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Nuclear reprogramming in zygotes

CHANCHAO LORTHONGPANICH[1], DAVOR SOLTER[1,2] and CHIN YAN LIM*[,1]

[1]*Mammalian Development Laboratory, Institute of Medical Biology, A*STAR, Singapore and*
[2]*Cancer and Stem Cell Biology Program, DUKE-NUS Graduate Medical School, Singapore*

ABSTRACT Nuclear reprogramming, the conversion of the epigenome of a differentiated cell to one that is similar to the undifferentiated embryonic state, can be facilitated by several methods, such as nuclear transfer, cell fusion, use of embryonic stem cell extracts, and more recently, by the introduction of exogenous transcription factors. Amongst these various strategies, somatic cell nuclear transfer (SCNT) is, by far, the most effective method of nuclear reprogramming. The majority of SCNT studies have been carried out using enucleated mature oocytes, as reprogramming is efficient and can be completed within hours following the introduction of the somatic cell nuclei into the recipient oocyte. Fertilized eggs, on the other hand, were regarded as poor recipients for nuclear transfer, as previous studies showed that embryonic blastomeres transferred into enucleated zygotes were unable to develop to blastocysts. However, more recent studies have demonstrated that the method of enucleation and the cell cycle phase of the embryos can impact the success of somatic cell reprogramming when zygotes were used as nuclear recipients. It is, therefore, timely to revisit and further explore the nuclear reprogramming capacity of zygotes as recipients for SCNT. Assessment of the various factors that influence the reprogramming capacity of zygotes in SCNT also provide hints of the mechanistic nature of nuclear reprogramming.

KEY WORDS: *nuclear transfer, reprogramming, zygote, embryo, SCNT*

During nuclear reprogramming, the cell-type specific epigenetic program of a differentiated cell is erased and the somatic genome re-acquires the potential to give rise to other cell types. In somatic cell nuclear transfer (SCNT), differentiated donor cells or nuclei are introduced into the cytoplasm of enucleated recipient cells to induce de-differentiation of the somatic genome to an embryonic state. Successful nuclear reprogramming will reset the transferred genome to totipotency. Thus, complete reprogramming of somatic genome via SCNT into embryos would result in normal embryonic development, giving rise to cloned animals that are genetically identical to the nuclear donor. The first mammal cloned from adult cell nuclei, Dolly the sheep, was successfully generated by SCNT in which nuclei of terminally differentiated mammary gland cells were transferred into enucleated mature oocytes and reprogrammed to a totipotent state (Wilmut *et al.*, 1997). Since this breakthrough work, many other mammals such as mice (Wakayama *et al.*, 1999), cattle (Kato *et al.*, 1998), pigs (Polejaeva *et al.*, 2000), cats (Shin *et al.*, 2002), rabbits (Chesne *et al.*, 2002; Li *et al.*, 2006a), dogs (Lee *et al.*, 2005), rats (Zhou *et al.*, 2003), buffalos (Shi *et al.*, 2007), ferrets (Li *et al.*, 2006b), and camels (Wani *et al.*, 2010) have been cloned by SCNT into enucleated oocytes (Table 1). However, in the majority of cloning experiments, reprogramming of the transplanted nuclei is often incomplete, resulting in embryonic arrest, high abortion rates, defective placentas, and neonatal abnormalities in the cloned animals (Hill *et al.*, 2000; Hill *et al.*, 2002; Ogura *et al.*, 2002; Ono and Kono, 2006; Young *et al.*, 1998).

The efficiency of SCNT is largely influenced by the reprogramming capacity of the recipient cytoplasm. Thus far, all successful mammalian cloning experiments were performed with the transfer of somatic cell/nuclei into enucleated metaphase II (MII) oocytes. These studies indicate that the ooplasm of MII oocytes contains all the necessary factors to efficiently reprogram the differentiated somatic cell nuclei and support embryonic development. It appears, however, that the reprogramming capacity of oocytes declines rapidly after ovulation, and aged oocytes cannot fully reprogram the transplanted nuclei (Hall *et al.*, 2007; Wakayama

Abbreviations used in this paper: ECNT, embryonic cell nuclear transfer; ES, embryonic stem; GV, germinal vesicle; ICM, inner cell mass; MII, metaphase II; SCNT, somatic cell nuclear transfer; ZGA, zygotic gene activation.

***Address correspondence to:** Chin Yan Lim. Institute of Medical Biology, 8A Biomedical Grove, #06-06 Immunos, Singapore 138648, Singapore. Fax: +65- 6464-2048. e-mail: chinyan.lim@imb.a-star.edu.sg

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TABLE 1

DIFFERENT SPECIES OF MAMMALS CLONED BY SCNT USING METAPHASE II OOCYTES AS RECIPIENT CYTOPLASTS

Species	Donor nuclei	No. manipulated	No. of live born (% of manipulated)	Reference
Sheep	Mammary gland cells	434	1 (0.2)	Wilmut et al., 1987
Cattle	Cumulus cells	99	5 (5)	Kato et al., 1998
	Oviductal cells	150	3(2)	
Mouse	Cumulus cells	2468	31 (1)	Wakayama et al., 1999
Pig	Granulosa cells	245	5 (2)	Polejaeva et al., 2000
Cat	Cumulus cells	87	1 (1)	Shin et al., 2002
Rabbit	Cumulus cells	775	6 (0.8)	Chesne et al., 2002
	Ear fibroblasts	975	14 (1)	Li et al., 2006a
Rat	Fetal fibroblasts	129	3 (2)	Zhou et al., 2003
Dog	Ear fibroblasts	1,095	2 (0.1)	Lee et al., 2005
Ferret	Cumulus cells	487	2 (0.4)	Li et al., 2006b
Buffalo	Fetal fibroblasts	236	2 (0.8)	Shi et al., 2007
	Granulosa cells	256	1 (0.3)	
Camel	Cumulus cells	77	1 (2)	Wani et al., 2010

et al., 2007). Early experiments investigating the nuclear reprogramming capacity of fertilized eggs also indicate that the zygotic cytoplasm does not support efficient reprogramming of the transferred nuclei (Howlett *et al*., 1987; McGrath and Solter, 1984; Robl *et al*., 1986; Tsunoda *et al*., 1987; Wakayama *et al*., 2000). It is unclear why this should be the case, given that epigenetic reprogramming of the male and female pronuclei occurs naturally upon fertilization during normal development. Were these reprogramming factors somehow degraded, sequestered or removed during the nuclear transfer process? What are the differences in the cytoplasmic contents of MII oocytes and zygotes that influence the ability to reprogram? In this review, we seek to highlight new insights into the mechanism of nuclear reprogramming gleaned from recent studies investigating the use of zygotes in nuclear transfer.

Nuclear transfer into zygotes

For more than two decades, zygotes have been regarded as poor recipients for nuclear transfer. Illmensee and Hoppe reported the first successfully cloned mouse via the transfer of inner cell mass (ICM) nuclei into microsurgically enucleated zygotes (Illmensee and Hoppe, 1981). However, the results have never been reproduced. In subsequent experiments by McGrath and Solter, nuclear transfer into zygotes was successful only when the authors performed pronuclei exchange between two fertilized zygotes but not when nuclei from cells at later embryonic stages were transferred (McGrath and Solter, 1983; McGrath and Solter, 1984). Embryos generated by pronuclear exchange developed into blastocysts *in vitro* which could give rise to live born mice *in vivo*. In contrast, zygotes reconstructed with nuclei from preimplantation blastomeres (4- and 8- cell stages) or ICM cells failed to form blastocysts in culture. These results led to the conclusion that mouse blastomere nuclei transferred into enucleated zygotes cannot support development *in vitro* (McGrath and Solter, 1984). The view that zygotes cannot efficiently reprogram differentiated

