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Hybrid Embryos Produced by Transferring Panda or Cat Somatic Nuclei Into Rabbit MII Oocytes Can Develop to Blastocyst In Vitro

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ABSTRACT The developmental potential of hybrid embryos produced by transferring panda or cat fibroblasts into nucleated rabbit oocytes was assessed. Both the panda–rabbit and the cat–rabbit hybrid embryos were able to form blastocysts in vitro. However, the rates of attaining the two-cell, four-cell, eight-cell, morula, or blastocyst stages for panda–rabbit hybrids were significantly greater than those of cat–rabbit hybrids ($P < 0.05$). Transferring the rabbit fibroblasts into nucleated rabbit oocytes, 31.0% of the blastocyst rate was obtained, which was significantly higher than that of both the panda–rabbit and the cat–rabbit hybrid embryos ($P < 0.05$). Whether or not the second polar body (PB_2) was extruded from the one-cell hybrid embryos (both panda–rabbit and cat–rabbit hybrids) significantly affected their developmental capacity. Embryos without an extruded PB_2 showed a higher capacity to develop into blastocysts (panda–rabbit: 19.2%; cat–rabbit: 4.3%), while embryos with extruded PB_2 could only develop to the morula stage. The hybrid embryos formed pronucleus-like structures (PN) in 2–4 hr after activation, and the number of PN in one-cell embryos varied from one to five. Tracking of the nucleus in the egg after fusion revealed that the somatic nucleus could approach and aggregate with the oocyte nucleus spontaneously. Chromosome analysis of the panda–rabbit blastocysts showed that the karyotype of the hybrid embryos ($2n = 86$) consisted of chromosomes from both the panda ($2n = 42$) and the rabbit ($2n = 44$). The results demonstrate that (1) it is possible to produce genetic hybrid embryos by interspecies nuclear transfer; (2) the developmental potential of the hybrid embryos is highly correlated to the donor nucleus species; and (3) the hybrid genome is able to support the complete preimplantation embryonic development of the hybrids. *J. Exp. Zool. 303A:689–697, 2005.*

INTRODUCTION

The production of hybrid embryos between species may be achieved by interspecies fertilization; however, the natural barrier of the zona pellucida preventing penetration by alien sperm has frustrated most attempts to do so. The techniques of micromanipulation, such as injection of a single spermatozoon into the perivitelline space, or transfer of a sperm head into the ooplasm (ICSI), could be used to overcome the natural barrier of interspecies fertilization. With these approaches, the interspecies fertilization rate remains, however, low and hybrid embryo developmental capacities are still very limited (Waksmundzka, '94; Kim et al., '99). Nuclear transfer (NT) technique developed in amphibians (Briggs and King, '52; McKinnell, '62) and mammals (McGrath and Solter, '83; Willadsen, '86) is an invaluable tool for studying fundamental questions about development and differentiation. This technique also permits the creation of genetic

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combinations that are otherwise impossible because of natural barriers to fertilization in mammals. Waksmundzka ('94) reported the first experiments of intergeneric NT in mammals, in which he transferred the rat spindle into nucleated MII mouse oocytes, but he found that the rat–mouse hybrid embryos could not develop beyond the eight-cell stage. Since the production of Dolly the sheep (Wilmut et al., '97), somatic NT has been applied to many other mammalian species (Cibelli et al., '98; Kato et al., '98; Wakayama et al., '98; Baguisi et al., '99; Polejaeva et al., 2000; Chesne et al., 2002; Shin et al., 2002). These experiments demonstrate that somatic cell nuclei from various differentiated states can be reprogrammed and are able to initiate another round of embryonic development after being introduced into enucleated recipient oocytes. On the other hand, researchers found that bovine and rabbit oocyte cytoplasm supports the development of embryos produced by the transfer of somatic nuclei from many different mammalian species (Chen et al., '99; Dominko et al., '99). This technique (i.e., interspecies NT) was recently proved practical in animal cloning (White et al., '99; Lanza et al., 2000; Loi et al., 2001). Chen et al. ('99, 2002) reported that embryos produced by transferring panda somatic cell nuclei into enucleated rabbit MII oocytes could develop to blastocyst in vitro and implant in vivo. The results suggest that factors exist in rabbit oocyte cytoplasm for nucleus reprogramming and dedifferentiation that are not species specific. The experiments described here were designed to answer the following questions: (1) Can rabbit oocytes and somatic cells from other species be used to produce genetic hybrid embryos by the technique of interspecies NT? (2) Can the rabbit genome and the genome from other species compatibly co-exist in these hybrid embryos?

