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Short Contribution

Cyclic changes of membrane conductivity in fertilized and activated eggs of teleost *(Misgurnus fossilis)* and their relation to the cell shape

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ABSTRACT Oscillations of membrane ionic conductivity, with a period similar to cell cycle duration, were observed in fertilized and activated loach *(Misgurnus fossilis)* eggs. In cleaving eggs the decrease in conductivity coincided with mitosis. Synchronously with the oscillations of membrane conductivity in activated as well as in fertilized eggs, rhythmic changes in blastodisc shape occurred. The blastodisc rounded up during the period of increasing membrane conductivity and flattened while conductivity decreased. Scanning microscopy of fertilized and activated eggs revealed differences in the surface relief of rounded and flattened blastodiscs.

KEY WORDS: *egg cleavage, cell cycle, membrane conductivity, cell surface relief*

Studies of eggs and developing embryos have demonstrated the important role of plasma membranes in the initiation of embryonic development (Whitaker and Steinhardt, 1982). The membrane changes observed after fertilization were found to be related both to the functioning of egg metabolic systems and to the organization of the cytoskeleton. However, little is known about the changes in membrane properties occurring during cleavage divisions. Following an earlier work (Woodward, 1968), changes in ion fluxes across the plasma membrane and in electrical membrane properties have been demonstrated in cleaving embryos of several species (Kvavilashvili *et al.*, 1971; de Laat and Bluemink, 1974; Medina and Brejestovski, 1988; Block and Moody, 1990). In most cases these data were analyzed only from one point of view: namely, the changes were considered as the consequence of cytokinesis. It was believed that these changes were caused by the addition of new membrane of different permeability to the cleavage furrows. The periodic character of these changes could be explained by temporary isolation of the new membrane from the external solution due to tight junctions (Woodward, 1968; de Laat and Bluemink, 1974). Such processes can provoke cyclic changes in recorded electrical and ionic properties of the plasma membrane during the cell cycle, having no direct relation to the cell cycle as such. To rule out the effect of factors related to cytokinesis, activated eggs realizing cell cycles without cleavage can be used. The purpose of the present work is to study the changes in membrane potential (E_m) and input resistance (R_{in}) of fertilized and activated loach eggs in order to try to link them to some other processes in the cell cycle.

Our continuous recordings of the R_{in} and E_m parameters in fertilized eggs incubated in fresh water or in Holtfreter solution revealed the existence of cyclic changes in R_{in} and E_m with a period coinciding with that of the cell division cycle (Fig. 1A). The period between the first cleavage divisions is equal to 33-35 min at 20-21°C. Cell cycle-dependent changes in R_{in} are between 1.3 and 2.5 fold. The E_m oscillations reach 5-10 mV. R_{in} decreases correspond to E_m augmentations and vice versa. The rise in R_{in} starts 5-10 min before the appearance of the first signs of cytokinesis, so that furrowing occurs when R_{in} is maximal. After completion of cytokinesis, R_{in} starts to decrease. Cyclic changes in R_{in} are observed in activated eggs as well as in fertilized eggs (Fig. 1B). In contrast to fertilized eggs, E_m values did not cycle in a majority of activated eggs. In only 4 out of 25 cases, were slight changes in E_m observed synchronously with the observed changes in R_{in}. In these cases, the increase in R_{in} was accompanied by a membrane depolarization of 5-10 mV, while its decrease was followed by a membrane hyperpolarization of the same magnitude. The period of such R_{in} changes increases gradually from 35 min during the first to 70-100 min at fourth cycle. The changes in R_{in} are significant: 2-6 fold. The mean values of R_{in} and E_m during the first hour after activation do not differ from those observed for fertilized eggs. However, in cleaving eggs, R_{in} is significantly lower and E_m higher (in absolute values) than observed in activated eggs. The R_{in} and E_m measured 4 hours after fertilization in Holtfreter solution were equal to 3±1 MΩ and -(36±8) mV, respectively, while during the same period, they were equal to 6±1 MΩ and -(10±5) mV in activated eggs.

