DIRECTIONAL TRANSPORTATION OF RIBONUCLEIC ACID IN *FUCUS* EGGS

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The *Fucus* eggs is almost completely spherical for several hours after fertilization. Then, a part of its surface bulges out to form the primary rhizoid. At this time, it is supposed that the substances that take part in the rhizoid formation are accumulated to the bulging region. Actually, in *Coccophora* eggs, it is reported that a rhizoidless embryo develoopes if a certain cytoplasmic elements are stratified by centrifugation at a distance from the presumptive rhizoid region (Nakazawa, 1960). In *Acetabularia*, the morphogenetic substance is directionally transported from the nucleus, that occupies the basalmost region, to the apical tip and there it takes part in the cap differentiation (Hämmerling, 1934). The nature of this substance seem to be a kind of RNA originated from the nucleus or to be a combination of RNA and protein (Werz, 1959, 1962). Similar intracellular transportation of RNA would be expected in *Fucus* eggs. The writer stayed at the Institute of Algology, Muroran, Japan extending over May to June, 1964, to investigate the above question. The result will be presented in this paper.

MATERIAL AND METHOD

Receptacles of *Fucus evanescens* were colected, washed several times with filtered sea water to remove diatoms and other miniature organisms attached on the surface. After being washed, they were kept in Petri dishes with filtered sea water. Eggs liberated and sank down to the bottom of the dish were taken up with a pipette and were cultured whit sterilized sea water contained in Petri dishes of 40 mm in diameter and 15 mm in depth. As the present species is hermaphrodite, i. e. both the oogonia and the antheridia develop in the same conceptacle, the liberated eggs are presumed to be fertilized already. Thus being cultured, soon the eggs sink and adhere to the bottom by the mucilage of its own. In half a day, most of these form the bulge of the primary rhizoid. At two stages, before and after the bulging, the eggs were fixed with Carnoy's fixative for 1 hour, washed with alcohol series, and soaked in water. These were divided into two lots. The one was stained directly by use of methyl gree-pyronine solution for 10 minutes, rinsed with water, immersed in tertiary butyl alcohol for 1 minute, then were transferred to 80 per cent alcohol, and were observed with a microscope. The other lot was treated with 0.2% RNase solution, adjusted at pH 7.1 by use of M/25 phosphate buffer, for 3 hours at 62°C, washed with water, and then was stained in the same method as above.

RESULTS WITH DISCUSSION

Before the bulging of the rhizoid pole, the nucleus stained blue, and the cytoplasm red mixed with some blue-stained particles distributed uniformly around the nucleus (fig. 2A). Different from this, in the eggs treated with RNase, the red or blue cytoplasmic staining could not be observed. This indicates that not only the red staining, but also the blue coloration of the cytoplasm should be attributed to the presence of RNA. Therefore, the blue-stained elements in the cytoplasm seems to be different from DNA, stained blue in the nucleus and cannot be digested by RNase.

In those eggs which bulged out the rhizoid protuberence, a peculiar staining pattern was observed. That is, the red and the blue staining of cytoplasmic RNA appeared not uniformly, but it is limited to the range of space from around the nucleus to the tip of the rhizoid bulging (figs. 1A, 2B). If two or three regions are bulging side by side (figs. 1B, 2C), oppositely (fig. 2D) or radially (fig. 2E), the peculiar staining ap-

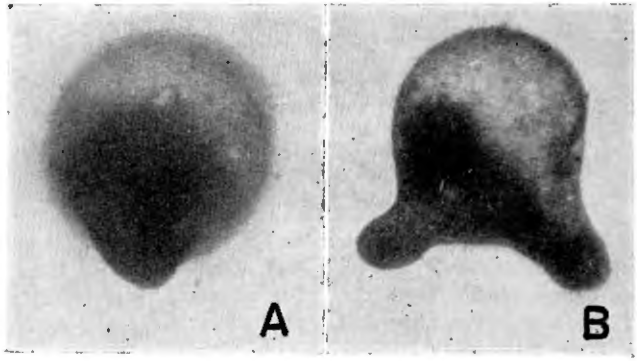

FIG. 1. Photographs of *Fucus evanescens* eggs, bulging the rhizoid, stained with methyl green-pyronine.

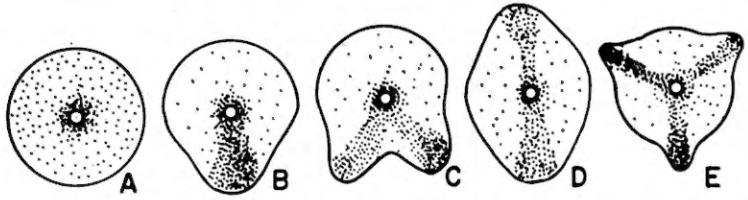

FIG. 2. Staining of ribonucleic acid in cytoplasm (in dots) of *F. evanescens* eggs. Note that the ribonucleic acid particles are transported from around the nucleus towards the rhizoid extremity.

pears correspondingly. If the bulging eggs are tested after being treated with RNase, the staining cannot be observed. The above observations strongly imply that cytoplasmic RNA, born in the nucleus, is uniformly distributed before bulging of the rhizoid pole, but later, after the bulging of the rhizoid protuberence, it is directionally transported to the tip of that bulging and there it takes part in the rhizoid elongation. This resembles the case of *Acetabularia* in which the nucleus-originated RNA is transported to the stemp tip (Werz, 1962). The facts that some cytoplasmic elements are stained blue with methyl green-pyronine and that they are also digested by RNase, may suggest that some RNA is also stained blue. Thus some Rna seems to have a similar property with DNA. It is well known that the so-called mesenger RNA is a high polymer reflecting bot the structure and the property of its template, the nuclear DNA. On the other hand, the peculiar blue staining of DNA with methyl green-pyronine is said to be attributed to its high polymerization (Lison, 1960). If so, the cytoplasmic RNA particles stained blue as above may represent such messengers, that may take part in the protein synthesis in the tip of the rhizoid.

SUMMARY

Fucus evanescens eggs were stained with methyl green-pyronine solution before and after bulging of the rhizoid pole. As a result, the following was made clear.

1) Before the bulging, red- and some blue-stained particles are demonstrated in the cytoplasm, and these particles are distributed uniformly in the cytoplasm. The nucleus is stained blue. After the bulging, the cytoplasmic staining occurs selectively from around the nucleus to the tip of the rhizoid protuberance in bulging. Both the red and the blue cytoplasmic staining did not take place if the eggs were tested after being treated with RNase for 3 hours at 62°C.

2) The above indicates that the stained cytoplasmic elements represent RNA originated from the nucleus, and it is directionally trans-

ported from around the nucleus to the tip of rhizoid to take part in the rhizoid elongation.

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