MATERIALS AND METHODS

Animal care and preparation of rabbit oocytes

Animal care and handling were conducted in accordance with policies on the care and use of animals promulgated by the ethical committee of the State Key Laboratory of Reproductive Biology, Institute of Zoology, The Chinese Academy of Sciences.

Female Japan Big-Eared white rabbits were superovulated by administration of follicle-stimulating hormone (FSH) and human chorionic gonadotropin (hCG) (Institute of Zoology, Academia Sinica). Each rabbit was injected intramuscularly with 1 mg of FSH two times daily for 3 d and with 100 IU hCG (intravenous) 12 hr after the last FSH injection. Rabbits were killed 14 hr after hCG injection. Cumulus masses were collected by flushing both oviducts with M2 medium (Sigma Chemical Co., St. Louis, MO) and treated shortly with 300 IU/ml hyaluronidase (SigmaChemical Co.) in M2 medium to separate the oocytes. The cumulus cells were removed mechanically by gently pipetting with a fine glass pipette. After washing in M2 medium three times, the cumulus-free eggs were transferred to M2 medium containing 7.5 μg/ml cytochalasin B (CB) (Sigma Chemical Co.), and used for micromanipulation.

Preparation of donor cells

The collection and use of giant panda tissues were approved by the Office of Wildlife Protection, the National Forestry Bureau of China. Abdominal muscles were collected within 30 min after the death of a 3-d-old triplet female panda (*Ailuropoda melanoleuca*). Tissues were cut into pieces and digested with 0.25% trypsin (Gibco BRL, New York, NY) for 30 min at 37°C. The digested cells and tissues were cultured in DMEM/F12 (Gibco BRL) supplemented with 20% FBS (Gibco BRL) in a humidified 5% CO_2+95% air incubator at 37°C. Cells were passaged at 70–80% confluence. The primary spindle-shaped cells confirmed as fibroblasts by immunochemical staining of vimentin proteins were isolated for further culture. Cells at 3–8 generations were used as donors.

Panda fibroblasts at 2–3 generations were used for transfecting the gene of green fluorescent protein (GFP). The $P^{EGFP\text{-}C1}$ plasmid DNA (Clontech Co., Mountain View, CA; Cat. No. 6084-1) was transferred into the panda fibroblasts with the help of cationic lipofectamine (LTI Co., Garthersburg, MD; Cat. No. 18324-012). After 48 hr of transfection, the fibroblasts were screened in DMEM/F12 medium containing 400–800 μg/ml G418 (LTI Co., Cat. No. 26140087) for 3–4 d. Only those $G418^-$ selected fibroblasts were used for NT.

Tissues from the ear skin of a female domestic cat (*Felis catus*, age of 3 yr) were collected after anesthetizing by intramuscular injection of 1 ml ''846'', a mixture of anesthetics for sedation, analgesia, and relaxing muscles (Changcun Agricultural and Animal Husbandry University, China). Cell culture was conducted by the same method as described above.

Nuclear transfer and activation

The first polar body of rabbit oocyte was aspirated using a 20–25 μm glass pipette. A single cell from panda, cat, or rabbit was placed in the perivitelline space and was put in close contact with the plasma membrane of an oocyte. The couplets were transferred to a fusion chamber containing 100 μl of fusion medium [0.25 M sorbitol, 0.5 mM Mg $(CH_3COO)_2$, 0.1 mM Ca$(CH_3COO)_2$, 0.5 mM Hepes, and 100 mg/100 ml BSA in deionized water]. Fusion was induced by double DC pulses of 1.4 kV/cm for 80 μs with an ECM2001 Electrocell Manipulator (BTX Inc., San Diego, CA). Couplets were then washed three times in M199 medium (Gibco BRL) supplemented with 10% FBS (Gibco BRL), and incubated in the same medium for 30 min at 38°C in humidified air containing 5% CO_2. Couplets were checked for fusion under an inverted microscope, and the fused couplets were activated by double DC pulses of 1.4 kV/cm for 40 μs in the fusion medium. The activated embryos were washed in M199+10% FBS at least three times before being transferred to the incubator for culturing.

