

Fusion of mammalian oocytes : SEM observations of surface changes

J. Fulka Jr ¹, B. Fléchon ² and J.-E. Fléchon ²

¹ Research Institute of Animal Production, 104 00 Prague 10, Uhřetíněves, Czechoslovakia;

² INRA, laboratoire de biologie cellulaire et moléculaire, 78350 Jouy-en-Josas, France

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Summary — Mouse oocytes at the germinal vesicle (GV) stage were fused with maturing oocytes in which GV's were no longer visible. The fused cells were fixed at different time-intervals after the initiation of fusion and prepared for scanning electron microscope (SEM) observation. Concomitantly, some fused cells were prepared for light microscope evaluation. Our SEM observations showed no significant differences in surface morphology between immature and maturing oocytes. However, immediately after fusion was initiated, dramatic changes occurred on the surface of the maturing oocytes. The microvilli were shortened or disappeared locally and the plasma membrane was deeply ruffled. One hour after fusion, when the giant cells were nearly spherical, the microvilli reappeared and the ruffling gradually disappeared. In some areas, the microvilli were extremely long. Three hours after fusion, the fused cells were perfectly round and their surfaces were generally covered with microvilli of equal length. No further ruffling was observed. It is suggested that cytoplasmic mechanisms regulate the surface morphology of the oocytes during fusion.

mouse oocyte — fusion — surface change — maturation

Résumé — **Fusion d'ovocytes de mammifères : observations de modifications de surface en microscopie électronique à balayage.** Des ovocytes de souris au stade vésicule germinative (VG) ont été fusionnés avec des ovocytes en cours de maturation où la VG n'est plus visible. Les cellules fusionnées ont été fixées à différents intervalles de temps après le début de la fusion et préparées pour l'observation au microscope électronique à balayage (MEB). Simultanément, des cellules fusionnées ont été observées en microscopie photonique. Au MEB, on n'observe pas de différences de morphologie de surface entre les ovocytes immatures et en cours de maturation. Cependant, immédiatement après fusion, des modifications se produisent sur les ovocytes en cours de maturation. Les microvillosités sont raccourcies ou disparaissent localement et la membrane plasmique est plissée. Une heure après la fusion, alors que les cellules géantes sont devenues à peu près sphériques, les microvillosités réapparaissent et les plissements disparaissent. De très longues microvillosités sont distribuées uniformément. Trois heures après la fusion les cellules géantes sont parfaitement rondes et leur surface est généralement couverte de microvillosités de taille égale. Il n'est plus possible d'observer de plissements. Il est probable que des mécanismes cytoplasmiques régulent la morphologie de surface des ovocytes pendant la fusion.

ovocyte de souris — fusion — modification de surface — maturation

INTRODUCTION

The method of mammalian oocyte fusion is widely used in reproductive and developmental biology (Tarkowski, 1982; Vasetzky & Sekirina, 1985). Its extensive use reveals specific cytoplasmic factors regulating the process of oocyte maturation (Balakier & Czolowska, 1977; Fulka Jr., 1983, 1985; Fulka Jr. *et al.*, 1985). On the other hand, changes taking place at the surface of the cells during fusion have been poorly described. From the observations of zona-free oocytes, it is clear that the surface of the immature oocyte is uniformly covered with microvilli (Nicosia *et al.*, 1978; Ebensperger & Barros, 1984) and that there is no polarity. Local changes begin to occur when the meiotic spindle moves from the center to the surface of the oocyte. A microvillus-free area is formed where the first polar body later becomes extruded (Longo & Chen, 1985; Okada *et al.*, 1986). The purpose of the present study was to describe surface changes occurring in the course of mouse oocyte fusion. These changes were correlated with light-microscopic observations of induced chromosome condensation in immature oocytes fused with maturing oocytes. In this preliminary morphological study, no attempt was made to analyze the mechanisms involved in surface changes.

MATERIAL AND METHODS

Immature oocytes at the germinal vesicle (GV) stage were released from large antral follicles of A/Ph-strain mice. The cumulus cells were then removed by repeated pipetting; only the oocytes containing intact germinal vesicles were used. They were kept for 2–3 h in M199 medium

(Pavlok & McLaren, 1972) containing 100–150 μg of dibutyrylcyclic AMP (Cho *et al.*, 1974) to prevent germinal vesicle breakdown (GVBD). Maturing oocytes were obtained by culture for 2–3 h in droplets of the medium without dbcAMP under an atmosphere of 5% CO_2 in the air and at 37.5 °C.

