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REVIEW

A developmental framework for induced pluripotency

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ABSTRACT

During development, cells transition from a pluripotent to a differentiated state, generating all the different types of cells in the body. Development is generally considered an irreversible process, meaning that a differentiated cell is thought to be unable to return to the pluripotent state. However, it is now possible to reprogram mature cells to pluripotency. It is generally thought that reprogramming is accomplished by reversing the natural developmental differentiation process, suggesting that the two mechanisms are closely related. Therefore, a detailed study of cell reprogramming has the potential to shed light on unexplained developmental mechanisms and, conversely, a better understanding of developmental differentiation can help improve cell reprogramming. However, fundamental differences between reprogramming processes and multi-lineage specification during early embryonic development have also been uncovered. In addition, there are multiple routes by which differentiated cells can re-enter the pluripotent state. In this Review, we discuss the connections and disparities between differentiation and reprogramming, and assess the degree to which reprogramming can be considered as a simple reversal of development.

KEY WORDS: Reprogramming, Development, iPSC, Stem cell

Introduction

More than 50 years ago, Conrad Waddington published his famous depiction of normal embryonic development, likening the process of differentiation to a ball rolling down a hill, from a pluripotent state to one of multiple differentiated states (Fig. 1) (Waddington, 1957). It has long been clear that the very early embryo must comprise cells with the potential to differentiate into all mature cell types in the body, and the idea has been that individual lineages gradually lose potency as they progress down their differentiation pathway (Fig. 2). The isolation of mouse embryonic stem cells (ESCs) in the early 1980s (Evans and Kaufman, 1981; Martin, 1981) gave rise to a new scientific field devoted to understanding how these cells can be maintained in a pluripotent state or differentiated towards a particular cell type or tissue, and provided a highly accessible system in which Waddington's ideas could be explored.

However, as early as 1962, Sir John Gurdon reported the first definitive example of cellular reprogramming by cloning a frog through somatic cell nuclear transfer (injecting the nucleus from a differentiated intestinal epithelial cell into an enucleated oocyte, Fig. 3), making a significant dent into the model that differentiation is terminal. More recently, cellular reprogramming of differentiated mature cells into ESC-like cells called induced pluripotent stem cells (iPSCs) was achieved with the introduction of specific transcription factors (Fig. 3) (Takahashi and Yamanaka, 2006) and further challenged the unidirectionality of developmental differentiation. Returning to Waddington's landscape, this can be likened to the ball traveling upwards (Hochedlinger and Plath, 2009; Yamanaka, 2009) (see purple ball in Fig. 1). These findings raise several questions: to what extent and by which mechanisms is differentiation reversible and what are the implications of such reversal?

Here, we review the various types of pluripotency occurring both naturally in the embryo and *in vitro* through experimental manipulations. We then discuss the different biological processes occurring during cell reprogramming and their similarities with developmental programs. Finally, we examine whether the potency achieved by reprogramming differentiated cells into iPSCs is comparable to that seen in ESCs.

Pluripotency *in vivo* and *in vitro*

Developing embryos at the blastocyst stage give rise to two distinct cell types: the inner cell mass (ICM) and the trophoblast. The pre-implantation embryo is composed of cells that each hold the inherited developmental capacity to generate all cell types in our body – including the germline – and that can contribute to all tissues of a chimeric mouse when transplanted into a host blastocyst. This broad developmental capacity is known as 'naïve' or 'ground state' pluripotency (Nichols and Smith, 2009) (Fig. 2). Around implantation, the ICM segregates into a bilaminar embryonic disc composed of two distinct epithelial layers: the dorsal epiblast and ventral hypoblast. After implantation, epiblast cells show a more restricted potency and are thought to be unable to contribute to chimeras when injected into a host blastocyst (Nichols and Smith, 2012). Subsequent gastrulation converts the bilaminar embryonic disc into three committed germ layers: ectoderm, mesoderm and endoderm (Fig. 2).

In the mouse, self-renewing cells lines can be derived *in vitro* from the different lineages of the embryo at pre- and post-implantation stages: cells taken from the ICM can be propagated in a dish as ESCs (Evans and Kaufman, 1981; Martin, 1981; Bradley et al., 1984), whereas cells taken from the post-implantation epiblast are cultured as EpiSCs (Brons et al., 2007; Tesar et al., 2007). In general, these cell lines retain similar lineage potential to their *in vivo* source cells and hence exhibit different types of potency. Murine ESCs display a naïve pluripotency whereas EpiSCs are considered to be in a 'primed' pluripotent state because, although they are able to generate derivatives of the three germ layers *in vitro*, they rarely contribute to chimera formation *in vivo* (Nichols and Smith, 2009) (Fig. 2). Human ESCs can also be derived from the ICM but, contrary to mouse ESCs, they spontaneously adopt a primed pluripotent state *in vitro* and exhibit properties including morphology, cytokine requirement and gene expression patterns that are reminiscent of murine EpiSCs (Nichols and Smith, 2009).

Mouse ESC self-renewal can be maintained with a combination of cytokines, including bone morphogenetic protein (BMP) and leukemia inhibitory factor (LIF). However, ESCs constitute a

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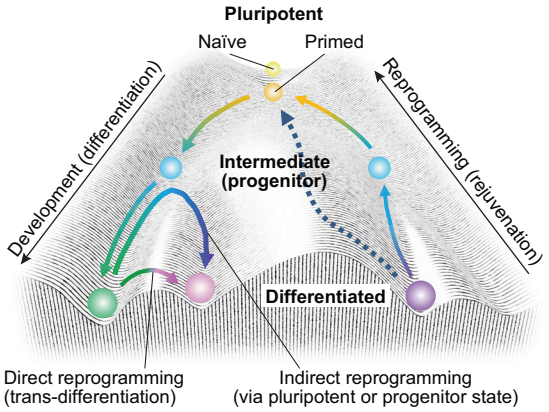

Fig. 1. Cell fate changes on Waddington's epigenetic landscape. Pluripotent stem cells (naïve in yellow and primed in orange) can commit to any somatic lineage (green, pink, purple) via a progenitor state (blue), not only during development but also in response to extrinsic cues *in vitro*. Direct reprogramming, or trans-differentiation, using tissue-specific transcription factors allows lineage-committed cells (green) to convert to another fate (pink), regardless of their germ layer origin, and bypasses the need for a pluripotent intermediate state. During indirect reprogramming, using a combination of OSKM expression and optimal conditions for the destined lineage, cells can be converted to another cell type via a transient pluripotent state. Finally, recently developed technologies can be used to revert mature somatic cells (purple) to pluripotency (orange or yellow) via a progenitor stage (blue) or directly (blue dashed arrow). Adapted, with permission, from Waddington (1957).

heterogeneous population *in vitro* as a result of the fluctuating expression of several pluripotency markers such as Nanog, Klf4, Tbx3 and Rex1 (Ying et al., 2003; Niwa et al., 2009). This heterogeneity can be abolished by reversibly inhibiting cell differentiation using chemical inhibitors for mitogen-activated protein kinase (MEK) and glycogen synthase kinase 3 (GSK3) (Ying et al., 2008), resulting in a homogeneous population of naïve pluripotent stem cells. Exposure to such small molecules also allows the generation of germline-competent ESCs derived from non-permissive mouse strains, such as non-obese diabetic (NOD) mice from which ESCs cannot easily be derived using standard culture methods (Hanna et al., 2009; Nichols et al., 2009). Thus, naïve pluripotency can be stabilized and acquired in cells with different genetic backgrounds (Kawase et al., 1994; Ohtsuka and Niwa, 2015). The nature of pluripotency is also variable across species. For example, similarly to mouse ESCs, rat ESCs exhibit and maintain naïve pluripotency under serum-free conditions with two kinase inhibitors (Buehr et al., 2008; Li et al., 2008). By contrast, ESCs derived from rhesus monkey blastocysts exhibit primed pluripotency and do not contribute to chimeric animals (Thomson et al., 1995; Tachibana et al., 2012). However, in some cases, the nature of the pluripotent state can be modulated. FAB-SCs, a mouse stem cell line established in a culture medium containing FGF and Activin, resembling the culture conditions used to derive primate ESCs, cannot contribute to chimeric mice or to teratoma formation, a hallmark of pluripotency (Chou et al., 2008). However, the primed-like state of FAB-SCs can be reverted to a naïve state by exposing the cells to medium containing serum and LIF. Together, these observations suggest that species and culture conditions significantly affect the nature of pluripotency.

Several routes to pluripotency: mechanisms of cell reprogramming

The generation of pluripotent stem cell lines by cell reprogramming can be achieved using several methods such as somatic cell nuclear transfer (SCNT) into an enucleated egg (Wakayama et al., 2001), fusion with a pluripotent stem cell (Tada et al., 2001), exposure to small chemical compounds (Hou et al., 2013), and transduction of reprogramming factors (Takahashi and Yamanaka, 2006) (Fig. 3). This latter technique for inducing pluripotency has also been used to convert a somatic cell directly into a different lineage, without inducing a stable pluripotent state (green and pink balls in Fig. 1), a process known as 'direct reprogramming' and reviewed extensively elsewhere (Zhou et al., 2008; Ieda et al., 2010; Vierbuchen et al., 2010; Huang et al., 2011; Sekiya and Suzuki, 2011).

In the cases of SCNT- and cell fusion-mediated reprogramming, somatic cell nuclei are suddenly exposed to toti- or pluripotent environments. Consequently, these reprogramming methods are rapid (Tada et al., 2001; Egli et al., 2011) (Fig. 3). By contrast, the transduction of the reprogramming factors POU domain class 5 transcription factor 1 (POU5F1, also known as OCT3/4), SRY (sex determining region Y)-box 2 (SOX2), Krüppel-like factor 4 (KLF4) and Myelocytomatosis oncogene (c-MYC) (together referred to as OSKM) results in a relatively slow reprogramming, presumably because the somatic cell nuclei are not immediately exposed to the cytoplasm of a pluripotent cell (Takahashi and Yamanaka, 2006). Consequently, reprogramming somatic cells to pluripotency using exogenous factors, such as OSKM or a cocktail of chemical compounds (Hou et al., 2013), progresses in a stepwise manner, via intermediate cells in which the changes induced by such reprogramming methods (erasure of somatic cell memories and activation of pluripotency-associated genes) are still ongoing. Some of these cells, which represent several immature stages, reflect

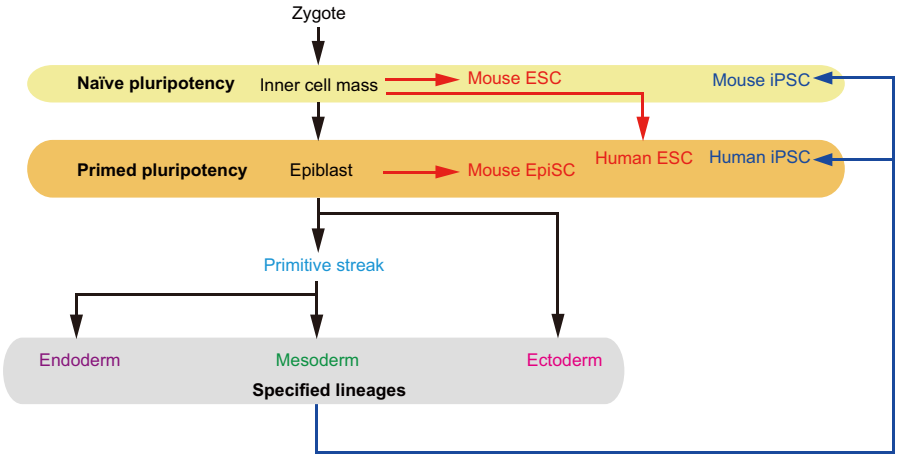

Fig. 2. Reprogramming of somatic cells toward pluripotency. A single zygote generates >200 mature cell types in the body derived from three lineages (endoderm, mesoderm and ectoderm; grey box), specified during embryonic development. Naïve pluripotency, a characteristic of the pre-implantation epiblast, is found in mouse ESCs and mouse iPSCs (yellow box). Primed pluripotency, found in post-implantation epiblasts, is also seen in mouse EpiSCs or human ESCs (orange box). Reprogramming of mouse cells achieves the whole spectrum of pluripotency seen *in vivo*.