Embryo culture and assessment of development

All the cat–rabbit and panda–rabbit hybrid embryos were cultured in M199+10% FBS at 38°C in humidified air containing 5% CO_2. Extrusion of the second polar body was checked 5–7 hr after activation. Embryos with extruding polar bodies were picked out and cultured in another drop of culture medium. The developmental stages of the embryos were observed twice a day at 12-hr intervals.

Estimation of embryo karyotype

The panda–rabbit hybrid embryos were assessed for karyotype at the blastocyst stage using an air-drying technique (Tarkowski, '66), with minor modifications. Briefly, embryos at the blastocyst stage were pre-incubated in 5 μg/ml colchicine (Sigma Chemical Co.) for 5–6 hr to maximize the number of cells in metaphase. Embryos were then exposed to a hypotonic solution consisting of 1% sodium citrate in deionized water for 30–50 min. Embryos were placed onto the pre-cooled glass slide and fixed with a mixture of glacial acetic acid and methanol (1:3). Slides were stained with Giemsa after being dried in air overnight. Chromosomes were counted under a light microscope.

Statistical analysis

Percentages of embryos at different stages were compared between groups using chi-square analysis. Differences were considered significant at $P < 0.05$.

RESULTS

Developmental capacity of cat–rabbit and panda–rabbit hybrid embryos in vitro

There was no significant difference in fusion rate after transferring panda, cat, or rabbit fibroblasts into rabbit oocytes ($P > 0.05$). The blastocyst rate of the panda–rabbit hybrid embryos was 8.4%, significantly greater than that of the cat–rabbit hybrid embryos (3.7%) ($P < 0.05$); however, blastocyst rates for both the panda–rabbit and the cat–rabbit hybrid embryos were significantly lower than that of the rabbit–rabbit embryos (31.0%, presumably tetraploid embryos) ($P < 0.05$). Most of the cat–rabbit hybrid embryos completed the first two cleavages normally, and these embryos began to compact at the 8- to 16-cell stage. Of the fused cat–rabbit embryos, 16.4% developed to the morula stage, at which point development was arrested, although a few (3.7%) reached the blastocyst stage. Blastocysts were formed about 7 d after activation. The panda–rabbit hybrid embryos possessed higher developmental potential than cat–rabbit hybrid embryos. About half of the fused embryos could develop to the morula stage, and 8.4% reached the blastocyst stage. The time of reaching the blastocyst stage for panda–rabbit hybrids was 5–6 d after activation (Fig. 1) (Table 1).

When GFP-panda fibroblasts were used to construct the hybrid embryos, the GFPs could immediately be tracked in the rabbit oocyte cytoplasm after fusion under blue laser light. This protein could also be detected in the blastomeres of hybrid embryos before the 8-cell stage, whereas it was not detected in the cells of morulae and blastocysts (Fig. 1). After fusion, most of the embryos could form pronucleus-like structures (PN) within 2–4 hr after activation. The number of the PN varied among these reconstructed embryos, 6.6% (11/167) of the one-cell embryos being found with 1PN, 33.5% (56/167) with 2PN, 43.1% (72/167) with 3PN, 12.0% (20/167) with 4PN, and 4.6% (8/167) with 5PN. Extrusion of the donor nucleus was not found before the first cleavage by GFP tracking. Observations with Hoechst staining of the nuclei showed that the

