Developmental Mechanics of Fucaceous Algae X. Structure of the Mucilage Surrounding *Coccophora* Eggs

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中沢信午*: フークス科藻類の発生力学 X. スギモクの卵をとりまく粘質物の構造

Received May 9, 1958

When fertilized *Fucus* eggs are forcibly elongated by sucking them into a capillary, having a diameter smaller than that of the eggs, and then jetting them slowly out of it, their developmental axis, the polarity, is determined parallel with the elongation⁷⁾. Another instance of axis determination by external morphology is known in *Sargassum*⁵⁾. That is, in this alga, the egg becomes elongated to an ovate form, pointed on one side, pressed laterally by each other at the time when it is discharged from the conceptacle, and the primary rhizoids are to be formed at the pointed end. In *Coccophora*, another fucoid, the majority of the eggs are spherical, and their polarity is determined by the entrance point of spermatozoid¹⁾. Some eggs, however, are more or less elongated as they are pressed by the surrounding mucilage, and their polarity axis is determined parallel with the elongation⁶⁾. The mechanism of that elongation is analysed in this paper from the structure of mucilage surrounding the egg.

Fig. 1. *Coccophora* egg (e) surrounded by mucilage, observed in Indian ink.

Eggs of *Coccophora Langsdorfii*, the material, were discharged from female receptacles cultured in glass vessels at the Marine Biological Station of Asamushi. For revealing its structure, the intact mucilage was stained with 0.01 per cent Congo red and with 0.001 per cent ruthenium red. Both of these were dissolved in sea water. Otherwise, the mucilage was immersed in 0.1 per cent tannic acid solution for ten hours, rinsed for ten minutes, then transferred to 0.1 per cent ferric chloride solution. By this method, the mucilage adsorbed tannic acid specifically, stained black with the iron, so that its structure was made clear.

The outer margin of the mucilage was revealed by putting eggs into sea water mixing Indian ink. By this means it is known that the egg, about 150 μ in diameter, is surrounded by a large mass of mucilage, about 800 μ in diameter, and a part of it

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is extended to form a stalk of some 100μ in length (Fig. 1). The mucilage can be stained with ruthenium red. The staining, however, occurs not in the overall mucilage mass but in a limited zone so as its extension is about $300\mu \times 700\mu$. Therefore the whole mucilage is divided into the inner and the outer parts, and merely the former is stained with ruthenium red. When the intact mucilage is immersed in ferric chloride after being treated with tannic acid, it is stained black. The staining appears only in the inner part which is stainable with ruthenium red as aforesaid. Upon this occasion, we can clearly distinguish the inner membrane staining deepest of all the other part of the mucilage. This membrane, i.e. the wall of the perioospheric space, is about 2.5μ in thickness and has a number of small pits piercing perpendicular (Fig. 3). The interval between the pits is about 1μ . The outside of that membrane is filled with a thick mucilaginous layer staining in a lighter tone so that it can be distinguished from the inner membrane as well as from the outer mucilage which is unstainable. That is to say, the mucilage mass is composed of three parts, i.e. 1) the inner membrane stained deep black, 2) the middle layer stained in a lighter tone, and 3) the outer mucilage unstainable by tannic acid-iron method. The middle layer consists of many sub-layers so that a number of striations running parallel with the surface can be perceived (Fig. 3). The middle layer extends towards one direction so as to form the axle of the stalk, in which the layer is also stained in a light black tint and there we can see many striations running longitudinally. On these striations, particularly in the stalk, there are a number of small particles staining black. The striations are much more closely compact in the stalk, so that there the striations look like a bundle of stripes. As a result, dynamically, the perioospheric space is more or less distorted to an elongated form. Herein, if the space is comparatively large enough, the egg is free from the shape of that space so that it takes a spherical form. But if

Fig. 2. Staining mucilaginous stalk with Congo red. A) eggs before immersing in Congo red solution, B) stained stalks, C) the same indicating a uniform relation between the egg axis and the direction of the stalk. Note that the stalk is composed of three separate zones, *a*, *b*, and *c*.

the space is comparatively small, the egg is pressed by the mucilage so that it comes to be elongated in the direction parallel with the longitudinal axis of that space, that is, parallel with the stalk. The egg before fertilization is so soft that its form can be altered even by a slight pressure. This relation is revealed when the mucilage is stained with Congo red.

Fig. 3. Schematic illustration of the structure of mucilage. a, outer mucilage; b, middle layer; c, inner membrane; d, perioospheric space; e, gap.

of such eggs is determined in relation to the position of the stalk. Its mechanism was stated above.

Summary

1. The mucilage which surrounds the egg of *Coccophora Langsdorffii* is stained with Congo red, with ruthenium red, and with ferric chloride preceded by treatment with tannic acid.
2. The mucilage is composed of the inner membrane, the middle layer, and the outer mucilage. The inner membrane has a number of small pits. The middle layer consists of many sublayers. The outer mucilage cannot be stained with any of the above agents.
3. The axle of the mucilaginous stalk is stained red, and its surrounding zone

When the intact mucilage is kept in 0.1 per cent Congo red dissolved in sea water, the dye is accumulated in the stalk gradually. At first, the stalk axle is stained red, then deep blue-violet particles begin to surround the axle (Fig. 2B, C). The particles are almost of the same spindle-form. What is noteworthy is that the staining appears to be separated into three principal zones, that is, 1) the neck zone which is the smallest, 2) the middle zone, and 3) the tail zone (Fig. 2B). These are separated from each other by unstainable clear gaps, which are also observed in the staining by tannic acid-iron method (Fig. 3). The colour differentiation of Congo red implies that the stalk is composed of the axle, charged negative, and the surrounding mucilage, charged positive electrically. That the egg was connected with the conceptacle by a stalk was reported by many authors^{2), 3), 4)}, and now its structure was made clear by the above staining experiments. In Figure 2 we can see that the axis of the elongated egg is all directed parallel with each stalk. This verifies that the polarity

deep blue-violet with Congo red. This reveals a positional relation between the egg and the stalk. It is known that the form of the elongated egg is controlled by the pressure, perpendicular to the stalk, of the inner membrane, so that the polarity axis of such eggs is determined parallel with the stalk.

The writer's sincere thanks are due to Prof. A. Kimura, Tohoku University, for his kind cooperation to the present research.

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