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Residual Nuclear Structures From *Zea mays* L.

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Abstract. Nuclei from *Zea mays L.* root tip meristematic cells were treated according to the conventional method for nuclear matrix isolation and according to a recently adapted procedure for isolation of nuclear shells from plant cells. In the first case, after high salt extraction of proteins and DNase I and RNase digestions, residual structures are obtained consisting of a periferal layer and an internal network. The obtained structures are very similar to the nuclear matrix preparations from animal cells. In case nuclei are swollen in EDTA first, digested with DNase II and RNase prior high salt treatment, structures devoid of internal network are obtained. The analogous residual structures were shown for *Phaseolus vulgaris L.* meristematic root cells nuclei (Galcheva-Gargova *et al.* 1988). The morphology and the protein composition of the two types of residual structures suggest that the organization of scaffold structures from plant nuclei is very similar to the one from animal cell nuclei.

Additional index words: Zea mays L.; internal nuclear matrix; nuclear shells; residual proteins.

The structure of the cell nucleus and the topology of the diverse nuclear components are dependent on a group of structures resistant to treatment with high salt buffers, nucleases and non-denaturing detergents, which are referred to as karyoskeleton, nuclear matrix, scaffold or cage (for review see Kaufman *et al.* 1986). The residual structures may comprise morphologically a residual nucleolus, a dense periferal layer and nonchromatin intranuclear framework. The different types of residual structures and their components have been isolated from wide variety of animal cells – different vertebrates including mammals, amphibians and birds, insects and moluscces (Krohne and Benavente 1986). Meanwhile there are very few analogous studies for plant cells. The

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works of Stoljarov (1984) and Ghosh and Dey (1986) revealed the presence of a fibrilar nonchromatin network in *Allium cepa* nuclei comparable to that reported for animal cells. Some evidence was presented for the fate of nuclear matrix during mitosis (Ghosh *et al.* 1986) and an initial characterization of its components was performed (Stoljarov 1984; Diaz de la Espina 1986).

In a previous study we have demonstrated that nuclear shells are isolated from plant cells by adapting a procedure for nuclear lamina preparation (Galcheva-Gargova *et al.* 1988).

In the present communication we report the isolation of two different types of residual structure from *Zea mays* root tip meristematic cell nuclei. Their morphology and protein composition were studied.

MATERIAL AND METHODS

Plant Material

Two days old seedlings of *Zea mays* L. (cv. Kneja 2L-611) grown in a thermostat at 28 °C were used. Nuclei were isolated as previously described (Müller *et al.* 1980), using 2 mm long root tip segments.

Isolation of residual structures

Nuclear matrix preparation was performed according to the method of Berezney and Coffey (1977). Briefly, the nuclei were incubated for 10 min in low magnesium buffer (LM – 0.2 mM $MgCl_2$, 10 mM Tris/HCl, pH 7.4). Next, the nuclear pellet was twice resuspended in high salt buffer (HSB – 2M NaCl, 0.2 mM $MgCl_2$, 10 mM Tris/HCl, pH 7.4) for 10 min each time. The pellet was further treated with Triton X-100 (1 % final concentration). After washing the nuclei twice with TM buffer (0.25 M sucrose, 0.05 M Tris/HCl, pH 7.4, 5 mM $MgCl_2$), the pellet was digested for 1 h at 25 °C with DNase I (Sigma) 200 $\mu g\ ml^{-1}$ and with pancreatic RNase (Merck) 50 $\mu g\ ml^{-1}$ in the presence of TM buffer and 1 mM PMSF. After subsequent washing with TM, the residual pellet was fixed for electron microscopy or dissolved in sample buffer for electrophoresis. All steps of the isolation were carried out at 0–4 °C, washings at 600 *g* for 10 min.

The method of Krachmarov *et al.* (1986) was used for the isolation of nuclear shells with some modifications. Briefly, the nuclei were washed twice with 20–30 volumes of solution I (0.25 M sucrose, 5 mM EDTA, 5 mM Tris/HCl, pH 7.0 and 0.5 mM PMSF). The pellet was incubated at 0 °C for 5 min each time and centrifuged at 600 *g* for 5 min. Next, the nuclear pellet was washed twice in the same manner with solution II (0.25 M sucrose, 0.1 mM EDTA, 5 mM

Abbreviations: EDTA-ethylenediaminetetraacetic acid; PMSF-phenylmethylsulfonylfluoride; SDS-sodium dodecylsulfate.

