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Identification of Embryonal Antigens of Maize: Globulins as Primary Reserve Proteins of the Embryo

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Abstract. Normal and γ-irradiated caryopses of *Zea mays* L. were germinated, and the degradation of embryonal antigens (EA) was followed in the endosperms, scutella and embryonic axes of the seedlings, using double immunodiffusion, immunoelectrophoresis and quantitative immunoprecipitation. The predominant transient EA were presumed to be storage proteins related to the reserve globulins of dicotyledonous seeds. Therefore globulins were isolated from maize scutella, purified by $(NH_4)_2SO_4$ fractionation and isoelectric precipitation, and the molecular weights of the polypeptide units were estimated by discontinuous sodium-dodecyl-sulphate slab electrophoresis. The globulins were found to be identical with the predominant EA and amounted to about 40% of the protein nitrogen in the embryos of mature, non-germinated caryopses. The presumed reserve function of the globulins and the characteristic time course of their degradation in embryonic axes and scutella of maize seedlings are discussed in relation to the two-step pattern of mobilization of nitrogen reserves in germinating cereal caryopses.

Key words: Antigen spectra – Embryo (proteins) – γ-Plantlets – Germination (seeds) – Globulins – Proteins (storage) – Seedling growth – Storage proteins – *Zea*.

Introduction

We have recently described a characteristic group of embryonal antigens (EA) and followed the pattern of their sequential accumulation in the developing maize caryopsis (Khavkin et al., 1977). These EA comprise at least two classes of proteins with apparently different physiological functions. The major EA are relatively abundant in mature, non-germinated or imbibing caryopses, but disappear rapidly during the 2nd and 3rd day of germination. These transient EA are globulins that resemble the well-known reserve globulins of the dicotyledons and some monocotyledons (for review see Derbyshire et al., 1976). In contrast, another class of the EA consisting of several minor components behave as constitutive proteins, and some of these EA are maintained in the caryopsis at least during 12 days of germination and seedling growth (Misharin et al., 1977).

Proceeding from the assumption that the EA include both storage and constitutive proteins, we have examined both classes of EA using immunological and other approaches. The presumed constitutive EA were studied by comparing antigen patterns in several normal and callus tissues of maize (Khavkin et al., 1978). More conclusive results have since been obtained concerning the identification of the transient EA since the storage function of proteins in seeds can be judged with reasonable confidence from correlations between the progress of germination and seedling growth and the kinetics of protein breakdown. γ-Irradiation of dry kernels at doses that totally prevent subsequent cell divisions is a convenient method to reduce seedling growth by decreasing the sink for metabolites liberated by mobilization of storage deposits, without any direct effects upon processes occurring in the endosperm. γ-Plantlets grow until the elongation of all cells that were already present in the meristems of the embryo is completed (Haber, 1968; Ivanov, 1974).

In this paper, we first describe the time course of the degradation of the EA as related to the progress of germination and early growth of normal and γ-irradiated maize caryopses and of the early growth of

Abbreviations: AG, AR and AS = antisera to globulins and to root and scutellum proteins, respectively; CA = common antigens; DAP = days after pollination; EA = embryonal antigens; ME = 2-mercaptoethanol; SDS-PAGE = discontinuous sodium-dodecyl-sulphate slab polyacrylamide-gel electrophoresis; TCA = trichloroacetic acid

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the resulting seedlings. Next, the globulin fraction from the scutella was isolated, purified, and separated into two subfractions, and the EA were compared with these preparations. Their subunits were characterized by the molecular weight using discontinuous sodium-dodecyl-sulphate slab polyacrylamide-gel electrophoresis (SDS-PAGE). Finally, reserve globulin components were detected among the EA of embryonic axes and scutella using a specific antiglobulin serum. A preliminary communication on some of these data has been presented elsewhere (Misharin et al., 1978).

Material and Methods

Plant Material

Maize (*Zea mays* L.) kernels (caryopses), hybrid Bukovinsky 3, were obtained commercially from the Krasnodar Corn Production Plant. To obtain γ-seedlings dry kernels were irradiated from a ^{60}Co source (100 R s^{-1}; total dose 200 kR). To investigate proteins of mature non-germinated caryopses we utilized either air-dry kernels or kernels presoaked for 0.5–2 h at +4–8° C, as the latter permitted a more convenient separation of the caryopsis into scutellum, embryonic axis and endosperm. In all other cases kernels were germinated at 27° C in the dark, and various parts of the seedlings were sampled for analysis: the entire upper part of the embryonic axis (the shoot) in non-germinated caryopses and 1-d-old seedlings; the endosperm, the scutellum, and either the entire embryonic axis (shoot) or separately the coleoptile, the mesocotyl, the mesocotyl node with the plumule, and the root in non-germinated caryopses and in 1–12-d-old seedlings. For the sake of convenience, we shall throughout the paper describe development as "days of germination", counted from the onset of imbibition and including germination proper (days 1–3) and early seedling growth.

To study EA development in embryogenesis, maize plants (inbreds C103 and W155) were grown in a greenhouse under xenon lamps, hand-pollinated, and the developing kernels were sampled at several intervals. All these procedures and the preparation of tissue extracts were performed essentially as described earlier (Khavkin et al., 1977). For total nitrogen determination samples were oven-dried at 105° C.

Determination of Total and Salt-soluble Protein

Scutella and embryonic axes were isolated from caryopses presoaked in the cold. To determine total protein content the tissue samples were blended with 5 volumes of 1 M NaCl in the cold, and the proteins were precipitated with trichloroacetic acid (TCA) to 5% final concentration and washed twice with 5% TCA. Salt-soluble proteins were extracted 5 times with 5-fold (w/v) volumes of ice-cold 0.1 M phosphate buffer, pH 7, containing 1 M NaCl and 0.01 M 2-mercaptoethanol (ME). An aliquot of this extract was precipitated with TCA to 5%, while another aliquot was subjected to dialysis until giving a negative reaction for Cl^-. Globulins were separated by centrifugation at 10,000 × *g* for 15 min at room temperature and washed twice with water, while albumins were precipitated from the supernatant with 5% TCA.

