Review

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Chromatin accessibility dynamics during cell fate reprogramming

Dongwei Li[1,2,3,4], Xiaodong Shu[1,3,4], Ping Zhu[2,*] & Duanqing Pei[1,3,4,5,**]

Abstract

Genome architecture and chromatin dynamics govern the fate and identify of a cell. Recent advances in mapping chromatin landscapes offer valuable tools for the acquisition of accurate information regarding chromatin dynamics. Here we discuss recent findings linking chromatin dynamics to cell fate control. Specifically, chromatin undergoes a binary off/on switch during iPSC reprogramming, closing and opening loci occupied by somatic and pluripotency transcription factors, respectively. This logic of a binary off/on switch may also be operational in cell fate control during normal development and implies that further approaches could potentially be developed to direct cell fate changes both *in vitro* and *in vivo*.

Keywords chromatin dynamics; reprogramming; stem cell

Subject Categories Chromatin, Transcription & Genomics; Post-translational Modifications & Proteolysis; Stem Cells & Regenerative Medicine

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See the Glossary for abbreviations used in this article.

Introduction

The discovery of the DNA double helix by Francis Crick and James Watson in 1953 marked a milestone in our quest to understand life's most basic events such as cell fate control. Fifty years later, the complete sequence of the human genome was obtained, which paved the way for rapid advances in almost all aspects of biological science and medicine. For example, sequencing of human tumor DNA has unraveled the mutation landscapes associated with different types of cancer and led to the development of many novel targeted therapies such as kinase inhibitors (LaCasse *et al*, 2008; Yap *et al*, 2009; Goldstein *et al*, 2011). On the other hand, sequencing of RNA obtained from single cells provides a rapid, yet increasingly informative dataset describing the state of each cell, ushering a new discipline called single cell biology (Tang *et al*, 2010; Buenrostro *et al*, 2015b). The genomic and transcriptomic information will continue to drive future advances in the life sciences.

Cell fate control is a fundamental question in biology. A fertilized egg, through a finite series of cell division, develops into an individual. All descendant cells share the same genomic sequence, however, they have distinct gene expression patterns and specific cell fates, suggesting that the genome encodes higher order information beyond the linear DNA sequence. We are starting now to understand these instructions within the realm of epigenetic regulation. Many components of the epigenetic machinery have been worked out over the years, including enzymes that modify the two components of the chromatin, DNA, and histones (Mirsky & Pollister, 1946). For example, DNA can be modified in many ways, but mostly on cytosine with methylation, and likewise histones are modified by methylation, acetylation, phosphorylation, and other marks (Murray, 1964; Ellgaard, 1967; Gutierrez & Hnilica, 1967; Chavez *et al*, 2010). Physically, DNA is wrapped around nucleosomes made of histones and further packaged into two types of chromatin: euchromatin, which is an active state and heterochromatin which is a repressed state, corresponding to on and off states controlling gene expression. It is this epigenetic machinery that controls the specific packaging and expression of the genome within each cell to specify its identity and fate.

Recent advances have made it possible to interrogate the genome beyond the primary DNA sequence, such as transcriptome sequencing (RNA-seq) (Morin *et al*, 2008), transcription factor binding site sequencing (ChIP-seq) (Johnson *et al*, 2007), chromatin accessibility sequencing (DNase-seq and ATAC-seq) (Boyle *et al*, 2008; Buenrostro *et al*, 2013; Buenrostro *et al*, 2015a), and 3C-based methods to study higher order chromatin conformation and associated gene expression (3C-seq, 4C-seq, 5C-seq, HiC-seq) (Dekker *et al*, 2002; Lieberman-Aiden *et al*, 2009). These approaches have provided a

1 CAS Key Laboratory of Regenerative Biology, South China Institutes for Stem Cell Biology and Regenerative Medicine, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou, China
2 Guangdong Cardiovascular Institute, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Guangzhou, China
3 Guangdong Provincial Key Laboratory of Stem Cell and Regenerative Medicine, South China Stem Cell and Regenerative Medicine, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou, China
4 Guangzhou Regenerative Medicine and Health GUANGDONG Laboratory, Guangzhou Institutes of Biomedicine and Health, Chinese Academic of Sciences, Guangzhou, China
5 Laboratory of Cell Fate Control, School of Life Sciences, Westlake University, Hangzhou, China
*Corresponding author. Tel: +86 2083827712; E-mail: tanganqier@163.com
**Corresponding author. Tel: +86 0571 86886859; E-mail: peiduanqing@westlake.edu.cn

