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Title

Effect of introducing somatic mitochondria into an early embryo on zygotic gene activation

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Supporting information list

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Materials and Methods for Supplemental data

Time-lapse imaging

Produced embryos (intact, BI-, and SM-embryos) were transferred into M16 medium in into individual culture dishes (Linkid micro25 (DNP. Tokyo, Japan)). The embryos were imaged every 1 hour from 10 hpi using an IX71 microscope (Olympus, Tokyo, Japan) in an atmosphere containing 5% CO_2 at 37°C.

Microinjection of zygotic mitochondria into zygotes

Embryos were treated in culture medium containing 1 μg/mL nocodazole for 1 hour in an atmosphere of 5% CO_2 at 37 °C. Next, the embryos were centrifuged at 35°C in culture medium containing 1 μg/mL nocodazole at 10,000 × *g* for 20 min to prepare the mitochondria-enriched regions of the embryos. After centrifugation, the mitochondria-enriched region was suctioned into a glass pipette and injected into the zygotes using a Piezo micromanipulator (Prime-Tech, Tokyo, Japan).

DMSO treatment

In vitro fertilization embryos were transferred to a drop of M16 medium supplemented with 2% DMSO (041-29351, Wako) and cultured in an atmosphere containing 5% CO_2 at 37°C.

Supplemental Figure legends

Fig. S1. Ultrastructure of isolated somatic mitochondria

A) Overview of the zygote showing somatic mitochondria introduction into the perivitelline space. Magnification × 2,000, Scale bar = 10 μm. B) Comparison of zygotic and somatic mitochondria. Magnification × 80,000, Scale bar = 200 nm. ZM: zygotic mitochondrion; SM: somatic mitochondrion. C, D) Ultrastructure of isolated somatic mitochondria in the perivitelline space. Magnification × 60,000 (C) and × 100,000 (D), Scale bar = 200 nm (C) and 200 nm (D).

Fig. S2. Localization of mitochondria in centrifuged zygotes after treatment with nocodazole

A) Fluorescence micrograph showing Mito tracker green-stained mitochondria in centrifuged zygotes after treatment with nocodazole. Scale bar = 60 μm. B) Overview of centrifuged zygote after treatment with nocodazole. Magnification × 2,000, Scale bar = 10 μm. C) Enlarged view of the blue frame. Magnification × 8,000, Scale bar = 2 μm. Organelles indicated with blue lines: lipid droplets. D) Enlarged view of the red frame. Magnification × 4,000, Scale bar = 5 μm. E) Enlarged view of the green frame. Magnification × 8,000, Scale bar = 2 μm. Arrowhead: zygotic mitochondria. F, G) Overview of the zygote immediately after introduction of mitochondria. Scale bar = 60 μm. Circles indicate regions with introduced mitochondria.

Fig. S3. Delay in first embryonic cleavage in embryos injected with somatic mitochondria

Black lines indicate average values (Intact: 20.083; BI: 20.333; SM: 23.352). Different letters indicate statistically significant differences ($P < 0.01$).

Fig. S4. Alteration in histone modifications in embryos with somatic mitochondria

A) Immunofluorescence detection of SIRT1 in late two-cell-stage embryos. Scale bars = 25 μm. B) $P < 0.01$ (nuclei n > 30) for comparison of nuclear signal intensities. Black lines indicate average signal intensities (Intact: 71.383; 2% DMSO: 38.934). C) Immunofluorescence detection of H3K4 trimethylation in late two-cell-stage embryos. Scale bars = 25 μm. D) $P < 0.01$ (nuclei n > 50) for comparison of nuclear signal intensities. Black lines indicate average signal intensities (Intact: 35.985; 2% DMSO: 54.943). E) Immunofluorescence detection of H3K27 acetylation in late two-cell-stage

embryos. Scale bars = 25 μm. F) $P < 0.01$ (nuclei n > 30) for comparison of nuclear signal intensities. Black lines indicate average signal intensities (Intact: 53.312; 2% DMSO: 48.113).