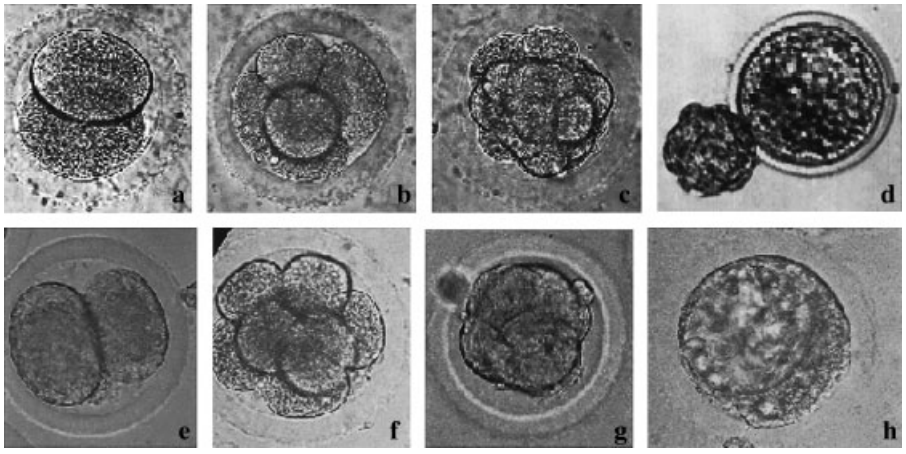

Fig. 1. Pre-implantation embryos of panda–rabbit and cat–rabbit hybrid embryos. (**a**–**d**) Two-cell, eight-cell, morula, and blastocyst of panda–rabbit hybrids. (**e**–**h**) Two-cell, eight-cell, morula, and blastocyst of cat–rabbit hybrids.

TABLE 1. Developmental capacity of cat--rabbit and panda--rabbit hybrid embryos in vitro

Cell type	No. of NT	Fused (%)	Two-cell (%)	Four-cell (%)	Eight-cell (%)	Morula (%)	Blastocyst (%)
Panda fibroblasts	612	347 (56.7)[a]	265 (76.4)[a]	223 (64.3)[a]	208 (59.9)[a]	171 (49.3)[a]	29 (8.4)[a]
Cat fibroblasts	342	189 (55.3)[a]	120 (63.5)[b]	104 (55.0)[b]	84 (44.4)[b]	31 (16.4)[b]	7 (3.7)[b]
Rabbit fibroblasts	111	71 (64.0)[a]	55 (77.5)[a]	40 (56.3)[a,b]	38 (53.5)[a,b]	31 (43.7)[a]	22 (31.0)[c]

Values with different superscripts within each column are significantly different (at least $P<0.05$). Fused (%): fused oocytes/nuclear transfer (NT) units; development rate of embryos at different stages: no. of embryos /no. of fused oocytes.

donor nucleus approached the oocyte nucleus spontaneously; most of the donor nuclei could get close to the oocyte nucleus within 4–5 hr, and aggregate into a new intermixing nucleus 7–8 hr after activation.

Effects of PB_2 extrusion on developmental potential of panda–rabbit hybrid embryos

About half of the embryos could extrude the PB_2, while the rest did not any show polar body in the perivitelline space. Occasionally, two polar bodies were observed in one embryo. The rate of development of two-cell, four-cell, eight-cell, morula, or blastocyst stages for embryos with extruded PB_2 was significantly lower than that of the embryos without extruded PB_2 ($P<0.05$). Embryos without extruded PB showed a higher capacity to develop to the blastocyst stage (19.2%). Although embryos with an extruded PB_2 were able to develop to the morula stage, no blastocysts were observed among the 81 hybrid embryos in this experiment (Table 2). Chromosome analysis showed that the number of chromosomes in these hybrid blastocysts was 86 (Fig. 2), which was different from the chromosome number of both the panda ($2n=42$) and the rabbit ($2n=44$).

Effects of PB_2 extrusion on developmental potential of cat–rabbit hybrid embryos

The rate of embryo development into two-cell, four-cell, or eight-cell stage for cat–rabbit hybrid embryos with extruded PB_2 was significantly lower than that of the embryos without extruded PB_2 ($P<0.05$); however, the rate of morula or blastocyst development was not significantly different in hybrid embryos with or without PB extrusion ($P>0.05$). Cat–rabbit hybrid embryos with extruded PB_2 could develop to the four- to six-cell stage with normal appearance, but most of the embryos remained at the eight-cell stage for 1–2 d with abnormal appearance and subsequently

TABLE 2. Effects of the second polar body (PB_2) extrusion on developmental potential of panda--rabbit hybrid embryos