Tris/HCl, pH 7.0, containing 0.5 mM PMSF). Further, the pellet was suspended in solution II, an equal volume of aqueous 0.5% Nonidet NP-40 was added and centrifuged through a cushion of 4 volumes of solution II at 3000 *g* for 15 min. The chromatin pellet was suspended in solution II and was digested with DNase II (Sigma) and pancreatic RNase (Merck) for 1 h at 25 °C. The enzyme reactions were stopped by the addition of an equal volume of ice cold 2 × High salt buffer (HSB: 2 M NaCl, 1 mM EDTA, 10 mM Tris/HCl pH 8.0). The pellet of the residual nuclear structures was isolated after purification from the bulk of proteins, DNA and RNA fragments by centrifugation through 10–15% linear sucrose gradient in HSB containing 1 mM PMSF for 30 min at 3500 *g*.

Light microscopy

The isolated nuclei were stained with 4% acetocarmine.

Electron microscopy

For electron microscopy the material was fixed with 5% glutaraldehyde in 0.1 M cocodylate buffer, pH 7.4 for 3 h and postfixed with 1% osmium tetroxide. Ultrathin sections were stained with both uranyl acetate and lead citrate according to Reynolds (1963).

Electrophoresis

Electrophoretic separation of proteins of the two type of residual structures was done in slab SDS containing 10% polyacrylamide gel (PAGE) according to Laemmli (1970).

RESULTS

Nuclei isolated from meristematic root tip cells of *Zea mays* were treated according to the scheme (Fig. 1). The purity of the nuclei was controlled by light microscopy (Fig. 2a) and an electron micrograph of isolated nuclei prior treatment is presented in Fig. 2b. Chromatin in diffuse and condensed state as well as nucleolus are seen. It was already suggested that nuclear ultrastructure in maize nuclei is a subtype of chromonematic organization (Koleva *et al.* 1989). After treatment of nuclei according to procedure A (Fig. 1), residual structures are obtained, presented on Fig. 2c. The shape of the nucleus is preserved and intranuclear network is seen. The preparation seemed very much alike the nuclear matrix structures reported for a large variety of animal cell nuclei.

If maize nuclei are first swollen in EDTA and decondensed chromatin is next digested with DNase II, RNase and high salt treated as in Fig. 1b, the obtained residual structures represent empty nuclear shells devoid of internal network

(Fig. 2d). They contain only a dense peripheral layer with some granules attached to it.

The protein composition of the two types of residual nuclear structures is presented in Fig. 3. A small number of proteins in 67–45 kD range were detected in the nuclear shell preparations (Galcheva-Gargova *et al.* 1988), while for the nuclear matrix a large number of bands are observed with different mobilities. In case the electrophoresis is run in 15% PAG (a condition to separate lower molecular mass polypeptides), very a few additional bands emerged (results not shown). The set of protein bands resolved in PAGE was found to be more complex in the presence of Mg^{2+} in the isolation procedure (Fig. 3b).

DISCUSSION

The presence or absence of an internal network in nuclear matrix preparations was shown to depend on the experimental protocol used. For a large variety of animal cells the effect of divalent cations (Galcheva-Gargova *et al.* 1982), the order in which the various preparation steps are applied and the extent of disulphide cross linking during preparation (Kaufman *et al.* 1981) and other factors have been studied (for review see Verheijen *et al.* 1988). Although the internal matrix is not an obligatory component of the residual structures, it may be generated during reactivation of nuclei and probably is not a static structure, but must display considerable dynamic activity (Lafond and Woodcock 1983).

The data for scaffolds from plant cells are very scanty. The isolation of nuclear shells from plant cells have suggested an organization of nuclear periphery probably similar to the one of animal cells. In this communication we have demonstrated that the presence of the internal network in residual nuclear structures from maize meristematic root tip cells is dependent on the presence of bivalent cations. In nuclear matrix preparations the main features of chromatin organization are preserved most probably because the association of chromatin proteins and DNA to some scaffolds depends on the presence of bivalent cations. Futher, our results show that as in a case of animal cells, the intranuclear matrix does not behave as a rigid structure. The low ionic strength and the swollen state of chromatin lead to isolation of residual structures with characteristic features of nuclear lamina preparations (Galcheva-Gargova *et al.* 1988).

