Review

Induced pluripotent stem cells: current progress and potential for regenerative medicine

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Lineage-restricted cells can be reprogrammed to a pluripotent state through overexpression of defined transcription factors. Here, we summarize recent progress in the direct reprogramming field and discuss data comparing embryonic stem (ES) and induced pluripotent stem (iPS) cells. Results from many independent groups suggest that mouse and human iPS cells, once established, generally exhibit a normal karyotype, are transcriptionally and epigenetically similar to ES cells and maintain the potential to differentiate into derivatives of all germ layers. Recent developments provide optimism that safe, viral-free human iPS cells could be derived routinely in the near future. An important next step will be to identify ways of assessing which iPS cell lines are sufficiently reprogrammed and safe to use for therapeutic applications. The approach of generating patient-specific pluripotent cells will undoubtedly transform regenerative medicine in many ways.

Generation of pluripotent stem cells

During cellular differentiation, cells become increasingly more specialized and simultaneously restricted in their developmental potential. Although totipotent and the more developmentally restricted pluripotent cells (see Glossary) exist only in the early embryo, the adult still contains numerous multipotent (e.g. hematopoietic stem cells) and unipotent stem cells [1]. In mammalian development, differentiation is considered to be unidirectional and reprogramming or transdifferentiation (see Glossary) is observed rarely [1]. Nuclear-transfer experiments over the past 50 years have established that, despite the decrease in developmental potential, the nucleus of most, if not all, adult cells retains nuclear plasticity and can be reset to an embryonic state. In accordance with this, cells (or nuclei) can be converted to a pluripotent state, using any of the following mechanisms: (i) exposure to factors in the oocyte through nuclear transfer (reviewed in [2]), (ii) exposure to factors expressed in pluripotent cells (i.e. embryonic stem [ES], embryonic germ [EG] and induced pluripotent stem [iPS] cells) through cell fusion [3,4], (iii) overexpression of defined transcription factors (direct reprogramming) [5–7] or (iv) in the case of testis cells, through the use of specific culture conditions [1,8] (Figure 1).

The first nuclear-transfer experiments were performed in 1952 by Briggs and King and were later followed by Gurdon to address the fundamental question of whether the nuclei of differentiated cells are equivalent to those of the early embryo [9,10]. A more detailed overview of the history of nuclear transfer can be found elsewhere [11]. In the late 1990s, two independent studies raised the possibility of generating human patient-specific stem cells. In 1997, Wilmut and colleagues reported the cloning of 'Dolly' the sheep, the first mammal to be cloned from an adult cell [12]. A year later, Thomson and colleagues reported the derivation of human ES cells [13]. This immediately led to speculations that one could use a combination of both

Glossary

Chimera formation: to test developmental potential *in vivo*, cells are frequently injected into diploid (3.5-day post-fertilization) blastocysts. Pluripotent cells will incorporate into the inner cell mass and contribute to all lineages of the developing mouse (chimera).
Embryonic stem cells: pluripotent cells derived from the inner cell mass of the blastocyst.
Germline contribution: in addition to the three germ layers, pluripotent cells can contribute to the germline of the mice when injected into diploid blastocysts.
Induced pluripotent stem (iPS) cells: pluripotent cells derived from any differentiated cell type through ectopic expression of transcription factors. Originally, through retroviral expression of Oct4, Sox2, Klf4 and C-Myc, as reported by Shinya Yamanaka [5]. Other combinations and ways of generating iPS cells have been developed over the past 2 years.
Multipotent cells: cells capable of differentiating into multiple cell types but within a certain lineage [hematopeoitic stem cells (HSCs)].
Pluripotent cells: cells capable of differentiating all germ layers (endoderm, mesoderm and ectoderm) and the germline but not extra embryonic tissues (e.g. inner cell mass, ES, EG, EpiSC, iPS cells).
Reprogramming: increase in developmental potential through nuclear transfer, cell fusion, ectopic transcription factor expression (direct reprogramming) and cell culture (Figure 1).
Tetraploid embryo complementation: diploid 2-cell embryos can be fused to generate a single tetraploid (4N) embryo. These 4N-embryos can develop normally to the blastocyst and can form extra-embryonic tissue but do not contribute to the embryo. Any cell injected into the 4N blastocysts has to be able to generate the entire embryo.
Totipotent cells: cells capable of developing into a complete organism or differentiating into any of its cells or tissues (e.g. zygote and early blastomeres).
Transdifferentiation: a controversial concept whereby multipotent or somatic stem cells would be able to also generate cell types outside of their primary lineage (e.g. HSCs contributing to non-hematopoeitic tissues).
Unipotent cells: cells that have the capacity to differentiate into only one type of cell (e.g. spermatogonial stem cells).

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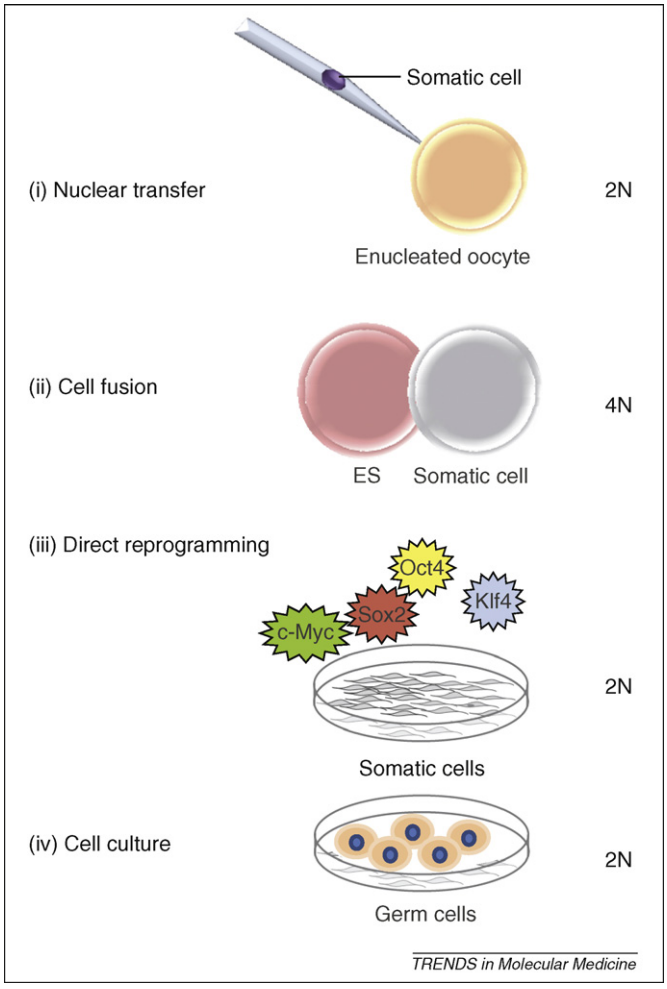

