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Cytochemical study of the embryo sac of *Nicotiana tabacum* L.

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Abstract

The mitotic cycle in the egg cell and physico-chemical state of chromatin in the egg cell and central cell of the tobacco embryo sac were studied. It was revealed that during egg cell formation a change in the mitotic cycle kinetics takes place, it consists in prolongation of the S-period as compared with that of somatic cells and G_1 - period as compared with that of male gametes. Egg cell and central cell nuclei differ in chromatin structure. Condensed chromatin dominates in the egg cell nucleus, diffuse chromatin in the central cell nucleus, but both show only weak metabolic activity.

INTRODUCTION

We studied the mitotic cycle in the developing egg cell and the physico-chemical state of chromatin in the egg and central cell nuclei.

MATERIAL AND METHODS

In the 4-nuclear embryo sac *Nicotiana tabacum* L. cv "Dubek 44" the DNA content in the lower micropylar nucleus, was determined by the method of two-wave cytophotometry. The working wave lengths 505-570 nm corresponded to the correlation of absorption coefficients 1 : 2 (Sherudilo, 1966). Mature egg cells (100) were used as a standard of ploidy, their nuclei give a clear Feulgen reaction (Fig. 1a). Analysis of the histogram was conducted according to Liberman and Malyuk (1970). The histogram (Fig. 1b) shows that the β-parameter is distributed normally, $\lambda = 1$ (at the marginal value of this criterion for the highly precise measurement of 1.36). To characterize the scattering of results the root-mean square deviation ($\delta = 60$) and variation coefficient (11%) values were estimated. The λ value of 0.5, testifies to the normal distribution law. The assymmetry coefficient of the histo-

gram (—3%) testifies to the absence of DNA synthesis. To determine the duration of the interphase of the mitotic cycle of the developing egg cells, 500 lower nuclei of the micropylar end of 4-nucleate embryo sac were photometered.

The physicochemical state of the chromatin of egg and central cell nuclei was studied by luminescent spectral analysis with the use of acridine orange (AO) as fluorochromate forming complexes with the chromatin. DNA localized in condensed chromatin, fluoresces in the complex with AO in green light with maximum at about 530 nm. Metabolically active DNA, a component of diffuse chromatin fluoresces with maximum at about 600-640 nm (Serdyuk, Konarev, 1970).

The RNA-AO complex always show red fluorescence. Free and unstably aggregated RNA was removed from the nuclei by RNA-ase treatment. The remaining red fluorescence is due to labile DNA. Thus, correlation of long wave (F_{600}) and short wave (F_{580}) fluorescence, i.e. the specificity coefficient $\alpha = \frac{600}{580}$ shows the degree of the metabolic activity of the cell nucleus (Rigler, 1966). Fluorochromation was combined with lipid removal and proteins acetylation. This makes possible determination of the character of the nucleic acids bound with proteins and the degree of their participation in lipoprotein complexes.

RESULTS AND DISCUSSION

Results of cytophotometric measurements of 500 nuclei were analyzed with consideration of the known haploid DNA value (5.41 ± 0.06 relative units), its dispersion and the error of individual measurement. The duration of interphase corresponds to the frequency of occurrence of cells with the definite DNA quality per cell within 1C to 2C. After mathematical processing of the data the DNA synthesis curve was built for the immediate predecessors of the female gametes nuclei (Fig. 1c). Analysis of this curve testifies to a uniform increase of the DNA quantity over the whole synthesis period. The longest interphase period is the synthetic one (S — 59%), the presynthetic one is shorter (G_1 — 26%) and the shortest is postsynthetic period (G_2 — 15%).

While comparing the parameters of the mitotic cycle of developing egg cells and somatic cells (Grif, Ivanov, 1975) with those of developing male gametes (Bannikova et al., 1978) it is necessary to note their peculiarities. In the mitotic cycle of the female gametes there is a protraction of the S-period as compared with that in somatic cells and the G_1-period as compared with that in male gametes, these changes correlate with functional peculiarities.

In the course of development of the egg cell the enlargment of nuclei and nucleoli always takes place as well as cytoplasm accumulation. The

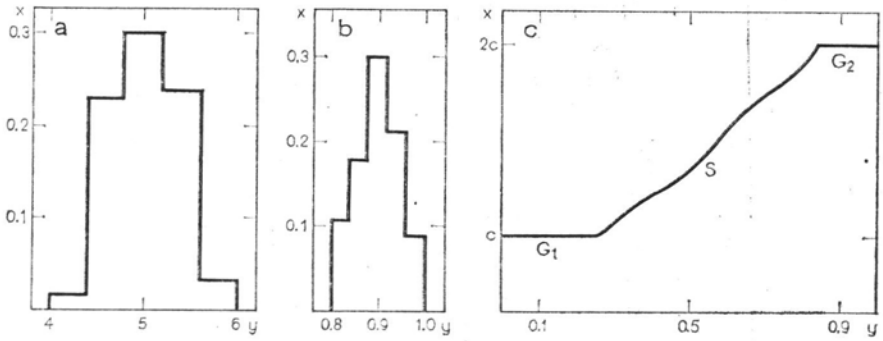

Fig. 1. a) DNA distribution in the egg cell of tobacco (x — frequencies accumulated; y — DNA quantity). b) β-parameter distribution (x — frequencies accumulated; y — β-parameter meanings). c) DNA synthesis in the lower micropylar nucleus of the 4-nucleate embryo sac of tobacco (x — DNA quantity; y —frequencies accumulated)

latter, is bound with the synthesis of the large amount of RNA and protein, which takes place in the G_1- and partly in the S-period. It results from the foregoing considerations that the increase in G_1 during egg cell development is the necessary condition of its formation (King, Barnhisel, 1967; Zetterberg, 1970).