One may suggest that the similar morphology of the two types of residual structures from *Zea mays* nuclei as well as from *Phaseolus vulgaris* nuclei (Galcheva-Gargova *et al.* 1988) to the analogous structures isolated from a variety of animal cells implies a similar pattern of topological chromatin organization in all eukaryotes. Besides, the two different methods applied in this study may turn useful as procedures for studying the components of the residual structures from different types of plant cells.

REFERENCES

Berezney, R., Coffey, D. S.: Nuclear matrix isolation and characterization of framework structure from rat liver nuclei. – J. Cell Biol. **73** : 616–673, 1977.

Diaz de la Espina, S. M.: Initial characterization of the nuclear matrix of plant system. – In: Abstract from First International Symposium Chromatin Structure of Plant Genes. P. 26. Frankfurt am Main 1986.

Galcheva-Gargova, Z., Petrov, P., Dessev, G.: Effect of chromatin decondensation on the intranuclear matrix. – Eur. J. Cell Biol. **28** : 155–159, 1982.

Galcheva-Gargova, Z., Marinova, E., Koleva, S.: Isolation of nuclear shells from plant cells. – Plant. Cell Environ. **11** : 819–825, 1988.

Ghosh, S., Dey, R.: Nuclear matrix network in *Allium cepa*. – Chromosoma **93** : 429–434, 1986.

Ghosh, S., Dey, R., Paweletz, N.: The nuclear matrix network in onion and its fate in mitosis. – In: Abstracts from First International Symposium Chromatin Structure of Plant Genes. P. 26. Frankfurt am Main 1986.

Kaufman, S. H., Gibson, W., Shaper, J. H.: Considerations in the isolation of rat liver nuclear matrix, nuclear envelope and pore complex lamina. – Exp. Cell Res. **132** : 105–123, 1981.

Kaufman, S. H., Fields, A. P., Shaper, J. H.: The nuclear matrix: current concepts and unanswered questions. – Meth. Achiev. Pathol. **12** : 141–171, 1986.

Koleva, S. T., Marinova, E. I., Dimitrov, B. D., Tourishcheva, M. S.: Investigation of histone proteins in plant nuclei possessing different ultrastructural organization. – Protoplasma **150:** 96–102, 1989.

Krachmarov, Ch., Tasheva, B., Markov, D., Hancock, R., Dessev, G.: Isolation and characterization of nuclear lamina from Ehrlich Ascites Tumor Cells. – J. Cell Biochem. **30** : 351–359, 1986.

Krohne, C., Benavente, R.: The nuclear lamine. A multigene family of protein in evolution and differentiation. – Exp. Cell Res. **167** : 1–10, 1986.

Laemmli, U. K.: Cleavage of structural proteins during the assembly of the head of bacteriophage t4. – Nature **287** : 680–685, 1970.

Lafond, R. E., Woodcock, C. L.: Status of nuclear matrix in mature and embryonic chick erythrocyte nuclei. – Exp. Cell Res. **147** : 31–39, 1983.

Müller, A., Phillips, G., Gigot, C.: Properties of condensed chromatin in barley nuclei. – Planta **149** : 67–77, 1980.

Reynolds, E. S.: The use of lead citrate at high pH as an electron opaque stain in electron microscopy. – J. Cell Biol. **17** : 208–211, 1963.

Stoljarov, S. D.: Isolation and characterization of nuclear matrix from onion *Allium cepa*. – Cytologia **26** : 874–878, 1984.

Verheijen, R., Van Venrooij, W.: The nuclear matrix: structure and composition. – J. Cell Sci. **90:** 11–36, 1988.

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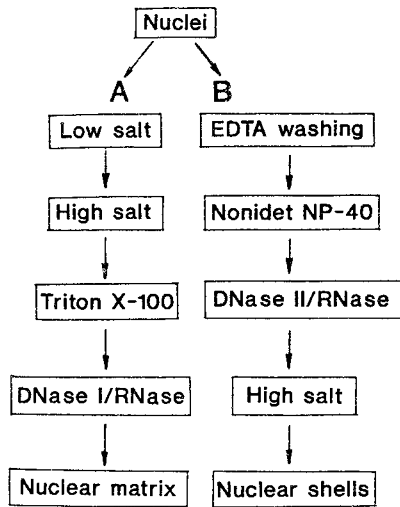

Fig. 1 Scheme of the experimental set up.

