CELL REGENERATION

REVIEW Open Access

Role of Oct4 in the early embryo development

Guangming Wu[1] and Hans R Schöler[1,2*]

Abstract

Oct4 is a key component of the pluripotency regulatory network, and its reciprocal interaction with Cdx2 has been shown to be a determinant of either the self-renewal of embryonic stem cells (ESCs) or their differentiation into trophoblast. Oct4 of maternal origin is postulated to play critical role in defining totipotency and inducing pluripotency during embryonic development. However, the genetic elimination of maternal *Oct4* using a Cre-lox approach in mouse revealed that the establishment of totipotency in maternal Oct4–depleted embryos was not affected, and that these embryos could complete full-term development without any obvious defect. These results indicate that Oct4 is not essential for the initiation of pluripotency, in contrast to its critical role in maintaining pluripotency. This conclusion is further supported by the formation of *Oct4*-GFP– and Nanog- expressing inner cell masses (ICMs) in embryos with complete inactivation of both maternal and zygotic *Oct4* expression and the reprogramming of fibroblasts into fully pluripotent cells by *Oct4*-deficient oocytes.

Keywords: Oct4, Oct4B, Totipotency, Pluripotency, Embryo, Development

Introduction

Life is like a journey of torch relay. From generation to generation, our bodies vanish at the end of our lives, but the germ cells are passed on to the next generation, ensuring the continuity and prosperity of our species. In comparison with the somatic cells, these germ cells possess many unique properties, of which the expression of Oct4 is the most important as it is required for the survival of primordial germ cells (PGCs) [1,2]. *Oct4* is also expressed specifically in the inner cell mass (ICM) and embryonic stem cells (ESCs), the cells derived from the ICM [3]. Interestingly, *Oct4* is expressed in mouse oocytes as a maternal transcript and protein [1,4-6]. As is typical for most maternal mRNAs, levels of *Oct4* mRNA drop dramatically after fertilization [6]. Zygotic *Oct4* expression is activated prior to the 8-cell stage, with a significant increase in both mRNA and protein levels [4,6]. *Oct4* expression is abundant and uniform in all cells of the embryo throughout the morula stage. However, as the outer cells of the embryo differentiate into the trophectoderm (TE), *Oct4* expression becomes downregulated and restricted to cells of the ICM in the blastocyst [5,7,8]. When cells of the primitive endoderm differentiate and migrate away from the ectoderm, their Oct4 protein levels transiently increase [4]. Oct4 expression then becomes downregulated in the primitive endoderm and maintained in the epiblast, concurrently with embryo implantation and gastrulation. *Oct4* expression finally becomes restricted to PGCs [9], which are first specified in the extraembryonic mesoderm at the base of the allantoic bud during gastrulation [9]. PGCs give rise to gametes, which can be fertilized to develop into a new fully functional organism of the next generation and complete one cycle of life (Figure 1).

Oct4, encoded by the gene Pou5f1, is a homeodomain transcription factor of the POU (Pit-Oct-Unc) family. The POU family of transcription factors can activate the expression of their target genes through binding to an octameric sequence motif of an ATGCAAAT consensus sequence. Oct4 protein consists of 3 domains: N-terminal domain, POU domain, and a C-terminal domain. The POU domain consists of two structurally independent subdomains: a 75 amino acid amino-terminal POU-specific (POU_S) region and a 60 amino-acid carboxyl-terminal homeodomain (POU_{HD}). Both domains make specific contact with DNA through a helix-turn-helix structure and are connected by a linker of 17 amino acids. Regions outside of the POU domain are not critical for DNA binding and exhibit little sequence conservation.

* Correspondence: office@mpi-muenster.mpg.de
[1]Max Planck Institute for Molecular Biomedicine, Department of Cell and Developmental Biology, Röntgenstrasse 20, 48149 Münster, Germany
[2]University of Münster, Medical Faculty, Domagkstr. 3, 48149 Münster, Germany

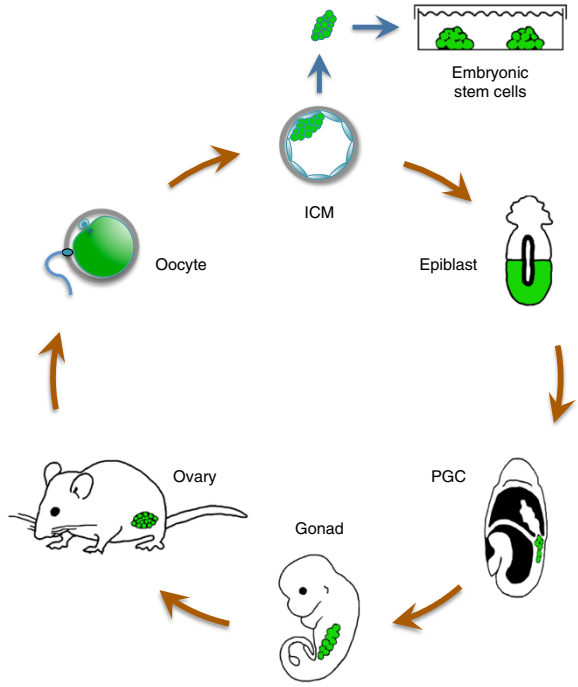

Figure 1 Oct4 expression during the mouse life cycle. Cells and tissues expressing Oct4 are marked in green. Oct4 is expressed in mouse oocytes as a maternal transcript and protein. Zygotic Oct4 expression is activated prior to the 8-cell stage and is abundant and uniform in all cells of the embryo throughout the morula stage. However, as the outer cells of the embryo differentiate into the TE, Oct4 expression is restricted to cells of the ICM in the blastocyst. After implantation, Oct4 expression is maintained in the epiblast. Finally, Oct4 expression becomes restricted to primordial germ cells (PGCs), which are first specified in the extraembryonic mesoderm at the base of the allantoic bud during gastrulation. PGCs give rise to gametes, which following fertilization will develop into a new organism of next generation. Oct4 expression is based on data previously reported by several studies [6,7,9-11].

The N-terminal domain (N-domain) is rich in Proline and acidic residues, while the C-terminal domain (C-domain) is rich in Proline, Serine, and Threonine residues. Both the N-domain and the C-domain play an important role in transactivation, but the activity of the C-domain is cell type specific and is regulated through phosphorylation, whereas that of the N-domain is not. The Oct4 POU-domain functions differently by serving as an interaction site for binding by cell type–specific regulatory factors [12,13]. *Oct4* has been deemed to be a critical regulator of cellular pluripotency, as shown by a zygotic *Oct4*-knockout study [14]. Loss of pluripotency in embryos, observed at the onset of somitogenesis, is coincident with reduction of *Oct4* and *Nanog* expression, and can be rescued by ectopic Oct4 expression [15]. *Oct4* can activate its own expression with its transcription factor partner *Sox2* through a positive autoregulatory loop in ESCs [16]. Studies on Oct4 interaction protein network have revealed that the Oct4 interactome includes many transcription factors and chromatin-modifying complexes with documented roles in self-renewal and pluripotency, and that acute depletion of Oct4 reduces the binding of Tcfcp2l1, Dax1, and Esrrb to target genes [17-19]. Depletion of Oct4 by siRNA leads to reduced binding of two key components of the bone morphogenetic protein (BMP) and leukemia inhibitory factor (LIF) signaling pathways, Smad1 and STAT3, to their respective targets. This result indicates that Oct4 plays a pivotal role in stabilizing the nucleoprotein complex and establishes a hierarchy of regulatory interactions between Oct4, STAT3, and Smad1 [20]. The core components of the pluripotency circuitry are formed by Oct4, Nanog, and Sox2, and *Nanog* expression is directly regulated by Oct4 and Sox2 [21], Sox2 is actually dispensable for the activation of Oct-Sox enhancers, and the forced expression of Oct4 could rescue Sox2-null ESCs [22]. Hence, *Oct4* is considered to be the genetic "master switch" in the establishment of totipotency-pluripotency during the life cycle of mammals [23], and it is presumed to be the most upstream gene in the molecular circuitry of pluripotency [24].