Isolation and Purification of Globulins

The following protocol is based mainly on Danielsson's procedure (for discussion see Derbyshire et al., 1976). Scutella isolated from non-germinated caryopses that had been presoaked for 0.5 h were sectioned into small pieces with a razor blade and defatted by stirring with 3 changes of 20 volumes of petroleum ether (reagent grade, b.p. 40–70° C) for 2 h in the cold. Then the samples were blended with 10 volumes of 0.05 M phosphate buffer, pH 7, containing 1 M NaCl and 0.01 M ME. After stirring for 1 h, the homogenate was centrifuged at 17,000 × *g* for 20 min, and the supernatant (salt-extracted proteins, preparation P1) was adjusted to 70% saturation with $(NH_4)_2SO_4$. A precipitate was allowed to form overnight at 4–6° C and collected by centrifugation at 17,000 × *g* for 20 min (preparation P2). It was next dispersed in an $(NH_4)_2SO_4$ solution (40% saturation), after 1 h the suspension was clarified by centrifugation, and the sediment (P3 fraction) was discarded while the supernatant was adjusted to 70% saturation with $(NH_4)_2SO_4$. The resulting precipitate (P4) was collected by centrifugation, dissolved in the homogenizing medium, and dialysed until the Cl^- reaction was negative. The supernatant fraction (P6) was discarded while the precipitate (P5) was used directly for immunization and analyses; however, in some cases P5 was redissolved and subjected to dialysis for a second time. To sub-fractionate the P5 globulins the precipitate was dissolved in the homogenizing medium and the solution was clarified by centrifugation and adjusted to pH 4.7 with 1 N HCl. Legumin-like globulins (P7) settled out, and were collected by centrifugation and dissolved in the homogenizing medium. The clarified supernatant (vicilin-like proteins, P8) was adjusted to pH 7 with $NaHCO_3$.

Polypeptide Separation with SDS-PAGE

Sample Preparation, Reduction and Alkylation of SH-Groups. Protein samples were precipitated in the cold with 10% TCA and washed with 0.1% TCA and then with acetone. Ca. 10 mg of precipitate were dissolved in 0.1 M tris(hydroxymethyl)aminomethane (Tris) buffer, pH 7.5, containing 8 M urea, reduced with 1% (v/v) ME, and treated with acrylamide as described by Cavins and Friedman (1968).

SDS-PAGE. The technique of Laemmli and Favre (1973) was employed with minor modifications. Vertical slab gels of 8 cm × 15.5 cm × 0.1 cm were prepared between 2-mm-thick glass plates; stacking gels of 3.5 cm inclued 1.5 -cm application wells and separation gels were of 12 cm length. The gels contained 5 and 10% acrylamide, respectively. The protein samples were solubilized in 0.125 M Tris-HCl, pH 6.8, containing 2% SDS, 20% glycerol and 0.001% bromophenol blue, and completely dissociated in a boiling water bath for 3 min. Samples (20–80 μg in 10–40 μl) were loaded on the stacking gel, and electrophoresis was carried out at 12 mA cm^{-2} for 5 h at room temperature. The gels were stained with Coumassie brilliant blue R-250 (Ferak, Berlin) according to Fairbanks (1977).

Molecular-weight Determination. The following proteins were reduced, alkylated, freeze-dried, and used as MW standards: human serum albumin (68,000 daltons), bovine glutamate dehydrogenase (53,000), human γ-globulin, H-chain (50,000), ovalbumin (43,000), γ-globulin, L-chain (23,500), pancreatic ribonuclease (13,700), and cytochrome c (12,400). A mixture containing ca. 250 μg of each of proteins in 1 ml was boiled for 2 min, and 10 μl were applied as an MW standard. The relative mobility values were plotted against log MW of the standards, and the MW of the globulin subunits were estimated from this plot.

The human serum albumin was obtained from Reanal, Hungary; the bovine glutamate dehydrogenase from Ferak, Berlin;

