

Pluripotency and nuclear reprogramming

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Embryonic stem cells are promising donor cell sources for cell transplantation therapy, which may in the future be used to treat various diseases and injuries. However, as is the case for organ transplantation, immune rejection after transplantation is a potential problem with this type of therapy. Moreover, the use of human embryos presents serious ethical difficulties. These issues may be overcome if pluripotent stem cells are generated from patients' somatic cells. Here, we review the molecular mechanisms underlying pluripotency and the currently known methods of inducing pluripotency in somatic cells.

Keywords: embryonic stem cell; induced pluripotent stem cell; transcription factor; retrovirus

1. INTRODUCTION

Embryonic stem (ES) cells are derived from the inner cell mass of blastocyst stage embryos and have the unique capacity to proliferate extensively while maintaining pluripotency. The isolation of the first ES cells from mouse embryos (Evans & Kaufman 1981; Martin 1981) led to the development of the revolutionary 'knockout mouse' technology (Doetschman *et al.* 1987; Hooper *et al.* 1987), which is widely used today. Since ES cells theoretically have the capacity to develop into any cell type, the generation of ES cell lines from human blastocyst embryos (Thomson *et al.* 1998) has offered the possibility of using these cells as a donor source for cell transplantation therapies. Potential clinical applications include treatment of degenerative diseases such as juvenile diabetes, Parkinson's disease and heart failure as well as spinal cord injury and burns. However, as is the case for organ transplantation, tissue rejection is a concern for ES cell transplantation. One possible means to avoid immune rejection is the reprogramming of the nuclei of differentiated cells to an ES cell-like, pluripotent state, and using these cells to generate appropriate donor cells for transplantation. Here, we will review the following three methods of inducing nuclear reprogramming and pluripotency in somatic cells: nuclear transfer, fusion with ES cells and reprogramming using defined factors (figure 1).

2. REPROGRAMMING BY NUCLEAR TRANSFER

Much progress has been made in the last 50 years in understanding how differentiation and pluripotency are controlled. First, in order for the somatic cells to regain pluripotency, it is essential that they maintain complete genomes. While it is now widely accepted that all cells in an organism possess the same genome, historically this has not always been the case. For instance, in his

1893 book 'The germ-plasma: a theory of heredity', Weismann proposed that somatic cells lose or irreversibly inactivate unnecessary genes in the course of differentiation. It took more than 50 years to disprove this theory (Gurdon & Byrne 2003).

In 1952, Briggs & King showed that nuclear transfer was possible in frogs by transplanting nuclei from blastula stage embryos into enucleated *Rana pipiens* eggs. The resulting embryos developed into normal hatched tadpoles (Briggs & King 1952). However, their later work showed that transplantation of nuclei from tail-bud stage somatic cells into enucleated eggs resulted in abnormal embryonic development, suggesting that differences in nuclear state affect the capacity of somatic cells to be reprogrammed (King & Briggs 1955, 1956). Gurdon and colleagues then succeeded in producing adult frogs by transferring tadpole intestine cell nuclei into enucleated *Xenopus laevis* eggs (Gurdon 1962; Gurdon & Uehlinger 1966), showing that the genes required for normal development can be activated even in differentiated cells. However, when they transferred nuclei from adult somatic cells, animals developed to tadpole, but not to adult stage (Laskey & Gurdon 1970; Gurdon *et al.* 1975; DiBerardino & Hoffner 1983). This raised the possibility that terminally differentiated cells are unable to activate genes required for full development to adulthood.

Nuclear transfer in mammals is more difficult than in frogs due to the small size of eggs. Nonetheless, in 1975, Bromhall reported development to the morula stage following nuclear transfer of rabbit morula cell nuclei into enucleated rabbit eggs, albeit with low efficiency (Bromhall 1975). In 1983, McGrath & Solter obtained live mice by transferring a zygotic donor nucleus into an enucleated zygote (McGrath & Solter 1983). In 1986, Willadsen fused cells of 8- or 16-cell embryos with enucleated sheep eggs and was able to obtain healthy cloned animals (Willadsen 1986). Successful nuclear transfer of embryonic donor cell nuclei was subsequently reported in rabbits (Stice & Robl 1988),

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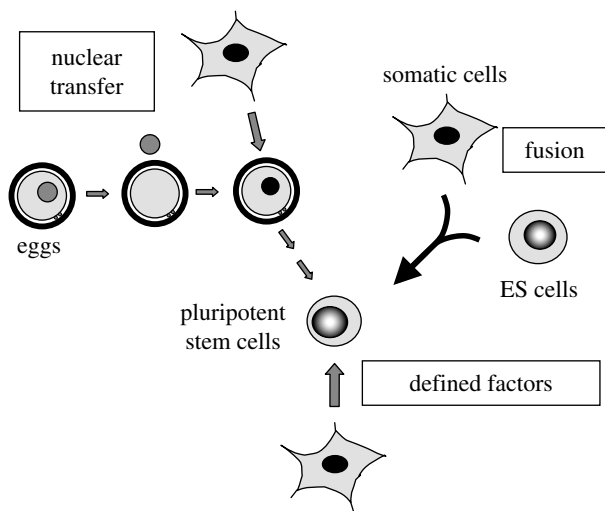


Figure 1. Three methods of inducing pluripotency in somatic cells.

mice (Cheong *et al.* 1993), pigs (Prather *et al.* 1989), cows (Sims & First 1994) and monkeys (Meng *et al.* 1997). However, it proved difficult to generate cloned animals by nuclear transfer from differentiated cells into eggs.

A breakthrough came in 1996, when Campbell & Wilmut performed nuclear transfer using day 9 embryo-derived differentiated cells and successfully obtained healthy cloned sheep. Their success was probably due to the fact that they induced their cells into a quiescent state prior to nuclear transfer (Wilmut *et al.* 2002). Using similar techniques, Wilmut was able to produce an adult sheep, famously known as 'Dolly', using nuclei derived from follicle cells (Wilmut *et al.* 1997). Subsequently, somatic cloning was successfully performed in other species, including the cow (Kato *et al.* 1998), mouse (Wakayama *et al.* 1998), goat (Baguisi *et al.* 1999), pig (Polejaeva *et al.* 2000), cat (Shin *et al.* 2002), rabbit (Chesne *et al.* 2002) and dog (Lee *et al.* 2005). Furthermore, Jaenisch and colleagues generated cloned mice from B lymphocytes that had undergone immunoglobulin rearrangement (Hochedlinger & Jaenisch 2002). These studies demonstrated that terminally differentiated cells are in fact able to reactivate all of the genes required for normal development, and that egg cytoplasm contains factors that are able to reprogramme somatic cell nuclei.

Given that embryos can now be cloned using somatic cell nuclei, derivation of ES cells from these cloned embryos might provide a means of avoiding immune rejection after transplantation therapy. However, many countries have laws prohibiting cloning of humans for ethical reasons. Cloned ES cells face even stronger ethical objections than ordinary human ES cells, since they involve generation of human embryos exclusively for the production of ES cells. Obtaining a sufficient number of human oocytes presents another challenging issue. As a solution to overcome this issue, Eggan and associates showed that reprogramming can be achieved by fertilized eggs, when mitosis is arrested (Egli *et al.* 2007). In 2005, a group in Korea reported that they had successfully generated cloned ES cells from skin

cells of patients suffering from spinal cord injury and juvenile diabetes (Hwang *et al.* 2004, 2005). However, in a much-publicized fraud scandal, their data were shown to be a fabrication; indeed, they were unable to generate a single cloned ES cell line from more than 2000 human eggs.

