

Do all roads lead to Oct4? The emerging concepts of induced pluripotency

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Pluripotent cells have the potential to differentiate into all of the cell types of an animal. This unique cell state is governed by an interconnected network of transcription factors. Among these, Oct4 plays an essential role both in the development of pluripotent cells in the embryo and in the self-renewal of its *in vitro* counterpart, embryonic stem (ES) cells. Furthermore, Oct4 is one of the four Yamanaka factors and its overexpression alone can generate induced pluripotent stem (iPS) cells. Recent reports underscore Oct4 as an essential regulator of opposing cell state transitions, such as pluripotency establishment and differentiation into embryonic germ lineages. Here we discuss these recent studies and the potential mechanisms underlying these contrasting functions of Oct4.

Oct4 in pluripotency

Cell identity is characterised by a stable, unique, self-sustaining gene expression pattern. Cell state transition represents a change from one cell identity to another. Such transitions occur in development during differentiation into progressively specialised cell types, which is accompanied by a gradual restriction of developmental potential. Pluripotency characterises the cells capable of giving rise to all of the cell types of an organism except for extraembryonic tissues [1,2]. In mouse embryos, pluripotent cells emerge in the epiblast during preimplantation development and, shortly after implantation, undergo differentiation into embryonic germ layers and the germline. The pluripotent cells in the preimplantation embryo are considered naïve as opposed to the primed pluripotent cells of the postimplantation embryo, because they have unbiased developmental potential and can give rise to germline-competent chimeras when reintroduced into a blastocyst [3]. The *in vitro* counterparts of this transient naïve

pluripotent cell population are ES cells. Similarly to the naïve epiblast, ES cells coexpress the pluripotency markers *Esrrb*, *Nanog*, *Rex1*, *Klf4*, *Klf2*, *Sox2*, *Tbx3*, *Tfcp2l1*, and *Oct4*, have two active X chromosomes in the case of female cells, and can give rise to fully ES cell-derived mice [4].

Although cell state transitions are typically perceived as moving from a less differentiated to a more differentiated state, groundbreaking work by Takahashi and Yamanaka demonstrated that the cell state transition from somatic cells to naïve pluripotency is also possible and can be induced by the overexpression of four transcription factors: *Oct4*, *Sox2*, *Klf4*, and *c-Myc* [5]. The derived cells were named iPS cells and they have since been obtained from different species and types of somatic cells. Although the method of reprogramming is firmly established, the molecular mechanisms underlying this process remain poorly characterised.

Among the four original reprogramming factors, the POU family transcription factor *Oct4* appears to be the most important pluripotency regulator. *Oct4* was found to be required for the formation of the naïve epiblast, because the inner cell mass of *Oct4*-null embryos lacks pluripotent characteristics [6]. In addition, abrogation of *Oct4* expression in ES cells leads to their differentiation along the trophoblast lineage [7]. Although the SRY-related HMG-box transcription factor *Sox2* has also been shown to possess a loss-of-function phenotype similar to that of *Oct4* in both embryos [8] and ES cells [9], ectopic expression of wild type levels of *Oct4* can rescue the *Sox2*-null phenotype in ES cells [9]. This result indicates that *Oct4* activation may be the sole essential function of *Sox2* in ES cell self-renewal. In contrast to *Oct4* and *Sox2*, other pluripotency factors seem to be individually dispensable for the maintenance of the naïve pluripotent state [10–15]. Overexpression of *Nanog* [16], *Esrrb* [17], *Klf4*, *Klf2* [18], *Tfcp2l1* [15], and *Tbx3* [19] leads to enhanced self-renewal of ES cells, illustrating a positive effect on the pluripotent network. In turn, overexpression of *Oct4* or *Sox2* leads to spontaneous ES cell differentiation [7,20].

Recent studies are revealing novel aspects of the biological functions of *Oct4*. Particularly, *Oct4* was found to regulate seemingly opposite processes of cell identity change: the induction of pluripotency from somatic cells, pluripotent cell differentiation into embryonic lineages,

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and transdifferentiation – that is, the conversion of one somatic cell type into another without a common progenitor. In this review, we discuss these recent studies and the potential molecular mechanisms underlying these contrasting roles of Oct4 and propose that Oct4 is an essential regulator of cell state transitions in development.

Oct4 in reprogramming

In addition to being an essential regulator of pluripotency, Oct4 is also central to nuclear reprogramming. Oct4 overexpression is sufficient to induce pluripotency when using somatic cell types expressing canonical reprogramming

factors endogenously [21–24] or when in combination with small molecules [25–27]. However, reprogramming with Oct4 alone exhibits decreased efficiency and delayed kinetics. Significantly, exogenous Oct4 was shown to be replaceable in initiating reprogramming; however, most of the factors and small molecules demonstrated to do this (Table 1) act by reactivating the endogenous *Oct4* locus. The nuclear receptors Nr5a1 and Nr5a2, which were shown to induce pluripotency in the absence of Oct4 [28], bind regulatory regions of the *Oct4* gene and activate its expression in ES cells and embryonic carcinoma (EC) cells [29,30]. Tet1 was recently demonstrated to replace exogenous Oct4

Table 1. Reprogramming cocktails without Oct4

Alternative reprogramming cocktail	Somatic cell type	Refs	Evidence of acting through endogenous Oct4?
KSM + BIX-01294	Primary mouse fetal neural progenitor cells	[64]	Yes
KSM + Nr5a1 KSM + Nr5a2	Mouse embryonic fibroblasts	[28]	Yes
KSM + Tet1	Mouse embryonic fibroblasts	[31]	Yes
KSM + Sall4 + Nanog KM + Lin28 + Sall4 + Esrrb + Nanog KM + Lin28 + Sall4 + Ezh2 + Nanog Lin28 + Sall4 + Esrrb + Nanog Lin28 + Sall4 + Esrrb + Dppa2	Mouse embryonic fibroblasts	[33]	Yes
KSM + Gata3	Mouse embryonic fibroblasts Mouse adult dermal fibroblasts Mouse gastric epithelial cells Mouse keratinocytes	[68]	No
KSM + Sox7 KSM + Pax1 KSM + Gata4 KSM + CEBPa KSM + HNF4a KSM + GRB2 KM + Gata3 + Sox1 KM + Gata3 + Sox3 KM + Gata6 + Sox1 KM + Gata6 + Sox3 KM + Pax1 + Sox1 KM + Pax1 + Sox3	Mouse adult dermal fibroblasts		
KSM + Gata6	Mouse adult dermal fibroblasts Mouse keratinocytes		
KM + Gata6 + Geminin	Mouse adult dermal fibroblasts Mouse embryonic fibroblasts		
KS ^{VP} M + Gata3 ^{VP}	Primary human foreskin fibroblasts	[69]	Yes
KSM + E-cadherin	Mouse embryonic fibroblasts	[54]	No
KSM + forskolin KSM + 2-methyl-5-hydroxytryptamine KSM + D4476	Mouse embryonic fibroblasts	[118]	No
VPA + CHIR99021 + 616452 + tranylcypromine + forskolin + DZNep	Mouse embryonic fibroblasts Mouse neonatal fibroblasts Mouse adult fibroblasts Adipose-derived stem cells	[43]	Act by direct substitution of Oct4 due to structural and functional homology
KSM + human Oct4 KSM + <i>Xenopus</i> Oct91 KSM + medaka Pou2 KSM + axolotl Oct4 KSM + axolotl Pou2	Mouse embryonic fibroblasts		
KSM + axolotl Oct4 KSM + axolotl Pou2 KM + axolotl Oct4 + axolotl Sox2 KM + axolotl Pou2 + axolotl Sox2	Primary human skin fibroblasts		
KSM + A-OD3 (TALE-based designer transcriptional activator of Oct4)	Mouse embryonic fibroblasts	[37]	Yes