Glossary

3C-seq	chromosome conformation capture sequencing
3D	3 dimensional
4C-seq	chromosome conformation capture on chip sequencing
5C-seq	chromosome conformation capture carbon copy sequencing
5mC	5 methylcytosine
ATAC-seq	Assay for Transposase-Accessible Chromatin using sequencing
ChIP-seq	chromatin immunoprecipitation and sequencing
CO	transition from closed to open chromatin
DbAs	DNA demethylation before accessibility
DNase-seq	DNase treatment and sequencing
DNMT	DNA methyltransferase
ESC	embryonic stem cell
FAIRE-seq	Formaldehyde Assisted Isolation of Regulatory Elements
H2AK119	histone 2A lysine 119
H3K27ac	histone 3 lysine 27 acetylation
H3K27me3	histone 3 lysine 27 trimethylation
H3K4me3	histone 3 lysine 4 trimethylation
H3K9me3	histone 3 lysine 9 trimethylation
HDAC3	histone deacetylase 3
HiC-seq	high-throughput chromosome conformation capture sequencing
ICM	inner cell mass
iPSC	induced pluripotent stem cell
Kdm	lysine demethylases
MEFs	mouse embryonic fibroblasts
MET	mesenchymal-to-epithelial transition
MNase-seq	micrococcal nuclease treatment and sequencing
NCoR	nuclear receptor co repressor
NOMe-seq	Nucleosome Occupancy and Methylome sequencing
OC	transition from open to closed chromatin
OSKM	Oct4, Sox2, Klf4, Myc
PNT	primed to naive transition
PRC2	polycomb repressive complex 2
SCNT	somatic cell nuclear transfer
SIN3A	SIN3 transcription regulator family member A
SUMO	small ubiquitin-related modifier
TETs	Ten-eleven translocation methylcytosine dioxygenases
TF	transcription factor
TSS	transcription start site

very rich dataset to describe the genome and its output in great details that impact both basic biology as well as pathophysiological mechanisms of diseases, most notably, cancer. In particular, ATAC-seq provides unique information about the accessibility of the entire genome based on the ability of the Tn5 transposon to insert into open chromatin loci, thus linking the linear DNA sequence and 3D genome structure. These approaches may help unravel critical principles in cell fate control.

Pluripotent stem cells, either derived from blastocysts such as ESCs, or induced from somatic cells such as iPSCs, have become attractive models to analyze cell fate control as they possess the potential to differentiate into any cell type (Evans & Kaufman, 1981; Martin, 1981). The process of inducing somatic cells to become pluripotent stem cells was first introduced by Takahashi and Yamanaka (2006). By introducing four transcription factors, namely Oct4, Sox2, Klf4, and Myc (OSKM) that are normally expressed in ESCs at high levels, into fibroblasts from 13.5 days mouse embryos (MEFs), Yamanaka and colleagues generated ESC-like colonies they named iPSCs. iPSCs were subsequently shown to be able to give rise to healthy mice when injected into 4n mouse embryos, proving that they are indistinguishable from ESCs (Takahashi & Yamanaka, 2006). In this review, we discuss recent advances in mapping the chromatin landscapes and dynamics during iPSC reprogramming and differentiation, and propose a binary logic of open/closed chromatin to control cell fate and identity.