TABLE 2

MAMMALIAN NUCLEAR TRANSFER INTO ZYGOTES

Species	Nuclear recipient	Enucleation method	Donor nuclei	No. manipulated	Developmental stage reached	No. live born (% of manipulated)	Reference
Mouse	I-zygote	CE*	Inner cell mass	179	Term	3 (2)	Illmensee and Hoppe, 1981
			Trophectoderm	369	Blastocyst	N/A	
Mouse	I-zygote	CE	Pronuclei	67	Term	10 (15)	McGrath and Solter, 1983
Mouse	I-zygote	CE	2-cell	174	Blastocyst	N/A	McGrath and Solter, 1984
			4-cell	84	Morula	N/A	
			8-cell	116	< Morula	N/A	
			Inner cell mass	101	< Morula	N/A	
Mouse	I-zygote	CE	8-cell	32	2-4 cell	N/A	Robl et al., 1986
Mouse	I-zygote	CE	2-cell	76	Blastocyst	N/A	Howlett et al., 1987
			8-cell	170	< Blastocyst	0 (0)	
Mouse	I-zygote	CE	2-cell	74	Term	3 (4)	Tsunoda et al., 1987
			4-cell	64	< Morula	N/A	
			8-cell	50	< Morula	N/A	
Mouse	I-zygote	CE	2-cell	31	Blastocyst	N/A	Smith et al., 1988
			4-cell	50	< Morula	N/A	
			8-cell	24	< Morula	N/A	
Rat	I-zygote	CE	Pronuclei	72	Term	9 (13)	Kono et al., 1988
			2-cell	63	2-cell	0 (0)	
			4-cell	23	2-cell	0 (0)	
			8-cell	52	2-cell	0 (0)	
Rabbit	I-zygote	CE	8-cell	196	Blastocyst	0 (0)	Modlinski and Smora, 1991
			16-cell	78	Blastocyst	N/A	
Monkey	I-zygote	CE	4- to 32-cell	15	< Blastocyst	N/A	Meng et al., 1997
Mouse	I-zygote	CE	Cumulus cells	164	< 8-cell	N/A	Wakayama et al., 2000
Rat	I-zygote	CE	2-cell	128	Blastocyst	0 (0)	Shinozawa et al., 2004
Mouse	I-zygote	SE	8-cell	129	Term	6 (5)	Greda et al., 2006
Mouse	M-zygote	CE	2-cell (M)	90	Term	12 (13)	Egli et al., 2007
			8-cell (M)	30	Term	2 (7)	
			ES cells (M)	1,093	Term	9 (0.8)	
			Tail fibroblasts (M)	775	Blastocyst	N/A	
Human	I-zygote	CE	Foreskin (I)	13	No cleavage	N/A	Fan et al., 2009
	M-zygote	CE	Foreskin (M)	92	< 8-cell	N/A	

I: interphase, M: metaphase, CE: complete enucleation, SE: selective enucleation.* It is unclear whether pronuclei were ruptured during enucleation. N/A: manipulated embryos were not transferred to pseudopregnant recipients.

nuclei was reinforced by many other unsuccessful attempts at cloning different mammals via the transfer of somatic, pre-implantation blastomere or embryonic stem (ES) cell nuclei into zygotes (Cheong *et al.*, 1993; Howlett *et al.*, 1987; Meng *et al.*, 1997; Modlinski and Smorag, 1991; Robl *et al.*, 1986; Shinozawa *et al.*, 2004; Smith *et al.*, 1988a; Tsunoda *et al.*, 1987; Wakayama *et al.*, 2000). In all these studies, the zygotes failed to support development to blastocysts when reconstructed with nuclei derived from cells later than two-cell stage blastomeres (Table 2).

However, the notion that zygotes are poor nuclear recipients has more recently been revised. Liveborn pups obtained from enucleated pronuclear zygotes reconstructed with interphase nuclei of 8-cell stage blastomeres were reported (Greda *et al.*, 2006). This was followed by a report of mice born from reconstructing metaphase zygotes, depleted of nuclear material, with chromosomes extracted from mitotically-arrested 2-cell, or 8-cell blastomeres or ES cells (Egli *et al.*, 2007). In addition, normal and polyspermic zygotes were shown to be capable of reprogramming the somatic genome, and supported development to blastocyst stage after reconstitution with mitotic donor cells (Egli *et al.*, 2007; Fan *et al.*, 2009). In these reports, donor nuclei at corresponding stages of the cell cycle were transferred into zygotic cytoplasts enucleated at pronuclear or mitotic phase. Recent experiments, however, have demonstrated that cell cycle synchrony between donor nucleus and the recipient zygote at the time of transfer is not requisite for successful reprogramming (Egli and Eggan, 2010). Using donor and recipient cells at different stages of the cell cycle, the authors demonstrated that the major determinant of nuclear transfer efficiency is the availability of nuclear reprogramming factors in the reconstructed embryo.

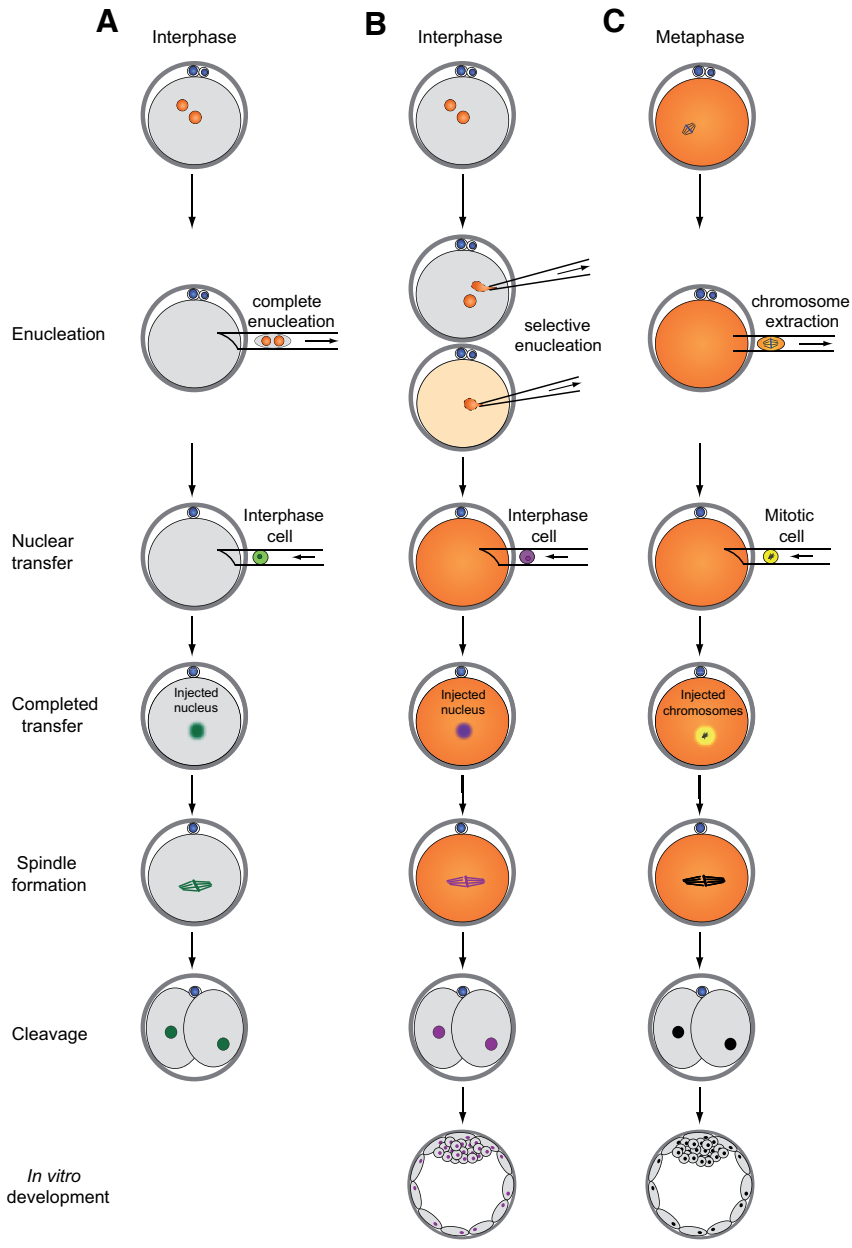


Fig. 1. Nuclear transfer into zygotic cytoplasts. *Pronuclear zygotes are enucleated using either wide-bore (15-20 μm) or narrow (1-2 μm) pipettes, resulting in complete* **(A)** *or selective* **(B)** *enucleation. Metaphase-arrested zygotes are enucleated by the removal of the mitotic spindle and chromosomes* **(C)**. *Zygotic cytoplasts at pronuclear or mitotic phases are reconstituted with donor nuclei at corresponding stages of cell cycle. Putative nuclear reprogramming factors (orange) are released into the cytoplasm upon breakdown of the pronuclear envelope during mitosis or through mechanical tearing by the enucleation pipette. Nuclei transferred into* **(A)** *cytoplasts will not be reprogrammed and the resultant embryos do not develop pass the first cleavage. Nuclei transferred into (B) and (C) cytoplasts will be reprogrammed and the cloned embryos can develop further.*

