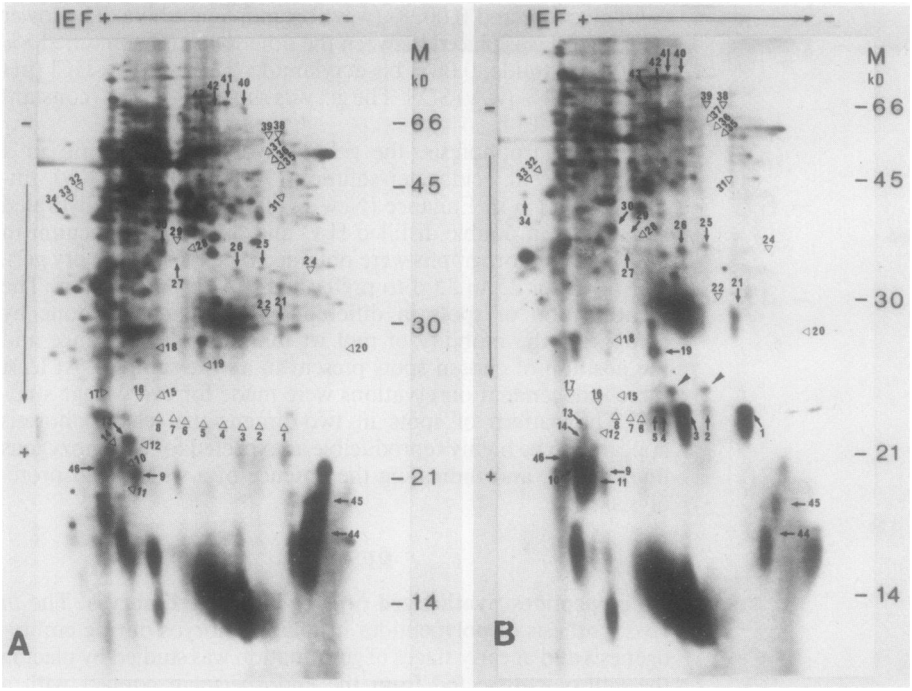

FIG. 4. *In vitro* protein synthesis with RNA from immature (20 DAP) (A) and dry embryos (B). Electrophoretic pattern of polypeptides synthesized *in vitro* in a cell free system directed by poly(A^+) RNA. Same symbols as in Figure 3.

stages examined could be due either to differences in the mRNA populations present or to co- or post-translational modifications of the proteins. To check these possibilities total RNA was prepared from young embryos (E20), dry embryos (D0) and dry embryos imbibed in water for 2 h (D2). Poly(A^+) RNA was prepared by oligo(dT)-cellulose chromatography and translated *in vitro* in a rabbit reticulocyte lysate system. The *in vitro* translation of poly (A^+) RNA from embryos rehydrated for 2 h is shown in Figure 3. Most of the polypeptides detected in the *in vivo* experiments in D2 embryos, as shown in Figure 1, appear to be present in the *in vitro* translation analysis of the same stage. However, 18 of these spots are lacking in the *in vitro* synthesis (white arrows). The same 18 polypeptides plus an additional three are lacking in the translation products of poly(A^+) RNA extracted from dry embryos (D0) as shown in Figure 4B. Instead, 12 of the polypeptides detected in D2 but not in immature embryos (E20) in the *in vivo* experiments (Figs. 1 and 2A, respectively) appear to be present in the *in vitro* experiments with E20 (Fig. 4A) indicating that their mRNA is already present at E20 but their level of translation is too low to be detected in the *in vivo* experiments. Unnumbered black arrows show two spots in D0 (Fig. 4B) and three in D2 (Fig. 3) *in vitro* pattern without correspondence in the *in vivo* synthesized polypeptides.

