Biochem. Physiol. Pflanzen **178**, 653—663 (1983)

Protein Antigens in the Differentiation of Maize Root and Scutellum

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Key Term Index: antigen, protein, differentiation; *Zea mays*

Summary

Two-dimensional immunoelectrophoresis and rocket-electrophoresis were used to investigate changes in the pattern of protein antigens in the differentiation of maize root cells. Differential changes in the pattern of cell antigens have been revealed. The appearance of two stage-specific antigens which were not organ-specific proteins is characteristic for mature root cells. Mitochondrial biogenesis in maize root and scutellum is accompanied by changes in mitochondrial antigen pattern.

Introduction

Growth and development of eukaryotic organisms accompanied by qualitative and quantiative changes in the pattern of cell proteins. Such changes, in turn, are the result of differential gene expression in the development (Korochkin 1981). Methods of immunochemical detection of individual protein species were used in present paper to study the changes in protein pattern of maize root cells being on several subsequent stages of the development. The appearance of new antigen in cells determined by two-dimensional immunoelectrophoresis (Laurell 1965) is indicative of new gene expression.

The immunochemical analysis has shown that growth and development of maize root cells was accompanied by differential changes in the pattern of cell antigens, however, quantitative changes predominated in the concentrations of individual protein antigens. The appearance of two stage-specific antigens which were not organ-specific proteins was characteristic for mature root cells.

Materials and Methods

Antigen protein preparation

Thoroughly washed maize seeds (hybrid Bukovinsky 3) were imbibed for 4 h and germinated at 27 °C. The roots of three-day etiolated seedlings were used for isolation of protein antigens. Total soluble proteins were extracted from freezed and grinded samples by 0.1 M tris-HCl buffer, *p*H 8.0, which contained 0.01 M 2-mercaptoethanol in the ratio 2 ml buffer per 1 g tissue. The extracting suspension was homogenized for 10 min at 2—4 °C and centrifuged at 20,000 · g for 20 min. The supernatants were used without additional purification for the immunoelectrophoretic analysis. Total soluble protein fractions, prepared for rabbit immunizations, were purified from low-molecular contaminations by gel-chromatography on Sephadex G-25 ("Pharmacia").

Mitochondrial fractions were prepared in the cold by differential centrifugation of root cell homogenates according to Ikuma and Bonner (1967) and Bourque and Naylor (1972). The grinding

medium contained 0.6 M mannitol, 0.005 M EDTA-Na_2, 0.04 M 2-mercaptoethanol and 0.05 M Tris-HCl buffer, *p*H 8.0. The wash medium contained 0.3 M mannitol, 0.01 M Tris-HCl buffer, *p*H 7.2.

Immunochemical methods

The immune antisera were prepared by injecting rabbits with total soluble proteins and mitochondrial proteins. 2 ml buffed saline containing 20 mg protein was mixed with 2 ml of complete Freund's adjuvant and injected subcutaneously at four locations on the rabbit's back. This was repeated twice at two-week intervals and the final bleeding was performed 15 d after the last injection.

The following antisera were obtained- anti-1- to total soluble proteins of root meristem, anti-2- to proteins of root elongation zone, anti-3- to proteins of mature two-day maize seedling, anti-M- to mitochondrial proteins of mature root cells. Immunoglobulin fractions of antisera were obtained by double precipitation with ammonium sulfate at 33% saturation. The precipitate was solved in buffed saline, *p*H 7.2, dialised against buffered saline. Immunoglobulin fractions with protein concentration 40 mg/ml were stored at —20 °C till the use. One-dimensional immunoelectrophoresis was performed at *p*H 8.6 1% agarose gel. The buffer was 0.025 M barbital — sodium barbital. For immunoelectrophoretic analysis of mitochondrial proteins 0.5% Triton X-100 ("Sigma") was added into agarose gels.