3. REPROGRAMMING BY FUSION WITH ES CELLS

Another strategy for reprogramming the somatic cell nuclei is fusion with ES, embryonic germ (EG) or embryonic carcinoma (EC) cells. In 1965, Harris showed that fusion of HeLa cells with Sendai virus was able to activate erythrocytes (Harris 1965; Harris & Watkins 1965; Harris *et al.* 1965). Their work facilitated studies in cell fate conversion by fusion with other cell types. In 1976, Miller & Ruddle demonstrated that thymocytes acquired pluripotency upon fusion with EC cells (Miller & Ruddle 1976), and similar results were later obtained by electrofusion with EG cells (Tada *et al.* 1997) and mouse ES cells (Tada *et al.* 2001). Reprogramming by spontaneous fusion with mouse ES cells was also demonstrated (Terada *et al.* 2002; Ying *et al.* 2002). In tetraploid cells generated by fusion of somatic cells with ES or EG cells, ES cell marker genes such as Oct3/4 acquire an ES cell-like epigenetic state, adopting the DNA methylation and histone modification patterns normally seen in ES cells (Kimura *et al.* 2004). The transplantation of these cells into nude mice results in formation of teratomas consisting of various tissues from all three germ layers, confirming the pluripotency of these cells. Finally, reprogramming by fusion with human ES cells was reported in 2005 (Cowan *et al.* 2005; Yu *et al.* 2005).

Thus, it appears that ES cells contain factors that induce pluripotency in somatic cells, though it is still controversial whether these factors reside in the nucleus (Do & Scholer 2004) or cytoplasm (Strelchenko *et al.* 2006) of ES cells. However, pluripotent cells generated by fusion contain both somatic cell and ES cell-derived chromosomes. As a result, rejection upon implantation remains an issue. In an effort to circumvent this problem, Tada and colleagues recently developed a system to remove whole chromosomes from tetraploid cells (Matsumura *et al.* 2007). In addition, other groups have been attempting to reprogramme the somatic cells with ES cell extracts (Taranger *et al.* 2005).

4. REPROGRAMMING WITH DEFINED FACTORS

The fact that somatic cell nuclei can be reprogrammed by transfer into oocytes or fusion with ES cells indicates that oocytes and ES cells contain reprogramming factors. It is probable that reprogramming factors largely overlap with those maintaining pluripotency in ES cells. Some of the candidate factors are described below.

(a) Oct3/4 (POU5F1)

POU5F1 is a POU family transcription factor specifically expressed in ES cells, early embryos and germ cells, and was originally designated as Oct3 (Okamoto *et al.* 1990) or Oct4 (Scholer *et al.* 1989). Oct3/4-null embryos die *in utero* at peri-implantation stages of

development (Nichols *et al.* 1998). Although these embryos are able to reach blastocyst stage, *in vitro* culture of the inner cell mass of homozygous mutant blastocyst produces only trophoblast lineages, and Oct3/4 mutant ES cells differentiate only into trophoblast cells, while cells expressing a single copy of Oct3/4 maintain their pluripotential ES cell state (Niwa *et al.* 2000). Likewise, knock-down of Oct3/4 by siRNA in human ES cells caused these cells to differentiate into trophoblast lineages (Zaehres *et al.* 2005). By contrast, twofold overexpression of Oct3/4 in ES cells causes differentiation into primitive endoderm and mesoderm (Niwa *et al.* 2000). Hence, the level of Oct3/4 expression is an important determinant of cell fate in mouse ES cells.

(b) *Sox2*

Sox2 is a Sox (SRY-related HMG-box) family transcription factor expressed in ES cells, early embryos, germ cells and neural stem cells (Koopman *et al.* 2004). The *Sox2*-null embryos die around implantation due to a failure in epiblast (primitive ectoderm) development (Avilion *et al.* 2003). Homozygous mutant blastocysts appear morphologically normal, but undifferentiated cells fail to proliferate when blastocysts are cultured *in vitro*, and only trophoblast and primitive endoderm-like cells are produced. Disruption of *Sox2* in mouse ES cells by either conditional knockout or RNAi resulted in rapid differentiation (Ivanova *et al.* 2006; Masui *et al.* 2007). These data demonstrate that *Sox2* is indispensable for maintaining pluripotency in both early embryos and ES cells. Another Sox family protein, *Sox15*, is also highly expressed in ES cells, but its role remains unclear (Maruyama *et al.* 2005).

(c) *Nanog*

Nanog is a homeobox protein specifically expressed in pluripotent cells and the inner cell mass of the blastocyst stage embryo (Chambers *et al.* 2003; Mitsui *et al.* 2003). *Nanog*-null embryos show disorganization of extraembryonic tissues at E5.5, with no discernible epiblast or primitive ectoderm (Mitsui *et al.* 2003). *Nanog*-deficient blastocysts appear to be morphologically normal, but the inner cell mass produces only parietal endoderm-like cells and not epiblast-derived cells when blastocysts are cultured *in vitro*. ES cells lacking *Nanog* can be derived, but they tend to differentiate into extraembryonic endoderm lineages even in the presence of LIF. Another group reported that even heterozygous *Nanog* mutant ES cells were unstable and susceptible to spontaneous differentiation (Hatano *et al.* 2005). RNAi-mediated knockdown of *Nanog* led to differentiation in both the mouse (Hough *et al.* 2006; Ivanova *et al.* 2006) and the human (Hyslop *et al.* 2005; Zaehres *et al.* 2005) ES cells. Importantly, overexpression of *Nanog* in mouse ES cells permits cells to self-renew in the absence of LIF. Likewise, overexpression of *Nanog* in human ES cells enabled growth without feeder cells (Darr *et al.* 2006). In addition, *Nanog*-overexpressing ES cells showed markedly increased reprogramming activity after fusion with somatic cells (Silva *et al.* 2006). *Nanog* expression is upregulated by Oct3/4 and *Sox2* (Kuroda *et al.* 2005; Rodda *et al.* 2005; Wu da & Yao 2005) and suppressed

by p53 (Lin *et al.* 2005), GCNF (Gu *et al.* 2005) and TCF3 (Pereira *et al.* 2006). Genome-wide chromatin immunoprecipitation analyses demonstrated that Oct3/4, *Sox2* and *Nanog* share many target genes in both the mouse and the human ES cells (Boyer *et al.* 2005; Loh *et al.* 2006).

(d) *STAT3*

Both the mouse and the human ES cells are usually derived and maintained on a feeder layer of mouse embryonic fibroblasts (MEFs) in order to promote self-renewal. Conditioned media from MEFs can also support self-renewal of mouse ES cells, indicating that MEFs secrete soluble factor(s) that inhibit differentiation (Smith & Hooper 1987). Indeed, MEFs are known to inhibit ES cell differentiation via production of the IL-6 family cytokine, leukaemia inhibitory factor (LIF; Smith *et al.* 1988; Williams *et al.* 1988). With the addition of recombinant LIF protein into the culture medium, mouse ES cells can be cultured without MEF feeder cells.

The LIF receptor is a heteromeric complex consisting of gp130 and the LIF receptor (LIFR, also known as LIFR β ; Ernst & Jenkins 2004). The tyrosine kinase Janus kinase (JAK) binds constitutively to the intracellular domain of this receptor complex in its inactive form. Upon LIF binding, JAK kinase phosphorylates tyrosine residues on both gp130 and LIFR. Phosphorylation of Y765/812/904/914 of the intracellular domain of gp130 and Y976/996/1023 of LIFR recruits signal transducers and activators of transcription (STAT) 1 and STAT3 through their SH2 domains (Stahl *et al.* 1995). The STAT proteins are then activated by JAK-mediated tyrosine phosphorylation to form homodimers and/or heterodimers and translocate into the nucleus, where they function as transcription factors (Auernhammer & Melmed 2000).