Figure 1. Strategies to induce nuclear reprogramming. Somatic cells have been converted to a pluripotent state, using all of the four displayed mechanisms. (i) Somatic cells can be reprogrammed through injection into a previously enucleated oocyte (nuclear transfer) (reviewed in [2]). (ii) When a pluripotent cell such as an ES cell is fused with a somatic cell, it will generate a tetraploid cell that has acquired a pluripotent state (cell fusion) [3,4]. (iii) Ectopic expression of the transcription factors Oct4, Sox2, Klf4 and c-Myc via viral (retro-, lenti- or adenovirus) or plasmid-based vectors is sufficient to reprogram somatic cells (direct reprogramming) [5–7]. (iv) Under specific culture conditions at a low frequency, it has been shown that germ cells can reprogram into pluripotent cells (culture-induced reprogramming) [1,8]. Abbreviations: 2N, diploid cell; 4N, tetraploid cell; ES, embryonic stem.

techniques to derive ES cells from patients and apply them for therapeutic purposes ('therapeutic cloning') [14]. Although proof-of-principle has been demonstrated in the mouse [15], human somatic cell-nuclear transfer (SCNT) has yet to be accomplished. There seem to be no conceptual obstacles to prevent SCNT working in humans. However, the procedure is technically challenging, inefficient and dependent on voluntary donation of a large number of unfertilized oocytes. The donation issue might be overcome by using fertilized embryos, as has been shown recently in the mouse [16], however, it is unlikely that SCNT could be performed on a large scale to derive pluripotent cell lines routinely for every patient.

The landmark discovery that lineage-restricted cells can be reprogrammed directly to a state of pluripotency through the ectopic expression of defined transcription factors has opened a new frontier in the field of regenerative medicine [5–7,17]. iPS cells, as they were termed by Shinya Yamanaka [5], have now been derived in various ways (Table 1). It is widely accepted that mouse and human iPS cells possess morphological, molecular and developmental features that closely resemble those of blastocyst-derived ES cells. Within 2 years of the original publications by Takahashi and Yamanaka, much progress had been made, including the recent derivation of integration-free murine iPS cells [17,18]. Although the exact mechanism of reprogramming remains unknown, only a few challenges, including generation of integration-free human iPS cells and improved ways of characterizing them, remain before iPS cells could be used routinely in pharmacological screens and regenerative medicine.

Pluripotent human ES cells had been derived before [13], however, the derivation of iPS cells is of such great importance because of the ease and reproducibility of generating them. In contrast to SCNT, direct reprogramming provides, for the first time, a realistic way of generating sufficient numbers of patient-specific pluripotent stem cells. Such cells could be used for regenerative and therapeutic purposes, as demonstrated in mouse models of, for example, sickle cell anemia and Parkinson's disease, respectively [19,20]. In addition, the iPS-cell technology facilitates the generation of disease-specific lines [21,22] that can be used as disease models in high-throughput screening and mechanistic studies [23].

One crucial point that still needs to be addressed in more detail is whether iPS cells are indeed identical to ES cells. If not identical, it is important to assess the levels of similarity that are sufficient for regenerative and screening purposes. This remains a relevant concern because even human ES cells often show some deficiencies regarding their differentiation potential [24].

In the following sections, we will review what is known about the molecular characteristics and developmental potential of iPS cells and compare them with ES cells. Furthermore, we will discuss recent insights into the mechanism of reprogramming, before concluding with a discussion of recent progress and future directions in the field of direct reprogramming.

Similarities and differences between iPS and ES cells

The finding that iPS cells have normal karyotypes, express genes that characterize ES cells and maintain the developmental potential to differentiate into advanced derivatives of all three primary germ layers – ectoderm, endoderm and mesoderm – is a major breakthrough in regenerative biology. Here, we summarize what is known currently, based on analysis of the genome, transcriptome, epigenome and developmental potential of ES and iPS cells.

Genome

Genomic integrity is of crucial importance for the derivation of high-quality iPS cells. This is particularly relevant for any therapeutic application because genomic alterations could result in diseases, such as cancer. Several groups have investigated the karyotype of mouse [6] and human [25,26] iPS cell lines. Some abnormalities were observed in a few lines but it appears that the majority of murine and human iPS cell lines exhibit a normal karyotype (40 and 46 chromosomes, respectively).

Table 1. Characteristics of induced pluripotent stem (iPS) cell lines produced in human and mouse models[a]

Animal	Reprogramming factors[b]	Cell type	Time (weeks)	Efficiency	Refs
Mouse	**Viral-integrating vectors**				
	O, K, S, M	MEFs	2–3	0.01–0.50%	[5–7,44,73]
	O, K, S, M	Hepatocytes	2–3	1–3%	[28]
	O, K, S, M	Gastric epithelial cells	2–3	1–3%	[28]
	O, K, S, M	B cells	2–3	0.01–0.10%	[34]
	O, K, S, M	β-cells	3–4	0.1–0.2%	[78]
	O, K, S, M	Neural stem cells	1–2	3–5%	[62,63]
	O, K, S	MEFs	3–4	0.001–0.010%	[57,60,73]
	O, K, S	Hepatocytes	2–3	0.5–1.0%	[28]
	O, K, M	Neural stem cells	1–2	0.1–1.0%	[62,63]
	O, K, S	Neural stem cells	2–3	0.1–1.0%	[63]
	O, M, S	Neural stem cells	3–4	0.1–1.0%	[63]
	O, K	Neural stem cells	2–3	0.1–0.2%	[63]
	O, M	Neural stem cells	3–4	0.1–0.2%	[63]
	Non-integrating viral vectors[c]				
	O, K, S, M	Hepatocytes	4–5	0.0001 to 0.0010%	[18]
	Plasmid-based vectors[c]				
	O, K, S, M	MEFs	3–4	0.0001 to 0.0010%	[17]
Human	**Viral-integrating vectors**				
	O, K, S, M	Dermal fibroblasts	4–5	0.001–0.002%	[21,22,25]
	O, K, L, N	Dermal fibroblasts	2–3	N.D.	[26]
	O, K, S, M	Bone marrow mesenchymal cells	2–3	N.D.	[22]
	O, K, N	Dermal fibroblasts	2–3	N.D.	[26]
	O, K, L,	Dermal fibroblasts	2–3	N.D.	[26]
	O, S, K[d]	Dermal fibroblasts	4	0.1–1.0%	[61]
	O, S[d]	Dermal fibroblasts	4	0.001–0.010%	[61]

Abbreviations: MEFs, mouse embryonic fibroblasts; N.D., not determined.
[a]The table shows the different combinations of transcription factors that induce nuclear reprogramming.
[b]O, Oct4; S, Sox2; M, c-Myc; K, Klf4; N, Nanog; L, Lin28.
[c]Integration-free clones.
[d]Valproic acid (VPA) was used in combination with the factors.