Extrusion of PB2	No. of fused (%)	Two-cell (%)	Four-cell (%)	Eight-cell (%)	Morula (%)	Blastocyst (%)
Extruded	81 (50.9)[a]	53 (65.4)[a]	47 (58.0)[a]	37 (45.7)[a]	29 (35.8)[a]	0 (0)[a]
Not extruded	78 (49.1)[a]	75 (96.2)[b]	60 (76.9)[b]	48 (61.5)[b]	43 (53.8)[b]	15 (19.2)[b]

Values with different superscripts within each column are significantly different (at least $P < 0.05$). Development rate of embryos at different stages: no. of embryos /no. of fused oocytes.

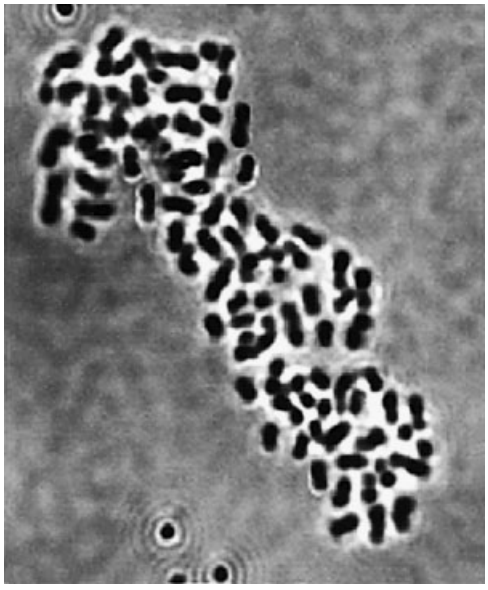

Fig. 2. Karyotype of panda–rabbit hybrid blastocyst ($2n = 86$).

degenerated. None of the 60 embryos reached the blastocyst stage. Embryos without extruded PB_2 could develop to the eight-cell stage normally. A small proportion of these embryos (20.3%) could develop into morulae, and 4.3% of these embryos developed into blastocysts (Table 3) (Figs. 3–5).

DISCUSSION

Previous studies in our laboratory have shown that panda somatic cell nuclei can undergo dedifferentiation and be reprogrammed after being introduced into enucleated rabbit MII oocytes; these somatic nuclei are able to support the development from reconstructed embryos into blastocysts (Chen et al., '99; 2002). In this experiment, we transferred rabbit fibroblasts into nucleated rabbit oocytes, which resulted in a 31.0% blastocyst rate after culturing in vitro. When transferring panda somatic cell nuclei into nucleated rabbit oocytes, 8.4% of these interspecies hybrid embryos developed to the blastocyst stage. Karyotype analysis confirmed that the panda–rabbit hybrid blastocysts ($2n = 86$) contained a total of 86 chromosomes, presumably resulting from the panda ($2n = 42$) and the rabbit ($2n = 44$). When cat fibroblasts were transferred into nucleated rabbit MII oocytes, most of these hybrid embryos could complete the first two cleavages normally, and 3.7% of the embryos developed to the blastocyst stage. The blastocyst rates of both the panda–rabbit and the cat–rabbit hybrid embryos were significantly lower than that of the rabbit–rabbit hybrid embryos, indicating that the developmental potential of the genetic hybrid embryos produced by somatic NT is affected by the donor nucleus species.

The developmental potential of the cat–rabbit hybrid embryos was significantly lower than that of panda–rabbit hybrids. For the cat–rabbit hybrid embryos, 44.4% of the fused embryos could complete the first three cleavages, but only 16.4% of these embryos developed to the morula stage. These findings suggest that there might be an eight-cell-to-morula block; this block may also be the cause of the low blastocyst rate of the cat–rabbit hybrid embryos. About half of the fused

TABLE 3. Effects of the second polar body (PB_2) extrusion on developmental potential of cat--rabbit hybrid embryos

Extrusion of PB_2	No. of fused (%)	Two-cell (%)	Four-cell (%)	Eight-cell (%)	Morula (%)	Blastocyst (%)
Extruded	60 (46.5)[a]	35 (58.3)[a]	31 (51.7)[a]	29 (48.3)[a]	7 (11.7)[a]	0 (0)[a]
Not extruded	69 (53.5)[a]	57 (82.6)[b]	57 (82.6)[b]	48 (69.6)[b]	14 (20.3)[a]	3 (4.3)[a]

Values with different superscripts within each column are significantly different (at least $P < 0.05$). Development rate of embryos at different stages: no. of embryos /no. of fused oocytes.