Maternal Oct4 expression is not critical for developmental competence of the oocyte

All biological processes occurring during the first cell cycle of the embryo rely on maternal factors, which have accumulated during the long growth phase of the germinal vesicle (GV) oocyte [25], because there is nearly no mRNA synthesis between the end of the mouse oocyte growth phase and the first zygotic cleavage [26]. But during the second cell cycle, there is a burst of transcription—what is known as zygotic genome activation (ZGA) [27]. Mature metaphase II (MII) oocytes have the capacity to reprogram somatic cells into cells of a totipotent state via nuclear transfer (NT) [28,29]. Therefore, activation of zygotic transcripts mediated by maternal regulatory factors provides the first step in the establishment of totipotency/pluripotency. *Oct4* is one of the 27 proven maternal-effect genes and is regarded to be functionally important for zygotic genome activation [30]. Maternal Oct4 is therefore widely accepted to play a role in igniting the establishment of totipotency and induction of pluripotency.

According to the chromatin organization, growing mouse oocytes could be classified into 2 types: SN (surrounded nucleolus) oocytes, with a ring of chromatin surrounding the nucleolus, and NSN (not surrounded nucleolus) oocytes, with chromatin dispersed throughout the nucleus, i.e., chromatin not surrounding the nucleolus. When they mature into metaphase II (MII) oocytes and are fertilized *in vitro*, only MII^{SN} oocytes (MII oocytes derived from SN oocytes) are developmentally competent to develop beyond the 2-cell stage, while MII^{NSN} oocytes (MII oocytes derived from NSN oocytes)

become arrested at the 2-cell stage [31]. By comparing the transcriptional profiles of these two types of mouse MII oocytes, Zuccotti et al. found that Oct4 was absent in MII^{NSN} oocytes, accounting for the downregulation of *Stella*, a maternal-effect factor required for the oocyte-to-embryo transition, and the upregulation of 18 Oct4-regulated genes implicated in the activation of adverse biochemical pathways such as oxidative phosphorylation, mitochondrial dysfunction, and apoptosis. Those authors concluded that the downregulation of Oct4 leads to the developmental arrest of MII^{NSN} oocytes and that maternal Oct4 emerges as a key regulator of the molecular events that govern the establishment of the developmental competence of mouse oocytes [32]. Moreover, another study claimed that Oct4 is a critical regulator of the maternal-embryonic transition at the 2-cell stage, as embryos invasively injected with Oct4-antisense morpholino oligonucleotides were found to arrest in various developmental stages prior to the blastocyst stage [33]. A later report from the same group using the same approach also showed developmental arrest between the 2- to 8-cell stages in morpholino-mediated Oct4, Nanog, or Sall4 knockdown, but developmental arrest remained obvious after co-injection of Oct4, Nanog, or Sall4 mRNA [34]. Although such a phenotype contradicts to genetic knockout studies [14,35,36], which indicated that homozygous null embryos could develop at least up to the blastocyst stage, the authors ignored the presence of maternal Oct4 protein and assumed that the apparent discrepancy was due to the presence of functional maternal transcript [34]. However, recently, numerous studies found that after genetic removal of maternal Oct4 in oocytes by crossing $Oct4^{flox/flox}/ZP3^{Cre/+}$female mice with wild-type male mice, these $Oct4^{flox/flox}/ZP3^{Cre/+}$female mice were found to be fully fertile [37-39]. All offspring had deletion of the maternal *Oct4* allele as confirmed by polymerase chain reaction (PCR) genotyping, clearly demonstrating that maternal *Oct4* is not critical for the establishment of totipotency-pluripotency [37]. The data in the study also showed the efficient deletion of the maternal Oct4 allele by genotyping of individual oocytes, the depletion of Oct4 mRNA by real-time reverse transcriptase PCR (RT-PCR), and the depletion of Oct4 protein by Western blot [37].

Oct4-null oocytes can reprogram somatic cells to a pluripotent state

Enucleated oocytes can also reprogram the nuclei of terminally differentiated somatic cells to a totipotent embryonic state after nuclear transplantation [28,29]. The nuclei can be reprogrammed by the recipient oocytes to express the pluripotent gene *Oct4* at a very high efficiency (88.7%) in just 2–3 days [40] and to give rise to pluripotent cells at an efficiency of up to 20% [41]. This compares favorably with the exciting 4-factor (Oct4, Sox2, Klf4, and c-Myc) technique of generating induced pluripotent stem cells (iPSCs) by Yamanaka and colleagues, which showed activation of internal *Oct4* expression after more than 2 weeks, with an efficiency of 0.01–0.1% [42]. A recent study revealed that Oct4 alone could reprogram neural stem cells into pluripotent iPSCs [43]. Could maternal *Oct4* in oocytes be essential in the reprogramming of somatic cells to pluripotent status? The answer is no. By conducting NT experiments using maternal *Oct4*-null oocytes from $Oct4^{flox/flox}/ZP3^{Cre/+}$female mice, it was found that all cloned embryos expressed pluripotency genes (*Oct4* and *Nanog*) in the ICM and the TE marker gene *Cdx2* in the TE by both immunocytochemistry and real-time RT-PCR [37]. Furthermore, ESC lines derived from cloned embryos using *Oct4*-knockout oocytes demonstrated full pluripotency by generating completely ESC-derived mice through the tetraploid complementation test, the most stringent test for pluripotency [37]. Clearly, the reprogramming engine in oocytes could work effectively to reprogram somatic cells into cells of a pluripotent state without the presence of maternal Oct4.

Reciprocal interaction between Oct4 and Cdx2 is not the initial cause sparking ICM/TE lineage separation

Previous studies have suggested that lineage commitment is controlled by the expression level of *Oct4* [14,44]. Repression of *Oct4* expression induced the differentiation of ESCs into TE cells, and a less than two-fold increase in *Oct4* expression caused the differentiation of ESCs into cells of primitive endoderm and mesoderm [44]. In the absence of Oct4, embryos could not form an ICM—i.e., the inner cells of morula-stage embryos rather were driven into trophoblast differentiation [14]. Therefore, these results indicated that *Oct4* plays a critical role in sustaining stem cell self-renewal and that up- or downregulation of *Oct4* expression induces divergent developmental programs, suggesting that *Oct4* is the master regulator of pluripotency and that it may also control lineage commitment during early embryonic development [14]. This hypothesis was supported by reports showing that Oct4 and Cdx2 could form a complex that reciprocally repressed their target genes and such an interaction determined the ICM/TE lineage separation in early embryos [45], while the interaction between Oct4 and the histone H3–specific methyltransferase ESET restricted the extraembryonic trophoblast lineage potential of pluripotent cells [46]. However, the expression of *Cdx2* in ESCs was found to be rapidly initiated by Ras activation (i.e., within 24 hours) without previous or simultaneous downregulation of *Oct4* expression [47], while ESCs with reduced Oct4 expression showed robust pluripotency and expressed naïve pluripotency genes, but were deficient

in differentiation in the absence of pluripotency culture requisites [48,49]. On the other hand, *Cdx2* deficiency did not interrupt TE-ICM lineage separation [50-52], and zygotic *Oct4* expression was shown not to be required for the initial repression of the TE genes *Cdx2* and *Gata3* in the ICM, indicating that other mechanisms are responsible for restricting the expression of these genes to the TE [53]. Finally, with a conditional knockout system, it was demonstrated that, similar to the reported zygotic Oct4 knockout observation [14,53], the genetic removal of both maternal and zygotic Oct4 did not prevent ICM-TE lineage separation [37-39], arguing against the notion that maternal Oct4 could partially compensate for the loss of zygotic Oct4 during cell fate specification in the blastocyst [33]. Therefore, the first lineage separation of the ICM-TE is not determined by the reciprocal interaction between Oct4 and Cdx2. More importantly, these studies indicated that maternal Oct4 is not at the root of pluripotency as a determinant of pluripotent cell lineage initiation, contrary to previous assumptions and views [23,24].