The table lists all studies that have reported reprogramming cocktails not containing Oct4. Abbreviations: K, Klf4; S, Sox2; M, c-Myc; ^{VP}, indicates a protein fused to a transcriptional activator VP.

in reprogramming by promoting 5mc–5hmc conversion at the *Oct4* regulatory regions and, thereby, to contribute towards the reactivation of the endogenous locus [31]. In addition, Tet1 together with Nanog was found to synergistically activate the endogenous *Oct4* locus in reprogramming intermediates [32]. *Oct4* is also not required to initiate reprogramming when the following transgene combinations are used: (i) Sox2, Sall4, Nanog, Klf4, C-Myc; (ii) Lin28, Sall4, Esrrb, Nanog, Klf4, c-Myc; (iii) Lin28, Sall4, Esrrb, Nanog; (iv) Lin28, Sall4, Esrrb, Dppa2; (v) Lin28, Sall4, Ezh2, Nanog, Klf4, c-Myc [33]; and (vi) Sall1, Sall4, Utf1, c-Myc, Nanog [34]. Notably, all of the combinations contain Sall4 as a reprogramming factor and Bayesian network analysis positions Sall4 upstream of *Oct4* in the sequence of events leading to the establishment of naïve pluripotency [33]. Moreover, Sall4 was previously reported to positively affect the expression of *Oct4* in both mouse and human ES cells [35,36], suggesting that Sall4 may activate *Oct4* during reprogramming. Importantly, it was recently demonstrated that an overexpression of a custom-made transcription activator targeting the *Oct4* enhancer leads to strong activation of the endogenous *Oct4* locus and efficient iPS cell generation in the absence of *Oct4* in the reprogramming cocktail [37]. This finding proves that any factor capable of activating *Oct4* expression could replace it in reprogramming.

Whereas Sox2, Klf4, and c-Myc can be replaced by their family members during reprogramming, octamer-binding POU family members Oct1 and Oct6 cannot replace Oct4 [38]. In addition, Oct1, Oct2, and Oct6 cannot sustain pluripotency in *Oct4*-null mouse ES cells [39]. The unique requirement for *Oct4* function in reprogramming, as opposed to other POU family members, was recently attributed to the linker region connecting the two DNA-binding domains of the protein [40]. Mutation of the key amino acids in this linker led to complete abrogation of *Oct4* reprogramming ability, while having no effect on its DNA binding, transactivation potential, or nuclear localization. Further analysis demonstrated that these key amino acids are located at the surface of the protein and are potentially involved in the recruitment of epigenetic modifiers to *Oct4* target genes [40].

Several reports investigated the evolutionary conservation of *Oct4* as a reprogramming factor. According to the current view, *Oct4* (also known as *Pou5f1*) and its paralog *Pou5f3* (also known as *Pou2*) arose by gene duplication at least as early as the last common ancestor of gnathostomes [41]. *Oct4* was subsequently lost in teleost fish, anurans, crocodilians, and birds. In turn, *Pou5f3* was lost in squamate reptiles and eutherian mammals, whereas marsupials, monotremes, urodeles, coelacanths, and turtles retained both genes [41,42]. Tested *Oct4* orthologues, human and axolotl, were shown to replace exogenous mouse *Oct4* in reprogramming [43]. Among the *Pou5f3* orthologues, the ability to initiate reprogramming in the mouse system was found for medaka, axolotl, and *Xenopus* but not zebrafish [43]. Importantly, human, axolotl and *Xenopus* but not zebrafish *Oct4* homologues can also maintain ES cell self-renewal in the absence of endogenous *Oct4* [44,45]. In turn, overexpression of mouse or human *Oct4* in combination with other Yamanaka factors in avian, zebrafish,

and fly somatic cells leads to the upregulation of endogenous pluripotency gene homologues and the formation of partially reprogrammed cells [46,47]. Together, these results demonstrate functional conservation between *Oct4* homologues.

In summary, *Oct4* is a powerful reprogramming factor with evolutionarily conserved functions that are non-redundant within the POU gene family.

Molecular mechanisms of *Oct4* in reprogramming

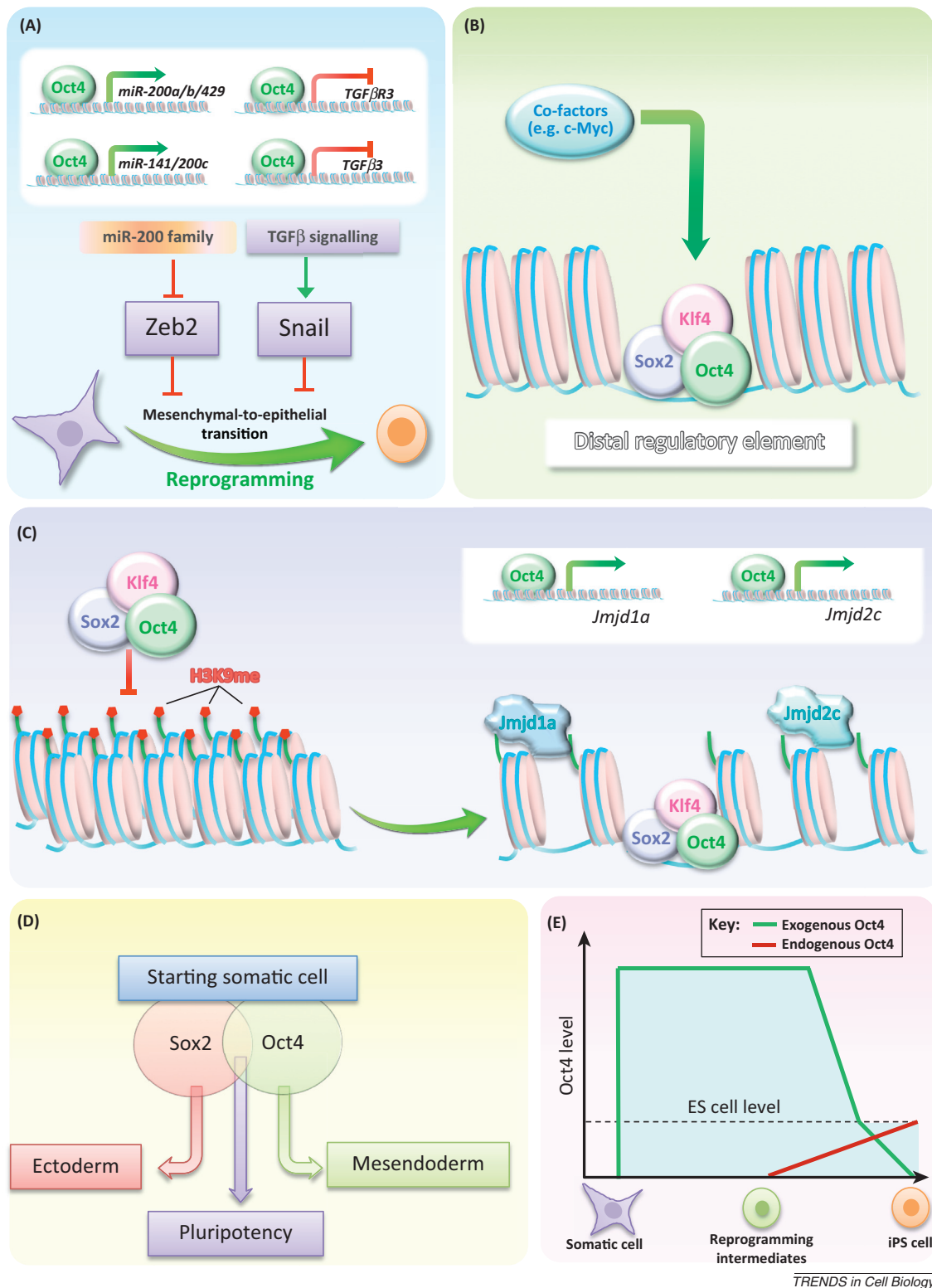
Despite its established importance for reprogramming, the precise mechanism of *Oct4* action during this process remains unclear. Experimental evidence suggests that *Oct4* participates in the induction of the mesenchymal-to-epithelial transition (MET) and in the derepression of somatic cell chromatin. In addition, *Oct4* in cooperation with Sox2 was proposed to prevent the acquisition of alternative cell states during reprogramming. Furthermore, *Oct4* dose and cellular localisation were proposed as important parameters of successful reprogramming.