Nevertheless, Aasen and colleagues showed that continuous passaging of human iPS cells resulted in frequent chromosomal abnormalities [karyotype 46,XY,t(17;20)(p13;p11.2)] starting as early as approximately passage 13 [27]. This finding suggests that the long-term culture of human iPS cells, similar to the situation for human ES cells, has to be monitored carefully for culture-induced genetic abnormalities.

The use of retroviral and lentiviral vectors for expressing the reprogramming transcription factors involves the risk of insertional mutagenesis, which would be a problem if the cells were used in regenerative medicine. However, Aoi and colleagues reported no common insertion sites in hepatocyte- and stomach-cell-derived iPS cells [28]. In addition, recent adenoviral and plasmid-based strategies have further confirmed the notion that insertional mutagenesis is not required for the reprogramming process [17,18]. However, even without viral integrations, it cannot be ruled out completely that genetic changes might occur as part of the reprogramming process. Finally, faithful reactivation of mouse telomerase reverse transcriptase (mTert) [29] and human hTert [22,25], as well as telomerase activity [25], suggests that telomere length can also be maintained in iPS cells. It is important to note that decreasing DNA sequencing costs will soon enable significantly higher resolution analysis of genomic integrity to be achieved routinely. This will readily identify even minor deletions, inversions or loss of individual alleles. Many of these improvements will benefit the characterization of future human iPS cells and ensure that they are safe to use for therapeutic applications.

Transcriptome

Whole-genome expression profiling is a widely used approach to compare and characterize different cell populations. Several groups have used gene-expression arrays to analyze iPS cells [5–7,30]. Furthermore, our group has used this approach to characterize the initial response to the four transcription factors (Oct4, Sox2, Klf4 and c-Myc) [30]. Ectopic expression of the POU domain transcription factor Oct4 (POU5F1), the transcription factor Sox2, Krueppel-like factor 4 (Klf4) and the c-Myc proto-oncogene protein in mouse embryonic fibroblasts initially induces a response that is characterized by downregulation of cell-type-specific transcription factors [29,30] and upregulation of genes involved in proliferation, DNA replication and cell-cycle progression. The latter (proliferation, DNA replication and cell-cycle progression) is probably a consequence of c-Myc overexpression [30]. In addition to their role in ES cells, a subset of these four factors has important roles in other cell types. Among others, Sox2 is expressed in the developing central nervous system and Klf4 is expressed in the epithelium of the kidney (see later). Consistent with this notion, we have observed that several neural and epidermal genes are upregulated in the initial phase of reprogramming [30], probably in response to the ectopic expression of Sox2 and Klf4 [31,32].

During the reprogramming process, many genes expressed in ES cells and linked to self-renewal are reactivated, including *Fgf4* (encoding fibroblast growth factor 4) and genes encoding proteins of the polycomb group [5,30]. However, a large fraction of pluripotency-related

genes are only upregulated during late stages of the process [29,30,33].

An elegant system for minimizing the heterogeneity of cells with different viral integrations was developed in the Jaenisch lab [30,34,35]. Inducible lentiviral vectors were used to generate iPS cells (primary iPS cells). These primary iPS cells were injected subsequently into blastocysts and so-called secondary somatic cells were derived. All secondary cells contained the same viral integration sites for the four doxycycline-inducible transgenes. This eliminated the heterogeneity caused by having varying numbers of cells that contain the right combination of factors. Interestingly, despite universal induction of transgene expression using these secondary mouse embryonic fibroblasts (MEFs) [30,34,35] or human fibroblast-like cells [36,37], the majority of cells do not complete the reprogramming process. In the mouse model, it seems that most cells respond by activating stress-induced and anti-proliferative genes, followed by differentiation and cell arrest [30]. These endogenous and potentially tumor-suppressive 'anti-reprogramming' mechanisms are a probable explanation for why the reprogramming efficiency remains low even in a system that guarantees that every cell contains the correct number of functional factors [30]. It still needs to be determined whether similar mechanisms are responsible for the low efficiency of human cell reprogramming.

Studies of murine and human iPS cells have shown that the established iPS cell lines express key markers of ES cells, using RT-PCR and immunocytochemistry [5–7]. In addition, global gene-expression analysis using microarrays has shown that mouse and human iPS cells cluster with their respective ES- and iPS-cell counterparts, rather than with the source population of somatic cells [5–7]. Nonetheless, most groups note that iPS cells are not identical to ES cells. Takahashi and colleagues compared the global gene-expression profile of human iPS and human ES cells for 32 266 transcripts [25]. Notably, 1267 (~4%) of the genes were detected with >5-fold difference in up- or downregulation between iPS cells and human ES cells. In a different study, the expression of key pluripotency-related genes, such as *OCT4*, *SOX2* and *REX1*, was approximately two-fold lower in the iPS cells compared with two human ES cell lines, HSF1 and H9 [38]. Pluripotent cells are highly susceptible to the levels of these transcription factors (TFs) [39] and there is a notable amount of normal transcriptional heterogeneity in human ES-cell cultures [24]. Therefore, the observed variation could reflect differences in the cultures rather than incomplete reprogramming. With the current data, it is difficult to discern between these possibilities. More work on human ES cells is thus required to better understand the extent of normal transcriptional variation within human and also mouse ES cells.

Epigenome

By regulating chromatin structure, epigenetic modifications have an essential role in controlling access to genes and regulatory elements in the genome [33]. The epigenetic status of a somatic cell and pluripotent stem cell is vastly different and conversion would require global 'epigenetic reprogramming' (Figure 2). Analysis of the epigenetic state therefore provides a meaningful way of determining the degree of reprogramming in iPS cells.

For instance, pluripotent stem cells contain a characteristic chromatin signature, termed 'bivalent domains' [40,41]. These are regions enriched for repressive histone H3 lysine 27 trimethylation (H3K27me3) and simultaneously for histone H3 lysine 4 trimethylation (H3K4me3), an activating mark [42]. It was assumed initially that bivalent domains might be ES-cell specific because they were first identified using chromatin-immunoprecipitation (ChIP) followed by hybridization to microarrays (ChIP-Chip) that featured key developmental regulators. All of these resolved either to a univalent (H3K4me3 only or H3K27me3 only) state or lost both marks in differentiated cells [40]. Using ChIP-seq (ChIP followed by high-throughput sequencing) technology, Mikkelsen and colleagues later showed that bivalent domains

