5 THE RELATION BETWEEN FURROWING RATE AND INITIAL DISTANCE BETWEEN THE MITOTIC APPARATUS AND THE SURFACE IN FLATTENED SAND DOLLAR EGGS

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The division mechanism of animal cells results from interaction between the mitotic apparatus (MA) and the surface. In flattened cells, furrows develop on the free margins but not on the flattened surfaces. When the MA of a flattened cell is excentric, the diametrically opposed furrows do not develop simultaneously; the later furrow appears on the more distant equatorial margin. The purpose of this investigation was to determine whether the furrows which develop in surfaces distant from the MA function at the same rate as furrows which develop in surfaces closer to the MA.

Fertilized sand dollar (Echinarachnius parma) eggs were flattened to 175 μm diameter against the bottom of an operation chamber with a rectangular fragment of coverslip positioned by a micromanipulator 15 to 30 minutes before the anticipated cleavage time. At this time, the MA is small and usually excentric. As the MA expanded, it did not center itself as it normally does, presumably because it was constrained by the upper and lower flattened surfaces which were about 60 μm apart. The rates of movement of the diametrically opposed furrow tips at 17°C were determined by measurements with an ocular micrometer and stopwatches. The period of measurement of each furrow was confined to the first 4 minutes of function, when physical contact between the cell surfaces and the glass surfaces used to achieve flattening did not impede the process.

The rate of movement of the furrow tips in flattened eggs is related to the initial distance between the MA and the equatorial margin. Furrows formed at extreme distances (105 μm or more from the MA) tended to stop and then regress. Furrows formed in more distant margins progressed more slowly than those in closer margins. In 37 cells in which the rates of both furrows were calculated, the correlation coefficient was 0.71537. The mean furrowing rate of the 10 fastest furrows was 12.13 $\pm$ 0.475 S.D. μm/min. The mean distance between the center of the spindle and the equatorial surface before division began was 76.25 $\pm$ 7.07 S.D. μm. The mean rate of the 10 slowest furrows was 2.89 $\pm$ 0.929 μm/min. The mean initial distance between the spindle and the surface which formed the slower furrow was 100.5 $\pm$ 9.19 S.D. μm.

The fact that, in the same flattened cell, furrowing begins later in the more distant margin has been attributed to the time required for a stimulus which originates in the MA to traverse the extra distance. These experiments afforded an opportunity to recalculate the rate of stimulus movement in cases where the time interval between appearance of the furrows in the near and far margins was greater than 2 min. The mean rate for 21 measurements at 17° C was 7.45 $\pm$ 3.19 S.D. μm/min. which is substantially in agreement with the previously published rate of 6.3 $\pm$ 1.8 μm/min. measured at 19° C. (Rappaport, J. Exp. Zool., 183: 115-120, 1973). This investigation was supported by NSF Grant PCM 7902624.

6 ROLE OF Ca^{2+}, KINASES AND PHOSPHODIESTERASES IN POLAR LOBE FORMATION AND CYTOKINESIS IN FERTILIZED EGGS OF ILYANASSA OBSOLETA

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Fertilized eggs of the marine mudsnail, Ilyanassa obsoleta (Nassarius obsoletus) repeatedly form and relax a constriction which resembles a cleavage furrow in a process called polar lobe formation before and during cytokinesis. Our previous work has demonstrated that microinjection of cAMP can cause a constriction to form quickly and that normal lobe formation and cytokinesis do not appear to require exogenous Ca^{2+}, because they can occur even in Ca-free sea water containing 10 mM EGTA (Conrad, G.W., and Davis, S.E. 1977. Devel. Biol. 61:184-201, and 1980. Devel. Biol. 74:152-172).