Facilitating the MET

Acquisition of an epithelial phenotype by mesenchymal cells, or the MET, is a hallmark of reprogramming initiation [48]. The importance of this process is illustrated by the abrogation of reprogramming in response to MET inhibition and by its enhancement on MET induction [49–52]. All four Yamanaka factors are involved in different aspects of MET regulation. *Oct4* was shown to down-regulate the epithelial-to-mesenchymal transition (EMT) regulator Snail via the repression of *Tgfb β 3* and *Tgfb β R3* [52] and, together with Sox2, to activate a specific cluster of the miR-200 miRNA family that in turn represses the expression of the EMT regulator Zeb2 [53], thereby facilitating the MET (Figure 1A). Interestingly, overexpression of the MET regulator E-cadherin was shown to replace exogenous *Oct4* in reprogramming [54]. This substitution, however, cannot be completely explained by compensation for the role of *Oct4* in MET induction, because chemical inhibition of the EMT cannot replace *Oct4* in the reprogramming cocktail [49,50]. E-cadherin overexpression may also favour reprogramming via β -catenin sequestration at the membrane and, thereby, inhibition of canonical Wnt-signalling, because Wnt signalling inhibition was shown to facilitate the initial reprogramming stages [55]. However, it is unknown whether this mechanism is connected to endogenous *Oct4* activation during reprogramming. In addition, E-cadherin was recently identified as an important downstream *Oct4* effector in establishing cell adhesion properties required to maintain a pluripotent state [56], which may represent another *Oct4* mechanism in reprogramming.

Overcoming epigenetic barriers

According to the current view, reprogramming initiation represents a stochastic process during which reprogramming factors bind the genome promiscuously and act as ‘pioneer’ factors opening up the repressed chromatin and recruiting other transcriptional activators and chromatin remodellers as well as the transcriptional machinery [57–59] (Figure 1B). Indeed, most of the genomic sites bound by



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Oct4, Klf4, and Sox2 at the initiation of reprogramming correspond to distal regulatory elements of genes embedded in repressed chromatin in fibroblasts [58]. Some derepression events may eventually occur leading to the activation of early pluripotency genes and, therefore, progression towards the pluripotent state [57,60]. In support of Oct4 pioneer activity, its overexpression in differentiated cells was shown to be sufficient to generate nucleosome-depleted regions at the unmethylated *Nanog* and *Oct4* regulatory sequences leading to basal gene reactivation [61].

Despite the pioneer activity of reprogramming factors, the chromatin of most pluripotency-associated genes is initially inaccessible for binding, which was recently attributed to the presence of the histone H3 lysine 9 trimethylation (H3K9me3) mark [58]. Consistently, global H3K9me3 depletion enhances reprogramming [58,62]. Because Oct4 was demonstrated to be a potent activator of the H3K9 demethylase *Jmjd1a* and *Jmjd2c* genes in mouse ES cells [63], it could also be involved in surpassing this epigenetic roadblock in reprogramming (Figure 1C). *Jmjd1a* and *Jmjd2c* are known to maintain Tcf1 and *Nanog* expression in ES cells through H3K9 demethylation at their regulatory regions [63]. Moreover, the expression of these two H3K9 demethylases increases during reprogramming [5] and their knock down inhibits efficient iPS cell generation [62]. Also in support of this idea, exogenous Oct4 can be replaced in the reprogramming cocktail by the H3K9 methylase G9a inhibitor BIX-01294 [64]. This may also occur at least in part through reactivation of endogenous *Oct4*, because G9a is thought to participate in heterochromatinisation of the *Oct4* locus [65]. However, in the absence of G9a, partial DNA methylation of the *Oct4* locus and *Oct4* repression still occur during ES cell differentiation [66], suggesting that additional mechanisms contribute towards Oct4 repression.

Seesaw model of reprogramming

Recently, a 'seesaw reprogramming and pluripotency model' was proposed suggesting that pluripotency could be established and maintained by counterbalancing the mutually exclusive lineage-specifying forces of Oct4 and Sox2 [67–69] (Figure 1D). Particularly, Oct4 could inhibit the ectodermal programme induced by Sox2. To counterbalance, Sox2 could inhibit the mesendodermal programme induced by Oct4. This model was proposed based on several observations. First, it was recently demonstrated that some mesendoderm-specific transcription factors (e.g., Gata6, Gata4, Sox7, Pax1, CEBPa, HNF4a) can replace exogenous Oct4 in the initiation of reprogramming, whereas some ectoderm-specific transcription factors (e.g., Sox1, Sox3, Otx2, Pax6, Gmnn) can replace exogenous Sox2 [68,69]. Second, Oct4 overexpression in ES cells in serum/leukaemia inhibitory factor (LIF) culture conditions was found to induce the predominant formation of mesendodermal lineages [7]. Third, Sox2 promotes neuroectodermal differentiation of pluripotent cells [70,71] and drives bipotential axial progenitors to a neuroectodermal cell fate in the mouse embryo [72].