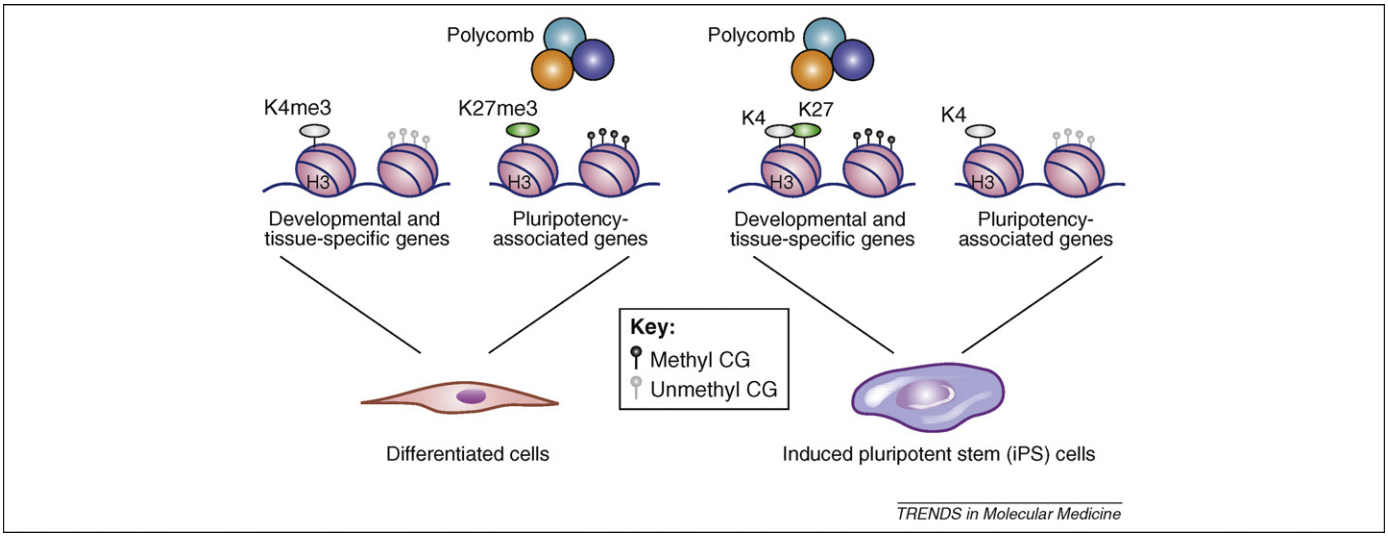

Figure 2. Epigenetic marks in somatic cells and pluripotent cells. In differentiated cells, many developmental and differentiation associated genes are active and show enrichment for H3K4me3 and lack of DNA methylation. Some early developmental genes have been silenced through polycomb-mediated H3K27me3 and all pluripotency-associated genes show high levels of DNA methylation. In pluripotent cells, these pluripotency-associated genes are active and show H3K4me3 and lack of DNA methylation. Many of the developmental genes show a 'bivalent' chromatin configuration and tissue-specific genes tend to be DNA methylated.

are more generally indicative of genes that remain in a poised state. Consequently, pluripotent cells were found to contain large numbers of bivalent domains (~2500) compared with, for instance, multipotent neural progenitor cells (NPCs) (~200) that still retain multilineage potential but are more restricted than ES cells [43].

Several of the murine iPS-cell studies have investigated a small number of representative loci for their chromatin- and DNA methylation patterns [5–7]. Two studies in mouse have investigated the epigenome of iPS cells at a larger scale. Maherali and colleagues used ChIP-Chip to investigate the presence of H3K4me3 and H3K27me3 in the promoter regions of ~16 500 genes in one iPS cell line [7]. The results suggested that iPS cells were highly similar in their epigenetic state to ES cells, with 94.4% of 957 'signature' genes (defined as genes that have a different epigenetic state between MEFs and ES cells) being reset to an ES-cell state in the respective iPS cell line. The authors also noted that the H3K4me3 pattern was similar across all samples, indicating that reprogramming was largely associated with changes in H3K27me3 rather than H3K4me3. Mikkelsen and colleagues have applied the more comprehensive ChIP-Seq technique to determine genome-wide chromatin maps in several iPS lines that were derived in distinct ways: (i) through drug selection using an Oct4–neomycin-resistance gene [6], (ii) through drug selection using a Nanog–neomycin-resistance gene [6], and (iii) by simply isolating reprogrammed cells through their morphological appearance [44]. Overall global levels of repressive H3K27me3 and the characteristic bivalent chromatin structure are restored across the different iPS cell lines [30]. The restoration of repressive chromatin marks appears crucial to stably silence lineage-specific genes that are active in somatic cells and inactive in undifferentiated pluripotent cells [30]. Failure to establish the repressive marks resulted in incompletely reprogrammed cells [30]. Notably, 'activating' H3K4me3 patterns are also crucial for complete reprogramming and have been observed to be restored genome-wide, in particular around the promoters of pluripotency-associated genes, such as *Oct4* and *Nanog*, in the fully reprogrammed iPS lines in this study [30].

A second component of the epigenetic machinery is DNA methylation, which is a stable and heritable mark that is involved in many biological processes, including gene regulation, genomic imprinting and X-chromosome inactivation. DNA-methylation patterns are dynamic during early embryonic development and are essential for normal post-implantation development [45]. Although overall DNA methylation levels remain stable during ES-cell differentiation, they are not static [43].

Notably, H3K4me3 and DNA methylation are mutually exclusive and so never present at the same time [43]. The re-establishment of H3K4me3 and the associated loss of DNA methylation in particular at 'ES-cell-associated transcript' (ECAT) genes seems to be a crucial and potentially rate-limiting step during reprogramming [30]. ECATs are expressed at high levels in ES cells and show low methylation levels at their promoters in pluripotent cells and high levels at their promoters in somatic cells [46]. Therefore, the loss of DNA methylation is essential for achieving complete reprogramming. For instance, in their initial study, Takahashi and colleagues found that iPS cells selected with F-box containing protein 15 (Fbx15) did not reactivate endogenous *Oct4* and *Nanog* genes and the respective promoters remained methylated [5]. Interestingly, loss of DNA methylation at this class of genes seems to be a rather late event in the reprogramming process because cells that have already acquired self-renewing properties still showed high levels of DNA methylation [30]. Consistent with the notion that DNA methylation might be a crucial step, 5-azacytidine, a well-known inhibitor of DNA methylation, can overcome this major roadblock and accelerates the reprogramming of lineage-restricted cells to iPS cells [30].

The resolution and comprehensiveness of the analyses of current techniques suggest that the overall epigenetic state of iPS cells is highly similar to that of ES cells, although cell line-to-line variation complicates the comparison. Furthermore, although comparisons have been made between iPS cell lines derived from different donor cell types and selection conditions, it still needs to be tested whether lines derived through different factor combinations, fewer factors or in combination with chemicals are reprogrammed to a similar extent. Furthermore, it is worth noting that current reprogramming protocols require cells to be kept in culture for an extended period of time with significant proliferation and possibly under selective pressure. Extended cell culture of differentiated cells is known to alter the epigenetic state of the respective cells over time [43,47]. In particular, imprinted genes can only be reset in the germline and are unstable in murine ES-cell cultures [48], although apparently not in human ES cells [49]. Because loss of imprinting has been associated with pathologies, including cancer [50], this warrants further investigation and it will be important to design effective ways to screen iPS cells routinely for a normal epigenetic state.

