Developmental Mechanics of Fucaceous Algae XV.
Effects of Ultracentrifuging at Later Stages upon the Development of Coccophora Eggs*

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In eggs of Fucus furcatus¹) and of Crystoseira barbata²), the polarity axis is determined by stratification of the intracellular materials brought about by means of centrifuging, so that the rhizoid tends to be formed at the centrifugal end when the centrifuged egg is cultured in normal sea water. But, centrifuging is not effective in some other fucoids, Fucus serratus³), Coccophora Langsdorffii⁴), Sargassum confusum⁵), and S. tortile⁶) in spite of the fact that almost the same stratification can be induced by centrifugal force. In Fucus furcatus, the duration of time in which the centrifugal force is effective for polarity determination is restricted to until 12 hours after fertilization. So that, after this the centrifugation is powerless for the determination¹). In Coccophora Langsdorffii, centrifuging experiments were carried out formerly only during the time before to 40 minutes after fertilization⁴). Therefore, it is questioned whether or not centrifuging is also invalid for polarity determination if the egg is centrifuged at a later stage. Coccophora eggs are spherical or a little elongated at or just after fertilization. Regardless of their original form, they are more or less elongated by their own morphogenetic movement to become ovate forms pointed towards an end. After this transformation, their polarity axis is determined, and later the rhizoids are formed at the pointed end. The writer here made an experiment to centrifuge egg strongly after the transformation stage, and inspected their later development.

Material and Method

The experiments were carried out in April 1960, at the Marine Biological Station of Asamushi, Aomori Prefecture, Japan. The material, Coccophora Langsdorffii, was collected from near the Station, and was cultured in glass vessels with filtered sea water. After liberation, eggs were artificially fertilized, and were strongly centrifuged at stages before and 1, 10, 15, and 20 hours after fertilization. After the centrifugation, the stratification was inspected by use of a microscope, and then they were cultured in filtered sea water contained in Petri dishes. Conveniently, the stratification of plastids is retained without being redistributed even until the stage of young embryos, so that the original direction towards which the egg was centrifuged can be distinguished with ease in later stages. Upon the centrifugation, the material was placed in glass tubes of 4 mm. in diameter and 12 mm. in length, the tubes containing the material were set in an air-turbine centrifuge of 15 mm. in radius, which was turned for five minutes at 25000 times gravity. In Coccophora, the development proceeds not always uniformly, but more or less differs according to the individual egg. Therefore, though centrifuged at the same time after fertilization, some eggs undergo stratification at the stage of the primary morphogenetic movement, but other
eggs at two-cell stage or another. Conveniently, however, as was remarked before, the position of plastid layer, which is still retained, indicates the stage at which the egg was centrifuged (Fig. 1 H, I, J, K, L, M).

**Observations**

The unfertilized egg is spherical or a little elongated containing one nucleus in the central region surrounded by a number of plastids (Fig. 1A). By means of centrifuging, the egg protoplasm is stratified into five layers (Fig. 1B). At the centripetal end, there come dark yellow substances which are presumed to be composed of oil or fat, stainable pink with Sudan III. This layer is liable to be thrown out of the egg cell into the space surrounding the latter when the egg is centrifuged before fertilization. Next to this, a transparent layer is stratified, then a dark brown layer composed of plastids and the nucleus, a colorless clear zone, and finally a layer composed of fine grey particles extending to the centrifugal end. The same stratification appears regardless of the form of the egg. Later than one hour after fertilization, the stratification takes place in a little different pattern. That is, the oil drops, which also appear at the centripetal end, are never thrown out of the egg (Fig. 1C), indicating that a certain change took place in the cell membrane after fertilization. Presumably, deposit of cellulose seems to be such a kind of change. One hour after fertilization most of the eggs still retain their original form as the time is earlier than the occurrence of the transformation. In the culture of these stratified eggs, it is known that the formation of the primary rhizoids takes place regardless of the site of stratification as was reported before

10 hours after fertilization, the egg undergoes the peculiar transformation into ovate forms pointed towards one end (Fig. 1D). Centrifuged at this stage, the stratification appears in various directions according to the orientation in which the egg is placed by chance upon the centrifugation (Fig. 1E, F, G). When these eggs were cultured, it was revealed that rhizoid differentiation was affected by centrifuging. In normal development, the presumptive part where the rhizoid pole is determined appears at the pointed end of the transformed egg

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<th>Table 1. Number of eggs centrifuged apically, laterally, and basally at the transformation stage, and ratio of their rhizoid formation.</th>
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basal end. The opposite part, the apical end, is the site where the embryo apex is differentiated later. If the egg is centrifuged basally, the oil cap appears at the apical end of the egg (Fig. 1E), so that this is called "basal centrifugation". But if it is centrifuged apically, the oil cap appears at the basal end (Fig. 1G), i.e. "apical centrifugation". In the same sense, "lateral" (Fig. 1F) and "oblique" centrifugations
Fig. 1. A, normal egg before fertilization; B, the same centrifuged, oil drops are arrowed; C, centrifuged after fertilization; D, transformation stage of a normal egg; E, the same centrifuged basally; F, centrifuged laterally; G, centrifuged apically. H, young embryo forming rhizoids resulting from an apical centrifuging; I, young embryo failing in rhizoid formation resulting from a basal centrifuging; J, young embryo forming rhizoids resulting from a lateral centrifuging. K, basal centrifuging at two-cell stage; L, embryo resulting from the same. M, embryo resulting from an apical centrifuging at two-cell stage. N, redistribution of the nucleus (arrowed) after stratification; O, a normal embryo.
are possible. In the culture of these centrifuged eggs, it was revealed that (1) the eggs apically or laterally centrifuged could form rhizoids normally at the presumptive rhizoid end (Fig. 1 H, J). However, (2) the eggs centrifuged basally could not form a rhizoid (Fig. 11, Tab. 1). In apical centrifugation, the apical half wanting plastids sometimes fails in cell division resulting in a half embryo reported in a previous paper7). Though a rhizoid is not formed in the egg centrifuged basally, irregular cell divisions take place. In this case, most of the plastids and the oily materials are retained without being redistributed. However, the nucleus is promptly replaced to the center, and undergoes division (Fig. 1 N). In the normal development, the primary segmentation wall is formed perpendicular to the long axis of the egg. If the egg is centrifuged at two-cell stage or later, the rhizoids are formed normally (Fig. 1 K, L, M). Thus, the ultracentrifuging experiments revealed the relation between the stages at which eggs were centrifuged basally and their later potency of rhizoid differentiation as shown in Fig. 2. That is, when eggs are centrifuged before fertilization, 70 per cent of them can form rhizoids at the presumptive rhizoid end. As the distinction