Immediately before fusion was induced, the cumulus-free oocytes were incubated for 5 min in pronase (0.5%). When the zonae were partially dissolved, the oocytes were transferred to a dbcAMP-supplemented medium and the zonae were definitively removed by pipetting. One immature and one maturing oocyte were then agglutinated in a solution of PHA (400 $\mu\text{g}/\text{ml}$) to achieve close contact between the two cells. Fusion was induced by incubating the agglutinated cells in polyethyleneglycol (PEG; M_R 1000; 50% solution; w/v) as described previously (Fulka Jr., 1985). After induction of fusion, the oocytes were washed 5 times in dbcAMP-supplemented medium and cultured in the latter for 15–30, 60 and 180 min, respectively. After these time-intervals, the cells showing fusion were removed from the culture medium, fixed in 2.5% glutaraldehyde and postfixed in 1% osmium tetroxide in 0.125 M cacodylate buffer. The specimens were dehydrated in alcohol, critical-point dried in freon and gold-coated.

Control (unfused) oocytes were also prepared. At least 10 specimens were observed in each experimental group. For light microscope observation, the cells were fixed in acetic alcohol (1/3), stained with orcein and examined under a phase-contrast microscope.

RESULTS

Light microscope observations

In the 15–30-min time-interval, the first fusing cells were observed. These cells assumed different shapes, probably depending on the area of contact established between the cells in the PHA solution.

According to our unpublished light microscope observations, immature oocytes always enter maturing ones following the movement of the GV. In all the specimens observed under phase-contrast, the fusing cells respectively contained a set of condensed chromosomes and the still intact germinal vesicle (Figs. 1, 2). At one hour post-fusion, the shape of the resulting giant cells was spherical or slightly oval. The GV from the immature oocyte was no longer visible, and 2 sets of chromosomes were present in the common cytoplasm (Fig. 3). One of the chromosome sets was that of the maturing oocyte, and the other resulted from premature condensation under the effect of the maturation-promoting factor (MPF) present in the maturing oocyte. At 3 post-fusion, the giant cells were spherical and the 2 sets of chromosomes joined together to form a common group (Fig. 4).

SEM observations

The cell surface was uniformly covered with microvilli and no microvillus-free areas were seen in control immature or maturing oocytes. On the other hand, almost immediately after the initiation of fusion, dramatic changes occurred in fusing oocytes. In most cases, the microvilli partially or almost completely disappeared on the maturing oocyte and its surface became deeply ruffled in the fusion area (Figs. 5, 6). Where present, the microvilli were shorter on the maturing oocyte. The surface of immature oocyte did not exhibit similar changes and its morphology remained unchanged. Protracted (stretched?) microvilli were seen around the sites of initial contact of both oocytes (Fig. 6). In the following two time-intervals (1—3 h

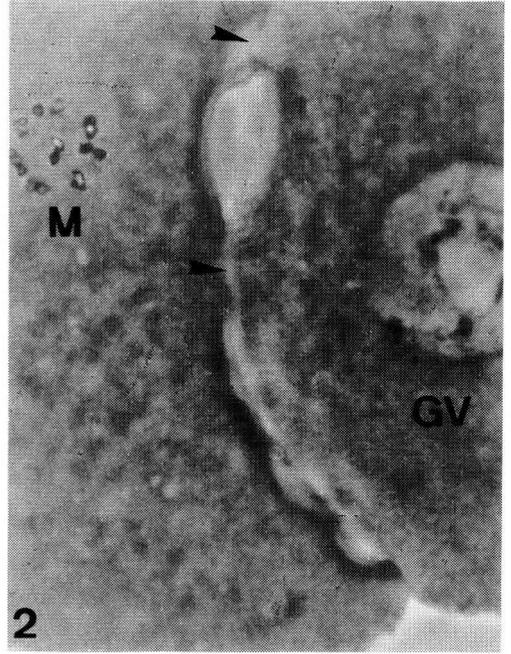
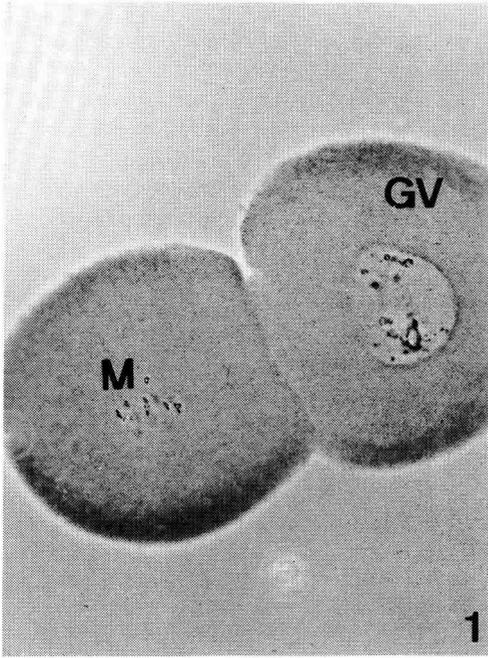
post-induction of fusion), the gradual reappearance of normal surface morphology was observed. One hour after fusion, the microvilli reappeared on the entire surface and the ruffling disappeared (while both sets of chromosomes were generally located in the centre of the giant cells). In some areas, long microvilli were also present, but they seemed to be randomly distributed (Fig. 7). In most fused cells it was impossible to distinguish the original hemispheres coming from different oocytes. The surface of fused cells was completely covered with almost uniform microvilli 3 h after induction of fusion; no ruffling was observed on the spherical giant cells (Fig. 8).