Developmental potential

Investigating a cell's transcriptional and epigenetic state is highly informative and it might ultimately be possible to characterize newly derived iPS cell lines based on their epigenome alone. However, to understand and select the most informative markers, it is important to use *in vivo* assays, analyzing the interplay between transcriptome, epigenome and developmental potential. Recently, Jaenisch and Young provided a detailed comparison of the different strategies for assessing developmental potential and their stringency [1]. *In vitro* differentiation is the least stringent assay and tetraploid-embryo complementation is the most stringent assay for testing developmental potential [1,51]. Although these strategies provide a wide array of functional assays for determining the developmental potential of pluripotent cells in the mouse, *in vitro* differentiation and teratoma formation are the only available methods for testing the potential of human iPS cells. This makes a comprehensive and careful characterization of the pluripotent epigenetic and transcriptional state of human iPS cells a crucial substitute for assessing their developmental potential

Murine induced pluripotent cells appear to have a developmental potential close to that of ES cells. Different

groups have evaluated developmental potential by teratoma and chimera formation [5–7]. Histological analysis demonstrated that iPS cells gave rise to teratomas comprising all three embryonic germ layers. Injection of iPS cells into diploid (2N) blastocysts, similar to ES cells, frequently gives rise to high-contribution chimeras (mice that show major tissue contribution of the injected iPS cells in the host mouse), as shown by many different research groups [5–7]. A subset of these chimeras has successfully demonstrated germline contribution. Only two reports have so far used the most stringent assay, tetraploid-embryo complementation [6,44]. Although several of the iPS cell lines in that study generated high-contribution chimeras, including one karyotypically abnormal line, varying degrees of development were observed in the tetraploid-complementation assay [6]. Some iPS cell lines without any apparent defects and with similar expression profiles continuously failed to support development of tetraploid (4N) embryos, whereas other iPS lines could generate mid–late-gestation 4N embryos. No viable term embryos using the tetraploid-complementation assay have been reported for any iPS cell line to date. Given the number of attempts and the generally lower efficiency of tetraploid versus diploid injections, it is difficult to draw any conclusions at this point. Several reasons not related to the reprogramming could be responsible for the failure, including leaky expression of viral vectors or small genetic lesions. These concerns could be addressed using newer adenoviral or plasmid-based iPS cells [17,18].

Mechanism of reprogramming

The most widely used set of reprogramming factors – Oct4, Sox2, Klf4 and c-Myc – was identified initially by screening 24 pre-selected factors in the murine system by Shinya Yamanaka's group [5]. The same combination of factors is sufficient for human cells [25]. A second team later identified a partially overlapping combination of factors – Oct4, Sox2, Nanog and Lin-28 [26] (Table 1). Below, we summarize what is known about the different reprogramming factors and then draw together recent developments and initial mechanistic insights.

Oct4

Oct4 (octamer-binding transcription factor 4, also known as Oct 3 and Pou5f1) was first described as a protein present in unfertilized oocytes, ES cells and primordial germ cells [52]. Its expression is essential for the development of the inner cell mass (ICM) *in vivo*, the derivation of ES cells and the maintenance of a pluripotent state [53]. The precise levels of Oct-3/4 govern three distinct fates of ES cells [39]. Within a narrow window of expression, ES cells retain an undifferentiated, pluripotent state. A less than twofold increase in expression causes differentiation into primitive endoderm and mesoderm, whereas repression of Oct-3/4 induces loss of pluripotency and differentiation into trophectoderm [39].

Sox2

SRY (sex-determining region Y)-box 2, known as Sox2, is a transcription factor involved in the self-renewal of ES cells. It has an important role in maintaining ES-cell pluripotency and heterodimerizes in a complex with Oct4 [54]. In human ES cells, SOX2 has 1279 binding sites and OCT4 has 623 binding sites. A total of 404 of these are overlapping and 87% (353) of these sites also overlap with NANOG (1687 binding sites) targets [55]. In addition to ES cells, *Sox2* is also expressed in the extra-embryonic ectoderm, trophoblast stem (TS) cells and the developing central nervous system (neural stem cells) [31,56]. In these cell lineages, *Sox2* expression is restricted to cells with stem-cell characteristics supporting their self-renewal capability and is no longer expressed in cells with a more restricted developmental potential [31]. Interestingly, forced expression of Oct4 can compensate for loss of Sox2 in ES cells [56] and, in direct reprogramming, Sox2 can be replaced by Sox1, Sox3 and, to a lesser extent, Sox15 or Sox18 [57].

c-Myc

c-Myc is a pleiotropic transcription factor that has been linked to several cellular functions, including cell-cycle regulation, proliferation, growth, differentiation and metabolism [58]. This factor tends to be highly expressed in the majority of rapidly proliferating cells and is generally low or absent during quiescence [59]. c-Myc also functions during both self-renewal and the differentiation of stem and progenitor cells, particularly in interactions between stem cells and the local microenvironment [56]. A large number of binding sites have been reported throughout the genome and c-Myc appears to be involved in recruiting chromatin-remodeling activities to promoters [59]. The role of c-Myc in reprogramming is not clear yet. It is dispensable for the generation of iPS cells in mouse and human [57,60–63] (Table 1) but the efficiency of reprogramming decreases dramatically. It can also be replaced by other family members, such as n-Myc and l-Myc, to reprogram somatic cells to an ES-like status [57].

Klf4

Krüppel-like factor 4 (*Klf4*) is a transcription factor expressed in a variety of tissues, including the epithelium of the intestine, kidney and the skin [64]. Depending on the target gene and interaction partner, Klf4 can both activate and repress transcription [32] and a growing body of evidence suggests that Klf4 can function both as an oncoprotein and tumor suppressor [65]. Constitutive expression of *Klf4* suppresses cell proliferation by blocking G_1–S progression of the cell cycle [65]. In human colorectal carcinoma, KLF4 appears to be downregulated, with evidence of hypermethylation and loss of heterozygosity [65]. Recently, it has been demonstrated that the forced overexpression of *Klf4* in ES cells inhibits differentiation in erythroid progenitors, suggesting a role for this factor in ES-cell function [66]. Its exact role in the reprogramming process is also not fully understood and it can be replaced with other Klf family members (Klf2 and Klf5) [57] or the unrelated factors Nanog and Lin28 [26].

Nanog

Nanog was first described as a factor that was involved in maintaining ES-cell self-renewal and pluripotency [67,68]. Smith and colleagues termed the factor Nanog, after the mythological Celtic land of the ever-young, 'Tir nan Og'.

