JCMM
Available online at
www.jcmm.ro
www.jcmm.org
Stem Cell Review Series

J. Cell. Mol. Med. Vol 9, No 2, 2005 pp. 320-330

Reprogramming mediated by stem cell fusion

Dominic J. Ambrosi [a b], Theodore P. Rasmussen [a, b, c *]

[a] Center for Regenerative Biology, University of Connecticut, Storrs, CT, USA
[b] Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT, USA
[c] Department of Animal Science, University of Connecticut, Storrs, CT, USA

Received: March 1, 2005; Accepted: April 22, 2005

Abstract

Advances in mammalian cloning prove that somatic nuclei can be reprogrammed to a state of totipotency by transfer into oocytes. An alternative approach to reprogram the somatic genome involves the creation of hybrids between somatic cells and other cells that contain reprogramming activities. Potential fusion partners with reprogramming activities include embryonic stem cells, embryonic germ cells, embryonal carcinoma cells, and even differentiated cells. Recent advances in fusion-mediated reprogramming are discussed from the standpoints of the developmental potency of hybrid cells, genetic and epigenetic correlates of reprogramming, and other aspects involved in the reprogramming process. In addition, the utility of fusion-mediated reprogramming for future cell-based therapies is discussed.

Keywords: embryonic stem cells • cell fusion • cell hybrid • reprogramming • epigenetics • nuclear transfer • therapeutic cloning • differentiation • transdifferentiation • dedifferentiation

Introduction

Nuclear reprogramming is the functional conversion of the genetic material contained within a differentiated somatic cell to a state of developmental pluripotency or multipotency. A

* Correspondence to: Theodore P. RASMUSSEN, Ph.D.,
Center for Regenerative Biology, 1392 Storrs Road, U-4243
University of Connecticut, Storrs, CT 06269-4243, USA.
Tel.: 860-486-8339,
Fax: 860-486-8809
E-mail: theodore.rasmussen@uconn.edu

Reprinted from:
Journal of Cellular and Molecular Medicine
Published by:
the CMM Foundation

notable early success with amphibians was recorded by J.B. Gurdon in studies that yielded clonal frogs produced by transfer of somatic nuclei into oocytes [1]. Decades elapsed before the creation of Dolly the sheep by somatic cell nuclear transfer (SCNT) [2]. The process of reprogramming, whereby a somatic nucleus acquires new developmental potential, is thought to occur through epigenetic mechanisms since gene expression is reset to an early embryonic state without alteration of DNA sequence. For this reason, a great deal of effort has been devoted to the investigation of the molecular-epigenetic machinery in cloned embryos, embryonic stem cells (ESCs), and animals. At present, the understanding of nuclear reprogramming at a molecular level is incomplete, but keen interest in this question is leading to rapid advances in our understanding. Areas of intense scrutiny include DNA methylation and its associated enzymology, mechanisms for the establishment, maintenance, and erasure of imprinted gene expression, and the management of developmentally-regulated gene expression through chromatin. This last area is characterized by considerable molecular complexity since histones, histone variants, histone-modifying enzymes and cofactors, and chromatin assembly and remodeling complexes all cooperate to assemble developmentally-regulated chromatin states that are thought to be extensively reorganized during the reprogramming process.

SCNT is highly effective for the reprogramming of somatic nuclei, but alternative approaches for reprogramming have garnered considerable interest. A number of recent studies have utilized reprogramming activities resident within cells of embryonic origin to bring about reprogramming of the somatic genome, either through direct fusion with somatic cells or through the use of extracts. In short, three experimentally-tractable approaches are now available to bring about reprogramming: [1] The use of the "reprogramming microenvironment" that naturally occurs within oocytes to confer developmental totipotency upon transplanted somatic nuclei (SCNT), [2] the use of fusions between somatic cells and stem cells to confer developmental potency to the resulting hybrids, and [3] the application of extracts from oocytes, stem cells, or other cells to directly alter the developmental potential of somatic cells.

Insights into reprogramming mechanisms from nuclear transfer

The production of Dolly the sheep by nuclear transfer in 1997 demonstrated that fully-differentiated mammalian somatic cells could be reprogrammed to a state of totipotency [2]. This was achieved by replacing the genome of an unfertilized oocyte with a somatic mammary cell nucleus. The success rate of mammalian cloning by SCNT is very low, however, with the majority of clones dying in utero or neonatally, often in conjunction with developmental problems such as large offspring syndrome. This low efficiency is improved by using somatic donor nuclei from hybrid genetic backgrounds [3, 4], but a major component of the inefficiency likely involves less-than-perfect nuclear reprogramming [5]. A surviving cloned animal is a highly rigorous operational assay for effective reprogramming, but such successes at the organismal level unfortunately provide little insight into the underlying molecular mechanisms that are involved in reprogramming processes. For this reason, much effort has been put forth into studying reprogramming on a molecular-epigenetic level.