The seesaw model implies the existence of tight control of the Oct4/Sox2 ratio, with deviations from such balance

directing cells into alternative fates in the context of both reprogramming and pluripotent cell maintenance [67–69]. Indeed, the importance of Oct4 and Sox2 levels for reprogramming and pluripotency maintenance is well established [7,9,20,73–80]. However, alterations of these levels in ES cells do not confirm the proposed idea of a balance shift towards a particular lineage-specifying force. Thus, pluripotent cells can be maintained with low levels of Oct4 and wild type levels of Sox2 without upregulating neuroectoderm markers and instead demonstrating enhanced self-renewal [80,81]. In addition, Sox2 loss in ES cells can be rescued by ectopic expression of wild type levels of Oct4 [9]. Also, Sox2 overexpression in ES cells can generate a mixture of lineages including trophectoderm, mesoderm, and neuroectoderm [20]. Moreover, the original study showing that Oct4 overexpression induces predominant mesoderm and endoderm differentiation in ES cells [7] was performed in the presence of serum containing BMP4, a powerful inhibitor of neuroectoderm differentiation [82,83]. Using the same Oct4 overexpression system but under neural differentiation conditions, Oct4 accelerated neural fate acquisition [84]. Furthermore, overexpression of Oct4 fused to a strong transactivation domain does not induce the expression of mesendodermal genes and does not cause differentiation of ES cells [85]. An alternative explanation for the capacity of lineage-specific master transcriptional regulators to replace conventional reprogramming factors could be their ability to act as pioneer factors. Indeed, it was demonstrated that Gata and HNF factors possess a strong ability to open repressive chromatin [59,86]. This may suggest that Oct4 and other reprogramming factors can be replaced as initiators of reprogramming by any transcription regulator or chromatin remodeller with pioneer activity. Another explanation for pluripotency induction with lineage specifiers could be their ability to upregulate endogenous pluripotency genes. In support of this idea, Gata3 was shown to activate endogenous *Oct4* in reprogramming [69], whereas Gmnn, an ectoderm specifier used to replace Sox2 in reprogramming [68], was reported to be an important positive regulator of pluripotency genes in ES cells [87]. To further evaluate the seesaw reprogramming model, one should compare the genomic occupancy of Oct4 and Sox2 with that of lineage specifiers during reprogramming and analyse the effect of lineage specifiers on the upregulation of the pluripotency network.

In summary, although there may be a place for a seesaw mechanism in pluripotent state regulation, it is unlikely to be a major determinant of cell fate.

Oct4 levels and cellular localisation in reprogramming

The functions of Oct4 in reprogramming are dose dependent (Figure 1E). Several lines of evidence indicate that transgenic Oct4 must be highly expressed in somatic cells and reprogramming intermediates for successful reprogramming [76–80,88,89]. Low levels of transgenic Oct4 led to the generation of iPS cells with aberrant methylation of the *Dlk-Dio3* locus, tumourigenicity in chimeric mice, and low capacity of tetraploid complementation. Importantly these could be corrected by increasing the levels of exogenous Oct4 in reprogramming [76]. Another study

reported that reprogramming intermediates with low levels of Oct4 are refractory to pluripotency induction, but can be rescued by increasing the OSKM transgene copy number [89]. Therefore, the presence of high Oct4 levels at the initial reprogramming stage may assist in opening chromatin, increasing the chances of reactivating early pluripotency genes.

Recently, it was shown that an ES cell level of Oct4 must be achieved at the late stages of reprogramming for cells to enter the pluripotent cell state [80]. This requirement specifically corresponds to the establishment of pluripotency, because reduced Oct4 levels are compatible with pluripotent cell self-renewal [80]. Notably, endogenous Oct4 activation is not a predictor of pluripotency acquisition, because Oct4 is activated earlier than most of the core pluripotency genes during reprogramming [33,73,88,90]. However, the reported reactivation of endogenous Oct4 may have been only partial and thus insufficient for reprogramming completion.

Besides expression levels, the cellular localisation of Oct4 may also have biological importance. It was shown that an Oct4 mutant actively exported from the nucleus can rescue self-renewal of Oct4-null ES cells, but inefficiently induces reprogramming [91]. Interestingly, the same study also described the weaker reprogramming capacity of an Oct4 mutant fused to a strong nuclear localisation signal. Although this result could be explained by the reduced transactivation ability of this mutant [91], the idea that Oct4 may have functions in the cytoplasm is intriguing. Oct4 was shown to be a nucleocytoplasmic shuttling protein [91,92] and was found in a complex with β -catenin at the membrane of ES cells [93]. However, the precise function of this complex and whether this occurs during reprogramming is unknown.

Oct4 in cell differentiation

In addition to its role in pluripotency establishment, Oct4 was recently implicated in a contrasting cell state transition; that is, cell differentiation. It was proposed to be involved in the acquisition of extraembryonic endoderm cell fate and in pluripotent cell differentiation into embryonic lineages.

Oct4 was shown to orchestrate the patterning of primitive endoderm during early mouse development by simultaneous activation of multiple primitive endoderm genes [94]. In this study, Oct4-null embryos lost the capacity of Gata6 expression by E4.0 and failed to acquire expression of Sox17, Sox7, and platelet-derived growth factor receptor α (Pdgfra), defining markers of the primitive endoderm lineage [94]. Another recent study described the cooperation of Oct4 with Sox17 for the activation of endoderm-specific genes during *in vitro* specification of extraembryonic endoderm [95].

Oct4 overexpression studies in mouse ES cells suggest that, depending on the culture environment used, increases in Oct4 levels could induce or enhance cell differentiation into various cellular lineages [7,70,80,84,96]. By contrast, Oct4 overexpression in human ES cells does not lead to spontaneous differentiation or loss of pluripotent cell identity [71]. Instead, Oct4-overexpressing human ES cells exhibit enhanced endoderm and decreased neural

differentiation capacity when placed in corresponding differentiation conditions [71]. It should be noted, however, that overexpression-induced differentiation does not indicate a requirement for Oct4 for specification of the formed lineages or proves a negative role of Oct4 in the specification of certain lineages, because overexpression phenotypes could represent neomorphic effects. In fact, when Oct4 is expressed constitutively at a wild type ES cell level in pluripotent cells, they can efficiently enter embryonic development and differentiate into progenitors of all three embryonic lineages and germline in mouse embryos without obvious bias [80]. This is somewhat opposed to both the hypothesis that Oct4 may act as a blocker of certain cellular lineages [70,71,97] and the seesaw model for pluripotency and reprogramming [67–69]. Importantly, it was recently demonstrated that mouse iPS cells and ES cells with decreased Oct4 levels can efficiently sustain self-renewal but are incapable of germ layer differentiation both *in vitro* and *in vivo* [80,81]. This proves the requirement of Oct4 for pluripotent cell differentiation. Importantly, the pattern of Oct4 expression in the embryo agrees with these newly uncovered roles in cell differentiation. In addition to pluripotent and germ cells, Oct4 is expressed in the primitive endoderm of the blastocyst stage [98,99] and in the progeny of all germ layers until the late somite stage [100,101]. Moreover, using conditional Oct4 knockout it was recently demonstrated that Oct4 is required for post-implantation mouse development [102].