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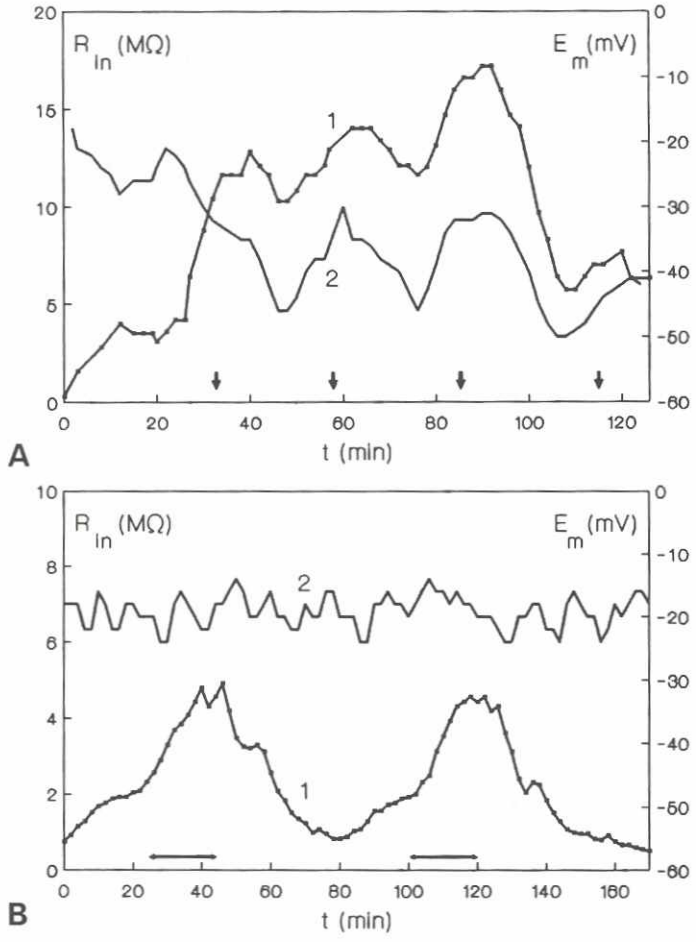

Fig. 1. Oscillations in membrane resistance R_{in} (1) and E_m (2) in fertilized (A) and activated (B) loach eggs incubated in tap water. *t - time after impalement of the egg with electrodes. Arrows* **(A)** *indicate the onset of the first, second, third and fourth cytokinesis, respectively. Horizontal bars* **(B)** *indicate the periods of blastodisc flattening in the third and the fourth cycle.*

Measurements of apparent membrane capacitance (C_m) performed on activated eggs simultaneously with R_{in} recordings demonstrated only a weak gradual decrease of about 20% within 2-3 hours, without any indication of periodic changes. The average value of C_m from 5 eggs (2 females) was 32±3 nF.

Synchronously with the oscillations of membrane conductivity of eggs, rhythmic changes in blastodisc shape occurred. 10-15 min before the onset of cytokinesis the rounded surface of the blastodisc and blastomeres becomes flattened and looks like a truncated segment (Fig. 2B). At the appearance of the first signs of cytokinesis or somewhat earlier, the blastodisc becomes rounded again and retains this shape until the next division cycle. Each subsequent flattening of the blastomeres sets in 7-10 min before cleavage starts. On the whole, the process of blastodisc flattening looks like a temporary surface contraction, which sometimes becomes apparent as a ligature of the egg at the boundary between the blastodisc and the yolky part. According to our histological observations of the 1st cell cycle, the flattening of the blastodisc coincides with metaphase-anaphase and the onset of cytokinesis of the first cleavage with telophase. First cleavage is completed at interphase of the next cell cycle. In the subsequent cleavage divisions, similar temporal relations between the changes in cell shape and the phases of the cell cycle are observed. Thus, the alteration in blastodisc shape appears to be a characteristic event at the onset of mitosis, when the majority of cells, including cultivated cells and embryonic blastomeres, become rounded. On the other hand, in embryos with a meroblastic type of cleavage, as in the case of the loach, the shape of the blastomeres during the transition from interphase to mitosis goes from rounded to flattened. In activated eggs the flattening and the subsequent rounding up (Fig. 2D) of the blastodisc surface take place without cytokinesis. At first they were synchronous with the corresponding changes observed in fertilized eggs, while afterwards, the period of these changes increased noticeably mounting to 100-120 min in third-fourth cycles instead of 34 min in the first cycle. The comparison of the changes in membrane properties with those in cell shape indicates that the rise in R_{in} coincides with the period of blastodisc or blastomere flattening, while the decrease of R_{in} occurs in phase with the rounding up of the blastodisc or blastomeres independently from the cell cycle duration (Fig. 1).