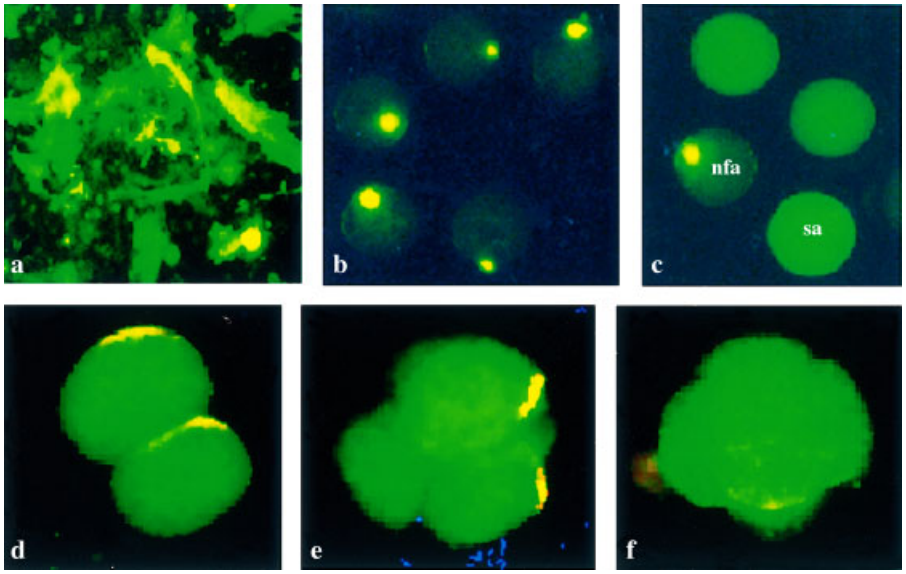

Fig. 3. Transferring green fluorescent protein (GFP)-panda fibroblasts into rabbit ooytes. (**a**) GFP infected panda fibroblast cells; (**b**) couplets of the GFP fibroblast and the rabbit oocyte; (**c**) fused couplets (fo: GFP-fibroblast fused into the oocyte, nfo: not fused oocyte); (**d–f**) two-cell, four-cell, and eight-cell embryos derived from transferring GFP-panda fibroblasts into rabbit MII oocytes, GFP could be detected in the blastomeres of these embryos.

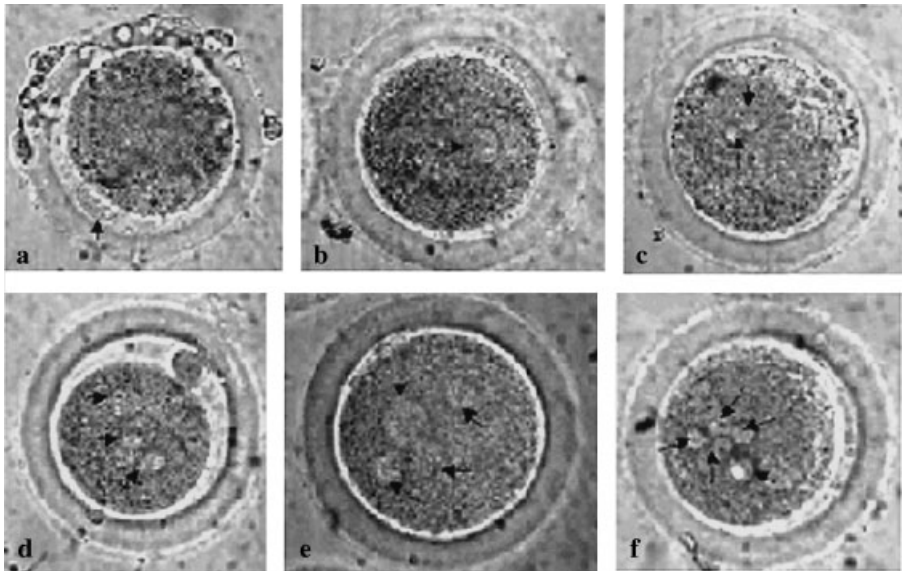

Fig. 4. Second polar body (PB_2) extrusion and pronucleus-like structure (PN) formation in the panda–rabbit hybrid embryos. (**a**) PB_2 extrusion after activation; (**b–f**) embryos with 1PN, 2PN, 3PN, 4PN, and 5PN (arrows) 4–5 hr after activation.

