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Report

Reprogramming after Chromosome Transfer into Mouse Blastomeres

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Summary

It is well known that oocytes can reprogram differentiated cells, allowing animal cloning by nuclear transfer. We have recently shown that fertilized zygotes retain reprogramming activities [1], suggesting that such activities might also persist in cleavage-stage embryos. Here, we used chromosome transplantation techniques to investigate whether the blastomeres of two-cell-stage mouse embryos can reprogram more differentiated cells. When chromosomes from one of the two blastomeres were replaced with the chromosomes of an embryonic or $CD4^+$ T lymphocyte donor cell, we observed nuclear reprogramming and efficient contribution of the manipulated cell to the developing blastocyst. Embryos produced by this method could be used to derive stem cell lines and also developed to term, generating mosaic "cloned" animals. These results demonstrate that blastomeres retain reprogramming activities and support the notion that discarded human preimplantation embryos may be useful recipients for the production of genetically tailored human embryonic stem cell lines.

Results and Discussion

Reprogramming by nuclear transfer allows the generation of animals and embryonic stem (ES) cell lines from somatic cells [2]. This approach, if successful with human cells, would allow the production of human stem cell lines from individual patients for personalized medicine or in vitro modeling of their condition [3–5]. However, attempts to produce human ES cell lines by nuclear transfer have thus far been unsuccessful, in part because of the limited availability of human oocytes.

Reprogramming by nuclear transfer is only successful under certain specific conditions, making it difficult to source the appropriate recipient cell types. Reprogramming and embryonic development can occur in animals after transfer of somatic nuclei into oocytes and zygotes in metaphase of the cell cycle but fail after transfer during interphase [6–8]. Nuclear transfer into embryonic blastomeres enucleated in interphase has also been attempted but has failed to demonstrate reprogramming activities [9, 10]. In particular, no development was observed after transfer of inner cell mass nuclei into two-cell-stage embryos enucleated in interphase [10].

Our results suggest that one key to successful reprogramming is the removal of the recipient cell genome at metaphase when the nuclear envelope is broken down and chromosomes are condensed (Figure 1A). This suggests that reprogramming might also be possible in cell types other than oocytes or zygotes, if and only if their genome is removed in mitosis. Because they are relatively large embryonic cells, we first considered whether the blastomeres in a two-cell mouse embryo harbored reprogramming activities.

To determine whether blastomeres contained reprogramming activities, we sought to stably but reversibly arrest them in mitosis for chromosome transfer studies. We isolated fertilized zygotes from superovulated mice, cultured them in vitro to the two-cell stage, and then observed the embryos for entry into the second mitosis. The two blastomeres usually entered mitosis between 48 and 54 hr after administration of the hormone trigger for ovulation. Shortly after mitotic entry, the embryos divided to the four-cell stage. To find the optimal conditions in which two-cell embryos could be arrested in mitosis, we cultured them in the presence of several nocodazole concentrations (see Table S1 available online). Mouse two-cell embryos required nocodazole concentrations similar to or slightly higher than those required by zygotes for mitotic arrest [1]. To determine whether cell-cycle arrest was compatible with embryo viability, we released embryos from the mitotic block and allowed them to develop in vitro to the blastocyst stage. We found that 32 of 35 embryos (91%) reached the blastocyst stage, indicating that mitotic arrest with nocodazole did not significantly compromise later development. This finding was consistent with previous studies, which also suggested that a transient arrest in mitosis by nocodazole is nontoxic to the embryo [11].

When two-cell embryos were treated with 0.1 μg/ml of nocodazole, we observed that they formed an irregular and unstable spindle that was presumably too disorganized to allow mitotic progression (Figure S1). Although the spindle was disorganized enough to cause mitotic arrest, it was still visible under Hoffman modulation contrast optics (Figure 1C). When these two-cell embryos were further treated with cytochalasin B to depolymerize the actin cytoskeleton, the spindle complex with attached chromosomes could still be identified and removed by micromanipulation (Figure 1D). In all cases, when the spindle was extracted from one of the two blastomeres, staining with the DNA dye Hoechst 33342 demonstrated that the chromosomes were also successfully removed. We initially removed the chromosomes from only one of the two blastomeres and left the other blastomere intact because this would allow a direct comparison of developmental potential between the transferred and the nontransferred blastomeres.

To optimize chromosome transfer into blastomeres and to determine whether they contained reprogramming activities, we arrested mouse ES cells in mitosis with nocodazole and injected their chromosomes into the enucleated blastomere (Figure 1E; Movie S1). To allow fate mapping of the blastomere