Among the many STAT proteins, STAT3 has been shown to be essential for the maintenance of pluripotency in mouse ES cells (Boeuf *et al.* 1997; Niwa *et al.* 1998; Matsuda *et al.* 1999; Raz *et al.* 1999). Using a tamoxifen-inducible form of STAT3 generated by fusion with the ligand-binding domain of the oestrogen receptor, it was shown that STAT3 activation is sufficient for self-renewal in the presence of foetal bovine serum (Matsuda *et al.* 1999). STAT3 also cooperates with Smad and Id proteins to support clonal expansion of mouse ES cells in the absence of serum (Ying *et al.* 2003).

The role of LIF appears to be different among different species. For example, LIF cannot promote self-renewal of human or monkey ES cells (Humphrey *et al.* 2004; Sumi *et al.* 2004). Human ES cells express relatively low levels of LIF signalling components (LIFR, JAK and STAT3), and high levels of suppressor of cytokine signalling (SOCS), which negatively regulates LIF signalling (Wei *et al.* 2005a). In monkey ES cells, suppression of LIF signalling by a dominant negative form of STAT3 does not cause them to differentiate (Sumi *et al.* 2004). Thus, human and monkey ES cells appear to maintain pluripotency using a LIF/STAT3-independent mechanism. Since contamination from feeder cells represents a potential problem for transplantation therapy, it will be useful

to determine the mechanisms by which feeder cells maintain pluripotency of human ES cells.

(e) *APC/β-catenin*

β-catenin is a dual-function protein that participates in cell–cell adhesion by linking cadherins to the actin cytoskeleton and also acts as an intracellular signalling molecule of the canonical Wnt signalling pathway (Reya & Clevers 2005). In the absence of Wnt activation, β-catenin is phosphorylated by a complex consisting of adenomatous polyposis coli gene (APC), Axin and glycogen synthase kinase (GSK) 3β. Phosphorylated β-catenin is degraded by the ubiquitin–proteasome system, thereby keeping the level of cytoplasmic β-catenin low. Upon binding of Wnt to its receptors, Frizzled and LRP5/6, GSK3β is inactivated through a poorly understood mechanism involving the direct interaction of Axin with LRP5/6, and/or the action of an Axin-binding molecule, dishevelled. As a result, β-catenin accumulates in the cytoplasm and travels to the nucleus, where it regulates transcription through association with lymphoid enhancer factor (LEF)/T-cell factor (TCF) transcription factors.

Neural differentiation of mouse ES cells was attenuated by the activation of Wnt signalling, either by overexpression of Wnt1 or treatment with lithium chloride, an inhibitor of GSK3β (Aubert *et al.* 2002). Moreover, Wnt3a mutant mice display ectopic neural tube formation at gastrulation stage (Yoshikawa *et al.* 1997). In addition, ES cells with a mutant form of APC were impaired in their ability to differentiate into the three germ layers (Kielman *et al.* 2002). Wnt also accelerates the proliferation of stem cells in the intestinal, epidermal and haematopoietic systems, and may be a common factor controlling stem cell proliferation (Reya *et al.* 2003). These data suggest that Wnt signalling promotes self-renewal of ES cells and other stem cells.

Comparisons of the gene expression profile of undifferentiated and differentiated human ES cells revealed that frizzled 5 is enriched in undifferentiated ES cells (Sato *et al.* 2003). In addition, 6-bromoindirubin-3'-oxime (BIO), a newly identified pharmacological inhibitor of GSK3β, was able to maintain mouse ES cell pluripotency in the absence of LIF (Sato *et al.* 2004). Most notably, BIO was also able to maintain human ES cells in an undifferentiated state, and to maintain the expression of Oct3/4, Rex1 and Nanog. Given that LIF has little effect on human ES cells and culture on feeder cells may result in cross-species contamination upon transplantation, BIO may provide a useful means of improving the culture techniques of human ES cells.

How Wnt/β-catenin signalling promotes self-renewal of mouse ES cells remains to be resolved. β-catenin is reported to upregulate the expression of STAT3 (Hao *et al.* 2006), and there appears to be a synergistic interaction between Wnt and LIF (Ogawa *et al.* 2006). On the other hand, BIO treatment permits LIF-independent self-renewal of mouse ES cells (Sato *et al.* 2004). This suggests that BIO may have additional functions besides GSK inhibition. In addition, it has been reported that Wnt facilitates neural differentiation of ES cells and induces the

expression of the mesoderm marker brachyury (Yamaguchi *et al.* 1999). Additional experiments are required to clarify the function of Wnt/β-catenin signalling in ES cells and other stem cells.

(f) *c-Myc*

c-Myc is a helix-loop-helix/leucine zipper transcription factor that associates with its partner protein, Max (Adhikary & Eilers 2005). c-Myc is regulated by STAT3 (Kiuchi *et al.* 1999) and plays important roles in self-renewal and maintenance of pluripotency in mouse ES cells (Cartwright *et al.* 2005): forced expression of stable c-Myc causes mouse ES cells to self-renew without LIF, whereas the dominant negative form of c-Myc induces differentiation even in the presence of LIF. GSK3β negatively regulates c-Myc activity by phosphorylation-dependent degradation by the proteasome (Sears *et al.* 2000). Thus, c-Myc is a common target for both the LIF and Wnt signalling pathways. c-Myc has a large number of binding sites in the genome (Fernandez *et al.* 2003; Li *et al.* 2003; Cawley *et al.* 2004) and is thought to modify chromatin structure (Knoepfler *et al.* 2006) and activate expression of some miRNAs (O'Donnell *et al.* 2005).

(g) *Klf4*

Klf4 is a Kruppel-like transcription factor (also known as gut-enriched Kruppel-like factor, GKLf) (Rowland *et al.* 2005) originally identified as a tumour suppressor that is deleted in gastrointestinal cancers (Zhao *et al.* 2004; Wei *et al.* 2005b). A conditional knockout mouse model supports a role for Klf4 in gastrointestinal cancers (Katz *et al.* 2005). Klf4, however, is overexpressed in squamous cell carcinoma and breast cancers (Foster *et al.* 1999, 2000). Moreover, induction of KLF4 in basal keratinocytes blocks the proliferation–differentiation switch and initiates squamous epithelial dysplasia (Foster *et al.* 2005). Thus, Klf4 is associated with both tumour suppression and oncogenesis. Ectopic expression of Klf4 suppresses cell proliferation, but ablation of only one of its target genes, p21, is sufficient to neutralize the cytostatic effect of Klf4 (Rowland *et al.* 2005). In p21-null cells, Klf4 promotes cell proliferation by downregulating p53 (Rowland *et al.* 2005). This may in part account for the dual function of Klf4 in cancers.

In addition to its well-known expression in gastrointestinal tract, testis and skin, we found that Klf4 is highly expressed in undifferentiated mouse ES cells (Tokuzawa *et al.* 2004, unpublished data). We also found that inactivation of STAT3 in mouse ES cells dramatically decreases Klf4 expression and that forced expression of Klf4 enables LIF-independent self-renewal (Tokuzawa *et al.* 2004, unpublished data). Another group also reported a positive effect of Klf4 in self-renewal of mouse ES cells (Li *et al.* 2005), and Klf4 is known to cooperate with Oct3/4 and Sox2 to activate the *Lefty1* core promoter (Nakatate *et al.* 2006).

