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Concurrent Microscopical, Cytochemical and Electron Microscopical Study on the Cell Polarity of *Equisetum* Spore Mother Cells in Syndetic Stage

By

Tosisuke HIRAOKA D. Sc.

Department of Botany, Faculty of Science, Kyoto University

ABSTRACT Concurrent microscopical, cytochemical and electron microscopical examinations of *Equisetum* spore mother cells were carried out to analyse the cell polarity peculiar to the syndetic stage of meiosis. The results obtained show that some physiological centre, which attracts the plastidsactive in reducing and starch synthesizing functions, is established in a certain region of cytoplasm ..... the plastid pole. This plastid pole stands in a definite spatial relationship to the base of the bouquet figure.

Introduction

In the spore mother cells of many fern plants, plastids show an unilateral localization with respect to the nucleus in the syndetic stage of meiosis, when two homologous chromosomes come in close side by side pairing (Hiraoka, 1949). As this peculiar plastid localization is found only in the syndetic stage, it may be taken as indicating the cell polarity characteristic to this stage (Hiraoka, 1952). In order to contribute something to the knowledge of the nature of this cell polarity, a concurrent microscopical, cytochemical and electron microscopical study of *Equisetum* spore mother cell in the syndetic stage was carried out. The results obtained will be given in the present paper.

Material and Methods

Fresh strobili in appropriate stage of development were collected from the plants (*Equisetum hiemale*) grown in the Botanical Garden, Faculty of Science, Kyoto University. The spore mother cells in the syndetic stage were mainly used as the material in the present investigation, but those in the pre-syndetic and post-syndetic stages were also examined for comparison's sake.

For the purpose of concurrent microscopic, cytochemical and electron microscopical study, following procedures were carried out:

1) Sections through the strobilus were fixed with Nawashin's fixative, paraffin sectioned and stained with Heidenhain's haematoxylin. This procedure was used for the observation of chromosome behavior in syndesis.

2) Fresh thin sections through the strobilus or spore mother cells liberated from them were mounted with liquid paraffin or with 10% sucrose solution, and observed immediately under a microscope. This procedure served for the observation of plastid behavior in intact spore mother cells.

3) In order to examine the localization of starch in the spore mother cells, two methods of detection were used. First, sections through the strobilus were fixed with Regard's fixative for 24 hours, treated with 3% potassium bichromate solution for 7 days (Romeis, 1932), paraffin sectioned, and then treated with iodine potassium iodide solution. Secondary, fresh sections were treated directly with chloralhydrate iodine solution (Molisch, 1923). In the former case starch grains turned brown black, and in the latter case they turned blue in color.

4) Fresh sections through the strobilus were treated with Meyer's modification of Fehling's solution, and processed according to Meyer's procedure (Tunmann, 1913). This reaction served for the detection of reducing sugar in the spore mother cells.

5) Fresh sections through the strobilus or fresh spore mother cells were treated with 10% silver nitrate solution in the presence of diffuse light (Hiraoka, 1951). In the case of positive reaction, the plastids turned black in color. This reaction was taken as an indication of general reducing power in the plastids.

6) Fresh sections through the strobilus were fixed with 2.5% potassium permanganate solution for 2 hours at room temperature (Mollenhauer, 1959), dehydrated with a series of ethyl alcohol, immersed in propylene oxide, and embedded in Epon (Luft, 1961). They were further sectioned with a JUM-5A ultramicrotome, and the ultrathin sections obtained were observed under a "Superscope" of Japan Electron Optics Co., Tokyo, Japan.

