CLONING AND STEM CELLS
Volume 10, Number 3, 2008

DOI: 10.1089/clo.2007.0084

Activation of Human Embryonic Gene Expression in Cytoplasmic Hybrid Embryos Constructed between Bovine Oocytes and Human Fibroblasts

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Abstract

Cross-species somatic all number transfer (SCNT) provides a potential solution to overcome the problem of oocyte shortage for therapeutic cloning. To further characterize the system, we constructed cytoplasm hybrid embryos between bovine oocytes and human fibroblasts and examined dynamics of human gene activation during preimplantation stages. Data from this study showed that human embryonic genes, *OCT4*, *SOX2*, *NANOG*, *E-CADHERIN*, as well as *β-ACTIN*, were activated by enucleated bovine oocytes. Activation of human genes was correlated with developmental potential of the embryos. The extent of human gene activation varied drastically and was incomplete in a large proportion of the embryos. Activation of human genes in the human–bovine cytoplasm hybrid embryos occurs in a temporal pattern resembling that of the bovine species. Results from this study suggest that human gene products are required for hybrid embryos to develop to later preimplantation stages. Facilitating human genome activation may improve successful rates in cross-species SCNT.

Introduction

Conventional somatic cell nuclear transfer (SCNT) transplants somatic nuclei into oocytes of the same species (Gurdon, 1962; Wilmut et al., 1997). In cross-species SCNT, somatic nuclei of one species are transplanted into enucleated oocytes of another species. This approach can also result in nuclear reprogramming and embryonic development (Chen et al., 2002; Dominko et al., 1999; Gomez et al., 2003; Lanza et al., 2000; Loi et al., 2001; Mastromonaco et al., 2007; White et al., 1999; Yin et al., 2006; see Tecirloglu et al., 2006, for more information). The resulting embryos are referred to as cytoplasmic hybrid embryos (Interspecies embryos, A report by the Academy of Medical Sciences, 2007; http://www.acmedsci.ac.uk). Nuclear transfer of human or mouse somatic cells into enucleated *Xenopus* oocytes respectively activated expression of human and mouse *Oct4* genes (Byrne et al., 2003). Cytoplasmic hybrid embryos were also constructed between bovine oocytes and somatic cells of the mouse, dog, sheep, goat, pig, monkey, yak, takin, horse, or humans (Chang et al., 2003; Chen et al., 2003; Dominko et al., 1999; Illmensee et al., 2006; Li et al., 2006; Mastromonaco et al., 2007; Tecirloglu et al. 2006) between cat oocytes and giant panda somatic cells (Chen et al., 1999), and between rabbit oocytes and somatic cells of the monkey or humans (Chen et al., 2002, 2003; Yang et al., 2003). Most of these cytoplasmic hybrid embryos developed to the blastocyst stage, and in a few cases, produced offsprings (Lanza et al., 2000; Loi et al., 2001).

Therapeutic cloning aims to derive patient-specific embryonic stem (ES) cells. Since human oocytes are precious and short in supply, animal oocytes have been used to reprogram human cells in nonclinic orientated studies. NtES cells were derived from cytoplasmic hybrid embryos constructed between rabbit oocytes and human fibroblasts. Isogeneity, karyotype, and DNA analyses showed that ntES cells contained human nuclei. Cell biology studies showed that in all aspects examined, ntES cells displayed characteristics similar to human ES cells (Chen et al., 2003). In a proof-in-principle experiment, mouse ntES cells were derived from blastocysts through fusion of mouse fibroblasts with enucleated bovine oocytes. Data from molecular, genetic, and

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embryonic analyses indicated that those ntES cells possess properties of mouse ES cells (Vogel, 2006).

A number of transcription factors, including OCT4, SOX2, and NANOG, play essential roles in early embryogenesis (Avilion, et al., 2003; Chambers et al., 2003; Hart et al. 2004; Mitsui et al., 2003; Nichols et al., 1998; Pesce and Schöler, 2001). They regulate downstream transcription factors, including homeodomain proteins (Boyer et al., 2005). OCT4, SOX2, and NANOG co-occupy a substantial portion of their target genes and collaborate to form regulatory circuitry (Boyer et al., 2005). E-CADHERIN mediates cell-to–cell adhesion, which is critical for embryonic compaction. Its transcripts and protein are found in the inner cell mass and trophoectoderm of blastocysts (Barcroft et al., 1998; Vestweber et al., 1987). In the absence of E-CADHERIN, development to the blastocyst stage is blocked *in vivo* (Larue et al., 1994; Riethmacher et al., 1995) and *in vitro* (Wianny and Zernicka-Goetz, 2000).

