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Role of the donor nuclei in cloning efficiency: can the ooplasm reprogram any nucleus?

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ABSTRACT Cloning efficiency has not been dramatically improved after the first success of somatic cell nuclear transfer (SCNT) in sheep in 1997. The reasons for the low efficiency of SCNT embryos must be attributed to the insufficient reprogramming of the donor nucleus in ooplasm. It has been clarified that the methylation and acetylation status are disordered in SCNT embryos and the gene expression pattern is different and widely varied in SCNT embryos, compared with fertilized embryos. In this paper, we focused on the role of the donor nuclei in cloning efficiency, and discuss whether ooplasm can reprogram any nucleus.

KEY WORDS: *cloning, donor nucleus, nuclear transfer, efficiency, reprogram*

Introduction

Despite several attempts to improve the cloning efficiency of somatic cell nuclear transfer (SCNT) since the first successful production of a sheep in 1997 (Wilmut *et al*., 1997), dramatic improvements have not yet been realized. Reprogramming the meiotic deacetylation process by inhibiting deacetylation with the potent and specific histone deacetylase inhibitor trichostatin A (TSA) has been effective for *in vitro* and *in vivo* cloning efficiency (Rybouchkin *et al*., 2006; Tsuji *et al*., 2009; Kishigami *et al.,* 2006), but has not induced sufficiently dramatic improvement. Although the low efficiency of SCNT embryos appears to be due to insufficient reprogramming of the donor nucleus in the ooplasm, this has not been clarified and overcoming this insufficient reprogramming presents several challenges. The nuclear reprogramming process is likely to be very complex; upon nuclear transfer, donor chromatin is exposed to the ooplasm (the first and most important reprogramming step), and after artificial activation of SCNT oocytes, reconstituted oocytes begin preimplantation development (the second reprogramming process), including zygotic genome activation, compaction, and the first differentiation into ICM and TE cell lineages. Postimplantation development is the last reprogramming step and is even more complex.

In the first reprogramming process, donor information should be reprogrammed from the somatic type to the embryonic type. Reprogramming factors in the ooplasm have also been examined, and several factors that promote cloning efficiency have been identified (Miyamoto *et al*., 2007, 2009; Jullien *et al*., 2010). In the second reprogramming process, SCNT embryos begin to cleave and develop to the blastocyst stage with a time schedule similar to that of fertilized embryos. Although the gene expression pattern of SCNT preimplantation embryos is largely different and varies widely compared with fertilized embryos (Li *et al*., 2006a,b, 2008), it is not known whether the different gene expression patterns are incompatible with successful SCNT cloning. The last reprogramming process involves very complex fero-maternal communication, an *in vivo* process that remains unclear. Administration of human chorionic gonadotrophin (hCG) to control the physiology of recipient females was recently reported to improve cloning efficiency (Tsuji *et al*., 2010).

Several studies have focused on determining which donor cell type or donor cell status is best for successful cloning (Table 1). The state of the donor cell is one of the most important factors for cloning efficiency. In the present study, we discuss mainly the role and effect of the donor nucleus type in cloning efficiency.

Cell cycle combination

For successful cloning, reconstituted oocytes must carry the diploid DNA contents after artificial activation. In nuclear-transferred blastomeres of preimplantation embryos, donor blastomeres at any cell cycle can be reprogrammed in MII ooplasm; G1 and M-phase donor blastomeres can be reprogrammed in MII ooplasm, and S-phase donor blastomeres can be reprogrammed in activated MII ooplasm, which might be because maturation promot-

Abbreviations used in this paper: SCNT, somatic cell nuclear transfer.

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ing factor (MPF) activity is decreased during S-phase.