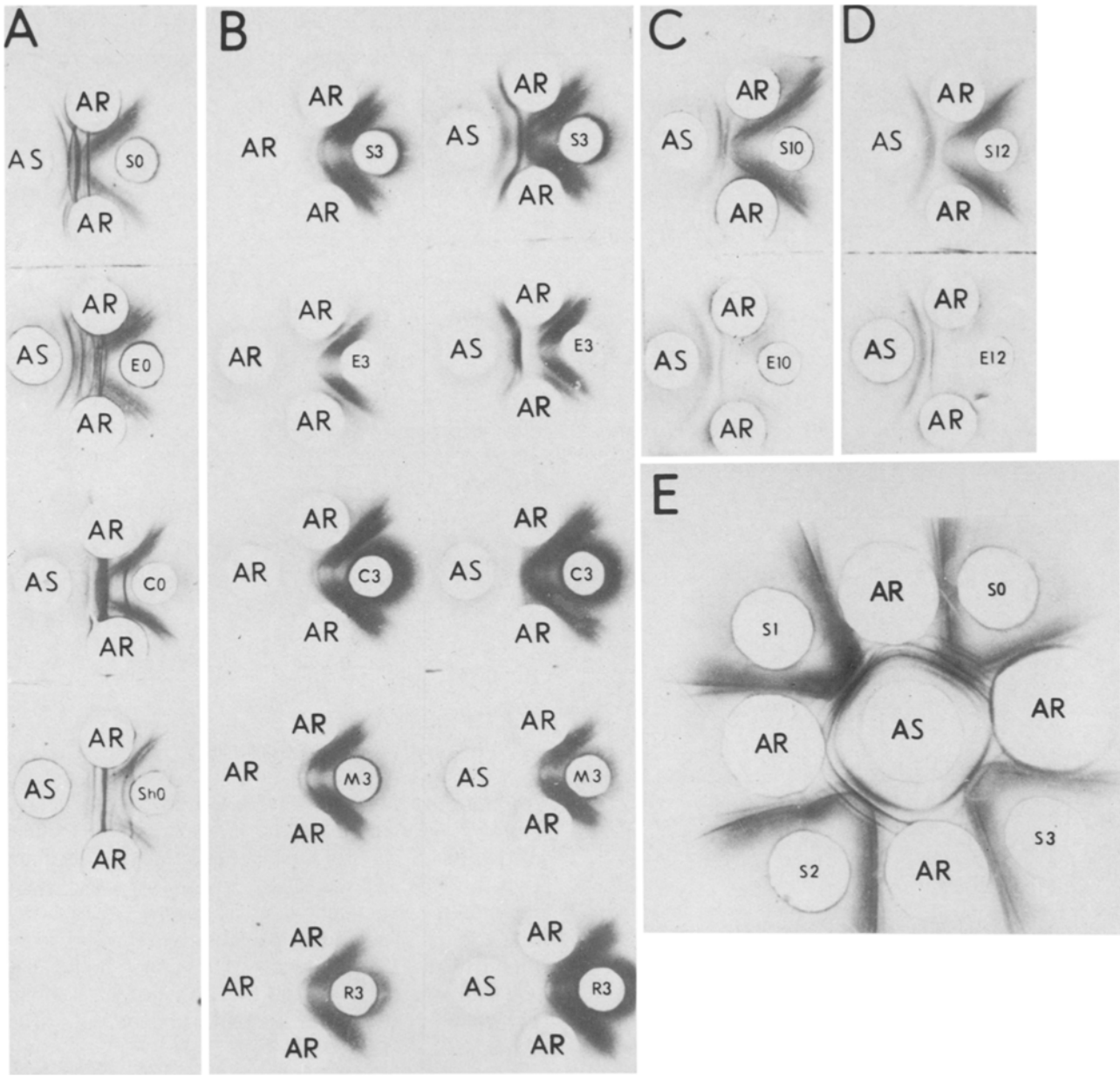

Fig. 1 A–E. The EA spectra in various parts of germinating caryopses and seedlings of maize (double immunodiffusion test with absorbing barrier). **A** non-germinated caryopses; **B** 3-d-old seedlings (shown also are control tests for the completeness of common antigen absorption); **C, D** 10- and 12-d-old seedlings; **E** changes of the scutellar EA in the course of germination. Protein samples: *C*, coleoptile; *E*, endosperm; *M* mesocotyl; *R*, root; *S*, scutellum; *Sh*, shoot (the upper part of the embryonic axis). The figures next to the letters stand for days of germination and seedling growth. Antisera: AS to proteins from the scutella of non-germinated caryopses; AR to proteins from 10-mm root tips of 3-d-old seedlings

the human γ-globulin was a local pharmaceutical preparation; the ovalbumin was obtained from the laboratory of Professor A.S. Zyperovich, Institute of Biochemistry, Ukrainian Academy of Sciences, Kiev, USSR; the bovine pancreatic ribonuclease (4 × recryst.) from U.K.; the cytochrome c from Polfa, Poland

Immunochemical Methods

Rabbit antisera were obtained by subcutaneous injections of proteins either from the scutella of mature, non-germinated caryopses (AS), or from 10-mm-long root tips of 3-d-old seedlings (AR), and of partially purified globulin preparations (AG = fraction P5, see above, section "Isolation and Purification of Globulins"). In addition, as globulin preparations usually contain traces of non-globulin proteins (see review by Millerd, 1975), AG was absorbed with the root proteins except for the absorbing barrier tests. Both the immunization protocols and the techniques of one-dimensional immunoelectrophoresis and double immunodiffusion with the AR absorbing barrier for the common antigens (CA) were described in detail earlier (Khavkin et al., 1977). In some

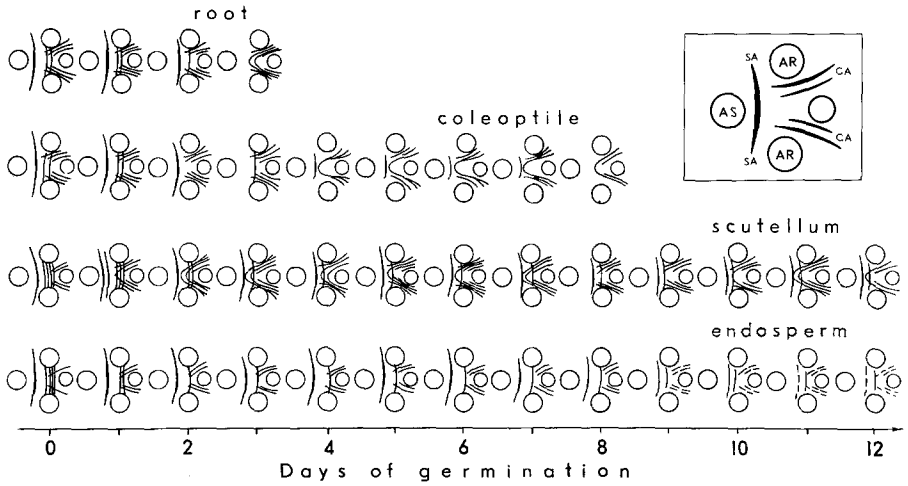

Fig. 2. A graphical presentation of the general temporal pattern of the EA spectra in germinating caryopses and seedlings of maize. The layout of the barrier absorption test is shown in the upper right corner. CA = common antigens; SA = specific antigens; antisera as in Figure 1