(h) *Induction of pluripotent stem cells by four factors*

To test these candidates for their pluripotency-inducing activity, we have developed a system in which induction of pluripotency can be detected as

Table 1. Pros and cons of three methods that induce pluripotency in somatic cells.

	use of human embryos	application to human cells	tumour formation	chromosomal abnormalities
nuclear transfer	yes	unknown	yes	normal
ES cell fusion	yes	yes	yes	tetraploid
iPS cell	no	unknown	yes	retroviral insertion c-Myc expression

marker gene expression. *Fbx15* is specifically expressed in ES cells and early embryos, but is dispensable for self-renewal of ES cells and development (Tokuzawa *et al.* 2003). We inserted a β -geo cassette (a fusion of β -galactosidase and the neomycin-resistant gene) into the mouse *Fbx15* locus by homologous recombination. ES cells homozygous for the β -geo knock-in ($Fbx15^{\beta_{geo}/\beta_{geo}}$) were resistant to an extremely high concentration of G418 (up to 12 mg ml^{-1}), whereas somatic cells derived from $Fbx15^{\beta_{geo}/\beta_{geo}}$ mice were sensitive to the selection. Thus, it is probable that even partial induction of pluripotency would render somatic cells resistant to G418 at normal concentrations (0.3 mg ml^{-1}).

We introduced candidate genes into $Fbx15^{\beta_{geo}/\beta_{geo}}$ MEFs by retrovirus-mediated transfection and cultured them in ES cell medium containing G418. With any single factor, we did not obtain G418-resistant colonies. However, by combining four factors (Oct3/4, Sox2, c-Myc and Klf4), we obtained multiple G418-resistant colonies. These cells resembled ES cells in both their morphology and proliferation capacity. Furthermore, when transplanted into nude mice, these induced pluripotent stem (iPS) cells produced teratomas containing tissues of all three germ layers. Thus, pluripotent cells can be generated from fibroblasts by introducing just a few defined factors (Takahashi & Yamanaka 2006).

Fbx15-selected iPS cells do not contribute adult or germ line chimeras, indicating that their reprogramming is partial. Recently, we and two other groups reported significant improvement in quality of iPS cells (Maherali *et al.* 2007; Okita *et al.* 2007; Wernig *et al.* 2007). The three groups used the same four factors, but used Nanog as a selection marker. Nanog-selected iPS cells showed more similar gene expression pattern to ES cells than did *Fbx15*-selected ones. These improved iPS cells also contributed to adult and germ line chimeras. These data showed that the combination of the four factors and proper selection can generate pluripotent cells that are indistinguishable from ES cells.

How the four factors induce pluripotency remains elusive. Our model is that c-Myc plays crucial roles. As a potent proto-oncogene, c-Myc should contribute to rapid proliferation of iPS cells. In addition, by binding numerous positions of genome and recruiting histone acetylase complexes, c-Myc should open up chromatin (Adhikary & Eilers 2005). However, overexpression of c-Myc alone in fibroblasts would induce p53-dependent apoptosis or senescence. We postulate that it is Klf4 that neutralizes the adverse effect of c-Myc by suppressing p53. Oct-3/4 and Sox2 then bind to their target sites and induce pluripotency by enhancing stemness genes and suppressing

differentiation-associated genes. Niwa and associates recently showed that Klf4 also functions as a cofactor of Oct-3/4 and Sox2 (Nakatake *et al.* 2006).

At present, however, we do not know whether the same four factors can induce iPS cells from human somatic cells. It is possible that additional factors are required to induce human iPS cells, but more work is necessary to determine whether or not this is the case. In addition, the use of retroviral vectors and the proto-oncogene c-Myc poses serious safety concerns that must be resolved prior to application of human iPS cells in regenerative medicine. In fact, we showed that approximately 20% of mice derived from Nanog-selected iPS cells developed tumours (Okita *et al.* 2007). Thus, the retroviral introduction of c-Myc should be avoided prior to clinical application. Once established, human iPS cells should also be useful in the fields of drug discovery and toxicology.

5. CONCLUSION

In this review, an overview of the three currently proposed methods of inducing pluripotency in somatic cells is given. These technologies each have their own pros and cons for future clinical applications (table 1). However, rather than discussing which approach is best at present, basic research should push ahead on all fronts so that the best possible technologies may be developed in the future (Yamanaka 2007).

6. NOTE ADDED IN PROOF

After we submitted this manuscript, we and others succeeded in producing iPS cells from human fibroblasts with the same four factors (Takahashi *et al.* 2007; Yu *et al.* 2007; Park *et al.* 2008). In addition, the c-Myc retrovirus was shown to be dispensable for iPS cell generation (Nakagawa *et al.* 2008). Furthermore, another group reported generation of human iPS cells with a partially different combination consisting of Oct3/4, Sox2, Nanog and Lin28 (Yu *et al.* 2007). These data collectively showed that nuclear reprogramming in human cells can be achieved by combinations of small numbers of factors.

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REFERENCES

- Adhikary, S. & Eilers, M. 2005 Transcriptional regulation and transformation by Myc proteins. *Nat. Rev. Mol. Cell Biol.* 6, 635–645. (doi:10.1038/nrm1703)