How Oct4 is activated in the embryo remains an open question

Oct4 is at the top of the pluripotency regulatory hierarchy in pluripotent cells [20,21]. It forms a positive feedback loop [16] and is essential to maintaining pluripotency [14], but is not required for initiating totipotency/pluripotency in embryos [37-39]. Therefore, understanding how early embryos activate *Oct4* expression is key in clarifying the oocyte reprogramming mechanism.

The upstream region of the transcriptional initiation site of the *Oct4* gene contains three regulatory elements for gene transcription: the distal enhancer (DE), proximal enhancer (PE), and TATA-less proximal promoter (PP) [9] as illustrated in Figure 2. The two enhancers exhibit a differential activation pattern according to the developmental stage of the mouse embryo. The DE drives *Oct4* expression in the ICM, ESCs, and PGCs, while the PE activates *Oct4* expression in epiblast cells. Each enhancer contains multiple potential binding sites for transcription factors that can either activate or repress *Oct4* expression. In addition, the methylation of these regions represses *Oct4* expression in differentiated cells. Several positive and negative regulators bind to the *Oct4* gene to regulate its expression. Of these, members of the orphan nuclear receptor superfamily, which can bind to Sp1 sites [54] and hormone response elements (HREs) in the PE and PP, are known to influence *Oct4* expression. Positive regulators of *Oct4* expression include *Nr5a2* [55], *SF1* (Steroidogenic Factor-1), and *RXR-β* (Retinoid X Receptor-β, also known as Nr2b2) [56,57]. Negative regulators include *GCNF* (Germ Cell Nuclear Factor) (also known as *Nr6a1*) [58], and *COUF-TFI/II* (Chicken Ovalbumin Upstream promoter-Transcription Factors-I/II), encoded by *Nr2f1* and *Nr2f2*, respectively [59,60]. The transcription factor TR2 can bind to the HRE of the *Oct4* gene to either activate or repress *Oct4* expression in P19 embryonal carcinoma (EC) stem cells and regulate the proliferation of the culture, based on whether there is SUMOylation on the Lys-238 of TR2 [61].

Recent studies have found that certain maternal factors are involved in the regulation of *Oct4* expression, providing clues on the mechanism underlying the initiation of totipotency/pluripotency. Cancer-associated factor Tpt1 has been reported to activate the transcription of *Oct4* and *Nanog* in transplanted somatic nuclei in the Xenopus oocyte [63], but another study failed to replicate this finding upon knockdown of Tpt1 by Small interfering RNA (siRNA) in the mouse embryos [37]. Components of the ATP-dependent BAF chromatin-remodeling complex have been shown to significantly increase reprogramming efficiency when used together with the Yamanaka's 4 factors [64]. Promyelocytic leukemia (Pml) protein was found to be required for *Oct4* gene expression and the maintenance of its open chromatin conformation in stem cells. In proliferating stem cells, Pml-nuclear body, along with the transcription factors TR2, SF1 and Sp1, and the Brg1-dependent chromatin remodeling complex (BRGC), associates with the Oct4 promoter to maintain a nucleosome-free region for gene activity [65]. Studies in search for master genes in the oocyte have revealed a novel oocyte-specific eukaryotic translation initiation factor 4E (*Eif4eloo*) [66] and a large number of oocyte-specific genes with yet unknown functions, such as those belonging to the homeodomain transcription factor Obox family [67]. The maternal transcription factor Sall4 binds to the *Oct4* DE, and it is regarded to be a transcriptional activator of *Oct4* expression based on evidence that reduction in *Sall4* mRNA level in blastocysts at merely 50% knockdown efficiency by *Sall4* siRNA injection into zygotes led to a 70% reduction in *Oct4* expression [68]. Single-cell expression analyses during *in vitro* cellular reprogramming have confirmed that *Sall4* is indeed an upstream activator of *Oct4* expression [69]. However, the efficient knockdown of *Sall4* by injecting *Sall4* siRNA into maternal *Oct4*-deficient zygotes—to avoid any possible effect of maternal Oct4 as a positive autoregulator—did not lead to any changes in *Oct4* expression at the blastocyst stage, arguing against such a role for *Sall4* as an upstream activator of *Oct4* expression *in vivo* [37]. Nr5a2 was found to maintain Oct4 expression at the epiblast stage of embryonic development, by binding to the PE and PP regions of *Oct4*, but to play no evident role in ESC self-renewal [55]. However, Nr5a2 can induce epiblast stem cells into ground state pluripotency, a basal proliferative state that is free of epigenetic

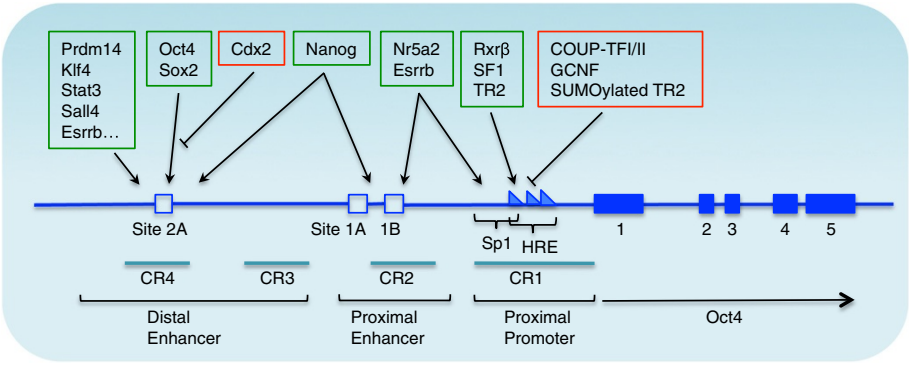

Figure 2 Genomic structure and transcriptional regulation of the mouse *Oct4* gene. The diagram represents ~24 kb of the genomic region surrounding the *Oct4* gene [62]. The gene has five exons, depicted as blue boxes. The identified upstream regulatory regions include the promoter, proximal enhancer, and distal enhancer. The sizes of the regulatory elements are stretched to enhance clarity. The transcription factors bind to these regions, and are shown above within colored boxes; they either activate (green box) or repress (red box) transcription. HRE = hormone responsive element; Sp1 = GC-rich site recognized by the Sp1/Sp3 family of transcription factors. CR1, CR2, CR3, and CR4 are conserved regions (CRs) at the 5' upstream region of the *Oct4* gene.

restriction [70], and replace Oct4 in the reprogramming of somatic cells into pluripotent cells [71]. Activation of *Zscan4* expression occurs during ZGA, with the gene being expressed in ESCs, whereas reduction in *Zscan4* transcript levels by siRNAs delays the progression from the 2-cell to the 4-cell stage, leading to blastocysts that fail to implant or proliferate in blastocyst outgrowth culture [72]. Zscan4 is essential for induction of iPSCs and its ectopic expression can activate early embryonic genes and improve the efficiency of iPSC generation [73]. However, knockdown of *Zscan4* in preimplantation embryos by siRNA against all 6 isoforms of *Zscan4* (*a-f*) had no impact on *Oct4* expression [37]. A component of an active DNA demethylase, activation-induced cytidine deaminase (*AID*), was also shown to be required for reprogramming [74]. A genome-scale RNA interference (RNAi) screen in ESCs identified components of the Paf1 complex with strong effects on *Oct4* expression, and showed that Paf1C overexpression blocks the differentiation of ESCs and that Paf1C knockdown causes expression changes in ESCs that are similar to those observed with Oct4 or Nanog depletion [75].