Localization of nuclear reprogramming factors

The major difference between these recent studies and previous unsuccessful attempts at using zygotes in cloning, lies in the breakdown of zygotic pronuclear structures before or upon enucleation. In the earlier studies, zygotes used as nuclear recipients were enucleated through the removal of intact pronuclei with wide-bore pipettes of 15-20 μm tip diameter (Fig. 1). This enucleation method results in the removal of all pronuclear material. In contrast, Greda *et al.* reported a technique of selective enucleation where a very thin pipette with a tip diameter of 1-2 μm was used to pull the pronuclear envelope out of the zygote with the chromatin attached (Greda *et al.*, 2006). This technique leads to tearing of the pronuclei, resulting in the release of pronuclear contents into the zygotic cytoplasm. In another study, Egli *et al.* used reversibly arrested mitotic zygotes as the nuclear recipient. Treatment of fertilized oocytes with nocodazole and MG-132 result in the zygotes arresting at metaphase. At this stage, the pronuclear envelope has broken down and condensed chromosomes attached to the mitotic spindle are aligned at the metaphase

plate. These zygotes can then be "enucleated" by removing the spindle alongside the attached chromosomes with a pipette (Egli *et al.*, 2007). Hence, in both selectively enucleated and metaphase zygotes, pronuclear contents were thought to become distributed in the cytoplasm (Fig. 1).

The finding that the cytoplasm of these zygotes now exhibits reprogramming activity previously thought to be lacking, led to the hypothesis that one or more factors necessary to support nuclear reprogramming are localized in the pronuclei. Hence, enucleation techniques that lead to the release of these factors into the cytoplasm will confer reprogramming capacity to the nuclear recipients (Fig. 2). In contrast, complete enucleation of intact pronuclei from interphase zygotes leads to the removal of these factors, leaving the cytoplasm unable to support nuclear reprogramming of the transferred somatic nuclei.

In the nuclear transfer experiments of Illmensee and Hoppe, it is unclear whether the technique of enucleation, with a pipette of 10 μm in diameter, resulted in pronuclei rupture and the release of nuclear contents into the cytoplasm (Illmensee and Hoppe, 1981). However, the hypothesized gain of reprogramming factors from leaked pronuclear material would not account for the reported differences in the developmental potential of zygotes reconstructed with nuclei of ICM versus trophectoderm (TE) cells. In addition, there are unresolved discrepancies in the pattern of glucose phosphate isomerase (GPI-1) activity in the reconstructed embryos. Oocyte-derived cytoplasmic maternal GPI-1 was inexplicably absent in these embryos, in contrast to studies that showed a distinct contribution of pre-existing maternal GPI-1 activity in reconstructed embryos (Gilbert and Solter, 1985). Hence, even in the light of recent reports of successful nuclear transfer into zygotes, the study carried out by Illmensee and Hoppe remains unproven and controversial.

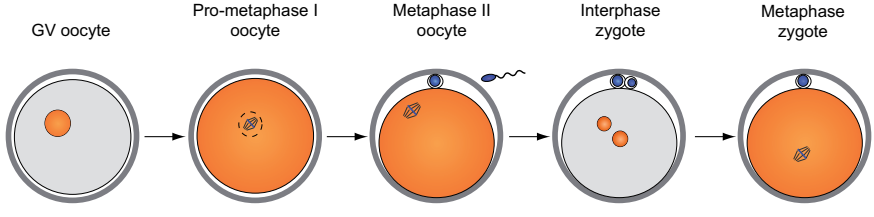
The hypothesis that reprogramming is mediated, at least in part, by molecules sequestered in the nucleus until nuclear envelope breakdown, is consistent with much of existing SCNT research. Most successful mammalian cloning were performed by SCNT into MII oocytes. This indicates the ooplasm of oocytes arrested at metaphase can support efficient nuclear reprogramming of transferred nuclei. More recently, early bovine zygotes were shown to support development of transferred somatic G1 phase nuclei when the maternal telophase II chromosomes and condensed sperm DNA were removed prior to pronuclei formation (Schurmann *et al.*, 2006). In addition, the cytoplasm of enucleated prometaphase I (pro-MI) stage oocytes was found to be capable of remodeling ES cell nuclei, resulting in pseudo-pronuclei formation (Gao *et al.*, 2002). This remodeling activity was, however, absent in germinal vesicle (GV) stage oocytes. Furthermore, selectively enucleated GV oocytes were able to support development to blastocysts when reconstructed with 2-cell blastomere nuclei, but complete enucleation abolished this ability (Mohammed *et al.*, 2008). Germinal vesicle material had also been shown to be required for male pronucleus formation upon fertilization of both mouse and porcine oocytes (Balakier and Tarkowski, 1980; Ogushi *et al.*, 2005). These findings suggest that vital reprogramming factors are contained within the germinal vesicles of these oocytes. The somatic nuclei reprogramming potential of other embryonic cell types, such as ES cells and 2-cell stage blastomeres, is also mediated by factors localized in the nucleus (Do and Scholer, 2004; Egli *et al.*, 2009; Landsverk *et al.*, 2002). However, whether reprogramming factors in these cells are the same as the factors active in oocytes and zygotes is not known.

Based on the successes of embryonic cell nuclear transfer (ECNT) in selectively enucleated or metaphase zygotes, it appears likely that some components of the pronuclei are essential to the nuclear reprogramming activity in zygotes (Egli *et al.*, 2007; Greda *et al.*, 2006). However, the capacity to completely reprogram and support preimplantation development after nuclear transfer likely requires multiple factors located in the nucleus as well as the cytoplasm. Recently, cytoplasmic lysates of mouse GV oocytes were shown to induce chromatin remodeling and DNA demethylation in somatic cell nuclei, and enhanced cloning efficiency when these nuclei were transferred into MII oocytes (Bui *et al.*, 2008). Thus, additional direct experiments will be required to determine whether factors localized in the nucleus are sufficient for the nuclear reprogramming capability of mammalian oocytes and zygotes, and to elucidate the identity of these factors.

Nuclear reprogramming during normal development

Epigenetic reprogramming is an important aspect of normal embryonic development. The male and female gametes are highly differentiated cells carrying genomes that have been epigenetically modified during the formation of primordial germ cells and gametogenesis. After fertilization, gamete-specific covalent modifications on the parental genomes have to be erased to allow incorporation of new epigenetic information permissive for the activation of embryonic transcription programs.

Reprogramming of the maternal genome is a process that takes place gradually over several cell divisions until blastocyst stage. Maternal DNA demethylation is a passive, replication-dependent process facilitated by the active exclusion of the oocyte-specific



Complete enucleation	-	+	+	-	+
Selective enucleation	+	NA	NA	+	NA

Fig. 2. Factors in the nuclei of nuclear recipients influence the success rate of reprogramming. *The release of nuclear components (orange) into the recipient cytoplasm, upon entry into mitosis or mechanical rupture of the nuclear envelope, is proposed to confer reprogramming capacity to the reconstructed cells. Successful nuclear transfer (NT) is indicated with (+), failed NT indicated with (-). NA, technique was not applicable; GV, germinal vesicle.*

DNA methyltransferase Dnmt1o from the nucleus in preimplantation embryos, except at the 8-cell stage (Doherty *et al.*, 2002; Grohmann *et al.*, 2005). The failure to maintain methylation on newly replicated DNA leads to gradual global demethylation except at certain protected regions such as imprinted genes, pericentric heterochromatin, and some endogenous retroviral loci (Armstrong *et al.*, 2006). The maternal genome is prepackaged with histones in the oocyte, and has a chromatin configuration containing high levels of H3 histones methylated on lysines 4, 9 and 27, and trimethlyated histone H4 lysine 20. (Erhardt *et al.*, 2003; Lepikhov and Walter, 2004; Liu *et al.*, 2004; van der Heijden *et al.*, 2005). In particular, DAPI-intense pericentric chromatin marked by H3K9me3 and H4K20me3 is present only on the maternal pronuclei (Puschendorf *et al.*, 2008; Santos *et al.*, 2005; van der Heijden *et al.*, 2005). These histone methylation states are maintained in the zygote, and might be involved in preserving the DNA methylation status of the maternal genome at this stage.