Two-dimensional (crossed) immunoelectrophoresis was performed according to LAURELL (1965) with slight modifications. Electrophoresis in the second dimension was performed at right angles to the primary electrophoretic run in 90×120×2 mm antibody-containing agarose gel, formed after primary electrophoretic separation by pouring 21.6 ml agarose contained 2 ml immunoglobulin fraction of antiserum on the remaining part of the glass plate. This electrophoresis was done at 4 °C and 4 volt/cm for 20 h.

Rocket-electrophoresis in an agarose gel containing specific antibodies was carried out as described by LAURELL (1966). Only 0.5% Triton X-100 was added into agarose gel for electrophoresis of mitochondrial proteins.

After electrophoresis, gels were washed with buffered saline and incubated in corresponding media for dehydrogenase zymogram identification (GEYER 1973). Dried immunoelectropherograms were obtained with 0.2% solution of Coomassie Brilliant Blue ("Sigma") in 7% acetic acid, 50% ethanol.

Results and Discussion

Rocket electrophoresis with zymogram identification of some antigens

Do cells at sequential stages of growth and development differ in enzyme content? The concentration of enzyme protein but not enzyme activity in vitro which depends on concentrations in the tissue of inhibitors, activators, coenzymes, ect. is of particular interest. Though the positive answer on this question has been received due to the numerous investigations with different eukaryotic organizms, we wanted to solve these questions with developing maize seedling using most direct and simple method. By this aim rocket-electrophoresis of total soluble proteins of root growth zones as well coleoptile and scutellum proteins was used. Agarose gel contained antibodies to the proteins of elongated root cell (anti-2). Each sample was applied in three different protein concentrations. Zymogram identification of glucose-6-phosphate dehydrogenase (EC 1.1.1.49), 6-phosphogluconate dehydrogenase (EC 1.1.1.44), glutamate dehydrogenase (EC 1.4.1.2) and malate dehydrogenase (EC 1.1.1.37) was carried out after rocket-electrophoresis. Rocket-electrophoresis is shown in Fig. 1. The height of the