- Aubert, J., Dunstan, H., Chambers, I. & Smith, A. 2002 Functional gene screening in embryonic stem cells implicates Wnt antagonism in neural differentiation. *Nat. Biotechnol.* **20**, 1240–1245. (doi:10.1038/nbt763)
- Auernhammer, C. J. & Melmed, S. 2000 Leukemia-inhibitory factor-neuroimmune modulator of endocrine function. *Endocr. Rev.* **21**, 313–345. (doi:10.1210/er.21.3.313)
- Avilion, A. A., Nicolis, S. K., Pevny, L. H., Perez, L., Vivian, N. & Lovell-Badge, R. 2003 Multipotent cell lineages in early mouse development depend on Sox2 function. *Genes Dev.* **17**, 126–140. (doi:10.1101/gad.224503)
- Baguisi, A. *et al.* 1999 Production of goats by somatic cell nuclear transfer. *Nat. Biotechnol.* **17**, 456–461. (doi:10.1038/8632)
- Boeuf, H., Hauss, C., Graeve, F. D., Baran, N. & Kedinger, C. 1997 Leukemia inhibitory factor-dependent transcriptional activation in embryonic stem cells. *J. Cell Biol.* **138**, 1207–1217. (doi:10.1083/jcb.138.6.1207)
- Boyer, L. A. *et al.* 2005 Core transcriptional regulatory circuitry in human embryonic stem cells. *Cell* **122**, 947–956. (doi:10.1016/j.cell.2005.08.020)
- Briggs, R. & King, T. J. 1952 Transplantation of living nuclei from blastula cells into enucleated frogs' eggs. *Proc. Natl Acad. Sci. USA* **38**, 455–463. (doi:10.1073/pnas.38.5.455)
- Bromhall, J. D. 1975 Nuclear transplantation in the rabbit egg. *Nature* **258**, 719–722. (doi:10.1038/258719a0)
- Cartwright, P., McLean, C., Sheppard, A., Rivett, D., Jones, K. & Dalton, S. 2005 LIF/STAT3 controls ES cell self-renewal and pluripotency by a Myc-dependent mechanism. *Development* **132**, 885–896. (doi:10.1242/dev.01670)
- Cawley, S. *et al.* 2004 Unbiased mapping of transcription factor binding sites along human chromosomes 21 and 22 points to widespread regulation of noncoding RNAs. *Cell* **116**, 499–509. (doi:10.1016/S0092-8674(04)00127-8)
- Chambers, I., Colby, D., Robertson, M., Nichols, J., Lee, S., Tweedie, S. & Smith, A. 2003 Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. *Cell* **113**, 643–655. (doi:10.1016/S0092-8674(03)00392-1)
- Cheong, H. T., Takahashi, Y. & Kanagawa, H. 1993 Birth of mice after transplantation of early cell-cycle-stage embryonic nuclei into enucleated oocytes. *Biol. Reprod.* **48**, 958–963. (doi:10.1095/biolreprod48.5.958)
- Chesne, P., Adenot, P. G., Viglietta, C., Baratte, M., Boulanger, L. & Renard, J. P. 2002 Cloned rabbits produced by nuclear transfer from adult somatic cells. *Nat. Biotechnol.* **20**, 366–369. (doi:10.1038/nbt0402-366)
- Cowan, C. A., Atienza, J., Melton, D. A. & Eggan, K. 2005 Nuclear reprogramming of somatic cells after fusion with human embryonic stem cells. *Science* **309**, 1369–1373. (doi:10.1126/science.1116447)
- Darr, H., Mayshar, Y. & Benvenisty, N. 2006 Overexpression of NANOG in human ES cells enables feeder-free growth while inducing primitive ectoderm features. *Development* **133**, 1193–1201. (doi:10.1242/dev.02286)
- DiBerardino, M. A. & Hoffner, N. J. 1983 Gene reactivation in erythrocytes: nuclear transplantation in oocytes and eggs of *Rana*. *Science* **219**, 862–864. (doi:10.1126/science.6600520)
- Do, J. T. & Scholer, H. R. 2004 Nuclei of embryonic stem cells reprogram somatic cells. *Stem Cells* **22**, 941–949. (doi:10.1634/stemcells.22-6-941)
- Doetschman, T., Gregg, R. G., Maeda, N., Hooper, M. L., Melton, D. W., Thompson, S. & Smithies, O. 1987 Targetted correction of a mutant HPRT gene in mouse embryonic stem cells. *Nature* **330**, 576–578. (doi:10.1038/330576a0)
- Egli, D., Rosains, J., Birkhoff, G. & Eggan, K. 2007 Developmental reprogramming after chromosome transfer into mitotic mouse zygotes. *Nature* **447**, 679–685. (doi:10.1038/nature05879)
- Ernst, M. & Jenkins, B. J. 2004 Acquiring signalling specificity from the cytokine receptor gp130. *Trends Genet.* **20**, 23–32. (doi:10.1016/j.tig.2003.11.003)
- Evans, M. J. & Kaufman, M. H. 1981 Establishment in culture of pluripotential cells from mouse embryos. *Nature* **292**, 154–156. (doi:10.1038/292154a0)
- Fernandez, P. C., Frank, S. R., Wang, L., Schroeder, M., Liu, S., Greene, J., Cocito, A. & Amati, B. 2003 Genomic targets of the human c-Myc protein. *Genes Dev.* **17**, 1115–1129. (doi:10.1101/gad.1067003)
- Foster, K. W. *et al.* 1999 Oncogene expression cloning by retroviral transduction of adenovirus E1A-immortalized rat kidney RK3E cells: transformation of a host with epithelial features by c-MYC and the zinc finger protein GKLf. *Cell Growth Differ.* **10**, 423–434.
- Foster, K. W., Frost, A. R., McKie-Bell, P., Lin, C. Y., Engler, J. A., Grizzle, W. E. & Ruppert, J. M. 2000 Increase of GKLf messenger RNA and protein expression during progression of breast cancer. *Cancer Res.* **60**, 6488–6495.
- Foster, K. W. *et al.* 2005 Induction of KLF4 in basal keratinocytes blocks the proliferation–differentiation switch and initiates squamous epithelial dysplasia. *Oncogene* **24**, 1491–1500. (doi:10.1038/sj.onc.1208307)
- Gurdon, J. B. 1962 The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. *J. Embryol. Exp. Morphol.* **10**, 622–640.
- Gurdon, J. B. & Byrne, J. A. 2003 The first half-century of nuclear transplantation. *Proc. Natl Acad. Sci. USA* **100**, 8048–8052. (doi:10.1073/pnas.1337135100)
- Gurdon, J. B. & Uehlinger, V. 1966 “Fertile”, intestine nuclei. *Nature* **210**, 1240–1241. (doi:10.1038/2101240a0)
- Gurdon, J. B., Laskey, R. A. & Reeves, O. R. 1975 The developmental capacity of nuclei transplanted from keratinized skin cells of adult frogs. *J. Embryol. Exp. Morphol.* **34**, 93–112.
- Gu, P., Lemenuet, D., Chung, A. C., Mancini, M., Wheeler, D. A. & Cooney, A. J. 2005 Orphan nuclear receptor GCNF is required for the repression of pluripotency genes during retinoic acid-induced embryonic stem cell differentiation. *Mol. Cell Biol.* **25**, 8507–8519. (doi:10.1128/MCB.25.19.8507-8519.2005)
- Hao, J., Li, T. G., Qi, X., Zhao, D. F. & Zhao, G. Q. 2006 WNT/beta-catenin pathway up-regulates STAT3 and converges on LIF to prevent differentiation of mouse embryonic stem cells. *Dev. Biol.* **290**, 81–91. (doi:10.1016/j.ydbio.2005.11.011)
- Harris, H. 1965 Behaviour of differentiated nuclei in heterokaryons of animal cells from different species. *Nature* **206**, 583–588. (doi:10.1038/206583a0)
- Harris, H. & Watkins, J. F. 1965 Hybrid cells derived from mouse and man: artificial heterokaryons of mammalian cells from different species. *Nature* **205**, 640–646. (doi:10.1038/205640a0)
- Harris, H., Watkins, J. F., Campbell, G. L., Evans, E. P. & Ford, C. E. 1965 Mitosis in hybrid cells derived from mouse and man. *Nature* **207**, 606–608. (doi:10.1038/207606a0)
- Hatano, S. Y., Tada, M., Kimura, H., Yamaguchi, S., Kono, T., Nakano, T., Suemori, H., Nakatsuji, N. & Tada, T. 2005 Pluripotential competence of cells associated with Nanog activity. *Mech. Dev.* **122**, 67–79. (doi:10.1016/j.mod.2004.08.008)
- Hochedlinger, K. & Jaenisch, R. 2002 Monoclonal mice generated by nuclear transfer from mature B and T donor cells. *Nature* **415**, 1035–1038. (doi:10.1038/nature718)