Reprogramming has been studied from two related perspectives: gene expression and chromatin analysis. Somatic nuclei do not express Oct4, a key transcription factor necessary for the maintenance of pluripotency [6]. For this reason, a great number of studies monitor the reactivation of Oct4 gene expression as a marker for the acquisition of pluripotency. The Oct4 transcription factor acts on a multitude of downstream target genes (including Dppa1-5, Pramel 4-7, & Ndp52l1) that are also expressed in pluripotent stem cells. A significant proportion of cloned blastocyst embryos fail to express Oct4 and these down-

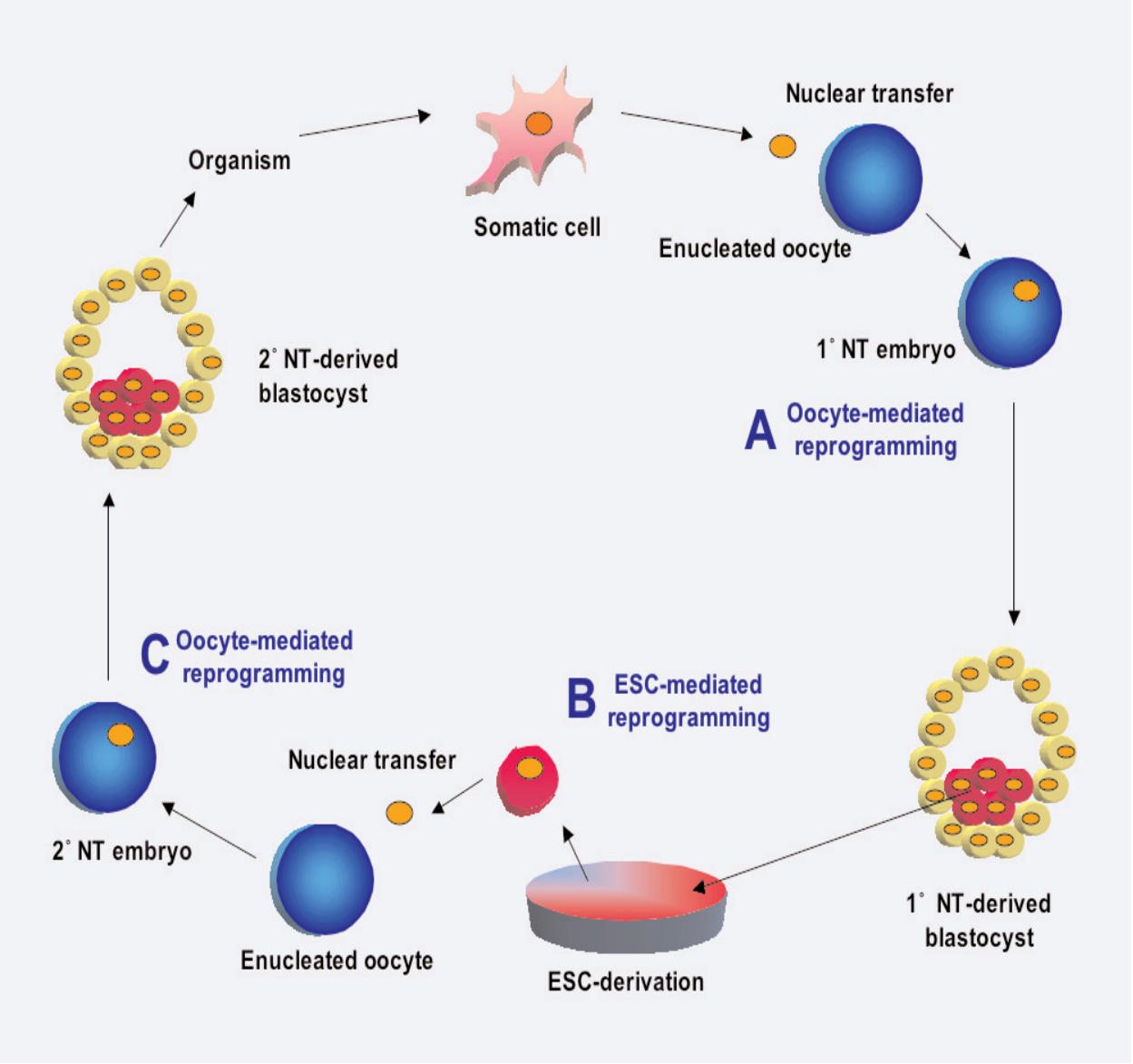

Fig. 1 Opportunities for reprogramming in serial nuclear transfer experiments. The diagram shows the essential steps that have allowed the successful production of cloned animals from somatic cell sources that are difficult to reprogram. In the first step, a somatic nucleus is transferred into an enucleated oocyte to yield a primary NT-embryo. After culture *in vitro* to the blastocyst stage, ES cells are derived. ES cell nuclei are then used for a second round of NT, and secondary NT blastocyst embryos can be transferred to surrogate females to yield live offspring. This approach contains three stages at which reprogramming of a genome of somatic origin can occur: (A) during the 1° round of NT, (B) during derivation and propagation of ES cells, and (C) during the 2° round of NT.

stream genes in a pattern similar to normal embryos, a finding that suggests that incomplete reprogramming is a major cause of embryo death shortly after implantation [7].