Results

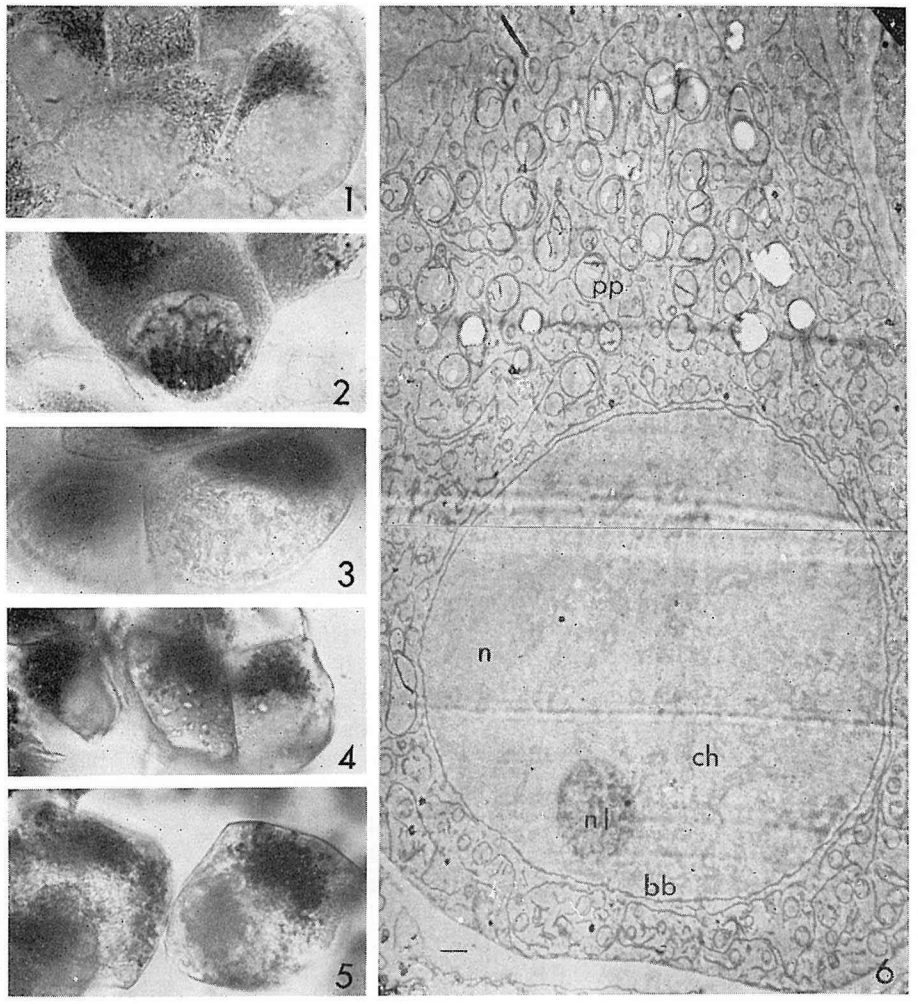
In *Equisetum hiemale*, the spore mother cell in the syndetic stage is rather of polyhedral shape. The nucleus is displaced from the central position and takes an eccentric one. In the broader region of cytoplasm, many faint green colored plastids are found to form a tight mass (Fig. 1). This mass of plastids is located close to the nuclear membrane. The region of this plastid grouping has been designated as the plastid pole by the present author (Hiraoka, 1949). In the pre-syndetic stage, as well as in the post-syndetic one, no such tight grouping of the plastids in the cytoplasm is found at all. Therefore, the formation of the plastid pole may be regarded as one of the cell conditions characteristic to the syndetic stage.

In the syndetic stage, the chromosomes take a polarized, regular bouquet arrangement in the nucleus. They are attached on a certain region of the nuclear membrane at least with one end and form the bouquet base. The base and the plastid pole take an opposite spatial relationship in the cell across the nucleus. When the median optical section of the nucleus in the middle of the syndetic stage is examined carefully, chromosome threads arranging in pairs are found running parallel with one another in the region not so remote from the bouquet base, while in the region remote from the bouquet base, they are found in somewhat irregular arrangement (Fig. 2). Thus, the chromosome syndesis begins from the bouquet base side and proceeds towards the plastid pole side (Darlington, 1937). It seems worth mentioning here that the chromosome syndesis takes place only in the period, when the plastid pole is formed in the cytoplasm, and that there is a definite spatial relationship between the plastid pole and the bouquet base.

