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Reprogramming Efficiency Following Somatic Cell Nuclear Transfer Is Influenced by the Differentiation and Methylation State of the Donor Nucleus

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Abstract

Reprogramming of a differentiated cell nucleus by somatic cell nuclear transplantation is an inefficient process. Following nuclear transfer, the donor nucleus often fails to express early embryonic genes and establish a normal embryonic pattern of chromatin modifications. These defects correlate with the low number of cloned embryos able to produce embryonic stem cells or develop into adult animals. Here, we show that the differentiation and methylation state of the donor cell influence the efficiency of genomic reprogramming. First, neural stem cells, when used as donors for nuclear transplantation, produce embryonic stem cells at a higher efficiency than blastocysts derived from terminally differentiated neuronal donor cells, demonstrating a correlation between the state of differentiation and cloning efficiency. Second, using a hypomorphic allele of DNA methyl-transferase-1, we found that global hypomethylation of a differentiated cell genome improved cloning efficiency. Our results provide functional evidence that the differentiation and epigenetic state of the donor nucleus influences reprogramming efficiency.

Keywords

Embryonic stem cells; DNA methylation; Nuclear transfer; Reprogramming

Introduction

Somatic cell nuclear transfer (NT) is an inefficient process. Development of NT-derived blastocysts into embryonic stem (ES) cells or adult animals appears to be influenced by the differentiation state of the donor genome [1,2]. For example, in the mouse model, the efficiency of ES cell derivation from NT-derived blastocysts varies from <10% when using

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well-defined differentiated donor cells, such as lymphocytes [3], natural killer (NK) cells [4], and neurons [5,6], to approximately 20% for fibroblast populations [7] and >50% for embryonic stem cells and their tumorigenic counterpart, embryonal carcinoma cells [8].

The low efficiency of postblastocyst development following NT of differentiated cells is, at least in part, due to faulty global reprogramming, leading to defects in early embryonic gene expression. During normal development, early embryos undergo a well-orchestrated series of DNA methylation and histone modification changes that are believed to play an important role in establishing a chromatin state permissive to early embryonic gene expression. In contrast, nuclear transfer-derived embryos typically show abnormal patterns of DNA methylation and histone modifications [9-12]. These abnormal patterns could explain the reported failure of many NT-derived embryos to reactivate expression of early embryonic genes [13,14]. The profiling of a small number of early embryonic genes following NT of cumulus cells showed that more than 30% of cloned embryos failed to express the complete gene set. In particular, *Oct-4*, a gene essential for the production of ES cells, failed to be re-expressed in a large number of somatic clones. The methylation status of the Oct-4 promoter correlates tightly with its ability to be expressed. The promoter is unmethylated in early embryos where it is expressed and densely methylated in differentiated cells where it is silenced [15-17]. The Oct4 promoter is inefficiently demethylated following nuclear transfer [18], consistent with faulty epigenetic reprogramming causing the abnormal expression of Oct4 and other essential pluripotency genes.

These data suggest that cellular differentiation influences the epigenetic state of the donor cell nucleus, which in turn determines the efficiency at which an enucleated oocyte can reprogram a donor cell into a pluripotent embryonic stem cell fate. Therefore, we hypothesized that somatic stem cells should be more efficiently reprogrammed than their differentiated counterparts. To test this, we analyzed the efficiency of reprogramming neural stem (NS) cells. We chose NS cells because they can be cultured as a homogenous population using recently established techniques [19] and because we have previously shown that differentiated neurons are inefficient donors [6]. We found that neural stem cell donors had as high an efficiency of producing NT-derived ES cells as ES cell donors, suggesting extremely efficient reprogramming of this somatic stem cell genome. Furthermore, we tested whether DNA methylation, a critical component of the epigenetic program, influences cloning efficiency. To test the effect of genomic hypomethylation on reprogramming efficiency, we used donor fibroblasts carrying a hypomorphic allele of the DNA methyltransferase Dnmt1, which results in the global hypomethylation of the donor genome. We found that the Dnmt1 hypomorphic donor cells were more efficiently reprogrammed into pluripotent ES cells than their wild-type counterpart.