In contrast, when somatic cells are used as donors for nuclear transfer, the cell cycle combination might be important for development. The G0-phase was thought to be the most adequate cell cycle for nuclear reprogramming of the donor nucleus (Campbell, *et al*., 2006; Wilmut *et al*., 1997), but it was later clarified that the G1-phase and M-phase are also reprogrammed in MII-phase oocytes and can develop to full-term (Table 1). Even if cells are not induced to the G0-phase by serum starvation or contact inhibition, up to approximately 60% cells of cultured somatic cells are in the G1 phase. When donor cells at the G0 and G1-phase are used, emission of the first polar body in reconstituted oocytes must be suppressed by cytochalasin B or a similar chemical to maintain the diploid status of the donor cell in the ooplasm, as in blastomere transfer. When M-phase cells are used as donor cells, as reported in mouse (Ono, 2001a,b) and rat (Zhou, 2003), emission of the

TABLE 1

THE EFFECTS OF DONOR CELL TYPE AND CELL CYCLE COMBINATION BETWEEN DONORS AND RECIPIENTS, ON DEVELOPMENTAL ABILITY OF NT EMBRYOS RECEIVING SOMATIC, EMBRYONIC, ES, EG AND PGC CELLS

		Donor cell type	animal (donor-recipient)	donor:recipient cell cycle(stage)	morula-Bl /activated (%)	live offspring /ET(%)	Ref.
Somatic cells	adult	mammary gland	sheep	ss1) 5 d:MII	11.7	3.4	Wilmut et al., 1997.
		oviduct	bovine	ss3:4d:MII	23	75	Kato et al., 1998.
		cumulus	bovine	ss3:4d:MII	49	83	Kato et al., 1998.
		cumulus	mouse	G0:MII	39.9-66.9	2-2.5	Wakayama et al., 1998.
		follicular epithelial cell	mouse	ss2:9d:MII	34	3	Kato et al., 1999a.
		tail tip	mouse	ss3:5d:MII	58.3	1	Wakayama and Yanagimachi, 1999a.
		tail tip	mouse	?:MII	49.5	1.1	Wakayama and Yanagimachi, 1999a.
		derived from 11 kinds of tissue(fibroblast)	bovine	G0:MII	30-53	15	Kato et al., 2000.
		fibroblast	gaur - bovine	?:MII	12	2[2)]	Lanza et al., 2000.
		granulosa cell	pig	ss48:72h:MII	?	7	Polejaeva et al., 2000.
		cumulus	mouse	G0:M(zygote)	0(4-cell)	0	Wakayama et al., 2000.
		granulosa cells	mouflon-sheep	G0:MII	30	14	Loi et al., 2001.
		cumulus	goat	ss:MII	7,8	1	Zou et al., 2001
		cumulus	rabbit	?:MII	47	1,6	Chesne et al., 2002.
		lymphocytes	mouse	?:MII	5	7-67[3)]	Hochedlinger and Jaenisch, 2002.
		lymphocytes	mouse	?:MII	5	1-10[4)]	Hochedlinger and Jaenisch, 2002.
		cumulus	cat	?:MII	?	33	Shin et al., 2002.
		fibroblast from mucosa	cat	?MII	?	0	Shin et al., 2002.
		fibroblast from heart	pig	MII	6	2	Yin et al., 2002.
		fibroblast	horse	? : MII	7	6	Galli et al., 2003.
		anterior pituitary	goat	ss5:6d: MII	3	17	Ohkoshi et al., 2003.
		fetal fibroblast	rat	M:MII	-	2	Zhou et al., 2003.
		fibroblast	african wild cat-domestic cat	ss 5d:MII	-	3	Gomez et al., 2004.
		Bone marrow mesenchyma stem cell	bovine	G0:MII	24	8	Kato et al., 2004.
		natural killer T cell	mouse	?:MII	71	1.1-1.6	Inoue et al., 2005.
		skin fibroblast	dog	?:MII	-	0,2	Lee et al., 2005.
		skin fibroblast	wolf-dog	?:MII	-	0,8	Kim et al., 2007.
		cultured cumulus	ferret	ss24h:MII	-	1.2-1.8	Li et al., 2006.
		fibroblast	rabbit	G0:S(2:cell)	24,5	1	Skrzyszowska et al., 2006.
		hematopoietic stem cells	mouse	?:MII	4.1-7.9	-	Sung et al., 2006.
		hematopoietic progenitor cells	mouse	?:MII	10.6	-	Sung et al., 2006.
		hematopoietic granulocytes	mouse	?:MII	34.5	1.1	Sung et al., 2006.
		antler stem cell	red deer	ss 4 d:MII	22	13	Berg et.al., 2007.
		tail tip	mouse	M:M(zygote)	3	-	Egli et.al., 2007.
		fibroblast	mouse	M:M(zygote)	-	?[5)]	Egli et.al., 2007.
		keratinocytes	mouse	?:MII	56	2	Li et al., 2007.
		cultured granulosa	buffalo	ss72h:MII	22,2	10	Shi et al., 2007.
		cumulus	sand cat-domestic cat	?:MII	15-16	0.3-1.8	Gomez et al., 2008.
		fibroblast	Pyrenean Ibex-domestic goat	72h:MII	65,3	0,6	Folch et al., 2009.
		cultured cumulus	camel	ss72h:MII	34-44	4	Wani et.al., 2010.
		iPS	mouse	M:MII	77.7	3.5	Kou et al., 2010.
	newborn/young	derived from 16 kinds of tissue(fibroblast)	bovine	G0:MII	25-47	14	Kato et al., 2000.
		Sertoli	mouse	?:MII	23-60	1.2-4.5	Ogura et al., 2000.
		neural stem cell	mouse	?:MII	-	0.5-1.1	Mizutani et al., 2006.
	fetus	fetal fibroblast	goat	ss 7d:MII	?	2,1	Baguisi et al., 1999.
		fetal fibroblast	goat	ss 7d:TeloII	?	5,2	Baguisi et al., 1999.
		genital ridge cells	pig	0:4d:MII	4-8	0,7	Betthauser, 2000.
		fibroblast	pig	0:4d:MII	4-8	0,3	Betthauser, 2000.
		fetal fibroblast	pig	16d:MII	1-31.2	0,9	Onishi et al., 2000.
		10 kinds of tissue(fibroblast)	bovine	G0:MII	23-46	8	Kato et al., 2000
		fibroblast	mouse	M:MII	29-37	0.7-3	Ono et al.,, 2001b.
		neural cells	mouse	?:MII	37	5.5	Yamazaki et al., 2001.
		neural cells, premature-early differentiated	mouse	?:MII	36	12	Yamazaki et al., 2001.
		neural cells, differentiated	mouse	?:MII	23	5	Yamazaki et al., 2001.
		fetal fibroblast	mule-horse	?:MII	-	0,3	Woods et al., 2003.
		keratinocytes	mouse	?:MII	54	2	Li et al., 2007.