- Hooper, M., Hardy, K., Handyside, A., Hunter, S. & Monk, M. 1987 HPRT-deficient (Lesch-Nyhan) mouse embryos derived from germline colonization by cultured cells. *Nature* **326**, 292–295. (doi:10.1038/326292a0)
- Hough, S. R., Clements, I., Welch, P. J. & Wiederholt, K. A. 2006 Differentiation of mouse embryonic stem cells after RNA interference-mediated silencing of Oct4 and Nanog. *Stem Cells* **24**, 1467–1475. (doi:10.1634/stemcells.2005-0475)
- Humphrey, R. K., Beattie, G. M., Lopez, A. D., Bucay, N., King, C. C., Firpo, M. T., Rose-John, S. & Hayek, A. 2004 Maintenance of pluripotency in human embryonic stem cells is STAT3 independent. *Stem Cells* **22**, 522–530. (doi:10.1634/stemcells.22-4-522)
- Hwang, W. S. *et al.* 2004 Evidence of a pluripotent human embryonic stem cell line derived from a cloned blastocyst. *Science* **303**, 1669–1674. (doi:10.1126/science.1094515)
- Hwang, W. S. *et al.* 2005 Patient-specific embryonic stem cells derived from human SCNT blastocysts. *Science* **308**, 1777–1783. (doi:10.1126/science.1112286)
- Hyslop, L. *et al.* 2005 Downregulation of NANOG induces differentiation of human embryonic stem cells to extra-embryonic lineages. *Stem Cells* **23**, 1035–1043. (doi:10.1634/stemcells.2005-0080)
- Ivanova, N., Dobrin, R., Lu, R., Kotenko, I., Levorse, J., DeCoste, C., Schafer, X., Lun, Y. & Lemischka, I. R. 2006 Dissecting self-renewal in stem cells with RNA interference. *Nature* **442**, 533–538. (doi:10.1038/nature04915)
- Kato, Y., Tani, T., Sotomaru, Y., Kurokawa, K., Kato, J., Doguchi, H., Yasue, H. & Tsunoda, Y. 1998 Eight calves cloned from somatic cells of a single adult. *Science* **282**, 2095–2098. (doi:10.1126/science.282.5396.2095)
- Katz, J. P., Perreault, N., Goldstein, B. G., Actman, L., McNally, S. R., Silberg, D. G., Furth, E. E. & Kaestner, K. H. 2005 Loss of Klf4 in mice causes altered proliferation and differentiation and precancerous changes in the adult stomach. *Gastroenterology* **128**, 935–945. (doi:10.1053/j.gastro.2005.02.022)
- Kielman, M. F. *et al.* 2002 Apc modulates embryonic stem-cell differentiation by controlling the dosage of beta-catenin signaling. *Nat. Genet.* **32**, 594–605. (doi:10.1038/ng1045)
- Kimura, H., Tada, M., Nakatsuji, N. & Tada, T. 2004 Histone code modifications on pluripotential nuclei of reprogrammed somatic cells. *Mol. Cell Biol.* **24**, 5710–5720. (doi:10.1128/MCB.24.13.5710-5720.2004)
- King, T. J. & Briggs, R. 1955 Changes in the nuclei of differentiating gastrula cells, as demonstrated by nuclear transplantation. *Proc. Natl Acad. Sci. USA* **41**, 321–325. (doi:10.1073/pnas.41.5.321)
- King, T. J. & Briggs, R. 1956 Serial transplantation of embryonic nuclei. *Cold Spring Harb. Symp. Quant. Biol.* **21**, 271–290.
- Kiuchi, N., Nakajima, K., Ichiba, M., Fukada, T., Narimatsu, M., Mizuno, K., Hibi, M. & Hirano, T. 1999 STAT3 is required for the gp130-mediated full activation of the *c-myc* gene. *J. Exp. Med.* **189**, 63–73. (doi:10.1084/jem.189.1.63)
- Knoepfler, P. S., Zhang, X. Y., Cheng, P. F., Gafken, P. R., McMahon, S. B. & Eisenman, R. N. 2006 Myc influences global chromatin structure. *EMBO J.* **25**, 2723–2734. (doi:10.1038/sj.emboj.7601152)
- Koopman, P., Schepers, G., Brenner, S. & Venkatesh, B. 2004 Origin and diversity of the *Sox* transcription factor gene family: genome-wide analysis in *Fugu rubripes*. *Gene* **328**, 177–186. (doi:10.1016/j.gene.2003.12.008)
- Kuroda, T., Tada, M., Kubota, H., Kimura, H., Hatano, S. Y., Suemori, H., Nakatsuji, N. & Tada, T. 2005 Octamer and Sox elements are required for transcriptional *cis* regulation of *Nanog* gene expression. *Mol. Cell Biol.* **25**, 2475–2485. (doi:10.1128/MCB.25.6.2475-2485.2005)
- Laskey, R. A. & Gurdon, J. B. 1970 Genetic content of adult somatic cells tested by nuclear transplantation from cultured cells. *Nature* **228**, 1332–1334. (doi:10.1038/2281332a0)
- Lee, B. C. *et al.* 2005 Dogs cloned from adult somatic cells. *Nature* **436**, 641. (doi:10.1038/436641a)
- Li, Z., Van Calcar, S., Qu, C., Cavenee, W. K., Zhang, M. Q. & Ren, B. 2003 A global transcriptional regulatory role for *c-Myc* in Burkitt's lymphoma cells. *Proc. Natl Acad. Sci. USA* **100**, 8164–8169. (doi:10.1073/pnas.1332764100)
- Li, Y., McClintick, J., Zhong, L., Edenberg, H. J., Yoder, M. C. & Chan, R. J. 2005 Murine embryonic stem cell differentiation is promoted by SOCS-3 and inhibited by the zinc finger transcription factor Klf4. *Blood* **105**, 635–637. (doi:10.1182/blood-2004-07-2681)
- Lin, T., Chao, C., Saito, S., Mazur, S. J., Murphy, M. E., Appella, E. & Xu, Y. 2005 p53 induces differentiation of mouse embryonic stem cells by suppressing *Nanog* expression. *Nat. Cell Biol.* **7**, 165–171. (doi:10.1038/ncb1211)
- Loh, Y. H. *et al.* 2006 The Oct4 and *Nanog* transcription network regulates pluripotency in mouse embryonic stem cells. *Nat. Genet.* **38**, 431–440. (doi:10.1038/ng1760)
- Maherali, N. *et al.* 2007 Directly reprogrammed fibroblasts show global epigenetic remodelling and widespread tissue contribution. *Cell Stem Cell* **1**, 55–70. (doi:10.1016/j.stem.2007.05.014)
- Martin, G. R. 1981 Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc. Natl Acad. Sci. USA* **78**, 7634–7638. (doi:10.1073/pnas.78.12.7634)
- Maruyama, M., Ichisaka, T., Nakagawa, M. & Yamanaka, S. 2005 Differential roles for Sox15 and Sox2 in transcriptional control in mouse embryonic stem cells. *J. Biol. Chem.* **280**, 24 371–24 379. (doi:10.1074/jbc.M501423200)
- Masui, S. *et al.* 2007 Pluripotency governed by Sox2 via regulation of Oct3/4 expression in mouse embryonic stem cells. *Nat. Cell Biol.* **9**, 625–635. (doi:10.1038/ncb1589)
- Matsuda, T., Nakamura, T., Nakao, K., Arai, T., Katsuki, M., Heike, T. & Yokota, T. 1999 STAT3 activation is sufficient to maintain an undifferentiated state of mouse embryonic stem cells. *EMBO J.* **18**, 4261–4269. (doi:10.1093/emboj/18.15.4261)
- Matsumura, H., Tada, M., Otsuji, T., Yasuchika, K., Nakatsuji, N., Surani, A. & Tada, T. 2007 Targeted chromosome elimination from ES-somatic hybrid cells. *Nat. Methods* **4**, 23–25. (doi:10.1038/nmeth973)
- McGrath, J. & Solter, D. 1983 Nuclear transplantation in mouse embryos. *J. Exp. Zool.* **228**, 355–362. (doi:10.1002/jez.1402280218)
- Meng, L., Ely, J. J., Stouffer, R. L. & Wolf, D. P. 1997 Rhesus monkeys produced by nuclear transfer. *Biol. Reprod.* **57**, 454–459. (doi:10.1095/biolreprod57.2.454)
- Miller, R. A. & Ruddle, F. H. 1976 Pluripotent teratocarcinoma-thymus somatic cell hybrids. *Cell* **9**, 45–55. (doi:10.1016/0092-8674(76)90051-9)
- Mitsui, K., Tokuzawa, Y., Itoh, H., Segawa, K., Murakami, M., Takahashi, K., Maruyama, M., Maeda, M. & Yamanaka, S. 2003 The homeoprotein *Nanog* is required for maintenance of pluripotency in mouse epiblast and ES cells. *Cell* **113**, 631–642. (doi:10.1016/S0092-8674(03)00393-3)
- Nakagawa, M. *et al.* 2008 Generation of induced pluripotent stem cells without *Myc* from mouse and human fibroblasts. *Nat. Biotechnol.* **26**, 101–106. (doi:10.1038/nbt1374)