Methylated xanthines are reported to raise cAMP levels by inhibiting phosphodiesterases. In fact, these compounds in both regular and Ca^{2+}-free sea water + EGTA accelerated the normal starting time for polar lobe formation and cytokinesis: 0.26 mM xanthine and 1 mM theophyline caused a 2-4 min acceleration, 1 mM theobromine caused a 5-10 min acceleration, and 1 mM caffeine caused as much as a 15 min acceleration in the starting time.

A number of phospholipid-interacting drugs are reported to inhibit Ca^{2+}-activated, phospholipid-dependent protein kinases. Chlorpromazine at 25 μM delays both polar lobe formation and cytokinesis, and at 100 μM stops both processes. Verapamil, D600, and nifedipine are Ca^{2+}-uptake inhibitors and verapamil, at least, also is an inhibitor of the protein kinase type above. Both verapamil and D600 at 100 μM cause a substantial delay in the normal starting times for polar lobe formation and cytokinesis, especially in Ca^{2+}-free sea water + EGTA. Nifedipine, even at 20 μM, causes a substantial delay in both the presence and absence of exogenous free Ca^{2+}.

Ouabain (10^{-3}M), veratridine (10^{-4}M), and sodium orthovanadate (2.5×10^{-3}M) had no effect on lobe formation and cytokinesis of Ilyanassa eggs in the presence or absence of exogenous Ca^{2+}. In addition, double-barrel microelectrodes were constructed with one barrel filled with 3 M KCl for measurement of membrane potential and the other barrel filled with a continuous column of antimony for measurement of pH. The final tips on these electrodes (3-5μ) were generously pulled by Dr. Klaus Beyenbach. When Ilyanassa eggs were impaled with the tips of such electrodes, the recordings showed that no detectable changes in membrane potential or intracellular pH occurred during polar lobe formation and cytokinesis.

From the inhibitor experiments, we conclude that both cyclic nucleotide activated-, and Ca^{2+}-activated protein kinases should be measured directly in these eggs during cell shape changes. Supported by NIH HDO7193.

7 REGENERATION FROM LONGITUDINALLY SPLIT FORELIMBS IN PLETHODON CINEREUS.

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When a urodele amphibian loses a limb or its tail, it grows a new one which is structurally and functionally identiaal to the one lost, a process known as epimorphic regeneration. Current studies are aimed at identifying 1) the locus of the pattern which regulates the genesis of new form during regeneration and 2) the mechanisms by which positional information required for pattern regulation is transmitted. Limb skin has been shown to be morphogenetically active during limb regeneration and, in the absence of the other morphogenetically active tissue (skeletal muscle), sufficient to regulate pattern for a complete limb regenerate. The converse also applies. A recent model (French, et al., Sci., 193: 969-981, 1976; Bryant, et al., Sci., 212: 993-1002, 1981) proposes that a complete circumference ("complete circle") of positional information (e.g., a normal circumference of skin) is necessary for limb regeneration to occur. Thus, if a longitudinally split limb were amputated, regeneration would not proceed because there is a gap in the normal "circle" of positional information. It was suggested that halved limbs would instead regenerate the circumferential base of the pattern and then produce normal distally complete regenerates (Bryant, Nature 263: 676-679, 1976). This contradicts the earlier works of Weiss (Arch. f. Entw.-mech. 107: 1-53, 1926) and Goss (J. Morph. 100: 547-564, 1957) who found that while split limbs could produce normal, distal regenerates they also regenerated half-limbs resembling the distal structure which had been amputated.

The split-limb experiments have been repeated in an attempt to resolve this apparent conflict in results. Red-backed salamanders, Plethodon cinereus, were collected and maintained as in earlier studies (Bull. MDIBL 20: 23-24, 1980). On the stage of a dissecting microscope, both the forelimbs of anesthetized animals were split longitudinally from between the second and third digits proximally to the elbow. The entire post-axial halves of the forelimbs, which included ulna, digits 3 and 4 and associated soft tissues, were discarded. Animals were placed in dilute amphibian saline for recovery and then returned to their covered fingerbowls. Following a 2-4 day period of