Yamanaka factors reprogram somatic epigenomes to pluripotent epigenomes

Reprogramming somatic cells to a pluripotent state requires the removal of somatic marks from the genome and the establishment of a pluripotent state. One direct way to assess this dramatic change is to map the epigenetic modifications during reprogramming. Extensive efforts have been devoted to map epigenetic marks, i.e., histone acetylation/methylation and DNA methylation, in three main reprogramming strategies including the classical Yamanaka approach (OSKM) (Takahashi & Yamanaka, 2006), the use of small chemicals (Hou *et al*, 2013; Cao *et al*, 2018) and expressing non-Yamanaka factors (seven factors) (Wang *et al*, 2019) (Fig 1). The classic Yamanaka approach has been applied mostly to mouse embryonic fibroblast (MEF)-virus infection reprogramming systems, MEFs-secondary reprogramming systems (i.e., MEFs carrying integrated doxycycline-inducible OSKM from iPSC-derived mice), or B-cell secondary reprogramming systems (i.e., B cells from iPSC-derived mice) (Stadtfeld *et al*, 2010; Cheloufi *et al*, 2015; Di Stefano *et al*, 2016; Chronis *et al*, 2017; Knaupp *et al*, 2017; Li *et al*, 2017). Among the major findings, it appears that H3K9me3 is a key barrier for reprogramming (Chen *et al*, 2013b; Wei *et al*, 2017). On the other hand, the role of DNA methylation in the reprogramming process appears to be more complex, as massive demethylation was observed, but the DNA modification enzymes, especially TET1, were found to both activate and inhibit reprogramming depending on the absence or presence of vitamin C (Chen *et al*, 2013a).

Several studies focused on the ignition of reprogramming by the Yamanaka factors. First, they appear to mediate chromatin remodeling by directly or indirectly binding to silent genomic loci to promote the expression of associated genes (Li *et al*, 2017; Zviran *et al*, 2019). These findings are consistent with those of pioneer factors that can initiate binding to chromatin at silent chromatin loci and then direct the binding of other transcription factors (Cirillo *et al*, 2002; Zaret & Carroll, 2011; Soufi *et al*, 2012; Soufi *et al*, 2015). The activation of pluripotency enhancers by pioneer factors appears to occur in a stepwise fashion (Fig 1) (Hansson *et al*, 2012; Polo *et al*, 2012; Cacchiarelli *et al*, 2015; Hochedlinger & Jaenisch, 2015; Chronis *et al*, 2017; Knaupp *et al*, 2017). Chronis *et al* (2017) further reported that during the initiation process of reprogramming, OSK bind to active somatic enhancers and initiate their inactivation.

The contributions of individual Yamanaka factors have also been investigated. For example, Oct4, the most important reprogramming