Oct4B expression in oocytes and embryos

After the finding that truncated isoforms of OCT4 are transcribed from the POU5F1 gene in human [76] and mouse [77], the originally described OCT4 is designated as OCT4A and the newly found truncated isoforms are variants of an OCT4 version named OCT4B [78]. OCT4B mRNAs encode proteins that have identical POU DNA-binding domains and C-domains but differ in their N-domains (Figure 3). Continued expression of Oct4B after the original Oct4 promoter was removed indicates that Oct4B transcription is regulated by an alternative promoter in the first intron [37], as presumed by an earlier study [79]. Other isoforms of Oct4B could be produced by alternative splicing or alternative translation initiation [80,81]. ESC-based complementation assays using ZHBTc4 ESCs, which has endogenous Oct4 inactivated by gene targeting and harbors a tetracycline-repressible Oct4 transgene to support ESC self-renewal [44], showed that OCT4B cannot rescue the self-renewal ability of ZHBTc4 ESCs in the presence of doxycycline, unlike OCT4A. Electrophonetic mobility shift assay showed that OCT4B does not bind to a probe carrying the OCT4 consensus binding sequence due to the repressive effect of the OCT4B N-domain. Furthermore, overexpression of OCT4B does not activate transcription from OCT4-dependent promoters [78]. However, Oct4B is involved in stress response [82] and acts as an antiapoptotic factor in cancer cells [83].

On the other hand, Oct4 expression in the adult has been reported in hematopoietic and mesenchymal stem cells [84-95], as well as progenitor cells from various somatic tissues including pancreatic islets [96], kidney [97,98], peripheral blood [89,99,100], endometrium of the uterus [101,102], thyroid [103], lung [104], brain [105,106], liver [107], and skin [108-111]. The wide expression of Oct4 in normal tissues suggests that *Oct4* may not only be crucial for the maintenance of pluripotency in embryonic cells, but also play an important role in the self-renewal of somatic stem cells and in maintenance of tissue homeostasis. As these studies did not distinguish the Oct4 isoforms, it is likely they actually detected Oct4B. A later study that conditionally deleted Oct4 from somatic cells *in vivo* found that Oct4 is dispensable for both the self-renewal and maintenance of somatic stem cells in the adult mammal [112]. Oct4 gene ablation in the intestinal epithelium, bone marrow (hematopoietic and mesenchymal lineages), hair follicle,

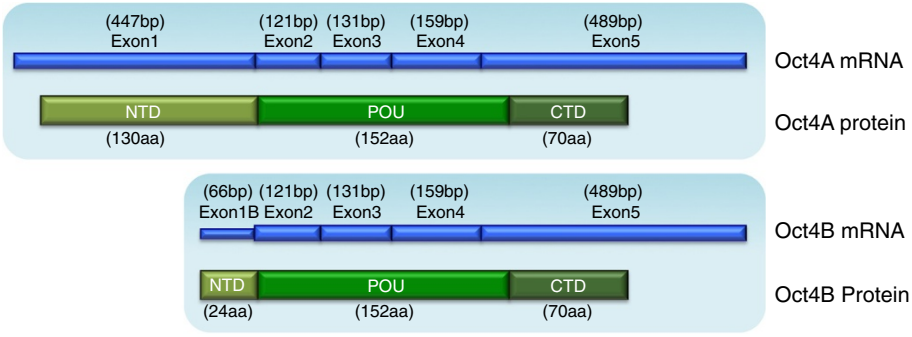

Figure 3 Schematic representation of the protein domains of the mouse Oct4 isoforms and the corresponding exons. Exon1B of Oct4B is in the intron 1–2 region of the *Oct4* gene. Modified from Guo et al. 2012 [81].

brain, and liver revealed no abnormalities in homeostasis or regenerative capacity [112]. Like many other publications claiming Oct4 expression in somatic stem cells, this study also noted low level of expression but that was regarded as false detection of Oct4 due to the noise of the detection methods, expression of pseudogenes, and expression of other POU-domain family members [112]. These conclusions are now in question. In our lab, the same Oct4floxed mice were used, in which two *LoxP* motifs had been inserted that span the proximal promoter and the Oct4A-unique first exon [2]. As the other 4 exons shared by *Oct4B* were not mutated, it is still possible that *Oct4*B can still be transcribed using an alternative promoter. After efficiently removing the floxed sequence of *Oct4* in oocytes by crossing the $Oct4^{flox/flox}$ mice with ZP3Cre transgenic mice, indeed, the expression of only Oct4B (not Oct4A) was detected at low levels by RT-PCR in the mutated oocytes and preimplantation embryos, which was confirmed by sequencing. Another recent report used a different Oct4-floxed mouse line to delete the entire Oct4 POU domain and C-domain, yet the authors of that study also observed full-term development of maternal Oct4–null embryos and TE/ICM lineage separation, as well as Nanog activation in maternal and zygotic Oct4–null embryos [39]. Taken together, these studies confirmed that (1) maternal Oct4 is indeed not essential for the establishment of totipotency. (2) The low levels of Oct4B expression could not rescue Oct4A-null embryos to maintain pluripotency *in vivo* [37,38]. The precise function of Oct4B in embryos and somatic stem cells remains to be clarified.

Conclusion

As summarized in Figure 4, new pieces of evidence clearly indicate that Oct4 is not the master regulator

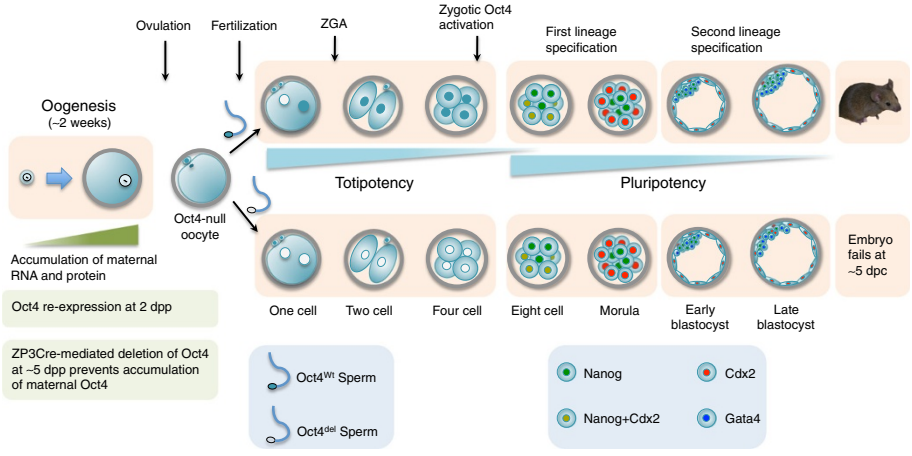

Figure 4 Development of maternal Oct4–depleted embryos. Totipotency in maternal Oct4–depleted embryos can be established in the absence of Oct4, and these embryos can maintain pluripotency and complete full-term development, supported by the zygotic activation of the paternal allele Oct4 gene at the late 4-cell stage. The lower panel shows that in the absence of both maternal and zygotic Oct4 expression, the Nanog-positive ICM and Cdx2-positive TE lineages are still established. However, this ICM cannot maintain pluripotency and complete the second lineage separation, and it fails to further develop at around the time of implantation. dpc: days post coitum; dpp: days post partum; ZGA: zygotic genome activation.

responsible for initiating totipotency-pluripotency in oocytes, and that maternal and zygotic Oct4–null blastocysts maintain the ability to activate Nanog and Oct4-GFP expression, indicating that unknown pathways other than the Oct4-centered pluripotency-regulating network are active in embryos and function upstream of Oct4 in driving pluripotency. However, to date no factors have proven to be essential for Oct4 activation in the preimplantation embryos. Further studies are required to elucidate how oocytes activate the pluripotent genes *Oct4* and *Nanog* on top of the Oct4/Sox2 autoregulatory loop in an effort to understand the establishment of totipotency in zygotes and in transplanted somatic cells.