Successful nuclear reprogramming must enable transformation of gene expression patterns from those of the donor nuclei to that of an embryo (Winger et al., 2000). In cross-species SCNT between mammalian species, although experimental data implied that donor nuclei were supporting development of cytoplasmic hybrid embryos in later preimplantation stages after degradation of maternal factors, gene activation of transplanted nuclei has not been experimentally demonstrated. In addition, we remain ignorant in the dynamics of gene activation in cytoplasmic hybrid embryos. It is not known whether in cross-species SCNT, embryonic genes are activated in dynamics similar to that in conventional SCNT, when the oocyte and nuclear donor are from different species and those species have different temporal patterns in genome activation, whether embryonic gene expression is activated according to the pattern of the oocyte species or the nuclear donor species. There is a need to determine if gene activation in cross-species SCNT is uniform and extensive, and how those parameters may influence developmental potential of the embryos. We addressed these questions in cytoplasmic hybrid embryos constructed between human fibroblasts and enucleated bovine oocytes.

Materials and Methods

Regulations

The study was performed according to guidelines issued by Chinese Ministry of Science and Technology and Ministry of Health. Cytoplasmic hybrid embryos between human somatic cells and animal oocytes were created for the purpose of derivation of human pluripotential stem cells. Human somatic cells were obtained from discarded skin tissue with informed consent from the patients. It is strictly prohibited to implant cytoplasmic hybrid embryos into the womb of any species or culture them for more than 14 days. Materials derived from cytoplasmic hybrid embryos are prohibited for clinic applications.

Human fibroblasts and bovine oocytes

Bovine ovaries were obtained from a local slaughterhouse and transported at 37°C to the laboratory. Those with antral follicles of 2–8 mm diameter were selected. Immature oocytes were aspirated using an 18-gauge needle attached to a 10-mL syringe. Only oocytes containing homogenous cytoplasm and with at least three layers of compact cumulus cells were selected for *in vitro* maturation. Cumulus–oocyte complexes (COC) were washed in HEPES-buffered Tissue Culture Medium 199 (TCM199; GIBCO/BRL, Grand Island, NY) containing 0.1% polyvinylalcohol, and matured in TCM199 supplemented with 10% fetal bovine serum (FBS; GIBCO/BRL), 10 μg/mL FSH, 1 μg/mL LH, and 1 mg/mL ρ-estradiol-17β. COCs (10–15) were incubated in 100 mL maturation medium under mineral oil at 38.5°C and 5% CO_2 in a humidified atmosphere for 19 h. Oocytes were washed in TCM-HEPES, and those at the metaphase II stage were selected for further experiments. It was found in previous studies that a dark ooplasm of the oocyte often suggests an accumulation of lipids and good developmental potential, a light-color ooplasm suggests a low density of organelles and poor developmental potential, while a black ooplasm indicates aging of the oocyte (Nagono et al., 2006; Wang and Sun, 2007). Human fibroblasts were cultured in 5% CO_2/95% air (37°C) in DMEM/F-12 (GIBCO/BRL), supplemented with 15% fetal bovine serum (FBS, Hyclone, Logan, UT), and 50 units/mL penicillin–streptomycin (GIBCO/BRL).

Micromanipulation

Oocytes were incubated in TCM199 supplemented with 10% FBS and 7.5 mL/mL cytochalasin B (CCB; Sigma, St. Louis, MO). Enucleation was performed by aspirating the first polar body and M-phase chromosomes with a 20-μm internal diameter pipette. The successful enucleation was confirmed by visualizing the karyoplast under the UV light. A single donor cell was inserted into the perivitelline space of each enucleated oocyte using the same pipette. After injection, reconstructed embryos remained in medium until after fusion.