Materials and Methods

Donor Cell Lines

The NS5, NSV6.5, and Cor1-5 neural stem cell lines were derived and cultured as described [19]. Cells were cultured on 0.2% gelatin-coated plates in NS-A (Euroclone, Pero, Italy, <http://www.euroclone.net>) plus N2 supplement (Gibco, Grand Island, NY, <http://www.invitrogen.com>), and 10 ng/ml fibroblast growth factor (FGF)/epidermal growth factor (R&D Systems Inc., Minneapolis, <http://www.rndsystems.com>). For differentiation, cells were transferred to polyornithine/laminin-coated plates. For glia differentiation, cells were cultured in NS-A medium plus 1% fetal bovine serum (FBS) for 6 days. For neuronal differentiation, cells were cultured in NS-A plus FGF alone for 7 days and then NS-A plus B27 for 6 days. Hypomorphic DNMT1 and wild-type tail tip fibroblasts were isolated from adult mice and cultured in Dulbecco's modified Eagle's medium plus 10% FBS, nonessential amino acids, β -mercaptoethanol, and penicillin/streptomycin.

Nuclear Transfer and ES Cell Derivation

Nuclear transfer was performed as previously described [20]. Oocytes were collected from superovulated (C57BL/6 × DBA/2 F1) females. Enucleation and nuclear transfer were done with a piezo-driven micromanipulator system (Primetech, Ibaraki, Japan, <http://www.primetech-jp.com>) on a Nikon microscope with inverted optics (Nikon, Tokyo, <http://www.nikon.com>). One to 3 hours after nuclear transfer, reconstructed oocytes were activated for 5 hours with 10 mM Sr^{2+} in Ca^{2+} -free medium in the presence of 5 $\mu\text{g}/\text{ml}$ of cytochalasin B. Resulting embryos were cultured to the blastocyst stage in KSOM medium (Specialty Media, Phillipsburg, NJ, <http://www.specialtymedia.com>). Resulting blastocysts were treated with acid Tyrode's solution to remove the zona pellucida and then cultured in ES medium supplemented with 5×10^{-5} M PD98059 MEK1 inhibitor (Cell Signaling Technology, Beverly, MA, <http://www.cellsignal.com>) on mouse embryonic fibroblasts (MEFs). Inner cell mass outgrowths were mechanically dissociated in the presence of trypsin and then replated on MEFs in ES medium.

Chimera Analysis

NT-derived ES cells were labeled with a ubiquitously expressed green fluorescent protein (GFP) by targeting an enhanced green fluorescent protein (eGFP)-puroR vector to the Rosa26 locus as previously described [8]. Chimeras were produced by injecting diploid blastocysts isolated from (C57BL/6 × DBA/2 F2) crosses. Three to eight cells were injected per blastocyst before transfer into day 2.5 pseudopregnant Swiss or (C57BL/6 × DBA/2 F1) females. Chimeras were allowed to develop to adults or were isolated by laparotomy at day 14.5. Day 14.5 embryos were analyzed under fluorescence microscopy, and representative GFP-positive embryos were fixed in 10% buffered formalin for 24 hours and embedded in paraffin. Five- μm sections were cut and immunostained using an avidin-biotin immunoperoxidase technique. Primary antibody (anti-GFP; 1:1,000; Molecular Probes Inc., Eugene, OR, <http://probes.invitrogen.com>) was incubated overnight at 4°C. Samples were subsequently incubated with biotinylated secondary antibodies (Vector Laboratories, Burlingame, CA, <http://www.vectorlabs.com>) for 30 minutes and then with avidin-biotin peroxidase complexes (Vector Laboratories) for 30 minutes. Diamino-benzidine was used as the chromogen and hematoxylin as the counterstain.

Northern and Bisulfite Sequencing

For Northern analysis, 10 μg per sample of total RNA isolated using Trizol (Invitrogen, Carlsbad, CA, <http://www.invitrogen.com>) was run in each lane and then transferred to Gene Screen (PerkinElmer Life and Analytical Sciences, Boston, <http://www.perkinelmer.com>). The resulting membranes were probed using cDNAs spanning the open reading frames of the corresponding genes and washed under high stringency conditions (0.2× standard saline citrate, 65°C for 45 minutes) before exposure to film. Bisulfite treatment of DNA was done using the CpGenome DNA Modification Kit (Chemicon, Temecula, CA, <http://www.chemicon.com>) following the manufacturer's instructions. The resulting modified DNA was amplified by nested polymerase chain reaction (PCR) using two forward (F) primers and one reverse (R) primer: Oct4 (F1, GTTGTTTTGTTTTGGTTTTGGATAT; F2, ATGGGTTGAAATATTGGGTTTATTTA; R, CCACCCTCTAACCTTAACCTCTAAC) and Nanog (F1, GAGGATGTTTTTAAGTTTTTTTT; F2, AATGTTTATGGTGGATTTTGTAGGT; R, CCCACACTCATATCAATATAATAAC). The first round of PCR was done as follows: 94°C for 4 minutes; five cycles of 94°C for 30 seconds, 56°C for 1 minute (−1°C per cycle), 72°C for 1 minute; and 30 cycles of 94°C for 30 seconds, 51°C for 45 seconds, and 72°C for 1 minute, 20 seconds. The second round of PCR was 94°C for 4 minutes; 30 cycles of 94°C for 30 seconds, 53.5°C for 1 minute, and 72°C for 1 minute 20 seconds. The resulting amplified products were gel-purified (Zymogen, Zymo Research, Orange, CA,

