PROTEIN PATTERNS OF DEVELOPING MITOCHONDRIA AT THE ONSET OF GERMINATION IN MAIZE (*ZEA MAYS* L.)

V. N. IVANOV and E. E. KHAVKIN

Siberian Institute of Plant Physiology and Biochemistry, USSR Academy of Sciences, Irkutsk 33, USSR

Received 2 April 1976

1. Introduction

The respiration increase during seed imbibition and the onset of germination is usually accompanied by significant mitochondriogenesis [1]. The latter process is manifested in characteristic development of organelle ultrastructure [2]. Changes in cytochrome spectra and respiration sensitivity to the electron transport inhibitors seem to suggest some rearrangement in the respiration chain [3]. An increase in the ratio of enzyme markers of mitochondrial matrix and inner membrane indicates the relative accumulation of matrix material [3]. However, it is not clear whether these developmental changes reflect differential de novo synthesis of mitochondrial proteins or merely rearrangements within mitochondria pre-existing in dormant seeds.

Two experimental approaches were initiated to answer this question. Quantitative immunochemical methods were applied in order to demonstrate changes in protein ratios and/or the origin of new antigens. Dual-label techniques were combined with SDS-disc-electrophoresis to evaluate de novo formation of polypeptides. The results show that alterations in the protein patterns may be attributed to the differential changes in synthesis and degradation rates of several groups of mitochondrial polypeptides.

2. Materials and methods

Thoroughly washed maize seeds (hybrid Bukovinsky 3) were imbibed for 4 h and germinated at 27°C. Mitochondria were isolated from the mature part (10–40 mm from the tip) of 3-day-old seedling roots or from the seed embryonal axes after 1–2 h imbibition [4]. Mitochondria were disintegrated by freeze-thawing in hypotonic medium, and about 70–75% of mitochondrial protein were solubilized in 1% Triton X-100 (final protein concentration 6 mg/ml). Two-dimensional (crossed) immunoelectrophoresis was performed using slightly modified procedure of Clarke and Freeman [5]. Agarose gel contained 0.1% Triton X-100 and antiserum against root mitochondria [6].

Roots were labelled by incubation of 3-day-old maize seedlings for 3 h in either D,L-[2-^{3}H]leucine (15 μCi/ml) or D,L-[1-^{14}C]leucine (24 μCi/ml) solution. To administrate label into the embryonal axes seeds were imbibed for 8 h in the solution of D,L-[1-^{14}C]leucine (80 μCi/ml). To prevent bacterial growth penicillin (20 μg/ml), streptomycin (20 μg/ml), and chloramphenicol (5 μg/ml) were added to incubation media. Mitochondria were isolated [4] from the combined ^{3}H- and ^{14}C-labelled samples. Proteins were precipitated by trichloroacetic acid (TCA) in cold, washed twice with 5% TCA containing non-labelled leucine and further with acetone, dissolved in 0.01 M Na-phosphate buffer, pH 7.2, containing 8 M urea, 1% SDS, and 1% 2-mercaptoethanol during 3–5 min at 95°C, and separated by SDS-disc-electrophoresis in 13%-polyacrylamide gels [7]. Gels were calibrated [7] using cytochrome *c*, ovalbumin, and bovine serum albumin as standards. After separation frozen gels were cut into 0.9 mm slices which were placed into scintillation vials containing 1 ml of 0.3% SDS solution, and incubated for 24 h at 40°C to ensure almost complete (95%) recovery of radioactivity in solution. ^{3}H and ^{14}C were counted in the same sample in a Wallac 81 000 liquid scintillation

counter (LKB) in 10 ml of dioxane counting solution using the external standard technique. $^{14}C/^{3}H$ ratios were determined to $< 10\%$ standard error.