Embryonic cells	blastocyst	inner cell mass (ICM) cells	mouse	G16):MII	23-64	8	Tsunoda and Kato, 1998.
		mural trophectoderm (TE) cells	mouse	G16):MI	32-62	11	Tsunoda and Kato, 1998.
		embryonic disc	sheep	ss 5 d:MII	6.7-21	11	Campbell et al., 1996.
		cultured embyonic disc (TNT4)	sheep	ss 2d : MII	9.2-50	15	Campbell et al., 1996.
	ES-cell		mouse	small size:MII	16.5	5	Wakayama et al., 1999b.
			mouse	large size:MII	36.8	3	Wakayama et al., 1999b.
			mouse	M:MII	34-88	2-6	Amano et al., 2001.
			mouse	M:MII	70	10	Ono et al., 2001a.
			mouse	M:MII	51[7)]	16[7)]	Ono et al., 2001a.
			mouse	M:M(zygote)	10	5	Egli et.al., 2007.
	EC-cell		mouse	?:MII	0-29.7	0[8)]	Chang et.al., 2010.
	PGCs	day 10.5	mouse	G1:M	-	0.4	Miki et al., 2005.
			mouse	M:M	-	0.4	Miki et al., 2005.
		day 11.5	mouse	G0:M(zygote)	14	-	Kato and Tsunoda, 1995a.
		day 12.5 male	mouse	G0:S(2:cell)	2	-	Kato and Tsunoda, 1992.
			mouse	G0:S(2:cell)	7	-	Kato and Tsunoda, 1992.
		day 15.5 male	mouse	G0:M(zygote)	57	-	Kato and Tsunoda, 1995a.
			mouse	G0:MII	58	(3)[10)]	Kato and Tsunoda, 1995b., Tsunoda and Kato, 1995.
			mouse	G0:MII	47-66	(57)[11)]	Kato et al., 1999b.

