CHARACTERIZATION OF PREY ADHESION AND EXTRUSOMES IN HELIOZOON *ACTINOPHRYS SOL*

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The heliozoon *Actinophrys sol* has numerous needle-like axopodia radiating from a spherical cell body. When prey organisms are added to the heliozoon, the prey cells become trapped by the axopodia and adhered to the cell surface. In *A. sol*, numerous extrusomes are present under the plasma membrane of axopodia and the cell body. The heliozoon extrusomes are known to discharge their contents during food uptake. In this study, we attempted to isolate extrusomes and adhesive substances from *A. sol*, and examined their adhesive activities to understand the role of extrusomes and the molecular mechanisms by which heliozoons capture prey organisms.
The supernatant of cell homogenate after freezing and thawing, showed strong adhesion to the prey flagellates. The adhesive substance was further extracted from the heat-treated *A. sol*. This fraction contained filamentous materials similar to the secreted contents of the extrusomes observed during feeding, and its adhesive activity was not inhibited by trypsin treatment.

INVOLVEMENT OF PLASMA MENBRANE PROTEIN IN CEUGLENOID MOVEMENT.

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We have recently proposed a possibility that motive force for cell shape changes of *Euglena gracilis* (euglenoid movement) is generated by a conformational change of the integral plasma membrane protein called IP-39. It is known that arrangement of IP-39 proteins in the plasma membrane becomes distorted when euglenoids are grown in a vitamin B_{12}-deficient medium. Here, we found that 1) euglenoid movement was induced by treatment with chlorpromazine or CTAB which are known to be incorporated into the plasma membrane and could induce shape changes of erythrocytes, and 2) motile activity of vitamin-B_{12} deficient cells was significantly lower than that of control cells, and 3) both motile activity and arrangement of the membrane proteins of vitamin-B_{12} deficient cells became restored after prolonged culture (>5 days). These results support the hypothesis that euglenoid cell shape change is mediated by expansion of the plasma membrane in which arrangement of IP-39 membrane protein is involved.

LOCALIZATION OF FULCIN AND A 260-KDA FILAMIN/ABP-RELATED PROTEIN IN MUSCLE AND NONMUSCLE CELLS

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Filamin, fulcin and a 260-kDa filamin/ABP related protein are extracted from chicken gizzard smooth muscle cells with a low-salt alkaline solution together with actin and α-actinin. Fulcin and the 260-kDa protein have significant homology with human ABP and chicken retina filamin. Despite the high homology, these proteins can be immunologically distinguishable one another.

Antibodies against fulcin and the 260-kDa filamin/ABP-related protein stained myotendinous junctions and Z-lines of isolated chicken leg muscle fibers. An anti-filamin antibody also stained the MTJ. By immunofluorescence microscopy, fulcin was revealed to localize at the adhesion plaques and on the actin fibers of cultured nonmuscle cells. In an isolated small intestinal epithelial cell, the localization of fulcin at the adherens junction and the rootlet was observed by a confocal microscope. The anti-fulcin antibody did not stain the terminal web and the apical tips of microvilli of the cell.

DEFENSIVE FUNCTION OF TRICHOCYSTS IN PARAMECIUM AGAINST A VARIETY OF PREDATORY PROTOZOA

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Defensive function of trichocysts in *Paramecium* has been verified against the haptorian ciliates such as *Dileptus margaritifer* and *Monodinium balbiani*, which have toxicysts as offensive extrusomes. Recently, defensive function of trichocysts was also verified against the heterotrich ciliate *Climacostomum virens*, which uses a well-developed oral apparatus to engulf other ciliates. To understand the whole extent of the effectiveness of trichocysts in *Paramecium* as defensive extrusomes, their defensive function should be tested against other groups of predators which are different in the mode of feeding.

In this work, we tested the defensive function of trichocysts in *P. tetraurelia* against heliozoans *Echinosphaerium akamae* and *E. nucleofilum*, and against a suctorian ciliate *Heliophrya enhardi*. These protozoa use extrusomes for capturing the prey. Trichocyst-non-discharge mutant cells were more vulnerable than wild-type cells in both species of heliozoans. There was no significant difference between mutant and wild-type cells in the suctorian. This work indicates that the trichocyst discharge may defend *Paramecium* from a variety of predators.

THE MECHANISM OF FACILITATED CELL MEMBRANE RESEALING

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Cells survive disruptions of cell membranes by means of a rapid resealing mechanism dependent on Ca^{2+}-regulated exocytosis. We measured resealing rates by observing fura-2 dye loss after wounding in individual 3T3 fibroblasts. A second disruption at the same site reseals more rapidly than the initial wound. This facilitated resealing was dependent upon both Ca^{2+} and protein kinase C (PKC). Activation of PKC by phorbol ester accelelated resealing of a first wound. The specific PKC inhibitors bisindolylmaleimide I and Gö-6976 suppressed the effect of phorbol ester on resealing rate as well as blocking facilitation to a second wound. Fluorescent dye loss from a pre-labeled FM1-43 endocytotic compartment was used to investigate the relationship between exocytosis, resealing and facilitation. Exocytosis of endocytotic compartments near the wounding site was correlated with successful resealing. The destaining did not occur when the resealing was inhibited by low external Ca^{2+} or by injected tetanus toxin. When the dye loaded cells were wounded twice, FM1-43 destaining at a second wound was smaller than at the first wound. Less destaining was also observed in phorbol ester treated cells, suggesting newly formed vesicles, which were FM1-43 unlabeled, were involved in the resealing at repeated woundings. Both facilitation and the acceleration of the resealing by phorbol ester were blocked by pre-treatment with brefeldin A, which inhibits secretion from the trans-Golgi network. Brefeldin A had no effect on the resealing rate of an initial wound. These results suggest that PKC activated by the first wound stimulates vesicle formation and delivery from the trans-Golgi network, resulting in an accelerated rapid resealing of a second disruption. Artificial decreases in membrane surface tension induced by a surfactant (Pluronic F68 NF) could facilitate resealing and restore resealing even when exocytosis was blocked by tetanus toxin. We conclude that resealing requires a decrease in surface tension and under natural conditions this is provided by Ca^{2+}-dependent exocytosis of new membrane near the site of disruption.

A NOVEL FIBROUS STRUCTURE IN MACRONUCLEUS OF *TETRAHYMENA THERMOPHILA*

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We found that a monoclonal antibody IR-2-1 originally raised for a purified 67kDa protein of endonuclear symbiotic bacterium *Holospora obtusa* of ciliate *Paramecium caudatum* cross-reacted with a novel fibrous structure in a macronucleus of the ciliate *Tetrahymena thermophila*. The antibody also labeled mitochondria, but did not label the macronucleus of *T. pyriformis*. Conforcal microscopy with mAb IR-2-1 showed that the fibrous structure formed branched net-work just beneath the nuclear envelope. Immunoblots with isolated macronuclei and mitochondria of *T. thermophila* showed that the antigen in the macronuclei was 86 kDa and that in the mitochondria was 86 and 40 kDa. In some strains, a short fibrous structure appeared in the morphologically differentiated macronuclear anlagen during nuclear differentiation, but in other strains the fibrous structure appeared after aging. So far all strains of *T. thermophila* examined eventually showed the fibrous structures in the macronucleus although timing of the appearance and ratio of the fibrous structure-bearing cells in a clone were different in strains. This fibrous structure differed morphologically from stress fiber found in *T. pyriformis* and was not labeled with anti-*Tetrahymena* actin antibody.