Electrofusion and culture of cytoplasmic hybrid embryos

The fibroblast/oocyte complex was equilibrated at room temperature in a fusion solution (0.25 M D-Sorbitol, 0.1 mM calcium acetate, 0.5 mM magnesium acetate, 0.5 mM HEPES, and 100 mg/mL of BSA; Sigma) for 3 to 5 min and placed between two electrodes (1.0 mm apart) in a cell fusion chamber of an electrofusion apparatus (Model ECM 830; BTX, San Diego, CA), overlaid with the fusion solution. Electrical pulses (1.6 kv/cm, 10 sec, two direct current pulses with a 1.0-sec interval) were applied to fuse the fibroblast and oocyte membranes. Reconstructed embryos were washed in CR1aa medium (114.7 mM NaCl, 3.1 M KCl, 26.2 M $NaHCO_3$, 0.4 M sodium pyruvate, 5 M L(+)-lactic acid hemicalcium salt, 1 M L-glutamine, 3 mg/mL BSA, 2% essential amino acid, and 1% nonessential amino acid) and examined for fusion under a microscope 30 min later. Fused embryos were cultured for 3 h in CR1aa medium before chemical activation. Activation was induced by incubation in CR1aa with 5 mM ionomycin (Sigma) for 5 min at 38.5°C. Embryos were extensively washed in CR1aa for five times and cultured for 4 h in 2.0 mM 6-dimethyl-aminopurine (6-DMAP, Sigma).

In vitro *culture of embryos*

Reconstructed embryos (5–10 per droplet) were placed into coculture with mouse fetal fibroblast cells (day 14) monolayers in a 100-mL droplet of CR1aa overlaid with mineral oil in a humidified atmosphere of 5% CO_2 in air at 38.5°C. The mouse fetal fibroblast cell monolayers were established at 48–72 h before introduction of embryos. After introduction of embryos, half of the medium was replaced with fresh medium every 48 h.

PCR

Embryos at the 16-cell stage were individually washed in DPBS for three times and moved to 10 μL cell lysis buffer II (cells to cDNA II kit, Ambion, Huntingdon, UK), heated 15 min at 75°C. Two microlitters of cell lysate were used as a template and amplified with gene-specific primers using a HotStarTaq DNA Ploymerase kit (Qiagen, Valencia, VA). Primer sets used were as follows: HUMSATA7A (328 bp, Gene bank accession number: M16037), 5′-TTCATTGGAATCGCGAATAC-3′ and 5′-CAAGAAGGCTTC AAA GCA CC-3′. Bovine Alu-like (385 bp, X05090), 5′-AGTCCAGTGGCTCAGTTGTG-3′ and 5′-GAAAGGTGACCGCTGAAGAA-3′. PCR reactions were carried out using following parameters: 94°C for 15 min, 40 cycles at 94°C for 40 sec, 57°C for 30 sec, and 72°C for 30 sec; final extension was at 72°C for 10 min.

RT-PCR

Reverse transcription was carried out using a cDNA II kit (Ambion) according to manufacturer's instructions. In brief, a single embryo was washed in DPBS for at least three times, submerged in 10 μL of ice-cold cell lysis buffer, and incubated at 75°C for 10 min. Genomic DNA was degraded by incubation with DNAse I for 15 min at 37°C. RNA was reverse transcribed using Moloney murine leukemia virus (M-MLV) reverse transcriptase and random hexamers following the manufacturer's instruction. One or 2 μL of cDNA template were amplified with gene-specific primers using a HotStarTaq DNA Ploymerase kit (Qiagen). PCR products were run on 2% agarose gels and stained with ethidium bromide. A negative control with no reverse transcriptase at the RT step was included in each experiment to monitor genomic contamination. The primer sets used to amplify cDNAs were listed in Table 1. RT-PCR products were cloned into a pMD18-T vector using a TA cloning kit (Takara, Dalian, China) and the sequence confirmed.

Results

We constructed cytoplasmic hybrid embryos between enucleated bovine oocytes and human fibroblasts isolated from skin tissues. A total of 28 nuclear transfer (nt) experiments were conducted. Morphology of cytoplasmic hybrid embryos at various preimplantation stages was shown in Figure 1, and the number and rate of cytoplasmic hybrid embryos developed to each preimplantation stage were presented in Table 2. Developmental rates varied drastically from experiment to experiment; 21.4% and 9% of total fused constructs (3315) developed to the 8- and 16-cell stages, respectively, and 0.87% of them developed to the blastocyst stage. Only 11 of the 28 experiments yielded blastocysts. The most successful experiment yielded three blastocysts from 186 fused constructs (1.61%). Parthenogenotes were used as controls routinely. Most of them developed to the blastocyst stage in 7 to 8 days after activation while nt-blastocysts were often formed in 8 to 9 days. As shown in Figure 1, two of the three nt-blastocysts appeared to resemble the morphology of pathenogenotes, while one of them (Fig. 1I) was obviously abnormal. Among many factors that could influence development of the embryos, quality of oocytes is of critical importance.