Nanog is thought to operate in concert with other crucial 'stemness' factors, such as Oct4 and Sox2, to establish the identity of ES cells [67]. Nanog expression is found in the interior cells of the compacted morula and the ICM of the blastocyst [67]. On implantation, Nanog expression is detected only in the epiblast and is eventually restricted to primordial germ cells [67]. It is of interest that it was reported initially that Nanog-deficient ES cells completely lose their self-renewal capability, differentiating into extra-embryonic cell lineages [67]. More recently, a slightly more refined role of Nanog has been proposed. According to new data, loss of Nanog predisposes ES cells to differentiation but does not mark commitment and is reversible [69]. Interestingly, Nanog is not an essential factor for iPS-cell generation and does not appear to notably affect the efficiency (Table 1).

Lin-28

Lin-28 is a conserved RNA-binding protein involved in developmental timing in *Caenorhabditis elegans* [70]. In mammals, Lin-28 (also known as zinc finger CCHC domain-containing protein 1) is expressed in ES cells and during early embryogenesis but its expression becomes restricted to several tissues during late embryogenesis and adult life. Several groups have demonstrated that Lin-28 operates as a 'translational enhancer' in embryonic and adult cells. It can increase the stability of specific mRNAs and contribute to the identity establishment of the tissue in which it is expressed [71]. Viswanathan and colleagues showed that Lin-28 blocks processing of the let7 miRNA, thus suggesting a role in controlling miRNA-mediated differentiation in stem cells [72].

Mechanistic insights

The mechanism of nuclear reprogramming is a complex process that remains largely elusive. It is unclear what the exact role of each of these factors is and whether the different combinations of factors act through a similar mechanism during conversion towards a reprogrammed state. Clonal cell lines have been derived that can be maintained in relatively stable 'partially reprogrammed' states using Oct4, Sox2, Klf4 and c-Myc [30]. These cells represent a powerful tool for the identification of transcriptional and epigenetic changes before reaching a pluripotent state. This has provided many general insights into the reprogramming process. Notably, the reactivation of genes in MEFs is strongly correlated with chromatin status. Genes that are in open or accessible chromatin (H3K4me3 and also H3K4me3–H3K27me3) respond much more readily to the ectopic expression of the four factors. By contrast, genes that are repressed by H3K27me3 or DNA methylation show inefficient and delayed reactivation. This is consistent with the observation that histone-deacetylation (HDAC) inhibitors [61,73] and DNA-methylation inhibitors [30] can distinctly increase the reprogramming efficiency.

Recent work using adenoviral vectors or simple plasmid-based transfection protocols has finally demonstrated that insertional mutagenesis is not required for the reprogramming process [17,18]. It remains to be tested whether this would, however, increase the lower efficiency of the two reported non-integrating strategies. Owing to its high reproducibility and simplicity (compared with the more complex handling and generation of adenovirus), the transient transfection technique appears easily scalable and would find widespread application if demonstrated in human cells [17,18].

Towards clinical applications of iPS cells

Based on the data that we have reviewed here, including the ability of iPS cells to generate all lineages of the embryo and to contribute to chimera formation, we can conclude that iPS cells have a developmental potency comparable to ES cells. Furthermore, iPS cells have already been differentiated into various functional cell types, including neurons, cardiomyocytes and hematopoeitic cells (Figure 3) [19–21]. Similar to previous proof-of principle experiments using a combination of gene targeting and nuclear transfer [15], the combination of direct reprogramming and gene targeting can be used for therapeutic purposes in the mouse [19,20]. For instance, Hanna and colleagues used a humanized sickle cell anemia mouse model and showed that it is possible to correct the defect by coupling gene targeting and direct reprogramming [19]. The differentiated iPS cells could rescue the disease phenotype when transplanted into the donor mice. Similarly, Wernig and colleagues showed that iPS-cell-derived dopaminergic neurons could alleviate the disease phenotype in a rat model of Parkinson's disease [20]. These murine experiments suggest that, in principle, human iPS cells could also be used for regenerative and therapeutic applications. Nonetheless, there are several issues that need to be addressed before iPS cells could find broader clinical applications. Many of these issues are, in fact, not unique

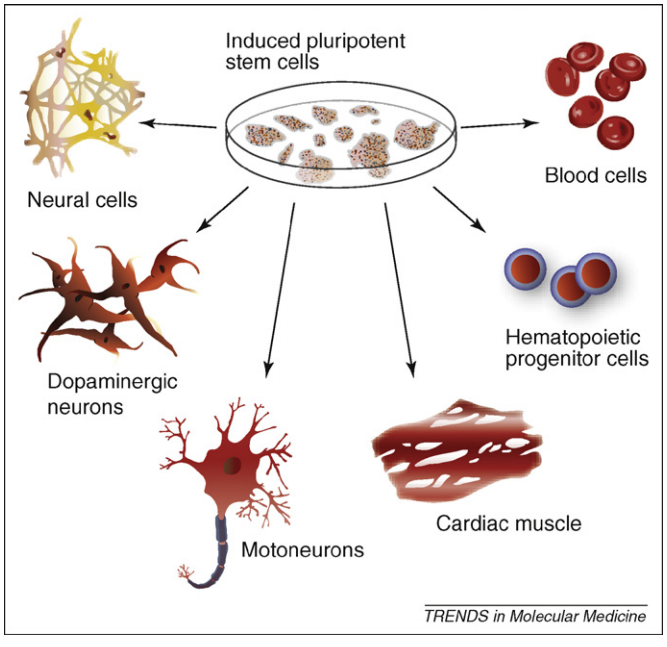

Figure 3. Directed differentiation of iPS cells. Mouse and human iPS cells have already been differentiated into a variety of disease-relevant cell types, including neural cells [5], dopaminergic neurons [20], motoneurons [21], cardiac muscle [79], hematopoietic progenitor cells and blood cells [19]. As pluripotent cells, iPS cells have the ability to generate all cell types and this is only limited currently by the ability to specifically direct the differentiation into other cell types.

to iPS cells but have already been noted for human ES cells: (i) it still needs to be demonstrated that human iPS cells can be generated without introduction of DNA to the genome (i.e. via adeno- or plasmid-based approaches); (ii) the manipulation of human ES and iPS cells is still significantly less efficient that in the mouse and this efficiency needs to be improved for routine gene targeting; (iii) some cell types can already be generated through directed differentiation of human ES/iPS cells, however, it will need a major effort to be able to generate all desired cell types; (iv) in addition, once such differentiation is possible, it still needs to be determined how comparable the *in vitro*-derived cell types are when compared with their *in vivo* counterparts and how to isolate them at sufficient purity; (v) along those lines, a general and efficient way of characterizing large numbers of human iPS and iPS-derived cells needs to be developed to provide a routine, high-throughput method for quality control.

Before moving any iPS cells into the clinic, they might probably find more immediate application in drug screens and basic research. However, some of the above-mentioned points also apply here, in particular, a better understanding of the quality and homogeneity of the iPS and iPS-derived cell types. This is relevant, for example, when differentiating disease-specific iPS cells (e.g. amyotrophic lateral sclerosis [ALS]-iPS cells) into the affected cell types (motor neurons) to investigate compounds that could ameliorate the phenotype. It is important to be able to distinguish culture- and reprogramming-induced phenotypes from the actual disease phenotype that the screen is targeting. The field of stem-cell biology and its translation towards clinical applications have made tremendous progress over recent years and there is reason for optimism, however, it might still take years before all of the issues raised here have been addressed satisfactorily.