How can Oct4 govern such different processes as pluripotency establishment, maintenance, and cell differentiation? Most probably the answer to this seeming paradox lies in the instructive role of the culture environment and cellular context. Oct4 together with Nanog and Sox2 comprises the core of the pluripotency network [103]. These master transcription factors together with Klf4, Esrrb, and Mediator were found to co-occupy the recently classified super-enhancers, which are regulatory sequences found associated with genes involved in the maintenance of ES cell identity [104]. Counterintuitively, under differentiation-inducing culture conditions, Oct4 participates in the repression of pluripotency genes [80]. It is known that the regulatory regions of pluripotency genes are also bound by transcriptional repressor complexes (e.g., Lsd1–NURD) [105]. Also, different Oct4 interactome studies in ES cells demonstrated its interaction with chromatin remodelling complexes, including Lsd1 and NURD [106–108]. Thus, on stimulation with a differentiation inducer, Oct4 may repress pluripotency genes via its association with chromatin repressor complexes. This is consistent with Oct4 typically being downregulated later than other naïve pluripotency-associated genes during ES cell differentiation [80,109,110]. In support of this view, it was demonstrated that Lsd1 and NURD are not required for the maintenance of ES cell self-renewal, but are important for ES cell differentiation [111–113]. Thus, it will be of future interest to assess the genomic occupancy of both Oct4 and chromatin repressor complexes at early stages of ES cell differentiation.

In addition to its potential role in the silencing of pluripotency genes on induction of cell differentiation, Oct4 is possibly involved in the upregulation of certain

lineage-specific genes, as was observed for extraembryonic endoderm specification [94,95]. However, the continuous requirement for Oct4 expression and the extent of Oct4 involvement in embryonic lineage differentiation requires further investigation. In particular, it would be important to address whether Oct4 has specific targets depending on the cell lineage being acquired. This could indicate whether Oct4 may act as a pioneer factor during cell differentiation, providing competence for the establishment of new gene expression patterns in development. This would unite the seemingly contradictory roles of Oct4 in the acquisition and loss of pluripotency.

Taken together, these results describe Oct4 as an essential pan-regulator of cell commitment, potentially involved in silencing of the pluripotency programme and the establishment of lineage-specific gene expression identities instructed by environmental signals.

Oct4 in transdifferentiation

In addition to its described roles in reprogramming, pluripotent cell self-renewal, and differentiation, Oct4 may also induce transdifferentiation. It has been proposed that human fibroblasts can transdifferentiate into multipotent haematopoietic progenitors when Oct4 is ectopically expressed in fibroblasts treated with cytokines [114]. Furthermore, overexpression of Oct4, c-Myc, Sox2, and Klf4 in combination with a culture environment supportive of the target cell lineage allowed the conversion of mouse fibroblasts into contracting patches of cardiomyocytes [115],

neural progenitors [116], and neural stem cells [117]. In all of these studies, the authors provide experimental evidence to exclude the possibility of Oct4 and other Yamanaka factors transiently inducing pluripotent cells that could later differentiate. However, a more extensive investigation utilising cells unable to establish pluripotency or using permanent labelling of cells that have passed through a pluripotent state is required to prove transdifferentiation. The possibility that Oct4 and other Yamanaka factors may induce transdifferentiation of various somatic cell types is a further indication of their pioneer activity in initiating the process that subsequently leads to a cell state change.

Concluding remarks

Oct4 is an essential transcriptional regulator with multiple and diverse functions during different stages of reprogramming, pluripotency maintenance, cell differentiation, and transdifferentiation (Figure 2). Although numerous recent studies demonstrate the replacement of exogenous Oct4 by other factors and compounds during reprogramming, most Oct4 substitutions appear to act by upregulating the endogenous *Oct4* locus (Table 1). This underscores the importance of Oct4 in reprogramming, and future investigations should focus on identifying and investigating its downstream functions.

Only recently was Oct4 implicated in cell differentiation, calling for a revaluation of our views on how the pluripotent state is controlled and how commitment is

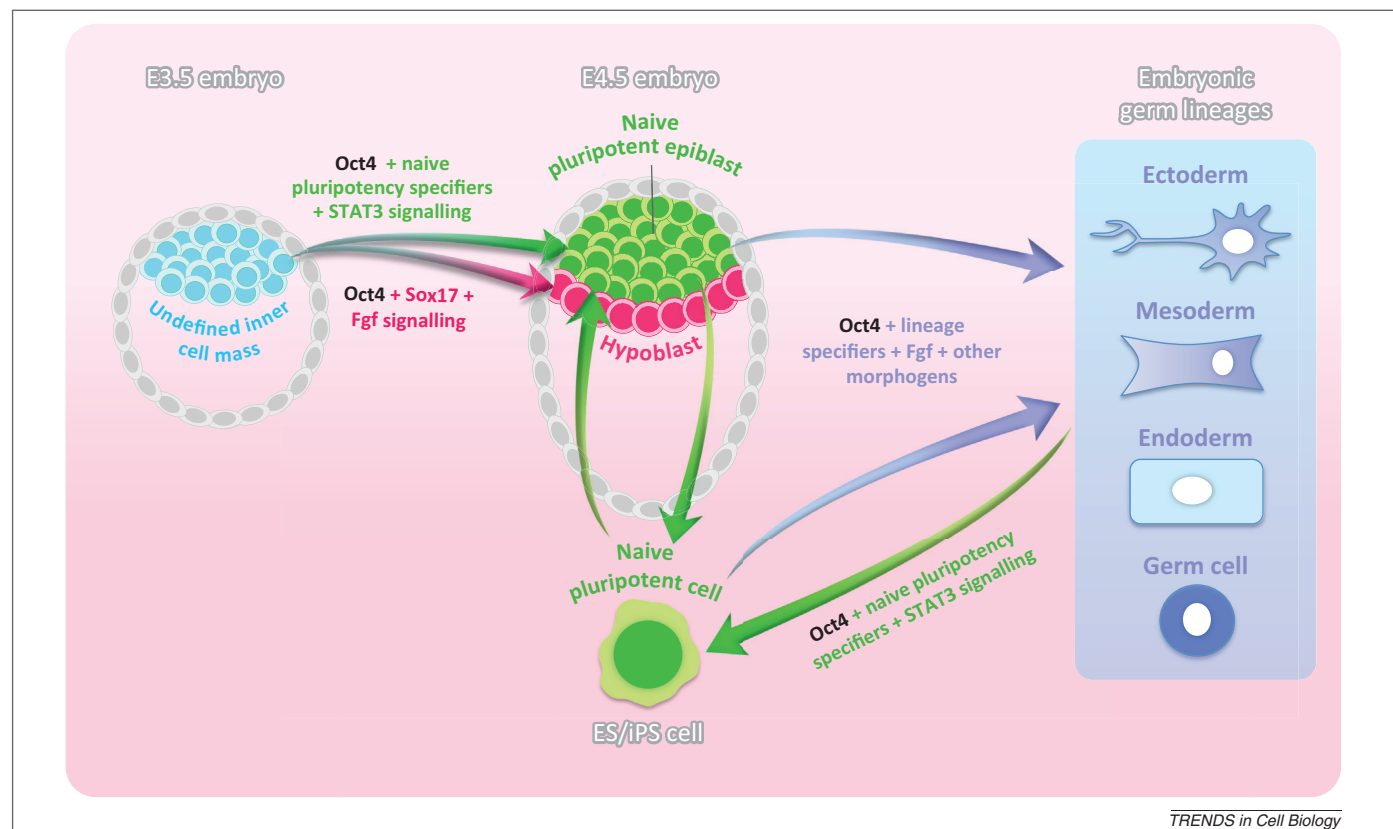


Figure 2. Oct4-controlled cell state transitions. Oct4, together with naive pluripotency specifiers (Nanog, Klf4, Esrrb, Sox2) and STAT3 signalling, governs the formation of the naive pluripotent cell compartment in mouse E4.5 blastocysts and directs the reprogramming of somatic cells into a naive pluripotent cell state. Together with Sox17 and Fgf signalling, Oct4 regulates specification of the primitive endoderm layer (hypoblast) at the blastocyst stage. Under differentiation-inducing culture conditions (on stimulation with Fgf and other morphogens), Oct4 in combination with lineage specifiers drives pluripotent cell commitment into all embryonic germ lineages.

