regeneration, as follows. The halved plumule regenerates a new shoot by the formation of a new growing point on the lateral side of the original apex. As the new growing point grows bigger, the relative position of the first leaf-primordia in reference to the whole stem becomes closer together, and eventually two leaf-primordia fuse together into a double-leaf during their growth.

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Growth of the Rhizoid and Behaviour of the Nucleus in *Dryopteris erythrosora*

by Yukio KATO*

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When the fern spore is cultured on the Knop's solution, the rhizoid and protonema apper out of the exine membrane. The protonematic cell, as a rule, contains a large number of chloroplasts, and it divides and elongates actively. On the contrary, no chloroplasts are usually found in the rhizoidal cell which only elongates without cell-division. That is, if the growth phases are classified into two, cell division and cell elongation, only one of these phases, cell elongation, is observed in the case of the rhizoid of *Dryopteris*. The elongation of rhizoid is just like to that of pollen tube. It has been believed that the vegetative nucleus of pollen tube has some rôle in the tube elongation. Bishop and McGowan (1) and Iwanami (3) suggested, however, that tube nucleus does not play a direct rôle in the tube elongation. Is there any relation between the growth and behaviour of nucleus, if it is assumed that the elongation of rhizoids is same with that of pollen tubes from the viewpoint of growth? In order to throw light on this point, some experiments here reported have been done.

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Material and Methods

The spores of *Dryopteris erythrosora* were mainly cultured in the 1/5 dilute solution. The culture of spores was, sometimes, carried out on an agar medium. The petri dishes, 5 cm in diameter, were filled with 10 c.c. Knop's solution or Knop's agar-medium. The media containing indole-3-acetic acid, naphthalene acetic acid, colchicine, sodium phosphate, etc. were also applied. About 15 days after sowing, germinating spores were put on a slide, and fixed or unfixed ones were investigated under the ordinary or phase contrast microscope. The material was fixed and stained with acetocarmine solution. According to this method, only the nucleus stains clearly. Whole length of rhizoid, the distance from the tip of rhizoid to the nucleus, the distance from the spore surface to the nucleus, the change of the nuclear shape affected by the chemicals in the media and the ramification of rhizoids were recorded in some details.

Results

1. Nuclear shape.

The rhizoidal nucleus in the Knop's solution is about $2.9-4.0 \times 0.25-0.5 \mu$ in size, and characteristic spindle-like, ellipsoidal or convex lens-like in shape (Figs. 1a-c), while the nucleus in the embryonic cell of protonema is usually round. Therefore, nuclear differentiation accompanied with cellular differentiation can be observed. In both stained and living materials, the nucleus of the rhizoidal cell is easily observed. In the 2% sodium phosphate solution

Fig. 1a-l. Nucleus of rhizoid and the change of nuclear shape.

a-b, spindle-shaped nucleus of rhizoid, fixed and stained with acetocarmine solution. Knop's solution. c, living state of rhizoidal nucleus, photographed by the use of the phase contrast microscope. Knop's solution. d-e, round nucleus of rhizoid, fixed and stained acetocarmine solution. 2% NaH_2PO_4 solution. f, living state of rhizoidal nucleus, photographed by the use of the phase contrast microscope. 2% NaH_2PO_4 solution. g-l, process of transformation from spindle-shaped to round nucleus.

the nuclear shape changes considerably. That is to say, the nucleus tends to be transformed into a round shape (Figs. 1d-f). The nuclear diameter is about $0.8-1.0\ \mu$ in this case. This change is obviously reversible. The process of nuclear change is shown in Figs. 1g-l. The nucleus becomes to be extremely slender filamentous in the solution containing indole-acetic acid or naphthalene acetic acid. In addition, triangle, heart-shaped or irregular shaped nuclei were also observed in these media.

2. The division of rhizoidal nucleus.

In order to induce the division of rhizoidal nucleus, NaH_2PO_4 , Vitamin B_1 , C, colchicine, sodium adenosine triphosphate, chloralhydrate, indole-acetic acid, naphthalene acetic acid, sucrose, glucose, adenylic acid, nicotinic acid, triiodobenzoic acid, sodium phyrophosphate, asparagic acid etc, were tested. Amitotic figures and dump-belled nuclei were frequently observed in these solutions, but a rhizoid having two nuclei was never found. Amitotic figures may be induced by the change of nuclear shape, as mentioned above. It seems to be probable that the differentiating rhizoid nucleus, at least in *Dryopteris*, does not divide.