panda–rabbit hybrid embryos (49.3%) reached the morula stage. However, only 8.4% reached the blastocyst stage. These results show that there might be a morula-to-blastocyst block for panda–rabbit hybrid embryos. In mammalian species, the maternal–zygotic transition (MZT) in genomic control usually occurs at earlier embryo stages and is often associated with developmental blocks (Frei et al., '89; Telford et al., '90). This MZT, which is species dependent and occurs at a particular stage of development, is characterized by a large increase in detectable transcription (Telford et al., '90; Campbell, '99). The completion of MZT leads the nucleus to take full control of embryo development. For somatic NT embryos, there is a similar transition associated with

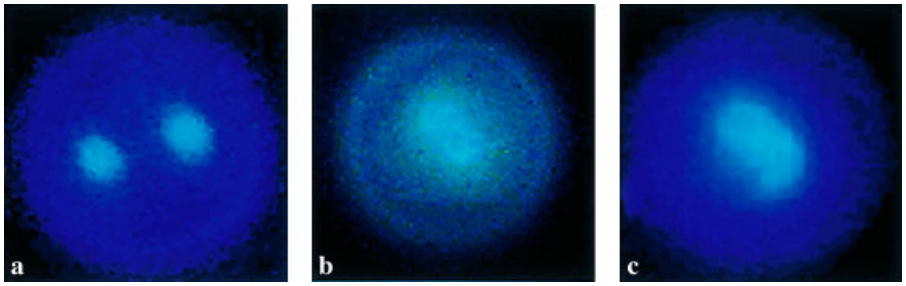

Fig. 5. The panda somatic nucleus in rabbit oocyte as revealed by Hoechst staining. (**a**) 30 min after fusing the panda fibroblast with rabbit oocyte; (**b**) two nuclei apposed 4 hr after fusion; (**c**) swelling and aggregation of the nuclei 7 hr after fusion.

nucleus reprogramming (Campbell, '99). Thus, the blocks in vitro suggest that the MZT for cat–rabbit hybrid embryos occurs before the eight-cell stage and in the panda–rabbit hybrid embryos it occurs before the morula stage. In another experiment, we transferred both panda and cat somatic nuclei into enucleated rabbit oocytes, and the time for development to blastocyst stage for panda–rabbit cloned embryos was 5 d (Chen et al., 2002), that for cat–rabbit cloned embryos was 6–7 d, and that for rabbit–rabbit cloned embryos was 4–5 d after activation (our unpublished data). The timing differences in embryogenesis among species may indicate the temporal and spatial differences in gene expression of the nuclei from different species. The lower developmental potential for cat–rabbit hybrid embryos may partly be attributed to the temporal and spatial differences in the gene expression of cat and rabbit nuclei, whereas the relatively higher developmental potentials for panda–rabbit hybrid embryos may be partly explained by their similar timing of embryogenesis.

In these interspecies genetic hybrid embryos, the donor nucleus was not extruded from the rabbit oocyte after fusion; on the contrary, it aggregated with the oocyte nucleus into a new intermixing nucleus and developed into a blastocyst. The results demonstrate that rabbit MII oocytes could not only dedifferentiate the somatic cell nuclei from other species but could also accept the exotic genomes to form an intermixing karyotype (genetic hybrid), and the hybrid genome was able to support the development from reconstructed hybrid embryos to blastocysts.

