EFFECTS OF REGIONAL REDUCTION OF TENSION AT THE SURFACE ON *ECHINARACHNIUS PARMA* EGG SURFACE BEHAVIOR

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The actin content of the contractile ring (CR) that constricts animal cells during cytokinesis appears to be enhanced. The mechanism of enhancement is unknown, but it is the subject of much speculation. White & Borisy (*J. Theoret. Biol.* 101: F289-F316, 1983) proposed that actin filaments accumulate to form the CR because they migrate up an assumed cortical tension gradient between the equatorial and polar regions of the cell. The circumferential accumulation of actin thus created is in turn presumed to result in localized surface constriction. The purpose of this investigation was to test the hypothesis by inducing regions of cortical relaxation in the cell surface and observing whether the predicted regions of overt contractile activity appear between them.

Cytochalasins reduce tension at the surface in echinoderm eggs (Usui & Yoneda, *Devel. Growth & Differ.* 24: F453-F465, 1983). The lowest concentration of Cytochalasin D (CD) that prevents both elongation and furrowing in totally immersed sand dollar (*Echinarachnius parma*) eggs is 1 µg/ ml. CD was mixed in 1% agar in filtered sea water (FSW). The mixture was drawn into the 175 µm o.d. tip of a glass pipet by capillary action. After the agar cooled and solidified, the pipet was backfilled with the same concentration of CD in FSW as was present in the agar, and the pipet was then immersed tip down in the same solution until use. All pipets were used on the day of preparation. For the experiments, 2 pipets were inserted in instrument collars that were attached to water-filled microinjection syringes. The pipet nozzles were positioned near the operation chamber floor and a portion of the CD-impregnated agar was extruded. The concave tip of the extruded agar was then pressed against diametrically opposite surfaces of the spherical cell or cell fragment. The dimensions of the agar and the cell were such that a circumferential band of surface was not in contact with the agar.

The optimum concentration of CD for these experiments (2 µg/ ml) was determined empirically. When agar containing 2 µg/ ml was pressed against the polar surfaces of a blastomere resulting from the first cleavage at the time it began the second cleavage, division was completed in normal time in 6 of 6 experiments. But when agar was pressed against the equatorial surface of a similar cell at the same point in the cell cycle, the furrow was blocked, stalled or delayed in 6 of 6 experiments. These control experiments show that 2 µg/ ml CD disrupts furrowing in surface it contacts, but it does not disrupt furrowing in cell surface it does not contact. In a third experiment, fertilized eggs were bisected by hand so that the mitotic apparatus developed in only one of the halves. When the nucleated control half began to cleave, CD impregnated agar was pressed against diammetrically opposed surfaces of the enucleated half until the nucleated half completed division. In 12 of 12 experiments, the enucleated half showed no capacity for autonomous shape change.

In these experiments, a substance with a demonstrated capacity for inducing cortical relaxation was applied to a normal cortex at the appropriate time in the cell cycle in a pattern that, according to the hypothesis, should create a near-normal tension gradient. Absence of furrowing activity strongly suggests that a simple cortical tension gradient is insufficient to establish a region of furrowing activity.

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