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Variations of total protein content and protein synthesis during microsporogenesis in *Larix europaea* L.

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Abstract

The variations of the total protein content and protein synthesis were analyzed in successive stages of microsporogenesis in *Larix europaea* L. Pure fractions of microsporocytes containing the cells in successive phases of meiosis and fractions of the anther wall cells were used to perform the assays. It was revealed, that the protein content and the intensity of protein synthesis undergo considerable changes in the course of meiosis. It was proved also, by means of electrophoretic separation of protein fractions, that no new proteins are synthesised during differentiation of microsporocytes of larch, and that the microsporogenesis process is related rather to disappearance of some protein fractions.

INTRODUCTION

Our knowledge of protein content and protein synthesis in cells undergoing meiosis in an anther is far from complete. The first information on this subject was obtained by means of microautoradiography by Albertini (1967) on *Rhoeo discolor* and by Sauter (1969) on *Paeonia*, and by biochemical methods in studies of isolated microsporocytes of *Lilium* (Linskens, 1966; Hotta et al., 1968).

MATERIAL AND METHODS

From anthers of *Larix europaea* L. pure fractions of microsporocytes were obtained and fractions of anther wall cells (Chwirot, 1980).

The protein content was assayed by the colorimetric method (Lowry et al., 1951) after precipitation with phosphotungstic acid and dissolution in 0.1 NaOH as well as in crude homogenate.

Electrophoretic separation of soluble proteins was performed by means of 7% polyacrylamide gel at pH 8.3-8.7. The gels were stained with Coomassie Brillant Blue G 250 (Blakesley, Boezi, 1977).

The intensity of protein synthesis was studied by using ^{14}C/U/leucine (UVVVR Czechoslovakia, spec. act. 180 mCi/mM). The incubation medium contained labelled L-leucine (7.5 μCi/ml) in 0.6 M mannitol buffered with Tris-H_2SO_4, pH 7.2. Incubation followed immediately after acquirement of the fraction and was performed at +8°C, near to natural conditions of microsporogenesis in larch. The incubation was stopped after 24 hours by means of 15% TCA. The samples were homogenized by hand and immediately washed with 50 ml of 15% TCA at room temperature in a suction filtration apparatus with a fine mesh filter (HAWP). Four samples for every meiotic stage were simultaneously incubated. The intensity of protein synthesis was assayed as the difference between radioactivity measured for a given sample and for the control, which consisted of microsporocytes in a corresponding stage of development treated with 15% TCA for 30 minutes just before incubation. Radioactivity was measured with a WZQ 605 Tesla Counter.

Protein content and radioactivity of microsporocyte samples were normalized to one cell of the fraction. In fractions of anther wall, containing different tissues, the protein content was normalized to dry weight.

The concentrations of cells of the microsporocyte fractions were measured in Thoma's chamber.

RESULTS AND DISCUSSION

The pattern of these variations was similar for protein denaturated in microsporocytes of *Larix europaea* during their development (Fig. 1). The pattern of these variations was similar for protein denaturated with phosphotungstic acid and for assayed in crude homogenate. The only difference caused by using phosphotungstic acid was a decrease of the total protein content of the order of 40-50%.

The highest values of protein level were found in the middle phase of diplotene, in the stages of young dyad and tetrad. When comparing the variations of protein content in the anther wall cells and in microsporocytes, it is seen that they are more dynamic in the latter (Fig. 1).

Protein synthesis was found in all developmental stages of microsporocytes, from diakinesis to microspores. The intensity of protein synthesis, measured as an increase of radioactivity in reference to control samples, was relatively low. However, the dynamics of its variations was strong. The highest level of protein synthesis was found at the end of diplotene, in the stages of dyad, telophase II and young tetrad.