1) serum starved; d: days, h: hours, 2) later-term abortion at day 202, 3) ntES cells were injected into 2n host blastocysts, 4) ntES-cells were injected into 4n blastocysts, 5) 5 chimeras after injection of ntES-cells into blastocysts. 6) ICM and TE cell were isolated from blastocysts previously treated with nocodazole followed by achidicolin. 7) serial nuclear transferred at 1-cell stage, 8) ntES-cells were injected into 4n bl;astocysts, 10) day 10.5 chimera after serial nuclear trasnfer at the 2-cell stage, 11) day10.5 fetuses.

second polar body after artificial activation is essential to maintain the diploid status in the ooplasm. This differs from blastomere transfer, because somatic cells at the S-phase do not develop in activated ooplasm. Also, when G0/G1-phase and M-phase donor cells are transferred to activated ooplasm, the *in vitro* developmental ability of reconstituted oocytes is significantly decreased (Tani *et al*., 2003).

The Table summarizes the effect of the cell cycle of the donor and recipient and of the donor cell types on the development of manipulated embryos. The first report of each animal species and the specific cell cycle combination of the donor and recipient was selected from the vast literature. As shown in Table, a G0/G1 donor with MII recipient ooplasm was used in almost all successful reports.

There have been some attempts to use fertilized oocytes such as zygotes and 2-cell embryos as SCNT recipients, but the success has been limited. Zygotes at the S-phase seem to support the development of SCNT up to the 4-cell stage and then development stops (Wakayama *et al*., 2000). Zygotes at the M-phase seem to support the development of SCNT to the blastocyst stage (Egli *et al*., 2007), but if embryonic stem (ES) cells are used, zygotes at the M-phase can develop to full term after embryo transfer to foster mothers (Egli *et al*., 2007).

When primordial germ cells at the arrested stage obtained from day 15.5 fetuses or at the mitotic phase obtained from day 11.5 fetuses were fused with enucleated zygotes at the M-phase (Kato and Tsunoda, 1995a) and/or enucleated blastomeres of one of the 2-cell embryos at the late S-phase (Kato and Tsunoda, 1992), reconstituted zygotes or chimeric 2-cell embryos(Kato and Tsunoda, 1992) developed to the blastocyst stage. Moreover, in rabbit, chimeric embryos, i.e., adult fibroblast cells fused with one enucleated blastomere of a 2-cell embryo, develop to chimeric offspring (Skrzyszowska *et al*., 2006).

Based on these studies, MII oocytes might possess some reprogramming factors in the ooplasm (Miyamoto *et al*., 2009), but gradually lose these reprogramming factors in the cytoplasm after fertilization or artificial activation (Tani *et al*., 2003). We examined the effect of ooplasm aging on the developmental potential of SCNT embryos in bovine (Tani *et al*., 2003) and in mouse (Liu *et al*., 2007). When bovine cumulus cells at the G0-phase and M-phase are fused every hour after activation for 6 h, the potential to develop into blastocysts after SCNT gradually decreased with time after activation. Ooplasm 2 h and more after activation did not support the development of G0-phase cumulus nuclei to blastocysts. When M-phase cumulus cells were fused, the ability to develop into blastocysts dramatically decreased beginning at 6h postactivation. These findings clearly demonstrate that some reprogramming factors present in the ooplasm decrease after artificial activation. We isolated some reprogramming factors in the ooplasm before activation, and identified one of the candidate reprogramming factors as TCTP (Tani *et al*., 2007), which expressed in ooplasm, but is no longer present in activated ooplasm as the same type. Although, oocytes injected with TCTP peptide after SCNT produce much healthier offspring in bovine than in non-injected oocytes, (Tani *et al*., 2007), the role played by TCTP in the whole reprogramming process remains unclear.