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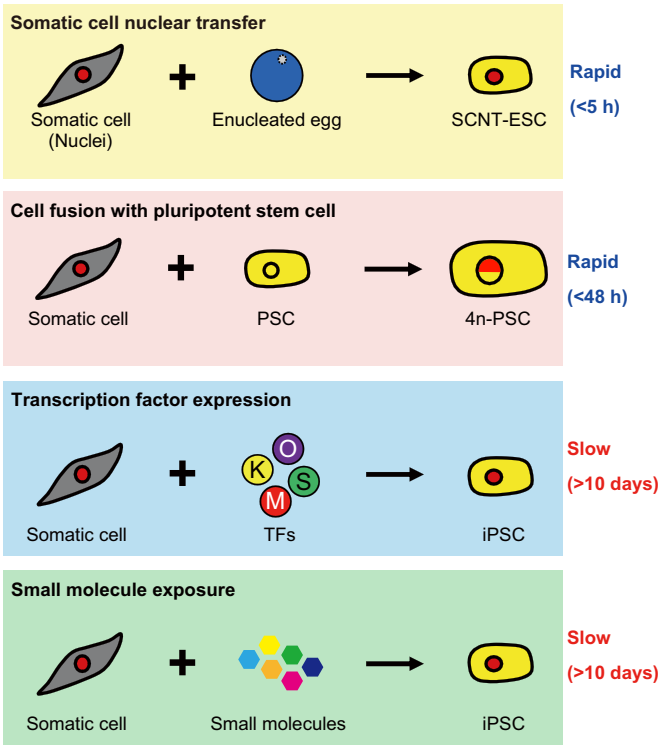

Fig. 3. Different paths to pluripotency. Reprogramming by somatic cell nuclear transfer (SCNT): the memory of a somatic cell nucleus can be reset by injecting it in an enucleated oocyte, generating an SCNT-embryonic stem cell (ESC). Cell fusion-mediated reprogramming: fusing a somatic cell with a pluripotent stem cell (PSC) erases the somatic cell identity. The resulting tetraploid cell (4n) exhibits pluripotent stem cell-like features. Transcription factor (TF)-mediated reprogramming: a small set of reprogramming factors, OCT3/4, SOX2, KLF4 and c-MYC (OSKM), transforms mature differentiated cells into induced pluripotent stem cells (iPSCs). Small molecule-mediated reprogramming: exposure to a set of small molecules also reprograms somatic cells to pluripotency.

developmental intermediates. These differences in the kinetics of reprogramming suggest that the cytoplasmic memories of pluripotency, such as transcripts found in the egg or in pluripotent stem cells, are key for a fast reprogramming process.

Another key difference between the different reprogramming techniques cited above is their efficiency, which is very low in the case of the transduction of reprogramming factors, where only a small minority of cells is successfully reprogrammed (Fig. 3). Such low efficacy can be explained by a stochastic model (Yamanaka, 2009): most cells initiate the reprogramming process but only a few are able to complete it. One of the first events required for a successful reprogramming is the pioneer activity of the OSKM factors. Soufi et al. mapped the binding sites of OSKM on the human genome in the initial phase of the reprogramming of fibroblasts to iPSCs (Soufi et al., 2012) and found that c-Myc facilitates the engagement of OSK with chromatin and their binding to the enhancer regions of genes that promote reprogramming. Additionally, histone H3 lysine 9 trimethylation, a mark of repressive chromatin, was found at many of the pluripotency loci, impeding OSKM accessibility and thus reducing reprogramming efficiency.

Another factor that determines the success rate of reprogramming is the stoichiometry of the reprogramming factors. For example, iPSC-derived secondary fibroblasts carrying doxycycline-inducible OSKM expression cassettes, which achieve a uniform expression of OSKM at levels required for iPSC generation, can be efficiently reprogrammed to pluripotency (Wernig et al., 2008). Additionally, it has been observed that reprogramming efficiency is enhanced by increasing levels of OCT3/4 and KLF4 (Papapetrou et al., 2009; Tiemann et al., 2011) as well as by lowering SOX2 expression (Yamaguchi et al., 2010). It has also been shown that in the early phase of reprogramming towards pluripotency, OCT3/4 and SOX2 promote the expression of mesendodermal and neuroectodermal genes, respectively (Shu et al., 2013), whereas KLF4 can induce the expression of epidermal genes in a context-dependent manner (Kim et al., 2015). Thus, unbalanced OSKM expression deviates the intermediate cells from the reprogramming path. Consequently, these cells are unable to ever become iPSCs (Polo et al., 2012; Tanabe et al., 2013). In addition to driving the reprogramming process, each of the OSKM factors has important developmental functions aside from promoting pluripotency (Payne et al., 1982; Nichols et al., 1998; Avilion et al., 2003; Jiang et al., 2008), which could be favored if OSKM stoichiometry is not optimal, and could thus have an inhibitory influence on reprogramming. Therefore, well-balanced expression of reprogramming factors leads the somatic cells toward pluripotency.

During embryonic development, an undifferentiated cell must choose between multiple fates, whereas in reprogramming to pluripotency, there is a single end point (the iPSC) but multiple possible starting states. Can the suppression of somatic gene expression and activation of pluripotency genes during reprogramming be likened to the reversal of the loss of pluripotency that leads to the commitment of a pluripotent cell to a specialized lineage during embryogenesis? Below, we discuss some processes shared by developmental maturation and reprogramming.

Transition between mesenchymal and epithelial fates during differentiation and reprogramming

During gastrulation, initiated by the formation of the primitive streak from the epiblast in mouse, pluripotent epiblast cells give rise to a structure with three committed germ layers (endoderm, mesoderm and ectoderm) (Tam and Loebel, 2007) (Fig. 2). The primitive streak constitutes a gate through which cells migrate from the epiblast into the interior of the embryo via an epithelial-to-mesenchymal transition (EMT) (Thiery et al., 2009; Chen et al., 2012). Therefore, the morphological process of EMT is coupled with the first cell fate decision – the generation of three germ layers – of the embryo.

Intriguingly, whereas EMT is thought to be important for cells to differentiate, the opposite process, mesenchymal-to-epithelial transition (MET), has been proposed as a requirement for the early phases of reprogramming (Li et al., 2010; Samavarchi-Tehrani et al., 2010) (Fig. 4). Indeed, following OSKM transduction in fibroblasts, the expression of mesenchyme-related transcription factors, such as Snai1, Snai2, Twist1, Twist2, Zeb1 and Zeb2, are repressed both in mouse and human cells (Samavarchi-Tehrani et al., 2010; Takahashi et al., 2014). At the same time, mesenchymal-specific surface proteins, Thy1 and Cd44, are repressed (O'Malley et al., 2013). Epithelial features, such as the expression of E-cadherin (Cdh1), arise almost as soon as mesenchymal identity is lost. These changes occur prior to the expression of Stage specific embryonic antigen 1 (SSEA-1, also known as Fut4) an early phase marker of intermediate reprogrammed cells in mouse. Additionally, inhibition of MET by transforming growth factor-beta (TGFβ) treatment or forced expression of Snai1 prevents the formation of cell-cell junctions

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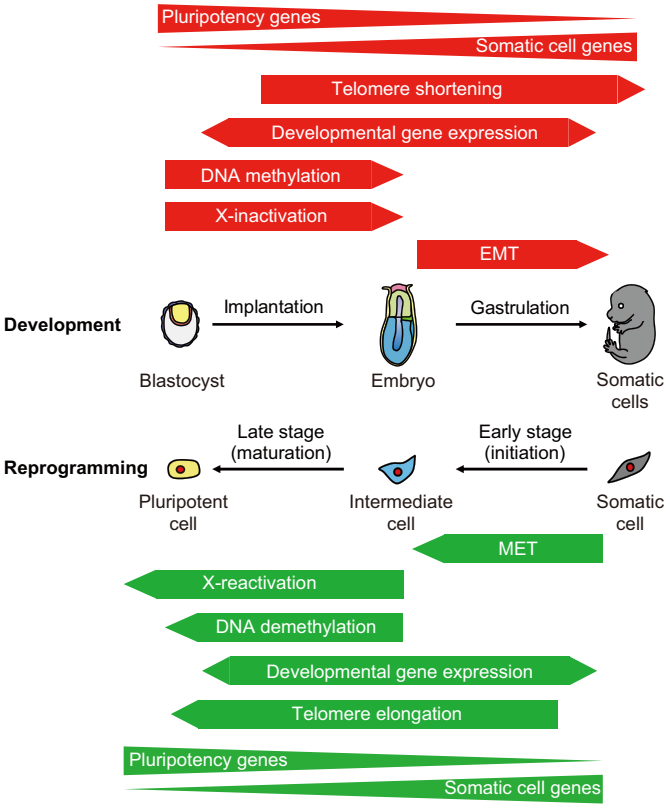

Fig. 4. Common characteristics of reprogramming and development. During embryonic development, a fertilized, pluripotent egg gives rise to a complete organism through cell differentiation. Conversely, the reprogramming process rejuvenates a mature cell and brings it to a pluripotent state. These two mechanisms share common events including changes in the expression of pluripotency and somatic genes, transitions between mesenchymal and epithelial states (through a mesenchymal-to-epithelial transition, MET, or an epithelial-to-mesenchymal transition, EMT), transient developmental genes expression, changes in telomere length, variations in X-chromosome activity, and evolution of the global DNA methylation status.

and drastically decreases the reprogramming efficiency (Ichida et al., 2009; Maherali and Hochedlinger, 2009). Conversely, the inhibition of TGFβ signaling by using a TGFβ1 receptor inhibitor or the ectopic expression of Smad7, an antagonistic molecule of the TGFβ pathway, facilitates mouse iPSC generation. Bone morphogenic protein 4 (Bmp4), which also inhibits TGFβ signaling, also increases the efficiency of mouse iPSC generation. In addition, Bmp4 can functionally replace Klf4 to achieve reprogramming by enhancing MET (Chen et al., 2010). Interestingly, the transduction of Klf4 alone is sufficient for the induction of Cdh1 expression in the early phases of reprogramming of mouse embryonic fibroblasts (MEFs) to pluripotency (Li et al., 2010; Samavarchi-Tehrani et al., 2010). Furthermore, forced expression of Klf4 can convert EpiSCs to the naïve state of pluripotency (Guo et al., 2009). In addition, ectopic expression of Cdh1 in EpiSCs restores their capacity to contribute to normal development after blastocyst injection (Ohtsuka et al., 2012). Therefore, the Klf4/Cdh1 axis is crucial for the acquisition of mouse naïve pluripotency. This suggests that, when the reprogramming process is activated, cells transit directly from a somatic to a post-gastrulation-like stage, thereby bypassing the progenitor stage usually seen in the developmental path *in vivo*. In summary, during development, EMT is coupled to lineage specification whereas promoting MET increases reprogramming efficiency. In this way, MET and EMT are defining processes for the entrance or exit of pluripotency, respectively.

miRNA circuits regulate reprogramming and development

Most miRNAs function as post-transcriptional repressors by annealing to target messenger RNAs. Therefore, miRNAs complement transcription factors to regulate gene expression networks during cell fate changes. Indeed, the inhibition of miRNA biogenesis by targeted disruption of Dicer, a ribonuclease that cleaves double-stranded RNAs and pre-miRNAs into short double-stranded RNA fragments, impaired normal embryonic development and reduced the differentiation potential of ESCs (Kanellopoulou et al., 2005). Interestingly, Dicer is also indispensable for the generation of iPSCs from MEFs (Kim et al., 2012). In this way, miRNAs are crucial for cell fate changes both during development and reprogramming. Here, we discuss three key examples of miRNAs and associated proteins involved in the regulation of pluripotency: let-7, miR-290/295 and miR-302/367.