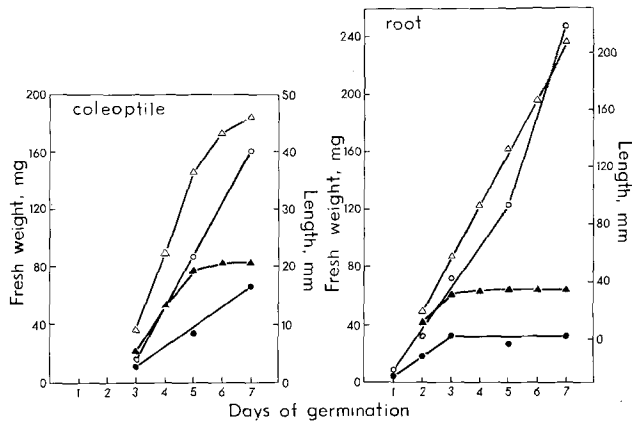

Fig. 3. Root and coleoptile growth of maize seedlings from normal (open symbols) and γ-irradiated (solid symbols) caryopses. ○,● fresh weight; △,▲ length

cases, when the CA concentration was relatively low, better resolution of the EA could be obtained in intragel CA absorption tests by introducing up to 20% (v/v) AR to agar gels before pouring on glass plates (Kabat and Mayer, 1964). All experiments included control tests for completeness of the CA absorption and for precipitation by non-immunized rabbit sera and cryoprecipitation. Using purified globulin preparations we determined the sensitivity of the double immunodiffusion test with the absorbing barrier to be between 1 and 5 μg of specific protein in the presence of 500–600 μg of common antigen proteins. The technique of Heidelberger was employed for quantitative immunoprecipitation of globulins: 50 μg of protein in 0.36 ml of cell-free tissue extract in buffered saline were added to 0.14 ml (2.7 mg) AG, and the protein content of washed precipitates was measured with Lowry Cu-Folin reagent (see Kabat and Mayer, 1964).

Analytical Methods

Total-nitrogen and protein-nitrogen content were determined by ammonia distillation after Kjeldahl digestion of air-dry tissue samples or protein precipitates. Protein content in tissue extracts and antisera was measured with the Cu-Folin reagent (Lowry et al., 1951) after TCA precipitation.

Results

The EA Patterns in the Course of Normal and Retarded Germination

Normal Caryopses. We have followed the EA degradation in the embryo and the endosperm in the course of the first 12 days of dark germination and seedling growth (Figs. 1, 2; see also Figs. 4, 8). In the endosperm three prominent EA disappeared in the very first 2 d, and no further qualitative changes were found in the EA spectrum, as traces of minor EA could be observed even on the 12th day of seedling growth after additional concentration of the tissue extracts.

The embryo exhibited a totally different pattern of EA degradation. During the 2nd and 3rd days of germination all EA had disappeared in the root and the mesocotyl, while several minor EA were retained in the coleoptile and the mesocotyl node (2–3-mm-long segment, including the intercalary meristem and the plumule with the developing leaves). The scutellum was last to lose the transient EA, and the minor components could still be traced up to the 12th day of seedling growth.

γ-Irradiated Caryopses. Under our experimental conditions, root and coleoptile growth in γ-seedlings were substantially slowed down starting on the 2nd day of germination, root growth stopping by the 3rd day and the elongation of the coleoptile by the 4th while

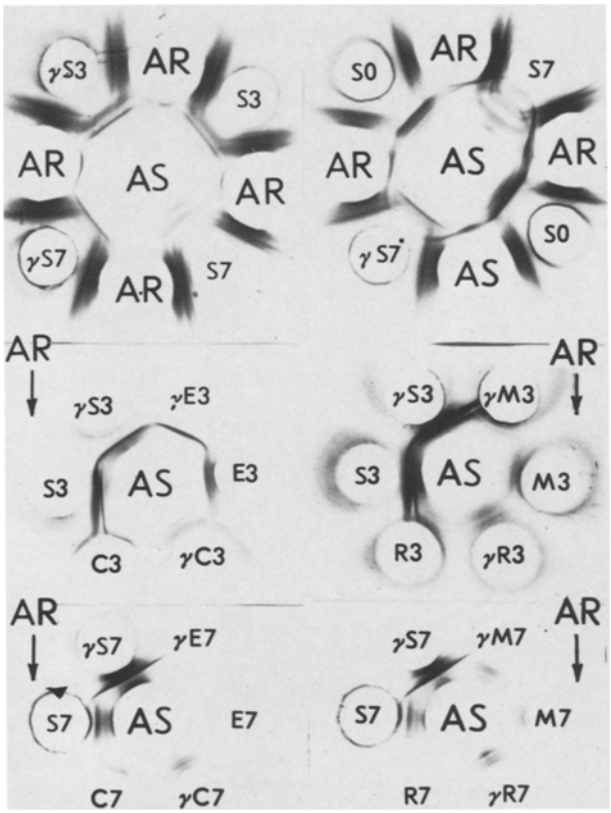

Fig. 4. EA spectra in various parts of 3- and 7-d-old normal and γ-seedlings of maize. The intra-gel absorption with AR is shown by an arrow; otherwise as in Figure 1

increase in thickness and in fresh weight of the latter organ continued until the 7th day of germination (Fig. 3). In agreement with these changes in the growth of the different parts of γ-seedlings, as compared to non-irradiated controls, the disappearance of the transient EA was delayed or altogether arrested.

The EA patterns of various organs of 3- and 7-d-old control and γ-seedlings are compared in Figure 4. In normal seedlings, the EA disappear first and entirely from the root and the mesocotyl, and it is in these same organs that growth retardation by γ-irradiation produces the most immediate effect on the EA spectra. By the 3rd day of germination the changes in the EA pattern are less prominent in the coleoptile, while no irradiation effects can be detected in the EA pattern of the scutellum. However, later on, when 7-d-old control and γ-seedlings were compared, the most striking differences in the EA composition were found in the scutellum and the coleoptile: the γ-plantlets had retained some of the transient EA while in the control seedlings only the minor EA were still present. Trace amounts of the transient EA were retained in the root and the mesocotyl of the γ-plantlets.

Thus, reduction and premature termination of seedling development slow down the disappearance of the major EA in the axial organs of the embryo and arrest EA breakdown in the scutellum. These temporal differences in EA behaviour agree well with the suggestion that the transient EA are storage proteins of the maize embryos.

Identification of Globulins among the EA

Globulins of Non-germinated Caryopses. In dicotyledonous seeds the reserve proteins are usually globulins located within the axis and the cotyledons of the embryo. Based upon this fact we assumed that principal protein reserves of maize embryos were also globulins. Therefore we estimated the globulin content in the embryo, and investigated some serological and chemical properties of these proteins.