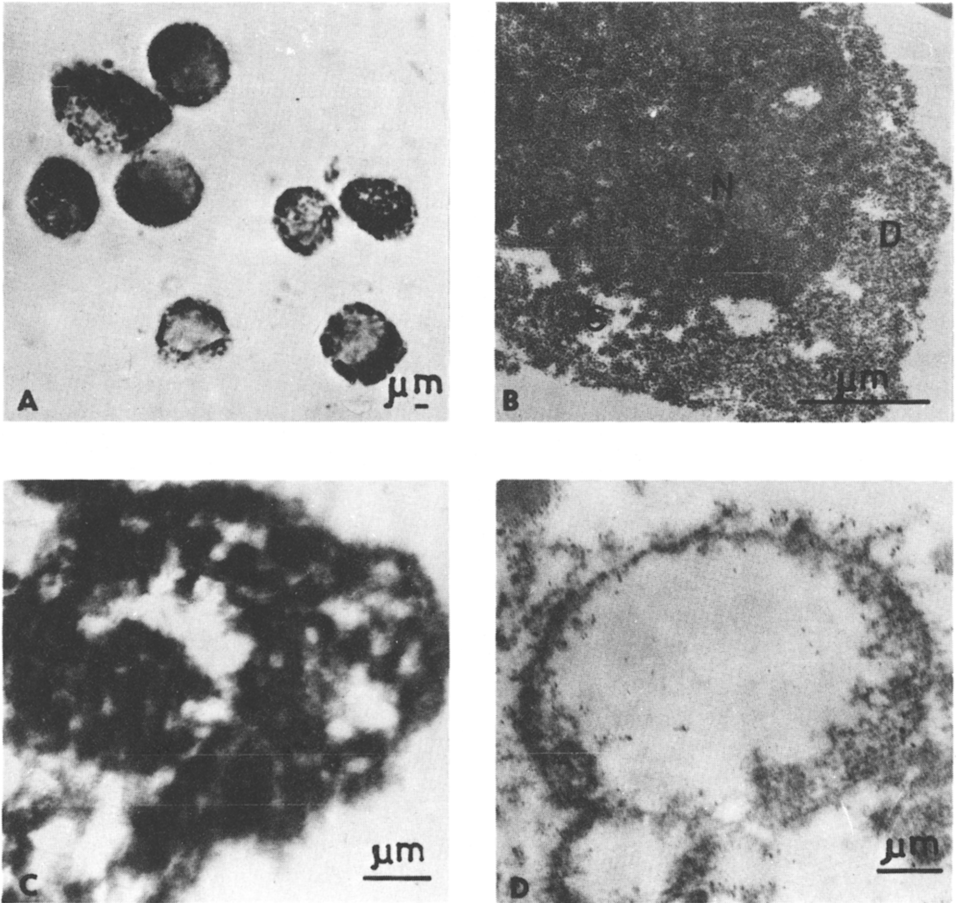

Fig. 2a. – Control of *Zea mays* L. nuclei by light microscopy; b – Electron microscopy of the same nuclei: N – nucleus, C – condensed and D – diffuse chromatin; c – Residual structures isolated in the presence of Mg^{2+} (see Fig. 1a.); d – Residual structures isolated in the absence of Mg^{2+} (see Fig. 1b.); bar – 1 µm.

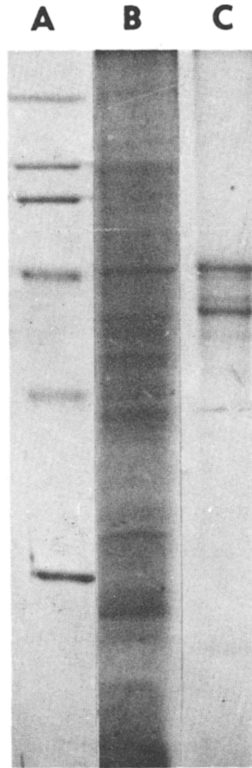

Fig. 3. SDS – PAG electrophoretic profiles: A – Marker proteins (myosin – 205 kD, β-galactosidase – 116 kD, phosphorylase B – 97.4 kD, bovine serum albumin – 66 kD, ovalbumin – 45 kD, carboxyanhydrase – 29 kD); B – Nuclear matrix proteins; C – Nuclear shells proteins.