- Nakatake, Y. *et al.* 2006 Klf4 cooperates with Oct3/4 and Sox2 to activate the Lefty1 core promoter in embryonic stem cells. *Mol. Cell Biol.* **26**, 7772–7782. (doi:10.1128/MCB.00468-06)
- Nichols, J., Zevnik, B., Anastassiadis, K., Niwa, H., Klewe-Nebenius, D., Chambers, I., Scholer, H. & Smith, A. 1998 Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. *Cell* **95**, 379–391. (doi:10.1016/S0092-8674(00)81769-9)
- Niwa, H., Burdon, T., Chambers, I. & Smith, A. 1998 Self-renewal of pluripotent embryonic stem cells is mediated via activation of STAT3. *Genes Dev.* **12**, 2048–2060.
- Niwa, H., Miyazaki, J. & Smith, A. G. 2000 Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. *Nat. Genet.* **24**, 372–376. (doi:10.1038/74199)
- O'Donnell, K. A., Wentzel, E. A., Zeller, K. I., Dang, C. V. & Mendell, J. T. 2005 c-Myc-regulated microRNAs modulate E2F1 expression. *Nature* **435**, 839–843. (doi:10.1038/nature03677)
- Ogawa, K., Nishinakamura, R., Iwamatsu, Y., Shimosato, D. & Niwa, H. 2006 Synergistic action of Wnt and LIF in maintaining pluripotency of mouse ES cells. *Biochem. Biophys. Res. Commun.* **343**, 159–166. (doi:10.1016/j.bbrc.2006.02.127)
- Okamoto, K., Okazawa, H., Okuda, A., Sakai, M., Muramatsu, M. & Hamada, H. 1990 A novel octamer binding transcription factor is differentially expressed in mouse embryonic cells. *Cell* **60**, 461–472. (doi:10.1016/0092-8674(90)90597-8)
- Okita, K., Ichisaka, T. & Yamanaka, S. 2007 Generation of germ-line competent induced pluripotent stem cells. *Nature* **448**, 313–317. (doi:10.1038/nature05934)
- Park, I. H., Zhao, R., West, J. A., Yabuuchi, A., Huo, H., Ince, T. A., Lerou, P. H., Lensch, M. W. & Daley, G. Q. 2008 Reprogramming of human somatic cells to pluripotency with defined factors. *Nature* **451**, 141–146. (doi:10.1038/nature06534)
- Pereira, L., Yi, F. & Merrill, B. J. 2006 Repression of Nanog gene transcription by Tcf3 limits embryonic stem cell self-renewal. *Mol. Cell Biol.* **26**, 7479–7491. (doi:10.1128/MCB.00368-06)
- Polejaeva, I. A. *et al.* 2000 Cloned pigs produced by nuclear transfer from adult somatic cells. *Nature* **407**, 86–90. (doi:10.1038/35024082)
- Prather, R. S., Sims, M. M. & First, N. L. 1989 Nuclear transplantation in early pig embryos. *Biol. Reprod.* **41**, 414–418. (doi:10.1095/biolreprod41.3.414)
- Raz, R., Lee, C. K., Cannizzaro, L. A., d'Eustachio, P. & Levy, D. E. 1999 Essential role of STAT3 for embryonic stem cell pluripotency. *Proc. Natl Acad. Sci. USA* **96**, 2846–2851. (doi:10.1073/pnas.96.6.2846)
- Reya, T. & Clevers, H. 2005 Wnt signalling in stem cells and cancer. *Nature* **434**, 843–850. (doi:10.1038/nature03319)
- Reya, T., Duncan, A. W., Ailles, L., Domen, J., Scherer, D. C., Willert, K., Hintz, L., Nusse, R. & Weissman, I. L. 2003 A role for Wnt signalling in self-renewal of haematopoietic stem cells. *Nature* **423**, 409–414. (doi:10.1038/nature01593)
- Rodda, D. J., Chew, J. L., Lim, L. H., Loh, Y. H., Wang, B., Ng, H. H. & Robson, P. 2005 Transcriptional regulation of Nanog by Oct4 and Sox2. *J. Biol. Chem.* **280**, 24 731–24 737. (doi:10.1074/jbc.M502573200)
- Rowland, B. D., Bernards, R. & Peeper, D. S. 2005 The KLF4 tumour suppressor is a transcriptional repressor of p53 that acts as a context-dependent oncogene. *Nat. Cell Biol.* **7**, 1074–1082.
- Sato, N., Sanjuan, I. M., Heke, M., Uchida, M., Naef, F. & Brivanlou, A. H. 2003 Molecular signature of human embryonic stem cells and its comparison with the mouse. *Dev. Biol.* **260**, 404–413. (doi:10.1016/S0012-1606(03)00256-2)
- Sato, N., Meijer, L., Skaltsounis, L., Greengard, P. & Brivanlou, A. H. 2004 Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor. *Nat. Med.* **10**, 55–63. (doi:10.1038/nm979)
- Scholer, H. R., Hatzopoulos, A. K., Balling, R., Suzuki, N. & Gruss, P. 1989 A family of octamer-specific proteins present during mouse embryogenesis: evidence for germline-specific expression of an Oct factor. *EMBO J.* **8**, 2543–2550.
- Sears, R., Nuckolls, F., Haura, E., Taya, Y., Tamai, K. & Nevins, J. R. 2000 Multiple Ras-dependent phosphorylation pathways regulate Myc protein stability. *Genes Dev.* **14**, 2501–2514. (doi:10.1101/gad.836800)
- Shin, T. *et al.* 2002 A cat cloned by nuclear transplantation. *Nature* **415**, 859. (doi:10.1038/nature723)
- Silva, J., Chambers, I., Pollard, S. & Smith, A. 2006 Nanog promotes transfer of pluripotency after cell fusion. *Nature* **441**, 997–1001. (doi:10.1038/nature04914)
- Sims, M. & First, N. L. 1994 Production of calves by transfer of nuclei from cultured inner cell mass cells. *Proc. Natl Acad. Sci. USA* **91**, 6143–6147. (doi:10.1073/pnas.91.13.6143)
- Smith, A. G. & Hooper, M. L. 1987 Buffalo rat liver cells produce a diffusible activity which inhibits the differentiation of murine embryonal carcinoma and embryonic stem cells. *Dev. Biol.* **121**, 1–9. (doi:10.1016/0012-1606(87)90132-1)
- Smith, A. G., Heath, J. K., Donaldson, D. D., Wong, G. G., Moreau, J., Stahl, M. & Rogers, D. 1988 Inhibition of pluripotential embryonic stem cell differentiation by purified polypeptides. *Nature* **336**, 688–690. (doi:10.1038/336688a0)
- Stahl, N., Farruggella, T. J., Boulton, T. G., Zhong, Z., Darnell Jr, J. E. & Yancopoulos, G. D. 1995 Choice of STATs and other substrates specified by modular tyrosine-based motifs in cytokine receptors. *Science* **267**, 1349–1353. (doi:10.1126/science.7871433)
- Stice, S. L. & Robl, J. M. 1988 Nuclear reprogramming in nuclear transplant rabbit embryos. *Biol. Reprod.* **39**, 657–664. (doi:10.1095/biolreprod39.3.657)
- Strelchenko, N., Kukhareno, V., Shkumatov, A., Verlinsky, O., Kuliev, A. & Verlinsky, Y. 2006 Reprogramming of human somatic cells by embryonic stem cell cytoplasm. *Reprod. Biomed. Online* **12**, 107–111.
- Sumi, T., Fujimoto, Y., Nakatsuji, N. & Suemori, H. 2004 STAT3 is dispensable for maintenance of self-renewal in nonhuman primate embryonic stem cells. *Stem Cells* **22**, 861–872. (doi:10.1634/stemcells.22-5-861)
- Tada, M., Tada, T., Lefebvre, L., Barton, S. C. & Surani, M. A. 1997 Embryonic germ cells induce epigenetic reprogramming of somatic nucleus in hybrid cells. *EMBO J.* **16**, 6510–6520. (doi:10.1093/emboj/16.21.6510)
- Tada, M., Takahama, Y., Abe, K., Nakatsuji, N. & Tada, T. 2001 Nuclear reprogramming of somatic cells by *in vitro* hybridization with ES cells. *Curr. Biol.* **11**, 1553–1558. (doi:10.1016/S0960-9822(01)00459-6)
- Takahashi, K. & Yamanaka, S. 2006 Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126**, 663–676. (doi:10.1016/j.cell.2006.07.024)
- Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K. & Yamanaka, S. 2007 Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* **131**, 861–872. (doi:10.1016/j.cell.2007.11.019)