Changes in ionic conductivity in the plasma membrane of cleaving and activated loach eggs, synchronous with the processes of the cell cycle, cannot be interpreted as a consequence of the structural reorganization of the cell surface in the cleavage furrow, because in activated eggs they took place in the complete absence of cytokinesis and furrow membrane formation. One of the reasons for such oscillations in membrane conductivity could be transient changes in the cell surface area. In the loach egg, internalization of the plasma membrane through endocytosis occurs at least up to the 4-cell stage (Ivanenkov *et al.*, 1990). However, measurements of membrane capacitance performed to get an estimation of membrane area failed to demonstrate periodic changes. So, we think that cyclic changes in membrane conductivity of fertilized and activated eggs reflect the alteration of specific characteristics of the egg membrane related to the cell cycle as such.

It is known that the transition from interphase to mitosis in dividing eggs of many animals is induced by the appearance of M-phase promoting factor (MPF) (Masui and Markert, 1971; reviewed in Guerrier *et al.*, 1990). With the exception of cell division, artificially activated eggs undergo almost all of the metabolic events characterizing fertilized eggs, including cycling MPF activity (Dabauvalle *et al.*, 1988). MPF could have an influence on plasma membrane channels by changing their phosphorylation level. However, other intermediate mechanisms between MPF activity, membrane conductivity and cell shape might exist. In amphibian and sea urchin eggs, several oscillatory activities have been recorded, in particular, cycling of intracellular pH and Ca (Bozhkova *et al.*, 1987; Grandin and Charbonneau, 1990; Whitaker and Patel, 1990).

Observations made on the amphibian egg indicate that modifications of cell shape are apparently associated with reorganization of the cortical cytoskeleton (Mabuchi, 1986). This is confirmed by the variations in the cell surface relief observed in the loach blastodisc. It is known that blastodisc surface exhibits numerous deep folds (Bozhkova *et al.*, 1983). Before the first flattening step, the folded area reached the equator of the egg

