THE EFFECT OF SHAPE ON THE DEVELOPMENTAL AXIS OF THE FUCUS EGG [1]

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The cleavage plane of many cells has been determined by artificially imposed elongation. In general the long axis of the division figure comes to lie in the long axis of the cell so that the plane of division is perpendicular to the long axis. The plane and sequence of division of many eggs has been altered in this way, but the primary developmental axis, or polarity, has not been affected. Lindahl (1936) has found, however, that when sea urchin eggs are stretched by passing through capillaries, the first end to emerge tends to become ventral (if it is not the animal or vegetal pole) so that in this case the dorso-ventral axis is determined, as it also is by centrifuging (Runnström, 1926; Lindahl, 1936). In the *Fucus* egg, which appears to be more plastic than most animal eggs, the primary axis may be determined by centrifuging (Whitaker, 1937, 1938). The experiments to be reported were undertaken to test the effect of elongation on the division plane and the developmental polarity of the egg of *Fucus furcatus.*

Method

This species of *Fucus* is hermaphroditic and fertilization takes place when the egg capsule dissolves and releases 8 eggs into sea water in which motile sperm are present. The time of fertilization of the eggs from a given capsule can be observed quite precisely. Immediately following fertilization the naked egg secretes a film of soft gelatinous material which gradually hardens to form a rigid cell wall. If a recently fertilized egg is gently sucked into a glass pipette of less diameter than the egg, it enters and becomes elongated to an extent which depends on the relative diameters of the egg and the pipette. When it is gently blown out into sea water, the elongated shape is retained, no doubt largely because of the hardening of the cell wall in the elongated shape. Unless the egg is sucked into the pipette before the wall hardens, it will not enter.

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Only spherical eggs were selected so that they were taken into the pipette entirely at random with respect to any pre-existing organization which might occur in the egg. The eggs were taken into the pipette at 10–20 minutes after fertilization. Each egg was gently blown out into an individual 1 cc. syracuse dish containing either of two media: normal sea water at pH 7.8–8.2, or sea water acidified to pH 6.0 by adding 5 parts of McIlvaine's buffer (secondary sodium phosphate-citric acid) to 95 parts of sea water. The acidified sea water was equilibrated with