Donor cell type

After the first report in which mammary gland cells were used as the donor cells, cumulus cells have been the most popular donor cell type in SCNT because of the experimental convenience (Table 1). Many attempts have been made to determine the most efficient donor cell types, especially for bovine and mouse. To examine which cell types are the most successful for SCNT in bovine, we compared the development potential of 39 cell types from adults, newborns, and fetuses of both sexes, but there was no big difference (Kato *et al*., 2000). When tissue or biopsy samples were used for the cell culture, fibroblast cells were the most common cell type to easily increase. Cells that were not identified as a specific cell before nuclear transfer were categorized as fibroblast cells. As shown in the table, although the developmental potential was higher in bovine compared with mice, the overall conclusion is that cloning efficiency is similar among somatic cell types. Although some types of stem cell, such as bone marrow mesenchymal stem cells (Kato *et al*., 2004), neural stem cells (Mizutani *et al*., 2006), and hematopoietic stem cells (Sung *et al*., 2006), might be adequate for cloning, the results do not indicate equivalent efficiency suggesting that the low

efficiency of SCNT must not depend on the cell type, but that ooplasm alone is also not sufficient for complete reprogramming of the somatic nucleus. To improve the efficiency of SCNT cloning, several possibilities are considered.

When male primordial germ cells on day 15.5 arrested at the G0-phase of the cell cycle were used as donor cells, nuclear-transferred oocytes developed to blastocysts, but after their transfer to recipient mice, they stopped developing at around day 10.5 due to the lack of a proper imprint (Tsunoda and Kato, 1995; Kato and Tsunoda, 1999b; Kato *et al*., 1999b). Later, Miki *et al.,*2005) demonstrated that PGCs on day 10.5 developed to term after nuclear transfer.

Based on these results, epigenetic modification of DNA such as methylation in imprinted genes might not be reprogrammed by the ooplasmic factor(s) alone. If donor cell chromatin undergoes epigenetic modification in genes important for development, such as imprinting before nuclear transfer, nuclear-transferred oocytes must be very difficult to completely reprogram. It might be suggested that imprinting status is more important for the success of cloning than the origin of the donor cells. It is possible that successful cloning requires the use of donor cells with an adequate methylation pattern that are then reprogrammed in the ooplasm and develop to term.

Cytoplasmic factors

In conducting nuclear transfer, cytoplasmic factors of donor cells often contaminate the recipient ooplasm. Somatic cytoplasmic factors affect the developmental potential of not only nuclear-transferred embryos but also parthenogenetic oocytes (Takeda *et al*., 2005, 2010) and fertilized oocytes (Thuan *et al*., 2006). Thuan *et al*. (2006) also demonstrated that the injection of cumulus cytoplasm into oocytes before fertilization induced a decrease in preimplantation development and impaired full-term development. When isolated mitochondria from ear epithelial cells in the G0-phase were injected into mouse oocytes, the developmental potential of parthenogenones into blastocysts was significantly decreased (Takeda *et al*., 2010), suggesting that mitochondrial heteroplasmy or foreign mitochondria introduction affects the developmental potential of parthenogenetic oocytes.

In SCNT, donor mitochondria comprise only about 1%, which is difficult to detect. In some cases, however, the amount of mitochondria from donor somatic cells increased and survived to become clones (Takeda *et al*., 2003). It has remained unclear how mitochondria heteroplasmy affects SCNT development.