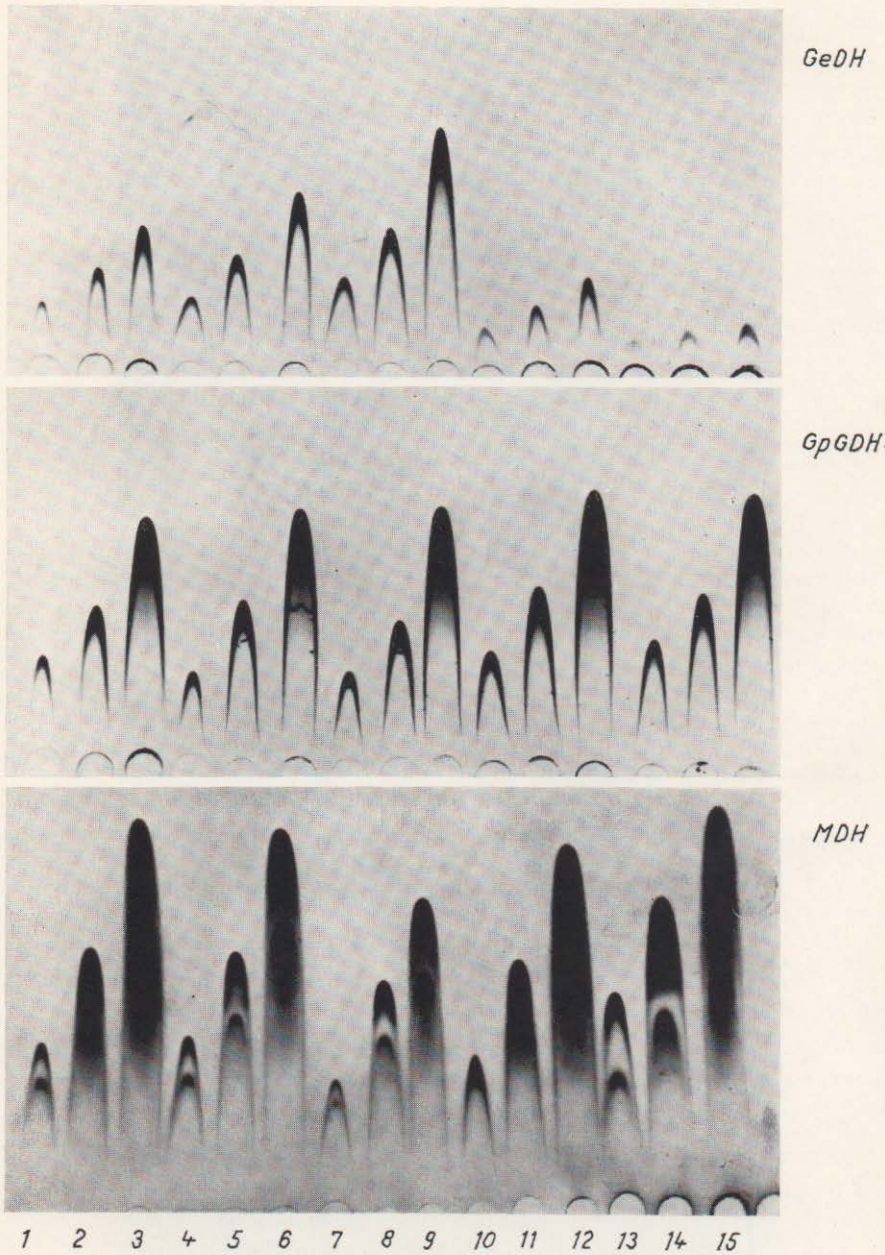
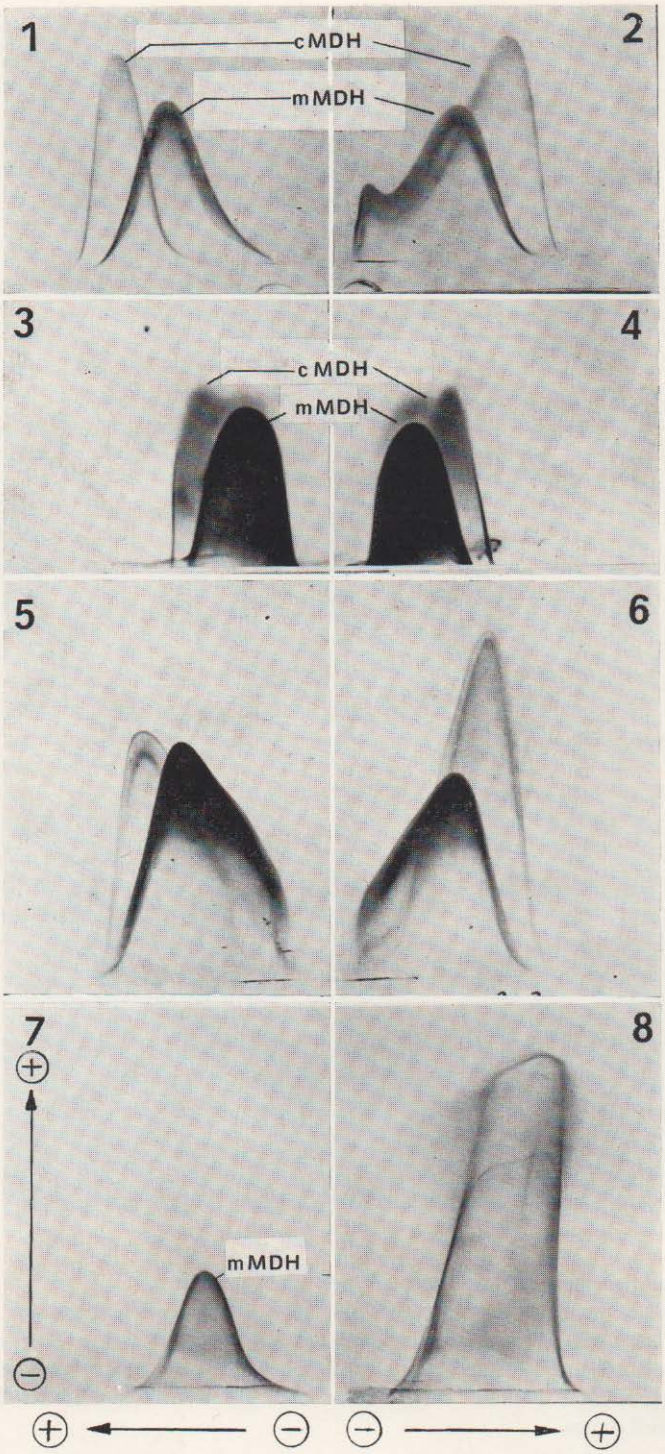