To examine the efficiency in embryo reconstruction and early development, we collected embryos at the 16-cell stage and performed PCR analyses using primers against either human-specific alphoid sequence or bovine-specific Alu-like sequence. Both primer pairs amplified a series of fragments, with major bands that migrated at approximately 328 and 385 bp for human and bovine, respectively. A no-template control and a species-specific negative control were included for each PCR experiment. As shown in Figure 2, the majority of the 16-cell stage embryos contained human alphoid sequences. These results indicated that enucleation and construction of the hybrid embryos in those experiments were highly efficient, which resulted in early development in most embryos. A small proportion of the embryos developed to

Table 1. Primers Used for RT-PCR

Gene	*Primer sequences*	*Location*	*Fragment size (bp)*	*GeneBank accession no.*
H-Oct-4	Forward 5′-GAGAGGCAACCTGGAGAATT-3′	722–742	174	DQ85156
	reverse 5′-TCAAAATCCTCTCGTTATGCA-3′	874–895		
H-Sox-2	Forward 5′-CCCCCGGCGGCAATAGCA-3′	825–842	468	NM003106
	reverse 5′-TCGGCGCCGGGGAGATACAT-3′	1253–1272		
H-Nanog	Forward 5′-CAAATGTCTTCTGCTGAGATGCC-3′	331–353	358	NM024865
	reverse 5′-CAGTTGTTTTTCTGCCACCTCTT-3′	667–689		
H-Ecadherin	Forward 5′-CACATTTGCCCAATTCCAG-3′	4351–4369	260	NM004360
	reverse 5′-CCAAAAAGTGATTGCAGGGT-3′	4591–4610		
H-β-actin	Forward 5′-TGCTTCTAGGCGGACTATGAC-3′	1172–1193	387	NM001101
	reverse 5′-CACGAAAGCAATGCTATCACC-3′	1558–1579		
H-B-β-actin	Forward 5′-CTGGAACGGTGAAGGTGACA-3′	1348–1367	140	NM001101
	reverse 5′-AAGGGACTTCCTGTAACAATGCA-3′	1465–1487		