Globulin content in the axis is somewhat higher than in the scutellum, and as a whole this fraction constitutes up to 40% of total embryonal protein (Table 1).

The crude globulin fraction (P2) isolated from the scutella of non-germinated caryopses exhibited most of the precipitin lines characteristic of the EA spectrum in the saline extract (P1) of the scutella (Fig. 5A). By further purification of P2 we obtained globulin preparations P4 and P5 that corresponded to the major EA of P1 while the P3 and P6 preparations were serologically entirely different from the globulins and seemed to correspond to the minor EA (see Fig. 5A). Preparations P7 and P8 were obtained by further subfractionation of P5 by isoelectric precipitation and can on this basis be provisionally

Table 1. Protein content in the embryos of non-germinated maize caryopses

Part of embryo	Fresh weight	Protein nitrogen (mg g^{-1} fr. wt.)[a]				Globulin as % of total
	(mg)	Total	Salt-soluble	Albumin	Globulin	
Axis	3.02	33.1 ± 0.1	30.3 ± 0.3	14.1 ± 1.6	15.6 ± 0.8	47
Scutellum	18.5	17.4 ± 0.1	11.6 ± 0.6	5.4 ± 0.7	6.5 ± 0.2	37

[a] Mean ± standard error of the mean of four experiments

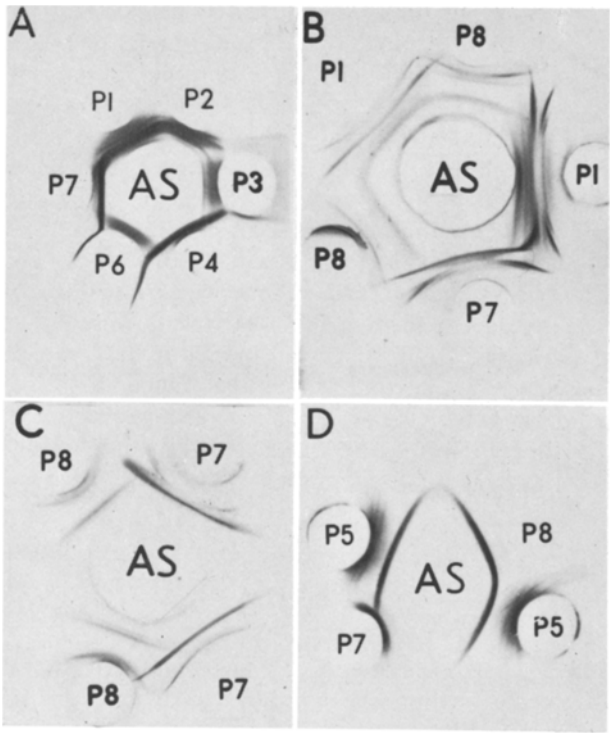

Fig. 5 A–D. The EA among the scutellar proteins of maize at successive stages of fractionation. Intra-gel absorption with AR. P1–P8 as described in Material and Methods: Isolation and Purification of Globulins. **A** first steps of globulin fractionation; **B**, **C** P7 and P8 isolated from crude P5; **D** same from twice-purified P5

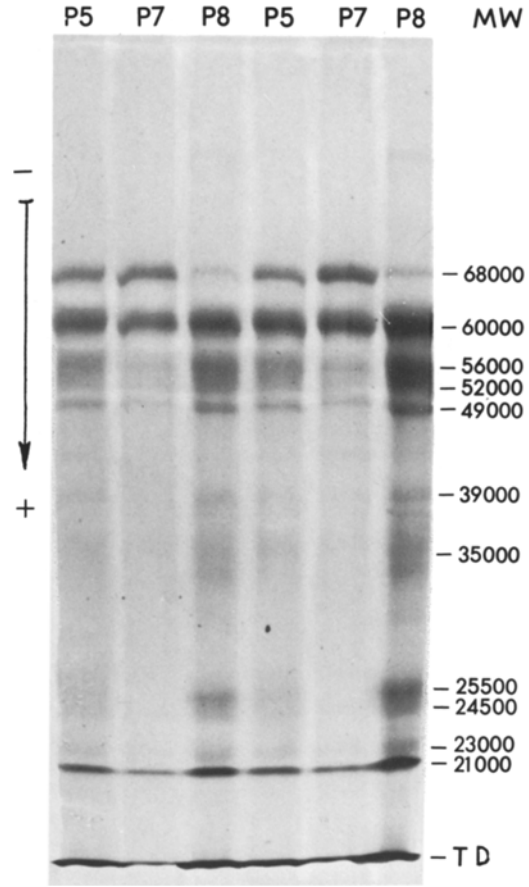

Fig. 6. SDS-PAGE separation and determination of molecular weight of maize globulins. *MW* Molecular weight (daltons); *TD* tracer dye; otherwise as in Figure 5