<http://www.zymoresearch.com>), sub-cloned into the TOPO TA vector (Invitrogen), and sequenced using the M13F&R primers.

Results

Nuclear Transfer of Neural Stem Cell Lines

An important experimental requirement for assessing cloning efficiency is homogeneity of the donor cell population. Because no good markers are available to directly isolate NS cells from animals, we used a cell culture method that has been shown to produce and maintain a homogenous population of NS cells [19]. These cells can be clonally expanded and passaged indefinitely and induced to differentiate into neurons and astrocytes both in vitro (supplemental online Fig. 1) and in vivo [19].

We used three lines for NT: NS5, NSV65, and Cor1-5. NS5 and NSV65 were derived from differentiation in vitro of strain 129 46C ES cells [21] and hybrid 129/B6 V6.5 ES cells [22], respectively. Cor1-5 is a clone of Cor1 [19] derived from the frontal cortex of an MF1 albino outbred E16.5 embryo. We performed nuclear transfer from these cells in seven independent experiments. The resulting embryos were cultured to the blastocyst stage and then explanted on to MEF-coated plates for ES cell derivation. The efficiency of ES derivation was recorded and compared with previous data from our laboratory on deriving ES cells after nuclear transfer from donor ES cells, embryonal carcinoma cells, or differentiated neurons (Table 1). In total, 14 embryonic stem cell lines were produced from 22 NS cell NT-derived blastocysts with an average efficiency of 64%. The lowest efficiency, 50%, was found when deriving ES cells from the outbred MF1 genetic background (Cor1-5 NS cell donors). These efficiencies are similar to what was previously seen when embryonic stem cells or embryonal carcinoma cells were used as donors (~50%) [8] and more than 6 times more efficient than previous work in our laboratory using differentiated neurons as donors (~8%, [6]). Furthermore, the efficiency of ES cell derivation from fertilization-derived blastocysts is 50%–80% (R.B., Z.W., and A.M., unpublished data). We conclude that ES cell derivation is significantly more efficient following nuclear transfer of neural stem cell nuclei than with nuclei from neurons or other differentiated cell types previously tested.

Methylation of Oct4 and Nanog Promoters in NS Cells

The transcription factors Oct4, Sox2, and Nanog have essential roles in early development and are required for the propagation of undifferentiated ES cells in culture [23]. Upon differentiation, Oct4 and Nanog are repressed, whereas Sox2 remains active in NS cells [19]. We performed Northern analysis to confirm the expression status of the three genes in NS and their corresponding NT-derived ES cells. Figure 1 shows that, as expected, the NS cells did not express Oct4 and Nanog but did express Sox2. In contrast, the NT-derived ES cells expressed all three genes.

Transcriptional silencing of Oct4 during differentiation has been correlated with de novo methylation of its promoter [15–17]. A possible explanation for the efficient derivation of ES cells from NS cells following nuclear transplantation could be that the promoters of essential early embryonic genes such as *Oct4* remain relatively hypomethylated in NS cells compared with their differentiated counterparts, thus facilitating nuclear reprogramming. We therefore investigated whether the promoters of Oct4 and Nanog were differentially methylated in NS cells relative to earlier and later stages of differentiation. For this, we performed bisulfite sequencing on DNA isolated from ES cells, NT ES cells, NS cells, and mature neural tissue. The results, depicted in Figure 2, show that both promoters were densely methylated in NS cells and adult brain relative to the wild-type and NT-derived ES cells. Therefore, the

methylation status of these two promoters cannot explain the high efficiency of deriving ES cells from NS cell NT blastocysts.