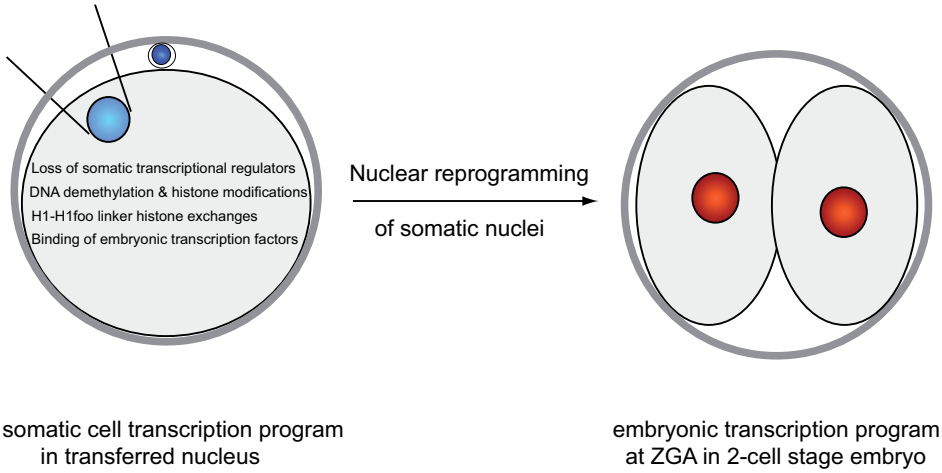


Fig. 3. Nuclear reprogramming of somatic nuclei has to occur in the mouse zygote before initiation of major zygotic gene activation (ZGA) at the 2-cell stage. *The transferred genome (blue) contains cell-specific epigenetic modifications and is associated with factors that direct a somatic cell transcription program. Nuclear reprogramming involves 1) loss of the somatic transcriptional regulators, 2) changes to the DNA methylation and histone modification patterns, 3) exchange of somatic H1 for oocyte-specific H1foo linker histones, and 4) binding of embryonic transcription factors. The reprogrammed chromatin (red) thus acquires epigenetic status permissive for expression of the embryonic transcription program during ZGA.*

The paternal genome, on the other hand, undergoes extensive remodeling in the zygote. Highly basic protamines that densely pack the sperm DNA become rapidly replaced by maternally provided histones in the zygote. This protamine-histone replacement allows the paternal genome to acquire a chromatin state that is enriched in hyper-acetylated histones (Adenot *et al.*, 1997). In addition, prior to the first DNA replication, the paternal chromatin contains only the replication-independent H3.3 variant, while the canonical H3.1/H3.2 histones are deposited at S phase (van der Heijden *et al.*, 2005). Different methylation modifications on histone H3 are also subsequently added throughout the pronuclear stages (Lepikhov and Walter, 2004; Liu *et al.*, 2004; Santos *et al.*, 2005). Constitutive heterochromatin in the paternal pronuclei become marked with histone H3K27 trimethylation, facilitating the recruitment of the polycomb group PRC1 complex for transcriptional silencing of pericentric major satellites (Puschendorf *et al.*, 2008). The paternal DNA also undergoes a rapid active demethylation process that is completed within hours after fertilization, before the onset of DNA replication (Mayer *et al.*, 2000; Oswald *et al.*, 2000; Rougier *et al.*, 1998). The demethylation mechanism is largely undefined though it has been suggested to operate via indirect, repair-mediated pathways involving cytidine deaminases AID and Apobec1, or Gadd45a-associated nucleotide-excision repair (Barreto *et al.*, 2007; Bhutani *et al.*, 2010; Morgan *et al.*, 2004; Morgan *et al.*, 2005; Santos *et al.*, 2002). In support of a DNA repair-induced demethylation mechanism, phosphorylated gH2A.X, a marker of DNA strand breaks, was shown to accumulate with dynamics coinciding with 5-methylcytosine depletion on the paternal genome (Wossidlo *et al.*, 2010). Interestingly, a recent study has also implicated the transcription elongator complex in a direct demethylation reaction that utilizes the s-adenosylmethionine radical (Okada *et al.*, 2010). In addition, members of the Tet (ten-eleven translocation) protein family were shown to catalyze the conversion of 5-methylcytosine (5mC) to 5-hydroxylmethylcytosine (5hmC), which could be an intermediate step in a direct DNA demethylation process (Ito *et al.,* 2010, Tahiliani *et al.,* 2009). Despite the identification of these proteins which are potentially involved in mediating active paternal genome demethylation, the actual mechanism of the process remains to be clearly elucidated.

The histone code hypothesis puts forward the idea that different combinations of covalent modification on the N-terminal domains of histones, are recognized by other proteins which mediate downstream processes such as transcriptional activation or repression (Strahl and Allis, 2000). Acetylation on specific lysine residues on H3 and H4 is proposed to result in open chromatin states permissive for transcription factor binding to cognate sequences to mediate gene activation. In contrast, methylation on H3K9 and H3K27, as well as DNA methylation, leads to transcriptional repression and gene silencing. The dramatic changes in the histone modification and DNA methylation status of the male and (to a lesser extent) female genomes are, therefore, presumably necessary to create diploid chromatin that is permissive for the initiation of an embryonic transcription program directed by specific transcription factors during zygotic gene activation (ZGA).

Reprogramming of transferred nuclei in zygotes

Successful cloning requires reprogramming of the transferred somatic nuclei by the recipient cell (Hiiragi and Solter, 2005). In contrast to reprogramming of parental genomes during normal embryonic development, reprogramming of the somatic nuclei in SCNT is a much more complicated process. Aside from changing a pre-existing differentiated cell-specific transcription program to one encoding the embryonic state, other processes such as

reversal of X-inactivation and telomere extensions must take place post-zygotically to restore totipotent developmental potential. For the purposes of this review, however, we will discuss only the reprogramming events that are required prior to the initiation of ZGA. For SCNT in mouse zygotes to be successful, the somatic genome must undergo changes to permit the precise expression of the embryonic transcription program initiated during ZGA at the two-cell stage. Hence, reprogramming of somatic nuclei requires 1) loss of differentiated cell-specific transcription and chromatin factors, particularly at repressed embryonic gene loci, 2) appropriate changes to DNA methylation and histone modification patterns, 3) chromatin remodeling via nucleosomal positioning and exchange of H1 linker histones, and 4) binding of embryonic transcription factors to activate embryonic gene expression (Fig. 3). These reprogramming events would have to occur in the time the reconstructed embryo takes to develop from the transcriptionally quiescent state of the zygote, to major ZGA at the 2-cell stage. For nuclear transfer into interphase zygotes, this process takes approximately 24 hrs, while reconstructed metaphase zygotes undergo the first cell cleavage just 120 min after chromosome transfer (Egli *et al.*, 2007).