activated. To date, the important differentiation-inducing signals have been identified and we have some knowledge of what chromatin regulators participate in the establishment of new gene expression patterns during differentiation. However, the mechanisms of interconnection between these 'inducers' and 'executioners' of cell differentiation remain unknown. Could Oct4 be involved in responding to differentiation-inducing stimuli by bringing about specificity to the activity of epigenetic regulators? This idea is intriguing and of interest to investigate in the future.

In conclusion, we have summarised our current understanding of the roles of Oct4 in various aspects of reprogramming, pluripotency, and cell differentiation. The emerging concept is that Oct4 is not merely a master pluripotency self-renewal factor, but, in addition, a key facilitator of cell state transitions occurring during cell differentiation, reprogramming, and transdifferentiation (Figure 2). In the future it would be important to define Oct4 targets and interaction partners during different cell state transitions. This will help in understanding how Oct4 function is regulated by the cellular context and/or extracellular signalling and what role Oct4 plays at different stages of reprogramming and in the initial specification of different lineages.

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References

- 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. U.S.A.* 78, 7634–7638
- Smith, A.G. (2001) Embryo-derived stem cells: of mice and men. *Annu. Rev. Cell Dev. Biol.* 17, 435–462
- 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
- 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
- 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
- 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
- Avilion, A.A. et al. (2003) Multipotent cell lineages in early mouse development depend on SOX2 function. *Genes Dev.* 17, 126–140
- 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
- Chambers, I. et al. (2007) Nanog safeguards pluripotency and mediates germline development. *Nature* 450, 1230–1234
- Martello, G. et al. (2012) Esrrb is a pivotal target of the Gsk3/Tcf3 axis regulating embryonic stem cell self-renewal. *Cell Stem Cell* 11, 491–504
- Masui, S. et al. (2008) Rex1/Zfp42 is dispensable for pluripotency in mouse ES cells. *BMC Dev. Biol.* 8, 45
- Jiang, J. et al. (2008) A core Klf circuitry regulates self-renewal of embryonic stem cells. *Nat. Cell Biol.* 10, 353–360
- Ye, S. et al. (2013) Embryonic stem cell self-renewal pathways converge on the transcription factor Tfc2l1. *EMBO J.* 32, 2548–2560
- Martello, G. et al. (2013) Identification of the missing pluripotency mediator downstream of leukaemia inhibitory factor. *EMBO J.* 32, 2561–2574
- Chambers, I. et al. (2003) Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. *Cell* 113, 643–655
- Festuccia, N. et al. (2012) Esrrb is a direct Nanog target gene that can substitute for Nanog function in pluripotent cells. *Cell Stem Cell* 11, 477–490
- Hall, J. et al. (2009) Oct4 and LIF/Stat3 additively induce Kruppel factors to sustain embryonic stem cell self-renewal. *Cell Stem Cell* 5, 597–609
- Niwa, H. et al. (2009) A parallel circuit of LIF signalling pathways maintains pluripotency of mouse ES cells. *Nature* 460, 118–122
- Kopp, J.L. et al. (2008) Small increases in the level of Sox2 trigger the differentiation of mouse embryonic stem cells. *Stem Cells* 26, 903–911
- Kim, J.B. et al. (2009) Oct4-induced pluripotency in adult neural stem cells. *Cell* 136, 411–419
- Kim, J.B. et al. (2009) Direct reprogramming of human neural stem cells by OCT4. *Nature* 461, 649–653
- Wu, T. et al. (2011) Reprogramming of trophoblast stem cells into pluripotent stem cells by Oct4. *Stem Cells* 29, 755–763
- Tsai, S.Y. et al. (2011) Single transcription factor reprogramming of hair follicle dermal papilla cells to induced pluripotent stem cells. *Stem Cells* 29, 964–971
- Yuan, X. et al. (2011) Brief report: combined chemical treatment enables Oct4-induced reprogramming from mouse embryonic fibroblasts. *Stem Cells* 29, 549–553
- Li, Y. et al. (2011) Generation of iPSCs from mouse fibroblasts with a single gene, Oct4, and small molecules. *Cell Res.* 21, 196–204
- Zhu, S. et al. (2010) Reprogramming of human primary somatic cells by OCT4 and chemical compounds. *Cell Stem Cell* 7, 651–655
- Heng, J.C. et al. (2010) The nuclear receptor Nr5a2 can replace Oct4 in the reprogramming of murine somatic cells to pluripotent cells. *Cell Stem Cell* 6, 167–174
- Gu, P. et al. (2005) Orphan nuclear receptor LRH-1 is required to maintain Oct4 expression at the epiblast stage of embryonic development. *Mol. Cell. Biol.* 25, 3492–3505
- Barnea, E. and Bergman, Y. (2000) Synergy of SF1 and RAR in activation of Oct-3/4 promoter. *J. Biol. Chem.* 275, 6608–6619
- Gao, Y. et al. (2013) Replacement of Oct4 by Tet1 during iPSC induction reveals an important role of DNA methylation and hydroxymethylation in reprogramming. *Cell Stem Cell* 12, 453–469
- Costa, Y. et al. (2013) NANOG-dependent function of TET1 and TET2 in establishment of pluripotency. *Nature* 495, 370–374
- Buganim, Y. et al. (2012) Single-cell expression analyses during cellular reprogramming reveal an early stochastic and a late hierarchic phase. *Cell* 150, 1209–1222
- Mansour, A.A. et al. (2012) The H3K27 demethylase Utx regulates somatic and germ cell epigenetic reprogramming. *Nature* 488, 409–413
- Zhang, J. et al. (2006) Sall4 modulates embryonic stem cell pluripotency and early embryonic development by the transcriptional regulation of Pou5f1. *Nat. Cell Biol.* 8, 1114–1123
- Yang, J. et al. (2010) A novel SALL4/OCT4 transcriptional feedback network for pluripotency of embryonic stem cells. *PLoS ONE* 5, e10766
- Gao, X. et al. (2013) Reprogramming to pluripotency using designer TALE transcription factors targeting enhancers. *Stem Cell Reports* 1, 183–197
- Nakagawa, M. et al. (2008) Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nat. Biotechnol.* 26, 101–106
- Niwa, H. et al. (2002) Phenotypic complementation establishes requirements for specific POU domain and generic transactivation function of Oct-3/4 in embryonic stem cells. *Mol. Cell. Biol.* 22, 1526–1536
- Esch, D. et al. (2013) A unique Oct4 interface is crucial for reprogramming to pluripotency. *Nat. Cell Biol.* 15, 295–301
- Frankenberg, S. and Renfree, M.B. (2013) On the origin of POU5F1. *BMC Biol.* 11, 56
- Frankenberg, S. et al. (2010) The evolution of class V POU domain transcription factors in vertebrates and their characterisation in a marsupial. *Dev. Biol.* 337, 162–170