More recently, the homeodomain protein Nanog has also been shown to be a key functional marker of stem cell gene expression as it is required for stem cell self renewal [8, 9].

Oct4 and nanog expression provide useful markers of pluripotency, but it is clear that SCNT brings about very broad changes in the transcriptional status of the genome. Since global alterations in chromatin can bring about such broad changes in gene expression, chromatin analyses have gained credence as useful assays for reprogramming. To this end, X-inactivation [10], imprinted gene expression, DNA methylation, and histone modifications have all been analyzed in the context of SCNT [11, 12]. Such studies show that incomplete reprogramming can be detected at many developmental stages. For instance, the majority of blastocysts derived by SCNT show severely altered DNA methylation within differentially methylated domains adjacent to the imprinted H19 and Snrpn genes and loss of monoallelic expression of imprinted genes [13].

A "two-step" method has been developed to improve the efficiency of mammalian reproductive cloning (Fig. 1), and has proved effective for reprogramming difficult cell types such as B and T cells [14]. In this approach, a somatic cell nucleus is transferred into an enucleated oocyte and the reconstructed embryo is allowed to develop to the blastocyst stage *in vitro*. Embryonic stem cells are derived from the blastocyst clones (ntESCs). Nuclei from ntESCs are then used for a second round of nuclear transfer. The resulting secondary blastocysts can then be implanted to yield live offspring [15]. This approach provides expanded opportunities for nuclear reprogramming since the somatic genetic material transits through two rounds of SCNT and preimplantation development, and resides in ESCs for a considerable number of cell divisions. Though ESC nuclei are efficiently reprogrammed in comparison to terminally-differentiated cells, mice generated by this approach are still not free of epigenetic defects [16]. For instance, mice cloned from cumulus cells or ESCs exhibit aberrant gene expression as judged by microarray analysis of liver and placenta RNA [16, 17].

Spontaneous fusion: nuisance or opportunity?

Interest was stirred in the field of adult stem cell research when engraftments of marked bone marrow stem cells (BMSCs) apparently displayed remarkable lineage plasticity following engraftment. However, subsequent studies demonstrated that fusion of ESCs and somatic cells occurs spontaneously under conditions of co-culture [18, 19]. These findings suggested alternative interpretations in which endogenous somatic cells acquired stem cell markers through simple fusion events. Though these findings raised concerns, later studies that controlled for cell fusion revealed that adult stem cells do possess a remarkable degree of developmental plasticity. For instance, bone marrow-derived cells possessed the ability to develop into cells with epithelial character, apparently without fusion as judged by a sensitive cre-lox strategy to detect fusion events [20]. Taken together, these results indicate a promising future for investigations of adult stem cell plasticity, but such research should be conducted in conjunction with robust methods to detect fusion events.

Cell fusion and reprogramming can be studied with rigor in cell culture systems. In one instance, hybrid cells were generated when mouse bone marrow cells were grown on mitotically-inactive fibroblast feeder cells. These hybrids contained genetic markers from both cell types and expressed endothelial markers when plated on matrigel matrix [21]. In another study, neurosphere cells derived from embryonic day 14.5 mouse forebrain spontaneously fused with ESCs under conditions of co-culture [19]. The fused cells had markers from both fusion partners, grew with ESC-like morphology, and contained a tetraploid complement of chromosomes. These neurosphere/ESC hybrids contributed readily to chimeras. Spontaneous cell fusion was also detected when hygromycin-sensitive mouse ESCs were co-cultured with hygromycin-resistant primary murine brain cells for 5 days [22]. The resulting hybrid cells formed hygromycin-resistant colonies and expressed the stem-cell specific Foxd3 gene

Table 1 Summary of fusion experiments demonstrating reprogramming

Year	Embryonic cell type	Somatic cell type	Method of Fusion	Reprogramming Assays	Ref.
1997	EGC[1]	Thymocyte	Electrofusion	Methylation and expression of imprinted genes, contribution to chimeras	[32]
1998	ESC[2]	Splenocyte	PEG[3]-mediated	Expression of embryonic antigens, embryoid body formation, contribution to chimeras, X-chromosome reactivation	[23]
2001	ESC	Thymocyte	Electrofusion	X-chromosome reactivation, somatic Oct4-GFP expression, contribution to chimeras	[24]
2002	ESC	Brain cells	Spontaneous fusion in culture	Contribution to chimeras	[19]
2002	ESC	Brain cells	Spontaneous fusion in culture	Contribution to chimeras, Foxd3 expression from somatic chromosomes	[22]
2003	ECC[4]	Human T-lymphoma cell	PEG-mediated	Oct4, Sox-2 expression from human chromosomes, CD45 down-regulation	[33]
2003	ESC	Thymocyte	Electrofusion	Gene expression in teratomas, dopaminergic neuronal differentiation and gene expression	[28]
2004	ESC	NSC[5]	PEG-mediated	Oct4-GFP expression from somatic chromosome, gene expression	[29]
2004	ESC	Thymocyte	Electrofusion	Histone modification patterns of hybrid cell genome	[26]
2005	ESC	Thymocyte	Electrofusion	Nanog expression from somatic chromosomes	[25]

1: mouse embryonic germ cells, 2: mouse embryonic stem cells, 3: polyethylene glycol, 4: mouse embryonal carcinoma cells, 5: neurosphere cells

from the chromosome originally present in the neural cells. Furthermore, these hybrid cells contributed to all three germ layers in chimeras.