Disassociation of somatic transcriptional regulators from transferred nuclei

The somatic nucleus carries not only chromosomal DNA but also proteins involved in maintaining the identity of the cell from which it was taken. Transcriptional activators are bound at somatic gene loci, while repressors and co-repressors are associated at embryonic gene regions to keep them transcriptionally silenced. The activation of the embryonic transcription program and reprogramming of somatic nuclei to a totipotent state requires a reciprocal binding of transcriptional activators and repressors. Hence, removal of somatic DNA- and chromatin- associated proteins is necessary to allow embryonic regulatory factors to access and activate or repress a new set of genes. Indeed, nuclear swelling is observed in somatic nuclei transferred into oocytes and interphase zygotes, indicative of extensive exchanges of proteins between the cytoplasm and the transferred nuclei. It has also been shown that transcription factors and chromatin-associated proteins such as TATA-box binding protein (TBP), histone deacetylase (HDAC2), and the ATP-dependent chromatin remodeler Brahma-related gene 1 (Brg1) are lost from the somatic nuclei and dispersed into the oocyte cytoplasm within 2 hrs of nuclear transfer, at the time of premature chromosome condensation (Gao *et al.*, 2007). Results from the chromosome transfer experiments performed by Eggan and colleagues also support the view that the loss of transcriptional activators and repressors during mitosis could be a critical reprogramming event (Egli *et al.*, 2008). During mitosis, many sequence-specific transcription factors such as HSF1, Sp1, and C/EBP, as well as some basal factors (TFIIB, TAF1 and TBP) were found to dissociate from the condensed chromosomes (Martinez-Balbas *et al.*, 1995; Sun *et al.*, 2007). Likewise, transcriptional repressors such as heterochromatin protein 1 (HP1), BMI-1 and other polycomb group proteins were also detached from the mitotic chromosomes (Hayakawa *et al.*, 2003; Minc *et al.*, 1999; Miyagishima *et al.*, 2003; Murzina *et al.*, 1999; Voncken *et al.*, 1999). Extensive dissociation of transcriptional regulators from the somatic nuclei and chromosomes upon nuclear transfer allows the DNA to become more accessible to other factors present in the recipient zygotes, thus enhancing the reprogramming process. Recently, it was found that the efficiency of reprogramming could be correlated to the degree of dissociation of TBP, TFIIB and HP1 from somatic nuclei transferred into MII-oocytes (Gao *et al.*, 2007). Hence, depletion of somatic DNA-transcriptional regulator interactions is likely to be an essential reprogramming event during SCNT.

Epigenetic modifications of DNA and histones

During reprogramming, the somatic nuclei also have to undergo extensive changes in DNA methylation and histone modification patterns to erase the existing differentiated cell-specific marks and acquire a new set of epigenetic information. However, the somatic genome, already packaged with histones, is unlikely to undergo the massive histone replacement process observed with the paternal genome. As extensive nucleosomal protein exchanges seems unlikely to occur during mitosis, the finding that the transfer of highly condensed mitotic chromosomes into metaphase zygotes can result in successful ECNT (Egli *et al.*, 2007), also argues against a large-scale histone replacement process. Instead, the somatic nuclei were observed to undergo dynamic changes in histone acetylation and methylation patterns which are developmentally regulated in reconstructed MII-oocytes (Wang *et al.*, 2007). These changes would have to be carried out by specific histone modifying enzymes localized in the nucleus. These specific histone modifiers could be dispersed into the zygotic cytoplasm upon pronuclear membrane breakdown, and contribute to the reprogramming activities observed in metaphase or selectively enucleated zygotes. DNA demethylation is another important aspect of epigenetic remodeling in the zygote. Demethylation of the *Pou5f1* promoter region was found to be critical for re-activation of Oct4 gene expression from mammalian somatic nuclei transferred into *Xenopus* oocytes (Simonsson and Gurdon, 2004). Thus, incomplete DNA demethylation is proposed to be a major cause of SCNT failure. Though the DNA methylation profiles of almost all cloned embryos/animals were found to be aberrant, analysis of DNA methylation in cloned bovine embryos provided a hint that active demethylation of the somatic nuclei potentially occurs in the zygote (Dean *et al.*, 2001; Kang *et al.*, 2001). It was observed that somatic pseudo-pronuclei, resulting from the transfer of highly methylated fibroblast nuclei into enucleated oocytes and activation, stained much less strongly with an antibody to 5-methylcytosine compared to the female pronucleus in a normal zygote (Dean *et al.*, 2001). While the extent and location of demethylation is unclear from the study, this observation nevertheless suggests that the somatic nuclei may be demethylated by the same proteins that mediate active paternal genome demethylation (Dean *et al.*, 2001). Given the observed differences in the reprogramming efficiency of MII-oocytes and zygotes, it will be interesting to further examine the DNA methylation profiles in cloned embryos derived from reconstructed interphase and metaphase zygotes.

Chromatin remodeling through H1 linker histone exchange

Linker histone H1 is generally thought to be a repressive component of chromatin due to its function in mediating higher order nucleosomal assembly. Apart from the somatic H1 isoform,

oocyte-specific H1 linker histones have been identified in amphibians and mammals (Smith *et al.*, 1988b; Tanaka *et al.*, 2001). During normal development, oocyte-specific H1foo linker histones are present in oocytes and embryos until ZGA, when they become replaced by somatic H1 on the chromatin. The oocyte-specific H1foo histones have different properties from the somatic H1, and are proposed to mediate a more fluid chromatin structure during early embryogenesis (Saeki *et al.*, 2005). Recently, it has been shown that H1 linker histones are rapidly replaced by oocyte-specific H1foo in somatic nuclei transferred into mammalian MII-oocytes (Bordignon *et al.*, 1999; Gao *et al.*, 2004; Teranishi *et al.*, 2004). Interestingly, the H1-H1foo exchange appears to be critical for pluripotency gene activation during SCNT in *Xenopus* oocytes (Jullien *et al.*, 2010). It is unclear if this pattern of H1 transition occurs during SCNT into enucleated mammalian zygotes. However, as it seems unlikely that extensive linker histone replacements would take place in condensed mitotic chromosomes, it would appear that H1-H1foo transition might not be essential for successful nuclear reprogramming in mammalian zygotes. The significance of the somatic to oocyte-specific H1 transition in nuclear reprogramming by mammalian oocytes is also uncertain given that the transition was observed in all cloned mouse embryos, of which less than 2-3% are able to develop to term (Gao *et al.*, 2004).

Activation of gene expression by embryonic transcription factors

Transcription factors are critical regulators of gene expression, and very likely play the principal role in directing cell-type specific transcription programs. The dominant action of a master transcription factor could potentially override the pre-existing cell-specific gene expression profile, and lead to a change in cell identity. This was demonstrated by the induction of a muscle-cell gene expression pattern in a range of different non-muscle cell types with the over-expression of a single transcription factor, MyoD (Weintraub *et al.*, 1989). More recently, ectopic expression of four transcription factors, Oct4, Sox2, Klf4, and c-Myc was found to be sufficient to revert differentiated fibroblasts to pluripotent, embryonic stem cell-like cells (Takahashi and Yamanaka, 2006). Further refinement of the induced pluripotent stem cell methodology reveals the requirement for only Oct4 and Sox2 in the reprogramming process (Huangfu *et al.*, 2008). These two transcription factors are maternally expressed in oocytes and zygotes, and should be readily available during SCNT to direct the activation of the embryonic gene expression program in the somatic nuclei. Successful nuclear transfer into zygotes suggest that these and other key embryonic transcription regulators might have been removed during complete enucleation of intact pronuclei (Egli *et al.*, 2007; Greda *et al.*, 2006). Breakdown of the nuclear envelope during selective enucleation or entry into mitosis would release these factors into the zygotic cytoplasm, thus conferring reprogramming capacity.

Although other reprogramming events such as DNA demethylation, changes to histone post-translational modifications and H1 linker histone exchanges occur during SCNT, these processes were often found to be partial and aberrant, even in the cloned animals that developed to term. These observations suggest that even if epigenetic reprogramming of the somatic nuclei is incomplete, master transcriptional regulators, present at sufficient levels in the nuclear recipients, are able to supersede unfavorable chromatin states, and activate appropriate embryonic gene expression for the development of reconstructed embryos (Egli *et al.*, 2008). If this hypothesis is true, one should then be able to successfully reprogram somatic nuclei in completely enucleated zygotic cytoplasts that are supplemented with the right set of transcriptional regulators. However, such experiments will be fraught with challenges in the identification of the appropriate transcription factors, control of delivery, expression and amounts of factors to be introduced, and accurate analysis of the developmental outcome.