- Taranger, C. K., Noer, A., Sorensen, A. L., Hakelien, A. M., Boquest, A. C. & Collas, P. 2005 Induction of dedifferentiation, genome-wide transcriptional programming, and epigenetic reprogramming by extracts of carcinoma and embryonic stem cells. *Mol. Biol. Cell* **16**, 5719–5735. (doi:10.1091/mbc.E05-06-0572)
- Terada, N. *et al.* 2002 Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. *Nature* **416**, 542–545. (doi:10.1038/nature730)
- Thomson, J. A., Itskovitz-Eldor, J., Shapiro, S. S., Waknitz, M. A., Swiergiel, J. J., Marshall, V. S. & Jones, J. M. 1998 Embryonic stem cell lines derived from human blastocysts. *Science* **282**, 1145–1147. (doi:10.1126/science.282.5391.1145)
- Tokuzawa, Y., Kaiho, E., Maruyama, M., Takahashi, K., Mitsui, K., Maeda, M., Niwa, H. & Yamanaka, S. 2003 Fbx15 is a novel target of Oct3/4 but is dispensable for embryonic stem cell self-renewal and mouse development. *Mol. Cell Biol.* **23**, 2699–2708. (doi:10.1128/MCB.23.8.2699-2708.2003)
- Wakayama, T., Perry, A. C., Zuccotti, M., Johnson, K. R. & Yanagimachi, R. 1998 Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. *Nature* **394**, 369–374. (doi:10.1038/28615)
- Wei, C. L. *et al.* 2005a Transcriptome profiling of human and murine ESCs identifies divergent paths required to maintain the stem cell state. *Stem Cells* **23**, 166–185. (doi:10.1634/stemcells.2004-0162)
- Wei, D., Gong, W., Kanai, M., Schlunk, C., Wang, L., Yao, J. C., Wu, T. T., Huang, S. & Xie, K. 2005b Drastic down-regulation of Kruppel-like factor 4 expression is critical in human gastric cancer development and progression. *Cancer Res.* **65**, 2746–2754. (doi:10.1158/0008-5472.CAN-04-3619)
- Wernig, M., Meissner, A., Foreman, R., Brambrink, T., Ku, M., Hochedlinger, K., Bernstein, B. E. & Jaenisch, R. 2007 *In vitro* reprogramming of fibroblasts into a pluripotent ES cell-like state. *Nature* **448**, 318–324. (doi:10.1038/nature05944)
- Willadsen, S. M. 1986 Nuclear transplantation in sheep embryos. *Nature* **320**, 63–65. (doi:10.1038/320063a0)
- Williams, R. L. *et al.* 1988 Myeloid leukaemia inhibitory factor maintains the developmental potential of embryonic stem cells. *Nature* **336**, 684–687. (doi:10.1038/336684a0)
- Wilmut, I., Schnieke, A. E., McWhir, J., Kind, A. J. & Campbell, K. H. 1997 Viable offspring derived from fetal and adult mammalian cells. *Nature* **385**, 810–813. (doi:10.1038/385810a0)
- Wilmut, I., Beaujean, N., De Sousa, P. A., Dinnyes, A., King, T. J., Paterson, L. A., Wells, D. N. & Young, L. E. 2002 Somatic cell nuclear transfer. *Nature* **419**, 583–587. (doi:10.1038/nature01079)
- Wu da, Y. & Yao, Z. 2005 Isolation and characterization of the murine Nanog gene promoter. *Cell Res.* **15**, 317–324. (doi:10.1038/sj.cr.7290300)
- Yamaguchi, T. P., Takada, S., Yoshikawa, Y., Wu, N. & McMahon, A. P. 1999 T (Brachyury) is a direct target of Wnt3a during paraxial mesoderm specification. *Genes Dev.* **13**, 3185–3190. (doi:10.1101/gad.13.24.3185)
- Yamanaka, S. 2007 Strategies and new developments in the generation of patient-specific pluripotent stem cells. *Cell Stem Cell* **1**, 39–49. (doi:10.1016/j.stem.2007.05.012)
- Ying, Q. L., Nichols, J., Evans, E. P. & Smith, A. G. 2002 Changing potency by spontaneous fusion. *Nature* **416**, 545–548. (doi:10.1038/nature729)
- Ying, Q. L., Nichols, J., Chambers, I. & Smith, A. 2003 BMP induction of Id proteins suppresses differentiation and sustains embryonic stem cell self-renewal in collaboration with STAT3. *Cell* **115**, 281–292. (doi:10.1016/S0092-8674(03)00847-X)
- Yoshikawa, Y., Fujimori, T., McMahon, A. P. & Takada, S. 1997 Evidence that absence of Wnt-3a signaling promotes neuralization instead of paraxial mesoderm development in the mouse. *Dev. Biol.* **183**, 234–242. (doi:10.1006/dbio.1997.8502)
- Yu, J., Vodyanik, M. A., He, P., Slukvin, I. I. & Thomson, J. A. 2005 Human embryonic stem cells reprogram myeloid precursors following cell–cell fusion. *Stem Cells* **24**, 168–176. (doi:10.1634/stemcells.2005-0292)
- Yu, J. *et al.* 2007 Induced pluripotent stem cell lines derived from human somatic cells. *Science* **318**, 1917–1920. (doi:10.1126/science.1151526)
- Zaehres, H., Lensch, M. W., Daheron, L., Stewart, S. A., Itskovitz-Eldor, J. & Daley, G. Q. 2005 High-efficiency RNA interference in human embryonic stem cells. *Stem Cells* **23**, 299–305. (doi:10.1634/stemcells.2004-0252)
- Zhao, W., Hisamuddin, I. M., Nandan, M. O., Babbin, B. A., Lamb, N. E. & Yang, V. W. 2004 Identification of Kruppel-like factor 4 as a potential tumor suppressor gene in colorectal cancer. *Oncogene* **23**, 395–402. (doi:10.1038/sj.onc.1207067)