In nuclear transfer, serial nuclear transfer at the late pronucleus (Ono *et al*., 2001b) or 2-cell stages in the mouse (Kato *et al*., 1999a), during which zygotic genome activation has occurred in the mouse, improves the development of SCNT embryos. The serial nuclear transfer has mainly two effective meanings; dilution of the donor cytoplasmic factors incorporated in the ooplasm, and fertilized cytoplasm after zygotic genome activation must be much better than parthenogenetic ooplasm (Kato *et al*., 1999a).

Direct reprogramming of somatic cells into pluripotency

The term “reprogramming” may be used to refer to either the induction of totipotency(Tsunoda and Kato, 2002), leading to successful cloning or the induction of pluripotent capabilities. Tada *et al.,*2001) demonstrated that somatic cells such as lymphocytes fused with ES cells become pluripotent. Activation of the Oct4 gene and reactivation of the X chromosome in lymphocyte nuclei in hybrid cells results in chimeric fetuses. These acquired characteristics in the lymphocytes are very similar to that of ES cells. Tada *et al*. (1997, 2001) also demonstrated that embryonic germ cells have more developmental potential than ES cells; the methylation status of embryonic germ cells is downregulated compared to that of ES cells and therefore the imprint of ES cells is erased after fusion in the hybrid cells. Although, it is not clear which factors contribute to controlling the methylation pattern and to erasing the imprint from ES cells, factor(s) including imprint status, in EG-cells and PGCs seemed to be dominant compared with that from ooplasm and ES-cells.

Somatic cells were recently directly reprogrammed (Mitalipov and Wolf, 2009) to induce pluripotency in cells *in vitro*, termed induced pluripotent stem (iPS) cells (Takahashi and Yamanaka, 2006) and MUSE cells (Kuroda *et al*., 2010). When iPS cells were used as donor cells, the cloning efficiency was slightly improved to a level between that of somatic cells and ES cells (Kou *et al*.,2010). MUSE cells can be isolated from cultured skin fibroblasts and bone marrow stromal cells, or directly from bone marrow aspirates. MUSE cells can be produced without gene transfer and are capable of self-renewal and expression of a set of genes associated with pluripotency. Moreover, they are nontumorigenic stem cells with the ability to generate multiple cell types of the three germ layers. At present, MUSE cells show low proliferation activity, but will be useful as candidate SCNT donors, especially in domestic animals, because the produced cloned animals are not transgenic.

One possibility is that clones are produced via nuclear transfer of ES cells (ntES-cells). Although ES cells have varied gene expression patterns (Furusawa *et al*., 2006), the cloning efficiency of ES cells is higher (12.3%-33%) than that of somatic cells (1.1%-3.4%, Mizutani *et al*., 2008; Amano *et al*., 2001), and may be useful for improving SCNT efficiency. But, three steps are needed for using ntES cells as donors for the second NT; first, NT with somatic cells, then ES cell establishment from SCNT blastocysts, and last, the second NT.

Because the direct reprogramming of somatic cells induces pluripotency, such as in ES cells or somatic cells *in vitro*, direct reprogrammed somatic cells might be interesting SCNT donor candidates.

Pre- and post-treatment of SCNT embryos

Treatment of reconstituted oocytes with TSA, which inhibits the activity of classical histone deacetylases, improves the potential of young to develop into mice. TSA treatment might also stimulate DNA demethylation (Cervoni & Szyf, 2001; Geiman & Robertson, 2002; Kishigami *et al*., 2006), leading to improved reprogramming.

The DNA methyltransferase inhibitor 5-aza-2‘-deoxycytidine (5-aza-dC) relaxes epigenetic marks of differentiated somatic cells. Pretreatment of somatic cells with 5-aza-dC, however, does not increase but rather decreases development potential due to its toxicity (Jones *et al*., 2001; Viggnon *et al*., 2002; Enright *et al*., 2003; 2005; Shi *et al*., 2003). Moreover, treatment of reconstituted oocytes with 5-aza-dC does not improve the developmental

potential of SCNT embryos. 5-aza-dC might be too toxic for cells and long-term culture is not adequate for cells and embryos (Tsuji *et al.*2009).