Conclusions

In this review, we have focused our discussion on the nuclear reprogramming capacity in zygotes. After two decades of unsuccessful attempts to use zygotes in mammalian cloning experiments, it has been demonstrated that soluble nuclear contents appeared to contain the major reprogramming activities (Egli *et al.*, 2007; Greda *et al.*, 2006). Given the complexity of somatic nuclei reprogramming, it is somewhat surprising to find that this process can be successfully completed in the short period of time from the introduction of mitotic chromosomes into metaphase zygotes to the activation of embryonic transcription in 2-cell stage embryos. However, the reprogramming process remains much less efficient in zygotes than in MII-oocytes. While a greater than 4% success rate (calculated as number of live-borns out of total reconstructed embryos) was reported when genomic material of 8-cell blastomeres was transferred into selectively enucleated or metaphase zygotes, SCNT into zygotes did not result in any live-born animals (Egli *et al.*, 2007; Greda *et al.*, 2006). It remains to be determined if the low success rates of reprogramming by zygotes can be improved by further technical advancements or are the results of the intrinsic properties of these recipient cells. Additional experiments are also required to address what cytoplasmic factors, if any, are vital complements to the nuclear factors. SCNT using zygotes provides a platform to assay for critical reprogramming factors and identify crucial processes correlated with normal development. A clear knowledge of the mechanistic nature of reprogramming is essential for our understanding of mammalian development. There is also significant medical interest in having a better comprehension of how cells can be modified from one cell type to another, with the ultimate goal of generating patient-specific cells that can be used in disease therapy via direct reprogramming.

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References

ADENOT, P.G., MERCIER, Y., RENARD, J.P. and THOMPSON, E.M. (1997). Differential H4 acetylation of paternal and maternal chromatin precedes DNA replication and differential transcriptional activity in pronuclei of 1-cell mouse embryos. *Development* 124: 4615-4625.

ARMSTRONG, L., LAKO, M., DEAN, W. and STOJKOVIC, M. (2006). Epigenetic modification is central to genome reprogramming in somatic cell nuclear

transfer. *Stem Cells* 24: 805-814.

BALAKIER, H. and TARKOWSKI, A.K. (1980). The role of germinal vesicle karyoplasm in the development of male pronucleus in the mouse. *Exp Cell Res* 128: 79-85.

BARRETO, G., SCHAFER, A., MARHOLD, J., STACH, D., SWAMINATHAN, S.K., HANDA, V., DODERLEIN, G., MALTRY, N., WU, W., LYKO, F. *et al.* (2007). Gadd45a promotes epigenetic gene activation by repair-mediated DNA demethylation. *Nature* 445: 671-675.

BHUTANI, N., BRADY, J.J., DAMIAN, M., SACCO, A., CORBEL, S.Y. and BLAU, H.M. (2010). Reprogramming towards pluripotency requires AID-dependent DNA demethylation. *Nature* 463: 1042-1047.

BORDIGNON, V., CLARKE, H.J. and SMITH, L.C. (1999). Developmentally regulated loss and reappearance of immunoreactive somatic histone H1 on chromatin of bovine morula-stage nuclei following transplantation into oocytes. *Biol Reprod* 61: 22-30.

BUI, H.T., WAKAYAMA, S., KISHIGAMI, S., KIM, J.H., VAN THUAN, N. and WAKAYAMA, T. (2008). The cytoplasm of mouse germinal vesicle stage oocytes can enhance somatic cell nuclear reprogramming. *Development* 135: 3935-3945.

CHEONG, H.T., TAKAHASHI, Y. and KANAGAWA, H. (1993). Birth of mice after transplantation of early cell-cycle-stage embryonic nuclei into enucleated oocytes. *Biol Reprod* 48: 958-963.

CHESNE, P., ADENOT, P.G., VIGLIETTA, C., BARATTE, M., BOULANGER, L. and RENARD, J.P. (2002). Cloned rabbits produced by nuclear transfer from adult somatic cells. *Nat Biotechnol* 20: 366-369.

DEAN, W., SANTOS, F., STOJKOVIC, M., ZAKHARTCHENKO, V., WALTER, J., WOLF, E. and REIK, W. (2001). Conservation of methylation reprogramming in mammalian development: aberrant reprogramming in cloned embryos. *Proc Natl Acad Sci USA* 98: 13734-13738.

DO, J.T. and SCHOLER, H.R. (2004). Nuclei of embryonic stem cells reprogram somatic cells. *Stem Cells* 22: 941-949.

DOHERTY, A.S., BARTOLOMEI, M.S. and SCHULTZ, R.M. (2002). Regulation of stage-specific nuclear translocation of Dnmt1o during preimplantation mouse development. *Dev Biol* 242: 255-266.

EGLI, D., BIRKHOFF, G. and EGGAN, K. (2008). Mediators of reprogramming: transcription factors and transitions through mitosis. *Nat Rev Mol Cell Biol* 9: 505-516.

EGLI, D., ROSAINS, J., BIRKHOFF, G. and EGGAN, K. (2007). Developmental reprogramming after chromosome transfer into mitotic mouse zygotes. *Nature* 447: 679-685.

EGLI, D., SANDLER, V.M., SHINOHARA, M.L., CANTOR, H. and EGGAN, K. (2009). Reprogramming after chromosome transfer into mouse blastomeres. *Curr Biol* 19: 1403-1409.

EGLI, D. and EGGAN, K. (2010). Recipient cell nuclear factors are required for reprogramming by nuclear transfer. *Development* 137: 1953-1963.

ERHARDT, S., SU, I.H., SCHNEIDER, R., BARTON, S., BANNISTER, A.J., PEREZ-BURGOS, L., JENUWEIN, T., KOUZARIDES, T., TARAKHOVSKY, A. and SURANI, M.A. (2003). Consequences of the depletion of zygotic and embryonic enhancer of zeste 2 during preimplantation mouse development. *Development* 130: 4235-4248.

FAN, Y., CHEN, X., LUO, Y., CHEN, X., LI, S., HUANG, Y. and SUN, X. (2009). Developmental potential of human oocytes reconstructed by transferring somatic cell nuclei into polyspermic zygote cytoplasm. *Biochem. Biophys. Res. Commun.* 382: 119-123.

GAO, S., CHUNG, Y.G., PARSEGHIAN, M.H., KING, G.J., ADASHI, E.Y. and LATHAM, K.E. (2004). Rapid H1 linker histone transitions following fertilization or somatic cell nuclear transfer: evidence for a uniform developmental program in mice. *Dev Biol* 266: 62-75.

GAO, S., GASPARRINI, B., MCGARRY, M., FERRIER, T., FLETCHER, J., HARKNESS, L., DE SOUSA, P. and WILMUT, I. (2002). Germinal vesicle material is essential for nucleus remodeling after nuclear transfer. *Biol Reprod* 67: 928-934.

GAO, T., ZHENG, J., XING, F., FANG, H., SUN, F., YAN, A., GONG, X., DING, H., TANG, F. and SHENG, H.Z. (2007). Nuclear reprogramming: the strategy used in normal development is also used in somatic cell nuclear transfer and parthenogenesis. *Cell Res* 17: 135-150.

GILBERT, S.F. and SOLTER, D. (1985). Onset of paternal and maternal Gpi-1 expression in preimplantation mouse embryos. *Dev Biol* 109: 515-517.

GREDA, P., KARASIEWICZ, J. and MODLINSKI, J.A. (2006). Mouse zygotes as recipients in embryo cloning. *Reproduction* 132: 741-748.

GROHMANN, M., SPADA, F., SCHERMELLEH, L., ALENINA, N., BADER, M., CARDOSO, M.C. and LEONHARDT, H. (2005). Restricted mobility of Dnmt1 in preimplantation embryos: implications for epigenetic reprogramming. *BMC Dev Biol* 5: 18.

HALL, V.J., COMPTON, D., STOJKOVIC, P., NESBITT, M., HERBERT, M., MURDOCH, A. and STOJKOVIC, M. (2007). Developmental competence of human *in vitro* aged oocytes as host cells for nuclear transfer. *Hum Reprod* 22: 52-62.

HAYAKAWA, T., HARAGUCHI, T., MASUMOTO, H. and HIRAOKA, Y. (2003). Cell cycle behavior of human HP1 subtypes: distinct molecular domains of HP1 are required for their centromeric localization during interphase and metaphase. *J Cell Sci* 116: 3327-3338.

HIIRAGI, T. and SOLTER, D. (2005). Reprogramming is essential in nuclear transfer. *Mol Reprod Dev* 70: 417-421.

HILL, J.R., BURGHARDT, R.C., JONES, K., LONG, C.R., LOONEY, C.R., SHIN, T., SPENCER, T.E., THOMPSON, J.A., WINGER, Q.A. and WESTHUSIN, M.E. (2000). Evidence for placental abnormality as the major cause of mortality in first-trimester somatic cell cloned bovine fetuses. *Biol Reprod* 63: 1787-1794.