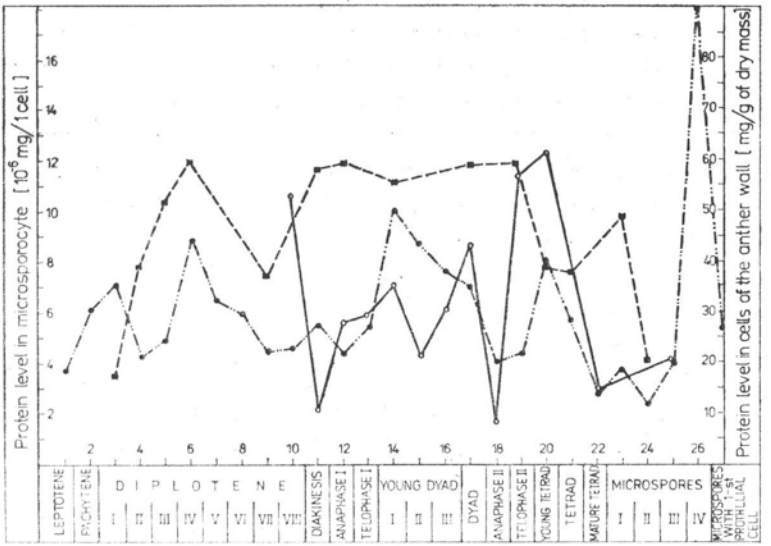

Fig. 1. Protein content in microsporocytes (●— . . —●) and in cells of the anther wall (■— —■); intensity of protein synthesis in microsporocytes (o——o) during microsporogenesis in *Larix europaea* L.

The lowest was noted in diakinesis, anaphase II and in the stage of splitting tetrad.

When comparing the variations of protein content with the corresponding changes of intensity of protein synthesis, it is generally found that the increase of synthesis intensity is associated with an increase of protein content in the microsporocytes. This phenomenon was particularly conspicuous in two developmental periods: from diakinesis to the stage of young dyad and from anaphase II to the stage of young tetrad.

At the time of developmental transition from young dyad to dyad stage a discrepancy appears between the intensity of protein synthesis and the protein level. The increase in intensity of synthesis processes is associated with a decrease in protein content in the cell, which takes place up to anaphase II. This can be due to the fact that the protein level in the cells is determined both by synthesis and degradation processes, the ratio of which can vary (Baxter, Stanners, 1978).

By means of electrophoresis 19 protein fractions were separated from the microsporocytes (Fig. 2a) and 22 protein fractions from the anther wall (Fig. 2b). Linskens (1966) obtained a similar number of protein fractions from *Lilium henryi* microsporocytes. Four protein fractions (Nos 6, 8, 22 and 23) occurring in the somatic cells of the

anther do not appear in cells undergoing meiosis. Two of these fractions are of the greatest electrophoretic mobility. As compared with the anther wall, which is built of four different tissues, the microsporocytes are characterized by a great number of protein fractions. Only 14 out of 19 protein fractions of the microsporocytes of *Larix europaea* occur in all stages of meiosis. Two fractions (Nos 11, 18) are characteristic of the prophase I stage only, and one (No 12) appears and disappears in different stages of meiosis.