Fusion-mediated reprogramming using ES cells

ESCs are pluripotent since they can give rise to every cell present in the embryo proper. For this reason, ESCs are a cell-type of choice to bring about reprogramming when used as a fusion partner with somatic cells. A number of recent studies demonstrate the existence of potent reprogramming activities within ESCs (Table 1).

In one study, hybrids were generated by fusion of male murine Hprt$^{-/-}$ ESCs with female mouse splenocytes. Fused cells were selected in HAT medium. The resulting hybrid cells exhibited near-tetraploid karyotypes and formed a variety of embryoid bodies containing cell-types corresponding to all three germ layers [23]. In addition, these cells contained synchronously-replicating X chromosomes, suggesting that the inactive X chromosome of splenocyte origin was reprogrammed to a pre-inactivation state. In another study, fusions of female mouse adult thymocytes harboring a silent GFP transgene under the control of the Oct4 promoter with male mouse ESCs yielded hybrids that expressed GFP [24]. The thymocyte/ESC hybrids contained reactivated X chromosomes of thymocyte origin as judged by fluorescent in situ hybridization for Xist RNA. In this study, Xist RNA was observed to bind unstably to all three X chromosomes, suggesting a state of partial X chromosome inactivation. The finding that all three X chromosomes in hybrids replicated synchronously and concurrently with autosomes strengthened evidence for a pre-X-inactivation state. Oct4 promoter activity (as assessed by reactivation of the silenced Oct4 promoter driven GFP transgene in thymocytes) was observed approximately 48 hours after fusion. In a developmental assay for potency, ESC/thymocyte hybrids contributed to all three germ layers in mouse chimeras. The methylation status of imprinted H19 and Igf2r genes was unaltered by fusion with ESCs, unlike the case of fusions of somatic cells with embryonic germ cells (EGCs, see next section), where methylated sites near imprinted genes were erased. The pluripotency factor Nanog is expressed in morula, blastocyst-stage embryos, and ESCs, but not differentiated somatic cells. The silent nanog gene is reactivated upon fusion of somatic cells with ESCs or when somatic nuclei are transplanted into oocytes [25].

Modifications upon histones play a key role in the epigenetic management of gene expression. Interestingly, the somatic genomes of hybrid cells (generated by fusion with ESCs) exhibit hyperacetylation of histones H3 and H4, global di- and tri-methylation of lysine 4 of H3, and hyperacetylation of lysine 4 of H3 within the Oct4 promoter. However, di- and tri-methylation of lysine 4 of H3 was also observed in the promoter region of several silent genes. This surprising finding shows that H3 lysine 4 methylation is not obligately correlated with transcriptional activity. Furthermore, this may indicate that reprogramming may be partial at the molecular level in cell hybrids [26].

Mice harboring the selectable gene-trap construct ROSA26βgeo contain cells with ubiquitously expressed neomycin drug resistance and β-galactosidase activity that allow them to be positively selected in the presence of neomycin and stained blue in the presence of the chromogenic substrate X-gal [27]. Hybrids between mouse Hprt^{-} Hm1 ES cells and thymocytes containing a selectable ROSA26βgeo transgene produced teratomas in SCID mice that contained differentiated cells of all three germ layers [28]. These hybrids retained chromosomes from both fusion partners as judged by the presence of specific polymorphisms. The fusion-derived cells could differentiate into neuronal-like cells that express the dopaminergic neuronal marker PitX3 when co-cultured with PA-6 stromal cells. When neuronally-differentiated hybrids were implanted into mouse brains, the cells expressed tyrosine hydroxylase, providing evidence that hybrid cells can differentiate into neuronal cells with dopaminergic characteristics.