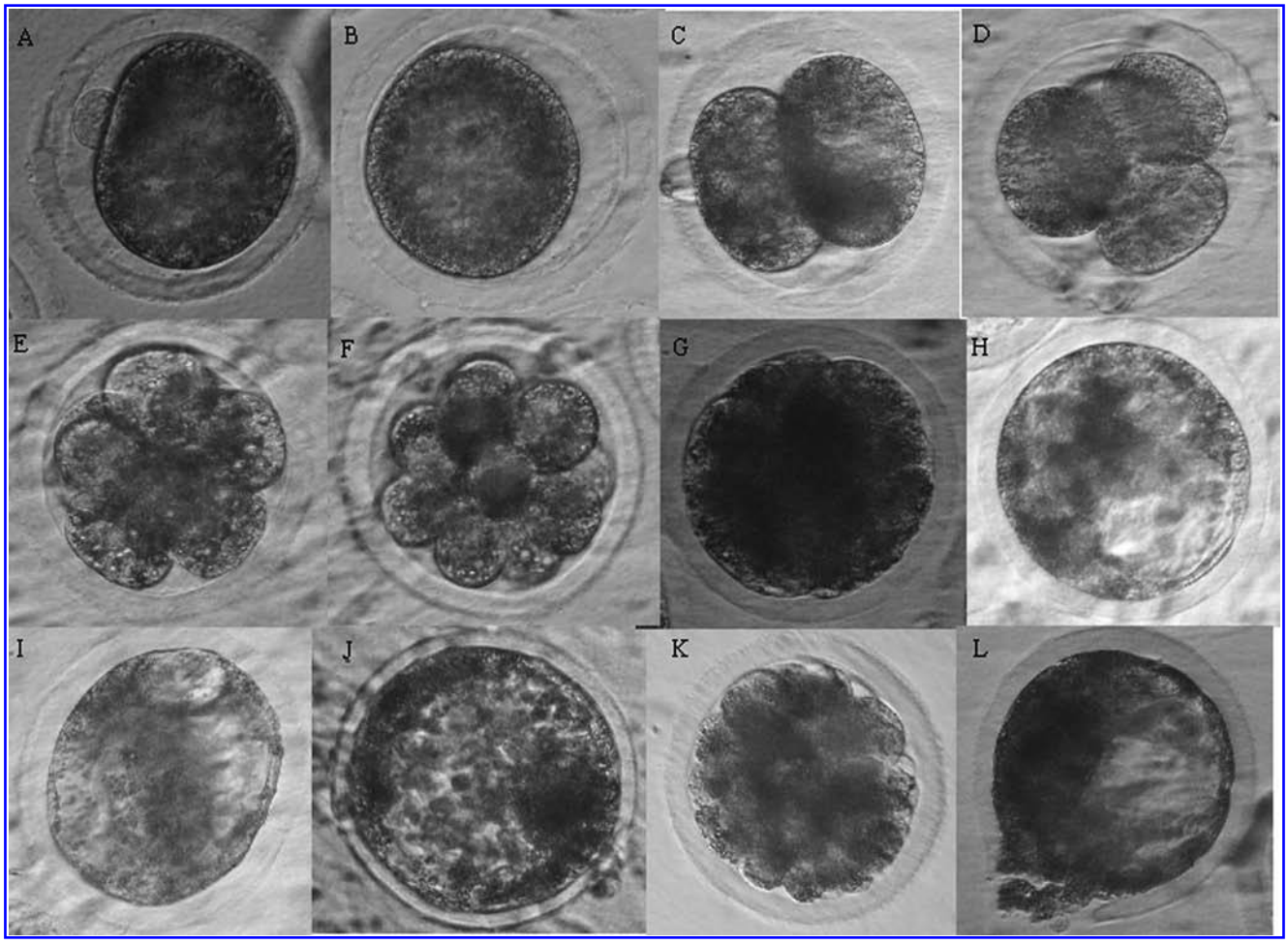

FIG. 1. Morphological development of cytoplasmic hybrid embryos constructed between human fibroblasts and enucleated bovine oocytes. (**A**) an oocyte, (**B**) a fused embryo, (**C–G**) cleaving embryos at the 2–cell, 4–cell, 8–cell, 16–cell, and the molura stages, respectively, (**H–J**) embryos at the blastocyst stage, (**K,L**) pathenogenotes at the molular and blastocyst stages, respectively.

the molura and blastocyst stages, and they were confirmed to contain only human genome as examined by both RT-PCR and PCR assays (Table 3, and data not shown).

Next, we examined dynamics of human gene activation and expression of a panel of embryonic genes during preimplantation stages. Besides OCT4, SOX2, NANOG, and E-CADHERIN, the four factors required for embryogenesis, human *β-ACTIN* was also selected to represent genes that are active in both embryonic and somatic cells. Primers were designed against human specific sequences and tested to ensure no crossreaction with the bovine species. In addition, a homologous fragment common in both human and bovine *β-ACTIN* cDNAs was also amplified to serve as the positive control for the RT-PCR assay. Each primer pair was to cross at least one intron, so that products derived from cDNA and genomic DNA can be easily distinguished. PCR products of the six genes listed above were also confirmed through sequencing (data not shown).

As shown in Figure 3, a proportion of cytoplasmic hybrid embryos expressed low levels of *OCT 4* (2/24) and *SOX 2* (3/47) immediately after fusion. Similarly, small proportions of fibroblasts before fusion expressed *OCT 4* and *SOX 2* as well (data not shown). These results were further confirmed using the real-time PCR assay (data not shown). Our data are consistent with previous reports (Takeda et al., 1992; Verlinsky and Kuliev, 1998), indicating that some somatic cells

TABLE 2. PREIMPLANTATION STAGE DEVELOPMENT OF CYTOPLASMIC HYBRID EMBRYOS

Exp	*NT*	*Fused*	*2-Cell*	*4-Cell*	*8-Cell*	*16-Cell*	*Blastocyst*
Total (28)	5406	3315 (61.3%)[a]	2166 (65.3%)[b]	1376 (41.5%)[b]	710 (21.4%)[b]	298 (9%)[b]	29 (0.87%)[b]

[a]Fused over NT.
[b]Developed over fused.