The behavior of the group of polypeptides located at around 23 to 25 kD is also interesting. While 8 of these polypeptides are detected in imbibed dry embryos in the *in vivo* experiments (D2), only 5 are detected in the corresponding *in vitro* experiment (Fig. 3) as well as in the same experiments carried out with poly(A^+) RNA prepared from dry seeds (Fig. 4B). None of the spots can be observed at the E20 stage in either *in vivo* (Fig. 2A) or *in vitro* (Fig. 4A) experiments of E20 stage. The other 3 polypeptides observed *in vivo* are not detectable in the *in vitro* experiments from either poly(A^+) or poly(A−) RNA (the last result is not shown). The analysis has been repeated in independent experiments and from independent batches of embryos. The absence of these 3 polypeptides from the *in vitro* pattern may be explained by differences in the efficiency of the translation for particular mRNAs in the rabbit reticulocyte system, or by post-translational modifications of the proteins occurring *in vivo* that modify the isoelectric point of the proteins.

***In Vivo* Protein Synthesis in Maturing Embryos.** To study when this specific group of 23 to 25 kD polypeptides is first synthesized, freshly dissected embryos were labeled *in vivo* at stages of embryogenesis later than the one studied previously, namely at 31, 42, and 50 DAP. The analysis of the polypeptides synthesized *in vivo* by two-dimensional electrophoresis is shown in Figure 5, gels A, B, and C, respectively. The sequential appearance of polypeptides that are present in the embryos after 2 h of rehydration and absent in the immature embryos can be observed at these stages of the maturation of the embryo. Twenty-eight of the D2 polypeptides are already present in E31 and the number increases to 36 in E50. The 23 to 25 kD polypeptides show a similar pattern. In this case, the spots corresponding to polypeptides 1, 3, 4, and 5 start to emerge very faintly from 31 DAP. These polypeptides, and also spots 2, 6, and 7 are present at 42 DAP, while in 50 DAP the full pattern observed after 2 h of imbibition is already visible. Eight polypeptides are detectable at these stages and not present at 20 DAP or at 2 h imbibition (they are marked as unnumbered black arrows in Fig. 5C) and only seven spots are not present in E50 as compared with the D2 stage (white arrows in Fig. 5C). The four spots present in E20 but not D2 or D8 (unnumbered black arrows, Fig. 2A) are also present through E50.

Effect of Abscisic Acid. Several mechanisms for the induction of the transcription of genes in embryonic plant systems have been proposed. These include induction by desiccation (2, 8) or by plant hormones (11, 22) such as ABA. Incubation of immature embryos (20 DAP) in the presence of 1 μM ABA induces the appearance of 32 polypeptides not present in the E20 *in vivo* pattern (Fig. 6). It is interesting to point out that of these 32 spots, 22 can be detected in the *in vivo* pattern from E50 and 2 h rehydrated mature embryos, D2. In particular if the eight spots present in the 23 to 25 kD region are considered, incubation with ABA induces the presence of four of them (spots 1, 3, 4, and 5), which are the same that can be detected in the *in vitro* translation of RNA from D0 and D2 embryos. However by comparing the polypeptides induced by ABA in immature embryos with those present at later stages of maturation (31, 42, and 50 DAP), a stage when high levels of ABA have been detected (18, 27), it appears that 10 ABA-induced polypeptides in E20 (unnumbered black arrows in Fig. 6) are not found in the *in vivo* patterns. No induction of these polypeptides can be observed at