3. Results

Fig.1 shows typical patterns of two-dimensional immunoelectrophoresis. At least twelve prominent precipitation arcs corresponding to the individual antigens were found in embryonal mitochondria of imbibing seeds while at least seventeen were present

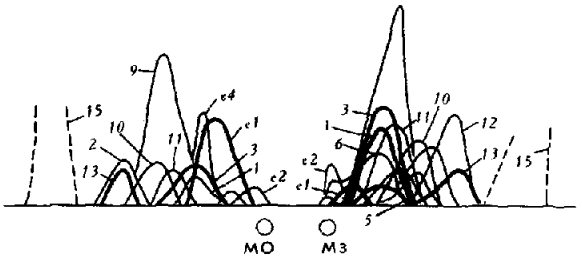

Fig.1. Two-dimensional immunoelectrophoretic analysis of mitochondrial antigens. (MO) embryonal axes mitochondria after 1–2 h seed imbibition, (M3) mitochondria of mature root cells. Each sample contained 250 μg protein.

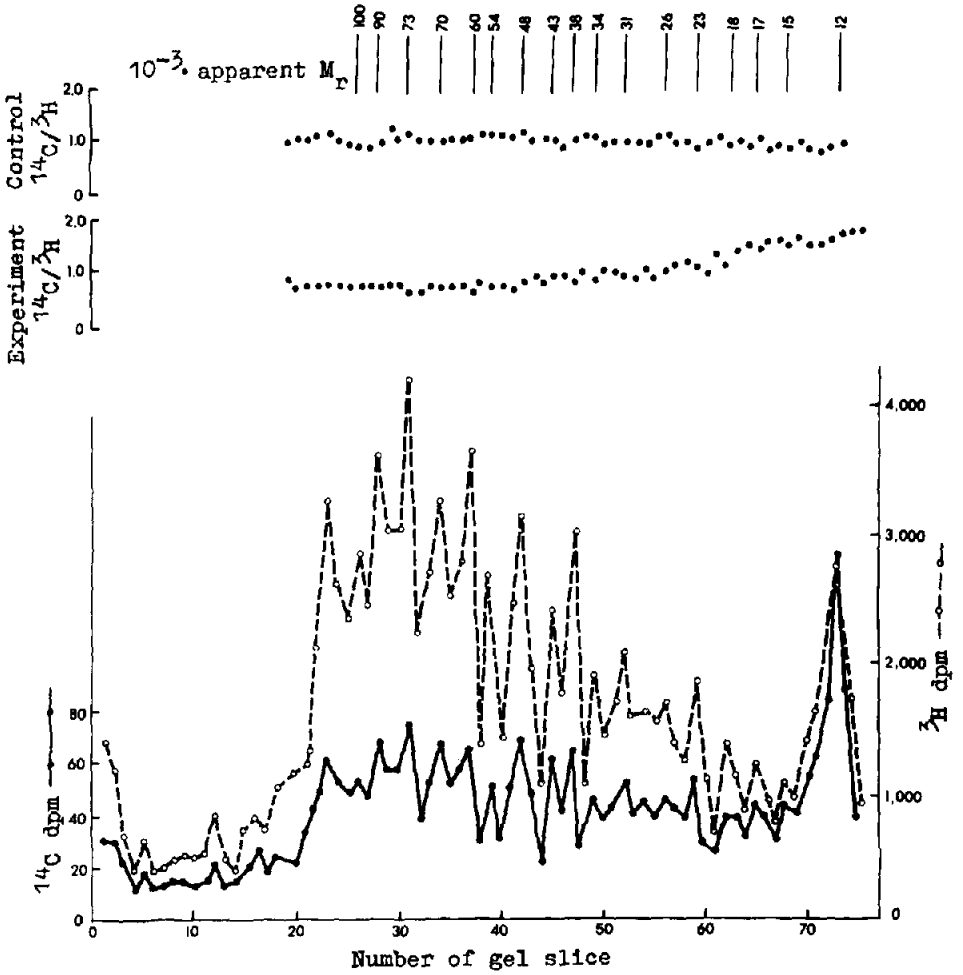

Fig.2. SDS-disc-electrophoretic analysis of mitochondrial polypeptides synthesized in embryo of imbibing seeds and mature root cells of seedlings. About 150 μg of mitochondrial protein (specific activity 800 dpm ^{3}H and 41 dpm ^{14}C per μg protein) were applied for separation. For other details, see Material and methods.

in the organelles from the mature root cells. Thus, a significant de novo synthesis or gross increase in content of at least five antigens including antigens 5 and 6 seems to have occurred with the onset of seed germination. The increase in concentration of several antigens is also revealed: 3–4-fold for antigen 3 identified by zymographical procedure to be the mitochondrial malate dehydrogenase, 1.5-fold for antigen 12, and 3-fold for antigen 13. At the same time antigen e1 concentration drastically decreased (5–8-fold). The localization of this antigen in the mitochondrial membrane was proved by electrophoretic separation of membrane fraction free of soluble proteins.