In intraspecies NT, the events that occur in the donor nucleus after transferring into an enucleated MII oocyte have been studied in a number of species (Czolowska and Modlinski, '84; Prather et al., '89; Collas and Robl, '91; Kanda et al., '91; Lai et al., 2001). The donor nucleus undergoes a series of remodeling, such as donor nuclear envelope breakdown, premature chromosome condensation, nuclear swelling, and formation of PN after activation (Campbell et al., '96; Lai et al., 2001). The number of PN in one-cell NT embryos varied from one to three and was correlated with the donor cells' stages (Lai et al., 2001). In our experiment, panda fibroblasts were injected into nucleated rabbit MII oocytes, and formation of PN was observed 2–4 hr after activation. The number of PNs in one-cell hybrid embryos varied from one to five, a more complex finding than what was seen in intraspecies cloned embryos derived from enucleated oocytes. The fibroblasts that we used in this experiment were not made quiescent by serum starvation; thus, the donor cells may have been in different stages of the cell cycle. However, the panda fibroblast cells were passaged at 70–80% confluence, and about 80% of the cells were at the G0/G1 stage, whereas the rest were at G2/M and S stage (our unpublished results). When transferring a G0/G1 stage cell nucleus into an MII rabbit oocyte, the somatic nucleus may decondense normally to form a PN in the cytoplasm after activation; at the same time, there are two possibilities regarding the fate of oocyte genetic material: extrusion of PB_2 and formation of one PN, or formation of two PNs without extrusion of PB_2. Regardless of the extrusion of PB_2, transferring a G0/G1 cell nucleus into a nucleated MII oocyte will form two or three PNs. In our experiment, about 75% of the reconstructed embryos were found with two or three PNs, which was consistent with the percentage of G0/G1 stage in donor cells. Embryos with four PNs might be the result of the G2/M-stage donor cells that were randomly selected in NT: two PNs might be derived from the two sets of chromosomes in the G2/M-stage cells, whereas the other two PNs might be derived from the MII oocyte nucleus. The reasons for the formation of one PN and five

PNs were not clear; however, the latter might be the result of abnormal chromosome separations in oocytes or in donor cells.

Normally, an MII oocyte will extrude the second polar body after activation. In this experiment, without post-activation treatment for suppression of polar body extrusion, only about half of the reconstructed oocytes extruded the PB_2 (panda–rabbit: 50.9%; cat–rabbit: 46.5%) after activation. The first polar body in the perivitelline space may affect the evaluation of the cell fusion and PB_2 extrusion for the NT couplets. In order to remove the first polar bodies from the oocytes, we preincubated the rabbit ooyctes in manipulation medium (M2 medium containing 7.5 μg/ml CB) for at least 15 min, and left these oocytes in the manipulation medium for about 1 hr until all the oocytes were manipulated. The low rate of PB_2 extrusion in the reconstructed oocytes may be partly due to this pre-activation treatment in CB.

Parthenogenetic development of MII oocytes may proceed in either of the following ways: extrusion of PB_2 and development of eggs with a single haploid pronucleus, or failure in PB_2 emission and the formation of two haploid pronuclei (Rougier and Werb, 2001). Transferring a diploid somatic nucleus into an MII oocyte without extruded PB_2 would result in a heterogenetic tetraploid karyotype ($2n+2n$), in which the two suits of chromosomes are from different species. In mammalian species, homogenetic tetraploid (in which the two suits of chromosomes are from the same species) embryos could develop to the blastocyst stage with correct segregation of the chromosomes during embryogenesis (Ozil and Modlinski, '86; Kaufman and Webb, '90; Curnow et al., 2000). In our experiment, the tetraploid hybrid embryos were able to develop into blastocysts, suggesting that the heterogenetic tetraploid embryos could also complete pre-implantation development with the correct segregation of chromosomes. However, transferring a diploid somatic nucleus into an MII oocyte with extruded PB_2 would form a heterogenetic triploid karyotype ($2n+n$). Our results show that embryos with extruded PB_2 possess lower developmental potential than those without extruded PB_2. During pre-implantation embryogenesis, the heterogenetic triploid karyotype might cause a disordered segregation of the chromosomes as several embryo cleavages. The loss of some chromosomes in the embryo cells, especially the rabbit chromosomes (of which only one set existed in the embryos), would probably be the cause of the low developmental potential of the hybrid embryos with extruded PB_2. However, a practicable experiment should be designed to verify this speculation.

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