NT of *Dnmt1* Hypomorphic Fibroblasts

Global demethylation is a hallmark of early development. During cleavage development of the early mouse embryo, the genome is globally demethylated and then remethylated in a stereotypical fashion [24]. However, following nuclear transfer, cloned embryos show variable and incomplete demethylation and premature remethylation [10-12]. Therefore, we asked whether a global decrease of DNA methylation in the donor cell prior to nuclear transfer would improve reprogramming efficiency.

We have previously generated a hypomorphic allele, *Chip*, of the DNA methyltransferase DNMT1 that, when heterozygous with a null allele of DNMT1, results in a globally hypomethylated genome [25]. *Chip*/null compound heterozygous mice survive but are runted and develop tumors [25]. Tail tip fibroblasts were derived from *Chip*/null and control mice. Bisulfite sequencing of the fibroblast DNA showed partial methylation of the Oct4 promoter and little to no methylation of the Nanog promoter in *Chip*/null mice as compared with wild-type controls (Fig. 3). Importantly, although the nanog promoter was unmethylated, nanog was not expressed in the *Chip*/null fibroblasts (supplemental online Fig. 2). Nuclei from the hypomethylated and wild-type fibroblasts were transferred into enucleated oocytes and cultured to the blastocyst stage that were explanted onto MEF-coated plates and cultured to derive ES cells. Strikingly, the globally hypomethylated donor fibroblasts showed a three-fold increase in the efficiency of ES cell derivation (Table 2). This suggests that DNA hypomethylation enhances the efficiency of ES derivation from NT blastocysts, presumably by altering the epigenetic state of the genome rendering it more susceptible to the “reprogramming factors” of the egg.

Pluripotency of NT-Derived ES Cells

To confirm pluripotency of the ES lines derived following NT of NS cells and hypomethylated fibroblasts, we produced chimeric mice. ES cells derived from the cor1-5 NS cells contributed to the coat color in adult mice in five of five ES lines tested. Coat color contribution varied from approximately 10% to approximately 50% (Fig. 4A). To further test contribution to chimeras, one of the ES lines from each of the Cor1-5 NS cells and the *chip*/null fibroblast experiments, was targeted with an eGFP reporter gene ubiquitously expressed from the ROSA26 locus during embryonic development [8]. These cells were injected into wild-type blastocysts, transferred to surrogate mothers, and allowed to develop to E14.5. Analysis of the resulting E14.5 embryos showed broad contribution of the NS cell-derived ES cells (Fig. 4B, 4C). In control experiments, the NS cells were labeled by infecting with lentivirus ubiquitously expressing eGFP from a CMV reporter [26], injected into blastocysts, and transferred to surrogate mothers as above. None of the resulting E14.5 embryos showed GFP expression (0 of 13 embryos), confirming that unlike ES cells, the neural stem cells cannot contribute to early embryonic development. NT ES cells derived from *Dnmt1* hypomorphic fibroblasts broadly contributed to E14.5 embryos (Fig. 4D-4F). We conclude that NT blastocysts from both NS cells and hypomethylated fibroblasts produce pluripotent ES cells at high efficiencies, similar to that seen with ES cell donors.

Discussion

The results described in this paper show that the differentiation and methylation status of the donor cell nucleus can strongly influence the efficiency of deriving ES cell lines from nuclear transfer-derived blastocysts. NS cell donors produced embryonic stem cell lines at a rate of greater than or equal to 50%, which is comparable to that seen for ES and embryonal

carcinoma cells [8]. In contrast, well-defined differentiated donor cells, such as lymphocytes [3], NK cells [4], and neurons [6] produced NT ES cell lines at a rate of <10%. Furthermore, using a hypomorphic allele of DNMT1, we found that global hypomethylation of fibroblasts prior to NT also improved the efficiency of ES cell derivation from NT-derived blastocysts (65% for hypomethylated vs. 25% for wild-type fibroblasts). Together, these results show that the epigenetic state of the donor genome plays an important role in determining cloning efficiency.