Abbreviations

ICM: Inner cell mass; ESCs: Embryonic stem cells; TE: Trophectoderm; PGCs: Primordial germ cells; Pit-Oct-Unc: POU; POU_S: POU-specific; POU_{HD}: Homeodomain; N-domain: N-terminal domain; C-domain: C-terminal domain; BMP: Bone morphogenetic protein; LIF: Leukemia inhibitory factor; GV: Germinal vesicle; ZGA: Zygotic genome activation; MII: Metaphase II; SN: Surrounded nucleolus; NSN: Not surrounded nucleolus; NT: Nuclear transfer; PCR: Polymerase chain reaction; RT-PCR: Reverse transcriptase PCR; iPSCs: Induced pluripotent stem cells; DE: Distal enhancer; PE: Proximal enhancer; PP: Proximal promoter; HREs: Hormone response elements; SF1: Steroidogenic Factor-1; RXR-Beta: Retinoid X Receptor-Beta; GCNF: Germ Cell Nuclear Factor; COUF-TFI/II: Chicken Ovalbumin Upstream promoter-Transcription Factors- I/II; BRGC: Brg1-dependent chromatin remodeling complex; Eif4eloo: Oocyte-specific eukaryotic translation initiation factor 4E; AID: Activation-induced cytidine deaminase; siRNA: Small interfering RNA; RNAi: RNA interference; CRs: Conserved regions; dpc: Days post coitum; dpp: days post partum.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

GW drafted the manuscript. HRS and GW outlined, edited and revised the manuscript and all authors read and approved the final manuscript.

Acknowledgements

This research was supported by the Max Planck Society, DFG grant SI 1695/1-2 (SPP1356) and NIH grant R01HD059946-01 from the Eunice Kennedy Shriver National Institute of Child Health & Human Development. We thank Areti Malapetsas for final editing.

Received: 17 January 2014 Accepted: 25 April 2014
Published: 29 April 2014

References

1. Scholer HR, Hatzopoulos AK, Balling R, Suzuki N, Gruss P: **A family of octamer-specific proteins present during mouse embryogenesis: evidence for germline-specific expression of an Oct factor.** *EMBO J* 1989, **8:**2543–2550.
2. Kehler J, Tolkunova E, Koschorz B, Pesce M, Gentile L, Boiani M, Lomeli H, Nagy A, McLaughlin KJ, Scholer HR, Tomilin A: **Oct4 is required for primordial germ cell survival.** *EMBO Rep* 2004, **5:**1078–1083.
3. Pesce M, Scholer HR: **Oct-4: gatekeeper in the beginnings of mammalian development.** *Stem Cells* 2001, **19:**271–278.
4. Palmieri SL, Peter W, Hess H, Scholer HR: **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* 1994, **166:**259–267.
5. Rosner MH, Vigano MA, Ozato K, Timmons PM, Poirier F, Rigby PW, Staudt LM: **A POU-domain transcription factor in early stem cells and germ cells of the mammalian embryo.** *Nature* 1990, **345:**686–692.
6. Yeom YI, Ha HS, Balling R, Scholer HR, Artzt K: **Structure, expression and chromosomal location of the Oct-4 gene.** *Mech Dev* 1991, **35:**171–179.
7. Okamoto K, Okazawa H, Okuda A, Sakai M, Muramatsu M, Hamada H: **A novel octamer binding transcription factor is differentially expressed in mouse embryonic cells.** *Cell* 1990, **60:**461–472.
8. Scholer HR, Dressler GR, Balling R, Rohdewohld H, Gruss P: **Oct-4: a germline-specific transcription factor mapping to the mouse t-complex.** *Embo J* 1990, **9:**2185–2195.
9. Yeom YI, Fuhrmann G, Ovitt CE, Brehm A, Ohbo K, Gross M, Hubner K, Scholer HR: **Germline regulatory element of Oct-4 specific for the totipotent cycle of embryonal cells.** *Development* 1996, **122:**881–894.
10. Scholer HR, Balling R, Hatzopoulos AK, Suzuki N, Gruss P: **Octamer binding proteins confer transcriptional activity in early mouse embryogenesis.** *Embo J* 1989, **8:**2551–2557.
11. Scholer HR, Ruppert S, Suzuki N, Chowdhury K, Gruss P: **New type of POU domain in germ line-specific protein Oct-4.** *Nature* 1990, **344:**435–439.
12. Campbell PA, Perez-Iratxeta C, Andrade-Navarro MA, Rudnicki MA: **Oct4 targets regulatory nodes to modulate stem cell function.** *Plos One* 2007, **2:**e553.
13. Babaie Y, Herwig R, Greber B, Brink TC, Wruck W, Groth D, Lehrach H, Burdon T, Adjaye J: **Analysis of Oct4-dependent transcriptional networks regulating self-renewal and pluripotency in human embryonic stem cells.** *Stem Cells* 2007, **25:**500–510.
14. Nichols J, Zevnik B, Anastassiadis K, Niwa H, Klewe-Nebenius D, Chambers I, Scholer H, Smith A: **Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4.** *Cell* 1998, **95:**379–391.
15. Osorno R, Tsakiridis A, Wong F, Cambray N, Economou C, Wilkie R, Blin G, Scotting PJ, Chambers I, Wilson V: **The developmental dismantling of pluripotency is reversed by ectopic Oct4 expression.** *Development* 2012, **139:**2288–2298.
16. Okumura-Nakanishi S, Saito M, Niwa H, Ishikawa F: **Oct-3/4 and Sox2 regulate Oct-3/4 gene in embryonic stem cells.** *J Biol Chem* 2005, **280:**5307–5317.
17. Ding J, Xu H, Faiola F, Ma'ayan A, Wang J: **Oct4 links multiple epigenetic pathways to the pluripotency network.** *Cell Res* 2012, **22:**155–167.
18. van den Berg DL, Snoek T, Mullin NP, Yates A, Bezstarosti K, Demmers J, Chambers I, Poot RA: **An Oct4-centered protein interaction network in embryonic stem cells.** *Cell Stem Cell* 2010, **6:**369–381.
19. Wang J, Rao S, Chu J, Shen X, Levasseur DN, Theunissen TW, Orkin SH: **A protein interaction network for pluripotency of embryonic stem cells.** *Nature* 2006, **444:**364–368.
20. Chen X, Xu H, Yuan P, Fang F, Huss M, Vega VB, Wong E, Orlov YL, Zhang W, Jiang J, Loh YH, Yeo HC, Yeo ZX, Narang V, Govindarajan KR, Leong B, Shahab A, Ruan Y, Bourque G, Sung WK, Clarke ND, Wei CL, Ng HH: **Integration of external signaling pathways with the core transcriptional network in embryonic stem cells.** *Cell* 2008, **133:**1106–1117.
21. Rodda DJ, Chew JL, Lim LH, Loh YH, Wang B, Ng HH, Robson P: **Transcriptional regulation of nanog by OCT4 and SOX2.** *J Biol Chem* 2005, **280:**24731–24737.
22. Masui S, Nakatake Y, Toyooka Y, Shimosato D, Yagi R, Takahashi K, Okochi H, Okuda A, Matoba R, Sharov AA, Ko MS, Niwa H: **Pluripotency governed by Sox2 via regulation of Oct3/4 expression in mouse embryonic stem cells.** *Nat Cell Biol* 2007, **9:**625–635.
23. Pesce M, Anastassiadis K, Scholer HR: **Oct-4: lessons of totipotency from embryonic stem cells.** *Cells Tissues Organs* 1999, **165:**144–152.
24. Jaenisch R, Young R: **Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming.** *Cell* 2008, **132:**567–582.
25. Bachvarova R, De Leon V: **Polyadenylated RNA of mouse ova and loss of maternal RNA in early development.** *Dev Biol* 1980, **74:**1–8.
26. Ram PT, Schultz RM: **Reporter gene expression in G2 of the 1-cell mouse embryo.** *Dev Biol* 1993, **156:**552–556.
27. Schultz RM: **Regulation of zygotic gene activation in the mouse.** *Bioessays* 1993, **15:**531–538.
28. Campbell KH, McWhir J, Ritchie WA, Wilmut I: **Sheep cloned by nuclear transfer from a cultured cell line.** *Nature* 1996, **380:**64–66.
29. Gurdon JB: **Adult frogs derived from the nuclei of single somatic cells.** *Dev Biol* 1962, **4:**256–273.
30. Li L, Zheng P, Dean J: **Maternal control of early mouse development.** *Development* 2010, **137:**859–870.