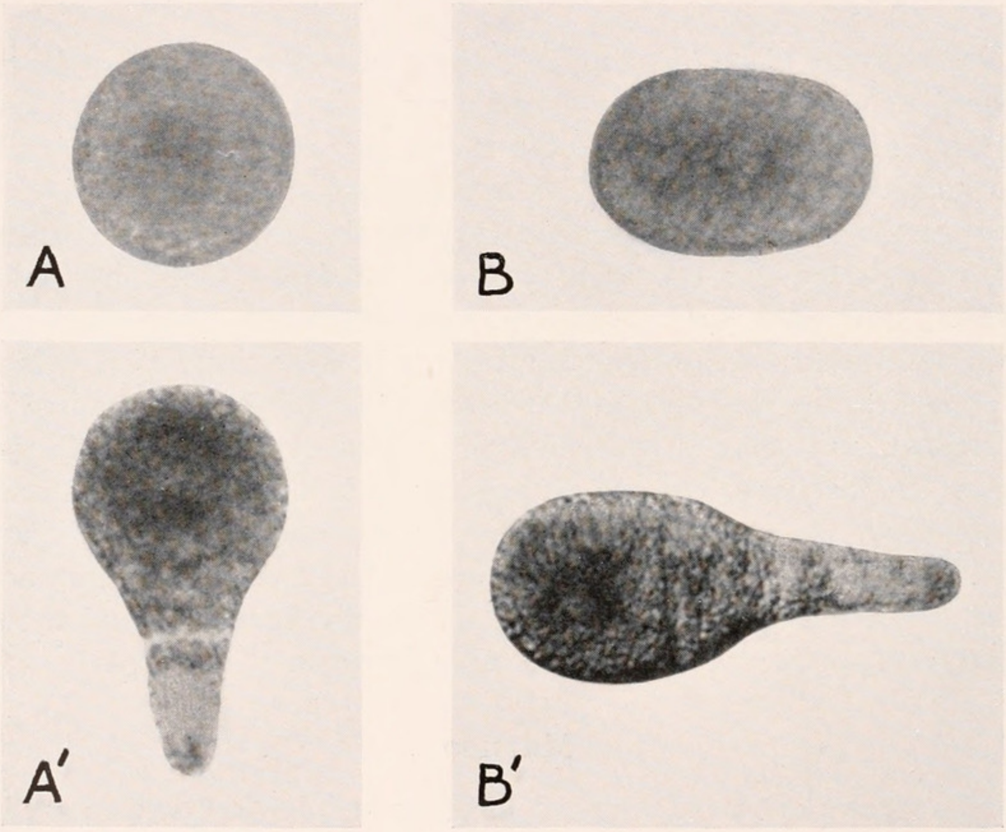

FIG. 1. Photomicrographs of *Fucus* eggs as seen from above. (*A*) Normal spherical egg soon after fertilization; (*A′*) similar egg after rhizoid has formed. (*B*) An egg which has been elongated soon after fertilization; (*B′*) an elongated egg which has developed in normal sea water at pH 8.0, showing typical formation of rhizoid at one end of long axis.

atmospheric CO_2 tension by vigorous aëration through a large scintered glass nozzle until the pH was stable as indicated by a glass electrode. The long and short axis of each egg was measured by means of an ocular micrometer, and the ratio, long axis/short axis, serves as a measure of the degree of elongation. The individual dishes were placed in moist chambers. All stages of the experiments were carried out in the dark or in dim red light in a humid constant temperature room at $15 \pm 1/4°$ C., until the final results were recorded 15–24 hours after fertilization.

Experiments were carried out in June, July, and August, 1936, at the Hopkins Marine Station, and in March and April, 1938 at Stanford University.

Results and Conclusions

One hundred and fourteen eggs, elongated so that the ratio, long axis/short axis, ranged from 1.07 to 1.8, were reared in normal sea water. It was clear at once that a very high proportion formed the rhizoid very close to one end of the long axis. An example is shown in Fig. 1, *B′*. The shape of the elongated egg is very nearly that of a cylinder with rounded ends (Fig. 1, *B*). The angle between the long axis of the egg and the extrapolated axis of the rhizoid was taken as a measure of the proximity of the point of rhizoid origin to an end of the long axis. The rhizoid ordinarily grows out quite straight. Ninety-six per cent of the eggs formed rhizoids within 45° of an end of the long axis, and 53 per cent formed them within 10°.

When the eggs are divided into catagories based on the degree of elongation, the results show that the effect is more marked the greater the elongation. Thus of the 33 most elongated eggs (long axis/short axis = 1.4–1.8), 100 per cent formed rhizoids within 45°, and 73 per cent within 10°, of an end of the long axis.

Ordinarily the two ends of the elongated egg were quite similar in size and shape, and there was not a high correlation between the end which left the pipette first or last, and the end which formed the rhizoid. The relations were observed in 93 eggs, and 60 per cent formed rhizoids near the ends first to leave the pipette. It is not clear what determines which end of the long axis will form the rhizoid. Farmer and Williams (1898) note in their classical work on the cytology of *Fucus* that the centrosomes appear to arise from the egg protoplasm near the egg nucleus rather than from the sperm, as in the typical animal egg. They also note that the two asters, at either end of the egg nucleus, are often if not usually unequal in size in the early stage. The elongation of the egg undoubtedly orients the division figure in the long axis, and it is possible that the asymmetry of the early aster may have something to do with determining which end forms the rhizoid.

From these experiments it can be concluded that elongation of the *Fucus* egg determines not only the plane of cell division, but the axis of differentiation as well. The differentiation in fact precedes the cell division, since the rhizoid protuberance forms some hours before the first cell division.

Elongated Eggs Reared in Acidified Sea Water

Forty-seven eggs which were elongated in the same manner were reared individually in dishes containing sea water acidified to pH 6.0. In this case the degrees of elongation did not cover quite so great a range, the ratio, long axis/short axis, being 1.2–1.6.