The LIN28/let-7/TRIM71 axis

The let-7 family is abundantly expressed in somatic cells, but not in pluripotent cells and promotes terminal differentiation during development (Ambros, 2011). In the mouse embryo, let-7 expression starts at embryonic day (E) 8.5 (mid-gastrulation) and remains elevated through the newborn stages (Schulman et al., 2005). Conversely, the levels of let-7 miRNA progressively decrease during reprogramming to iPSCs in mouse and human (Melton et al., 2010; Worringer et al., 2013). LIN28, a factor that regulates stem cell renewal, inhibits the biogenesis of let-7 during mouse embryogenesis and in pluripotent stem cell lines (Heo et al., 2008, 2009; Viswanathan et al., 2008; West et al., 2009; Viswanathan and Daley, 2010) and improves the reprogramming efficiency of mouse and human cells (Yu et al., 2007; Yusa et al., 2009; Tanabe et al., 2013). The same pattern is observed for a target of let-7, TRIM71 (Worringer et al., 2013). TRIM71 is expressed in epiblasts and embryonic ectoderm by E9.5 and its expression pattern is the opposite to that of let-7 (Rybak et al., 2009). TRIM71 and let-7 functionally repress each other in a negative feedback loop. In fact, forced expression of the gene encoding TRIM71 along with OSK or OSKM facilitates iPSC generation and cancels out the negative effects of let-7 on reprogramming (Worringer et al., 2013). During embryonic development, LIN28 plays important roles in primordial germ cell specification (West et al., 2009). In the embryo from E8.5 to E10.5, LIN28 expression is reduced and results in global let-7 activation. *Trim71* knockout mouse embryos show abnormalities at E9.5 and die as a result of neural tube closure defects (Maller Schulman et al., 2008). In mouse ESCs, TRIM71 is dispensable for self-renewal but its loss led to upregulation of genes required for neural development (Mitschka et al., 2015) suggesting that TRIM71 is associated with the priming of neural differentiation rather than with pluripotency. Thus, the activation of the LIN28/let-7/TRIM71 axis is important for early development whereas its repression is crucial for successful reprogramming.

The miR-290/295 and miR-302/367 clusters

The miR-290/295 and miR-302/367 clusters are abundantly expressed in pluripotent cells. miR-290 and 295 are transcribed specifically in early embryos and pluripotent cells whereas the miR-302/367 cluster is expressed not only in pluripotent cells but also at early stages of lung development. To study the expression of miR-290 and miR-302 *in vivo* in more detail, Parchem et al. developed a dual color reporter system using endogenous miR-290 and miR-302

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promoters and found that miR-290 was expressed in pluripotent cells from E3.5 to E6.5 (Parchem et al., 2014). By E7.5 (gastrulation) and at later stages, miR-290 was expressed in extra-embryonic tissues but not in the epiblast. By contrast, the expression of miR-302 started from E5.5, just after implantation, and continued until the gastrulation stage. Finally, the expression of miR290 and miR302 is attenuated after gastrulation, when let-7 begins to be expressed. The analysis of such reporter systems also demonstrated specific expression of miR-290 in naïve ESCs and of miR-302 in primed EpiSCs.

The miR-290/295 cluster plays important roles in embryonic development as knockout mouse embryos exhibit partially penetrant lethality (Medeiros et al., 2011). In addition, knockout adult female mice show infertility as a result of premature ovarian failure (Medeiros et al., 2011). The miR-302/367 cluster is a target of the transcription factor GATA binding protein (Gata) 6 in the lung and assumes key roles in proliferation and differentiation of lung endoderm progenitors (Tian et al., 2011). In ESCs, these miRNA clusters both inhibit BMP/TGFβ-dependent EMT by repressing the translation of TGFβ receptor II (TgfbrII) and promote rapid ESC proliferation by accelerating the G1 to S transition via control of p21CIP1 (Cdkn1a) and cyclin D expression (Wang et al., 2008; Subramanyam et al., 2011).

Interestingly, the analysis of transcription activator-like effector nuclease (TALEN)-mediated knockout cells revealed that the miR-302/367 cluster is crucial for iPSC generation from human cells (Zhang et al., 2013). Furthermore, ectopic expression of OSK stochastically produced intermediates that express either or both miR-290 and miR-302 during reprogramming. These random patterns are very different from the patterns seen in early embryogenesis. Of note, the addition of Sal-like 4 (Sall4), a pluripotency-associated transcription factor, to OSK drastically increased the number of miR-290^{-} miR-302^{+} intermediate reprogrammed cells, akin to epiblast cells between E5.5 and gastrulation, and iPSC colony formation. These observations suggest that there are high heterogeneities in the intermediate reprogrammed cells induced by OSK, and that the intermediates such as miR-290^{-} miR-302^{+} cells that mimic the reversion of development preferentially become iPSCs. Additional factors, including Sall4, can help homogenize the intermediate state and facilitate the reprogramming efficiency. Taken together, these data suggest that the reversal of early embryonic miRNA circuits is an effective way to reprogram a cell to pluripotency.

Transient expression of developmental genes during reprogramming

During the reprogramming of somatic cells to pluripotency, somatic genes should be suppressed and pluripotency-associated genes upregulated. However, intermediates on the way to reprogramming can be identified, suggesting that a transient reprogrammed state might exist. It is possible that such a state reflects transient developmental intermediates, which can display a unique gene expression signature (such as, for example, the expression of the key mesoderm transcription factor T, also known as Brachyury, which defines mesoderm during gastrulation but is then downregulated; Marcellini et al., 2003).

Several types of reporter lines carrying fluorescent proteins driven by the promoters of pluripotency genes, such as Oct3/4 and Nanog, have been established to distinguish cells undergoing reprogramming from those that are not (Maherali et al., 2007; Okita et al., 2007; Wernig et al., 2007; Park et al., 2008). However, these reporter genes are activated at the late phase of the reprogramming process. To capture intermediates at the early phase of the process, cells can be labeled with antibodies against surface antigens such as SSEA-1 in mice and Tumor-related antigen-1-60 (TRA-1-60) in humans, facilitating the sorting and purification of cells at early stages of reprogramming (Buganim et al., 2012; Polo et al., 2012; O'Malley et al., 2013; Tanabe et al., 2013). Thus, by removing non-reprogrammed cells early during the re-acquisition of pluripotency, researchers can also remove the associated noise, which improves study of the molecular events of reprogramming.

Although SSEA-1 is expressed at the early phase of reprogramming, its expression is sustained throughout the entire process. The analysis of SSEA-1^{+} intermediates derived from MEFs in the late phase of reprogramming showed transient expression of miR-302a, which is normally expressed in mouse EpiSCs and in the post-implantation epiblast but not in ESCs (Polo et al., 2012). These data suggest that during reprogramming, cells transition through a primed epiblast-like state to reach a naïve pluripotent state. SSEA-1^{+} cells have also been shown to express mesendodermal markers such as Forkhead box A2 (*FoxA2*), Mix1 homeobox-like 1 (*Mixl1*) and Eomesodermin homolog (*Eomes*) but not ectodermal genes, suggesting that reprogramming takes them through a fate resembling the primitive streak, which is mainly composed of mesendodermal cells in embryos (Polo et al., 2012).

During the reprogramming of human somatic cells towards iPSCs, TRA-1-60^{+} intermediates exhibit transient expression of mesendodermal but not ectodermal genes (Takahashi et al., 2014). Additionally, a recent study has shown that ∼40% of 3771 human endogenous retroviruses type-H (HERV-Hs) transcripts, which are detected in both human pluripotent stem cells and mesendodermal cells, are transiently hyper-activated during reprogramming (Friedli et al., 2014; Lu et al., 2014; Ohnuki et al., 2014; Wang et al., 2014). Furthermore, human ESCs and iPSCs expressing aberrantly high levels of HERV-H transcripts show defective directed differentiation down the neural lineage (Koyanagi-Aoi et al., 2013; Ohnuki et al., 2014). A more recent study observed a transient signature reminiscent of pre-implantation stages marked by the expression of UTF1, DPPA3 and miR-371 during reprogramming even in human cells (Cacchiarelli et al., 2015). Taken together, these data suggest that both mouse and human intermediate reprogrammed cells go through mesendodermal and epiblast-like states. However, MEF-derived SSEA-1^{+} intermediates also transiently express several genes associated with the epidermis, a much more specialized tissue derived from the ectoderm, suggesting that reprogramming is not a simple reversal of the normal developmental process (O'Malley et al., 2013; Kim et al., 2015).

Such transient expression of developmental genes in intermediate reprogrammed cells is thought to be directly or indirectly regulated by reprogramming factors. Recently, the roles of each reprogramming factor during the re-acquisition of pluripotency have been investigated. During reprogramming, OCT3/4 enhances the expression of mesendodermal genes and inhibits that of ectodermal genes (Montserrat et al., 2013; Shu et al., 2013). SOX2 shows the opposite effect, as it correlates with an upregulation of ectodermal genes and a downregulation of mesendodermal genes (Montserrat et al., 2013; Shu et al., 2013).

In the native primitive streak of mouse embryos high levels of Oct3/4 and low levels of Sox2 were observed around E7.5 (Teo et al., 2011). In addition, in differentiating human ESCs OCT3/4 contributes to BMP-induced specification of a primitive streak-like state (Wang et al., 2012), whereas SOX2 – again showing an opposite effect – represses this process (Wang et al., 2012).

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Furthermore, conditional loss-of-function experiments revealed that Oct3/4 is required for normal primitive streak development in gastrulating mouse embryos (DeVeale et al., 2013). In the late phase of reprogramming, which proceeds independently of exogenous reprogramming factor expression, the expression of endogenous OCT3/4 reaches a level similar to that of ESCs and iPSCs. By contrast, the expression of endogenous SOX2, a late reprogramming marker in both mouse and human, slowly increases (Buganim et al., 2012; Takahashi et al., 2014). These different timings of expression create an OCT3/4^{high} SOX2^{low} transition state and probably result in a primitive streak-like state characterized by high mesendodermal and low ectodermal gene expression.

The activation of endogenous SOX2 is the final piece of the reconstitution of the pluripotency transcription factor network during reprogramming (Buganim et al., 2012). Furthermore, the high levels of OCT3/4 and SOX2 contribute to the maintenance of pluripotency not only in ESCs and iPSCs, but also in the ICM and the epiblast in blastocysts. In this way, reprogramming factors regulate lineage-specifying factors and induce the activation of other developmental genes during reprogramming. Taken together, these findings suggest that OCT3/4 and SOX2 regulate the expression of other developmental genes both during differentiation and reprogramming.

Intriguingly, OCT3/4 and SOX2 can be replaced with a cocktail of lineage specifiers, including GATA binding protein 3 (GATA3) and zinc finger protein 521 (ZFP521), to achieve cell reprogramming (Montserrat et al., 2013; Shu et al., 2013), suggesting that these non-pioneer factors can promote dedifferentiation in the early phase of reprogramming.

Altogether, this body of work shows that, during reprogramming, OSKM factors not only induce pluripotency genes but also regulate the expression of lineage specifiers required during development. This suggests that cell differentiation and reacquisition of pluripotency might proceed through similar genetic programs and that reprogramming factors are not devoted to the establishment and maintenance of pluripotency but also play a role in cell differentiation. Equally, lineage specifiers can contribute to the reprogramming process.

