Some Experiments on the Polarity of Spores in *Dryopteris erthrosora* and *Equisetum arvense*

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The spores of *Dryopteris* are unicellular and soy bean-shaped in dry conditions. When they are placed in the dilute Knop's solution and kept at about 27°C, they begin to germinate within 7–8 days. Two distinct cells—one containing a number of chloroplasts, the other greatly enlarged containing very few of them—grow out of the exine membrane. The one continues the cell division successively and a prothallium is formed, while the other, without cell division, becomes the rhizoid. The present paper treats some questions concerning the essential properties for the determination of polarity. Nakazawa (1952) studied in detail on the polarity of *Equisetum arvense*. However, the spore of this fern is very different from that of *Dryopteris* in regards the essential nature of spores themselves. That is, the former is morphologically apolar spore, while the latter is polar spore. The differentiation patterns of rhizoid are affected by environmental conditions in the case of *Dryopteris*, *Osmunda* and *Equisetum* (Kato 1955, 1956 and in the press), but it still remains the problem whether the original polarity which was latent before the influence is changeable. This question seems to be concerned with the essence of polarity in general, and it is the major subject of this report.

Experimental results

1. Experiments with *Dryopteris*

The dry spore is soy bean-shaped, being about 50 μ long about 30 μ wide. If the spore is immersed in a concentrated KOH solution, the spore coat is dissolved by strong alkali, and the original form of spore is more clearly observable. When the spores are sown in a dilute Knop's agar medium, they begin to germinate. At first the spore becomes enlarged owing to the absorption of water. In the dark, they never germinate. The experiments of unilateral illumination show that both the rhizoid and protonema are formed in random direction. It seems to be probable that the place of rhizoid formation in a spore is not, at least in a dilute Knop's agar medium, altered by the unilateral illumination. The internal structure of spore is usually invisible owing to the opaque thicken membrane. If the spore is cultured in a medium containing tryptophane, it becomes greatly enlarged prior to the first division. Therefore, the spores cast off the spore-coat, as shown in

Fig. 3 a and b (Kato 1956). In the first experiment, spores of such a state were used mainly. By immersing the spore in a concentrated KOH solution, the structure of membrane can be analyzed in detail. In this solution the membrane is differentiated in many layers. Fig. 1 shows the structure of exine membrane, spore membrane and cytoplasm. Each layer in the spore membrane is uniform respectively. The third layer is rather thin and contains abundantly plasm-like substances. Cytoplasm can be divided into two parts, outer and inner, but no membrane between the two is present. Inside of the outer cytoplasm, there lies the cortical layer.

A conspicuous partial differentiation of spore membrane was observed (Fig. 3 c and d, and Fig. 2 a). This portion seems to be an original region where the rhizoid is to be formed in the future when the spore is germinated. Partial thickening of the spore membrane has been observed in *Equisetum* spore by Nakazawa (1952). By him it was called the "rhizoid point", but in the present case it is called the "rhizoid-bearing region", or "RBR" by abbreviation. There are two types as regards the rhizoid formation: i.e., 1) the rhizoid is formed at the RBR (Fig. 2 c). 2) it is formed at some part out of the RBR (Fig. 2 f). The results observed are summarized in Table 1.

As mentioned previously, tryptophane and naphthalene acetic acid (NAA) can change the mode of rhizoid formation. Therefore, it is natural that the rhizoid is formed at the part out of the RBR. It is interesting to note that, if the spore is immersed with a concentrated KOH solution for a long time, the rhizoid-bearing cell alone is coloured red selectively (Fig. 2 e). In

Fig. 1. Schematic drawing of the structure of exine membrane (ex), spore membrane (sp) and cytoplasm (cy) in *Dryopteris erthrosora*. "cot" represents the cortical cytoplasm.

Table 1

	No. of spores with rhizoid formed at the RBR	No. of spores with rhizoid formed at the part out of the RBR
Number of cases	91	9

non-germinating spore, the RBR and its neighbourhood is in particular strongly coloured (Fig. 2 b). Differential colouration was evidently observed.

The partial difference of colouration in RBR can not be attributed only to the partial difference of thickness of the spore membrane. For, it was observed that the colouration extended to the cytoplasm of neighbourhood of the RBR. The special staining of the rhizoid-forming cell may be probably due to some physico-chemical properties. Though Nakazawa (1952) did not observe the differential colouration with concentrated KOH solution,

he described that RP is coloured selectively with dyes such as methylene blue, neutral red, Nile blue, ruthenium red and safranin. In the present ma-

Fig. 2. Spores observed in concentrated KOH solution (*Dryopteris erthrosora*). a-e, spores cultured in the medium containing tryptophane. f-h, spores cultured in the medium containing naphthalene acetic acid. rbr, rhizoid-bearing regions. ch, chloroplasts. rh, rhizoid.

a certain rôle in the formation of rhizoid. In *Equisetum*, the rhizoid cell was formed usually at the RP. Such RP is naturally situated on the top of the rhizoid cell deeply stained with certain dyes (Nakazawa). The results of the present experiment showed that the top of rhizoid is more deeply coloured than the rest. This is, however, limited in a very early stage of rhizoid development, and in the later stages all the parts of rhizoid are uniformly coloured (Fig. 2 d). When the rhizoid was elongated greatly, its cytoplasm and membrane did not quite exhibit the alkaline reaction.