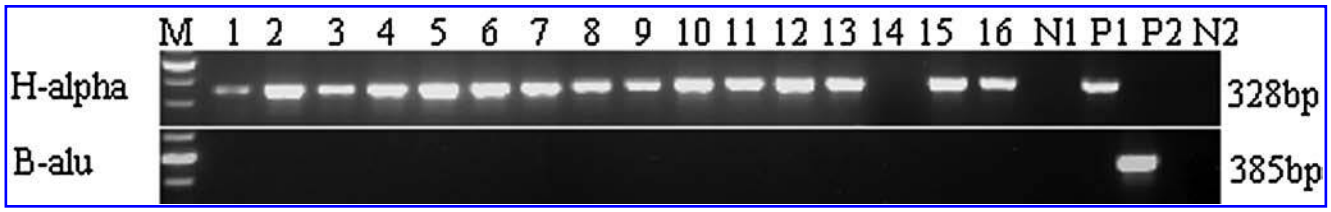

FIG. 2. Most cytoplasmic hybrid embryos at the 16–cell stage contained human genome as shown by the PCR assay using primers against human alphoid repetitive sequences (HUMSATA7A) or bovine *Alu*-like repetitive sequences (B-Alu). M, molecular weight markers; 1–16, embryos; P1, human genomic DNA; P2, bovine genomic DNA; N1, negative control for reverse transcription; N2, no template was added during PCR.

express low levels of *OCT 4* and *SOX 2* constitutively. Human *β-ACTIN* transcripts were detected in a proportion but not all cytoplasmic hybrid embryos. We assume that this is a variation resulted from the assay. When 1/10 of a cytoplasmic hybrid embryo was used in each reaction as the template, *β-ACTIN* mRNA contributed by one somatic cells may not always been detected. Expression of *NANOG*, *E-CADHERIN* was never detected at this stage. This expression pattern of the five human genes in freshly fused embryos was generally maintained as embryos cleaved through the two-cell and four-cell stages. At the eight-cell stages, expression of *NANOG*, and *E-CADHERIN* begun. The expression frequencies of *OCT 4*, *SOX 2*, *NANOG*, and *E-CADHERIN* increased most significantly in between the 8- and 16-cell stages. By the 16-cell stage, all five human genes were activated (Table 3). These results suggest that human embryonic genes are activated in between the 8- to 16-cell stages in the cytoplasmic hybrid embryos constructed between human fibroblasts and enucleated bovine oocytes.

It was also demonstrated in Figure 3 that activation and expression of human genes were not uniform. In a small proportion of cytoplasmic hybrid embryos, all five human genes were activated. In others, however, only zero to four of the five genes were expressed. These results indicated that human gene activation in a large proportion of cytoplasmic hybrid embryos was incomplete. In those cytoplasmic hybrid embryos where human genes were partially activated, the combination of expressed genes was almost random, suggesting that gene activation in nt-embryos might be a randomized event in general.

Importantly, developmental potential of the cytoplasmic hybrid embryos appears to be positively correlated with the extent of human gene activation. As shown in Table 3, embryos with more human genes activated developed farther than those with less genes activated. In the three blastocysts analyzed, all five human genes were activated. These results suggest that sustained development of the cytoplasmic hybrid embryos depends on gene products encoded by transplanted human nuclei. Embryogenesis ceases if human nuclei are not activated or not sufficiently activated. This observation is consistent with previous findings in mice that zygotic gene products are essential for embryonic development after maternal products are degraded (Nothias et al. 1995).

Discussion

We demonstrated in this study that human somatic nuclei can be activated by enucleated bovine oocytes to express pluripotential genes. Activation of human genes in human–bovine cytoplasmic hybrids appears to follow the temporal order of the bovine species. Expression of human pluoripotential genes is essential for sustained development of the hybrid embryos.

Due to functional importance, some genes and genetic pathways are preserved during evolution and become common in different species, which forms the base for cross-species SCNT. As shown in this study, the cytoplasm of bovine oocytes was able to activate human embryonic genes, albeit in a low efficiency, indicating that the nuclear reprogramming mechanism is partially conserved between human and bovine. This result suggests that, with further im-