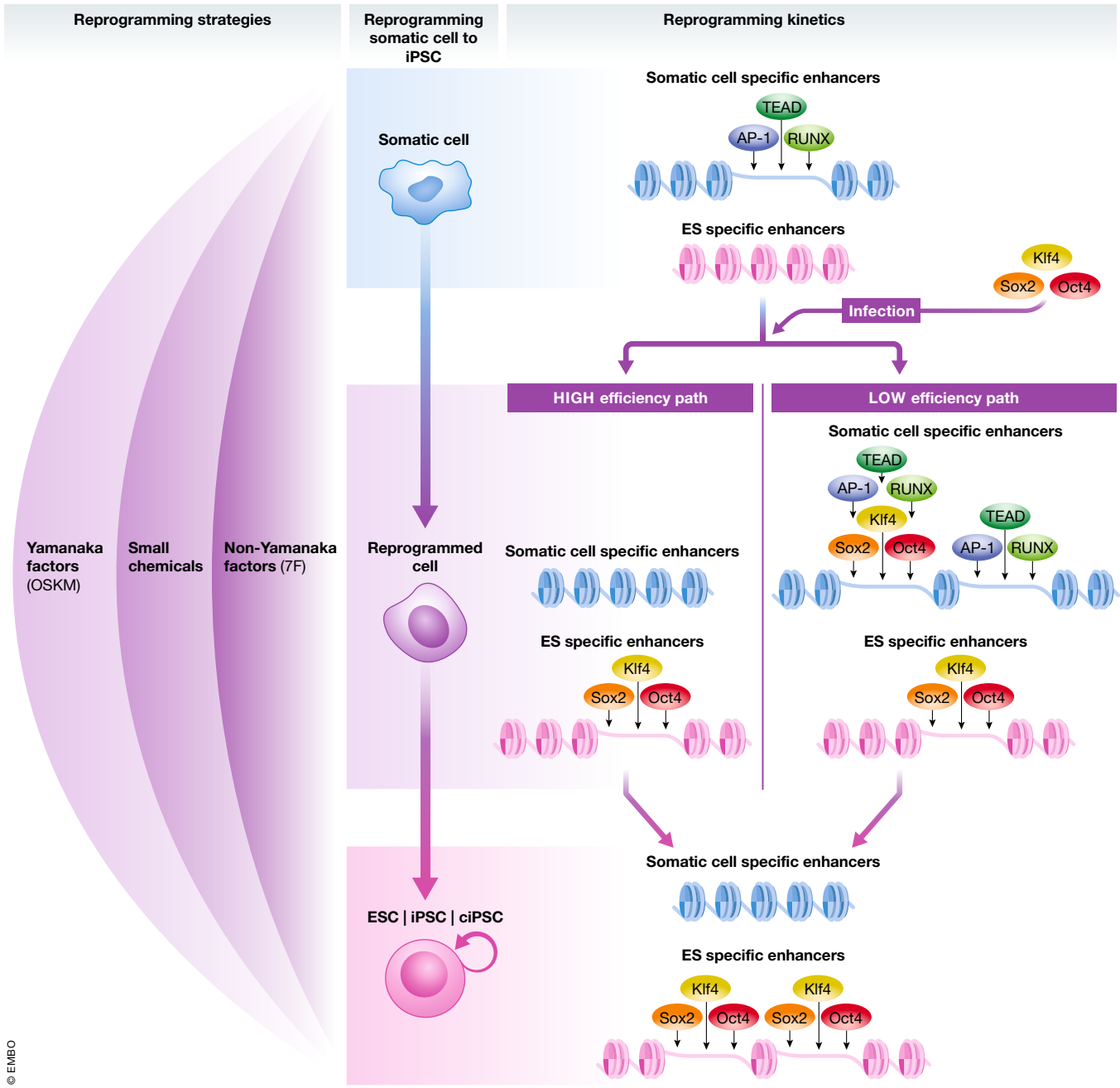

Figure 1. Hypothetic model of OSK binding to somatic and pluripotent enhancers.

Three main approaches for reprogramming are shown on the left. The stages of reprogramming are shown in the middle. Reprogramming kinetics are shown on the right. Upon entry into somatic cells, OSK find ESC-specific pluripotent enhancers (left arrow "high efficiency path") or both somatic and pluripotent enhancers (right arrow "low efficiency path"), resulting in differences in reprogramming efficiencies.

factor, binds to condensed heterochromatin and promotes its opening (Chen *et al*, 2020a). Mechanistically, Oct 4 appears to activate the pluripotency network-related genes in a stepwise fashion, ie., early, intermediate, and late during reprogramming, suggesting that Oct4 binds to these loci gradually to activate their expression, often accompanied by H3K27me3/H3K4me3/H3K27ac modifications (Chen *et al*, 2016). Oct4 also binds to some NCoR targets at early stages of reprogramming to promote H3K27ac deacetylation and silencing of somatic cell identity (Penalosa-Ruiz *et al*, 2020). However, in a relatively low efficiency reprogramming system, Oct4 also induces the expression of inappropriate genes during MEF reprogramming, perhaps reflecting off-target effects that lower reprogramming efficiency (Chronis *et al*, 2017; Cossec *et al*, 2018; Velychko *et al*, 2019). A recent study further suggests that Oct4

appears to be detrimental for reprogramming, as SKM are sufficient to induce iPSCs with better developmental potentials (Velychko *et al*, 2019). These studies suggest that each reprogramming factor may also have detrimental effects on reprogramming (Chen *et al*, 2011b).

Like Oct4, Sox2 binds heterochromatin and facilitates chromatin opening at loci containing pluripotency genes at an early reprogramming stage (Soufi *et al*, 2012; Cheloufi *et al*, 2015; Li *et al*, 2017; Malik *et al*, 2019). Sox2 targets were more strongly enriched for nucleosomes than Oct4 and Klf4 targets, suggesting a stronger pioneer function for Sox2 (Soufi *et al*, 2015). Single factor overexpression experiments in MEFs showed that Sox2 can induce more opening and closing loci than Klf4 and Oct4. The combination of Sox2 and Klf4 further contributes to the opening of most chromatin during early stages of reprogramming and promotes the mesenchymal-to-epithelial transition (MET) process (Li *et al*, 2010; Chen *et al*, 2011b; Li *et al*, 2017).

