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RELATIONSHIPS BETWEEN PLASMA MEMBRANE DEPOLARIZATION, NUCLEAR MEMBRANE BREAKDOWN, AND THE APPEARANCE OF CYTOPLASMIC FACTORS DURING THE FIRST MEIOTIC DIVISION IN RANA PIPIENS OOCYTES

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The present study examines the relationship among three events which occur in Rana pipiens oocytes following hormone-induced reinitiation of meiosis: (i) plasma membrane depolarization, (ii) nuclear membrane breakdown, and (iii) the appearance of specific cytoplasmic factors. Preventing plasma membrane depolarization with a voltage clamp did not prevent nuclear breakdown. Transfer of cytoplasm from hormone-induced oocytes into naive oocytes resulted in both depolarization and nuclear breakdown. Depolarization of injected oocytes was dependent on protein synthesis, whereas nuclear breakdown was not. These findings, together with other recent evidence, suggest that different cytoplasmic factors may initiate protein synthesis-independent nuclear membrane events and protein synthesis-dependent plasma membrane events.

INTRODUCTION

The vertebrate oocyte is arrested in first meiotic prophase until hormonal release at the time of ovulation. The oocyte then completes one and one-half meiotic divisions and is again arrested at second meiotic metaphase. Fertilization allows completion of the meiotic divisions and is followed by the cleavage phase of development. During the meiotic divisions, the oocyte plasma membrane undergoes a depolarization by the end of the first meiotic division and repolarizes following the completion of the second meiotic division after fertilization (1-5). Studies with Rana pipiens oocytes indicate that the depolarization is due to down-regulation of an electrogenic Na^+, K^+ pump (6) and a decrease in plasma membrane K^+ permeability (1). The onset of depolarization coincides with the swelling and subsequent breakdown of the large nucleus (some 8-12 h after hormonal reinitiation of meiosis, ref. 1). Since it is well established that specific cellular events can be

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initiated by changes in transmembrane potential (e.g. as in nerve and muscle), we examined the extent to which nuclear membrane breakdown depends on plasma membrane depolarization. Previous studies have shown that plasma membrane depolarization (1), increased protein synthesis (7), and increased protein phosphorylation (8), events which occur during the first meiotic division, can occur in enucleated oocytes, indicating that these events are under cytoplasmic control. Transfer of small amounts of cytosol from hormonally-induced oocytes into prophase-arrested oocytes has been shown to initiate cellular events (as described above) which are the hallmarks of meiotic maturation (9,10,11). Therefore, we have examined the role of cytoplasmic factors in inducing plasma membrane depolarization and nuclear membrane breakdown.

MATERIALS AND METHODS

Fully grown Rana pipiens follicles, arrested at first meiotic prophase, were stripped of their follicular envelopes and follicle cells by the method of Masui (12), with the modifications described elsewhere (1). The resulting denuded oocytes were kept in a modified amphibian Ringer's solution (1) at 20-24°C.

Progesterone (Sigma Chemical Co.) was used to reinitiate the meiotic divisions (12), and was prepared in 95% ethanol; when indicated, 1 µl was added per ml of Ringer's solution. Cytoplasmic transfers were carried out using calibrated micropipettes according to the method of Masui and Markert (9). Cycloheximide (Sigma Chemical Co.) was used, when indicated, at a concentration of 3.6 µM.

Standard electrophysiological techniques were used as described elsewhere (6).

RESULTS AND DISCUSSION

The coincidence of nuclear breakdown and plasma membrane depolarization (1) suggested a possible causal relationship between the two events. We have previously shown that depolarization occurs in the absence of the nucleus (1). Thus, nuclear breakdown per se is not essential for depolarization. Previous studies in this (1, 13) and other laboratories (10, 14) have shown that natural variations in or experimental manipulation of the resting potential of Rana and Xenopus oocytes measured prior to hormonal reinitiation of meiosis has little influence on the ability of the hormone-induced oocyte to undergo nuclear breakdown. However, regardless of the initial resting potential,

Table 1

The Effect of a Continuous Voltage Clamp[a] on Progesterone-Induced Depolarization and Nuclear Breakdown in Rana oocytes

Preparation	E(mV)[b] #1, #2, #3	g (μS)[c] #1, #2, #3	Nuclear Breakdown #1, #2, #3
Prophase-arrested oocyte	-50, -63, -73	6.1, 2.3, 9.7	.....
Progesterone-Induced Oocyte, Continuous Voltage Clamp, @ 7-12 h	-59, -73, -88	0.46, 0.27, 1.1	+ + +
Progesterone-Induced Oocyte, @ 7-12h, Clamp Removed	-25, -19, -37	.....	.....