Fig. 1. *Rocket-electrophoresis with zymogram identification of enzymes.*
6pGDH — 6-phosphogluconate dehydrogenase, GeDH — glutamate dehydrogenase, MDH — malate dehydrogenase. Total soluble proteins of meristem (1—3), elongation zone (4—6) and mature cells (7—9) of roots, coleoptiles (10—12) and scutellums (13—15) of 2-day maize seedlings were separated by rocket-electrophoresis. Proteins of each sample were applied in three different dilutions. Agarose gel contained anti-2 serum.


1
cMDH
mMDH
2
3
cMDH
mMDH
4
5
6
7
mMDH
8

rocket referred to the amount of total soluble protein applied on the gel characterizes the concentration of enzyme protein in total soluble protein. The results of this experiments suggest that the concentrations of glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and malate dehydrogenase (2 antigens — mitochondrial and cytoplasmic) in total soluble protein changed slightly during growth and development of root cells and remained on the same level in coleoptile and scutellum. In contrast to these enzymes, a noticable increase (almost 3 times) in the concentration during the differentiation of root cells is characteristic of glutamate dehydogenase, and on the contrary its concentration is sharply decreased in the total protein isolated from 2-day coleoptile and scutellum (to 28 and 14% accordingly with respect to the concentration in mature root cells).

Two-dimensional immunoelectrophoresis with zymogram identification of malate dehydrogenase

The existence of 2 main groups of isoenzymes — mitochondrial and cytoplasmic which are distinguished by several characteristics including immunochemical properties has been established for malate dehydrogenase in different eukaryotic organisms (DÖLKEN et al. 1974; YANG and SCANDALIOS 1974). Rocket-electrophoresis is not sufficient to study in details changes of antigen with malate dehydrogenase activity during the development of maize roots. It is necessary to use two-dimensional immunoelectrophoresis. The results of zymogram identification of antigens with malate dehydrogenase activity are shown on Fig. 2.

Two antigens with malate dehydrogenase activity were identified by anti-2 serum and only one antigen with less electrophoretic mobility was identified by anti-M serum. Relatively constant ratios between mitochondrial and cytoplasmic antigens are characteristic for growing maize root (Fig. 2: 3, 4), however, in some other cases it can change. Thus, this ratio decreases in the scutellum of germinating maize seeds in comparison with one in the scutellum of ripening maize seeds (Fig. 2: 5, 6). Concentration of mitochondrial malate dehydrogenase in mitochondrial malate dehydrogenase in mitochondrial protein can change also. For example, this concentration increases almost in 5—6 times for scutellum mitochondrial protein in 4 d after the beginning of seed germination (Fig. 2: 7, 8). Such changes of the malate dehydrogenase concentration in mitochondrial protein reflect the degree of mitochondrial differentiation. In weakly differen-

Fig. 2. *Malate dehydrogenase isoenzymes in antigen patterns of total soluble and mitochondrial proteins.* cMDH — cytoplasmic, mMDH — mitochondrial isoenzyme. Agarose gel contained anti-2 serum (1, 2, 3, 4, 5, 6) or anti-M serum (7, 8). Proteins of the following sample were studied: 1 — embryonic axises from developing maize seeds in 38 d after pollination. 2, 3 — meristematic and 4 — mature root cells of 2-day maize seedlings, 5 — embryonic scutellums from developing maize seeds in 38 d after pollination, 6 — scutellums of 2-day maize seedlings, 7 — mitochondria from scutellum of dormant maize seeds, 8 — mitochondria from scutellum of 4-day maize seedlings.