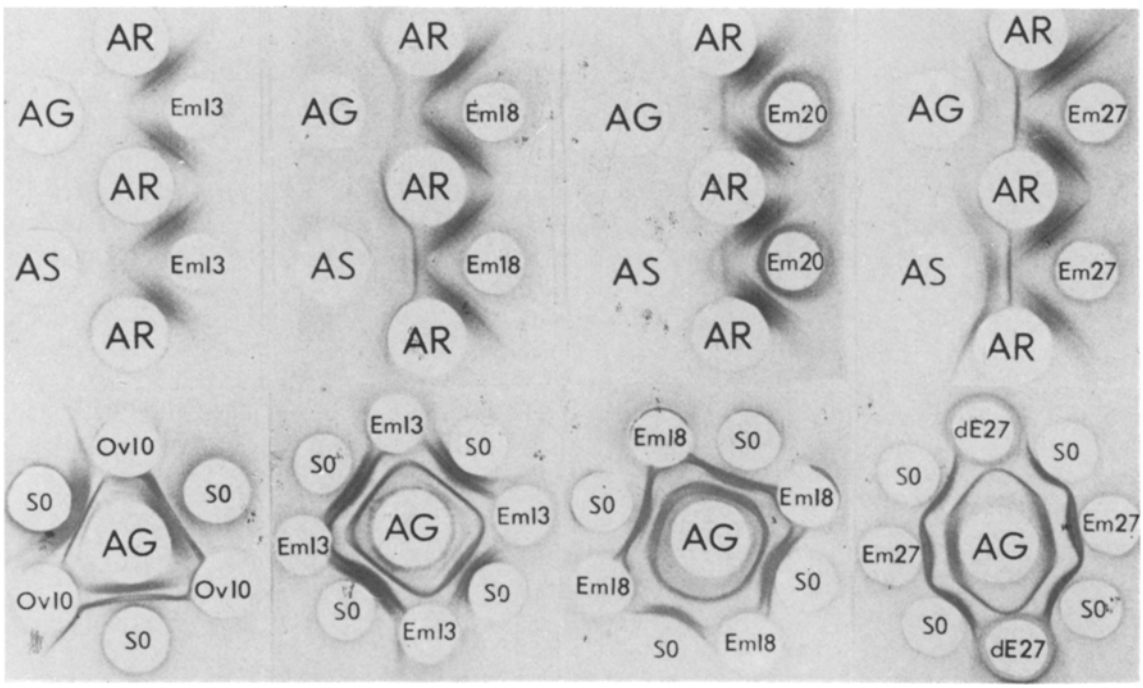

Fig. 7. Globulins among the EA of developing maize caryopses. AG, antiserum to the scutellar globulins. dE = developing endosperm; Em embryo; Ov ovary. Figures next to letters = days after pollination

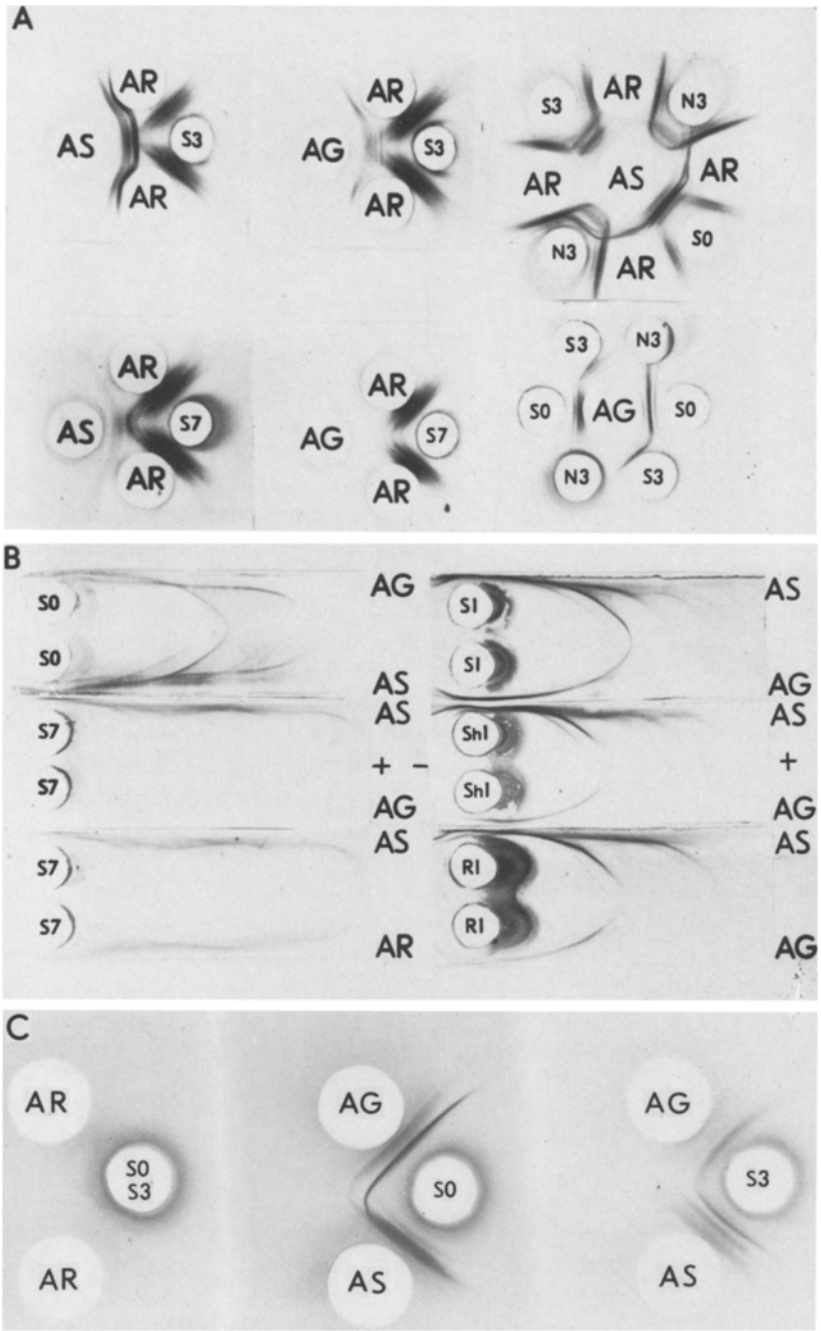

Fig. 8A–C. Globulins among the EA of non-germinated and germinated caryopses and in seedlings of maize.
A double immunodiffusion with absorbing barrier;
B immunoelectrophoresis;
C classic Ouchterlony test of identity (common antigens removed first by batch and then by intragel absorption with AR). *N* mesocotyl node; otherwise as in Figures 1 and 7

designated as legumin- and vicilin-like globulins. The antigen patterns of P7 and P8 depend to a large extent on the degree of the preliminary purification of P5: repeated salt fractionation and dialysis of P4 produce P7 and P8 preparations practically free of other antigens, yet the solubility and mobility of P7 may decline after these procedures (compare Fig. 5B-D).