[a] Oocytes were voltage-clamped and the chamber containing the oocyte was perfused with Ringer's solution containing 3.2 μM progesterone. Membrane conductance was measured prior to exposure to progesterone and after nuclear breakdown. Nuclear breakdown was apparent as a white area on the black animal hemisphere and usually appeared during the voltage clamp; nuclear breakdown was confirmed by microdissection (see ref. 1). + indicates the occurrence of nuclear breakdown.

[b] Plasma membrane potentials. Prophase-arrest values represent initial resting potentials. Final clamping voltages were maintained 9 to 15 mV more negative than the initial potentials for the three oocytes shown. Each experiment (#1, #2, #3) represents one oocyte from a different frog.

[c] Plasma membrane conductance, in μSiemens.

nuclear breakdown was always accompanied by depolarization. As shown in Table 1, preventing depolarization using a voltage clamp did not prevent nuclear breakdown, nor did it prevent the occurrence of the conductance decrease which normally accompanies depolarization (4, 6, 15). Thus, the present study shows that the change in membrane potential is not prerequisite to nuclear breakdown. Interestingly, it has been shown that the induction of nuclear breakdown is a post-transcriptional event (16), and that the hormonal induction of both nuclear breakdown and depolarization can be inhibited by treating oocytes with cycloheximide, a protein synthesis inhibitor which works at the level of translation (1). Thus it appears that properties of the nuclear and plasma membranes are under translational control.

One known translational event is the appearance of cytoplasmic protein factors within the first few hours after exposure to hormones which release

Table 2

Effect of Cytoplasmic Transfer on the Plasma Membrane Potential and Nucleus of Prophase-Arrested Rana Oocytes

Treatment	E(mV)[a]	Nuclear Breakdown % @ 24 h
Untreated	-50.6 ± 4.2(8)[b]	0
Sham injection[c]	-50.0 ± 5.0(3)	0
Exogenous progesterone (3.2μM)	- 0.6 ± 2.4(5)	100
"Competent" cytoplasm[d]	+ 0.6 ± 1.1(9)	100
"Competent" cytoplasm[e] + cycloheximide	-44.0 ± 4.6(5)	100

[a] Sibling oocyte resting potentials were measured 18-20 h after treatment.
[b] Mean ± SEM (no. of oocytes)
[c] Injection of whole cytoplasm (60 nl) from prophase-arrested oocytes into prophase-arrested oocytes. This volume is less than 2% of the volume of prophase-arrested oocytes.
[d] Injection of whole cytoplasm (60 nl) from donor oocytes (24 h after progesterone treatment) into prophase-arrested oocytes.
[e] Prophase-arrested oocytes were injected as in (d) and transferred to Ringer's containing 3.6 μM cycloheximide.

the prophase block (9,17). These factors ("maturation promoting factors"), when injected in small quantities into prophase-arrested oocytes, initiate events which are major features of hormone-induced meiotic maturation: an increase in the rate of protein synthesis (11), nuclear breakdown (9), plasma membrane depolarization (10), and arrest at second meiotic metaphase (9). These factors are produced even in the absence of the nucleus (9). Inhibition of protein synthesis with cycloheximide prevents the appearance of these factors, but does not prevent the ability of these factors to induce nuclear breakdown (17). Table 2 presents data which show that microinjection of cytoplasm from oocytes undergoing the second meiotic division ("competent" cytoplasm) into prophase-arrested oocytes results in both nuclear breakdown and plasma membrane depolarization identical to that measured after hormone-stimulation. Transfer of cytoplasm from prophase-arrested oocytes was without effect. The two events were again found to be coupled temporally, and suggested that the same cytoplasmic factor(s) might be altering both nuclear and plasma membrane properties. However, Table 2 shows that cycloheximide

treatment prevented depolarization in all oocytes injected with competent cytoplasm, whereas cycloheximide treatment did not prevent nuclear breakdown. The dependence of cytoplasmic factor-induced depolarization on protein synthesis is particularly interesting in light of recent evidence (18) that the cytoplasmic activity which induces the increase in protein synthesis can be separated from the activity which induces nuclear breakdown. Thus, it may be that two distinct cytoplasmic factors induce alterations in the nuclear and plasma membranes: one factor can cause dissolution of the nuclear membrane, independent of protein synthesis, whereas the action of the other factor requires protein synthesis in order to induce those events which result in plasma membrane depolarization: down-regulation of an electrogenic Na^+, K^+ pump (6) and a decrease in plasma membrane K^+ permeability (1).

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