TABLE 3. HUMAN GENE ACTIVATION IN CYTOPLASMIC HYBRID EMBRYOS

Gene	*Fused*	*2-Cell*	*4-Cell*	*8-Cell*	*16-Cell*	*Morula*	*Blastocyst*
H-Oct-4	2/24 (8)[a]	1/24 (4)	1/36 (3)	3/24 (13)	12/24 (50)	3/4 (75)	3/3 (100)
H-Sox-2	3/47 (6)	12/44 (27)	8/36 (22)	10/41 (24)	32/44 (72)	3/4 (75)	3/3 (100)
H-Nanog	0/47 (0)	0/44 (0)	0/36 (0)	6/41 (15)	25/44 (57)	3/4 (75)	3/3 (100)
H-Ecadherin	0/47 (0)	0/44 (0)	0/36 (0)	2/41 (5)	23/44 (52)	2/4 (50)	3/3 (100)
H-β-actin	10/24 (42)	19/24 (79)	22/36 (61)	11/24 (49)	13/24 (54)	4/4 (100)	3/3 (100)
H-B-β-actin	24/24	23/24	36/36	24/24	24/24	4/4	3/3

[a]Numbers in brackets represent percentages (developed embryos over fused embryos).

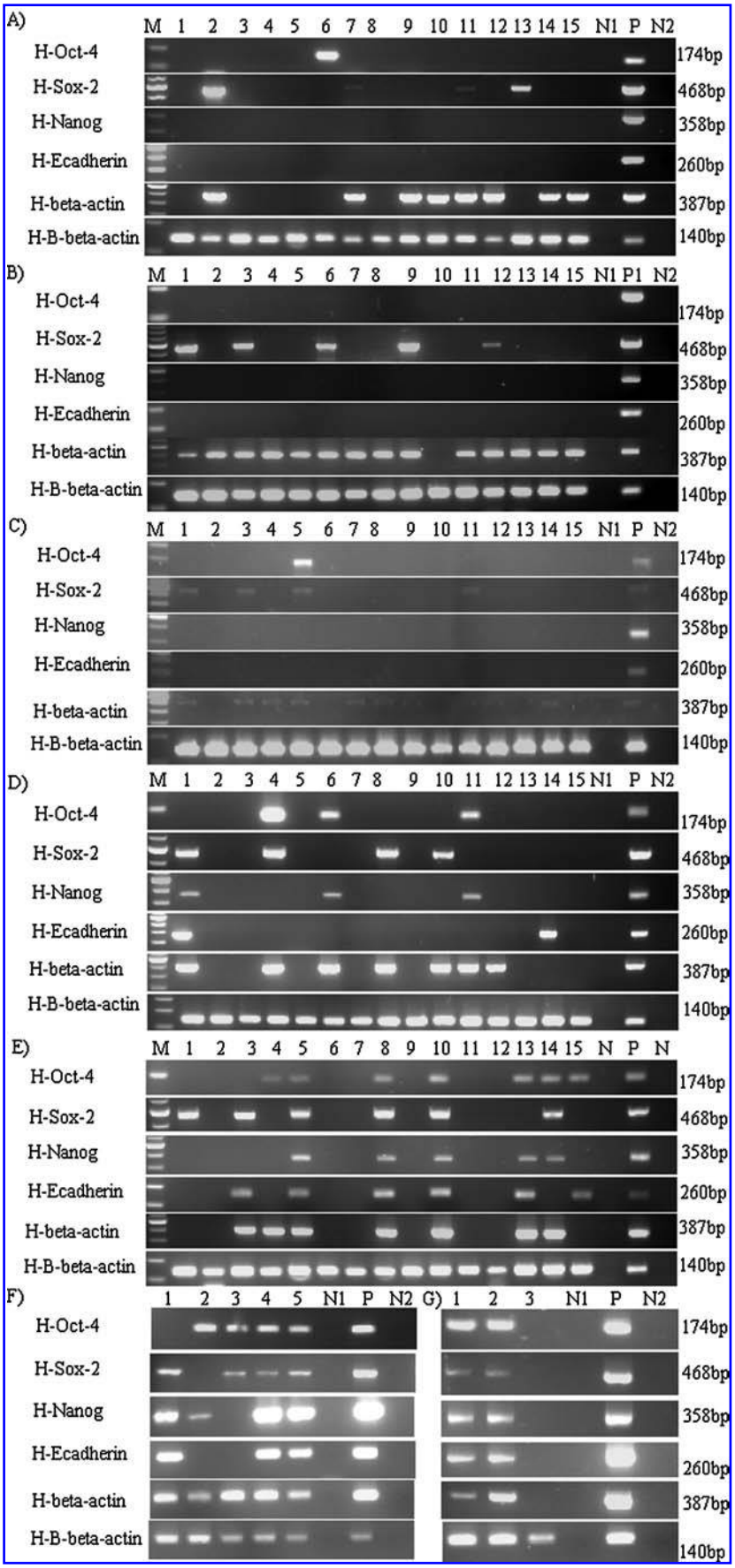

FIG. 3. RT-PCR results showing expression of human *OCT 4*, *SOX 2*, *NANOG*, *E-CADHERIN*, and *β-ACTIN* in cytoplasmic hybrid embryos during pre-implantation stages. (**A**) Immediately after fusion; (**B**) the 2–cell, (**C**) 4–cell, (**D**) 8–cell, (**E**) 16–cell stages, (**F**) 1–4, molura; 5, blastocyst, (**G**)1–2, blastocysts, 3, pathenogenote. H-B-β-actin, a homologous fragment in the human and bovine β-actin genes. See Figure 2 legend for more details.

provement, bovine oocytes may be used to substitute human eggs in studies aiming to understand mechanism controlling early development and to derive human pluoripotential stem cells with desired genotypes.

Embryogenesis in the earliest stages depends on maternal products stored within oocytes and shifts to become dependent on embryonic gene expression later. The timing when the embryonic genome is activated is species specific. It occurs at the 4- to 8-cell stage in humans (Braude et al., 1988; Taylor, et al., 1997) and at the late 8- to 16-cell stage in bovine (Frei et al., 1989). The temporal pattern of human gene activation in human–bovine cytoplasmic hybrids was similar to that of the bovine instead of humans. It was found in previous study that development of bovine embryos to the 16-cell stage was mainly supported by peptides and mRNAs stored in the ooplasm (Memili and first, 1998). These maternal products of the bovine species seem to dictate the timing of human genome activation in human-bovine cytoplasm hybrids. This finding is consistent with the notion that the onset of zygotic genome activation depends on maternally inherited proteins and is under maternal control (Schultz, 1993).

The efficiency of SCNT must be improved before the human–bovine hybrid system is practically useful. In cross-species SCNT between bovine and primate species (humans and monkey), preimplantation developmental rates vary drastically from experiment to experiment. In some experiments, 5% of the reconstructed embryos developed to the blastocyst stage while in other experiment, none did (data not shown). The current SCNT protocol needs to be optimized to achieve more efficient and consistent nuclear reprogramming.