Concluding remarks

The generation of induced pluripotent stem cells is likely to have a major impact on regenerative medicine. Recent developments allow for cautious optimism that the remaining obstacles to medical applications, such as the use of viruses or even any kind of exogenous DNA, will soon be overcome for human cell types (Box 1). Transcription-factor-mediated reprogramming has exciting biomedical applications, such as the generation of customized pluripotent stem cells for therapeutic purposes and, more immediately, the generation of disease-specific iPS cell lines for drug screening and disease modeling [19–21]. However, the utility of iPS technology goes beyond these reprogramming applications. Reprogramming somatic cells to convert them into iPS cells provides a method for amplifying single somatic cells '*in vivo*' and can be applied to ask mechanistic questions, as has been done using monoclonal mice derived by nuclear transfer [74–76].

To realize the full therapeutic potential of iPS technology, it will be necessary to develop novel and improved quality assessments that can be used readily to determine the exact cellular state of future reprogrammed cells, regardless of whether they are iPS cells or somatic cells that have been converted directly into other somatic cells [77]. New technologies, including genome-wide epigenetic profiling and stem-cell-focused reference epigenome mapping efforts, such as the NIH Roadmap for Epigenetics, will certainly be valuable to allow progress in this exciting field.

Box 1. Outstanding questions

- Can integration-free approaches be translated into human cells?
- Is the mechanism of human and mouse iPS-cell generation the same?
- Oct4 and Sox2 are the core factors across all combinations of factors needed for iPS reprogramming. What is the exact role of the non-essential factors (Klf4, c-Myc, Lin-28 and Nanog)?
- Does insertional mutagenesis increase reprogramming efficiency in non-integrating strategies?
- Human ES cells are distinct from and more variable than mouse ES cells. What variations exist in human iPS cells and what is the cause of this variation?
- How will future iPS cells be screened for quality?
- How can the iPS-derived cell types best be evaluated before application?
- Is it possible to reprogram human cells with small molecules alone?
- Can this approach be automated and scaled to enable routine generation of human iPS cells for every patient?

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References

1 Jaenisch, R. and Young, R. (2008) Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming. *Cell* 132, 567–582
2 Hochedlinger, K. and Jaenisch, R. (2006) Nuclear reprogramming and pluripotency. *Nature* 441, 1061–1067
3 Tada, M. *et al.* (2001) Nuclear reprogramming of somatic cells by *in vitro* hybridization with ES cells. *Curr. Biol.* 11, 1553–1558
4 Cowan, C.A. *et al.* (2005) Nuclear reprogramming of somatic cells after fusion with human embryonic stem cells. *Science* 309, 1369–1373
5 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
6 Wernig, M. *et al.* (2007) *In vitro* reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature* 448, 318–324
7 Maherali, N. *et al.* (2007) Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. *Cell Stem Cell* 1, 55–70
8 Kanatsu-Shinohara, M. *et al.* (2004) Generation of pluripotent stem cells from neonatal mouse testis. *Cell* 119, 1001–1012
9 Briggs, R. and King, T.J. (1952) Transplantation of living nuclei from blastula cells into enucleated frogs' eggs. *Proc. Natl. Acad. Sci. U. S. A.* 38, 455–463
10 Gurdon, J. (1962) The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. *J. Embryol. Exp. Morphol.* 10, 622–640
11 Meissner, A. and Jaenisch, R. (2006) Mammalian nuclear transfer. *Dev. Dyn.* 235, 2460–2469
12 Campbell, K.H. *et al.* (1996) Sheep cloned by nuclear transfer from a cultured cell line. *Nature* 380, 64–66
13 Thomson, J.A. *et al.* (1998) Embryonic stem cell lines derived from human blastocysts. *Science* 282, 1145–1147
14 Hochedlinger, K. and Jaenisch, R. (2003) Nuclear transplantation, embryonic stem cells, and the potential for cell therapy. *N. Engl. J. Med.* 349, 275–286
15 Rideout, W.M., 3rd *et al.* (2002) Correction of a genetic defect by nuclear transplantation and combined cell and gene therapy. *Cell* 109, 17–27
16 Egli, D. *et al.* (2007) Developmental reprogramming after chromosome transfer into mitotic mouse zygotes. *Nature* 447, 679–685