The differential staining between the protonema cell and rhizoid cell indicates that the plasm is polarized according to the morphological polarity. Germinated spores were thus stained with various dyes in this study. For

material, the RBR could not be detected unless the spore is immersed in alkaline solution. Therefore, so far as the present material is concerned, it is still uncertain that the RBR is selectively stainable with certain dyes. The redcoloured substances are not abundantly found in the cytoplasm of rhizoid itself, although the basal part of rhizoid may be deeply stained (Fig. 2 c). The fact suggests that the redcoloured substances may play

the experiment, 20 reagents were applied. Most of them being dissolved in distilled water in 0.01%. In all cases, the material was stained without being fixed. The spores were transferred with pipette on the slide glass, and then a drop of stain solution was added to it. In this experiment, the miniature strata of spore could not be distinguished. The results obtained is as follows; azur eosin and light green stain the plasm of the protonema cell but not the rhizoid cell. Fast green strains both the rhizoid and protonema. Janus green, methylene blue, methylviolet, safranin, Nile blue, neutral red, iodine green etc. stain only the plasm of the rhizoid cell. In the next series of experiments, the spore cultured in a medium containing naphthalene acetic acid were used exclusively.

NAA promotes the formation of lateral rhizoid, but it hinders the formation of rhizoid which is to be developed directly from the spore (Kato 1957). It may be considered that, if the RBR is the centre of rhizoid formation, numerous RBRs are expected to be formed by the addition of NAA of a large quantity in the medium. In fact, the experiment has proved this true. The spore shown in Fig. 2 f has one rhizoid and one RBR, both of which being red-coloured in concentrated KOH solution. Figs. 2 h and g represent respectively a spore having two rhizoids and one RBR and a germinating spore with two RBRs (in one cell). The present result is considerably different from the case of *Equisetum* spores observed by Nakazawa. The presence of RBR is now questionable in the dormant stage of mature spore, but it may newly be formed later owing to the environmental condition in the case of *Dryopteris*.

2. Experiments with *Equisetum*

As a result of the author's previous experiment with colchicine and chloralhydrate treatment, it was known that the rhizoid-differentiation does not quite occur in *Equisetum* spore (Kato 1956). That is to say, in the colchicine treatment, the spores develop into giant globes of about 10-15 times their normal volume (Figs. 4 b and c). As the cell division is perfectly prevented, the germination does not occur. In the chloralhydrate and Na-salt of adenosine triphosphate treatment, only cell multiplication without rhizoid-differentiation takes place (Figs. 4 d-g).

If we eliminate the chemical gradients which are based on the polarity

Fig. 3. Spores cultured in the medium containing tryptophane (*Dryopteris erthrosora*). a and b, spores casting off the exine membrane. c and d, rhizoid-bearing regions and cytoplasm of its neighbourhood.

and which in their turn are responsible for the cell-differentiation, we can bring about cancerous growth. However, it is still questionable whether or not the chemical gradients in these abnormal spores were really eliminated. This point will be clarified if the spore is coloured with alkaline solu-

Fig. 4. a, normal germinating spore with rhizoid (*Equisetum arvense*). b and c, giant globes without rhizoids, cultured in the colchicine-containing medium. d-g, cell aggregates without rhizoid-differentiation, cultured in the medium containing Na-salts of adenosine triphosphate.

tion and vitally with various dyes used in the preceding experiment. In April 1956, the experiments using Nile blue, safranin and neutral red were carried out. Fresh spores were treated with a concentrated KOH solution. In this solution the spore was coloured in reddish brown as a whole, and the "Rhizoid Point" was clearly observed (Fig. 5 a). After germination, however, the colouration begins always from the base of spore and gradually extends to the apex (Fig. 5 b). In two cell stage, the colouration is most intensive in the basal cell of protonema, the least in the apical cell, and there is a gradient between the cells (Fig. 5 c). The giant globe induced by the colchicine treatment is coloured uniformly as a whole (Fig. 5 d). In cancerous cell aggregates induced by Na-salt of adenosine triphosphate or chloralhydrate treatments, there is a remarkable gradient. Young prothallia, showing longitudinally polarized growth (without rhizoid-differentiation), is especially remarkable (Figs. 5 e-f). Essentially the same results were obtained in the vital staining by the use of Nile blue (Figs. 5 h-k). In this case the colouration is most intensive in the rhizoid cell and the basal cell of protonema, the least in the apical cell. These phenomena seem to show that the polarity is of essential for the rhizoid-differentiation and the chemical gradient is not experimentally eliminated. It is interesting to note that the RBR was not often seen in the giant globe obtained by the colchicine treatment. This may be due to the severe extension of spore membrane.