HILL, J.R., SCHLAFER, D.H., FISHER, P.J. and DAVIES, C.J. (2002). Abnormal expression of trophoblast major histocompatibility complex class I antigens in cloned bovine pregnancies is associated with a pronounced endometrial lymphocytic response. *Biol Reprod* 67: 55-63.

HOWLETT, S.K., BARTON, S.C. and SURANI, M.A. (1987). Nuclear cytoplasmic interactions following nuclear transplantation in mouse embryos. *Development* 101: 915-923.

HUANGFU, D., OSAFUNE, K., MAEHR, R., GUO, W., EIJKELENBOOM, A., CHEN, S., MUHLESTEIN, W. and MELTON, D.A. (2008). Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. *Nat Biotechnol* 26: 1269-1275.

ILLMENSEE, K. and HOPPE, P.C. (1981). Nuclear transplantation in Mus musculus: developmental potential of nuclei from preimplantation embryos. *Cell* 23: 9-18.

ITO, S., D'ALESSIO, A.C., TARANOVA, O.V., HONG, K., SOWERS, L.C. and ZHANG, Y. (2010). Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification. *Nature* 466: 1129-1133.

JULLIEN, J., ASTRAND, C., HALLEY-STOTT, R.P., GARRETT, N. and GURDON, J.B. (2010). Characterization of somatic cell nuclear reprogramming by oocytes in which a linker histone is required for pluripotency gene reactivation. *Proc Natl Acad Sci USA* 107: 5483-5488.

KANG, Y.K., KOO, D.B., PARK, J.S., CHOI, Y.H., CHUNG, A.S., LEE, K.K. and HAN, Y.M. (2001). Aberrant methylation of donor genome in cloned bovine embryos. *Nat Genet* 28: 173-177.

KATO, Y., TANI, T., SOTOMARU, Y., KUROKAWA, K., KATO, J., DOGUCHI, H., YASUE, H. and TSUNODA, Y. (1998). Eight calves cloned from somatic cells of a single adult. *Science* 282: 2095-2098.

LANDSVERK, H.B., HAKELIEN, A.M., KUNTZIGER, T., ROBL, J.M., SKALHEGG, B.S. and COLLAS, P. (2002). Reprogrammed gene expression in a somatic cell-free extract. *EMBO Rep* 3: 384-389.

LEE, B.C., KIM, M.K., JANG, G., OH, H.J., YUDA, F., KIM, H.J., HOSSEIN, M.S., KIM, J.J., KANG, S.K., SCHATTEN, G. *et al.* (2005). Dogs cloned from adult somatic cells. *Nature* 436: 641.

LEPIKHOV, K. and WALTER, J. (2004). Differential dynamics of histone H3 methylation at positions K4 and K9 in the mouse zygote. *BMC Dev Biol* 4: 12.

LI, S., CHEN, X., FANG, Z., SHI, J. and SHENG, H.Z. (2006a). Rabbits generated from fibroblasts through nuclear transfer. *Reproduction* 131: 1085-1090.

LI, Z., SUN, X., CHEN, J., LIU, X., WISELY, S.M., ZHOU, Q., RENARD, J.P., LENO, G.H. and ENGELHARDT, J.F. (2006b). Cloned ferrets produced by somatic cell nuclear transfer. *Dev Biol* 293: 439-448.

LIU, H., KIM, J.M. and AOKI, F. (2004). Regulation of histone H3 lysine 9 methylation in oocytes and early pre-implantation embryos. *Development* 131: 2269-2280.

MARTINEZ-BALBAS, M.A., DEY, A., RABINDRAN, S.K., OZATO, K. and WU, C.

(1995). Displacement of sequence-specific transcription factors from mitotic chromatin. *Cell* 83: 29-38.

MAYER, W., NIVELEAU, A., WALTER, J., FUNDELE, R. and HAAF, T. (2000). Demethylation of the zygotic paternal genome. *Nature* 403: 501-502.

MCGRATH, J. and SOLTER, D. (1983). Nuclear transplantation in the mouse embryo by microsurgery and cell fusion. *Science* 220: 1300-1302.

MCGRATH, J. and SOLTER, D. (1984). Inability of mouse blastomere nuclei transferred to enucleated zygotes to support development *in vitro*. *Science* 226: 1317-1319.

MENG, L., ELY, J.J., STOUFFER, R.L. and WOLF, D.P. (1997). Rhesus monkeys produced by nuclear transfer. *Biol Reprod* 57: 454-459.

MINC, E., ALLORY, Y., WORMAN, H.J., COURVALIN, J.C. and BUENDIA, B. (1999). Localization and phosphorylation of HP1 proteins during the cell cycle in mammalian cells. *Chromosoma* 108: 220-234.

MIYAGISHIMA, H., ISONO, K., FUJIMURA, Y., IYO, M., TAKIHARA, Y., MASUMOTO, H., VIDAL, M. and KOSEKI, H. (2003). Dissociation of mammalian Polycomb-group proteins, Ring1B and Rae28/Ph1, from the chromatin correlates with configuration changes of the chromatin in mitotic and meiotic prophase. *Histochem Cell Biol* 120: 111-119.

MODLINSKI, J.A. and SMORAG, Z. (1991). Preimplantation development of rabbit embryos after transfer of embryonic nuclei into different cytoplasmic environment. *Mol Reprod Dev* 28: 361-372.

MOHAMMED, A.A., KARASIEWICZ, J. and MODLINSKI, J.A. (2008). Developmental potential of selectively enucleated immature mouse oocytes upon nuclear transfer. *Mol Reprod Dev* 75: 1269-1280.

MORGAN, H.D., DEAN, W., COKER, H.A., REIK, W. and PETERSEN-MAHRT, S.K. (2004). Activation-induced cytidine deaminase deaminates 5-methylcytosine in DNA and is expressed in pluripotent tissues: implications for epigenetic reprogramming. *J Biol Chem* 279: 52353-52360.

MORGAN, H.D., SANTOS, F., GREEN, K., DEAN, W. and REIK, W. (2005). Epigenetic reprogramming in mammals. *Hum Mol Genet* 14 Spec No 1: R47-58.

MURZINA, N., VERREAULT, A., LAUE, E. and STILLMAN, B. (1999). Heterochromatin dynamics in mouse cells: interaction between chromatin assembly factor 1 and HP1 proteins. *Mol Cell* 4: 529-540.

OGURA, A., INOUE, K., OGONUKI, N., LEE, J., KOHDA, T. and ISHINO, F. (2002). Phenotypic effects of somatic cell cloning in the mouse. *Cloning Stem Cells* 4: 397-405.

OGUSHI, S., FULKA, J., JR. and MIYANO, T. (2005). Germinal vesicle materials are requisite for male pronucleus formation but not for change in the activities of CDK1 and MAP kinase during maturation and fertilization of pig oocytes. *Dev Biol* 286: 287-298.

OKADA, Y., YAMAGATA, K., HONG, K., WAKAYAMA, T. and ZHANG, Y. (2010). A role for the elongator complex in zygotic paternal genome demethylation. *Nature* 463: 554-558.

ONO, Y. and KONO, T. (2006). Irreversible barrier to the reprogramming of donor cells in cloning with mouse embryos and embryonic stem cells. *Biol Reprod* 75: 210-216.

OSWALD, J., ENGEMANN, S., LANE, N., MAYER, W., OLEK, A., FUNDELE, R., DEAN, W., REIK, W. and WALTER, J. (2000). Active demethylation of the paternal genome in the mouse zygote. *Curr Biol* 10: 475-478.

POLEJAEVA, I.A., CHEN, S.H., VAUGHT, T.D., PAGE, R.L., MULLINS, J., BALL, S., DAI, Y., BOONE, J., WALKER, S., AYARES, D.L. *et al.* (2000). Cloned pigs produced by nuclear transfer from adult somatic cells. *Nature* 407: 86-90.