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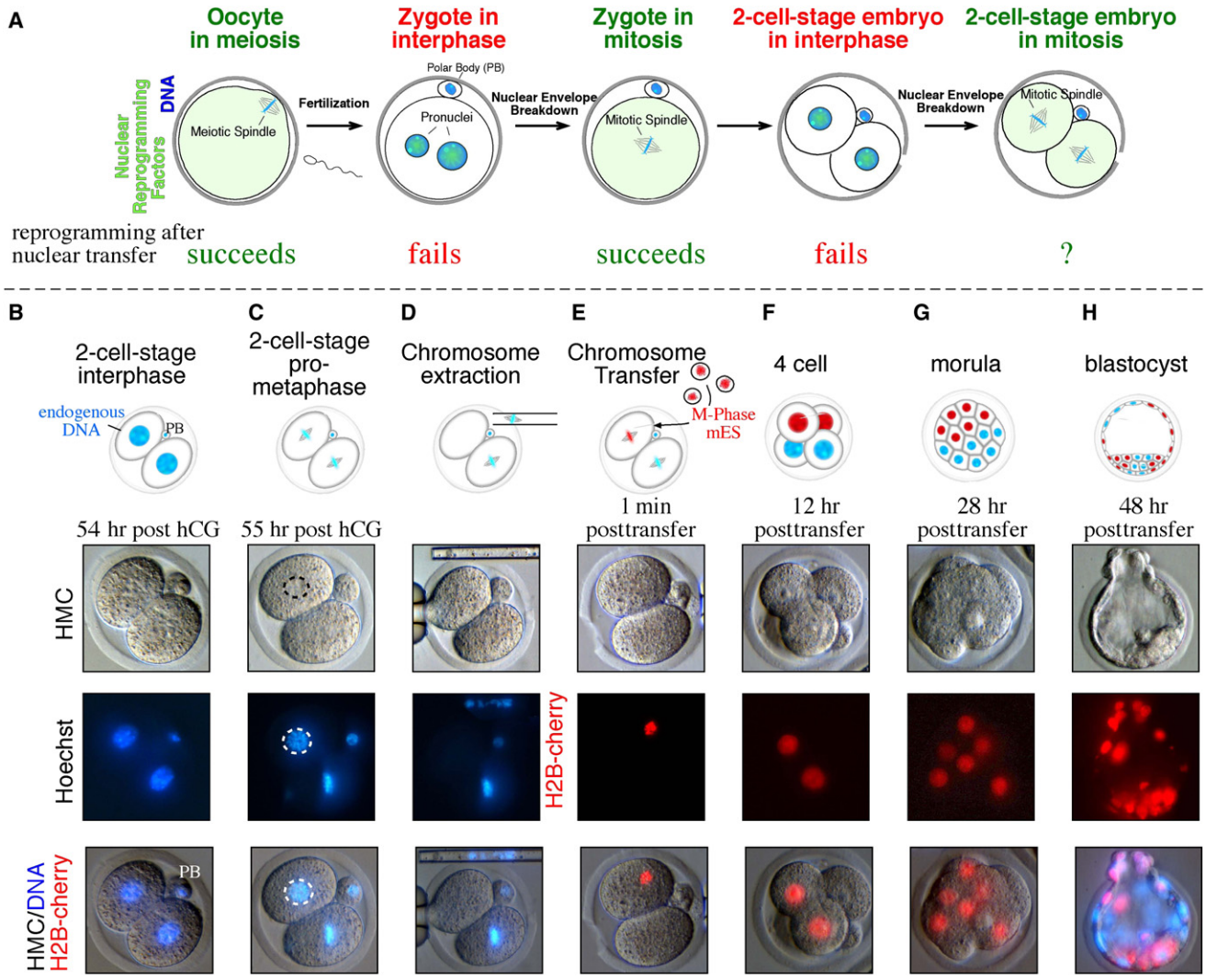

Figure 1. Chromosome Transfer into Mitotic Blastomeres

(A) Stages of development from the unfertilized oocytes to the two-cell-stage embryo. Oocytes in meiosis and zygotes in mitosis are suitable for nuclear transfer, but zygotes in interphase or even two-cell-stage embryos in interphase are not. Whether two-cell-stage embryos in mitosis can be used for transfer of a genome from a more differentiated cell is addressed here.

(B) Two-cell-stage embryo in interphase 54 hr post hCG (human chorionic gonadotropin, a hormone stimulating ovulation).

(C) Blastomeres in mitosis 55 hr post hCG.

(D) One blastomere had the genome removed in mitosis.

(E) One of the two blastomeres transferred with mouse embryonic stem (ES) cells expressing histone H2B-cherry.

(F) A four-cell-stage embryo 12 hr posttransfer.

(G) Morula at 28 hr posttransfer, composed of ten cells, six of which are derived from the transferred blastomere.

(H) Blastocyst at 48 hr posttransfer.

that had undergone nuclear transplant, we used donor cells that expressed a histone H2B-cherry fluorescent fusion protein. The descendants of the nuclear transfer blastomere all possessed red fluorescent chromosomes and nuclei, allowing their identification within the embryo (Figures 1E–1H). Upon release from mitotic arrest, both the transferred and the unmanipulated blastomeres completed mitosis, and the embryos cleaved to the four-cell stage. As expected, two cells within the embryo displayed H2B-cherry fluorescence, indicating that they carried the donor cell chromosomes (Figure 1F). We found that these four-cell embryos continued to develop efficiently in vitro to both the morula and blastocyst stages (Figures 1G and 1H; Table 1).

To exclude the possibility that this development depended on the presence of the nonmanipulated blastomere, we generated embryos that were derived entirely from the donor genome. To do so, we fused the two blastomeres at the two-cell stage, which resulted in a tetraploid two-cell-stage embryo (Figure 2A). At mitosis, the tetraploid genome was removed and replaced by a diploid genome of an ES cell. These manipulated embryos cleaved to the two-cell stage (27 of 50, or 54%) and efficiently proceeded in development to the blastocyst stage (21 of 27, or 78%). All nuclei of both trophectoderm and inner cell mass (ICM) expressed H2B-cherry, indicating that they were derived entirely from the injected donor chromosomes (Figure 2B). Cavitation of the blastocyst