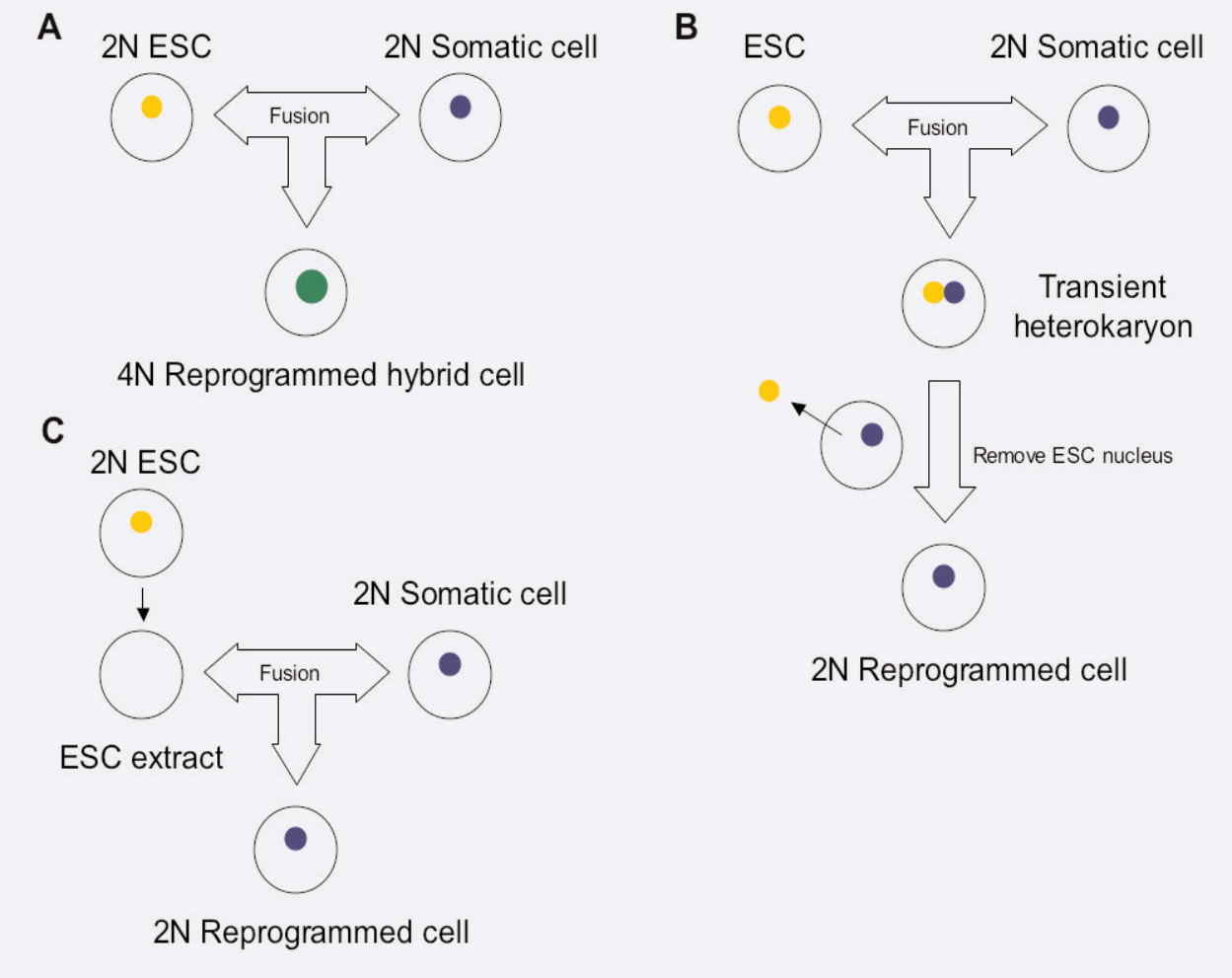

Fig. 2 Approaches to generate diploid reprogrammed cells. A: Simple fusion. The resulting hybrid, though reprogrammed, contains a 4N complement of chromosomes of both somatic and ESC origin, and will be rejected upon engraftment in the absence of immunomodulatory drugs. B: ESCs are fused with somatic cells to yield a transient heterokaryon. The ESC nucleus is removed prior to synkaryon formation. C: A membrane-bound derivative from ESCs is prepared that lacks a nucleus. Alternatively, a reprogramming extract from ESCs is prepared that lacks DNA.

Cell fusion has also been used as a tool to investigate the subcellular location of reprogramming activities by performing fusions utilizing karyoplasts and cytoplasts derived from ESCs [29]. ESC karyoplasts and cytoplasts were fused to neurosphere cells harboring a silent Oct4-GFP transgene and ROSA26βgeo. Neomycin-resistant colonies with stem cell-like morphologies were obtained from karyoplast but not cytoplast fusions, indicating that the major reprogramming activity within ESCs is nuclear in this system. Furthermore, the ESC karyoplast/neurosphere hybrids also reactivated the Oct4-GFP reporter, suggesting the acquisition of pluripotentiality. RT-PCR assays also showed that the pluripotency markers nanog and Rex1 were reactivated, while the ectoderm marker GluR6 was silenced. These results show that these hybrids do not simply contain the union of gene expression patterns of the two fusion partners, showing that *bona fide* reprogramming likely occurred.