of the base and the apex is not always easy in spherical eggs before actual transformation, the observation was restricted only to the eggs whose form was originally ovate pressed by the mucilaginous coat surrounding them. For this purposes, these eggs were cultured isolated from other ones. The percentage of forming rhizoids at the presumptive end rises when the eggs are centrifuged after fertilization but before the transformation to 88 per cent. But when they are centrifuged at transformation stage, it sinks down to only two to four per cent, then rises again when centrifuged at two-cell stage or later.

When the embryo resulting from the centrifuged egg is immersed in sea water where 0.01 per cent brilliant green is dissolved, the cytoplasm is stained vitally. At this time the staining appears from the basal pole or the rhizoids, which is also the case in the abnormal embryo without being centrifuged8). In addition, it is noteworthy that even in the normal embryo failing in rhizoid formation resulting from the basal centrifuging (Fig. 11), the vital staining also tends to appear from the presumptive rhizoid pole, i.e. the basal end.
Discussion

The most notable facts observed in the above experiments are that rhizoid formation is effected by direction of the stratification when the egg is ultracentrifuged after morphogenetic transformation. That is, when the plastid layer was stratified laterally or in basal regions, the rhizoids were formed normally. However, the rhizoids are not differentiated when the plastid layer was stratified in apical regions. This relation is illustrated in Figure 3, and it will be explained as follows. Judging from the facts above, it seems that the localization of intracellular materials takes part in the differentiation of rhizoids. Though the main material for this is not clear at present, it is certain that it is one of the substances stratified by ultracentrifuging. But it is also clear that the main factor is not concerned with the nucleus. Because the nucleus is promptly replaced to the central region before occurrence of the cleavage in whatever direction it was stratified (Fig. 1 N). Therefore, it must be cytoplasmic materials that take part in the rhizoid formation. However, it is not true that the differentiation of rhizoids is conditioned only by intracellular materials. If it were so, the rhizoid would be formed on any side of the ovate egg corresponding to the direction of the centrifugation. But the fact is different. That is, the rhizoid formation is inhibited when the plastid layer is stratified to the apical region, i.e. far from the presumptive rhizoid pole where the rhizoids were to be formed in normal development. Therefore, it seems that rhizoid formation is also dependent upon the cortical cytoplasm which is not moved by centrifugal force. That is to say, there must be two main factors controlling rhizoid formation. One is the polar differentiation of the cortex of the egg cell which determines the site of the differentiation. The other is a certain intracellular cytoplasmic materials movable by centrifugal force whose localization is important for actual formation of rhizoid. It seems that the rhizoids cannot be formed if the intracellular materials are distributed too far from the presumptive rhizoid pole. In normal cases, the materials are almost uniformly distributed in the cell, so that rhizoid differentiation takes place at the presumptive part. But, if the materials are taken off from the presumptive rhizoid pole by means of centrifuging, the rhizoid formation fails. It is known that the presumptive rhizoid pole is determined at or sometimes before fertilization\(^6,9\). It is also reported that in *Coccophora* the rhizoids are formed but their site of appearance is not determined by centrifuging when the egg is centrifuged before or just after fertilization\(^6,9\). At this time, it is considered that the powerlessness of centrifuging in determination is attributed to prompt redistribution of the materials necessary for rhizoid formation to their original location corresponding to the presumptive rhizoid pole. When the egg is centrifuged at two-cell stage, it cannot affect the rhizoid formation even though the plastid layer is stratified apically in each cell. Therefore the plastids do not seem to be the main factor for rhizoid formation.

Summary

Eggs of *Coccophora Langsdorfi* were cultured after being ultracentrifuged at
25000 time gravity for five minutes at the time before and 1, 10, 15, and 20 hours after fertilization. As a result, the following was revealed.

(1) When the plastid layer is stratified in apical regions of the egg after transformed to ovate forms, the rhizoid cannot be formed. However, when the same layer is stratified laterally or in basal regions, normal rhizoids are differentiated at the basal, i.e. the pointed end.

(2) Therefore, distribution of the intracellular materials is a factor in the actual formation of rhizoids. But, as the site of the nucleus stratified is not concerned with this, distribution of certain components of the cytoplasm seems to take part in the rhizoid differentiation. It is, however, not the only factor. It seems that (a) the site of the rhizoid differentiation is determined by the cortical layer of the cytoplasm immovable by centrifuging and (b) its actual formation is performed by a certain kind of cytoplasmic elements movable by centrifuging.

(3) When the egg is centrifuged at two-cell stage or later, the rhizoid formation is not influenced by it.

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References