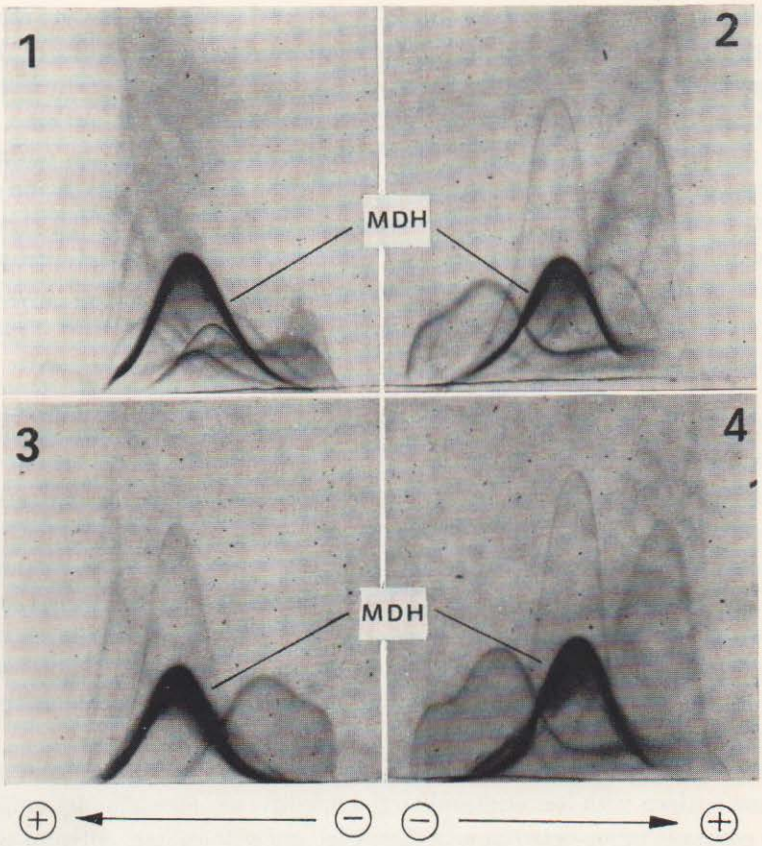


Fig. 3. *Malate dehydrogenase identification among mitochondrial antigens.*
MDH — antigen with malate dehydrogenase activity revealed by zymogram staining. Mitochondrial proteins of the following samples were investigated: 1 — mature root cells, 2, 4 — scutellums from 2-day maize seedlings, 3 — embryonic scutellum from the developing maize seeds 41 d after pollination. Gels contained anti-M serum.

tiated organelles the content of matrix protein is considerably less than in mature, actively functioning organelles (IVANOV and KHAVKIN 1976a). The identification of malate dehydrogenase antigen among other mitochondrial antigens is shown in Fig. 3. While the malate dehydrogenase antigen was identified by zymogram staining, other mitochondrial antigens were stained with Coomassie Brilliant Blue. It was revealed that organ specificity exists in the concentration of individual mitochondrial antigens of root and scutellum of 3-day maize seedlings.

Thus, studying of some antigens with enzyme activity and mitochondrial antigens allowed to show the differential changes of concentrations of these antigens in germinating maize seedlings. What changes can be expected in more complex systems, e.g. total cell proteins at some sequential stages of growth and development?