One recent study reported that Klf4 binds to 30% of its binding sites in ESCs at early reprogramming stage and promotes chromatin opening, which leads to metabolic changes and cell-junction organization (Di Giammartino *et al*, 2019). Another study shows Klf4 binding to poised chromatin loci that are related to MET (Chen *et al*, 2020a), which is consistent with the report that Klf4 activates MET during early phases of reprogramming (Li *et al*, 2010; Chen *et al*, 2011b). The study by Di Giammartino *et al* (2019) shows that Klf4 is also involved in the rebuilding of pluripotent enhancer-promoter contacts and activation of pluripotency-associated genes during iPSCs formation.

Lastly, c-Myc facilitates NCoR/SMRT-HDAC3 binding to both somatic and pluripotent loci to mediate histone deacetylation in the early phase of reprogramming (Zhuang *et al*, 2018; Zviran *et al*, 2019). In addition, c-Myc activates the expression of chromatin modifiers such as PRC2 members and Wdr5, which in turn enhances the reprogramming process (Neri *et al*, 2012; Rao *et al*, 2015; Thomas *et al*, 2015; Fagnocchi & Zippo, 2017). Although several studies suggest that c-Myc is dispensable for reprogramming (Martinez-Fernandez *et al*, 2009; Chen *et al*, 2010; Nakagawa *et al*, 2010), inhibition of endogenous c-Myc expression significantly reduces reprogramming (Zviran *et al*, 2019) indicating a positive role of c-Myc in iPSCs formation.

The binding of Yamanaka factors to their targets is inhibited by additional factors. For example, suppression of histone chaperone CAF-1 decreases H3K9me3 levels and increases accessible chromatin that promotes the binding of Sox2 to pluripotency-specific targets and enhances reprogramming efficiency (Cheloufi *et al*, 2015). In the B-cell reprogramming system, a pulse of C/EBPα induces the conversion of B cells to Bα' cells that can be efficiently reprogrammed into iPSCs with the Yamanaka factors OSKM (Di Stefano *et al*, 2014). Mechanistically, C/EBPα stimulates the expression of chromatin modifying enzymes such as Lsd1 and Brd4 and enhances chromatin accessibility at loci enriched with pluripotency factors such as Oct4, Sox2, and Klf4 (Di Stefano *et al*, 2016).

In addition to those Yamanaka factors, Esrrb, Tfap2c, Dppa2, and Dppa4 can facilitate chromatin opening and improve reprogramming efficiency (Adachi *et al*, 2018; Hernandez *et al*, 2018; Pastor *et al*, 2018). Together, these results support the general hypothesis that reprogramming factors orchestrate global epigenetic changes necessary for the erasure of somatic marks and the establishment of pluripotent ones.

Epigenetic modifications and chromatin dynamics during iPSC formation

Epigenetic modifications such as histone modification and DNA methylation during reprogramming have been extensively studied. Huang *et al* (2015) reported that also nucleosome profiles are different between somatic cells and pluripotent stem cells. Nucleosome reorganization and histone modifications are highly coordinated with cell fate transition (Voss & Hager, 2014; Yadav *et al*, 2018; Ninova *et al*, 2019; Penalosa-Ruiz *et al*, 2019). Histone H3K9me3 modification, that is often associated with repressive heterochromatin, is a barrier in somatic cell reprogramming that inhibits the transition of pre-iPSCs into mature iPSCs (Chen *et al*, 2013b; Wei *et al*, 2017). Heterochromatin protein 1 (HP1) recognizes H3K9 methylation and recruits the H3K9 methyltransferase Suv39h1 to promote H3K9me3 modification and heterochromatin formation. BMP4 stimulates the activity of the Suv39h1/Setdb1/Ehmt2 complex, promotes H3K9me3 on Oct4/Nanog promoters, and thus inhibits the activation of pluripotency genes (Chen *et al*, 2013b). On the other hand, vitamin c (Vc) induces demethylation of H3K9me3 by stimulating the activity of the histone demethylase Kdm3/4 and enhances the efficiency of somatic cell reprogramming (Wang *et al*, 2011). H3K9me3 is also a blockade in somatic cell nuclear transfer (SCNT) mediated reprogramming (Jullien *et al*, 2010; Matoba *et al*, 2014), indeed, injection of H3K9me3 demethylase Kdm4 mRNA into enucleated oocytes could greatly improve SCNT efficiency (Matoba *et al*, 2014; Chung *et al*, 2015; Liu *et al*, 2016; Liu *et al*, 2018; Chen *et al*, 2020b). Those results suggest that H3K9me3 is associated with closed chromatin to maintain cell identity, thus being an obstacle for somatic reprogramming.