PUSCHENDORF, M., TERRANOVA, R., BOUTSMA, E., MAO, X., ISONO, K., BRYKCZYNSKA, U., KOLB, C., OTTE, A.P., KOSEKI, H., ORKIN, S.H. *et al.* (2008). PRC1 and Suv39h specify parental asymmetry at constitutive heterochromatin in early mouse embryos. *Nat Genet* 40: 411-420.

ROBL, J.M., GILLIGAN, B., CRITSER, E.S. and FIRST, N.L. (1986). Nuclear transplantation in mouse embryos: assessment of recipient cell stage. *Biol Reprod* 34: 733-739.

ROUGIER, N., BOURC'HIS, D., GOMES, D.M., NIVELEAU, A., PLACHOT, M., PALDI, A. and VIEGAS-PEQUIGNOT, E. (1998). Chromosome methylation patterns during mammalian preimplantation development. *Genes Dev* 12: 2108-2113.

SAEKI, H., OHSUMI, K., AIHARA, H., ITO, T., HIROSE, S., URA, K. and KANEDA, Y. (2005). Linker histone variants control chromatin dynamics during early embryogenesis. *Proc Natl Acad Sci USA* 102: 5697-5702.

SANTOS, F., HENDRICH, B., REIK, W. and DEAN, W. (2002). Dynamic reprogramming of DNA methylation in the early mouse embryo. *Dev Biol* 241: 172-182.

SANTOS, F., PETERS, A.H., OTTE, A.P., REIK, W. and DEAN, W. (2005). Dynamic chromatin modifications characterise the first cell cycle in mouse embryos. *Dev Biol* 280: 225-236.

SCHURMANN, A., WELLS, D.N. and OBACK, B. (2006). Early zygotes are suitable recipients for bovine somatic nuclear transfer and result in cloned offspring. *Reproduction* 132: 839-848.

SHI, D., LU, F., WEI, Y., CUI, K., YANG, S., WEI, J. and LIU, Q. (2007). Buffalos (Bubalus bubalis) cloned by nuclear transfer of somatic cells. *Biol Reprod* 77: 285-291.

SHIN, T., KRAEMER, D., PRYOR, J., LIU, L., RUGILA, J., HOWE, L., BUCK, S., MURPHY, K., LYONS, L. and WESTHUSIN, M. (2002). A cat cloned by nuclear transplantation. *Nature* 415: 859.

SHINOZAWA, T., MIZUTANI, E., TOMIOKA, I., KAWAHARA, M., SASADA, H., MATSUMOTO, H. and SATO, E. (2004). Differential effect of recipient cytoplasm for microtubule organization and preimplantation development in rat reconstituted embryos with two-cell embryonic cell nuclear transfer. *Mol Reprod Dev* 68: 313-318.

SIMONSSON, S. and GURDON, J. (2004). DNA demethylation is necessary for the epigenetic reprogramming of somatic cell nuclei. *Nat Cell Biol* 6: 984-990.

SMITH, L.C., WILMUT, I. and HUNTER, R.H. (1988a). Influence of cell cycle stage at nuclear transplantation on the development *in vitro* of mouse embryos. *J Reprod Fertil* 84: 619-624.

SMITH, R.C., DWORKIN-RASTL, E. and DWORKIN, M.B. (1988b). Expression of a histone H1-like protein is restricted to early*Xenopus* development. *Genes Dev* 2: 1284-1295.

STRAHL, B.D. and ALLIS, C.D. (2000). The language of covalent histone modifications. *Nature* 403: 41-45.

SUN, F., FANG, H., LI, R., GAO, T., ZHENG, J., CHEN, X., YING, W. and SHENG, H.Z. (2007). Nuclear reprogramming: the zygotic transcription program is established through an «erase-and-rebuild» strategy. *Cell Res* 17: 117-134.

TAHILIANI, M., KOH, K.P., SHEN, Y., PASTOR, W.A., BANDUKWALA, H., BRUDNO, Y., AGARWAL, S., IYER, L.M., LIU, D.R., ARAVIND, L. *et al.* (2009). Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science* 324: 930-935.

TAKAHASHI, K. and YAMANAKA, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126: 663-676.

TANAKA, M., HENNEBOLD, J.D., MACFARLANE, J. and ADASHI, E.Y. (2001). A mammalian oocyte-specific linker histone gene H1oo: homology with the genes for the oocyte-specific cleavage stage histone (cs-H1) of sea urchin and the B4/H1M histone of the frog. *Development* 128: 655-664.

TERANISHI, T., TANAKA, M., KIMOTO, S., ONO, Y., MIYAKOSHI, K., KONO, T. and YOSHIMURA, Y. (2004). Rapid replacement of somatic linker histones with the oocyte-specific linker histone H1foo in nuclear transfer. *Dev Biol* 266: 76-86.

TSUNODA, Y., YASUI, T., SHIODA, Y., NAKAMURA, K., UCHIDA, T. and SUGIE, T. (1987). Full-term development of mouse blastomere nuclei transplanted into enucleated two-cell embryos. *J Exp Zool* 242: 147-151.

VAN DER HEIJDEN, G.W., DIEKER, J.W., DERIJCK, A.A., MULLER, S., BERDEN, J.H., BRAAT, D.D., VAN DER VLAG, J. and DE BOER, P. (2005). Asymmetry in histone H3 variants and lysine methylation between paternal and maternal chromatin of the early mouse zygote. *Mech Dev* 122: 1008-1022.

VONCKEN, J.W., SCHWEIZER, D., AAGAARD, L., SATTLER, L., JANTSCH, M.F. and VAN LOHUIZEN, M. (1999). Chromatin-association of the Polycomb group protein BMI1 is cell cycle-regulated and correlates with its phosphorylation status. *J Cell Sci* 112 (Pt 24): 4627-4639.

WAKAYAMA, S., SUETSUGU, R., THUAN, N.V., OHTA, H., KISHIGAMI, S. and WAKAYAMA, T. (2007). Establishment of mouse embryonic stem cell lines from somatic cell nuclei by nuclear transfer into aged, fertilization-failure mouse oocytes. *Curr Biol* 17: R120-121.

WAKAYAMA, T., RODRIGUEZ, I., PERRY, A.C., YANAGIMACHI, R. and MOMBAERTS, P. (1999). Mice cloned from embryonic stem cells. *Proc Natl Acad Sci USA* 96: 14984-14989.

WAKAYAMA, T., TATENO, H., MOMBAERTS, P. and YANAGIMACHI, R. (2000).

Nuclear transfer into mouse zygotes. *Nat Genet* 24: 108-109.

WANG, F., KOU, Z., ZHANG, Y. and GAO, S. (2007). Dynamic reprogramming of histone acetylation and methylation in the first cell cycle of cloned mouse embryos. *Biol Reprod* 77: 1007-1016.

WANI, N.A., WERNERY, U., HASSAN, F.A., WERNERY, R. and SKIDMORE, J.A. (2010). Production of the first cloned camel by somatic cell nuclear transfer. *Biol Reprod* 82: 373-379.

WEINTRAUB, H., TAPSCOTT, S.J., DAVIS, R.L., THAYER, M.J., ADAM, M.A., LASSAR, A.B. and MILLER, A.D. (1989). Activation of muscle-specific genes in pigment, nerve, fat, liver, and fibroblast cell lines by forced expression of MyoD. *Proc Natl Acad Sci USA* 86: 5434-5438.

WILMUT, I., SCHNIEKE, A.E., MCWHIR, J., KIND, A.J. and CAMPBELL, K.H. (1997). Viable offspring derived from fetal and adult mammalian cells. *Nature* 385: 810-813.

WOSSIDLO, M., ARAND, J., SEBASTIANO, V., LEPIKHOV, K., BOIANI, M., REINHARDT, R., SCHOLER, H. and WALTER, J. (2010). Dynamic link of DNA demethylation, DNA strand breaks and repair in mouse zygotes. *EMBO J* 29: 1877-1888.

YOUNG, L.E., SINCLAIR, K.D. and WILMUT, I. (1998). Large offspring syndrome in cattle and sheep. *Rev Reprod* 3: 155-163.

ZHOU, Q., RENARD, J.P., LE FRIEC, G., BROCHARD, V., BEAUJEAN, N., CHERIFI, Y., FRAICHARD, A. and COZZI, J. (2003). Generation of fertile cloned rats by regulating oocyte activation. *Science* 302: 1179.