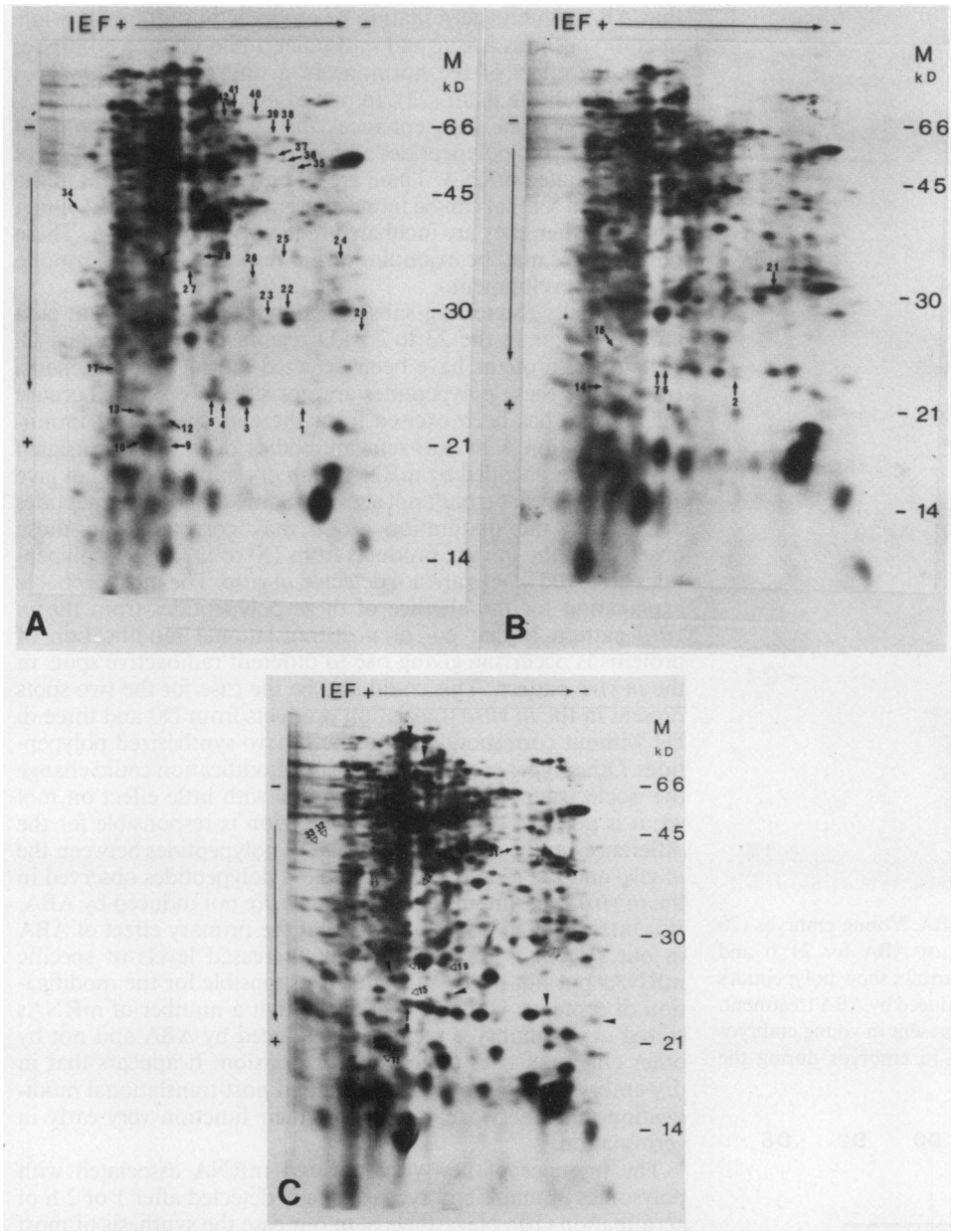

FIG. 5. *In vivo* protein synthesis in immature embryos 31 (A), 42 (B), and 50 (C) DAP. Numbered black arrows in A, B, and C show the sequential appearance of polypeptides present in D2 and not present in young embryos. In C, white arrows show polypeptides present in D2 and not in E50 and unnumbered black arrows show stage specific polypeptides.

early imbibition by *in vivo* labeling of 24 h air-desiccated immature (20 DAP) embryos (results not shown).

DISCUSSION

Changes in the relative abundance of mRNA species during development have been reported in different plant systems (2, 11, 26). A previous study in maize reported the patterns of protein synthesis in embryos after 6 and 24 h of germination (24). In the present study we have used short incubation periods (2 h) to study the *de novo* synthesis of polypeptides at very early stages of germination (between 2 and 8 h of imbibition) in *Z. mays* and the results have been compared with embryos at several stages of embryogenesis.

We have used maize embryos isolated from the endosperm in order to enhance the uptake of labeled aminoacids (9) and to distinguish clearly between embryo and endosperm mRNA (5, 15, 21). Endosperm at the early stages of embryogenesis is actively engaged in storage protein synthesis.

Throughout the period of maize ontogeny studied a number of labeled polypeptides can be clearly resolved by two-dimensional gel electrophoresis. In this group of spots we have identified three sets of expressed polypeptides (Fig. 7) that can be characterized as follows.