DISCUSSION

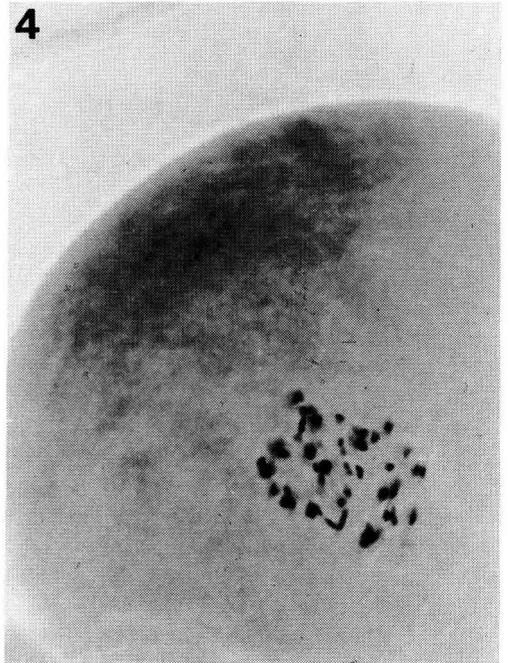
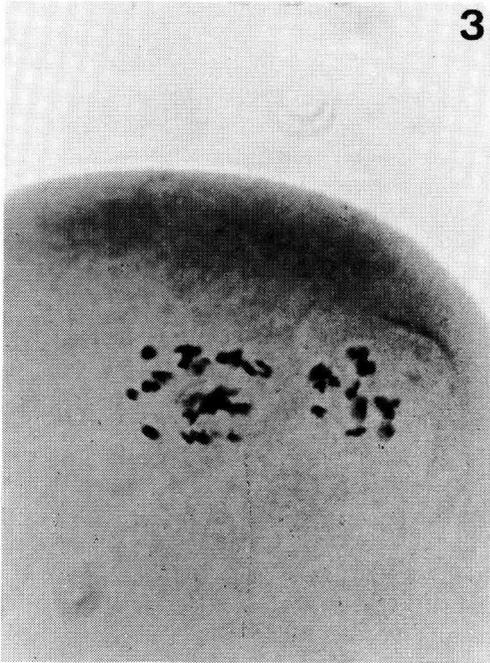
The present results deal with the surface features observed during fusion of 2 different types of oocytes. We did not expect such specific changes; however, they did not seem to be artifacts as they were repeatedly observed. The differences between the 2 kinds of oocytes were stage-specific, as the treatments with PHA and PEG were the same. A possible shrinkage effect of PEG (Gulyas, 1986) was eliminated by several washes.

The selective surface changes were not induced by cAMP, as they occurred in maturing oocytes in contact with this drug for a much shorter time than immature oocytes. Could PHA be involved in these surface effects?

On the other hand, shortening and disappearance of the microvilli during the first time-interval after initiation of fusion somewhat resembled the changes observed when the 2nd meiotic spindle moves to the oocyte periphery (microvillus-free and ruf-



1 2
3 4



fled areas) (Longo, 1985; Longo & Chen, 1985; Nicosia *et al.*, 1978; Okada *et al.*, 1986). The fertilization cone observed by Shalgi *et al.* (1978) also showed a similar surface morphology. Formation of microvillus-free cones was also observed by Soltynska *et al.* (1986) after fusion of thymocytes with activated mouse oocytes. When non-activated oocytes were fused with thymocytes the cones did not form, but the surfaces over the introduced nuclei were smooth and without microvilli. Immature oocyte-maturing oocyte fusion may in some respects resemble the process of fertilization, but the cells are of the same size. So a stronger trigger may explain why the entire surface of the maturing oocyte was ruffled.