Histone H3K27me3 modification, that is associated with reduced DNA accessibility, is another repressive chromatin modification. The activation of pluripotent genes during somatic reprogramming involves two waves of H3K27 modification: the first is the removal of H3K27me3 to induce chromatin opening, followed by the formation of H3K27ac at the pluripotent promoters to form an active state (Polo *et al*, 2012; Hussein *et al*, 2014; Zviran *et al*, 2019). The MEF-specific genes are silenced in a reversed fashion: The removal of H3K27ac is followed by the accumulation of repressive histone modifications such as H3K27me and H3K9me3 (Chen *et al*, 2013b; Cheloufi *et al*, 2015; Wei *et al*, 2017; Zviran *et al*, 2019). In line with the global loss of H3K27me3, the expression of the H3K27me3 demethylases Kdm6 and Kdm7 is upregulated in the early stages of reprogramming (Hussein *et al*, 2014). KDM6 depletion leads to H3K27me3 accumulation and inhibits chromatin opening and iPSCs formation (Mansour *et al*, 2012). Taken together, these results suggest that the dynamics of H3K27me3 correlate with the shutdown of the somatic program and the establishment of pluripotency.

DNA methylation is an important epigenetic regulation during mammalian early embryo development. Dynamic DNA methylation changes occur during early embryogenesis: a global DNA demethylation process from fertilization to the ICM stage is followed by the re-methylation of DNA at epiblast stage (Li & Zhang, 2014; Messerschmidt *et al*, 2014; Okae *et al*, 2014; Wang *et al*, 2014; Guo *et al*,

2014a; Guo *et al*, 2014b; Guo *et al*, 2017; Iurlaro *et al*, 2017; Zhang *et al*, 2018; Zhu *et al*, 2018; Zeng & Chen, 2019). In somatic reprogramming, DNA methylation functions as a barrier for pluripotency acquisition (Mikkelsen *et al*, 2008; Wang *et al*, 2013; Hu *et al*, 2014). Demethylation of enhancers of pluripotency genes occurs gradually and in a cascaded manner by TF-guided recruitment of TET proteins to designated chromatin loci (Li *et al*, 2018; Sardina *et al*, 2018; Schwarz *et al*, 2018). Tet2-dependent DNA demethylation occurs before chromatin opening at distinct chromatin loci and was termed DbAs (demethylation before accessibility) (Sardina *et al*, 2018). Zviran *et al* (2019) also reported that DNA demethylation precedes chromatin opening and promotes OSK-mediated activation of pluripotency enhancers contributing to efficient reprogramming. *De novo* DNA methylation and the silencing of MEF-specific genes occur at later stages of reprogramming. However, deletion of DNMT does not prevent iPSCs formation, indicating DNA re-methylation is not required for reprogramming (Pawlak & Jaenisch, 2011; Schwarz *et al*, 2018). These studies signify that DNA methylation is required for maintaining somatic cell identity but dispensable for establishing pluripotency.

In conclusion, chromatin modifications such as H3K9me3 \H3K27me3\DNA methylation function to maintain a somatic chromatin state that must be removed during somatic reprogramming (Fig 2).

An interface between puripotent and somatic states

Reprogramming by defined factors has revealed unexpected principles about cell fate control. On a practical note, reprogramming can be fast and efficient when performed under optimized conditions, in contrast to the general belief that it is slow and inefficient (Chen *et al*, 2010; Chen *et al*, 2011a) . Initially, reprogramming was investigated with the hope to understand the underlying signaling pathways and epigenetic changes as discussed above. On a conceptual level, we propose that there is an interface between somatic and pluripotent fates that both differentiating and reprogramming cells must cross (Fig 3). The initial evidence came from our investigation of c-JUN in reprogramming. Based on the fact that c-JUN inhibits reprogramming mediated by the Yamanaka factors, we proposed that c-JUN behaves as a guardian of somatic fate and its inhibition opens the gate to pluripotency at the interface (Liu *et al*, 2015). We further propose that c-JUN and its related AP1 family of TFs, with the help of additional networks of factors, cooperate to specify somatic fates as opposed to the pluripotent state guarded by the Oct4-Sox2-Nanog system.