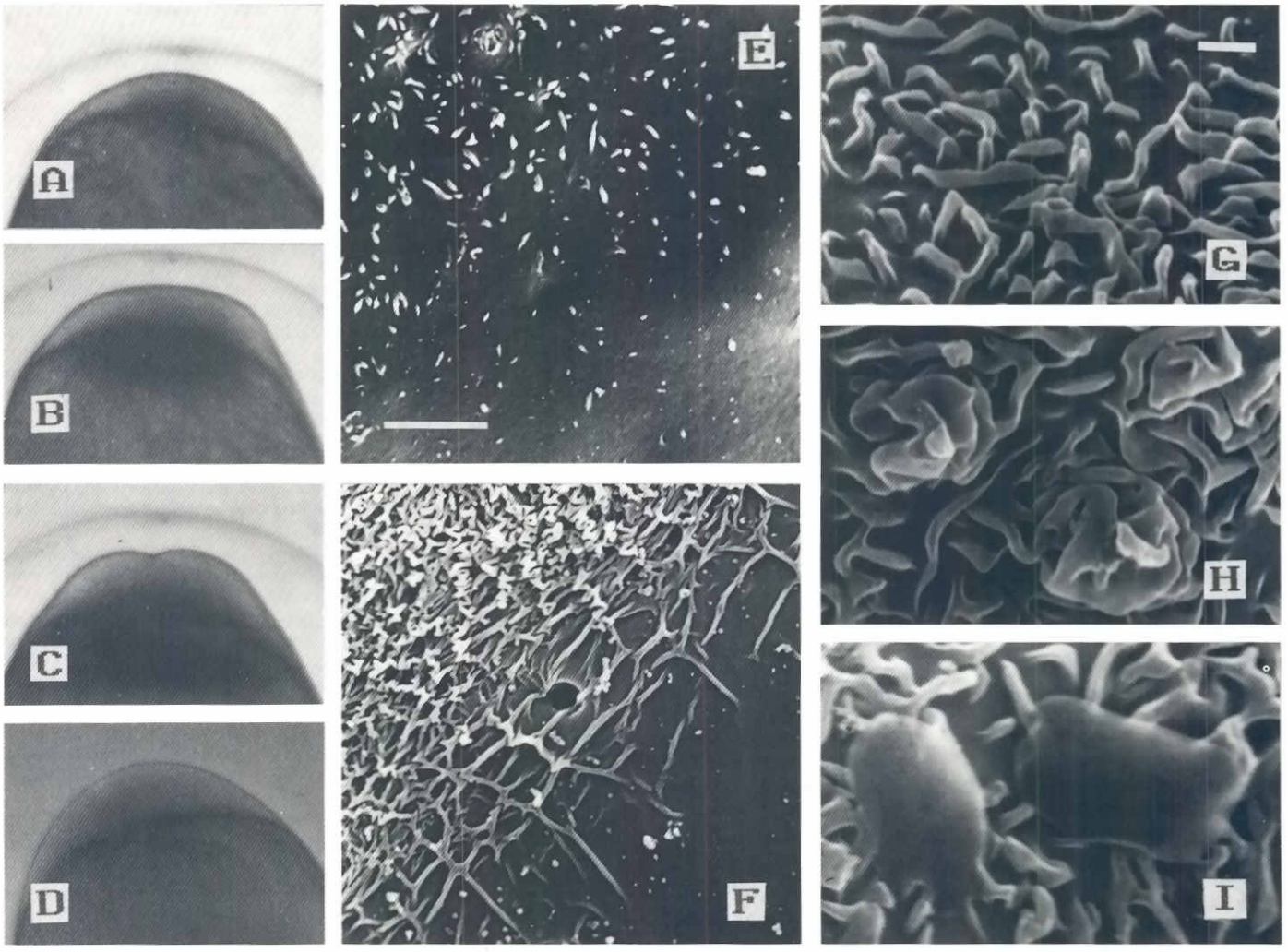

Fig. 2. Changes in the blastodisc shape (A-D) and surface topography (E-I) of fertilized (A-C, E, F) and activated (D, G-I) loach eggs. **(A-C)** *At 70, 80 and 92 min after fertilization respectively,* **(D)** *at 95 min after activation (t=19°C).* **(E, F)** *Surface of blastodisc-yolky boundary at 40 and 60 min after fertilization, before and during first flattening of the blastodisc;* **(G-I)** *blastodisc surface during the second cycle of blastodisc flattening* **(G)** *and blastodisc rounding up* **(H, I).** *Bar= μm for E, F and 1 μm for G-I.*