- 43 Tapia, N. *et al.* (2012) Reprogramming to pluripotency is an ancient trait of vertebrate Oct4 and Pou2 proteins. *Nat. Commun.* 3, 1279
- 44 Niwa, H. *et al.* (2008) Platypus Pou5f1 reveals the first steps in the evolution of trophoblast differentiation and pluripotency in mammals. *Evol. Dev.* 10, 671–682
- 45 Morrison, G.M. and Brickman, J.M. (2006) Conserved roles for Oct4 homologues in maintaining multipotency during early vertebrate development. *Development* 133, 2011–2022
- 46 Rossello, R.A. *et al.* (2013) Mammalian genes induce partially reprogrammed pluripotent stem cells in non-mammalian vertebrate and invertebrate species. *Elife* 2, e00036
- 47 Lu, Y. *et al.* (2012) Avian-induced pluripotent stem cells derived using human reprogramming factors. *Stem Cells Dev.* 21, 394–403
- 48 Esteban, M.A. *et al.* (2012) The mesenchymal-to-epithelial transition in somatic cell reprogramming. *Curr. Opin. Genet. Dev.* 22, 423–428
- 49 Ichida, J.K. *et al.* (2009) A small-molecule inhibitor of TGF- β signaling replaces Sox2 in reprogramming by inducing nanog. *Cell Stem Cell* 5, 491–503
- 50 Maherali, N. and Hochedlinger, K. (2009) TGF- β signal inhibition cooperates in the induction of iPSCs and replaces Sox2 and c-Myc. *Curr. Biol.* 19, 1718–1723
- 51 Samavarchi-Tehrani, P. *et al.* (2010) Functional genomics reveals a BMP-driven mesenchymal-to-epithelial transition in the initiation of somatic cell reprogramming. *Cell Stem Cell* 7, 64–77
- 52 Li, R. *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
- 53 Wang, G. *et al.* (2013) Critical regulation of miR-200/ZEB2 pathway in Oct4/Sox2-induced mesenchymal-to-epithelial transition and induced pluripotent stem cell generation. *Proc. Natl. Acad. Sci. U.S.A.* 110, 2858–2863
- 54 Redmer, T. *et al.* (2011) E-cadherin is crucial for embryonic stem cell pluripotency and can replace OCT4 during somatic cell reprogramming. *EMBO Rep.* 12, 720–726
- 55 Ho, R. *et al.* (2013) Stage-specific regulation of reprogramming to induced pluripotent stem cells by Wnt signaling and T cell factor proteins. *Cell Rep.* 3, 2113–2126
- 56 Livigni, A. *et al.* (2013) A conserved Oct4/POU domain-dependent network links adhesion and migration to progenitor maintenance. *Curr. Biol.* 23, 2233–2244
- 57 Buganim, Y. *et al.* (2013) Mechanisms and models of somatic cell reprogramming. *Nat. Rev. Genet.* 14, 427–439
- 58 Soufi, A. *et al.* (2012) Facilitators and impediments of the pluripotency reprogramming factors' initial engagement with the genome. *Cell* 151, 994–1004
- 59 Zaret, K.S. and Carroll, J.S. (2011) Pioneer transcription factors: establishing competence for gene expression. *Genes Dev.* 25, 2227–2241
- 60 Papp, B. and Plath, K. (2013) Epigenetics of reprogramming to induced pluripotency. *Cell* 152, 1324–1343
- 61 You, J.S. *et al.* (2011) OCT4 establishes and maintains nucleosome-depleted regions that provide additional layers of epigenetic regulation of its target genes. *Proc. Natl. Acad. Sci. U.S.A.* 108, 14497–14502
- 62 Chen, J. *et al.* (2013) H3K9 methylation is a barrier during somatic cell reprogramming into iPSCs. *Nat. Genet.* 45, 34–42
- 63 Loh, Y.H. *et al.* (2007) Jmjd1a and Jmjd2c histone H3 Lys 9 demethylases regulate self-renewal in embryonic stem cells. *Genes Dev.* 21, 2545–2557
- 64 Shi, Y. *et al.* (2008) A combined chemical and genetic approach for the generation of induced pluripotent stem cells. *Cell Stem Cell* 2, 525–528
- 65 Feldman, N. *et al.* (2006) G9a-mediated irreversible epigenetic inactivation of Oct-3/4 during early embryogenesis. *Nat. Cell Biol.* 8, 188–194
- 66 Athanasiadou, R. *et al.* (2010) Targeting of *de novo* DNA methylation throughout the Oct-4 gene regulatory region in differentiating embryonic stem cells. *PLoS ONE* 5, e9937
- 67 Loh, K.M. and Lim, B. (2011) A precarious balance: pluripotency factors as lineage specifiers. *Cell Stem Cell* 8, 363–369
- 68 Shu, J. *et al.* (2013) Induction of pluripotency in mouse somatic cells with lineage specifiers. *Cell* 153, 963–975
- 69 Montserrat, N. *et al.* (2013) Reprogramming of human fibroblasts to pluripotency with lineage specifiers. *Cell Stem Cell* 13, 341–350
- 70 Thomson, M. *et al.* (2011) Pluripotency factors in embryonic stem cells regulate differentiation into germ layers. *Cell* 145, 875–889
- 71 Wang, Z. *et al.* (2012) Distinct lineage specification roles for NANOG, OCT4, and SOX2 in human embryonic stem cells. *Cell Stem Cell* 10, 440–454
- 72 Takemoto, T. *et al.* (2011) Tbx6-dependent Sox2 regulation determines neural or mesodermal fate in axial stem cells. *Nature* 470, 394–398
- 73 Silva, J. *et al.* (2008) Promotion of reprogramming to ground state pluripotency by signal inhibition. *PLoS Biol.* 6, e253
- 74 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
- 75 Yamaguchi, S. *et al.* (2011) Sox2 expression effects on direct reprogramming efficiency as determined by alternative somatic cell fate. *Stem Cell Res.* 6, 177–186
- 76 Carey, B.W. *et al.* (2011) Reprogramming factor stoichiometry influences the epigenetic state and biological properties of induced pluripotent stem cells. *Cell Stem Cell* 9, 588–598
- 77 Papapetrou, E.P. *et al.* (2009) Stoichiometric and temporal requirements of Oct4, Sox2, Klf4, and c-Myc expression for efficient human iPSC induction and differentiation. *Proc. Natl. Acad. Sci. U.S.A.* 106, 12759–12764
- 78 Tiemann, U. *et al.* (2011) Optimal reprogramming factor stoichiometry increases colony numbers and affects molecular characteristics of murine induced pluripotent stem cells. *Cytometry A* 79, 426–435
- 79 Nagamatsu, G. *et al.* (2012) Optimal ratio of transcription factors for somatic cell reprogramming. *J. Biol. Chem.* 287, 36273–36282
- 80 Radzishchanskaya, A. *et al.* (2013) A defined Oct4 level governs cell state transitions of pluripotency entry and differentiation into all embryonic lineages. *Nat. Cell Biol.* 15, 579–590
- 81 Karwacki-Neisius, V. *et al.* (2013) Reduced Oct4 expression directs a robust pluripotent state with distinct signaling activity and increased enhancer occupancy by Oct4 and Nanog. *Cell Stem Cell* 12, 531–545
- 82 Finley, M.F. *et al.* (1999) BMP-4 inhibits neural differentiation of murine embryonic stem cells. *J. Neurobiol.* 40, 271–287
- 83 Ying, Q.L. *et al.* (2003) BMP induction of Id proteins suppresses differentiation and sustains embryonic stem cell self-renewal in collaboration with STAT3. *Cell* 115, 281–292
- 84 Shimozaki, K. *et al.* (2003) Involvement of Oct3/4 in the enhancement of neuronal differentiation of ES cells in neurogenesis-inducing cultures. *Development* 130, 2505–2512
- 85 Hammachi, F. *et al.* (2012) Transcriptional activation by Oct4 is sufficient for the maintenance and induction of pluripotency. *Cell Rep.* 1, 99–109
- 86 Cirillo, L.A. *et al.* (2002) Opening of compacted chromatin by early developmental transcription factors HNF3 (FoxA) and GATA-4. *Mol. Cell* 9, 279–289
- 87 Yang, V.S. *et al.* (2011) Geminin escapes degradation in G1 of mouse pluripotent cells and mediates the expression of Oct4, Sox2, and Nanog. *Curr. Biol.* 21, 692–699
- 88 Theunissen, T.W. *et al.* (2011) Nanog overcomes reprogramming barriers and induces pluripotency in minimal conditions. *Curr. Biol.* 21, 65–71
- 89 Polo, J.M. *et al.* (2012) A molecular roadmap of reprogramming somatic cells into iPS cells. *Cell* 151, 1617–1632
- 90 O'Malley, J. *et al.* (2013) High-resolution analysis with novel cell-surface markers identifies routes to iPS cells. *Nature* 499, 88–91
- 91 Oka, M. *et al.* (2013) Differential role for transcription factor Oct4 nucleocytoplasmic dynamics in somatic cell reprogramming and self-renewal of embryonic stem cells. *J. Biol. Chem.* 288, 15085–15097
- 92 Plachta, N. *et al.* (2011) Oct4 kinetics predict cell lineage patterning in the early mammalian embryo. *Nat. Cell Biol.* 13, 117–123
- 93 Faunes, F. *et al.* (2013) A membrane-associated beta-catenin/Oct4 complex correlates with ground-state pluripotency in mouse embryonic stem cells. *Development* 140, 1171–1183
- 94 Frum, T. *et al.* (2013) Oct4 cell-autonomously promotes primitive endoderm development in the mouse blastocyst. *Dev. Cell* 25, 610–622
- 95 Aksoy, I. *et al.* (2013) Oct4 switches partnering from Sox2 to Sox17 to reinterpret the enhancer code and specify endoderm. *EMBO J.* 32, 938–953

