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Notes on Fucales 10. Inhibition of rhizoid formation and division by gossypitrin in *Fucus* eggs

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Eggs of *Fucus evanescens*, just after fertilization, were cultured in sea water containing gossypitrin, a flavonoid. As a result, in culture with 10^{-3} *M* gossypitrin, rhizoid development was completely inhibited while cell divisions took place twice up to 4-cell stage. When the egg was transferred from natural sea water to 5×10^{-4} *M* gossypitrin-sea water earlier than 8 hr after fertilization, cell division ceased at 4-cell stage and rhizoid occurred in 65% eggs. While cell divisions took place normally and rhizoid development occurred in more than 70% eggs when transferred to gossypitrin-sea water later than 10 hr after fertilization. It those eggs, undergoing cell division without rhizoid in gossypitrin-sea water, are transferred to normal sea water, rhizoid begins to develop.

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According to personal communications of Dr. TOSHIO NAKABAYASHI, Shizuoka University, gossypitrin, a flavonoid, inhibited in body weight of rats when it was fed successively for 10 days at 100 mg a day. The present author received gossypitrin from him and it was tested for *Fucus* eggs. A part of this experiment was preliminarily described formerly (NAKAZAWA 1969). Later, similar experiments were repeated extending over 1969 to 1972. As a result, it was found that gossypitrin inhibits formation of rhizoid without disturbing cell divisions up to 4-cell stage, but further division is inhibited. These experiments were carried out at the Institute of Algological Research of the Hokkaido University, Muroran.

Material and Method

Gossypitrin (MW=480) was taken out and purified by Dr. NAKABAYASHI from sporangiophores of *Equisetum arvense*. By dissolving this agent in natural sea water, a series of culture media were prepared: 0.5, 1.0, 2.5, 5.0, 7.5 and 10.0 times 10^{-4} *M* gossypitrin-sea waters. For preparation of these media, 48 mg gossypitrin was put into 100 m*l* natural sea water, and it was warmed to 80°C for complete dissolution. By this process, the medium first turned to yellowish green, then gradually to orange color, which was cooled to 18°C. Thus was obtained the stock medium (10^{-3} *M*), which was diluted to make the above series.

Fresh spores of *Fucus evanescens*, just after being liberated from receptacles and naturally fertilized, were sown in these

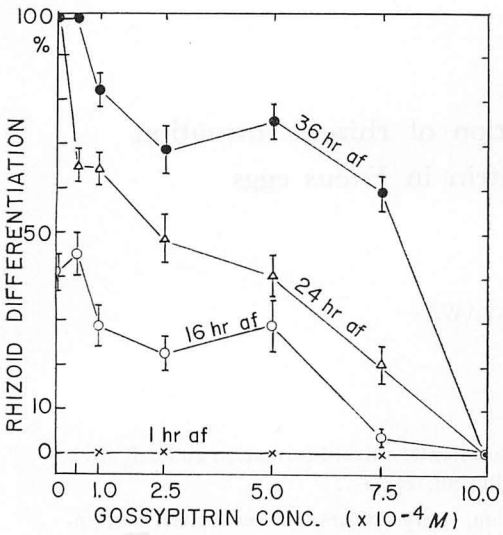

Fig. 1. Rhizoid formation in *Fucus evanescens* eggs cultured with sea water containing gossypitrin at various concentrations. 1 hr af=one hour after fertilization.

media contained in petri dishes, 70 mm in diameter and 15 mm in height. Depth of the medium was about 4 mm. These cultures were placed under diffuse light of the laboratory, at about 18°C. Thus, eggs sank and adhered to the bottom of the dish. One hour after being sown, the eggs were examined under a microscope, and it was confirmed that there was no rhizoid formed yet. Density of the eggs was about 1 or 2 individuals per 1 mm^2.

Next, eggs were kept first in natural sea water, then transferred to 5×10^{-4} *M* gossypitrin-sea water at various times after fertilization, and were observed 36 hr after being fertilized to know the time when gossypitrin was most effective.

Results with Discussion

Observation at 16 hr after being sown revealed that 34 of 84 (i.e. $34\times100/84=40.4\pm5.3\%$) eggs formed rhizoid in control culture. However, it was $45.7\pm5.1\%$ in 0.5×10^{-4} *M*, $29.3\pm4.9\%$ in 1×10^{-4} *M*, $22.3\pm5.0\%$ in 2.5×10^{-4} *M*, $29.1\pm6.1\%$ in 5×10^{-4} *M*, $3.1\pm1.7\%$ in 7.5×10^{-4} *M*, and 0% in 10×10^{-4} *M* of gossypitrin. Likewise, examined at 24 hr and 36 hr after sowing, the results are indicated in Figure 1.