We have observed incomplete activation of genes in the transplanted nuclei. This is not unique to cross-species SCNT. It was also observed in SCNT between the same species (Ng and Gurdon, 2005a). The incomplete activation of transplanted nuclei may be related to incomplete erasure of the somatic epigenetic program. Previous studies showed that erasure of the somatic epigenetic markers, including DNA methylation, histone acetylation (Armstrong et al., 2006; Blelloch et al., 2006; Ng and Gurdon, 2005b; Santos et al., 2003; Simonsson and Gurdon, 2004) and transcription profiles (Sun et al., 2007), was incomplete in most SCNT embryos. Mechanistically, residual epigenetic markers on chromatin could block the access/binding of new factors and interfere with reprogramming. Effort to facilitate a more complete erasure of the existing epigenetic program in the somatic nucleus may improve embryonic gene activation and development.

It has been consistently observed in nonparallel experiments that percentages of preimplantation development resulting from human–bovine SCNT are significantly lower than that from bovine–bovine SCNT (data not shown), indicating that incompatibility in genetic components between human and bovine attributes to the low efficiency. The problem could potentially be solved through introducing human factors into the cytoplasm of bovine oocytes. Oocytes tolerate well and incorporate efficiently exogenous components (Forbes et al., 1983; Gao et al., 2007), which creates an opportunity for engineering the cytoplasm of the oocyte for desired composition. Since bovine oocytes are abundantly available for extensive tests, with increasing knowledge in the mechanism, pathways, and components underlying nuclear reprogramming, it is entirely possible that we can use bovine oocytes as basic devices, redesign them, and turn them into effective units for reprogramming human cells.

Acknowledgements

This work was supported by grants from the Projects of Development Plan of the State Key Fundamental Research of China (2001CB509903), the National High Technology Research and Development Project of China (2001AA216121), Science and Technology Commission of Shanghai Municipality (01DJ14003), Chinese Academy of Sciences (No: KSCX-2-3-08), and Shanghai Municipal Education Commission (303042802).

Author Disclosure Statement

The authors declare that no competing financial interests exist.

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