In and out of pluripotency: epigenetic regulation

The epigenetic marks acquired during lineage commitment constitute one of the barriers separating cells in Waddington's landscape (Waddington, 1957) (Fig. 1). Indeed, recent genome-wide studies have uncovered crucial differences between the epigenetic landscape of lineage-committed cells and that of pluripotent cells, suggesting that the exit from and the re-entry to the pluripotent state is under epigenetic control.

DNA methylation

DNA methylation is an important epigenetic mark that regulates gene expression and genome stability. In mammals, two *de novo* DNA methyltransferases (Dnmt), 3a and 3b, are conserved. Double knockout of these two genes disrupted *de novo* DNA methylation in ESCs and led to smaller embryos with abnormal morphology at E8.5 and E9.5 and death before E11.5 (Okano et al., 1999). However, Dnmt3a/3b are dispensable for mouse iPSC generation (Pawlak and Jaenisch, 2011), even if ESCs lacking Dnmt3a/3b and iPSCs derived from Dnmt3a/3b knockout MEFs exhibit poor differentiation phenotypes (Chen et al., 2003; Pawlak and Jaenisch, 2011). These data suggest that *de novo* DNA methylation is essential for cellular differentiation during normal embryonic development, but not for reprogramming.

DNA demethylation is mediated by the family of tet methylcytosine dioxygenase (Tet) enzymes (Tet1, 2 and 3) which catalyze the conversion of 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC), 5-hmC to 5-fC (5-formylcytosine), and 5-fC to 5-caC (5-carboxylcytosine). Tet1-deficient ESCs have reduced levels of 5-hmC and global gene expression changes (Dawlaty et al., 2011). Nevertheless, these mutant cells contributed to the development of live-born pups in tetraploid complementation experiments, suggesting that they were pluripotent. By contrast, knockdown of Tet1 caused the downregulation of Nanog expression and LIF/Stat3 activity, and impaired mouse ESCs self-renewal (Ito et al., 2010; Freudenberg et al., 2011). *Tet1* knockout mice are viable, fertile and grossly normal, though some have a slightly smaller body size at birth. *Tet1/2* double knockout mice are also viable and fertile, but show lower 5-hmC and higher 5-mC levels and aberrant methylation statuses at various imprinted loci (Dawlaty et al., 2013). Depletion of all Tet enzymes in ESCs caused loss of 5-hmC and impaired ESC differentiation (Dawlaty et al., 2014). These results demonstrate that Tet-mediated 5-hmC maintenance and DNA demethylation is crucial for the differentiation of ESCs and embryonic development.

Another component of the active DNA demethylation machinery, the DNA repair enzyme thymine DNA glycosylase (Tdg), is essential for normal mouse embryonic development (Cortázar et al., 2011; Cortellino et al., 2011). By using Tet1/2 or Tdg-deficient MEFs, Hu et al. showed that the Tet/Tdg axis is essential for mouse iPSC generation (Hu et al., 2014): in the absence of these enzymes, the expression of miR-200, crucial for MET, is not induced as a result of insufficient demethylation of its promoter. Forced expression of miR-200 restores efficient reprogramming in knockout MEFs. Importantly, after the blockage of the MET step is overcome by the reintroduction of miR-200, Tet1/2 and Tdg were found to be dispensable for the activation of pluripotency genes such as Oct3/4. Thus, the role of DNA methylation appears to differ between development and reprogramming but active DNA demethylation is required for both cell fate changes. Taken together, the roles of DNA methylation and demethylation during reprogramming share more common ground with pluripotent stem cell lines than with early embryogenesis.

X-chromosome activity

In female mammalian somatic cells, X-chromosome inactivation (XCI) ensures gene dosage compensation, which normalizes the expression of genes on the X chromosome to that observed in male cells. During mouse embryonic development, there are two waves of XCI: paternal XCI and random XCI. Shortly after fertilization, the paternal X chromosome is silenced. This inactivation continues in extra-embryonic tissues, but is erased in the ICM (Mak et al., 2004; Okamoto et al., 2004) (Fig. 4). Subsequently, random inactivation of either the maternal or paternal X-chromosome occurs. Therefore, all somatic cells derived from the ICM have a randomly inactivated X-chromosome (Xi). The non-coding X-inactivation specific transcript (Xist) plays an important role in triggering random chromosome-wide silencing (Brockdorff et al., 1991; Brown et al., 1991). Xist is transcribed only from the Xi in somatic cells, and its monoallelic expression is highly correlated with the onset of XCI in female development and female ESC differentiation in mouse (Penny et al., 1996; Marahrens et al., 1997).

The randomly inactivated X-chromosome in female somatic cells can be reactivated during transcription factor-mediated reprogramming (Maherali et al., 2007). A recent study dissected the kinetics of X-chromosome reactivation during the

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reprogramming of MEFs (Pasque et al., 2014). After the transduction of reprogramming factors into MEFs, the Xi colocalizes with EZH2, an enzyme of the Polycomb-group family that leads to chromatin condensation by the addition of three methyl groups to lysine 27 of histone 3 (H3K27me3). During the later phase of reprogramming, Xist is silenced and the localization of EZH2 to Xi is lost. At this point, late reprogramming markers such as Rex1 and Dppa4 start to be expressed, immediately followed by the biallelic expression of X-linked genes. Silencing of Xist is necessary but not sufficient for this process, which also requires DNA demethylation of the inactivated X-chromosome. The high persistence of DNA methylations that inactivate the X-chromosome during reprogramming in mouse might partly explain why a substantial time for X-chromosome reactivation is needed.

Interestingly, Tchieu et al. argued that reactivation of the X chromosome does not occur in human iPSCs (Tchieu et al., 2010). Conversely, Tomoda et al. demonstrated that X-chromosome reactivation can occur in human iPSCs by extending the time of post-reprogramming culture and using SNL feeder cells that overexpress LIF (Tomoda et al., 2012). Thus, the Xi found during embryogenesis can be gradually reactivated over time by cell reprogramming (Fig. 4).

Telomere elongation during development and reprogramming

Embryonic pluripotent cells initially have long telomeres, conserved repetitive TTAGGG sequences at each end of the chromatids that protect the chromosome from degradation and fusion with other chromosomes (O'Sullivan and Karlseder, 2010). These progressively shorten with each cell division and are associated with replicative senescence. Telomerase, a ribonucleoprotein polymerase, maintains the telomere ends, therefore playing a crucial role in cellular senescence. Its expression is normally silenced during differentiation, as a consequence of which telomeres progressively shorten with each cell division (Blasco, 2005) (Fig. 4). Furthermore, telomerase dysregulation in somatic cells can contribute to oncogenesis (Collins, 2006). Therefore, telomere shortening is associated with cellular senescence and constitutes a fundamental step in the developmental process.

Telomerase-dependent telomere elongation occurs after reprogramming of MEF-derived iPSCs and continues until a telomere length similar to that in ESCs is reached (Marion et al., 2009b) (Fig. 4). However, it was demonstrated that telomere elongation is dispensable for mouse iPSC generation (Tejera et al., 2010; Kinoshita et al., 2014). Interestingly, MEFs derived from third generation (G3) Telomerase RNA component (*Terc*) knockout mice showed significantly low reprogramming efficiency and massive apoptosis. Furthermore, this diminished efficiency could be rescued by reintroduction of telomerase or suppression of p53 (Trp53), suggesting that increased DNA damage induced by the short telomeres reduced iPSC generation efficiency (Marión et al., 2009a). Accordingly, reducing the DNA damage response significantly facilitated reprogramming toward iPSCs (Banito et al., 2009; Hong et al., 2009; Kawamura et al., 2009; Li et al., 2009; Marión et al., 2009a; Utikal et al., 2009). In *Terc* knockout mice, shortened telomeres exhibit a DNA damage response that is followed by chromosomal fusions, aneuploidy and apoptosis (Blasco et al., 1997; Rajaraman et al., 2007). Similarly to its role during development, telomerase activity contributes to cell maturation: iPSCs derived from G3 *Terc* knockout tail-tip fibroblasts (TTFs) had low differentiation potential and showed neither a contribution to chimeric mice nor teratoma formation activity (Le et al., 2014). These G3 $Terc^{-/-}$ iPSCs also exhibited senescence-associated phenotypes, including the elevation of p21CIP1 expression, mitochondrial dysfunction and telomere shortening. Furthermore, restoring mitochondria function by ectopic expression of PGC-1α (also known as PPARGC1A) increased their differentiation potential. Taken together, countering DNA damage by telomere elongation promotes early development and reprogramming.

Comparing iPSCs and ESCs

The above sections discuss some of the commonalities between reprogramming and embryonic development. In this section, we consider the debate on whether iPSCs and ESCs are equivalent cell types generated by different methods.

Dozens of reports have claimed that, despite the fact that they both share pluripotency, iPSCs differ from ESCs by their epigenetic relics of somatic origin, including DNA methylation and gene expression (Chin et al., 2009; Deng et al., 2009; Doi et al., 2009; Marchetto et al., 2009; Ghosh et al., 2010; Kim et al., 2011; Lister et al., 2011; Ohi et al., 2011). However, many others have demonstrated that there are no differences between ESCs and iPSCs, including their somatic epigenetic memory (Guenther et al., 2010; Newman and Cooper, 2010; Bock et al., 2011; Rouhani et al., 2014). Resolution of this argument is considered to be important for the study of developmental processes using these cell types as well as for their clinical application.

When iPSC colonies emerge, the reprogramming process is usually not complete. Thus, in early passages, iPSCs could show differences of gene expression and epigenetic status compared with ESCs that have been maintained for a longer period of time *in vitro*. Indeed, it has been demonstrated that continuous passaging of mouse iPSCs abrogates transcriptional, epigenetic and functional differences between iPSCs and ESCs, further approaching a homogeneous population (Polo et al., 2010). Additionally, tetraploid complementation of mouse iPSCs produced normal pups, suggesting that selected fully reprogrammed iPSCs have bona fide naïve pluripotency, indistinguishable from that of ESCs (Boland et al., 2009; Kang et al., 2009; Zhao et al., 2009). Thus, these data suggest that transcription factor-mediated reprogramming can achieve the full spectrum of pluripotency in mice.

Regarding the similarities in transcriptional output and epigenetic modifications between human iPSCs and ESCs, conflicting results have been reported. Indeed, studies that used 2-6 ESC lines and 2-12 iPSC lines found notable differences in gene expression and/or DNA methylation between ESCs and iPSCs (Chin et al., 2009; Deng et al., 2009; Doi et al., 2009; Marchetto et al., 2009; Ghosh et al., 2010; Kim et al., 2011; Lister et al., 2011; Ohi et al., 2011), whereas studies that used 20-36 ESC lines and 12-68 iPSC lines found ESCs and iPSCs to be fairly similar in terms of chromatin structure, gene expression and DNA methylation profiles (Guenther et al., 2010; Newman and Cooper, 2010; Bock et al., 2011). Therefore, the number of cell lines analyzed might influence the conclusions regarding whether ESCs and iPSCs are equivalent. However, it has also been demonstrated that in human cells, certain reprogramming events, including X-chromosome reactivation and re-suppression of transiently activated genes, were incomplete in iPSCs at the early passages (Tomoda et al., 2012; Ohnuki et al., 2014), indicating that the reprogramming process might take longer in human cells compared with murine cells. Thus, certain iPSC lines might be only partially re-programmed, and thus cannot be compared with ESCs. For example, Koyanagi-Aoi et al.

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performed comparison analyses of gene expression, miRNA expression and DNA methylation between ten ESC lines and 39 iPSC lines (Koyanagi-Aoi et al., 2013). Although they saw no clear differences between the two cell types, they did find that some iPSC lines exhibited distinct expression signatures that related to defects in differentiation. However, genes that were abundantly expressed in differentiation-defective iPSCs included many HERV-H-related genes, suggesting that these differentiation-defective iPSCs are partially reprogrammed cells. Therefore, we argue that mature iPSCs, which exclude incompletely reprogrammed clones, are probably indistinguishable from ESCs based on gene expression profiles and epigenetic status. However, because genetic background variations including single nucleotide polymorphisms affect PSC characteristics, a definitive comparison between cell lines derived from different sources is difficult.