In those eggs not forming rhizoid being cultured with gossypitrin, for instance at concentration of 5.0×10^{-4} *M*, nuclear and cell divisions took place successively into 2, 3 or 4 cells, so far as cultured with gossypitrin of the same concentration. When transferred to control sea water, rhizoid began to bulge out of one or two cells of the cloven egg and it was elongated (Fig. 2).

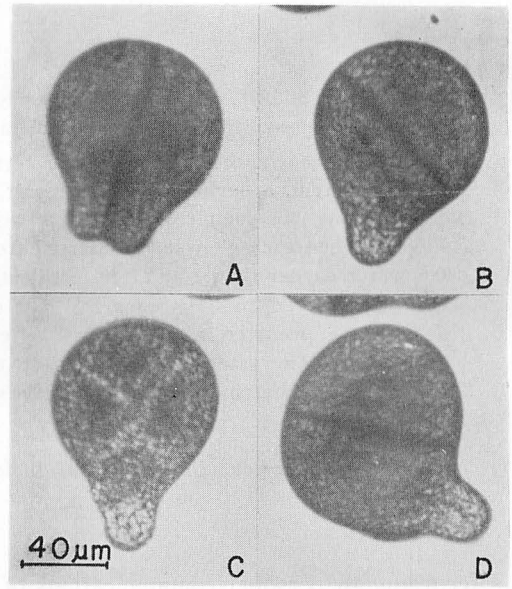

Fig. 2. Rhizoid formation in *Fucus* eggs when transferred from gossypitrin-sea water to normal sea water after cleavage without rhizoid formation. (a) Two rhizoids bulged out from one region previously cut into two by a septum, (b, c, d) one rhizoid bulging after cleavage.

In the next experiment, eggs were first sown in petri dishes containing control sea water just after fertilization. Then they were dividedly transferred to 5×10^{-4} *M* gossypitrin-sea water at (a) 4 hr, (b) 6 hr, (c) 8 hr, (d) 10 hr and (e) 12 hr after fertilization, and cultured in the same way. Observation at 36 hr after fertilization revealed that in control culture 99% eggs formed normal embryos consisting of more than 50 cells and one rhizoid. While, in (a) culture rhizoid formation was $44\times100/80=55\%$ and most of the individuals including rhizoidless ones were cloven into 2 to

4 cells. Likewise, in (b) culture, rhizoid was 65.5% and cell division was the same. In (c) culture, rhizoid was 65.7% and cell division was also the same. In (d) culture, rhizoid was 70.3% with normal cell division, and in (e) culture, rhizoid was 76% with normal cell division. The difference in percentage between (c) and (d) cultures is not very remarkable, while the degree of cell division differs clearly between the two. These implies that the critical time for inhibitory effect of gossypitrin for rhizoid formation as well as for further cell divisoin is between 8 hr and 10 hr after fertilization. Because, if the egg is transferred to gossypitrin earliear than 8 hr after fertilization, cell division ceases at 4-cell stage, and rhizoid formation is lowered to 65%. While cell divisions continue normally and rhizoid formation is increased to 70% if they are transferred to gossypitrin later than 10 hr after fertilization. This is consistent with report of QUATRANO (1968) that proteins for cell division and rhizoid development are synthesized between 8 hr and 10 hr after fertilization. Thus occurrence of rhizoid is inhibited by gossypitrin as well as the cell division, while the latter takes place up to the 4-cell stage even in the egg which does not form a rhizoid in gossypitrin. Therefore, the rhizoid formation and the cell division are controlled by independent factors as reported formerly (NAKAZAWA 1972).

Gossypitrin was first taken out from yellow flowers of *Gossypium* (PERKIN 1916). Later, the same was obtained from *Chrysanthemum* flowers (GEISSMAN and STEELINK 1957) and from *Equisetum* stroboli (NAKABAYASHI 1958, KUTNEY and HALL 1971). Considering from the present experiments, it seems that gossypitrin takes part in regulation of growth in morphogenesis of these plants.

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中沢信午: Fucales ノート 10. ゴシピトリンによるヒバマタ卵の仮根形成と細胞分裂の抑制

ヒバマタ (*Fucus evanescens*) の卵を受精直後からフラボノイドの一種ゴシピトリンを含む海水で培養した結果，この物質 10^{-3} *M* を含む場合に仮根形成は完全に阻害されたが，細胞分裂は 2 回だけ続けられて止まった。また受精後 8 時間より以前に 5×10^{-4} *M* ゴシピトリン海水にうつすと分裂は 4 細胞で止まり，仮根形成は 65% にすぎないが，受精後 10 時間以後にゴシピトリン海水にうつすと分裂は正常に進行し，仮根形成も 70% 以上に上昇する。またゴシピトリン海水中で仮根なしの分裂がおこった卵を正常海水にもどすと，仮根形成がはじまる。
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