This is not true in the case of *Osmunda*. In *Osmunda japonica*, the rhizoidal nucleus divides even in the Knop's-agar medium (Kato, in press). The fact that auxin

Fig. 2. Relation between distance from the spore surface to the nucleus and the length of rhizoid.

is ineffective for the induction of the division in differentiated rhizoidal cells of *Dryopteris erythrosora* must be noticed.

3. The number of rhizoids.

Indole-acetic acid and naphthalene acetic acid make increase the number of rhizoids. The number of rhizoids per spore in a very early stage of germination is only one with a few exceptions in the case of Knop's solution, while it is usually two in the case of the other medium containing monogen (detergent) and it varies from one to four in the case of the medium containing indole-acetic acid. Naphthalene acetic acid prevents the formation of rhizoids from the spore, but rather promotes the formation of lateral rhizoids from the protonema (Kato, in press). When the dormant spores are treated by radioactive cobalt (total dose, 415.4r), germinating spores with two or three rhizoids were frequently observed.

Fig. 3. a-j. Position of the nucleus and elongation of the rhizoid. sp—spore, p—protonema, r—rhizoid, n—nucleus, a-d, rhizoids in the Knop's solution. a, normal rhizoid. b, spore having two rhizoids. c, nucleus present in the tip. d, spore which the nucleus is invisible. e-g, ramification of rhizoids in the colchicine solution. h-j, swelling of rhizoids in the indole-acetic acid solution.

4. The relation between the length of rhizoids and the position of nuclei.

Figure 2 shows the relation between the length of rhizoids and the distance from the spore surface to the nucleus. As shown in the figure, the relation is exhibited by a straight line. This line shows that the distance from the tip of rhizoid to the nucleus is always constant (about 9-10 μ). Such constancy is ascertained also in the case of the spore with two rhizoids (Fig. 3b). However, the nucleus is seldom present in the tip (Fig. 3c) or is not found at any part of the considerably long rhizoid (Fig. 3d).

The lateral rhizoids are resulting from an unequal division in the protonematic cell (Fig. 4). The distance from the tip to the nucleus increases abruptly, and later holds constant value. This fact shows that the nucleus does not dislocate, until the length of rhizoids becomes to be about 7 μ . As soon as the rhizoid elongates up to 7 μ , the nucleus moves from place to place.

As the rhizoid grows, the increase of distance from the tip to the nucleus becomes smaller, while the distance from the spore surface to the nucleus becomes larger (Fig. 4). Although the velocity of movement of nucleus does not, in the strict sense, coincide with the elongation rate, the tendency to coincide holds true always. Finally, it is concluded that the constancy is found not only in the case of rhizoid from the spore but also in that of the lateral

Fig. 4. Relation between length of the lateral rhizoid and distance from spore surface and tip to the nucleus. Black circles and white ones show the distance from the base of protonemal cell to the nucleus and from the tip to the nucleus, respectively. Schematic figures in the square show elongating process of lateral rhizoids and position of nuclei.

rhizoid from the protonema in the early stage of germination. This suggests that the nucleus has a rôle in the elongation.

Indole-acetic acid induces the swelling of rhizoids, and inhibits their growth (Figs. 3h-j). In this case, the constancy of distance mentioned above is not recognizable. Usually the nucleus takes its random position in the rhizoid. In the spores cultured in the medium containing 0.01 % colchicine, the ramification of rhizoids occurs markedly. About 25 days after sowing the spores with ramified and smooth rhizoid were 60 and 40 %, respectively. Even spores having seven branches were often observed (Figs. 5a-d). In order to investigate the rôle of nucleus for the elongation, agar culture medium was applied to this study. Figures 5e-h and 6a-c show the spores drawn every day. Both the branches of rhizoidal cell without nucleus and the original cell with nucleus may elongate well (Figs. 6a-c). The branches appear even from the proximal portion containing no nucleus (Figs. 5e-h).

From these experiments presented here, it may be concluded that(1) the nucleus is not indispensable or requisite for the elongation, and that (2) the elongation occurs chiefly in the apical zone of rhizoids.

At the stage of filamentous protonema, the distance from the tip to the nucleus increases as the rhizoidal elongation proceeds. The distance was about 15-25 μ in a stage of protonema investigated. It was found that the rule of constancy can not be equally applied to the young and old prothallia.