Under this frame of a somatic/pluripotent interface, one may view the classic Yamanaka reprogramming as OSKM, upon transduction in a somatic cell, pushing cell identity through the interface by shutting down the somatic program to achieve a pluripotent

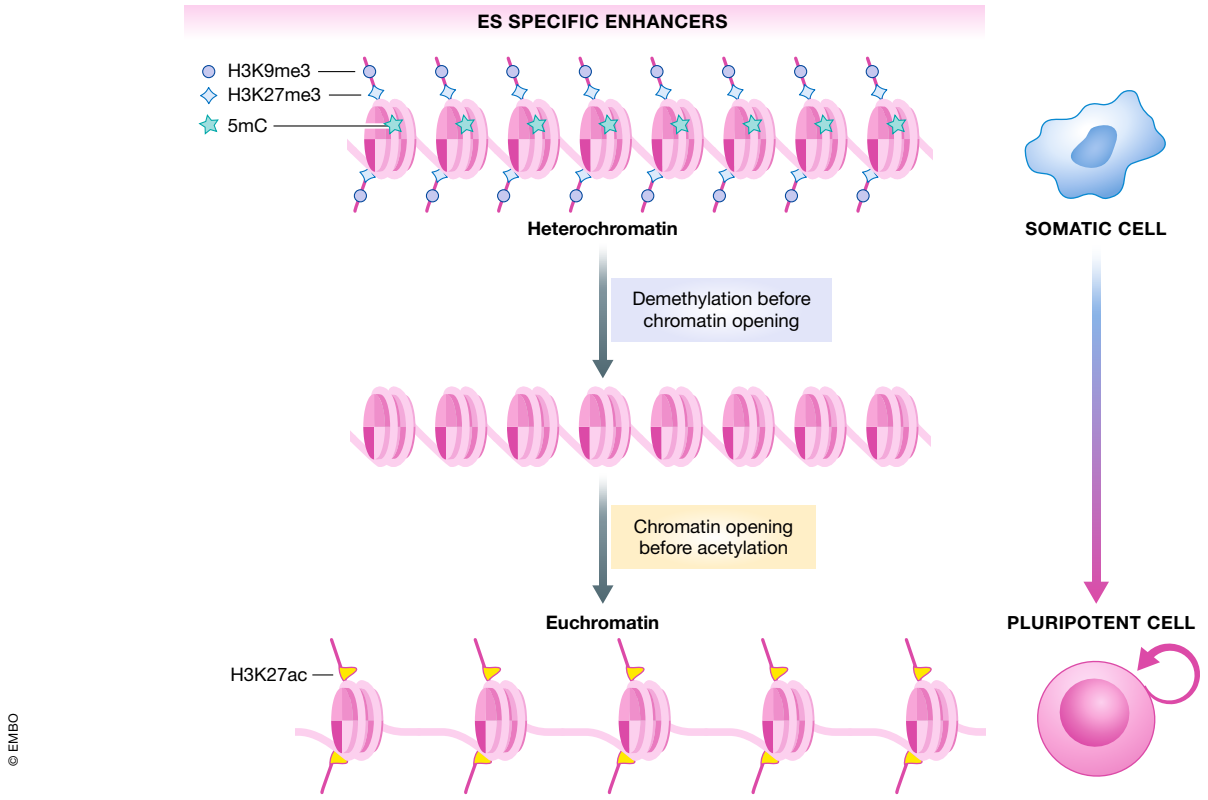

Figure 2. Epigenetic modification is coupled with chromatin accessibility dynamics during reprogramming.