Recently, human SCNT-ESCs were generated (Tachibana et al., 2013; Chung et al., 2014; Yamada et al., 2014) and a comparison of the gene expression profile, the epigenetic status and the genetic alterations was performed between isogenic human SCNT-ESCs and iPSCs derived from the same somatic cell cultures (Johannesson et al., 2014; Ma et al., 2014). Ma et al. concluded that iPSCs have inherent abnormalities because SCNT-ESCs are more similar to ESCs than iPSCs (Ma et al., 2014). Although imprinting loss occurred in both SCNT-ESC and iPSC lines at similar frequencies, only in iPSCs did some imprinted genes, such as *DIRAS3*, *MEG3* and *PEG3*, have aberrant DNA methylation status and defective XCI. By contrast, Johannesson et al. concluded that human SCNT-ESCs and iPSCs show no significant differences in gene expression, DNA methylation or frequency of *de novo* coding mutations (Johannesson et al., 2014).

Recent technologies have allowed the analysis of the expression profile of human pre-implantation embryos and ESCs at the single-cell level (Yan et al., 2013) and two recent papers have revealed significant differences in the global DNA methylation status of ESCs and blastocysts (Guo et al., 2014; Smith et al., 2014). These studies demonstrate that human ESCs in a dish are not identical to embryos in their natural milieu. Hence, a satisfying description of proper human pluripotent stem cells is needed before we can determine whether factor-induced reprogramming causes abnormalities.

Conclusions

In this Review, we have summarized common features observed between transcription factor-induced reprogramming of differentiated somatic cells toward iPSCs and normal embryonic development (Fig. 4). Although there are holistic differences between these opposite phenomena, including their kinetics, at the unicellular level and within a defined species, both processes involve similar mechanisms directing cell fate changes. Thus, we predict that a more detailed understanding of cell reprogramming will help us to understand human embryonic development.

In particular, understanding the mechanisms by which pluripotency is established and defining the naïve state of human pluripotency *in vitro* is a much sought after goal (Pera, 2014). In the past few years, several strategies to create human naïve pluripotency have been proposed (Chan et al., 2013; Gafni et al., 2013; Takashima et al., 2014; Theunissen et al., 2014; Valamehr et al., 2014; Ware et al., 2014) and naïve human ESCs, which exhibited global DNA hypomethylation except on imprinted gene loci (a feature similar to that of pre-implantation embryos), were recently obtained using the inhibitors of MEK, GSK3 and protein kinase C (PKC) (Takashima et al., 2014). These studies open the way to a deeper understanding of the naïve state of human pluripotency, which will bring a definitive end to the controversial comparison between murine and human cells and shed light on the mechanisms of reprogramming.

In this Review, we introduced the similarities and differences between transcription factor-induced reprogramming toward pluripotency and embryonic development. OSKM-mediated reprogramming requires reversing development, but not necessarily by simply going backwards along the differentiation process. Knowledge of developmental biology gives us hints as to the mechanisms of cell fate changes and determination that might also apply to the reprogramming process. However, if a novel approach for reprogramming of somatic cells is discovered, it might very well go through an entirely different route than the reversion of the developmental path (Fig. 1, broken arrow).

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Competing interests

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References

Ambros, V. (2011). MicroRNAs and developmental timing. *Curr. Opin. Genet. Dev.* **21**, 511-517.

Avilion, A. A., Nicolis, S. K., Pevny, L. H., Perez, L., Vivian, N. and Lovell-Badge, R. (2003). Multipotent cell lineages in early mouse development depend on SOX2 function. *Genes Dev.* **17**, 126-140.

Banito, A., Rashid, S. T., Acosta, J. C., Li, S., Pereira, C. F., Geti, I., Pinho, S., Silva, J. C., Azuara, V., Walsh, M. et al. (2009). Senescence impairs successful reprogramming to pluripotent stem cells. *Genes Dev.* **23**, 2134-2139.

Blasco, M. A. (2005). Telomeres and human disease: ageing, cancer and beyond. *Nat. Rev. Genet.* **6**, 611-622.

Blasco, M. A., Lee, H.-W., Hande, M. P., Samper, E., Lansdorp, P. M., DePinho, R. A. and Greider, C. W. (1997). Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. *Cell* **91**, 25-34.

Bock, C., Kiskinis, E., Verstappen, G., Gu, H., Boulting, G., Smith, Z. D., Ziller, M., Croft, G. F., Amoroso, M. W., Oakley, D. H. et al. (2011). Reference Maps of human ES and iPS cell variation enable high-throughput characterization of pluripotent cell lines. *Cell* **144**, 439-452.

Boland, M. J., Hazen, J. L., Nazor, K. L., Rodriguez, A. R., Gifford, W., Martin, G., Kupriyanov, S. and Baldwin, K. K. (2009). Adult mice generated from induced pluripotent stem cells. *Nature* **461**, 91-94.

Bradley, A., Evans, M., Kaufman, M. H. and Robertson, E. (1984). Formation of germ-line chimaeras from embryo-derived teratocarcinoma cell lines. *Nature* **309**, 255-256.

Brockdorff, N., Ashworth, A., Kay, G. F., Cooper, P., Smith, S., McCabe, V. M., Norris, D. P., Penny, G. D., Patel, D. and Rastan, S. (1991). Conservation of position and exclusive expression of mouse Xist from the inactive X chromosome. *Nature* **351**, 329-331.

Brons, I. G. M., Smithers, L. E., Trotter, M. W. B., Rugg-Gunn, P., Sun, B., Chuva de Sousa Lopes, S. M., Howlett, S. K., Clarkson, A., Ahrlund-Richter, L., Pedersen, R. A. et al. (2007). Derivation of pluripotent epiblast stem cells from mammalian embryos. *Nature* **448**, 191-195.

Brown, C. J., Ballabio, A., Rupert, J. L., Lafreniere, R. G., Grompe, M., Tonlorenzi, R. and Willard, H. F. (1991). A gene from the region of the human X inactivation centre is expressed exclusively from the inactive X chromosome. *Nature* **349**, 38-44.

Buehr, M., Meek, S., Blair, K., Yang, J., Ure, J., Silva, J., McLay, R., Hall, J., Ying, Q.-L. and Smith, A. (2008). Capture of authentic embryonic stem cells from rat blastocysts. *Cell* **135**, 1287-1298.

Buganim, Y., Faddah, D. A., Cheng, A. W., Itskovich, E., Markoulaki, S., Ganz, K., Klemm, S. L., van Oudenaarden, A. and Jaenisch, R. (2012). Single-cell expression analyses during cellular reprogramming reveal an early stochastic and a late hierarchic phase. *Cell* **150**, 1209-1222.

Cacchiarelli, D., Trapnell, C., Ziller, M. J., Soumillon, M., Cesana, M., Karnik, R., Donaghey, J., Smith, Z. D., Ratanasirintrawoot, S., Zhang, X. et al. (2015). Integrative analyses of human reprogramming reveal dynamic nature of induced pluripotency. *Cell* **162**, 412-424.

Chan, Y.-S., Göke, J., Ng, J.-H., Lu, X., Gonzales, K. A. U., Tan, C.-P., Tng, W.-Q., Hong, Z.-Z., Lim, Y.-S. and Ng, H.-H. (2013). Induction of a human pluripotent state with distinct regulatory circuitry that resembles preimplantation epiblast. *Cell Stem Cell* **13**, 663-675.

Chen, T., Ueda, Y., Dodge, J. E., Wang, Z. and Li, E. (2003). Establishment and maintenance of genomic methylation patterns in mouse embryonic stem cells by Dnmt3a and Dnmt3b. *Mol. Cell. Biol.* **23**, 5594-5605.

Chen, J., Liu, J., Yang, J., Chen, Y., Ni, S., Song, H., Zeng, L., Ding, K. and Pei, D. (2010). BMPs functionally replace Klf4 and support efficient reprogramming of mouse fibroblasts by Oct4 alone. *Cell Res.* **21**, 205-212.

Chen, J., Han, Q. and Pei, D. (2012). EMT and MET as paradigms for cell fate switching. *J. Mol. Cell Biol.* **4**, 66-69.

Chin, M. H., Mason, M. J., Xie, W., Volinia, S., Singer, M., Peterson, C., Ambartsumyan, G., Aimiuwu, O., Richter, L., Zhang, J. et al. (2009). Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. *Cell Stem Cell* **5**, 111-123.

Chou, Y.-F., Chen, H.-H., Eijpe, M., Yabuuchi, A., Chenoweth, J. G., Tesar, P., Lu, J., McKay, R. D. G. and Geijsen, N. (2008). The growth factor environment defines distinct pluripotent ground states in novel blastocyst-derived stem cells. *Cell* **135**, 449-461.

Chung, Y. G., Eum, J. H., Lee, J. E., Shim, S. H., Sepilian, V., Hong, S. W., Lee, Y., Treff, N. R., Choi, Y. H., Kimbrel, E. A. et al. (2014). Human somatic cell nuclear transfer using adult cells. *Cell Stem Cell* **14**, 777-780.

Collins, K. (2006). The biogenesis and regulation of telomerase holoenzymes. *Nat. Rev. Mol. Cell Biol.* **7**, 484-494.

Cortázar, D., Kunz, C., Selfridge, J., Lettieri, T., Saito, Y., MacDougall, E., Wirz, A., Schuermann, D., Jacobs, A. L., Siegrist, F. et al. (2011). Embryonic lethal phenotype reveals a function of TDG in maintaining epigenetic stability. *Nature* **470**, 419-423.

Cortellino, S., Xu, J., Sannai, M., Moore, R., Caretti, E., Cigliano, A., Le Coz, M., Devarajan, K., Wessels, A., Soprano, D. et al. (2011). Thymine DNA glycosylase is essential for active DNA demethylation by linked deamination-base excision repair. *Cell* **146**, 67-79.

Dawlaty, M. M., Ganz, K., Powell, B. E., Hu, Y.-C., Markoulaki, S., Cheng, A. W., Gao, Q., Kim, J., Choi, S.-W., Page, D. C. et al. (2011). Tet1 is dispensable for maintaining pluripotency and its loss is compatible with embryonic and postnatal development. *Cell Stem Cell* **9**, 166-175.

Dawlaty, M. M., Breiling, A., Le, T., Raddatz, G., Barrasa, M. I., Cheng, A. W., Gao, Q., Powell, B. E., Li, Z., Xu, M. et al. (2013). Combined deficiency of Tet1 and Tet2 causes epigenetic abnormalities but is compatible with postnatal development. *Dev. Cell* **24**, 310-323.

Dawlaty, M. M., Breiling, A., Le, T., Barrasa, M. I., Raddatz, G., Gao, Q., Powell, B. E., Cheng, A. W., Faull, K. F., Lyko, F. et al. (2014). Loss of Tet enzymes compromises proper differentiation of embryonic stem cells. *Dev. Cell* **29**, 102-111.

Deng, J., Shoemaker, R., Xie, B., Gore, A., LeProust, E. M., Antosiewicz-Bourget, J., Egli, D., Maherali, N., Park, I.-H., Yu, J. et al. (2009). Targeted bisulfite sequencing reveals changes in DNA methylation associated with nuclear reprogramming. *Nat. Biotechnol.* **27**, 353-360.

DeVeale, B., Brokhman, I., Mohseni, P., Babak, T., Yoon, C., Lin, A., Onishi, K., Tomilin, A., Pevny, L., Zandstra, P. W. et al. (2013). Oct4 is required ~E7.5 for proliferation in the primitive streak. *PLoS Genet.* **9**, e1003957.

