Differential Treatment of *Acetabularia* with Cytochalasin B
and N-Ethylmaleimide with Special Reference to Their Effects
on Cytoplasmic Streaming

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Summary

Cytoplasmic streaming in the stalk of *Acetabularia ryukyuensis* at the vegetative stage was reversibly inhibited by cytochalasin B (CB) of 50 μg/ml and irreversibly by N-Ethylmaleimide (NEM) above concentrations of 0.25 mM. After the endoplasm and the chloroplasts were pushed forward one end of the stalk by gentle centrifugation at about 500 × g for 3 minutes, numerous ectoplasmic striations remained *in situ* in the stalk cortex. The striations ran in parallel with the longitudinal axis of the stalk at unequal intervals. The endoplasm streamed back only along these striations.

By combining centrifugation and a double chamber technique, the endoplasm and the cortex of the stalk were treated separately with CB or NEM. CB treatment of the cortex arrested streaming; when treatment was restricted to the endoplasm, streaming continued at an normal rate. NEM treatment restricted to the cortex permitted normal streaming rates. Treatment restricted to the moving endoplasm inhibited streaming.

These results suggest that microfilaments and a moiety, possibly myosin, play an active role in the streaming. Microfilaments must reside in the cortex, especially in the ectoplasmic striations, while the putative myosin must reside in the moving endoplasm.

*Keywords: Acetabularia ryukyuensis; Cytochalasin B; Cytoplasmic streaming; Differential treatment; N-Ethylmaleimide.*

1. Introduction

In the stalk of *Acetabularia*, a marine coenocytic alga, the cytoplasm and chloroplasts move along numerous ectoplasmic striations arranged in parallel to the longitudinal axis of the stalk. The streaming direction differs with the
striation, i.e., acropetal and basipetal directions. Such a streaming pattern was named multistriate streaming by Kamiya (1962). Most of the recent advances in our understanding of cytoplasmic streaming have been made through observations and experiments with characean cells. Rotational cytoplasmic streaming in characean cells is generally understood to be caused by the active shearing force generated at the interface between the stationary ectoplasm and the moving endoplasm (Kamiya and Kuroda 1956, Kamiya 1959). On the inner surface of the stationary chloroplasts which are embedded in the cell cortex, bundles of microfilament are arrayed in parallel with the streaming direction (Nagai and Rehun 1966, Palevitz and Hepler 1975, Nagai and Hayama 1979 b). In the moving endoplasm is the factor which interacts with microfilaments to produce the active shearing force. The factor has been accepted to be myosin molecules which are anchored on some characteristic organelles in the endoplasm (Chen and Kamiya 1975, Nagai and Hayama 1979 a, b).

It should be interesting to apply this concept to the mechanism of the multistriate streaming in Acetabularia for a general understanding of the cytoplasmic streaming in plant cells. The present work was conducted to clarify whether the microfilaments and other components such as myosin molecules play an active role in the streaming of this alga and whether they are separately localized, namely the microfilaments in the ectoplasm and the myosin molecules in the moving endoplasm.

2. Materials and Methods

Acetabularia ryukyuensis, was collected at Amami Island and cultured in our laboratory with artificial sea water (Aquamarine, Yashima Chemicals, Japan). The alga was illuminated alternately for 12 hours periods of dark and light with fluorescent lamps at about 1,300 lux. The temperature was kept at 23 °C.

Stalks of each alga at the vegetative stage were cut into suitable lengths after ligation with silk thread at appropriate points. In a few hours after amputation, the ligated stalk fragments showed generally the same rate and pattern of streaming as the normal stalk. They are referred to hereafter simply as stalks.

The rate of cytoplasmic streaming was measured by the usual method using an ocular micrometer and a stopwatch.

Drugs were administered to the stalks mounted between a glass slide and a cover slip by replacing the culture medium with test solutions dissolved in the culture medium. Cytochalasin B (Aldrich Chemicals, U.S.A.) was initially dissolved in dimethyl-sulfoxide (DMSO) at a final concentration of 0.5% and subsequently diluted with the culture medium to the required concentration. N-Ethylmaleimide (Sigma Chemicals, U.S.A.) was directly dissolved in the culture medium.

For centrifugation and differential treatment of stalks, a double chamber, the same as constructed by Nagai and Kamiya (1977), was used. All methods for the treatment were the same as reported previously.

The streaming was observed using both a Nikon light microscope and a Zeiss photomicroscope II with differential interference optics.