Fusion-mediated reprogramming using other embryonic cells

Primordial germ cells (PGCs) contain interesting reprogramming activities since they undergo imprint erasure shortly after migration to the genital ridge but prior to the establishment of male or female gonadal fates. PGCs can be identified by expression of an Oct4-GFP transgene, which allows their purification by fluorescence activated cell sorting (FACS). Analysis of DNA contained within PGCs prepared in this way revealed demethylation at multiple genetic loci including both imprinted and non-imprinted genes [30, 31]. This finding makes PGC cells and their cultured derivatives, embryonic germ cells (EGCs), attractive candidates for intentional fusion with somatic cells to bring about reprogramming. Indeed, when PGCs marked with ROSA26βgeo were electrofused to thymocytes, several imprinted and non-imprinted genes underwent demethylation, in essence adopting a state observed in normally-derived EGCs [32]. In overall morphology, the hybrids resembled EGCs, but inspection of the hybrid karyotype revealed a tetraploid complement of chromosomes. When EGC/T-cell hybrids were injected into host blastocysts and implanted into pseudopregnant mothers, β-galactosidase expression was widely observed in chimeric embryos at days E9.5 and E10.5. Though EGCs clearly contain potent demethylating activities, it remains unclear if the expected loss of imprints in PGC/somatic fusions would have consequences for cell-based therapies.

Mouse embryonal carcinoma cells (ECCs) can also reprogram somatic cells. For instance, human T-lymphoma cells were reprogrammed upon fusion with mouse embryonal carcinoma cells. In this instance, the hybrids expressed human Oct4 and Sox-2 genes, showing that human genes involved in pluripotency were derepressed by the hybridization event. In addition, ECC/T-lymphoma hybrids expressed reduced levels of CD45 surface protein, suggesting that lymphocytic characteristics of the hybrids may have been altered [33].

Prospects for the future

Custom human embryonic stem cells (hESCs) could in theory be harvested from embryos created by SCNT. Though such cells might have complete, or nearly complete, autologous immune characteristics upon engraftment, this approach would require a ready supply of oocytes, be labor-intensive, technically challenging, and expensive. Therefore, much interest has been focused on alternative methods to bring about similar reprogramming without using SCNT. One approach would be to use reprogramming activities resident within embryonic stem cells. Such activities could be brought into contact with a patient's somatic cells by fusion. The resulting tetraploid cells may themselves be of utility for cell-based therapy, but these cells may have unforeseen problems that arise from their tetraploid chromosome complement, including intrinsic tendencies to form tumors. Therefore, approaches to remove the stem cell genome from hybrids, or utilize reprogramming extracts prepared from stem cells, will need to be developed and refined.

Cell fusion with ESCs is an attractive strategy since a single human ES cell line with proven reprogramming activity could be used for hundreds, if not thousands, of fusion-mediated reprogramming events. However, hybrid cell karyotypes can be unstable, and ESC/splenocyte hybrids lose chromosomes from the somatic partner, especially if the cells are not maintained under selection [34]. For these reasons, much interest has focused on ways to alleviate this problem by removing the nucleus of the reprogramming cell either before, or shortly after fusion. One approach, in development by Paul Verma at the Monash Institute of Reproduction, fuses stem cells to themselves to create tetraploid ESCs which are then fused to somatic cells [35]. Chemical inhibition is applied long enough to keep the tetraploid nuclei from fusing to the diploid somatic nuclei, but hopefully without inhibiting the reprogramming of the somatic genome. The heavier tetraploid nucleus is then removed from the hybrid cell by centrifugation (Fig. 2B).

Though removal of the ESC genome after fusion but before synkaryon formation could address the ploidy problem, the recent finding that the nucleoplasm of ES cells may contain the bulk of reprogramming activities [29] suggests that reprogramming may be difficult to achieve with such methods. Another approach is to use "reprogramming cocktails". Cell-free extracts or artificial preparations could be delivered directly to somatic cells or applied in cell culture. The source of such extracts may not even need to be mammalian: amphibians can regenerate lost limbs through a process thought to proceed through the de-differentiation of cells at the wound site. When mouse C2C12 myotubes are exposed to extracts derived from the limbs of regenerating newts, a significant number of myotube cells reenter the cell cycle and lose expression of the muscle markers MyoD, myogenin, and Troponin T, suggesting that reprogramming or de-differentiation can be elicited by exposure to newt extracts [36].

Novel approaches are being developed that utilize cell extracts that can directly reprogram somatic cells to bring about transdifferentiation. For instance, extracts from human activated T cells remodel the chromatin of the IL-2 promoter in resting T cells and other non-T cell lines resulting in derepression of the IL-2 gene [37]. Though this is encouraging, it remains to be seen if this strategy of "direct reprogramming" will be effective in altering cell identity to the extent that a patient's somatic cells could be rendered useful for cell-based therapies. In a similar study, the application of cytoplasmic and nuclear extracts from T-cells caused 293T fibroblasts to express lymphoid genes such as T-cell and IL-2 receptor genes [38]. Finally, synthetic small molecules may one day perform some reprogramming functions, and a recent report details a small molecule called reversin that can transform mammalian myoblasts into cells with characteristics of adipocytes or osteoblasts [39].

Conclusions

Recent experiments that make use of spontaneous or intentional fusions of somatic cells with ESCs, ECCs, EGCs, or others, have shown that somatic nuclei can be reprogrammed through cell fusion. Such reprogramming consists of the epigenetic remodeling of the somatic genome to yield a new (and typically more embryonic) state of chromatin organization and gene expression. Reprogramming of somatic/embryonic cell hybrids can be operationally assessed by performing assays of developmental potential such as chimera formation. In addition, the combined use of polymorphisms and reporter genes, especially those driven by Oct4 transcriptional regulatory sequences, has shown that embryonic genes can be activated within the genome of somatic origin within hybrids.