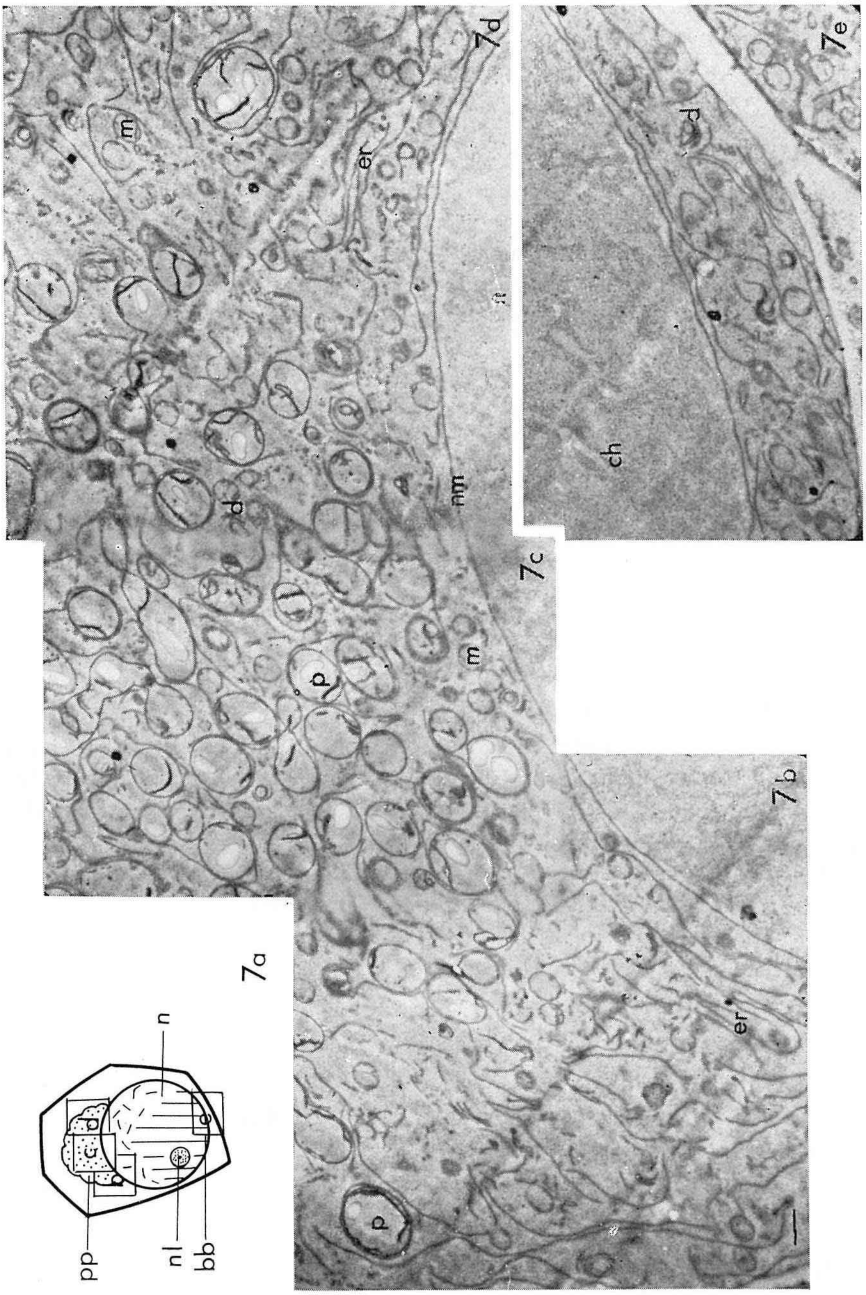
When the spore mother cells in the syndetic stage are treated with a modified Fehling's reagent, the plastid pole is the only region which turns deeply brown in the cell ····· a positive reaction color for reducing sugar (Fig. 3). From this coincidence in position of the plastid pole and the region positive to the reaction, we may say that these plastids contain some reducing sugar, though the brown coloration exhibited by an individual plastid is rather diffuse. When the spore mother cells in the same stage are treated with chloralhydrate iodine solution or iodine potassium iodide solution, starch grains are detectable in the plastids which gather at the plastid pole to form a tight mass (Fig. 4). In the early period of the syndetic stage starch grains are found only in some of the plastids, and the plastids positive in the starch reactions increase in number during the syndetic stage. From the results of these cytochemical tests, we may say that the synthetic activity of producing starch from sugar is remarkable in those plastids which gather at the plastid pole in the syndetic stage.

When the spore mother cells in the syndetic stage are treated with a silver nitrate solution in diffuse light, the plastids which gather at the plastid pole turn black. Thus the plastid pole is the only region in the spore mother cell, which reduces silver nitrate to form the black product (Fig. 5). This result shows that the plastids in the syndetic stage have a reducing power strong enough to reduce silver nitrate.

Electron microscopical examinations of the spore mother cells in the syndetic stage permit more detailed morphological analysis of the plastid pole region. Fig. 6 gives a general view of a spore mother cell in the syndetic stage. The nucleus (n) takes an eccentric positon in the cell. In the bouquet base region of the nucleus (bb), the chromosomes (ch) show some indication of the parallel bouquet arrangement, and they also show an evidence of side by side pairing (compare Fig. 2 with Fig. 6). In the plastid pole many plastids, some of which contain starch and others do not, are found gathering to form a group. Besides these plastids, other kinds of cytoplasmic bodies such as mitochondria with poorly developed tubuli (Frey-Wyssling and Mühlethaler, 1965), dictyosomes (Mollenhauer



and Morré, 1965) and elements or cisternae of endoplasmic reticulum (Palade, 1955) are also seen. It is clearly shown that the bouquet base and the plastid pole stand in opposite spatial position across the nucleus. Fig. 7 is a median section of another spore mother cell in this stage, and shows the distribution of various kinds of cytoplasmic bodies in the plastid pole and the bouquet base region of the cytoplasm. The major cytoplasmic bodies which are found exclusively in the central part of the plastid pole region are the plastids (Fig. 7c). Thus, these plastids form a group in this plastid pole region. The peripheral region of the cytoplasm surrounding this plastid group is occupied by numbers of cisternae of endoplasmic reticulum running nearly parallel with one another, though similar cisternae are scattered randomly in the whole cytoplasm