As has been shown recently, these surface changes are closely correlated with the microfilament system; that is, with actin. Immunofluorescent localization of actin is enhanced in the region associated with the oocyte microvillus-free area (Longo & Chen, 1985; Howlett *et al.*, 1985). Microfilaments are assembled along the hybrid plasma membrane after thymocyte—oocyte fusion (Szöllösi *et al.*, 1986). Similarly, this activity may also be involved in the process of oocyte—oocyte fusion and may correspond to the mechanisms regulating the engulfment of incorporation of the immature oocyte. Tubulin may also play a role in this process: both groups of chromosomes move together and form a common spindle after fusion between oocytes and blastomeres (Szöllösi *et al.*, 1980). Such reorganization may also affect

the surface morphology. In fact, the chromosomes themselves may act on the assembly of microtubules and on the egg cortex (Maro *et al.*, 1986). It would be interesting to determine why maturing and immature oocytes behave differently after fusion, perhaps as a result of the accumulation of both sets of chromosomes in the former.

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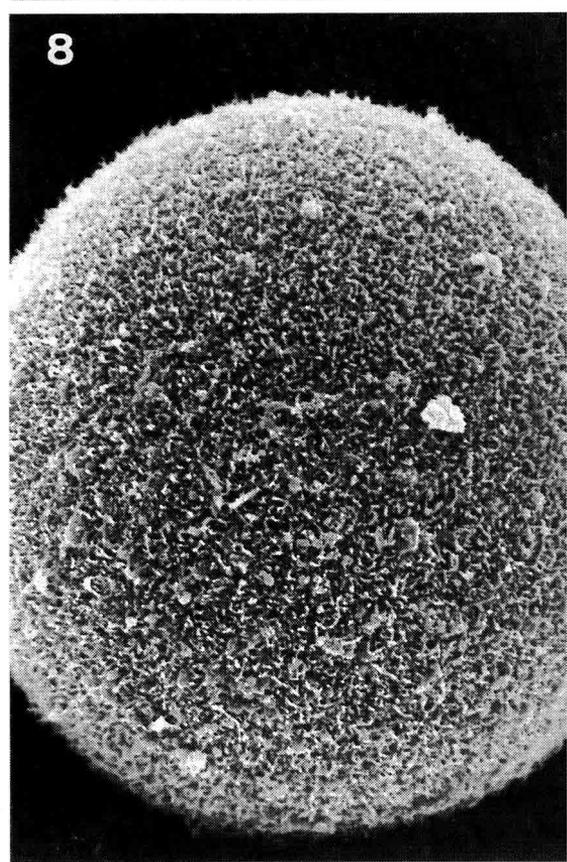
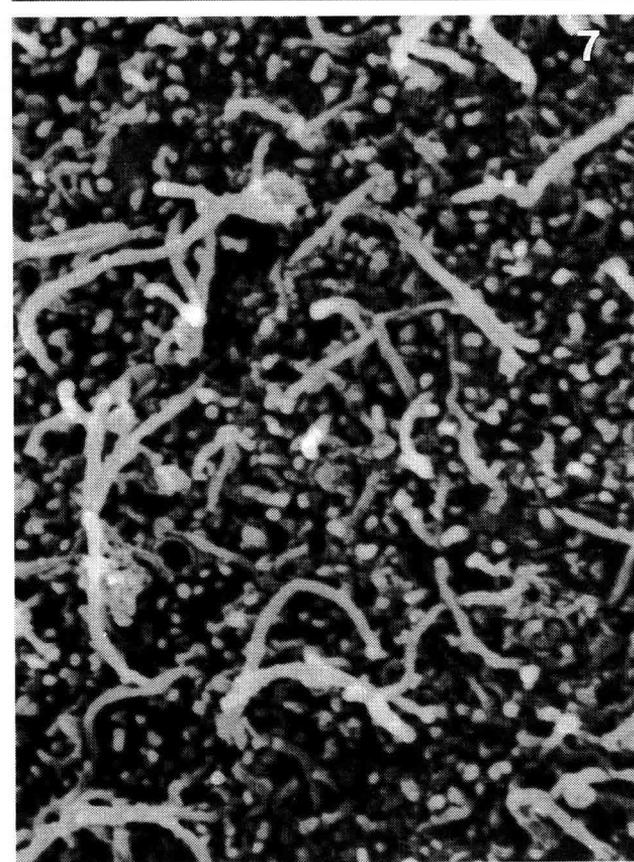
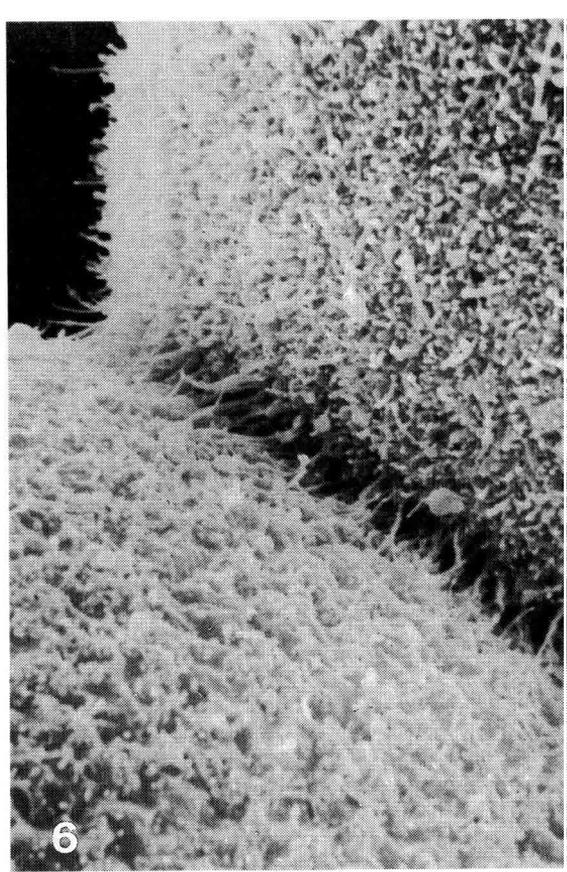