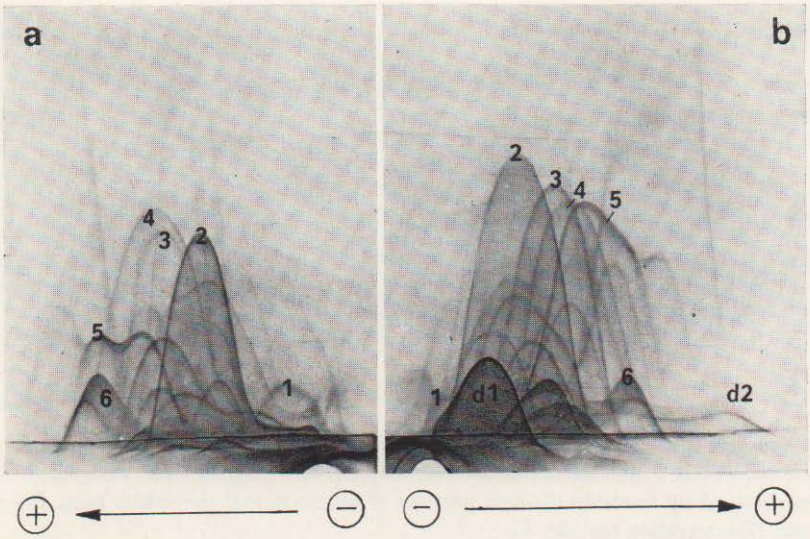


Fig. 4. *Antigens of meristem (a) and mature (b) root cells of two-day maize seedlings on two-dimensional immunoelectrophoregrams.*
Gel contained anti-3 serum. Main antigens: 1—6, d1, d2.

Two-dimensional immunoelectrophoresis of total soluble cell proteins

The use of two-dimensional immunoelectrophoresis has shown similarity in antigen pattern of root growth zones (meristem, elongation zone and mature cells). Identification of the same antigen but taken from different developmental stages was based on its electrophoretic mobility and shape of its precipitation peak. The appearance of 2 new antigens d_1 and d_2 which are absent in meristematic cells is characteristic of elongated and mature cells (Fig. 4). The appearance of d_1 antigen in cells of elongation zone was shown as well by Krøll's procedure (Krøll 1968) consisted in co-electrophoresis of comparable protein in first direction followed by electrophoresis in gels containing antibodies. As a result of such resolution antigen which is characteristic only for one sample the form of precipitation line doesn't change in comparison with usual two-dimensional immunoelectrophoresis (Fig. 5, d_1 antigen). The antigens d_1 and d_2 were stage-specific but not organ-specific proteins since they were present in coleoptiles, too.

The presence of a relatively small number of antigens in different tissues and organs of maize seedlings is one of the important features revealed when studying antigen spectra of total soluble proteins. The number of main antigens equal to 17 is shown on Fig. 4 where the antigen pattern of mature root cells is given. Besides that, 10—15 antigens in minor concentrations can be revealed additionally. Thus, a great number of antigens can be revealed. The complicated form of precipitation lines is characteristic of some antigens. There are precipitation lines with 2 and even 3 peaks (Fig. 4). It obviously illustrates the complexity of individual antigens, which appears to represent

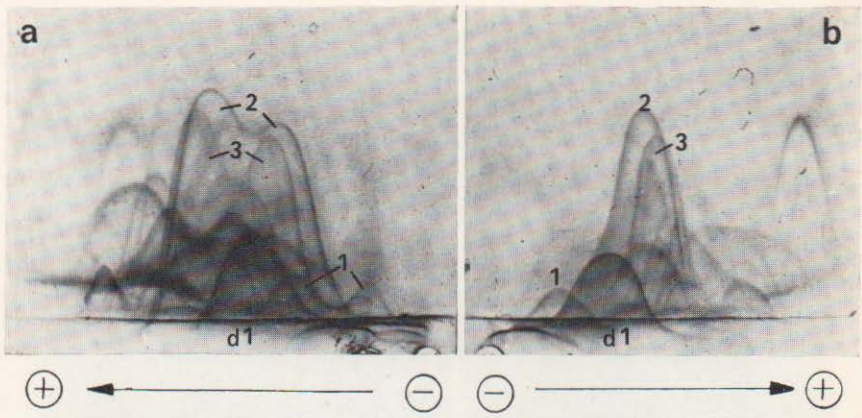


Fig. 5. *Comparison of antigen pattern of meristem and elongation zone by Krøll's procedure.*
a — typical pattern of 2-dimensional immunoelectrophoresis of meristematic antigens. b — electrophoregram obtained by co-electrophoresis proteins of meristem and elongation zone. Gel contained anti-2 serum. (Enumeration see Fig. 4).