Embryonic set. This group with 7 members is expressed in young (20 DAP) and in mature (50 DAP) embryos. Three members of this group (E2 in Fig. 7) are also found in the mRNA stored in dry embryos but disappear by 8 h of germination.

Maturation Set. This group has 44 members appearing in the embryo maturation period (31–50 DAP). Eight of them (M1 in Fig. 7) are not observed in the mRNA of dry embryo, 16 have disappeared 8 h after imbibition (M2 in Figs. 7) and 20 of them are still present in embryos after 8 h of imbibition (M3 in Fig. 7). Some polypeptides of this maturation set can be induced prematurely in young embryos by incubation with ABA.

Germination Set. This group has 13 members appearing during the first hours of germination, 6 of them only apparent in 8 h imbibed embryos (G2 in Fig. 7), and 7 expressed after 2 h imbibition (G1 in Fig. 7). Presumably, the mRNAs for 3 (spots 11, 19, and 29) of this last group are already present in the stored mRNA of dry embryos.

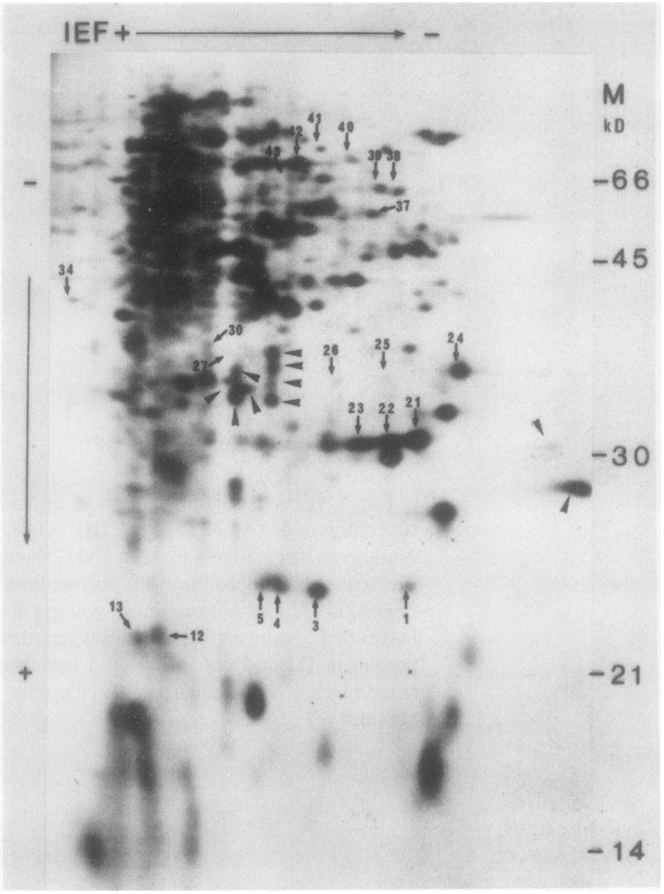

FIG. 6. Induction of protein synthesis by ABA. Young embryos (20 DAP) were incubated in the presence of 1 μM ABA for 21 h and subsequently labeled for 2 h. Numbered black arrows show polypeptides which are not synthesized at this stage but are induced by ABA treatment. Unnumbered black arrows show polypeptides present in young embryos after induction with ABA and not identified in embryos during the maturation process.