7a
7b
7c
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(Figs. 7b, 7d and 7e). Both mitochondria and dictyosomes are distributed sporadically in the whole cytoplasm (Figs. 7b, 7c, 7d and 7e), and thus in the plastid pole region they are found scattered amidst the plastids and the cisternae of endoplasmic reticulum.

Conclusion

In *Equisetum hiemale*, the spore mother cells in the syndetic stage are characterized by an establishment of a distinct uniaxial cell polarity. This cell polarity is indicated by various phenomena, such as the displaced localization of the cell nucleus, the formation of the plastid pole in the cytoplasm, the existence of a definite spatial relationship between the plastid pole and the bouquet base, the bouquet arrangement of the chromosomes in the nucleus, and finally the occurrence of chromosome pairing (Hiraoka, 1952). The present electron microscopical observation reveals the presence of various kinds of cytoplasmic bodies in the *Equisetum* spore mother cells. In the syndetic stage, all the plastids gather exclusively in the plastid pole to form a tight mass. Many cisternae of the endoplasmic reticulum in nearly parallel arrangement are localized in the peripheral part of the cytoplasm surrounding this plastid mass, but they are also distributed randomly in other regions of the cytoplasm. Both the mitochondria and the dictyosomes do not show any definite localization with respect to the plastid pole, but are sporadically found in the whole cytoplasm. This difference in behavior of these four kinds of cytoplasmic bodies in the syndetic stage gives us a definite evidence to show that the formation of the plastid pole in the cytoplasm is not a mere result of the nuclear displacement. Moreover, the present concurrent cytochemical examinations reveal that those plastids which gather at the plastid pole exhibit the starch forming activity and the reducing power strong enough to reduce silver nitrate. From all these results obtained, we may draw some conclusion as to the nature of the polar organization of the *Equisetum* spore mother cell in the syndetic stage. In this stage, there is established some physiological centre which attracts the plastids active in reducing and synthetic functions in the plastid pole region of the spore mother cell.

Acknowledgement

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Explanation of Figures

Fig. 1. Intact spore mother cells of *Equisetum* in syndetic stage, mounted in 10% sucrose solution. Notice the tight mass of plastids at the plastid pole. 720x.

Fig. 2. Spore mother cells in syndetic stage, fixed with Nawashin's fixative, paraffin sectioned and stained with Heidenhain's haematoxylin. Notice the bouquet arrangement of chromosomes. 900x.

Fig. 3. Spore mother cells in syndetic stage, treated with a modified Fehling's reagent. Notice deep brown color in the plastid pole. 900x.

Fig. 4. Spore mother cells in syndetic stage, fixed with Regard's fixative, treated with 3% potassium bichromate solution, paraffin sectioned and treated with iodine potassium iodide solution. Notice positive starch reaction in the plastids, gathering at the plastid pole. 900x.

Fig. 5. Spore mother cells in syndetic stage, treated with 10% silver nitrate solution. Notice black positive silver reaction in the plastids, gathering at the plastid pole. 900x.

In Figs. 1—5, plastid pole stands on the upper side of the figures.

Fig. 6. Electron micrograph showing the general view of a *Equisetum* spore mother cell in syndetic stage. Abbreviations: pp, plastid pole. bb, bouquet base. n, nucleus. nl, nucleolus. ch, chromosome. Black line at the bottom of the figure shows 1μ.

Fig. 7a. Schematic diagram showing due positions of Figs. 7b—7e in a *Equisetum* spore mother cell in syndetic stage. Abbreviations: pp, plastid pole. bb, boupuet base. n, nucleus. nl, nucleolus. b, area shown in Fig. 7b. c, area shown in Fig. 7c. d, area shown in Fig. 7d. e, area shown in Fig. 7e.

Figs. 7b, 7c, 7d, and 7e. Electron micrographs of a spore mother cell in syndetic stage, showing the distribution of various kinds of cytoplasmic bodies in the plastid pole region and the bouquet base region of cytoplasm. Abbreviations: p, plastid. er, endoplasmic reticulum. m, mitochondrion. d, dictyosome. n, nucleus. nm, nuclear membrane. ch, chromosome. Black line at the bottom of Fig. 7b shows 1μ.