Nuclear reprogramming is used here to describe the transition of the donor genome from an epigenetic state that is characteristic for the somatic cell to one that is characteristic of the early embryo. It can be assessed by evaluating abnormalities in gene expression, DNA methylation, and histone modifications in NT-derived embryos or adult clones [13,14,24,27,28]. Reprogramming can also be measured functionally by evaluating clone development at several different levels, including (a) the rate of blastocyst formation, (b) the fraction of survival to birth or to adulthood after implantation of NT-derived embryos into the uterus, and (c) as the frequency of deriving pluripotent ES cells after explantation of cloned blastocysts in culture. However, a number of parameters make it difficult to compare results between experiments and, therefore, functionally quantify the efficiency of reprogramming. These parameters are especially prevalent during cleavage development. For example, damage induced during the NT process leads to a block in early cleavage development and is variable from experiment to experiment. Also, the cell cycle stage of the donor cells critically effects cleavage development. Donor cells need to be in G0, G1, or G2 for successful cleavage development of clones [29-31]. Thus, when fibroblasts arrested at G0 by serum starvation are used as nuclear donors, the rate of development to the blastocyst stage has been reported to be as high as 70%, whereas ES cells that are rapidly cycling give rates in the 10%–15% range. The NS cells used in this study cycle at a rate similar to that of ES cells (R.B., unpublished observation) and also show a low rate of blastocyst development. Therefore, to control for experimental variables associated with poor cleavage development but not necessarily epigenetic reprogramming, we used the efficiency of ES cell derivation from explanted cloned blastocysts as the criterion for reprogramming efficiency. Blocks in development due to the nuclear transfer manipulations and cell cycle asynchrony occur prior to the blastocyst stage and do not appear to significantly effect postblastocyst development into ES cells. This is reflected by the high degree of reproducibility when comparing efficiencies of development from the blastocyst stage onward using similar donor cells [7,22]. Furthermore, unlike the efficiency of producing term pups from NT embryos, the derivation of ES cells from NT embryos is not strongly influenced by genetic background ([22] vs. [7] and this study). Therefore, the differences in the efficiencies of ES derivation from NT blastocysts described here likely reflect differences in the efficiency of reprogramming between donor epigenetic states rather than damage inherent to NT manipulations, cell cycle asynchrony, or the genetics of the donor nucleus. Furthermore, relative to full-term development of clones, the production of NT ES cells occurs in high enough numbers to make meaningful numerical comparisons possible.

During cleavage, the genome undergoes a stereotypical wave of demethylation followed by remethylation. Although it has been shown that global demethylation and histone acetylation are abnormal in cloned embryos [9-12], a clear causal relationship of these abnormal epigenetic conformations with reprogramming efficiency or clone survival has not been established. To test whether the level of DNA methylation affects reprogramming efficiency, we compared the rate of ES cell generation from blastocysts derived from wild-type fibroblasts with that of cloned blastocysts derived from fibroblasts carrying a *Dnmt1* hypomorphic allele. Our results show that global hypomethylation did not affect the cleavage rate of cloned embryos but significantly increased the fraction of blastocysts that generated ES cells. These results are consistent with the conclusion that hypomethylation

sensitizes the somatic donor genome to the reprogramming activities of the egg cytoplasm. Previous attempts to improve reprogramming efficiency by changing the chromatin state or the DNA methylation level using drugs such as the demethylating drug 5-aza-2-deoxycytidine were unsuccessful [32,33]. This likely reflects the toxicity associated with these drugs [34] rather than the effect on the epigenetic state of the donor nucleus.

Expression of Oct4 and Nanog is essential for early embryonic development and the establishment and maintenance of ES cell lines. Oct4 often fails to be expressed appropriately following nuclear transfer [13,14], and its expression correlates tightly with the methylation status of its promoter [15-17]. When the methylation patterns of the Oct4 and Nanog promoters were analyzed in the different cell types, we found dense methylation in both the NS cells and differentiated neural tissue, consistent with gene inactivity, in contrast to the lack of methylation and active transcription in ES cells. Thus, the higher reprogramming efficiency of NS cells did not correlate with hypomethylation of these genes. It is possible that other genes not analyzed in our present study are differentially methylated in NS versus differentiated cells. Alternatively, other types of epigenetic modification may be involved that make the promoters of Oct4, Nanog, and similar pluripotency genes more amenable to demethylation and reactivation in NS cells than neurons.