Preparations of P5, P7 and P8 that had been purified, most extensively were subjected to SDS-PAGE analysis (Fig. 6). Bands with the MW of 21,000, 60,000 and presumably 49,000 and 56,000 daltons were common of P7 and P8; a 68,000 band was characteristic of P7; while subunits of 23,000, 24,500–25,500, 35,000, 39,000 and 52,000 daltons were specific for P8. Some weak bands in the P7 and P8 spectra seem to arise because of reciprocal contamination during isoelectric precipitation.

Demonstration of Globulins Among the EA of Developing and Germinating Caryopses using AG. When glob-

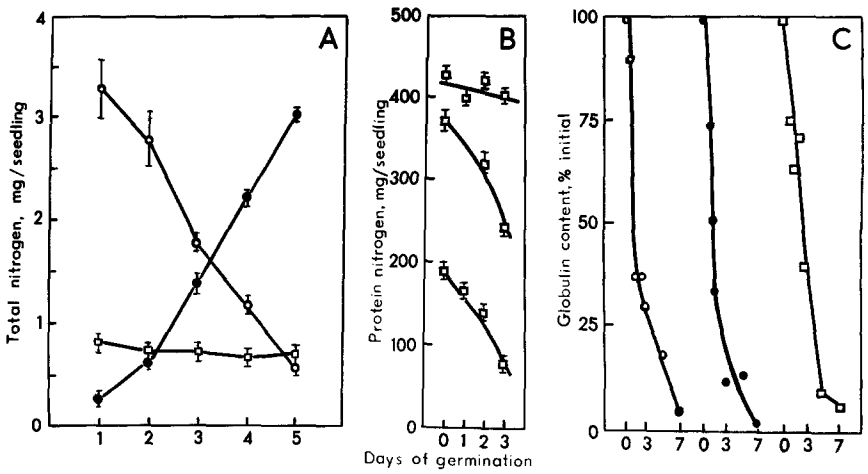

Fig. 9A–C. Redistribution of nitrogen in relation to globulin degradation during germination and early seedling growth in maize.
A total nitrogen: ○ endosperm, ● embryonic axis, □ scutellum;
B protein nitrogen in scutellum: top curve = total, center curve = salt-soluble, bottom curve = globulins precipitated by dialysis.
C specific globulins precipitated with AG: symbols as in **A**.
Vertical bars in **A** and **B** = standard errors

ulins are purified to the P5 step to produce AG, it cannot be ruled out that some components are lost; however, the antigen spectra are sufficiently simplified to permit direct identification of the major globulins among the EA (see Figs. 7, 8).

Earlier it has been shown that the genotype of different maize lines does not substantially affect the EA patterns (Khavkin et al., 1978). Therefore the data on developing caryopses of C103 and W155 inbreds can be related to the previously reported data for the Bukovinsky 3 hybrid as the temporal patterns of caryopsis development (as estimated by the dimensions and morphology of the embryo). Also, EA accumulation was practically the same in experiments conducted in 1974–1975 and in 1977–1978. EA were absent from the unpollinated ovary, and on the 10th to 13th day after pollination (DAP) the first of the EA that did not belong to globulins could be found. One of two EA found by the 18th–20th DAP in the embryo was precipitated with AG. On the 27th DAP all globulins appeared in the embryo, while no antigens had yet been precipitated by AG in the endosperm (Fig. 7).

The utilization of AG in the study of non-germinated and germinated caryopses provided clear evidence that the transitory EA are globulins (compare Figs. 1, 2 with Fig. 8). Two principal globulins of low electrophoretic mobility (Fig. 8b) can be associated with those designated earlier as the α- and β-EA (Khavkin et al., 1977). Thus, by their behaviour during germination it may be concluded that the major embryonal proteins with a reserve function are globulins.

Redistribution of Nitrogen in the Germinating Caryopsis

We related the time course of globulin breakdown in the embryo to the general pattern of nitrogen mobilization and redistribution during germination and early seedling growth (Fig. 9). These processes appear to proceed in two stages. 1. The first 3 d of germination are characterized by a slow loss of total nitrogen from the endosperm and its slow accumulation in the rapidly growing axial organs, while their specific globulin content drops to 30% and 12%, respectively, of the initial value. In the scutella, rapid decrease in salt-soluble protein and globulin content occurs between the 2nd and the 3rd day of germination. 2. The following days are characterized by rapid translocation of nitrogen from the endosperm to the axis, while the residual 40% of the globulins in the scutellum are degraded. This quantitative pattern accords well with the qualitative data presented in Figures 1, 2 and 8. It is noteworthy, however, that the total nitrogen content of the scutellum shows no significant changes during the entire period studied.

Discussion

The evidence presented above indicates that characteristic proteins accumulate in the course of the development of the maize caryopsis mainly in the embryo, but also in the endosperm. The most abundant ones among these proteins are globulins. The MW values of the globulin subunits of maize, determined by SDS-PAGE are largely in accordance with the values compiled by Millerd (1975) and Derbyshire et al. (1976) and also reported in recent papers by Barker et al. (1976) and Hall et al. (1977). Luthe and Peterson (1977) reported major components 21,000 and 36,000 daltons and a minor polypeptide of 56,000 in oat globulin preparations; these values are also in line with some of data for maize.

The temporal patterns of accumulation and degradation of globulins in developing and germinating maize caryopses are consistent with the previous time-

course studies on the increase and decrease in the α- and β-EA content as estimated by cross immunophoresis (Khavkin et al., 1977). As a whole, the massive phase-specific accumulation of globulins in developing maize embryos appears to be quite similar to the system investigated in rice (Horikoshi and Morita, 1975) and in more detail in peas and beans (see review by Millerd, 1975). While the globulin content in embryos of maize is lower than that in the embryos of legumes (up to 70–90%), it satisfies the 5% criterion of storage proteins suggested by Derbyshire et al. (1976).