- 96 Zeineddine, D. *et al.* (2006) Oct-3/4 dose dependently regulates specification of embryonic stem cells toward a cardiac lineage and early heart development. *Dev. Cell* 11, 535–546
- 97 Teo, A.K. *et al.* (2011) Pluripotency factors regulate definitive endoderm specification through eomesodermin. *Genes Dev.* 25, 238–250
- 98 Palmieri, S.L. *et al.* (1994) Oct-4 transcription factor is differentially expressed in the mouse embryo during establishment of the first two extraembryonic cell lineages involved in implantation. *Dev. Biol.* 166, 259–267
- 99 Guo, G. *et al.* (2010) Resolution of cell fate decisions revealed by single-cell gene expression analysis from zygote to blastocyst. *Dev. Cell* 18, 675–685
- 100 Downs, K.M. (2008) Systematic localization of Oct-3/4 to the gastrulating mouse conceptus suggests manifold roles in mammalian development. *Dev. Dyn.* 237, 464–475
- 101 Yeom, Y.I. *et al.* (1996) Germline regulatory element of Oct-4 specific for the totipotent cycle of embryonal cells. *Development* 122, 881–894
- 102 Deveale, B. *et al.* (2013) Oct4 is required ~e7.5 for proliferation in the primitive streak. *PLoS Genet.* 9, e1003957
- 103 Young, R.A. (2011) Control of the embryonic stem cell state. *Cell* 144, 940–954
- 104 Whyte, W.A. *et al.* (2013) Master transcription factors and mediator establish super-enhancers at key cell identity genes. *Cell* 153, 307–319
- 105 Whyte, W.A. *et al.* (2012) Enhancer decommissioning by LSD1 during embryonic stem cell differentiation. *Nature* 482, 221–225
- 106 Pardo, M. *et al.* (2010) An expanded Oct4 interaction network: implications for stem cell biology, development, and disease. *Cell Stem Cell* 6, 382–395
- 107 van den Berg, D.L. *et al.* (2010) An Oct4-centered protein interaction network in embryonic stem cells. *Cell Stem Cell* 6, 369–381
- 108 Ding, J. *et al.* (2012) Oct4 links multiple epigenetic pathways to the pluripotency network. *Cell Res.* 22, 155–167
- 109 Palmqvist, L. *et al.* (2005) Correlation of murine embryonic stem cell gene expression profiles with functional measures of pluripotency. *Stem Cells* 23, 663–680
- 110 Glover, C.H. *et al.* (2006) Meta-analysis of differentiating mouse embryonic stem cell gene expression kinetics reveals early change of a small gene set. *PLoS Comput. Biol.* 2, e158
- 111 Kaji, K. *et al.* (2006) The NuRD component Mbd3 is required for pluripotency of embryonic stem cells. *Nat. Cell Biol.* 8, 285–292
- 112 Wang, J. *et al.* (2009) The lysine demethylase LSD1 (KDM1) is required for maintenance of global DNA methylation. *Nat. Genet.* 41, 125–129
- 113 Reynolds, N. *et al.* (2012) NuRD suppresses pluripotency gene expression to promote transcriptional heterogeneity and lineage commitment. *Cell Stem Cell* 10, 583–594
- 114 Szabo, E. *et al.* (2010) Direct conversion of human fibroblasts to multilineage blood progenitors. *Nature* 468, 521–526
- 115 Efe, J.A. *et al.* (2011) Conversion of mouse fibroblasts into cardiomyocytes using a direct reprogramming strategy. *Nat. Cell Biol.* 13, 215–222
- 116 Kim, J. *et al.* (2011) Direct reprogramming of mouse fibroblasts to neural progenitors. *Proc. Natl. Acad. Sci. U.S.A.* 108, 7838–7843
- 117 Thier, M. *et al.* (2012) Direct conversion of fibroblasts into stably expandable neural stem cells. *Cell Stem Cell* 10, 473–479
- 118 Hou, P. *et al.* (2013) Pluripotent stem cells induced from mouse somatic cells by small-molecule compounds. *Science* 341, 651–654