The morphology of SCNT blastocysts is not a criteria of embryo quality, because even SCNT embryos with a visually-perfect morphology under a microscope develop at a very low rate to full-term. The developmental ability of SCNT embryos and ES cell nuclear-transferred embryos gradually decreases *in vivo* (Amano *et al.*, 2001; Yabuuchi *et al.*, 2001) Many attempts to improve the potential of SCNT have focused mainly on the preimplantation stages, and not focused on the postimplantation stages. Ewes carrying SCNT clone pregnancies have significantly lower serum progesterone levels than ewes carrying control pregnancies, suggesting that a low serum P4 level is one reason for the low potential of SCNT embryos to reach full term (Alexander *et al.*, 2008). Enhancement of the recipient by daily injection of hCG from day 3.5 to day 6.5 of pregnancy after embryo transfer of SCNT significantly increases the implantation and fetal development rates compared to controls (Tsuji *et al.*, 2010). The potential of SCNT embryos to develop to full term, however, was not greater than that of controls, even if hCG administration was continued to day 11.5 or day 17.5 and progesterone was administered from day 7.5 to day 17.5 after hCG injection. These findings demonstrated that injection of hCG to recipients protects the *in vivo* development of SCNT embryos until day 10.5, but other treatment is necessary to support the progression of the embryos to full-term development.

Selection of SCNT embryos before transfer

Although the morphologic appearance of SCNT embryos does not differ from that of *in vitro*-fertilized embryos, the potential to develop to term dramatically differs between SCNT and IVF embryos (Li *et al.*, 2005, 2006a, b, 2008; Kato *et al.*, 2007.). Markers that will be useful for predicting the potential of NT embryos to develop into young are needed. We examined the relation between the morphology of embryos with gene expression of development-related genes, such as Oct 4, Nanog, Stat3, FGF4, Stella, and Sox2 (Li *et al.*, 2006a,b). In that study, six kinds of blastocysts were produced; *in vivo* fertilized/*in vivo*-developed, *in vivo* fertilized/*in vitro*-developed, pronuclear exchanged, morula blastomere NT, ES-NT, and cumulus cell NT. Based on the small variations in the gene expression levels among the *in vivo*-developed blastocysts, and the significant differences in gene expression between *in vivo*-developed (high developmental potential), and ES cell and cumulus cell NT blastocysts (low developmental potential), the downregulation of Sox2 and Oct4 genes is considered to be a candidate marker for the low potential of NT embryos to develop into young. A method of preselecting donor cells before SCNT is needed (Furusawa *et al.*, 2006)

Interspecies nuclear transfer

The use of SCNT methods has been extended to a wide variety of fields. Interspecies nuclear transfer of endangered species is a new purpose for SCNT (Beyhan *et al.*, 2007). Many attempts have been taken to produce animals by interspecies nuclear transfer, developmental potential of interspecies nuclear-transferred embryos was very low, and full-term development was very limited (Beyhan *et al.*, 2007). The interactions between the donor nucleus and recipient ooplasm should be matched for development. Mitochondrial interaction, transcription, and translation, which occur in reconstituted ooplasm, must be overcome. As shown in the table, several experiments using interspecies SCNT have succeeded.

Conclusion

Despite continuing problems with SCNT, it is also clear that SCNT can be used to produce healthy cloned animals. After the first successful SCNT more than 10 years ago, researchers have tried several methods to improve the efficiency of SCNT. Nevertheless, the efficiency remains low. It is still unclear if the successful cloning was derived from the elite donor cells were happened to be selected as nuclear donor, or some kinds of donor nucleus happened to be reprogrammed in ooplasm. Ooplasm reprogramming should be further investigated as a factor in the direct reprogramming of somatic cells to become pluripotent. If ooplasm which can reprogram any donor nucleus, it will be much useful for extended to a wide variety of fields more than expection, although it is still in the beginning.

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