Based on the work with hybrids outlined in this review, we propose three conditions that should be satisfied for proof of reprogramming in hybrid cells: (1) The gene expression in hybrids must consist of more than the sum of individual patterns of gene expression present in unfused parental cells. (2) Gene silencing or activation in hybrids is best demonstrated with polymorphisms that mark the "parental" origin of reprogrammed gene expression. (3) Reprogrammed hybrid cells should exhibit unique developmental potential or biological characteristics.

References

1. **Gurdon JB.** Adult frogs derived from the nuclei of single somatic cells. *Dev Biol.* 1962; 4: 256–73.
2. **Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KH.** Viable offspring derived from fetal and adult mammalian cells. *Nature* 1997; 385: 810–3.
3. **Eggan K, Akutsu H, Loring J, Jackson-Grusby L, Klemm M, Rideout WM 3rd, Yanagimachi R, Jaenisch R.** Hybrid vigor, fetal overgrowth, and viability of mice derived by nuclear cloning and tetraploid embryo complementation. *Proc Natl Acad Sci USA*. 2001; 98: 6209–14.
4. **Rideout WM 3rd, Wakayama T, Wutz A, Eggan K, Jackson-Grusby L, Dausman J, Yanagimachi R, Jaenisch R.** Generation of mice from wild-type and targeted ES cells by nuclear cloning. *Nat Genet.* 2000; 24: 109–10.
5. **Jaenisch R Eggan K, Humpherys D, Rideout W, Hochedlinger K.** Nuclear cloning, stem cells, and

genomic reprogramming. *Cloning Stem Cells* 2002; 4: 389–96.