In this medium the results are quite different. Seventy per cent formed rhizoids between 46° and 90° from an end of the long axis, i.e., formed rhizoids nearer an end of a short axis. An example is

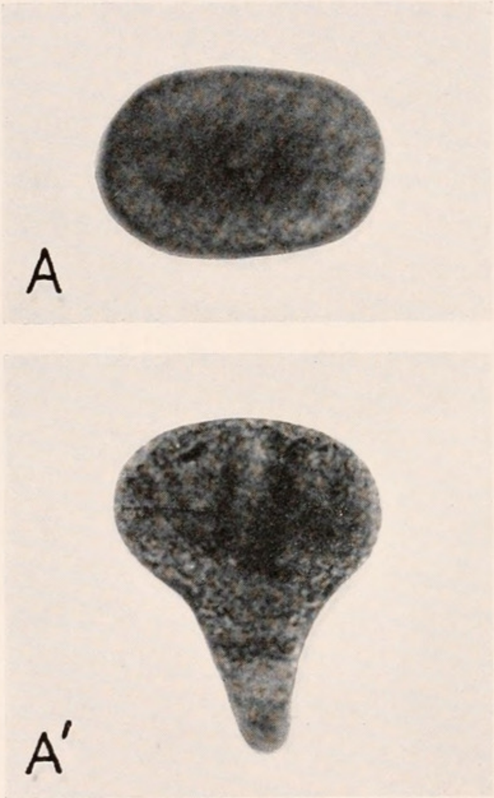

FIG. 2. Photomicrographs of *Fucus* eggs. (*A*) An egg which has been elongated soon after fertilization. (*A'*) Side view of a similar egg after developing in acidified sea water at pH 6.0, showing formation of rhizoid at end of short axis. The rhizoid formed toward the bottom of the culture dish.

shown in Fig. 2, *A'*. Thirty per cent formed rhizoids within 45° and 13 per cent within 10° of an end of the long axis. The determination in this case is not so sharp as it is at pH 7.8–8.2, but it is clearly centered about the short instead of the long axis of the egg.

It has been noted previously (Whitaker, 1937) that an egg developing on the bottom of a dish in medium at pH 6.0 tends very strongly to form the rhizoid toward the bottom of the dish, while at pH 8.0 the egg is more nearly indifferent to the bottom of the dish. There is now evidence that this phenomenon at pH 6.0 is largely due to the fact that the bottom of the dish is an impediment to diffusion, so that the egg is developing in a gradient of products diffusing from itself. When two eggs develop in close proximity they tend strongly to form rhizoids toward each other when the medium is at pH 6.0,[2] but not when it is at pH 8.0 (Whitaker, 1937; Whitaker and Lowrance, 1937). Acidity of the medium strongly intensifies the response of the eggs to their common diffusion pattern, and in like fashion it greatly intensifies the response of an egg to the bottom of the dish. All of the elongated eggs in medium at pH 6.0 formed rhizoids toward the bottom of the dish. Since an elongated egg naturally lies on the bottom of the dish with the long axis parallel to the bottom, a rhizoid forming toward the center of concentration of substances diffusing from the egg tends to form toward the lower end of the vertical short axis.

It appears highly probable that the response of the elongated eggs at pH 7.8–8.2 is essentially a shape effect, and that at pH 6.0 the shape effect is largely overcome or superseded by the response of the egg to the bottom of the dish. Some shape effect appears to persist as a resultant, to spread the positions of rhizoid origin, as noted. At pH 6.0, 13 per cent of the eggs still formed rhizoids within 10° of an end of the long axis, while at pH 7.8–8.2 no eggs formed rhizoids within 10° of an end of a short axis.

Summary

1. When recently fertilized eggs of *Fucus furcatus* are gently sucked into a small pipette while the cell wall is hardening, and are then blown out into sea water, an elongated shape is retained.

2. Elongated eggs reared in normal sea water at pH 7.8–8.2 form rhizoids at or near one end of the long axis. The axis of differentiation, as well as the plane of cell division, is thus determined by the shape imposed on the cell. The exactness of determination increases with greater elongation of the egg.

3. When *Fucus* eggs develop in sea water acidified to pH 6.0, they acquire a very strong tendency to form rhizoids toward the bottom of the culture dish (which blocks diffusion to form a gradient of products from the egg). This tendency largely overcomes the shape effect when elongated eggs are reared at pH 6.0, and most of them form rhizoids toward the bottom of the dish near the lower end of the vertical short axis.

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[2] Acidified with either citric acid-secondary sodium phosphate, or with HCl–$NaHCO_3$.

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