31. Zuccotti M, Giorgi Rossi P, Martinez A, Garagna S, Forabosco A, Redi CA: **Meiotic and developmental competence of mouse antral oocytes.** *Biol Reprod* 1998, **58:**700–704.
32. Zuccotti M, Merico V, Sacchi L, Bellone M, Brink TC, Bellazzi R, Stefanelli M, Redi CA, Garagna S, Adjaye J: **Maternal Oct-4 is a potential key regulator of the developmental competence of mouse oocytes.** *BMC Dev Biol* 2008, **8:**97.
33. Foygel K, Choi B, Jun S, Leong DE, Lee A, Wong CC, Zuo E, Eckart M, Reijo Pera RA, Wong WH, Yao MW: **A novel and critical role for Oct4 as a regulator of the maternal-embryonic transition.** *Plos One* 2008, **3:**e4109.
34. Tan MH, Au KF, Leong DE, Foygel K, Wong WH, Yao MW: **An Oct4-Sall4-Nanog network controls developmental progression in the pre-implantation mouse embryo.** *Mol Syst Biol* 2013, **9:**632.
35. Mitsui K, Tokuzawa Y, Itoh H, Segawa K, Murakami M, Takahashi K, Maruyama M, Maeda M, Yamanaka S: **The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells.** *Cell* 2003, **113:**631–642.
36. Sakaki-Yumoto M, Kobayashi C, Sato A, Fujimura S, Matsumoto Y, Takasato M, Kodama T, Aburatani H, Asashima M, Yoshida N, Nishinakamura R: **The murine homolog of SALL4, a causative gene in Okihiro syndrome, is essential for embryonic stem cell proliferation, and cooperates with Sall1 in anorectal, heart, brain and kidney development.** *Development* 2006, **133:**3005–3013.
37. Wu G, Han D, Gong Y, Sebastiano V, Gentile L, Singhal N, Adachi K, Fischedick G, Ortmeier C, Sinn M, Radstaak M, Tomilin A, Scholer HR: **Establishment of totipotency does not depend on Oct4A.** *Nat Cell Biol* 2013, **15:**1089–1097.
38. Frum T, Halbisen MA, Wang C, Amiri H, Robson P, Ralston A: **Oct4 Cell-Autonomously Promotes Primitive Endoderm Development in the Mouse Blastocyst.** *Dev Cell* 2013, **25:**610–622.
39. Le Bin GC, Muñoz-Descalzo S, Kurowski A, Leitch H, Lou X, Mansfield W, Etienne-Dumeau C, Grabole N, Mulas C, Niwa H, Hadjantonakis A-K, Nichols J: **Oct4 is required for lineage priming in the developing inner cell mass of the mouse blastocyst.** *Development* 2014, **141:**1001–1010.
40. Boiani M, Eckardt S, Scholer HR, McLaughlin KJ: **Oct4 distribution and level in mouse clones: consequences for pluripotency.** *Genes Dev* 2002, **16:**1209–1219.
41. Yang X, Smith SL, Tian XC, Lewin HA, Renard JP, Wakayama T: **Nuclear reprogramming of cloned embryos and its implications for therapeutic cloning.** *Nat Genet* 2007, **39:**295–302.
42. Takahashi K, Yamanaka S: **Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors.** *Cell* 2006, **126:**663–676.
43. Kim JB, Sebastiano V, Wu G, Arauzo-Bravo MJ, Sasse P, Gentile L, Ko K, Ruau D, Ehrich M, van den Boom D, Meyer J, Hubner K, Bernemann C, Ortmeier C, Zenke M, Fleischmann BK, Zaehres H, Scholer HR: **Oct4-induced pluripotency in adult neural stem cells.** *Cell* 2009, **136:**411–419.
44. Niwa H, Miyazaki J, Smith AG: **Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells.** *Nat Genet* 2000, **24:**372–376.
45. Niwa H, Toyooka Y, Shimosato D, Strumpf D, Takahashi K, Yagi R, Rossant J: **Interaction between Oct3/4 and Cdx2 determines trophectoderm differentiation.** *Cell* 2005, **123:**917–929.
46. Yuan P, Han J, Guo G, Orlov YL, Huss M, Loh YH, Yaw LP, Robson P, Lim B, Ng HH: **Eset partners with Oct4 to restrict extraembryonic trophoblast lineage potential in embryonic stem cells.** *Genes Dev* 2009, **23:**2507–2520.
47. Lu CW, Yabuuchi A, Chen L, Viswanathan S, Kim K, Daley GQ: **Ras-MAPK signaling promotes trophectoderm formation from embryonic stem cells and mouse embryos.** *Nat Genet* 2008, **40:**921–926.
48. Karwacki-Neisius V, Goke J, Osorno R, Halbritter F, Ng JH, Weisse AY, Wong FC, Gagliardi A, Mullin NP, Festuccia N, Colby D, Tomlinson SR, Ng HH, Chambers I: **Reduced oct4 expression directs a robust pluripotent state with distinct signaling activity and increased enhancer occupancy by oct4 and nanog.** *Cell Stem Cell* 2013, **12:**531–545.
49. Radzisheuskaya A, Chia Gle B, dos Santos RL, Theunissen TW, Castro LF, Nichols J, Silva JC: **A defined Oct4 level governs cell state transitions of pluripotency entry and differentiation into all embryonic lineages.** *Nat Cell Biol* 2013, **15:**579–590.
50. Blij S, Frum T, Akyol A, Fearon E, Ralston A: **Maternal Cdx2 is dispensable for mouse development.** *Development* 2012, **139:**3969–3972.
51. Strumpf D, Mao CA, Yamanaka Y, Ralston A, Chawengsaksophak K, Beck F, Rossant J: **Cdx2 is required for correct cell fate specification and differentiation of trophectoderm in the mouse blastocyst.** *Development* 2005, **132:**2093–2102.
52. Wu G, Gentile L, Fuchikami T, Sutter J, Psathaki K, Esteves TC, Arauzo-Bravo MJ, Ortmeier C, Verberk G, Abe K, Scholer HR: **Initiation of trophectoderm lineage specification in mouse embryos is independent of Cdx2.** *Development* 2010, **137:**4159–4169.
53. Ralston A, Cox BJ, Nishioka N, Sasaki H, Chea E, Rugg-Gunn P, Guo G, Robson P, Draper JS, Rossant J: **Gata3 regulates trophoblast development downstream of Tead4 and in parallel to Cdx2.** *Development* 2010, **137:**395–403.
54. Marin M, Karis A, Visser P, Grosveld F, Philipsen S: **Transcription factor Sp1 is essential for early embryonic development but dispensable for cell growth and differentiation.** *Cell* 1997, **89:**619–628.
55. Gu P, Goodwin B, Chung AC, Xu X, Wheeler DA, Price RR, Galardi C, Peng L, Latour AM, Koller BH, Gossen J, Kliewer SA, Cooney AJ: **Orphan nuclear receptor LRH-1 is required to maintain Oct4 expression at the epiblast stage of embryonic development.** *Mol Cell Biol* 2005, **25:**3492–3505.
56. Barnea E, Bergman Y: **Synergy of SF1 and RAR in activation of Oct-3/4 promoter.** *J Biol Chem* 2000, **275:**6608–6619.
57. Pikarsky E, Sharir H, Ben-Shushan E, Bergman Y: **Retinoic acid represses Oct-3/4 gene expression through several retinoic acid-responsive elements located in the promoter-enhancer region.** *Mol Cell Biol* 1994, **14:**1026–1038.
58. Gu P, LeMenuet D, Chung AC, Mancini M, Wheeler DA, Cooney AJ: **Orphan nuclear receptor GCNF is required for the repression of pluripotency genes during retinoic acid-induced embryonic stem cell differentiation.** *Mol Cell Biol* 2005, **25:**8507–8519.
59. Ben-Shushan E, Sharir H, Pikarsky E, Bergman Y: **A dynamic balance between ARP-1/COUP-TFII, EAR-3/COUP-TFI, and retinoic acid receptor: retinoid X receptor heterodimers regulates Oct-3/4 expression in embryonal carcinoma cells.** *Mol Cell Biol* 1995, **15:**1034–1048.
60. Schoorlemmer J, van Puijenbroek A, van Den Eijnden M, Jonk L, Pals C, Kruijer W: **Characterization of a negative retinoic acid response element in the murine Oct4 promoter.** *Mol Cell Biol* 1994, **14:**1122–1136.
61. Park SW, Hu X, Gupta P, Lin YP, Ha SG, Wei LN: **SUMOylation of Tr2 orphan receptor involves Pml and fine-tunes Oct4 expression in stem cells.** *Nat Struct Mol Biol* 2007, **14:**68–75.
62. Ovitt CE, Scholer HR: **The molecular biology of Oct-4 in the early mouse embryo.** *Mol Hum Reprod* 1998, **4:**1021–1031.
63. Koziol MJ, Garrett N, Gurdon JB: **Tpt1 activates transcription of oct4 and nanog in transplanted somatic nuclei.** *Curr Biol* 2007, **17:**801–807.
64. Singhal N, Graumann J, Wu G, Arauzo-Bravo MJ, Han DW, Greber B, Gentile L, Mann M, Scholer HR: **Chromatin-Remodeling Components of the BAF Complex Facilitate Reprogramming.** *Cell* 2010, **141:**943–955.
65. Chuang YS, Huang WH, Park SW, Persaud SD, Hung CH, Ho PC, Wei LN: **Promyelocytic leukemia protein in retinoic acid-induced chromatin remodeling of Oct4 gene promoter.** *Stem Cells* 2011, **29:**660–669.
66. Evsikov AV, Graber JH, Brockman JM, Hampl A, Holbrook AE, Singh P, Eppig JJ, Solter D, Knowles BB: **Cracking the egg: molecular dynamics and evolutionary aspects of the transition from the fully grown oocyte to embryo.** *Genes Dev* 2006, **20:**2713–2727.
67. Rajkovic A, Yan C, Yan W, Klysik M, Matzuk MM: **Obox, a family of homeobox genes preferentially expressed in germ cells.** *Genomics* 2002, **79:**711–717.
68. Zhang J, Tam WL, Tong GQ, Wu Q, Chan HY, Soh BS, Lou Y, Yang J, Ma Y, Chai L, Ng HH, Lufkin T, Robson P, Lim B: **Sall4 modulates embryonic stem cell pluripotency and early embryonic development by the transcriptional regulation of Pou5f1.** *Nat Cell Biol* 2006, **8:**1114–1123.
69. Buganim Y, Faddah DA, Cheng AW, Itskovich E, Markoulaki S, Ganz K, Klemm SL, van Oudenaarden A, Jaenisch R: **Single-Cell Expression Analyses during Cellular Reprogramming Reveal an Early Stochastic and a Late Hierarchic Phase.** *Cell* 2012, **150:**1209–1222.
70. Guo G, Smith A: **A genome-wide screen in EpiSCs identifies Nr5a nuclear receptors as potent inducers of ground state pluripotency.** *Development* 2010, **137:**3185–3192.
71. Heng JC, Feng B, Han J, Jiang J, Kraus P, Ng JH, Orlov YL, Huss M, Yang L, Lufkin T, Lim B, Ng HH: **The Nuclear Receptor Nr5a2 Can Replace Oct4 in the Reprogramming of Murine Somatic Cells to Pluripotent Cells.** *Cell Stem Cell* 2010, **6:**167–174.