Early amphibian cloning experiments demonstrated an inverse correlation between developmental age of embryonic endodermal cells and cloning efficiency [35-37]. More recently, Yamazaki et al. showed that clones derived from NT of neural cells isolated close to the ventricle developed more efficiently than those isolated close to the pial surface of late stage embryo brains [38]. Neural stem/progenitor cells reside in the ventricular zone. Do and Scholer have shown recently that the neural sphere cells from E14 embryos are not more efficiently reprogrammed than cumulus cells following fusion to ES cells [39]. However, neurospheres consist of cells at different stages of differentiation, with only a small fraction being neural stem cells [40]. In studies, a heterogeneous population of donor cells was used, and therefore, the exact identity of the donor cells that produced the clones or pluripotent ES cell hybrids could not be ascertained. The NS cells used were a homogeneous, cultured population of NS cells, as shown both by functional tests and marker analysis [19]. It will be interesting to repeat these cloning experiments with NS cells isolated directly from animals. However, at present, no markers exist that allow for the direct purification of a homogeneous population of NS cells.

In this study, we have identified two parameters, the differentiation status and methylation state of the donor cell, that strongly influence the efficiency of ES cell derivation following NT. Because nuclear transfer-derived ES cells can be used to dissect mechanisms of disease [8,41] as well as a potential therapy for disease [42], it is of practical significance to identify means of improving reprogramming efficiency. It will be important to determine whether other somatic stem cells, which are more accessible than NS cells, and alternative means of altering the epigenetic state of the donor genome would also result in more efficient reprogramming.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Oct4 and Nanog are not expressed in neural stem (NS) cells but are expressed in derivative NT ES cells. Northern analysis of Oct4, Nanog, and Sox2 expression in wild-type ES cells, NS cells, and NT-derived ES cells from the three independent neural stem cell lines, NS5, NSV6.5, and Cor1-5. Nanog shows two bands. The upper band corresponds to the expected size for the Nanog transcript. The nature of the lower band is unknown. Abbreviations: ES, embryonic stem; NSC, neural stem cell; NT, nuclear transfer; WT, wild-type.

Figure 2.

Bisulfite sequencing of the Oct4 and Nanog promoters in the three NS cell lines and NT-derived ES cells from the Cor1-5 NS cell line. Wild-type ES cells and whole adult brain are shown as controls. Differentially methylated CpG sites are depicted below the bar graphs as closed circles along the promoters of Oct4 and Nanog. Bars represent the percentage of sequence reads that showed methylation for each site. Number of reads for each line is shown in brackets. Four independent cell lines were used in the Cor1-5 analysis. Abbreviations: ES, embryonic stem; NT, nuclear transfer; WT, wild-type.

Figure 3. Bisulfite sequencing of hypomethylated (chip/c) versus wild-type fibroblasts. Abbreviation: WT, wild-type.

Figure 4.

NT-derived ES cells are pluripotent. NT-derived ES cells from both neural stem (NS) cell (A–C) and hypomethylated tail tip fibroblast (D–F) donors are pluripotent. Representative NT ES cell lines were targeted with a ubiquitously expressed green fluorescent protein (GFP) (R26-GFP). Resulting ES cells were injected into wild-type blastocysts to produce chimeras. Chimerism was analyzed in adults by coat color contribution (A) and anti-GFP antibody staining in E14.5 embryos. The NS cells were derived from outbred mice with white coat color and were injected into wild-type 129/B6 F2 blastocysts, which produce tan, brown, and black coat color adults. Therefore, any white coat color must derive from the NT ES cells. Darkly labeled cells represent positive GFP staining. Contribution to all three germ layers was seen including ectoderm ([A], melanocytes; [D], neural tissue), mesoderm ([B, E], cartilage), and endoderm ([C], intestine; [F], liver). Abbreviations: ES, embryonic stem; NSC, neural stem cell; NT, nuclear transfer; TT, tail tip.

Table 1

Nuclear transfer of NS cells

Cell line (genetic background)	No. pronuclei	No. of two-cell embryos	No. of four-cell embryos	No. of blastocysts	No. of NT ES cell lines (% of blasts)
NS5 (129)	93	73	23	5	5 (100)
NSV6.5 (B6/129)	46	22	13	5	3 (60)
Cor1-5 (MF1)	234	102	75	12	6 (50)
NS cell total	373	197	111	22	14 (64)

Abbreviations: ES, embryonic stem; NS, neural stem.

Table 2

Nuclear transfer of tail tip fibroblasts

Cell line (genetic background)	No. pronuclei	No. of two-cell embryos	No. of four-cell embryos	No. of blastocysts	No. of NT ES cell lines (% of blasts)
WT (B6/129)	95	42	35	12	3 (25%)
WT (129)	103			18	4 (22%)
Chip/C (129)	66	24	16	11	7 (65%)

Abbreviations: ES, embryonic stem; NT, nuclear transfer; WT, wild type.