Spectra of mitochondrial polypeptide synthesis in embryos of imbibing seed and mature root cells were almost similar and consisted of 18–20 peaks of radioactive polypeptides of mol. wt. around $115 \cdot 10^3$ to $12 \cdot 10^3$. To compare the radioactivity distribution in several experiments, $^{14}C/^{3}H$ ratio values for separate gel slices were normalized by dividing by the mean value for the total gel. In the control experiment when mature root cells were labelled by [^{3}H]leucine as well as by [^{14}C]leucine, normalized $^{14}C/^{3}H$ value was about 1. When embryonal proteins were ^{14}C-labelled, and mature root cell proteins were labelled with ^{3}H, normalized $^{14}C/^{3}H$ were significantly over 1 for mitochondrial polypeptides of mol. wt. $< 25 \cdot 10^3$ and especially for the most fast-moving fraction of mol. wt. $\leqslant 12 \cdot 10^3$ (fig.2). Thus, synthesis of low mol. wt. mitochondrial polypeptides are relatively abundant in imbibing seeds as compared to mature cells.

4. Discussion

The above and previously published [6,8] data seem to fit into the following concept of protein pattern changes during mitochondriogenesis at the successive phases of maize development.

A decline in respiration and an inactivation of mitochondria in the embryos of ripening and dormant seeds is accompanied by significant decrease in the relative content of matrix antigens and by disappearance of several mitochondrial antigens.

The reactivation of mitochondria after seed dormancy is broken can be related to significant alterations in the patterns of mitochondrial antigens and of labelled proteins. Both qualitative and quantitative changes are observed during imbibition while mostly quantitative change occur during subsequent germination. The relative accumulation of matrix antigens (about 20-fold increase in antigen 3/antigen e1 ratio) is in line with our previous data concerning matrix/inner membrane ratio based on enzyme marker concentrations [3].

However, the nature of proteins which are formed de novo in germinating seeds has not been elucidated. Marked increase of label incorporation into low mol. wt. components may be related to some extent to the de novo synthesis of cytochrome *c* which is deficient in dormant peanut mitochondria [9]. However, an increase reported above is also compatible with an activation of mitochondrial translation producing first the low mol. wt. components, [10] as it was noted in germinating *Neurospora conidia* [11].

References

[1] Malhotra, S. S., Solomos, T. and Spencer, M. (1973) Planta 114, 169–184.
[2] Chrispeels, M. J., Vatter, A. E. and Hanson, J. B. (1966) J. R. Microsc. Soc. 85, 29–44.
[3] Varakina, N. N., Zeleneva, I. V., Polikarpochkina, R. T. and Khavkin, E. E. (1974) Ontogenez 5, 61–69 (Russ.).
[4] Ikuma, H. (1970) Plant Physiol. 45, 773–781.
[5] Clarke, H. G. M. and Freeman, T. (1968) Clin. Sci. 35, 403–413.
[6] Ivanov, V. N., Misharin, S. I. and Khavkin, E. E. (1974) Ontogenez 5, 492–500 (Russ.).
[7] Maizel, J. V. (1971) in: Methods in Virology (Maramorosch, K. and Korowski, H., eds.) 5, 179–246, Acad. Press, New York and London.
[8] Ivanov, V. N. and Khavkin, E. E. (1975) in: Abstr. XII Intern. Bot. Con., p. 359, Nauka Publ. House, Leningrad.
[9] Wilson, S. B. and Bonner, W. D. (1971) Plant Physiol. 48, 340–344.
[10] Malhotra, S. S. and Spencer, M. (1973) Plant Physiol. 52, 575–579.
[11] Hawley, E. S. and Greenawalt, J. W. (1975) Eur. J. Biochem. 54, 585–601.