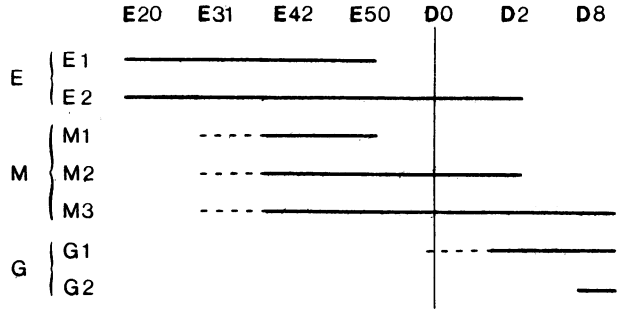

FIG. 7. Schematic diagram of sets of polypeptides expressed during embryogenesis and early germination of dissected embryos of *Zea mays* L. E, Embryonic set. Subset E1, 4 polypeptides (unnumbered black arrows in Fig. 2A). Subset E2, polypeptides 44, 45, and 46. M., Maturation set. Subset M1, 8 polypeptides (unnumbered black arrows in Fig. 5C). Subset M2, polypeptides 1 to 10, 20, 28, 31, and 35 to 37. Subset M3, polypeptides 12 to 17, 21 to 27, 30, 34, and 38 to 43. G, Germination set. Subset G1, polypeptides 11, 15, 18, 19, 29, 32, and 33. Subset G2, 6 polypeptides (unnumbered black arrows in Fig. 2B).

It has been reported that when the maize embryo progresses from the cell division phase to the maturation stage, the level of ABA in the seed rises, preventing the viviparous germination of the embryo (18, 27). A number of other studies have reported that ABA inhibits germination of embryos in culture (1) while in embryogenesis, tissue and molecular differentiation continues under the effect of the hormone in a way similar to embryos remaining in the plant (22). In our case as in other systems (11, 22) this hormone also enhances the synthesis of a group of embryo-specific polypeptides and increases steady-state levels of their associated mRNA. There are 22 polypeptides present in the maturation set that can be induced prematurely in excised young embryos when they are incubated in the presence of ABA. These polypeptides may be examples of the response to this hormone in maize development.

A specially interesting subset of polypeptides is present as a group of spots in the 23 to 25 kD region. In other systems (2, 11) storage proteins have been detected during embryogenesis. In our case these polypeptides are not storage proteins because the embryo has been excised from the endosperm and immunoprecipitation wiht anti-zein antibodies of *in vitro* translated polypeptides encoded by mRNA from dry embryo does not give any positive result (result not shown). Some of these polypeptides detected in the maturation group may correspond to those observed in the *in vitro* products from D0 or D2, but 3 polypeptides observed *in vivo* are not detected *in vitro*. The most probable explanation for the absence of these polypeptides from the *in vitro* pattern is that co- or post-translational modification of proteins is occurring giving rise to different radioactive spots in the *in vivo* pattern. This could also be the case for the two spots present in the *in vitro* translation products from D0 and three in D2 without correspondence to the *in vivo* synthesized polypeptides. Other types of post-translational modification could change the isoelectric point of the polypeptide with little effect on mol wt; it is possible that such a modification is responsible for the differences in the 23 to 25 kD group of polypeptides between the *in vivo* and the *in vitro* patterns. The 3 polypeptides observed in the *in vivo* experiments but not *in vitro* are not induced by ABA. It is interesting to note therefore that the primary effect of ABA in our system is the induction of increased levels of specific mRNAs but not of the machinery responsible for the modification of proteins. Our results indicate that a number of mRNAs stored in dry embryos seem to be induced by ABA and not by other effects such as desiccation or excision. It appears that in dry embryos both the translational and post-translational modification machinery are in place for their function very early in germination.

The presence of newly synthesized mRNA associated with polysomes in maize embryos is already detected after 1 or 2 h of germination (10). Nevertheless, in our case the synthesis of most of the E50 polypeptides during the first 2 h of imbibition of dry embryos suggests a significant role for stored mRNA shortly after the onset of imbibition. This could be explained as a consequence of stored mRNA (6, 24, 25) reported to have a long half-life and being abundant enough to be translated after this short period of incubation. After 8 h of imbibition the protein pattern changes and at least 6 new polypeptides appear, these are probably encoded by newly synthesized mRNA.

Some spots are detected in young embryos in the *in vitro* translation products but are missing in the *in vivo* pattern at the same stage. These polypeptides are clearly identified in the mature embryo. This effect suggests the existence of a lag period between the appearance of the mRNA in the cytoplasm of the cells and the detection of the final product. These results suggest the existence of mechanisms of control at the different levels of gene expression during embryogenesis and early germination. The characterization of gene expression at defined stages of embryogenesis and germination will be an important information for understanding the mechanisms of control of plant cell development.

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