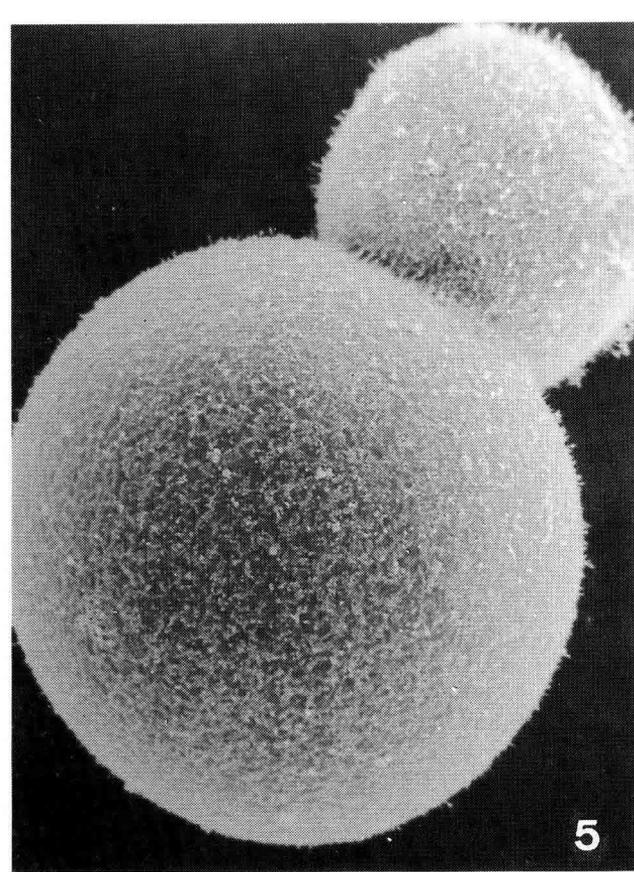
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Fig. 1. Fusing oocytes fixed 15 min after induction of fusion. In the cytoplasm of maturing oocyte the circular bivalent stage chromosomes are visible (M), whereas in the immature oocyte, an intact germinal vesicle is still present (GV). x 1 400.

Fig. 2. Fusing oocytes fixed 15 min after induction of fusion. Two fusing sites are clearly seen between the immature and maturing oocytes (arrows). x 800.

Fig. 3. Cell fixed 60 min after induction of fusion. In the cytoplasm of fused cell two groups of chromosomes are visible. x 800.

Fig. 4. Three hours after induction of fusion a common group of chromosomes is formed. x 800.



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Fig. 5. Oocytes fixed ≤ 30 min after induction of fusion. The maturing oocyte shows very short microvilli and deep ruffling. The surface of immature oocyte is unchanged. x 1 400.

Fig. 6. Close view of the contact between both cells. **M**, maturing oocyte; **I**, immature oocyte. x 5 600.

Fig. 7. Fused oocytes 60 min after PEG treatment. Short and long microvilli are still present. x 11 000.

Fig. 8. Fused cell fixed 3 h after induction of fusion. Its surface is uniformly covered with microvilli, no ruffling is observed. x 1 800.