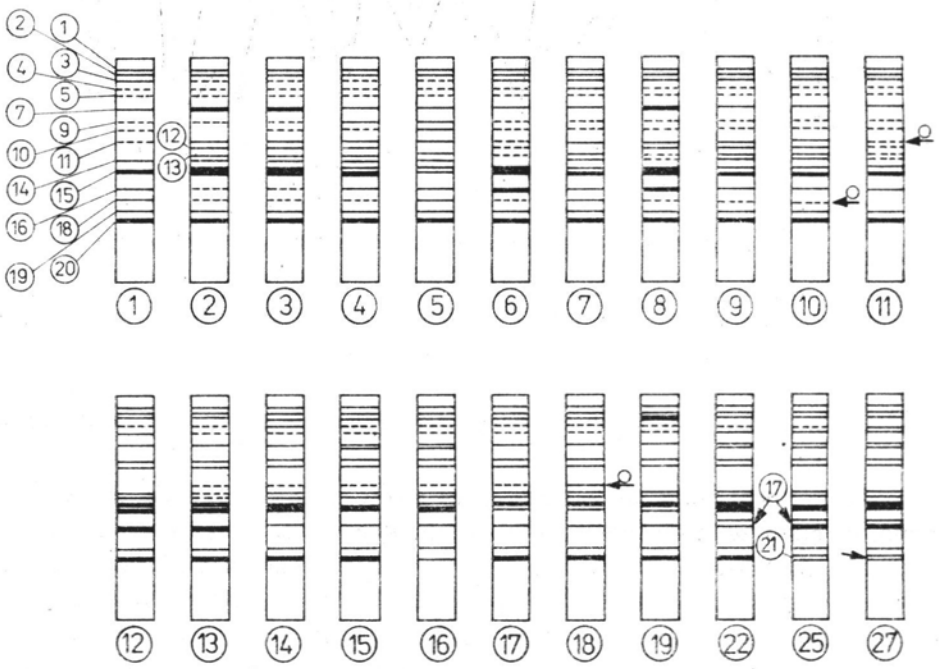

Fig. 2a. Separation of soluble proteins from the cell fractions isolated from microsporocytes of *Larix europaea* L. anthers
Designations of gel slabs are the same as those of the developmental stages in Fig. 1.

It is noteworthy that in the course of meiosis one does not observe the appearance of new proteins in microsporocytes, but the process of differentiation is related to disappearance of some proteins. The first new protein fraction occurs only at the beginning of the haploid phase, in microspores originating from the splitting tetrads. The degree of variability of the protein pattern, expressed as the ratio of the number of fractions occurring periodically or alternately to those occurring continuously during the meiosis process, shows that more intensive differentiation processes take place in cells undergoing meiosis than in those of the anther wall.

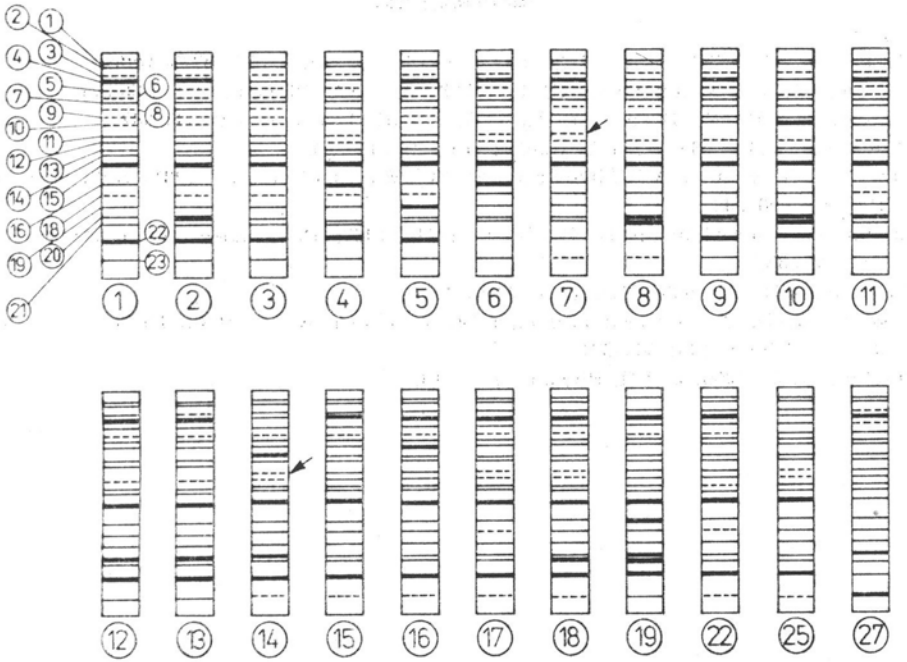

Fig. 2b. Separation of soluble proteins from the cell fractions isolated from the anther wall cells of *Larix europaea* L.
Designations as in Fig. 1.

The disappearance of fraction No 11 from the cells of the anther wall in the course of diplotene followed by the disappearance of this fraction from the microsporocytes at the end of prophase I deserves special attention, considering the metabolism of interaction between anther wall cells and microsporocytes.

A confrontation of the results of these investigations concerning the total protein content, protein synthesis intensity and the protein pattern in developing microsporocytes of *Larix europaea* points to the stages of microsporogenesis which are important for protein metabolism. These are the end stages of prophase I and the stage of young dyad.

Considering the fact that exactly in the same stages of development of microsporocytes an increase of the level of endogeneous IAA was observed (Górska-Brylass et al., 1981) and a considerable increase of activity of some enzymes (Chwirot, unpublished) was observed, it seems that these stages are of particular interest and deserve special attention in future investigations.

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