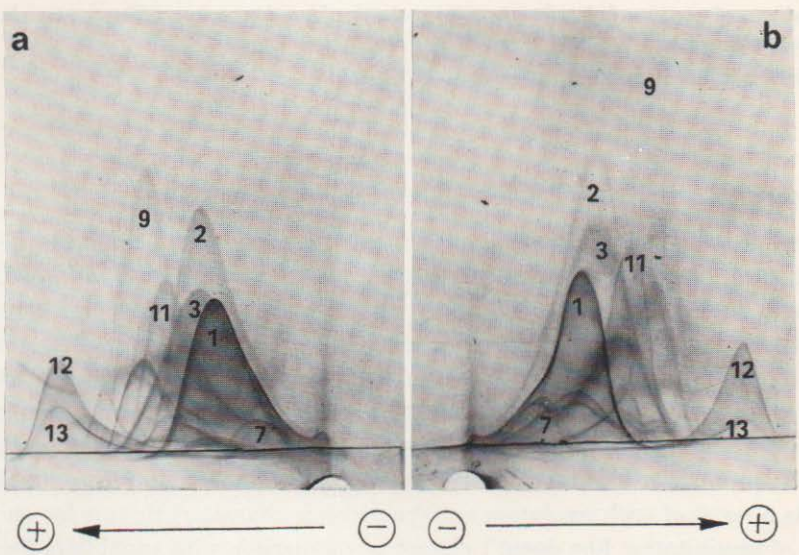


Fig. 6. *Pattern of mitochondrial antigens obtained by two-dimensional immunoelectrophoresis.*
The comparison of mitochondrial proteins of meristematic (a) and elongated root cells (b). Gels contained anti-M serum.

the whole protein family. Members of a such a protein family are characteristic of immunochemical similarity and differences of electrophoretic mobility and other features.

The existence of tissue- and organ-specific proteins has been established for many eukaryotic species (KOROCHKIN 1981). On the other hand, no organ-specific antigens were found in maize active functional organs and tissues (IVANOV et al. 1974). Each organ can be characterized by its antigen pattern. Qualitatively antigen pattern