Doi, A., Park, I.-H., Wen, B., Murakami, P., Aryee, M. J., Irizarry, R., Herb, B., Ladd-Acosta, C., Rho, J., Loewer, S. et al. (2009). Differential methylation of tissue- and cancer-specific CpG island shores distinguishes human induced pluripotent stem cells, embryonic stem cells and fibroblasts. *Nat. Genet.* **41**, 1350-1353.

Egli, D., Chen, A. E., Saphier, G., Ichida, J., Fitzgerald, C., Go, K. J., Acevedo, N., Patel, J., Baetscher, M., Kearns, W. G. et al. (2011). Reprogramming within hours following nuclear transfer into mouse but not human zygotes. *Nat. Commun.* **2**, 488.

Evans, M. J. and Kaufman, M. H. (1981). Establishment in culture of pluripotential cells from mouse embryos. *Nature* **292**, 154-156.

Freudenberg, J. M., Ghosh, S., Lackford, B. L., Yellaboina, S., Zheng, X., Li, R., Cuddapah, S., Wade, P. A., Hu, G. and Jothi, R. (2011). Acute depletion of Tet1-dependent 5-hydroxymethylcytosine levels impairs LIF/Stat3 signaling and results in loss of embryonic stem cell identity. *Nucleic Acids Res.* **40**, 3364-3377.

Friedli, M., Turelli, P., Kapopoulou, A., Rauwel, B., Castro-Diaz, N., Rowe, H. M., Ecco, G., Unzu, C., Planet, E., Lombardo, A. et al. (2014). Loss of transcriptional control over endogenous retroelements during reprogramming to pluripotency. *Genome Res.* **24**, 1251-1259.

Gafni, O., Weinberger, L., Mansour, A. A., Manor, Y. S., Chomsky, E., Ben-Yosef, D., Kalma, Y., Viukov, S., Maza, I., Zviran, A. et al. (2013). Derivation of novel human ground state naive pluripotent stem cells. *Nature* **504**, 282-286.

Ghosh, Z., Wilson, K. D., Wu, Y., Hu, S., Quertermous, T. and Wu, J. C. (2010). Persistent donor cell gene expression among human induced pluripotent stem cells contributes to differences with human embryonic stem cells. *PLoS ONE* **5**, e8975.

Guenther, M. G., Frampton, G. M., Soldner, F., Hockemeyer, D., Mitalipova, M., Jaenisch, R. and Young, R. A. (2010). Chromatin structure and gene expression programs of human embryonic and induced pluripotent stem cells. *Cell Stem Cell* **7**, 249-257.

Guo, G., Yang, J., Nichols, J., Hall, J. S., Eyres, I., Mansfield, W. and Smith, A. (2009). Klf4 reverts developmentally programmed restriction of ground state pluripotency. *Development* **136**, 1063-1069.

Guo, H., Zhu, P., Yan, L., Li, R., Hu, B., Lian, Y., Yan, J., Ren, X., Lin, S., Li, J. et al. (2014). The DNA methylation landscape of human early embryos. *Nature* **511**, 606-610.

Hanna, J., Markoulaki, S., Mitalipova, M., Cheng, A. W., Cassady, J. P., Staerk, J., Carey, B. W., Lengner, C. J., Foreman, R., Love, J. et al. (2009). Metastable pluripotent states in NOD-mouse-derived ESCs. *Cell Stem Cell* **4**, 513-524.

Heo, I., Joo, C., Cho, J., Ha, M., Han, J. and Kim, V. N. (2008). Lin28 mediates the terminal uridylation of let-7 precursor MicroRNA. *Mol. Cell* **32**, 276-284.

Heo, I., Joo, C., Kim, Y.-K., Ha, M., Yoon, M.-J., Cho, J., Yeom, K.-H., Han, J. and Kim, V. N. (2009). TUT4 in concert with Lin28 suppresses microRNA biogenesis through pre-microRNA uridylation. *Cell* **138**, 696-708.

Hochedlinger, K. and Plath, K. (2009). Epigenetic reprogramming and induced pluripotency. *Development* **136**, 509-523.

Hong, H., Takahashi, K., Ichisaka, T., Aoi, T., Kanagawa, O., Nakagawa, M., Okita, K. and Yamanaka, S. (2009). Suppression of induced pluripotent stem cell generation by the p53–p21 pathway. *Nature* **460**, 1132-1135.

Hou, P., Li, Y., Zhang, X., Liu, C., Guan, J., Li, H., Zhao, T., Ye, J., Yang, W., Liu, K. et al. (2013). Pluripotent stem cells induced from mouse somatic cells by small-molecule compounds. *Science* **341**, 651-654.

Hu, X., Zhang, L., Mao, S.-Q., Li, Z., Chen, J., Zhang, R.-R., Wu, H.-P., Gao, J., Guo, F., Liu, W. et al. (2014). Tet and TDG mediate DNA demethylation essential for mesenchymal-to-epithelial transition in somatic cell reprogramming. *Cell Stem Cell* **14**, 512-522.

Huang, P., He, Z., Ji, S., Sun, H., Xiang, D., Liu, C., Hu, Y., Wang, X. and Hui, L. (2011). Induction of functional hepatocyte-like cells from mouse fibroblasts by defined factors. *Nature* **475**, 386-389.

Ichida, J. K., Blanchard, J., Lam, K., Son, E. Y., Chung, J. E., Egli, D., Loh, K. M., Carter, A. C., Di Giorgio, F. P., Koszka, K. et al. (2009). A small-molecule inhibitor of tgf-Beta signaling replaces sox2 in reprogramming by inducing nanog. *Cell Stem Cell* **5**, 491-503.

Ieda, M., Fu, J.-D., Delgado-Olguin, P., Vedantham, V., Hayashi, Y., Bruneau, B. G. and Srivastava, D. (2010). Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. *Cell* **142**, 375-386.

Ito, S., D'Alessio, A. C., Taranova, O. V., Hong, K., Sowers, L. C. and Zhang, Y. (2010). Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification. *Nature* **466**, 1129-1133.

Jiang, J., Chan, Y.-S., Loh, Y.-H., Cai, J., Tong, G.-Q., Lim, C.-A., Robson, P., Zhong, S. and Ng, H.-H. (2008). A core Klf circuitry regulates self-renewal of embryonic stem cells. *Nat. Cell Biol.* **10**, 353-360.

Johannesson, B., Sagi, I., Gore, A., Paull, D., Yamada, M., Golan-Lev, T., Li, Z., LeDuc, C., Shen, Y., Stern, S. et al. (2014). Comparable frequencies of coding mutations and loss of imprinting in human pluripotent cells derived by nuclear transfer and defined factors. *Cell Stem Cell* **15**, 634-642.

Kanellopoulou, C., Muljo, S. A., Kung, A. L., Ganesan, S., Drapkin, R., Jenuwein, T., Livingston, D. M. and Rajewsky, K. (2005). Dicer-deficient mouse embryonic stem cells are defective in differentiation and centromeric silencing. *Genes Dev.* **19**, 489-501.

Kang, L., Wang, J., Zhang, Y., Kou, Z. and Gao, S. (2009). iPS cells can support full-term development of tetraploid blastocyst-complemented embryos. *Cell Stem Cell* **5**, 135-138.

Kawamura, T., Suzuki, J., Wang, Y. V., Menendez, S., Morera, L. B., Raya, A., Wahl, G. M. and Belmonte, J. C. I. (2009). Linking the p53 tumour suppressor pathway to somatic cell reprogramming. *Nature* **460**, 1140-1144.

Kawase, E., Suemori, H., Takahashi, N., Okazaki, K., Hashimoto, K. and Nakatsuji, N. (1994). Strain difference in establishment of mouse embryonic stem (ES) cell lines. *Int. J. Dev. Biol.* **38**, 385-390.

Kim, K., Zhao, R., Doi, A., Ng, K., Unternaehrer, J., Cahan, P., Hongguang, H., Loh, Y.-H., Aryee, M. J., Lensch, M. W. et al. (2011). Donor cell type can influence the epigenome and differentiation potential of human induced pluripotent stem cells. *Nat. Biotechnol.* **29**, 1117-1119.

Kim, B.-M., Thier, M.-C., Oh, S., Sherwood, R., Kanellopoulou, C., Edenhofer, F. and Choi, M. Y. (2012). MicroRNAs are indispensable for reprogramming mouse embryonic fibroblasts into induced stem cell-like cells. *PLoS ONE* **7**, e39239.

Kim, S.-I., Oceguera-Yanez, F., Hirohata, R., Linker, S., Okita, K., Yamada, Y., Yamamoto, T., Yamanaka, S. and Woltjen, K. (2015). KLF4 N-terminal variance modulates induced reprogramming to pluripotency. *Stem Cell Rep.* **4**, 727-743.