Fig. 5. a-h. Ramification of rhizoids reveals by colchicine treatment. e-h, ramification and elongation of the rhizoid, sketched every day.

Fig. 6. a-c. Ramification of rhizoids induced by colchicine. a-c, elongation of rhizoid, sketched every day.

Fig. 7. Relation between the developmental stage of prothallium and distance from the tip of rhizoid to the nucleus. Schematic figures show the developmental process of prothallium and the position of nucleus.

There is a great variability of the distance in these stages (Fig. 7). Generally speaking, the distance from the tip to the nucleus is larger in old stages than in young stages. For instance, it varies from 13 to 70 μ in a stage of prothallium. The relation is shown in Figure 7.

Discussion

The literatures concerning the rôle of the nucleus in cellular differentiation of plants have been summarized in a recent monograph of Bünning (2). In fern, a marked polarity of the plasm configuration is observed in the protonematic cells. Beside the observation of the plasm configuration, the shape and position of the nucleus and the qualities of the chloroplasts within the different cells were especially observed in the protonematic cells by Reuter (7).

As to the shape of the nucleus, the spherical and oval forms, as well as the spindle-shaped one in cells developed under certain conditions are observed. These qualities of the nuclei and plastids are in correlation with the configuration of plasm. In general, the nucleus is oval in young cells and spindle-shaped in the elongated cells. In the rhizoid of the present material, the spindle-shaped nucleus was observed in the Knop's solution. Thus, the cellular differentiation may be accompanied by the nuclear differentiation.

It is considered that the position of nucleus in the cytoplasm may be changed by the surface tension, the position of vacuole and the protoplasmic density of a cell. The cytoplasm of a rhizoid is abundant in and near the apical portion. The nucleus is usually located in the most active metabolic region. Hence, the nucleus of root hair and of pollen tube are located in and near apical portion. Tanaka (8) have recently reported that the pollen tube nucleus plays an important rôle during the growth of pollen tube. The distance between the tube tip and the nucleus was kept nearly constant during the growth of the tube except in certain stage, and also, in the bifurcated pollen tubes, the position of the nucleus had a certain relation to the growth of the tube. He suggested that the nucleus acts as a center of synthesis of cytoplasm in some way. However, this does not necessarily mean that the nucleus is indispensable for elongation of the tube-like structure. In fact, Bishop and McGowan (1) and Iwanami (3) described that the vegetative nucleus may not act directly on the elongation of pollen tube. In the development of foliar sclerids, translocation of the nucleus of initial cell occurs in certain early stages, giving rise to the protuberances which soon elongate to form ramification (5). Kitamura (5) mentioned that there are found no instances in which the nucleus translocates into the elongating branches and that the nucleus play no rôle for the elongation of protuberances. These suggestions can be applied to the rhizoid elongation in fern. Yamazaki (9) reported that the uneven big rhizoid is formed by the colchicine treatment, contrary to the flat smooth rhizoid in the control medium. However, the relation between the growth of rhizoid and the behaviour of nucleus were not investigated.

Summary

1. The quality and behaviour of nucleus have been studied in the rhizoid of *Dryopteris erythrosora*.
2. Very striking change of nuclear shape has been observed in a medium containing 2 % sodium phosphate, and this change was reversible.
3. In *Dryopteris*, the cell-differentiation may be accompanied by the nuclear differentiation.
4. It has been discussed that the nucleus of rhizoids is not indispensable for the elongation.

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Electron-microscopical Study on Fine Structures of Diatom Frustules XVI

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Chaetoceros Eibenii Grunow (Text-fig. 1; Pl. VI, Figs. 1a-c), Ikari, Bot. Mag. Tokyo, 39: 52, Figs. 1, 2 (1925); Mills, Index Diat. p. 385 (1933); Cupp, Bull. Scrips Inst. Ocean. 5: 106, Fig. 61 (cf. p. 7, Fig. C8) (1942); Desikachary, Mikrosk. 9: 174, Figs. 25, 27, 28 (1954)

Syn. *Chaetoceros borealis* Bailey, Helmcke and Krieger, Diat. Elektr. Bild, 2: 9, pls. 120-122 (1954)

L.M.S.¹⁾ Cells cylindrical, broadly elliptical in valve view, about 39 μ long and about 27 μ broad. Valve surface slightly convex, with an inconspicuous central spine (cs). Mantle (m) moderately low, but distinct. Setae (st) with swollen bases (bs),

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1) Light-microscopic structure.