6. **Nichols J Zevnik B, Anastassiadis K, Niwa H, Klewe-Nebenius D, Chambers I, Scholer H, Smith A.** Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. *Cell* 1998; 95: 379–91.
7. **Bortvin A, Eggan K, Skaletsky H, Akutsu H, Berry DL, Yanagimachi R, Page DC, Jaenisch R.** Incomplete reactivation of Oct4-related genes in mouse embryos cloned from somatic nuclei. *Development* 2003; 130: 1673–80.
8. **Chambers I, Colby D, Robertson M, Nichols J, Lee S, Tweedie S, Smith A.** Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. *Cell* 2003; 113: 643–55.
9. **Mitsui K, Tokuzawa Y, Itoh H, Segawa K, Murakami M, Takahashi K, Maruyama M, Maeda M, Yamanaka S.** The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. *Cell* 2003; 113: 631–42.
10. **Eggan K, Akutsu H, Hochedlinger K, Rideout W 3rd, Yanagimachi R, Jaenisch R.** X-Chromosome inactivation in cloned mouse embryos. *Science* 2000; 290: 1578–81.
11. **Shi W, V Zakhartchenko, E Wolf.** Epigenetic reprogramming in mammalian nuclear transfer. *Differentiation* 2003; 71: 91–113.
12. **Bourc'his D, Le Bourhis D, Patin D, Niveleau A, Comizzoli P, Renard JP, Viegas-Pequignot E.** Delayed and incomplete reprogramming of chromosome methylation patterns in bovine cloned embryos. *Curr Biol*. 2001; 11: 1542–6.
13. **Mann MR, Chung YG, Nolen LD, Verona RI, Latham KE, Bartolomei MS.** Disruption of imprinted gene methylation and expression in cloned preimplantation stage mouse embryos. *Biol Reprod.* 2003; 69: 902–14.
14. **Hochedlinger K, R Jaenisch.** Monoclonal mice generated by nuclear transfer from mature B and T donor cells. *Nature* 2002*;* 415: 1035–8.
15. **Rideout WM 3rd, Hochedlinger K, Kyba M, Daley GQ, Jaenisch R.** Correction of a genetic defect by nuclear transplantation and combined cell and gene therapy. *Cell* 2002; 109: 17–27.
16. **Humpherys D, Eggan K, Akutsu H, Hochedlinger K, Rideout WM 3rd, Biniszkiewicz D, Yanagimachi R, Jaenisch R.** Epigenetic instability in ES cells and cloned mice. *Science* 2001; 293: 95–7.
17. **Humpherys D, Eggan K, Akutsu H, Friedman A, Hochedlinger K, Yanagimachi R, Lander ES, Golub TR, Jaenisch R.** Abnormal gene expression in cloned mice derived from embryonic stem cell and cumulus cell nuclei. *Proc Natl Acad Sci USA*. 2002; 99: 12889–94.
18. **Terada N, Hamazaki T, Oka M, Hoki M, Mastalerz DM, Nakano Y, Meyer EM, Morel L, Petersen BE, Scott EW.** Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. *Nature* 2002; 416: 542–5.
19. **Ying QL, Nichols J, Evans EP, Smith AG.** Changing potency by spontaneous fusion. *Nature* 2002; 416: 545–8.
20. **Harris RG, Herzog EL, Bruscia EM, Grove JE, Van Arnam JS, Krause DS.** Lack of a fusion requirement for development of bone marrow-derived epithelia. *Science* 2004; 305: 90–3.
21. **Que J, El Oakley RM, Salto-Tellez M, Wong N, de Kleijn DP, Teh M, Retnam L, Lim SK.** Generation of hybrid cell lines with endothelial potential from spontaneous fusion of adult bone marrow cells with embryonic fibroblast feeder. *In Vitro Cell Dev Biol Anim.* 2004; 40: 143–9.
22. **Pells S, Di Domenico AI, Gallagher EJ, McWhir J.** Multipotentiality of neuronal cells after spontaneous fusion with embryonic stem cells and nuclear reprogramming *in vitro*. *Cloning Stem Cells* 2002; 4: 331–8.
23. **Matveeva NM, Shilov AG, Kaftanovskaya EM, Maximovsky LP, Zhelezova AI, Golubitsa AN, Bayborodin SI, Fokina MM, Serov OL.** *In vitro* and *in vivo* study of pluripotency in intraspecific hybrid cells obtained by fusion of murine embryonic stem cells with splenocytes. *Mol Reprod Dev.* 1998; 50: 128–38.
24. **Tada M, Takahama Y, Abe K, Nakatsuji N, Tada T.** Nuclear reprogramming of somatic cells by *in vitro* hybridization with ES cells. *Curr Biol*. 2001; 11: 1553–8.
25. **Hatano SY, Tada M, Kimura H, Yamaguchi S, Kono T, Nakano T, Suemori H, Nakatsuji N, Tada T.** Pluripotential competence of cells associated with Nanog activity. *Mech Dev*. 2005; 122: 67–79.
26. **Kimura H, Tada M, Nakatsuji N, Tada T.** Histone code modifications on pluripotential nuclei of reprogrammed somatic cells. *Mol Cell Biol*. 2004; 24: 5710–20.
27. **Zambrowicz BP, Imamoto A, Fiering S, Herzenberg LA, Kerr WG, Soriano P.** Disruption of overlapping transcripts in the ROSA beta geo 26 gene trap strain leads to widespread expression of β-galactosidase in mouse embryos and hematopoietic cells. *Proc Natl Acad Sci USA*. 1997; 94: 3789–94.
28. **Tada M, Morizane A, Kimura H, Kawasaki H, Ainscough JF, Sasai Y, Nakatsuji N, Tada T.** Pluripotency of reprogrammed somatic genomes in embryonic stem hybrid cells. *Dev Dyn*. 2003; 227: 504–10.
29. **Do JT, HR Scholer.** Nuclei of embryonic stem cells reprogram somatic cells. *Stem Cells* 2004; 22: 941–9.
30. **Monk M, M Boubelik, S Lehnert.** Temporal and regional changes in DNA methylation in the embryonic, extraembryonic and germ cell lineages during mouse embryo development. *Development* 1987; 99: 371–82.
31. **Hajkova P, Erhardt S, Lane N, Haaf T, El-Maarri O, Reik W, Walter J, Surani MA.** Epigenetic reprogramming in mouse primordial germ cells. *Mech Dev*, 2002; 117: 15–23.
32. **Tada M, Tada T, Lefebvre L, Barton SC, Surani MA.** Embryonic germ cells induce epigenetic reprogramming of somatic nucleus in hybrid cells. *EMBO J*. 1997; 16: 6510–20.

33. **Flasza M, Shering AF, Smith K, Andrews PW, Talley P, Johnson PA.** Reprogramming in inter-species embryonal carcinoma-somatic cell hybrids induces expression of pluripotency and differentiation markers. *Cloning Stem Cells* 2003; 5: 339–54.
34. **Serov OL, Matveeva NM, Kizilova EA, Kuznetsov SB, Zhelezova AI, Golubitsa AN, Pristiazhniuk IE, Puzakov MV.** "Chromosome memory" of parental genomes in embryonic hybrid cells. *Ontogenez* 2003; 34: 216–27.
35. **Dennis C.** Developmental reprogramming: take a cell, any cell. *Nature* 2003; 426: p. 490–1.
36. **McGann CJ, SJ Odelberg, MT Keating.** Mammalian myotube dedifferentiation induced by newt regeneration extract. *Proc Natl Acad Sci USA*. 2001; 98: 13699–704.
37. **Landsverk HB, Hakelien AM, Kuntziger T, Robl JM, Skalhegg BS, Collas P.** Reprogrammed gene expression in a somatic cell-free extract. *EMBO Rep*. 2002; 3: 384–9.
38. **Hakelien AM, Landsverk HB, Robl JM, Skalhegg BS, Collas P.** Reprogramming fibroblasts to express T-cell functions using cell extracts. *Nat Biotechnol*. 2002; 20: 460–6.
39. **Chen S, Zhang Q, Wu X, Schultz PG, Ding S.** Dedifferentiation of lineage-committed cells by a small molecule. *J Am Chem Soc*. 2004; 126: 410–1.