When speaking of storage proteins in cereals, globulins are usually given far less consideration than prolamins, even though it is globulins that constitute the bulk of protein, for example, in oat caryopses (Brohult and Sandgren, 1954; Luthe and Peterson, 1977). Different globulin components are localized preferentially in the cereal embryo and endosperm (Brohult and Sandgren, 1954; Horikoshi and Morita, 1975), however, their respective physiological roles have not yet been elucidated. We feel it reasonable to relate the existence of different types of storage protein in cereals to the phenomenon of the two-step mobilization of storage substances in germinating seeds. This problem was noted by Sachs as early as in the 19th century and later by Haberlandt and Purievich, particularly in relation to nitrogen reserves (for review, see Lehmann and Aichele, 1931).

At the very beginning of germination, before any noticeable breakdown of endosperm protein in cereals, castor beans, etc., can be seen the growth of the seedling is provided for by nitrogen reserves in the embryonic tissues (compare also Rost, 1972). Our data seem to indicate that it is embryonal globulins that serve as the immediate protein reserves for the growing axial organs of cereal seedlings. As in the case of legumes (for references see Chrispeels and Boulter, 1975), globulins completely disappear first from the growing axis. In the scutellum some decrease in globulin content seems to proceed from the very beginning of imbibition; however, from the scutellum, as from the legume cotyledons, globulins disappear completely only much later. The period of globulin degradation in the embryonic axes of maize and the breakdown of the bulk of globulins in maize scutella coincide with the beginning of nitrogen mobilization in the endosperm, while residual scutellar globulins degrade when the development of the seedling is already also supported by nitrogen released from the endosperm.

This is the pattern of gradual mobilization of storage proteins that we visualize in the germinating maize caryopsis. We would conclude by emphasizing how important it appears to understand the specific features of degradiation of intracellular protein reserves of the cereal *embryo* since the intracellular localization of protein deposits, the specificity of proteolytic enzymes, and the mechanisms triggering proteolysis and their hormonal regulation have been investigated in the embryo to a far lesser extent than have the protein bodies and protein breakdown in the aleurone layer and the starchy endosperm (for a recent review see Ashton, 1976).

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References

Ashton, F.M.: Mobilization of storage proteins of seeds. Ann. Rev. Plant Physiol. **27**, 95–117 (1976)

Barker, R.D.J., Derbyshire, E., Yarwood, A., Boulter, D.: Purification and characterization of the major storage proteins of *Phaseolus vulgaris* seeds, and their intracellular and cotyledonary distribution. Phytochemistry **15**, 751–757 (1976)

Brohult, S., Sandgren, E.: Seed proteins. In: The Proteins. Chemistry, biological activity and methods, vol. 2, pt. A, pp. 487–512, Neurath, H., Bailey, K., eds. New York: Academic Press 1954

Cavins, J.F., Friedman, M.: Specific modification of protein sulphhydryl groups with α,β-unsaturated compounds. J. Biol. Chem. **243**, 3357–3360 (1968)

Chrispeels, M., Boulter, D.: Control of storage protein metabolism in the cotyledons of germinating mung beans: role of endopeptidase. Plant Physiol. **55**, 1031–1037 (1975)

Derbyshire, E., Wright, D.J., Boulter, D.: Legumin and vicilin, storage proteins of legume seeds. Phytochemistry **15**, 3–24 (1976)

Fairbanks, G., Steck, T.L., Wallach, D.F.H.: Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. Biochemistry **10**, 2606–2617 (1971)

Haber, A.H.: Ionizing radiations as research tools. Ann. Rev. Plant Physiol. **19**, 463–489 (1968)

Hall, T.C., McLeester, R.C., Bliss, F.A.: Equal expression of the maternal and paternal alleles for the polypeptide subunits of the major storage globulin of the bean *Phaseolus vulgaris* L. Plant Physiol. **59**, 1122–1124 (1977)

Horikoshi, M., Morita, Y.: Localization of γ globulin in rice seed and changes in γ globulin content during seed development and germination. Agr. Biol. Chem. **39**, 2309–2314 (1975)

Ivanov, V.B.: Cellular fundamentals of plant growth. [In Russ.] Moscow: Nauka 1974

Kabat, E.A., Mayer, M.M.: Experimental immunochemistry. Springfield: Thomas 1964

Khavkin, E.E., Misharin, S.I., Ivanov, V.N., Danovich, K.N.: Embryonal antigens in maize caryopses: the temporal order of antigen accumulation during embryogenesis. Planta **135**, 225–231 (1977)

Khavkin, E.E., Misharin, S.I., Monastyreva, L.E., Polikarpochkina, R.T., Sukhorzhevskaja, T.B.: Specific proteins maintained in maize callus cultures. Z. Pflanzenphysiol. **86**, 273–277 (1978)

Laemmli, U.K., Favre, M.: Maturation of the head of bacteriophage T4 I. DNA packing events. J. Mol. Biol. **80**, 575–599 (1973)

Lehmann, E., Aichele, F.: Keimungsphysiologie der Gräser. Stuttgart: Enke 1931

Lowry, O.H., Rosenbrough, N.J., Farr, A.L., Randall, R.J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**, 265–276 (1951)

Luthe, D.S., Peterson, D.M.: Cell-free synthesis of globulin by developing oat (*Avena sativa* L.) seeds. Plant Physiol. **59**, 836–841 (1977)

Millerd, A.: Biochemistry of legume seed proteins. Ann. Rev. Plant Physiol. **26**, 53–72 (1975)

Misharin, S.I., Antipina, A.I., Reimers, F.E., Khavkin, E.E.: Differential degradation of embryonal antigens in the embryo of germinating maize seeds. [In Russ.] Dokl. Akad. Nauk SSSR **232**, 248–251 (1977)

Misharin, S.I., Mozgova, E.A., Reimers, F.E., Khavkin, E.E.: Reserve globulins among maize embryonal antigens. [In Russ.] Dokl. Akad. Nauk SSSR 1978

Rost, T.L.: The ultrastructure and physiology of protein bodies and lipids from hydrated dormant and nondormant embryos of *Setaria lutescens* (Gramineae). Am. J. Bot. **59**, 607–616 (1972)

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