72. Falco G, Lee SL, Stanghellini I, Bassey UC, Hamatani T, Ko MS: **Zscan4: a novel gene expressed exclusively in late 2-cell embryos and embryonic stem cells.** *Dev Biol* 2007, **307:**539–550.
73. Hirata T, Amano T, Nakatake Y, Amano M, Piao Y, Hoang HG, Ko MS: **Zscan4 transiently reactivates early embryonic genes during the generation of induced pluripotent stem cells.** *Sci Rep* 2012, **2:**208.
74. Bhutani N, Brady JJ, Damian M, Sacco A, Corbel SY, Blau HM: **Reprogramming towards pluripotency requires AID-dependent DNA demethylation.** *Nature* 2010, **463:**1042–1047.
75. Ding L, Paszkowski-Rogacz M, Nitzsche A, Slabicki MM, Heninger AK, de Vries I, Kittler R, Junqueira M, Shevchenko A, Schulz H, Hubner N, Doss MX, Sachinidis A, Hescheler J, Iacone R, Anastassiadis K, Stewart AF, Pisabarro MT, Caldarelli A, Poser I, Theis M, Buchholz F: **A genome-scale RNAi screen for Oct4 modulators defines a role of the Paf1 complex for embryonic stem cell identity.** *Cell Stem Cell* 2009, **4:**403–415.
76. Takeda J, Seino S, Bell GI: **Human Oct3 gene family: cDNA sequences, alternative splicing, gene organization, chromosomal location, and expression at low levels in adult tissues.** *Nucleic Acids Res* 1992, **20:**4613–4620.
77. Mizuno N, Kosaka M: **Novel variants of Oct-3/4 gene expressed in mouse somatic cells.** *J Biol Chem* 2008, **283:**30997–31004.
78. Lee J, Kim HK, Rho JY, Han YM, Kim J: **The human OCT-4 isoforms differ in their ability to confer self-renewal.** *J Biol Chem* 2006, **281:**33554–33565.
79. Papamichos SI, Lambropoulos AF, Kotoula V: **OCT4B expression in PBMNCs suggests the existence of an alternative OCT4 promoter.** *Genes Chromosomes Cancer* 2009, **48:**1112–1114.
80. Gao Y, Wang X, Han J, Xiao Z, Chen B, Su G, Dai J: **The novel OCT4 spliced variant OCT4B1 can generate three protein isoforms by alternative splicing into OCT4B.** *J Genet Genomics* 2010, **37:**461–465.
81. Guo CL, Liu L, Jia YD, Zhao XY, Zhou Q, Wang L: **A novel variant of Oct3/4 gene in mouse embryonic stem cells.** *Stem Cell Res* 2012, **9:**69–76.
82. Farashahi Yazd E, Rafiee MR, Soleimani M, Tavallaei M, Salmani MK, Mowla SJ: **OCT4B1, a novel spliced variant of OCT4, generates a stable truncated protein with a potential role in stress response.** *Cancer Lett* 2011, **309:**170–175.
83. Asadi MH, Mowla SJ, Fathi F, Aleyasin A, Asadzadeh J, Atlasi Y: **OCT4B1, a novel spliced variant of OCT4, is highly expressed in gastric cancer and acts as an antiapoptotic factor.** *Int J Cancer* 2011, **128:**2645–2652.
84. D'Ippolito G, Diabira S, Howard GA, Menei P, Roos BA, Schiller PC: **Marrow-isolated adult multilineage inducible (MIAMI) cells, a unique population of postnatal young and old human cells with extensive expansion and differentiation potential.** *J Cell Sci* 2004, **117:**2971–2981.
85. D'Ippolito G, Diabira S, Howard GA, Roos BA, Schiller PC: **Low oxygen tension inhibits osteogenic differentiation and enhances stemness of human MIAMI cells.** *Bone* 2006, **39:**513–522.
86. Goolsby J, Marty MC, Heletz D, Chiappelli J, Tashko G, Yarnell D, Fishman PS, Dhib-Jalbut S, Bever CT Jr, Pessac B, Trisler D: **Hematopoietic progenitors express neural genes.** *Proc Natl Acad Sci USA* 2003, **100:**14926–14931.
87. Izadpanah R, Trygg C, Patel B, Kriedt C, Dufour J, Gimble JM, Bunnell BA: **Biologic properties of mesenchymal stem cells derived from bone marrow and adipose tissue.** *J Cell Biochem* 2006, **99:**1285–1297.
88. Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M, Du J, Aldrich S, Lisberg A, Low WC, Largaespada DA, Verfaillie CM: **Pluripotency of mesenchymal stem cells derived from adult marrow.** *Nature* 2002, **418:**41–49.
89. Johnson J, Bagley J, Skaznik-Wikiel M, Lee HJ, Adams GB, Niikura Y, Tschudy KS, Tilly JC, Cortes ML, Forkert R, Spitzer T, Iacomini J, Scadden DT, Tilly JL: **Oocyte generation in adult mammalian ovaries by putative germ cells in bone marrow and peripheral blood.** *Cell* 2005, **122:**303–315.
90. Lamoury FM, Croitoru-Lamoury J, Brew BJ: **Undifferentiated mouse mesenchymal stem cells spontaneously express neural and stem cell markers Oct-4 and Rex-1.** *Cytotherapy* 2006, **8:**228–242.
91. Moriscot C, de Fraipont F, Richard MJ, Marchand M, Savatier P, Bosco D, Favrot M, Benhamou PY: **Human bone marrow mesenchymal stem cells can express insulin and key transcription factors of the endocrine pancreas developmental pathway upon genetic and/or microenvironmental manipulation in vitro.** *Stem Cells* 2005, **23:**594–603.
92. Nayernia K, Lee JH, Drusenheimer N, Nolte J, Wulf G, Dressel R, Gromoll J, Engel W: **Derivation of male germ cells from bone marrow stem cells.** *Lab Invest* 2006, **86:**654–663.
93. Pallante BA, Duignan I, Okin D, Chin A, Bressan MC, Mikawa T, Edelberg JM: **Bone marrow Oct3/4+ cells differentiate into cardiac myocytes via age-dependent paracrine mechanisms.** *Circ Res* 2007, **100:**e1–e11.
