PRELIMINARY OBSERVATIONS ON IONIC CURRENTS IN SAND DOLLAR EGGS DUIRNG THE CELL CYCLE

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Evidence has been accumulating that ion channel activity and/or expression changes during the cell cycle in a variety of systems (for reviews see: Moody, W. *Topics in Dev. Biol.* 39: 159-185, 1998; Jaffee, L.F. and R. Nucitelli *Ann. Rev. Biophys. Bioeng.* 6:445-476, 1977). Changes in ion channel activity during cell cycle may be related to the changes in cytosolic Ca, the dramatic remodeling of cell shape and volume, or the considerable changes in membrane traffic that occur during the cell cycle. The purpose of this study was to investigate cell cycle-related ion currents in sand dollar (*Echinarachnius parma*) eggs, which have long served as a model system for cell division.

Gametes were obtained by spawning sand dollars by injecting 0.5 ml KCl into the body cavity. Eggs were fertilized by mixing ~0.2ml packed eggs with 1-2 drops of sperm in a 120 mm petrie dish. Fertilization was monitored by the appearance of the fertilization membrane which occurred within 1-2 min after sperm addition. After fertilization, the jelly coat, fertilization membrane, and hyaline layer were quickly removed by washing the eggs several times in 1 M glycine. Ionic currents were recorded from the stripped eggs in sea water using two microelectrode voltage clamp (TEVC). Temperature was controlled at 17° C. Electrode resistance was 20 - 40 MOhms when filled with 3 M KCl or 4 M KAc.

In the initial experiments, membrane potential (V_m) was recorded continuously with a single microelectrode starting 10 to 20 min after fertilization. V_m was -74.1 ± 2.4 mV measured 51 ± 4 min after fertilization. Usually, ~60 min after fertilization, oscillations in V_m began with amplitudes of about 4 mV and periods of ~1.5 min (Fig. 1). In recordings from cells that divided normally during recording, the membrane hyperpolarized transiently during cytokinesis.

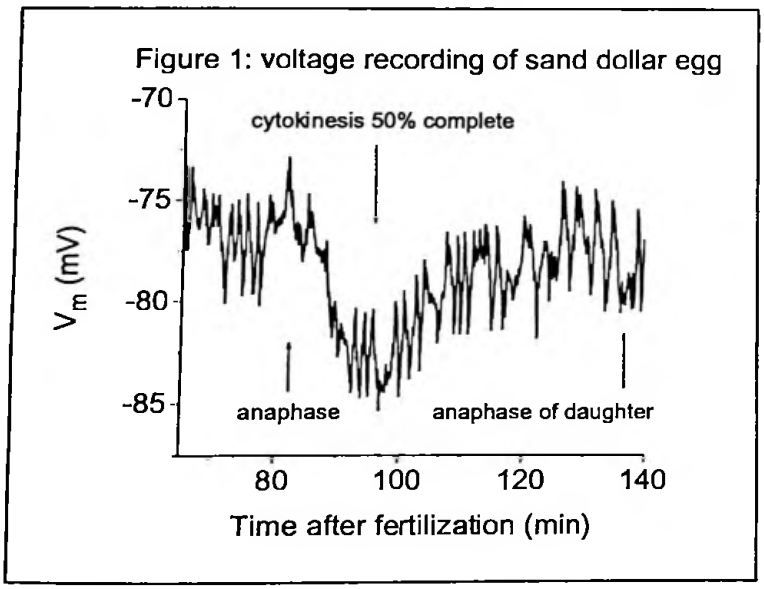
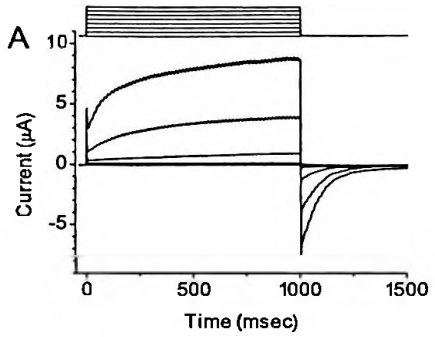
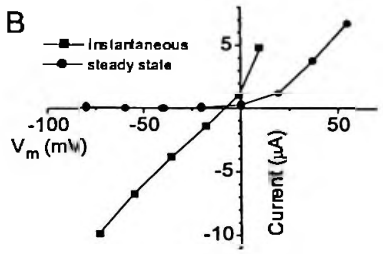


Figure 1: voltage recording of sand dollar egg

Voltage-clamp analysis revealed the presence of an outward current that activated slowly upon depolarization (Fig. 2A). The steady-state I-V curve was outwardly rectifying, but the instantaneous IV measured by tail current analysis was approximately linear (Fig. 2B). The E_{rev} was -10 mV. These data suggest that the current may be cation non-selective or a Cl current.

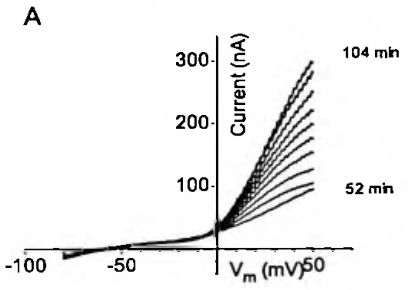
Figure 2: Time-dependent outward current in sand dollar eggs.

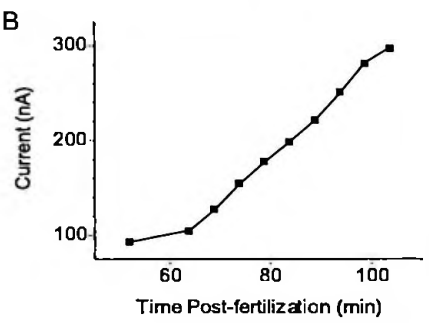




This current was the predominant current observed. The current was monitored continuously during the cell cycle using a 1-sec duration voltage ramp from -80 mV to +50 mV. The current increased substantially between 50 and 100 min after fertilization (Fig. 3). However, this current is unlikely to be responsible for the hyperpolarization observed during anaphase in Fig. 1, because this current would tend to depolarize the cell towards -10 mV. Voltage-clamped eggs did not divide normally, even though current-clamped eggs usually did divide normally. This suggests the possibility that inhibiting the voltage oscillations (Fig. 1) or other ionic currents by voltage-clamp may prevent the egg from executing its normal cell cycle program. The slowly

Figure. 3: Outward current increases with time.





developing outward current observed in Fig. 3 may represent a response of the cell to inappropriate conditions at a cell cycle checkpoint. Because recent data has implicated Cl channels and regulation of cell volume to be a hallmark feature of apoptosis (Maeno et al. *Proc. Natl. Acad. Sci.* 97:9487-9492, 2000), we hypothesize that inhibition of mitosis by voltage-clamp may drive the cell into an apoptotic pathway. These preliminary studies illustrate a number of interesting features of ionic currents in sand dollar eggs during cell cycle that warrant additional study.

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