Kinoshita, T., Nagamatsu, G., Saito, S., Takubo, K., Horimoto, K. and Suda, T. (2014). Telomerase reverse transcriptase has an extratelomeric function in somatic cell reprogramming. *J. Biol. Chem.* **289**, 15776-15787.
Koyanagi-Aoi, M., Ohnuki, M., Takahashi, K., Okita, K., Noma, H., Sawamura, Y., Teramoto, I., Narita, M., Sato, Y., Ichisaka, T. et al. (2013). Differentiation-defective phenotypes revealed by large-scale analyses of human pluripotent stem cells. *Proc. Natl. Acad. Sci. USA* **110**, 20569-20574.
Le, R., Kou, Z., Jiang, Y., Li, M., Huang, B., Liu, W., Li, H., Kou, X., He, W., Rudolph, K. L. et al. (2014). Enhanced telomere rejuvenation in pluripotent cells reprogrammed via nuclear transfer relative to induced pluripotent stem cells. *Cell Stem Cell* **14**, 27-39.
Li, P., Tong, C., Mehrian-Shai, R., Jia, L., Wu, N., Yan, Y., Maxson, R. E., Schulze, E. N., Song, H., Hsieh, C.-L. et al. (2008). Germline competent embryonic stem cells derived from rat blastocysts. *Cell* **135**, 1299-1310.
Li, H., Collado, M., Villasante, A., Strati, K., Ortega, S., Cañamero, M., Blasco, M. A. and Serrano, M. (2009). The Ink4/Arf locus is a barrier for iPS cell reprogramming. *Nature* **460**, 1136-1139.
Li, R., Liang, J., Ni, S., Zhou, T., Qing, X., Li, H., He, W., Chen, J., Li, F., Zhuang, Q. et al. (2010). A mesenchymal-to-epithelial transition initiates and is required for the nuclear reprogramming of mouse fibroblasts. *Cell Stem Cell* **7**, 51-63.
Lister, R., Pelizzola, M., Kida, Y. S., Hawkins, R. D., Nery, J. R., Hon, G., Antosiewicz-Bourget, J., O'Malley, R., Castanon, R., Klugman, S. et al. (2011). Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. *Nature* **471**, 68-73.
Lu, X., Sachs, F., Ramsay, L., Jacques, P.-É., Göke, J., Bourque, G. and Ng, H.-H. (2014). The retrovirus HERVH is a long noncoding RNA required for human embryonic stem cell identity. *Nat. Struct. Mol. Biol.* **21**, 423-425.
Ma, H., Morey, R., O'Neil, R. C., He, Y., Daughtry, B., Schultz, M. D., Hariharan, M., Nery, J. R., Castanon, R., Sabatini, K. et al. (2014). Abnormalities in human pluripotent cells due to reprogramming mechanisms. *Nature* **511**, 177-183.
Maherali, N. and Hochedlinger, K. (2009). Tgfbeta signal inhibition cooperates in the induction of iPSCs and replaces Sox2 and cMyc. *Curr. Biol.* **19**, 1718-1723.
Maherali, N., Sridharan, R., Xie, W., Utikal, J., Eminli, S., Arnold, K., Stadtfeld, M., Yachechko, R., Tchieu, J., Jaenisch, R. et al. (2007). Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. *Cell Stem Cell* **1**, 55-70.
Mak, W., Nesterova, T. B., de Napoles, M., Appanah, R., Yamanaka, S., Otte, A. P. and Brockdorff, N. (2004). Reactivation of the paternal X chromosome in early mouse embryos. *Science* **303**, 666-669.
Maller Schulman, B. R., Liang, X., Stahlhut, C., DelConte, C., Stefani, G. and Slack, F. J. (2008). The let-7 microRNA target gene, Mlin41/Trim71 is required for mouse embryonic survival and neural tube closure. *Cell Cycle* **7**, 3935-3942.
Marahrens, Y., Panning, B., Dausman, J., Strauss, W. and Jaenisch, R. (1997). Xist-deficient mice are defective in dosage compensation but not spermatogenesis. *Genes Dev.* **11**, 156-166.
Marcellini, S., Technau, U., Smith, J. C. and Lemaire, P. (2003). Evolution of Brachyury proteins: identification of a novel regulatory domain conserved within Bilateria. *Dev. Biol.* **260**, 352-361.
Marchetto, M. C. N., Yeo, G. W., Kainohana, O., Marsala, M., Gage, F. H. and Muotri, A. R. (2009). Transcriptional signature and memory retention of human-induced pluripotent stem cells. *PLoS ONE* **4**, e7076.
Marión, R. M., Strati, K., Li, H., Murga, M., Blanco, R., Ortega, S., Fernandez-Capetillo, O., Serrano, M. and Blasco, M. A. (2009a). A p53-mediated DNA damage response limits reprogramming to ensure iPS cell genomic integrity. *Nature* **460**, 1149-1153.
Marion, R. M., Strati, K., Li, H., Tejera, A., Schoeftner, S., Ortega, S., Serrano, M. and Blasco, M. A. (2009b). Telomeres acquire embryonic stem cell characteristics in induced pluripotent stem cells. *Cell Stem Cell* **4**, 141-154.
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.
Medeiros, L. A., Dennis, L. M., Gill, M. E., Houbaviy, H., Markoulaki, S., Fu, D., White, A. C., Kirak, O., Sharp, P. A., Page, D. C. et al. (2011). Mir-290-295 deficiency in mice results in partially penetrant embryonic lethality and germ cell defects. *Proc. Natl. Acad. Sci. USA* **108**, 14163-14168.
Melton, C., Judson, R. L. and Blelloch, R. (2010). Opposing microRNA families regulate self-renewal in mouse embryonic stem cells. *Nature* **463**, 621-626.
Mitschka, S., Ulas, T., Goller, T., Schneider, K., Egert, A., Mertens, J., Brüstle, O., Schorle, H., Beyer, M., Klee, K. et al. (2015). Co-existence of intact stemness and priming of neural differentiation programs in mES cells lacking Trim71. *Sci. Rep.* **5**, 11126.
Montserrat, N., Nivet, E., Sancho-Martinez, I., Hishida, T., Kumar, S., Miquel, L., Cortina, C., Hishida, Y., Xia, Y., Esteban, C. R. et al. (2013). Reprogramming of human fibroblasts to pluripotency with lineage specifiers. *Cell Stem Cell* **13**, 341-350.
Newman, A. M. and Cooper, J. B. (2010). Lab-specific gene expression signatures in pluripotent stem cells. *Cell Stem Cell* **7**, 258-262.
Nichols, J. and Smith, A. (2009). Naive and primed pluripotent states. *Cell Stem Cell* **4**, 487-492.
Nichols, J. and Smith, A. (2012). Pluripotency in the embryo and in culture. *Cold Spring Harb. Perspect. Biol.* **4**, a008128.
Nichols, J., Zevnik, B., Anastassiadis, K., Niwa, H., Klewe-Nebenius, D., Chambers, I., Schöler, H. and Smith, A. (1998). Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. *Cell* **95**, 379-391.
Nichols, J., Jones, K., Phillips, J. M., Newland, S. A., Roode, M., Mansfield, W., Smith, A. and Cooke, A. (2009). Validated germline-competent embryonic stem cell lines from nonobese diabetic mice. *Nat. Med.* **15**, 814-818.
Niwa, H., Ogawa, K., Shimosato, D. and Adachi, K. (2009). A parallel circuit of LIF signalling pathways maintains pluripotency of mouse ES cells. *Nature* **460**, 118-122.
Ohi, Y., Qin, H., Hong, C., Blouin, L., Polo, J. M., Guo, T., Qi, Z., Downey, S. L., Manos, P. D., Rossi, D. J. et al. (2011). Incomplete DNA methylation underlies a transcriptional memory of somatic cells in human iPS cells. *Nat. Cell Biol.* **13**, 541-549.
Ohnuki, M., Tanabe, K., Sutou, K., Teramoto, I., Sawamura, Y., Narita, M., Nakamura, M., Tokunaga, Y., Watanabe, A., Yamanaka, S. et al. (2014). Dynamic regulation of human endogenous retroviruses mediates factor-induced reprogramming and differentiation potential. *Proc. Natl. Acad. Sci. USA* **111**, 12426-12431.
Ohtsuka, S. and Niwa, H. (2015). The differential activation of intracellular signaling pathways confers the permissiveness of embryonic stem cell derivation from different mouse strains. *Development* **142**, 431-437.
Ohtsuka, S., Nishikawa-Torikai, S. and Niwa, H. (2012). E-cadherin promotes incorporation of mouse epiblast stem cells into normal development. *PLoS ONE* **7**, e45220.
Okamoto, I., Otte, A. P., Allis, C. D., Reinberg, D. and Heard, E. (2004). Epigenetic dynamics of imprinted X inactivation during early mouse development. *Science* **303**, 644-649.
Okano, M., Bell, D. W., Haber, D. A. and Li, E. (1999). DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell* **99**, 247-257.
Okita, K., Ichisaka, T. and Yamanaka, S. (2007). Generation of germline-competent induced pluripotent stem cells. *Nature* **448**, 313-317.
O'Malley, J., Skylaki, S., Iwabuchi, K. A., Chantzoura, E., Ruetz, T., Johnsson, A., Tomlinson, S. R., Linnarsson, S. and Kaji, K. (2013). High-resolution analysis with novel cell-surface markers identifies routes to iPS cells. *Nature* **499**, 88-91.
O'Sullivan, R. J. and Karlseder, J. (2010). Telomeres: protecting chromosomes against genome instability. *Nat. Rev. Mol. Cell Biol.* **11**, 171-181.
Papapetrou, E. P., Tomishima, M. J., Chambers, S. M., Mica, Y., Reed, E., Menon, J., Tabar, V., Mo, Q., Studer, L. and Sadelain, M. (2009). Stoichiometric and temporal requirements of Oct4, Sox2, Klf4, and c-Myc expression for efficient human iPSC induction and differentiation. *Proc. Natl. Acad. Sci. USA* **106**, 12759-12764.
Parchem, R. J., Ye, J., Judson, R. L., LaRussa, M. F., Krishnakumar, R., Blelloch, A., Oldham, M. C. and Blelloch, R. (2014). Two miRNA clusters reveal alternative paths in late-stage reprogramming. *Cell Stem Cell* **14**, 617-631.
Park, I.-H., Zhao, R., West, J. A., Yabuuchi, A., Huo, H., Ince, T. A., Lerou, P. H., Lensch, M. W. and Daley, G. Q. (2008). Reprogramming of human somatic cells to pluripotency with defined factors. *Nature* **451**, 141-146.
Pasque, V., Tchieu, J., Karnik, R., Uyeda, M., Sadhu Dimashkie, A., Case, D., Papp, B., Bonora, G., Patel, S., Ho, R. et al. (2014). X chromosome reactivation dynamics reveal stages of reprogramming to pluripotency. *Cell* **159**, 1681-1697.
Pawlak, M. and Jaenisch, R. (2011). De novo DNA methylation by Dnmt3a and Dnmt3b is dispensable for nuclear reprogramming of somatic cells to a pluripotent state. *Genes Dev.* **25**, 1035-1040.
Payne, G. S., Bishop, J. M. and Varmus, H. E. (1982). Multiple arrangements of viral DNA and an activated host oncogene in bursal lymphomas. *Nature* **295**, 209-214.
Penny, G. D., Kay, G. F., Sheardown, S. A., Rastan, S. and Brockdorff, N. (1996). Requirement for Xist in X chromosome inactivation. *Nature* **379**, 131-137.
Pera, M. F. (2014). In search of naivety. *Cell Stem Cell* **15**, 543-545.
Polo, J. M., Liu, S., Figueroa, M. E., Kulalert, W., Eminli, S., Tan, K. Y., Apostolou, E., Stadtfeld, M., Li, Y., Shioda, T. et al. (2010). Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. *Nat. Biotechnol.* **28**, 848-855.
Polo, J. M., Anderssen, E., Walsh, R. M., Schwarz, B. A., Nefzger, C. M., Lim, S. M., Borkent, M., Apostolou, E., Alaei, S., Cloutier, J. et al. (2012). A molecular roadmap of reprogramming somatic cells into iPS cells. *Cell* **151**, 1617-1632.
Rajaraman, S., Choi, J., Cheung, P., Beaudry, V., Moore, H. and Artandi, S. E. (2007). Telomere uncapping in progenitor cells with critical telomere shortening is coupled to S-phase progression in vivo. *Proc. Natl. Acad. Sci. USA* **104**, 17747-17752.
Rouhani, F., Kumasaka, N., de Brito, M. C., Bradley, A., Vallier, L. and Gaffney, D. (2014). Genetic background drives transcriptional variation in human induced pluripotent stem cells. *PLoS Genet.* **10**, e1004432.

Rybak, A., Fuchs, H., Hadian, K., Smirnova, L., Wulczyn, E. A., Michel, G., Nitsch, R., Krappmann, D. and Wulczyn, F. G. (2009). The let-7 target gene mouse lin-41 is a stem cell specific E3 ubiquitin ligase for the miRNA pathway protein Ago2. *Nat. Cell Biol.* **11**, 1411-1420.

Samavarchi-Tehrani, P., Golipour, A., David, L., Sung, H.-k., Beyer, T. A., Datti, A., Woltjen, K., Nagy, A. and Wrana, J. L. (2010). Functional genomics reveals a BMP-driven mesenchymal-to-epithelial transition in the initiation of somatic cell reprogramming. *Cell Stem Cell* **7**, 64-77.

Schulman, B. R. M., Esquela-Kerscher, A. and Slack, F. J. (2005). Reciprocal expression of lin-41 and the microRNAs let-7 and mir-125 during mouse embryogenesis. *Dev. Dyn.* **234**, 1046-1054.

Sekiya, S. and Suzuki, A. (2011). Direct conversion of mouse fibroblasts to hepatocyte-like cells by defined factors. *Nature* **475**, 390-393.

Shu, J., Wu, C., Wu, Y., Li, Z., Shao, S., Zhao, W., Tang, X., Yang, H., Shen, L., Zuo, X. et al. (2013). Induction of pluripotency in mouse somatic cells with lineage specifiers. *Cell* **153**, 963-975.

Smith, Z. D., Chan, M. M., Humm, K. C., Karnik, R., Mekhoubad, S., Regev, A., Eggan, K. and Meissner, A. (2014). DNA methylation dynamics of the human preimplantation embryo. *Nature* **511**, 611-615.

Soufi, A., Donahue, G. and Zaret, K. S. (2012). Facilitators and impediments of the pluripotency reprogramming factors' initial engagement with the genome. *Cell* **151**, 994-1004.