94. Pochampally RR, Smith JR, Ylostalo J, Prockop DJ: **Serum deprivation of human marrow stromal cells (hMSCs) selects for a subpopulation of early progenitor cells with enhanced expression of OCT-4 and other embryonic genes.** *Blood* 2004, **103:**1647–1652.
95. Ren H, Cao Y, Zhao Q, Li J, Zhou C, Liao L, Jia M, Zhao Q, Cai H, Han ZC, Yang R, Chen G, Zhao RC: **Proliferation and differentiation of bone marrow stromal cells under hypoxic conditions.** *Biochem Biophys Res Commun* 2006, **347:**12–21.
96. Wang R, Li J, Yashpal N: **Phenotypic analysis of c-Kit expression in epithelial monolayers derived from postnatal rat pancreatic islets.** *J Endocrinol* 2004, **182:**113–122.
97. Sagrinati C, Netti GS, Mazzinghi B, Lazzeri E, Liotta F, Frosali F, Ronconi E, Meini C, Gacci M, Squecco R, Carini M, Gesualdo L, Francini F, Maggi E, Annunziato F, Lasagni L, Serio M, Romagnani S, Romagnani P: **Isolation and characterization of multipotent progenitor cells from the Bowman's capsule of adult human kidneys.** *J Am Soc Nephrol* 2006, **17:**2443–2456.
98. Gupta S, Verfaillie C, Chmielewski D, Kren S, Eidman K, Connaire J, Heremans Y, Lund T, Blackstad M, Jiang Y, Luttun A, Rosenberg ME: **Isolation and characterization of kidney-derived stem cells.** *J Am Soc Nephrol* 2006, **17:**3028–3040.
99. Romagnani P, Annunziato F, Liotta F, Lazzeri E, Mazzinghi B, Frosali F, Cosmi L, Maggi L, Lasagni L, Scheffold A, Kruger M, Dimmeler S, Marra F, Gensini G, Maggi E, Romagnani S: **CD14 + CD34low cells with stem cell phenotypic and functional features are the major source of circulating endothelial progenitors.** *Circ Res* 2005, **97:**314–322.
100. Tondreau T, Meuleman N, Delforge A, Dejeneffe M, Leroy R, Massy M, Mortier C, Bron D, Lagneaux L: **Mesenchymal stem cells derived from CD133-positive cells in mobilized peripheral blood and cord blood: proliferation, Oct4 expression, and plasticity.** *Stem Cells* 2005, **23:**1105–1112.
101. Cervello I, Martinez-Conejero JA, Horcajadas JA, Pellicer A, Simon C: **Identification, characterization and co-localization of label-retaining cell population in mouse endometrium with typical undifferentiated markers.** *Hum Reprod* 2007, **22:**45–51.
102. Matthai C, Horvat R, Noe M, Nagele F, Radjabi A, van Trotsenburg M, Huber J, Kolbus A: **Oct-4 expression in human endometrium.** *Mol Hum Reprod* 2006, **12:**7–10.
103. Thomas T, Nowka K, Lan L, Derwahl M: **Expression of endoderm stem cell markers: evidence for the presence of adult stem cells in human thyroid glands.** *Thyroid* 2006, **16:**537–544.
104. Ling TY, Kuo MD, Li CL, Yu AL, Huang YH, Wu TJ, Lin YC, Chen SH, Yu J: **Identification of pulmonary Oct-4+ stem/progenitor cells and demonstration of their susceptibility to SARS coronavirus (SARS-CoV) infection in vitro.** *Proc Natl Acad Sci USA* 2006, **103:**9530–9535.
105. Okuda T, Tagawa K, Qi ML, Hoshio M, Ueda H, Kawano H, Kanazawa I, Muramatsu M, Okazawa H: **Oct-3/4 repression accelerates differentiation of neural progenitor cells in vitro and in vivo.** *Brain Res Mol Brain Res* 2004, **132:**18–30.
106. Davis SF, Hood J, Thomas A, Bunnell BA: **Isolation of adult rhesus neural stem and progenitor cells and differentiation into immature oligodendrocytes.** *Stem Cells Dev* 2006, **15:**191–199.
107. Beltrami AP, Cesselli D, Bergamin N, Marcon P, Rigo S, Puppato E, D'Aurizio F, Verardo R, Piazza S, Pignatelli A, Poz A, Baccarani U, Damiani D, Fanin R, Mariuzzi L, Finato N, Masolini P, Burelli S, Belluzzi O, Schneider C, Beltrami CA: **Multipotent cells can be generated in vitro from several adult human organs (heart, liver, and bone marrow).** *Blood* 2007, **110:**3438–3446.
108. Dyce PW, Zhu H, Craig J, Li J: **Stem cells with multilineage potential derived from porcine skin.** *Biochem Biophys Res Commun* 2004, **316:**651–658.
109. Dyce PW, Wen L, Li J: **In vitro germline potential of stem cells derived from fetal porcine skin.** *Nat Cell Biol* 2006, **8:**384–390.
110. Kues WA, Petersen B, Mysegades W, Carnwath JW, Niemann H: **Isolation of murine and porcine fetal stem cells from somatic tissue.** *Biol Reprod* 2005, **72:**1020–1028.

111. Yu H, Fang D, Kumar SM, Li L, Nguyen TK, Acs G, Herlyn M, Xu X: **Isolation of a novel population of multipotent adult stem cells from human hair follicles.** *Am J Pathol* 2006, **168:**1879–1888.
112. Lengner CJ, Camargo FD, Hochedlinger K, Welstead GG, Zaidi S, Gokhale S, Scholer HR, Tomilin A, Jaenisch R: **Oct4 expression is not required for mouse somatic stem cell self-renewal.** *Cell Stem Cell* 2007, **1:**403–415.

doi:10.1186/2045-9769-3-7
Cite this article as: Wu and Schöler: **Role of Oct4 in the early embryo development.** *Cell Regeneration* 2014 **3**:7.