Egg cell. The DNA of the egg cell chromatin is characterized by a stable structural state. Green luminescence dominates in the spectrum of these nuclei after RNA-ase treatment (Fig. 2-Ib): this fact testifies that DNA is firmly bound in the chromatin structures. The arm visible in the red part of the spectrum (Figs 2-Ib, d, f) shows that a small part of the DNA is included in the labile component. But the low specificity coefficient (0.70 ± 0.02) indicates the dominance of condensed chromatin in the egg cell nucleus. There is a large amount of RNA in the egg cell nucleus revealed by the intense red luminescence (Fig. 2-Ia) and its considerable decrease after treatment with RNA-se. RNA is weakly bound in the chromatin structures.

Central cell. The major part of the DNA of the central cell is characterized by a labile structural state. Luminescence spectra of this nucleus after treatment with acetic anhydride and RNA-ase have a high peak in the red area (Fig. 2-IIf). The high value of the specificity coefficient (1.2 ± 0.03) also shows that diffuse chromatin dominates in the central cell nucleus. The increase of luminescence intensity as compared with the control after treatment of the preparations with RNA-ase and lipid solvents (Fig. 2-IId) proves that DNA is tightly bound with the lipids. There is a smaller amount of RNA in the central cell nucleus than in the egg cell nucleus. RNA as well as DNA are in a labile structural state.

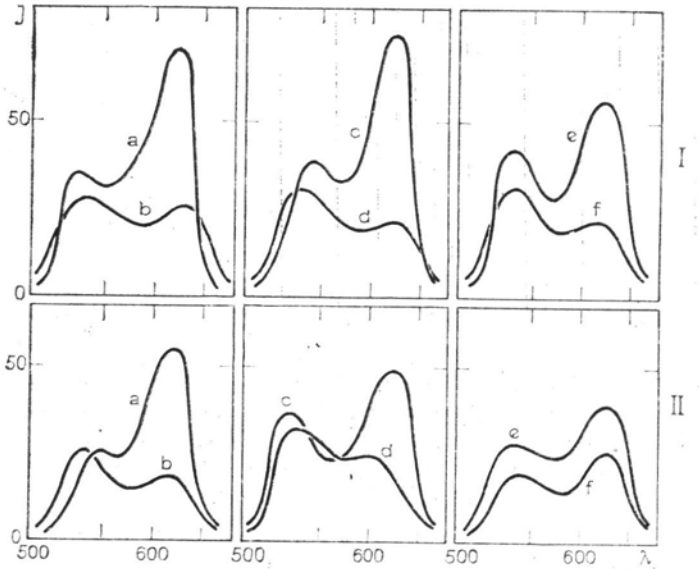

Fig. 2. Curves of fluorescence spectra: I — egg cell nucleus; II — central cell nucleus of the embryo sac of tobacco
a — control; b — RNA-ase treatment; c — lipid solvents treatment; d — lipid solvents and RNA-ase treatment; e — acetic anhydride treatment; f — acetic anhydride and RNA-ase treatment.

Our data indicate that the egg cell and central cell nuclei are in different states in the mature embryo sac. The egg cell nucleus is characterized by the dominance of condensed chromatin where DNA is tightly bound with proteins and is functionally inactive. The central cell nucleus is characterized by diffuse chromatin. The presence of labile RNA there, may result in intensive RNA synthesis and functional activity of the nucleus, but the small amount of RNA in the nucleus and cytoplasm of the central cell shows that its synthesis is considerably reduced and the high specificity coefficient reflects only the diffuse structure of the chromatin, and is not a factor of its matrix activity. Thus egg and central cell nuclei differ considerably in the chromatin structure whereas they show similar weak metabolic activity.

The RNA amount in the nucleus of the tobacco egg cell exceeds that in the nucleus of the central cell. This is a special feature of the egg cell of tobacco. RNA was perhaps synthesized in the process of egg cell development, because the low value of the specificity coefficient of chromatin in the nucleus of the mature egg cell shows the impossibility of this synthesis. This suggestion is confirmed by investigations on the mitotic cycle in the egg cell.

Some RNA was found in the nuclei of animal eggs (ref. in J o s t, 1975). Synthesis of different types of RNA during oogenesis, which are

in disbalance with each other (Davidson et al., 1966) may be one of the factors causing RNA accumulation in the egg cell nucleus (Brown, Littna, 1966). Similar processes are, perhaps, inherent to the egg cell nuclei of plants, because the general principles of gametes formation, should obviously be the same.

We may conclude that the developing egg cell of tobacco undergoes considerable transformations connected first of all with mitotic cycle transformations in the mature embryo sac. The egg and central cell nuclei differ considerably in the structural state of their chromatin and in RNA content, but they are functionally similar. The data obtained may be important for explanation of some essential moments of the fertilization process.

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