17 Okita, K. *et al.* (2008) Generation of mouse induced pluripotent stem cells without viral vectors. *Science* 322, 949–953
18 Stadtfeld, M. *et al.* (2008) Induced pluripotent stem cells generated without viral integration. *Science* 322, 945–949
19 Hanna, J. *et al.* (2007) Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. *Science* 318, 1920–1923
20 Wernig, M. *et al.* (2008) Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. *Proc. Natl. Acad. Sci. U. S. A.* 105, 5856–5861
21 Dimos, J.T. *et al.* (2008) Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. *Science* 321, 1218–1221
22 Park, I.H. *et al.* (2008) Disease-specific induced pluripotent stem cells. *Cell* 134, 877–886
23 Rubin, L.L. (2008) Stem cells and drug discovery: the beginning of a new era? *Cell* 132, 549–552
24 Osafune, K. *et al.* (2008) Marked differences in differentiation propensity among human embryonic stem cell lines. *Nat. Biotechnol.* 26, 313–315
25 Takahashi, K. *et al.* (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131, 861–872
26 Yu, J. *et al.* (2007) Induced pluripotent stem cell lines derived from human somatic cells. *Science* 318, 1917–1920
27 Aasen, T. *et al.* (2008) Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. *Nat. Biotechnol.* 26, 1276–1284
28 Aoi, T. *et al.* (2008) Generation of pluripotent stem cells from adult mouse liver and stomach cells. *Science* 321, 699–702
29 Stadtfeld, M. *et al.* (2008) Defining molecular cornerstones during fibroblast to iPS cell reprogramming in mouse. *Cell Stem Cell* 2, 230–240
30 Mikkelsen, T.S. *et al.* (2008) Dissecting direct reprogramming through integrative genomic analysis. *Nature* 454, 49–55
31 Avilion, A.A. *et al.* (2003) Multipotent cell lineages in early mouse development depend on SOX2 function. *Genes Dev.* 17, 126–140
32 Rowland, B.D. and Peeper, D.S. (2006) KLF4, p21 and context-dependent opposing forces in cancer. *Nat. Rev. Cancer* 6, 11–23
33 Brambrink, T. *et al.* (2008) Sequential expression of pluripotency markers during direct reprogramming of mouse somatic cells. *Cell Stem Cell* 2, 151–159
34 Hanna, J. *et al.* (2008) Direct reprogramming of terminally differentiated mature B lymphocytes to pluripotency. *Cell* 133, 250–264
35 Wernig, M. *et al.* (2008) A drug-inducible transgenic system for direct reprogramming of multiple somatic cell types. *Nat. Biotechnol.* 26, 916–924
36 Maherali, N. *et al.* (2008) A high-efficiency system for the generation and study of human induced pluripotent stem cells. *Cell Stem Cell* 3, 340–345
37 Hockemeyer, D. *et al.* (2008) A drug-inducible system for direct reprogramming of human somatic cells to pluripotency. *Cell Stem Cell* 3, 346–353
38 Lowry, W.E. *et al.* (2008) Generation of human induced pluripotent stem cells from dermal fibroblasts. *Proc. Natl. Acad. Sci. U. S. A.* 105, 2883–2888
39 Niwa, H. *et al.* (2000) Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. *Nat. Genet.* 24, 372–376
40 Bernstein, B.E. *et al.* (2007) The mammalian epigenome. *Cell* 128, 669–681
41 Bernstein, B.E. *et al.* (2006) A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell* 125, 315–326
42 Mikkelsen, T.S. *et al.* (2007) Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. *Nature* 448, 553–560
43 Meissner, A. *et al.* (2008) Genome-scale DNA methylation maps of pluripotent and differentiated cells. *Nature* 454, 766–770
44 Meissner, A. *et al.* (2007) Direct reprogramming of genetically unmodified fibroblasts into pluripotent stem cells. *Nat. Biotechnol.* 25, 1177–1181
45 Rougier, N. *et al.* (1998) Chromosome methylation patterns during mammalian preimplantation development. *Genes Dev.* 12, 2108–2113
46 Imamura, M. *et al.* (2006) Transcriptional repression and DNA hypermethylation of a small set of ES cell marker genes in male germline stem cells. *BMC Dev. Biol.* 6, 34
47 Jones, P.A. *et al.* (1990) *De novo* methylation of the MyoD1 CpG island during the establishment of immortal cell lines. *Proc. Natl. Acad. Sci. U. S. A.* 87, 6117–6121
48 Humpherys, D. *et al.* (2001) Epigenetic instability in ES cells and cloned mice. *Science* 293, 95–97
49 Rugg-Gunn, P.J. *et al.* (2007) Status of genomic imprinting in human embryonic stem cells as revealed by a large cohort of independently derived and maintained lines. *Hum. Mol. Genet.* 16, R243–R251
50 Holm, T.M. *et al.* (2005) Global loss of imprinting leads to widespread tumorigenesis in adult mice. *Cancer Cell* 8, 275–285
51 Eggan, K. *et al.* (2001) Hybrid vigor, fetal overgrowth, and viability of mice derived by nuclear cloning and tetraploid embryo complementation. *Proc. Natl. Acad. Sci. U. S. A.* 98, 6209–6214
52 Schöler, H.R. *et al.* (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
53 Nichols, J. *et al.* (1998) Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. *Cell* 95, 379–391
54 Yuan, H. *et al.* (1995) Developmental-specific activity of the FGF-4 enhancer requires the synergistic action of Sox2 and Oct-3. *Genes Dev.* 9, 2635–2645
55 Boyer, L.A. *et al.* (2005) Core transcriptional regulatory circuitry in human embryonic stem cells. *Cell* 122, 947–956
56 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
57 Nakagawa, M. *et al.* (2008) Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nat. Biotechnol.* 26, 101–106
58 Schmidt, E.V. (1999) The role of c-myc in cellular growth control. *Oncogene* 18, 2988–2996
59 Murphy, M.J. *et al.* (2005) More than just proliferation: Myc function in stem cells. *Trends Cell Biol.* 15, 128–137
60 Wernig, M. *et al.* (2008) c-Myc is dispensable for direct reprogramming of mouse fibroblasts. *Cell Stem Cell* 2, 10–12
61 Huangfu, D. *et al.* (2008) Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. *Nat. Biotechnol.* 26, 1269–1275
62 Eminli, S. *et al.* (2008) Reprogramming of neural progenitor cells into induced pluripotent stem cells in the absence of exogenous sox2 expression. *Stem Cells* 26, 2467–2474
63 Kim, J.B. *et al.* (2008) Pluripotent stem cells induced from adult neural stem cells by reprogramming with two factors. *Nature* 454, 646–650
64 Segre, J.A. *et al.* (1999) Klf4 is a transcription factor required for establishing the barrier function of the skin. *Nat. Genet.* 22, 356–360
65 Zhao, W. *et al.* (2004) Identification of Krüppel-like factor 4 as a potential tumor suppressor gene in colorectal cancer. *Oncogene* 23, 395–402
66 Li, Y. *et al.* (2005) Murine embryonic stem cell differentiation is promoted by SOCS-3 and inhibited by the zinc finger transcription factor Klf4. *Blood* 105, 635–637
67 Chambers, I. *et al.* (2003) Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. *Cell* 113, 643–655
68 Mitsui, K. *et al.* (2003) The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. *Cell* 113, 631–642
69 Chambers, I. *et al.* (2007) Nanog safeguards pluripotency and mediates germline development. *Nature* 450, 1230–1234
70 Moss, E.G. *et al.* (1997) The cold shock domain protein LIN-28 controls developmental timing in *C. elegans* and is regulated by the lin-4 RNA. *Cell* 88, 637–646
71 Polesskaya, A. *et al.* (2007) Lin-28 binds IGF-2 mRNA and participates in skeletal myogenesis by increasing translation efficiency. *Genes Dev.* 21, 1125–1138
72 Viswanathan, S.R. *et al.* (2008) Selective blockade of microRNA processing by Lin28. *Science* 320, 97–100

73 Huangfu, D. *et al.* (2008) Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. *Nat. Biotechnol.* 26, 795–797

74 Hochedlinger, K. and Jaenisch, R. (2002) Monoclonal mice generated by nuclear transfer from mature B and T donor cells. *Nature* 415, 1035–1038

75 Serwold, T. *et al.* (2007) Early TCR expression and aberrant T cell development in mice with endogenous prerearranged T cell receptor genes. *J. Immunol.* 179, 928–938

76 Koralov, S.B. *et al.* (2005) Direct *in vivo* VH to JH rearrangement violating the 12/23 rule. *J. Exp. Med.* 201, 341–348

77 Zhou, Q. *et al.* (2008) *In vivo* reprogramming of adult pancreatic exocrine cells to beta-cells. *Nature* 455, 627–632

78 Stadtfeld, M. *et al.* (2008) Reprogramming of pancreatic β cells into induced pluripotent stem cells. *Curr. Biol.* 18, 890–894

79 Shi, Y. *et al.* (2008) Induction of pluripotent stem cells from mouse embryonic fibroblasts by Oct4 and Klf4 with small-molecule compounds. *Cell Stem Cell* 3, 568–574