First Cleavage of the Mouse Embryo Responds to Change in Egg Shape at Fertilization

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Supplemental Experimental Procedures

Egg Collection and Culture

Oocytes and zygotes were collected from F1 (C57BL/6 × CBA) females superovulated as described previously [S1]. Females were mated with F1 males. All embryos were collected in M2 medium containing 200 IU/ml of hyaluronidase and washed in fresh M2. Cultures were performed in drops of KSOM medium with 4 mg/ml BSA under paraffin oil in an atmosphere of 5% CO2 at 37.5°C as previously described [S1]. In certain experiments eggs were cultured in the presence of 1 μg/ml cytochalasin D (Sigma) or 0.25 μg/ml nocodazole (Sigma).

Time-Lapse Video Microscopy

Real-time video microscopy and imaging were performed and analyzed on either a Leica inverted microscope with an Orca-Hamamatsu camera using OpenLab RTI software or a Nikon inverted microscope with a Princeton Instruments camera using IP Lab software. Zygotes were obtained 16.5–17 hr after hCG and placed in 0.8 ml of M2 under paraffin oil in a glass-bottomed dish on a heating stage at 37°C. Images were recorded at 5 min intervals over 20 hr on two focal planes, one occupied by the second polar body and the other by the fertilization cone. The images were first analyzed for two types of movement: that of the second polar body and for any rotation of the egg within the zona that could be followed by the relationship of distinctive markers within the egg cytoplasm or membrane to distinct reference points on the zona itself. The rotation about an axis in the x-y plane was estimated by measuring the change in angle of the second polar body (PB) from the z axis. The angle θ subtended by the second PB from the *z* axis was calculated from the distance (*d*) from the center of the embryo in the x-y plane as $\theta = (\sin^{-1}[d/r])/2$. The angular displacement due to rotation about an axis in the x-y plane was calculated as $\Delta\theta = (\sin^{-1}(d_2/r) - \sin^{-1}[d_1/r])/2$. If during imaging the egg had rotated within the zona for more than 15°, it was excluded as its analysis could not be precise.

Measurements of Embryo Dimensions

Ovulated oocytes were obtained 14 hr after hCG injection, stained for chromatin with Hoechst 33342 (5 μg/ml) in M2 for 8 min and added to droplets of M2 under paraffin oil on glass-bottomed dishes. Oocytes were positioned with the metaphase II spindle on top and photographed. Zygotes were obtained 15.5 hr after hCG. After transfer to glass-bottomed dishes, embryos were positioned with a holding pipette with the second polar body on top, and photographed. Zygotes were then cultured and photographed 4–5 hr later as described above. For some groups of zygotes, the zona pellucida was removed using short exposure to acid Tyrode's solution.

Artificial Activation

Metaphase II oocytes were obtained 15.5 hr after hCG and treated with 7% ethanol for 8 min as previously described [S2]. Activated oocytes were then cultured for 2 hr to allow them to extrude the second polar body, photographed, returned to culture for a further 2 hr, and photographed again.

In Vitro Fertilization

HTF medium [S3] for in vitro fertilization was equilibrated overnight at 37.5°C in 5% CO_2 in atmosphere. The contents of caudae epididymes from mature F1 males were released into 1 ml of equilibrated fertilization medium and held for 2 hr to allow capacitation. Oocytes were collected 12.5 hr after hCG and placed in fertilization medium together with sperm suspension (approximately 10^6 spermatozoa/ml). Eggs with a single sperm that entered the egg labeled with Hoechst as described before (but without a fertilization cone or visible signs of polar body extrusion) were selected for analysis.

Immunostaining

For immunocytochemical staining, eggs were fixed with 3.7% paraformaldehyde in PBS containing 0.1% Triton X-100 for 40 min at 37°C. The samples were then extracted with 0.25% Triton X-100 for 20 min, washed for three periods of 10 min in 0.1% Tween 20 in PBS, incubated for 10 min in NH_4Cl, washed again for three periods of 5 min in 0.1% Tween in PBS, and then processed for immunofluorescence. Tubulin was visualized using a rat monoclonal antibody to tyrosinated α-tubulin (YL1/2) and FITC-conjugated goat anti-rat IgG (Harlan Sera Lab). Actin was visualized using Rhodamine phalloidin (20 μg/ml; Molecular Probes). DNA was visualized using DAPI (100 μg/ml). Mouse Par6 was visualized using an antibody obtained from Lin and colleagues [S4]. Prior to examination embryos were washed in PBS and placed on cover glasses coated with 10% Concanavalin A or 0.01% Poly-L-lysine. Then they were mounted on slides as described before. Fluorescent micrographs were captured using a Zeiss AxioVert inverted microscope supplied with an AxioCam camera and processed using AxioVision 3.0 software (Zeiss) or with a confocal (BioRad MRC1024) scanning head.

Modification of Embryo Shape

To impose a shape, eggs were drawn into a mouth-controlled pipette such that they were slightly squeezed. They were subsequently released into KSOM containing sodium alginate (0.5%) such that their change in shape was maintained upon solidification of this matrix by adding a few drops of a 1.5% $CaCl_2$ (0.3g CaCl2 dissolved in 20.0 ml 0.15M NaCl) solution. Eggs were oriented such that either the fertilization cone, the second polar body, or both were on one end of the long axis. In the last group, the animal pole and fertilization cone could be either on the same or opposite ends of the long axis. Control zygotes were also placed in sodium alignate for the same length of time, but their shape was not changed. The embryos that developed into the morulae/blastocysts were transferred to the uteri of the foster mothers together with MF1 embryos as carriers. Only those pregnancies that resulted in development of carrier embryos were taken into account in the analysis of the success of survival.

Statistical Analysis

To compare the ratio data from the nine independent groups (Table 1), a Kruskal-Wallis nonparametric test was performed. This global test was used to determine whether there were any differences amongst the groups. If a statistically significant result was obtained, then Mann-Whitney U tests were performed to determine where the (pairwise) differences were between the groups. Statistical significance for the Kruskal-Wallis test was set at the 0.05 level, while for the Mann-Whitney U tests the statistical significance level was set at the 0.0014 level to adjust for multiple comparisons (i.e., 36 pairwise tests).

Supplemental References

S1. Piotrowska, K., and Zernicka-Goetz, M. (2001). Role for sperm in spatial patterning of the early mouse embryo. Nature *409*, 517–521.

S2. Piotrowska, K., and Zernicka-Goetz, M. (2002). Early patterning of the mouse embryo—contributions of sperm and egg. Development *129*, 5803–5813.

S3. Nakagata, N., and Takeshima, T. (1993). Cryopreservation of mouse spermatozoa from inbread and F1 hybrid strains. Exp. Anim. *42*, 317–320.

S4. Lin, D., Edwards, A.S., Fawcett, J.P., Mbamalu, G., Scott, J.D., and Pawson, T. (2000). A mammalian PAR-3-PAR-6 complex implicated in Cdc42/Rac1 and aPKC signalling and cell polarity. Nat. Cell Biol. *2*, 540–547.