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Discussion

Polarity of the spore in *Dryopteris erthrosora* and *Equisetum arvense* appears as the differential staining activity between the protonema cell and of the rhizoid cell, partial thickening of the spore membrane and partial accumulation of internal material. Polarity brings about the concentration gradients of substances required for the embryonic growth. By the environmental conditions, the original polarity can be modified even in the morphologically polar spore. According to Nakazawa (1952), the essence of polarity in *Equisetum* spores seems to be related to the cortical region coloured with prussian blue reaction. It is labile, being changed by the illuminated direction of the spore, but it is stable against the mechanical stratification induced by centrifuging. Although such importance of cortical region for the polarity is not indicated in the present material, this may be true also in *Dryopteris*.

The classical works are apt to consider something as essential to the polarity. However, the partial aggregation of plastids and chondriosomes is a result of the original polarization existent already and the internal materials are aggregated owing to the polarity. Differential staining of the plasm may be considered as a secondary affair rather than as the essential factor of the polarity. It is evidently found that partial thickening of spore membrane when the spore is immersed in a concentrated KOH solution.

RBR is formed in the case of lateral rhizoid formation so that it may be the centre of the rhizoid formation rather than of the original polarity. This consideration is partially evidenced by the formation of RBR in the secondary rhizoid. It is a well known fact that the anatomical and morphological polarity may often be reversed by applying electric or centrifugal

Fig. 5. Staining spores with concentrated KOH solution and Nile blue. a-f, KOH treatment. g-h, staining by Nile blue. a and g, dry spores. b, c and h, germinating spores. d and i, giant globes obtained by the colchicine treatment. e, f, j and k, cell aggregates obtained by the Na-salt of adenosine triphosphate treatments.

forces. Chemical and electric gradients within the cell, which are often considered to be the essence of polarity, are merely the corollaries and manifestation of the polarity. The ultimate cause of these chemical gradients, i.e., the polarity induced within the spore, cannot be a chemical gradient but only a specific protoplasmic structure. Even if a polar protoplasmic structure is the cause of polarity, we need not suppose that the polar structure is present throughout the entire protoplasm; it may apparently be confined to the peripheral part closely adjoining the cell wall (cortical cytoplasm).

The importance of unequal cell division for the differentiation of tissues and organs has been mentioned by Bloch (1946) and Bünning (1952).

Idioblasts, stomata and hair initials, root hair initials, pollen grains etc.,

Fig. 6. Scheme showing the process of rhizoid formation in the spore.

are produced by unequal division. In ferns, unequal division of the spore is of very importance for rhizoid-differentiation (Kato 1955). The polar region of denser protoplasm is formed as a consequence of polar synthesis of new protoplasm, but not as that of a polar regrouping of the existing protoplasm. However, after the unequal division, synthesis of special substances proceeds at a rapid rate. The theory of polarity based on the partial accumulation of the chemical substances seems to be not appropriate for explanation of the case in *Dryopteris* and *Equisetum*. But it is apparent that the accumulation of chemical substances in the rhizoid-bearing part shows the presence of metabolic gradients in the spore. The plasmic differentiation and partial accumulation of the internal material are the essential factors of the cell-differentiation but not of the polarity. Fig. 6 is a scheme showing the process of the rhizoid formation, hypothetical in part. It shows that the environmental factors bring about the changes of cortical region and this causes the original polarity to establish. In consequence of the polarity, it appears partial aggregations of chloroplasts, differential staining of protonema and rhizoid cells, partial accumulation of red-coloured substances, partial thickening of spore membrane and others. The term "protoplasmic gradient" in this figure means chemical gradients, physical and electric

potential differences of the protoplasmic structure. These phenomena result in an inequality of the daughter cells. This principle of rhizoid-differentiation is similarly applicable to the morphologically apolar as well as polar spore of ferns. It must be the object of further analysis to determine how the polarity manifests the different physiological activities on the cell surface and in the cytoplasm as well as the submicroscopical and visible morphological properties of spore cells. Our approaches to these difficult biochemical problems may be expected from the investigation.

Summary

Some experiments were made on the polarity of *Dryopteris erthrosora* and *Equisetum arvense*. The results obtained are as follows :

1. When *Dryopteris* spores were cultured in a medium containing tryptophane, the hypertrophy of spore occurs prior to the first division. If the spore, the exine membrane of which being taken away by this method, is immersed in a concentrated KOH solution, the partial thickening of spore membrane was clearly seen as in *Equisetum* spore. The thickening place is now called the "rhizoid-bearing region" or "RBR" by abbreviation. The rhizoid is usually formed in the RBR. This is true in the tryptophane-containing medium. In the case of the secondary or lateral rhizoid which is formed at the spore cultured in the naphthalene acetic acid-containing medium, the RBR is so found that it stands for the centre of rhizoid formation rather than that of the original polarity. The partial and localized accumulation of some substances coloured in the KOH solution was found within the spore.

2. In *Equisetum*, the giant spore obtained by the colchicine treatment does not show the gradients in regard to the distribution of red coloured-substances and to the vital staining with Nile blue, while the cell aggregates without rhizoids induced by Na-salt of adenosine triphosphate or chloralhydrate show an obvious gradient mentioned above.

3. The cause and mechanism of the rhizoid-differentiation in ferns were discussed and a working hypothesis was proposed in the present paper.

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