where the surface became gradually smoother, except for the presence of a number of single folds (Fig. 2E). Temporary flattenings (contractions) of the blastodisc occurring prior to cell division are followed by a deepening of the folds near the animal pole, by the appearance of sharper boundaries between the folded surface of the blastodisc and the smooth surface of the yolk and by a displacement of this boundary towards the animal pole (Fig. 2F). At the blastodisc and yolky part boundary, the folds interact, forming star-like or network-like clusters. During cytokinesis, the deepening of the furrow was followed by the appearance, at its bottom, of a domain with a much smoother surface, which had a number of lamellar formations and spherical bodies, as has been described by Bozhkova *et al.* (1983). On the basis of these features, the furrow region differs from the preexisting surface of the egg and apparently corresponds to the new membrane formed during cytokinesis (Bluemink and de Laat, 1973). Polar differences in the egg surface topography, similar to the normal ones, were observed in the activated egg as well. However, in the absence of cytotomy, no new membrane was formed, while cyclic changes in the relief of the preexisting surface became more distinct. At each cycle of shape changes, the rounding up and augmentation of the blastodisc was followed by a partial smoothing (swelling) of the surface folds (Fig. 2H) and the appearance of «thicker» (spherical) folds or blebs (Fig. 2I) on the egg surface. During flattening of the blastodisc, surface folds became sharper again (Fig. 2G). The changes were more distinct during the second than during the first cycle of shape changes. The relation between changes in the state of cytomatrix and properties of the membrane is not yet clear. In the membrane of the cleaving loach embryo (2-256 cells), stretch-activated ionic channels have been described which were activated by membrane tension during patch clamp studies (Medina and Brejestovsky, 1988). Perhaps the enhancement of ionic transport observed

during cell cycle interphase occurs due to stretch-activation of the ionic channels. Ionic transport from the egg might be necessary to equilibrate the increase in local osmotic pressure provoked by solation of the actin network in egg blastodisc, and to restrict the excessive accumulation of ooplasm.

Experimental Procedures

Mature eggs of the loach *(Misgurnus fossilis)* were obtained and fertilized by sperm or artificially activated by tap water. In most experiments the embryos were incubated in dechlorinated tap water at 19-21°C or in Holtfreter solution, containing 59 mM NaCl, 0.7 mM KCl, 0.9 mM $CaCl_2$, 2.5 mM Tris-HCl, pH 7.6. In certain cases artificial pond water containing 1 mM NaCl, 0.25 mM KCl, 0.2 mM $CaCl_2$, 0.2 mM $MgCl_2$ and 0.1 mM Na_2HPO_4 was used instead of tap water.

To prepare the specimens for SEM, the fertilization membrane of eggs incubated in tap water was punctured by a needle. Eggs were fixed in Hanks solution, containing 2% glutaraldehyde (Fluka, Switzerland), or in solution containing 2% glutaraldehyde, 0.15 M cacodylic buffer (Sigma, USA), pH 7.4 for 24 h at 4°C. The preparations were dehydrated in acetone series and dried at the critical point of carbon dioxide in EIKO Critical Point Dryer DX-1 (Japan). The dried samples were shadowed with gold and examined under a TEMSCAN-100 Cx11 microscope (Japan). Observations of developing embryos were made under Biolam microscope (LOMO, USSR) with 52.5x fold magnification.

Measurements of electrical characteristics were performed by means of conventional electrophysiological equipment for intracellular studies (Experimetria, Hungary) with two microelectrodes being introduced into the yolk of an egg. Since there is no permeability barrier between the blastodisc and the yolky part, we assume that R_{in} values actually reflect the average R_{in} of the blastodisc and yolk membranes. The microelectrodes were filled with 2.5 M KCl (R_{in}= 10-50 MΩ). The long-term measurements were made by periodically injecting rectangular hyperpolarizing current pulses I (0.2-10 nA) through one electrode while recording the membrane potential E_m and electrotonic potential V_1 with the second one. E_m and V_1 were displayed on the pen recorder Endim 622.01 (DDR). The long time constant τ of the loach egg membrane required a pulse duration of 1-3 sec or more. The condition of measurements in the linear part of voltage-current curves for eggs with different resistances required current pulses producing potentials less negative than -30 mV. During the experiment the control of the linear part of voltage-current relationships was fulfilled by the periodical half diminution of current pulses. R_{in} was calculated as V_1/I. C_m was estimated from the membrane time constant of egg.

To visualize mitosis, fertilized eggs were fixed with Bouin solution. Serial sections of 5-7 μm were stained with methyl blue-eosin. More than 10 eggs were examined in each phase.

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