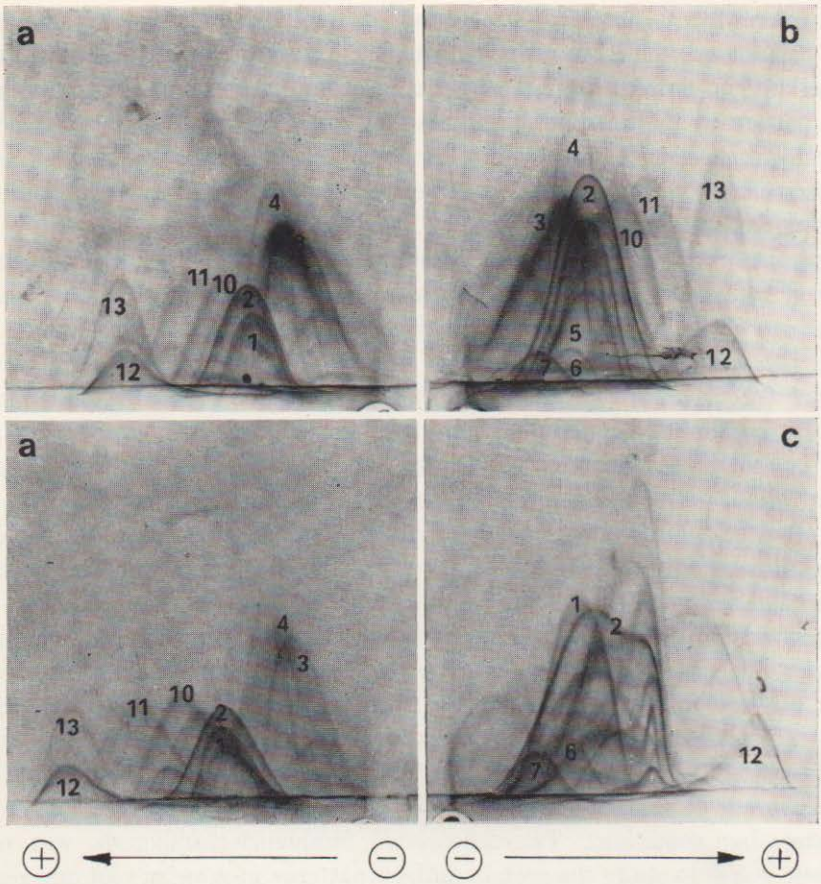


Fig. 7. *Mitochondrial antigen pattern obtained by two-dimensional immunoelectrophoresis.* The comparison of mitochondrial proteins from scutellum of dormant seeds (a), one-day (b), two-day (c) scutellum of germinating seeds. Gels contained anti-M serum. (Antigen numbers 1—13 should be read as s1 etc., s = scutellum).

are similar, but quantitative changes in concentration of individual antigens are mainly observed.

The problem of cell differentiation is closely connected with biogenesis of cell organelles. Mitochondrial antigen pattern changes during cell differentiation should be considered.

Two-dimensional immunoelectrophoresis of mitochondrial proteins

The study of mitochondrial antigen pattern by two-dimensional immunoelectrophoresis revealed that some sequential stages of cell growth and development were not characterized by qualitative changes. For example mitochondrial antigen pattern is relatively stable in meristem, elongation zone and mature cells of maize root (number of mitochondrial antigen is about 20), but the concentrations of individual mitochondrial antigens change (Fig. 6).

It is interesting to note that the periods of qualitative changes of mitochondrial antigen pattern were found in maize ontogenesis. This is the simplification of the mitochondrial antigen pattern at final stages of seed maturation and the complication of antigen pattern at the onset of germination of seeds. The results of two-dimensional immunoelectrophoresis of mitochondrial proteins isolated from scutellums of germinating maize seeds are shown on Fig. 7. The amount of protein applied on gels was proportional to mitochondrial protein content of 100 scutellums.

During the first day of germination considerable changes took place in mitochondrial proteins of scutellums, some new antigens appeared (including s5, s6, s7 antigens). The content of most mitochondrial antigens per scutellum increased 2—4 times. Between the first and the second day of germination the transformation of antigen pattern of mitochondria continued, and s3 and s4 antigen concentrations sharply decreased. During the 3rd d of germination a simultenous increase of mitochondrial antigen content in scutellum was observed. During the 4th d of germination the processes of partial mitochondrial degradation began, it led to significant simplification of the pattern of mitochondrial antigens isolated after 5 days of germination. Similar changes in the pattern of mitochondrial antigens were observed formerly in embryonal axes during seed germination (Ivanov and Khavkin 1976b).

Dodecyl sulfate polyacrylamide gel electrophoresis allow high resolution fractionation of proteins and has become a widespread standard technique in protein research (Maizel 1971). However, sodium dodecyl sulfate causes denaturation of the proteins and generally interferes with the assignment of function or immunochemical specificity to the separated bands. Useful supplement of this technique is two-dimensional immunoelectrophoresis of proteins which allow also high resolution of proteins but in native, nondenaturating conditions. Two-dimensional immunoelectrophoresis was used in the present paper to study the protein antigen patterns in growing root and scutellum cells.

The process of cell differentiation in maize root and scutellum is accompanied by the appearance of new antigens. This is evidence for differential expression of genes in organism development. However, the number of stage-specific antigens is small. The main changes consist in changes of concentration of enzyme proteins, mitochondrial antigen proteins, and other antigen proteins. However, such concentration changes may be the result of differentional gene expression in bounds of a gene family (McCarty 1980).

Acknowledgements

The author gratefully acknowledges E. E. Khavkin and S. I. Misharin for useful discussion.

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Received January 11, 1983; *accepted* April 4, 1983

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