Subramanyam, D., Lamouille, S., Judson, R. L., Liu, J. Y., Bucay, N., Derynck, R. and Blelloch, R. (2011). Multiple targets of miR-302 and miR-372 promote reprogramming of human fibroblasts to induced pluripotent stem cells. *Nat. Biotechnol.* **29**, 443-448.

Tachibana, M., Sparman, M., Ramsey, C., Ma, H., Lee, H.-S., Penedo, M. C. and Mitalipov, S. (2012). Generation of chimeric rhesus monkeys. *Cell* **148**, 285-295.

Tachibana, M., Amato, P., Sparman, M., Gutierrez, N. M., Tippner-Hedges, R., Ma, H., Kang, E., Fulati, A., Lee, H. S., Sritanaudomchai, H. et al. (2013). Human embryonic stem cells derived by somatic cell nuclear transfer. *Cell* **153**, 1228-1238.

Tada, M., Takahama, Y., Abe, K., Nakatsuji, N. and Tada, T. (2001). Nuclear reprogramming of somatic cells by in vitro hybridization with ES cells. *Curr. Biol.* **11**, 1553-1558.

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.

Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Sasaki, A., Yamamoto, M., Nakamura, M., Sutou, K., Osafune, K. and Yamanaka, S. (2014). Induction of pluripotency in human somatic cells via a transient state resembling primitive streak-like mesendoderm. *Nat. Commun.* **5**, 3678.

Takashima, Y., Guo, G., Loos, R., Nichols, J., Ficz, G., Krueger, F., Oxley, D., Santos, F., Clarke, J., Mansfield, W. et al. (2014). Resetting transcription factor control circuitry toward ground-state pluripotency in human. *Cell* **158**, 1254-1269.

Tam, P. P. L. and Loebel, D. A. F. (2007). Gene function in mouse embryogenesis: get set for gastrulation. *Nat. Rev. Genet.* **8**, 368-381.

Tanabe, K., Nakamura, M., Narita, M., Takahashi, K. and Yamanaka, S. (2013). Maturation, not initiation, is the major roadblock during reprogramming toward pluripotency from human fibroblasts. *Proc. Natl. Acad. Sci. USA* **110**, 12172-12179.

Tchieu, J., Kuoy, E., Chin, M. H., Trinh, H., Patterson, M., Sherman, S. P., Aimiuwu, O., Lindgren, A., Hakimian, S., Zack, J. A. et al. (2010). Female human iPSCs retain an inactive X chromosome. *Cell Stem Cell* **7**, 329-342.

Tejera, A. M., Stagno d'Alcontres, M., Thanasoula, M., Marion, R. M., Martinez, P., Liao, C., Flores, J. M., Tarsounas, M. and Blasco, M. A. (2010). TPP1 is required for TERT recruitment, telomere elongation during nuclear reprogramming, and normal skin development in mice. *Dev. Cell* **18**, 775-789.

Teo, A. K. K., Arnold, S. J., Trotter, M. W. B., Brown, S., Ang, L. T., Chng, Z., Robertson, E. J., Dunn, N. R. and Vallier, L. (2011). Pluripotency factors regulate definitive endoderm specification through eomesodermin. *Genes Dev.* **25**, 238-250.

Tesar, P. J., Chenoweth, J. G., Brook, F. A., Davies, T. J., Evans, E. P., Mack, D. L., Gardner, R. L. and McKay, R. D. G. (2007). New cell lines from mouse epiblast share defining features with human embryonic stem cells. *Nature* **448**, 196-199.

Theunissen, T. W., Powell, B. E., Wang, H., Mitalipova, M., Faddah, D. A., Reddy, J., Fan, Z. P., Maetzel, D., Ganz, K., Shi, L. et al. (2014). Systematic identification of culture conditions for induction and maintenance of naive human pluripotency. *Cell Stem Cell* **15**, 471-487.

Thiery, J. P., Acloque, H., Huang, R. Y. J. and Nieto, M. A. (2009). Epithelial-mesenchymal transitions in development and disease. *Cell* **139**, 871-890.

Thomson, J. A., Kalishman, J., Golos, T. G., Durning, M., Harris, C. P., Becker, R. A. and Hearn, J. P. (1995). Isolation of a primate embryonic stem cell line. *Proc. Natl. Acad. Sci. USA* **92**, 7844-7848.

Tian, Y., Zhang, Y., Hurd, L., Hannenhalli, S., Liu, F., Lu, M. M. and Morrisey, E. E. (2011). Regulation of lung endoderm progenitor cell behavior by miR302/367. *Development* **138**, 1235-1245.

Tiemann, U., Sgodda, M., Warlich, E., Ballmaier, M., Schöler, H. R., Schambach, A. and Cantz, T. (2011). Optimal reprogramming factor stoichiometry increases colony numbers and affects molecular characteristics of murine induced pluripotent stem cells. *Cytometry A* **79A**, 426-435.

Tomoda, K., Takahashi, K., Leung, K., Okada, A., Narita, M., Yamada, N. A., Eilertson, K. E., Tsang, P., Baba, S., White, M. P. et al. (2012). Derivation conditions impact x-inactivation status in female human induced pluripotent stem cells. *Cell Stem Cell* **11**, 91-99.

Utikal, J., Polo, J. M., Stadtfeld, M., Maherali, N., Kulalert, W., Walsh, R. M., Khalil, A., Rheinwald, J. G. and Hochedlinger, K. (2009). Immortalization eliminates a roadblock during cellular reprogramming into iPS cells. *Nature* **460**, 1145-1148.

Valamehr, B., Robinson, M., Abujarour, R., Rezner, B., Vranceanu, F., Le, T., Medcalf, A., Lee, T. T., Fitch, M., Robbins, D. et al. (2014). Platform for induction and maintenance of transgene-free hiPSCs resembling ground state pluripotent stem cells. *Stem Cell Rep.* **2**, 366-381.

Vierbuchen, T., Ostermeier, A., Pang, Z. P., Kokubu, Y., Südhof, T. C. and Wernig, M. (2010). Direct conversion of fibroblasts to functional neurons by defined factors. *Nature* **463**, 1035-1041.

Viswanathan, S. R. and Daley, G. Q. (2010). Lin28: A microRNA regulator with a macro role. *Cell* **140**, 445-449.

Viswanathan, S. R., Daley, G. Q. and Gregory, R. I. (2008). Selective blockade of microRNA processing by Lin28. *Science* **320**, 97-100.

Waddington, C. H. (1957). *The Strategy of the Genes. A Discussion of Some Aspects of Theoretical Biology*. London: George Allen & Unwin.

Wakayama, T., Tabar, V., Rodriguez, I., Perry, A. C. F., Studer, L. and Mombaerts, P. (2001). Differentiation of embryonic stem cell lines generated from adult somatic cells by nuclear transfer. *Science* **292**, 740-743.

Wang, Y., Baskerville, S., Shenoy, A., Babiarz, J. E., Baehner, L. and Blelloch, R. (2008). Embryonic stem cell–specific microRNAs regulate the G1-S transition and promote rapid proliferation. *Nat. Genet.* **40**, 1478-1483.

Wang, Z., Oron, E., Nelson, B., Razis, S. and Ivanova, N. (2012). Distinct lineage specification roles for NANOG, OCT4, and SOX2 in human embryonic stem cells. *Cell Stem Cell* **10**, 440-454.

Wang, J., Xie, G., Singh, M., Ghanbarian, A. T., Raskó, T., Szvetnik, A., Cai, H., Besser, D., Prigione, A., Fuchs, N. V. et al. (2014). Primate-specific endogenous retrovirus-driven transcription defines naive-like stem cells. *Nature* **516**, 405-409.

Ware, C. B., Nelson, A. M., Mecham, B., Hesson, J., Zhou, W., Jonlin, E. C., Jimenez-Caliani, A. J., Deng, X., Cavanaugh, C., Cook, S. et al. (2014). Derivation of naive human embryonic stem cells. *Proc. Natl. Acad. Sci. USA* **111**, 4484-4489.

Wernig, M., Meissner, A., Foreman, R., Brambrink, T., Ku, M., Hochedlinger, K., Bernstein, B. E. and Jaenisch, R. (2007). In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature* **448**, 318-324.

Wernig, M., Lengner, C. J., Hanna, J., Lodato, M. A., Steine, E., Foreman, R., Staerk, J., Markoulaki, S. and Jaenisch, R. (2008). A drug-inducible transgenic system for direct reprogramming of multiple somatic cell types. *Nat. Biotechnol.* **26**, 916-924.

West, J. A., Viswanathan, S. R., Yabuuchi, A., Cunniff, K., Takeuchi, A., Park, I.-H., Sero, J. E., Zhu, H., Perez-Atayde, A., Frazier, A. L. et al. (2009). A role for Lin28 in primordial germ-cell development and germ-cell malignancy. *Nature* **460**, 909-913.

Worringer, K. A., Rand, T. A., Hayashi, Y., Sami, S., Takahashi, K., Tanabe, K., Narita, M., Srivastava, D. and Yamanaka, S. (2013). The let-7/LIN-41 pathway regulates reprogramming to human induced pluripotent stem cells by controlling expression of prodifferentiation genes. *Cell Stem Cell* **14**, 40-52.

Yamada, M., Johannesson, B., Sagi, I., Burnett, L. C., Kort, D. H., Prosser, R. W., Paull, D., Nestor, M. W., Freeby, M., Greenberg, E. et al. (2014). Human oocytes reprogram adult somatic nuclei of a type 1 diabetic to diploid pluripotent stem cells. *Nature* **510**, 533-536.

Yamaguchi, S., Hirano, K., Nagata, S. and Tada, T. (2010). Sox2 expression effects on direct reprogramming efficiency as determined by alternative somatic cell fate. *Stem Cell Res.* **6**, 177-186.

Yamanaka, S. (2009). Elite and stochastic models for induced pluripotent stem cell generation. *Nature* **460**, 49-52.

Yan, L., Yang, M., Guo, H., Yang, L., Wu, J., Li, R., Liu, P., Lian, Y., Zheng, X., Yan, J. et al. (2013). Single-cell RNA-Seq profiling of human preimplantation embryos and embryonic stem cells. *Nat. Struct. Mol. Biol.* **20**, 1131-1139.

Ying, Q.-L., Nichols, J., Chambers, I. and 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.

Ying, Q.-L., Wray, J., Nichols, J., Batlle-Morera, L., Doble, B., Woodgett, J., Cohen, P. and Smith, A. (2008). The ground state of embryonic stem cell self-renewal. *Nature* **453**, 519-523.

Yu, J., Vodyanik, M. A., Smuga-Otto, K., Antosiewicz-Bourget, J., Frane, J. L., Tian, S., Nie, J., Jonsdottir, G. A., Ruotti, V., Stewart, R. et al. (2007). Induced pluripotent stem cell lines derived from human somatic cells. *Science* **318**, 1917-1920.

Development (2015) 142, 3274-3285 doi:10.1242/dev.114249

Yusa, K., Rad, R., Takeda, J. and Bradley, A. (2009). Generation of transgene-free induced pluripotent mouse stem cells by the piggyBac transposon. *Nat. Methods* **6**, 363-369.

Zhang, Z., Xiang, D., Heriyanto, F., Gao, Y., Qian, Z. and Wu, W.-S. (2013). Dissecting the roles of miR-302/367 cluster in cellular reprogramming using TALE-based repressor and TALEN. *Stem Cell Rep.* **1**, 218-225.

Zhao, X.-Y., Li, W., Lv, Z., Liu, L., Tong, M., Hai, T., Hao, J., Guo, C.-l., Ma, Q.-W., Wang, L. et al. (2009). iPS cells produce viable mice through tetraploid complementation. *Nature* **461**, 86-90.

Zhou, Q., Brown, J., Kanarek, A., Rajagopal, J. and Melton, D. A. (2008). In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. *Nature* **455**, 627-632.