During reprogramming, silenced ESC-specific enhancers are activated by two steps, first by demethylation of H3K9me3/H3K27me3/5mC to remove epigenetic barriers; second by opening ESC-specific enhancers and acetylation of the reprogrammed chromatin to keep it active.

state. However, OSK have been shown to be dispensable for the inactivation of somatic enhancers (Li *et al*, 2017; Zviran *et al*, 2019). This suggests that there must be other, OSK-induced factors that function to shut down the somatic program (Pei, 2009). Indeed, this hypothesis was validated by screening candidate genes resulting in the identification of Sap30, a member of the SIN3A complex, that promotes H3K27ac deacetylation, facilitates chromatin open to close transition (OC) and inactivates key somatic genes (Li *et al*, 2017). Mechanistically, Sap30 binds to the TSS of somatic TFs and removes H3K27ac to suppresses somatic genes such as c-Jun/Fosl1/Fosl2/Zeb2/Runx1. Overexpression of these factors blocks reprogramming, suggesting that they are gatekeepers for somatic cell identities.

Recently, SUMO, a post-translational modifier, has been reported as a cell fate keeper that maintains cell type-specific transcriptional programs by binding to transcription start sites (TSSs) enriched with the active epigenetic modifications H3K4me3 and H3K27ac (Borkent *et al*, 2016; Cossec *et al*, 2018). Depleting Ubc9 to disrupt sumoylation led to higher reprogramming efficiency by downregulating the expression of fibroblast related genes, such as Zeb1/Zeb2/Snai1 and upregulating the pluripotency-associated genes Nanog/Sall4/Dppa3/Nr0b1. At the chromatin level, downregulation of Ubc9 facilitates the closing of MEF-specific open enhancers and reduces OSK binding to fibroblast-specific genes like Snai1/Twist1/Zeb2. On the other hand, it promotes OSK binding to ESC super-enhancers and stimulates the expression of the pluripotency-associated genes Oct4/Sox2/Sall1 and the MET-related genes Cdh1/Epcam/Cldn1 (Cossec *et al*, 2018).

In addition to AP-1 families and SUMO (Cossec *et al*, 2018), NCoR complex (Zhuang *et al*, 2018; Zviran *et al*, 2019) and EHMT1/2 also have been reported to sustain fibroblastic gene expression and inhibit MET during iPSC formation (preprint: Vidal *et al*, 2019). Loss of HMGN nucleosome-binding proteins enhances the kinetics of iPSCs formation by accelerating the closing of MEF-specific open chromatin, while the differentiation potential of iPSCs is unaffected (He *et al*, 2018), suggesting that HMGNs function to stabilize cell fate rather than determining cell identity. Mybl2 is a somatic cell gatekeeper that inhibits the opening of chromatin to activate MET genes in the early stage of reprogramming (Ward *et al*, 2018). Mbd3 maintains the somatic cell fate and depletion of Mbd3 strongly promotes MEF reprogramming to iPSCs (Luo *et al*, 2013; Rais *et al*, 2013). ChIP-seq data analysis shows that Mbd3 directly binds to OKSM target sites and inhibits gene activation. Chd1 is another protein that promotes pluripotency by its ability to open chromatin to activate an ESCs regulatory network (Gaspar-Maia *et al*, 2009).

Overall, the identities of somatic cells and pluripotent stem cells are clearly maintained by transcription factors, chromatin remodeling factors, and epigenetic modifying enzymes (Fig 3). During cell fate transition, the active/open chromatin of cell type-specific regulatory elements should be closed and new loci need to open to form specific, new cell identities.

A binary logic for cell fate control

As discussed above, the conversion of somatic to pluripotent cells involves the activation of pluripotency gene regulatory networks and the inactivation of somatic networks. While signaling and epigenetic studies have clearly demonstrated several novel mechanisms that play a role, no unifying principle has emerged that explains the entire reprogramming process.

Direct assessment of chromatin states represents a new approach in analyzing cell fate control. There are several techniques to identify accessible chromatin, such as DNase-seq (Boyle *et al*, 2008; Hesselberth *et al*, 2009), FAIRE-seq (Giresi *et al*, 2007; Simon *et al*, 2012), MNase-seq (Schones *et al*, 2008), ATAC-seq (Buenrostro *et al*, 2013; Buenrostro *et al*, 2015b) (Cusanovich *et al*, 2015), and NOMe-seq (Kelly *et al*, 2012; Pott, 2017; Clark *et al*, 2018). Among these methods, the assay for transposase-accessible chromatin (ATAC-seq), which uses a hyperactive Tn5 transposase to label

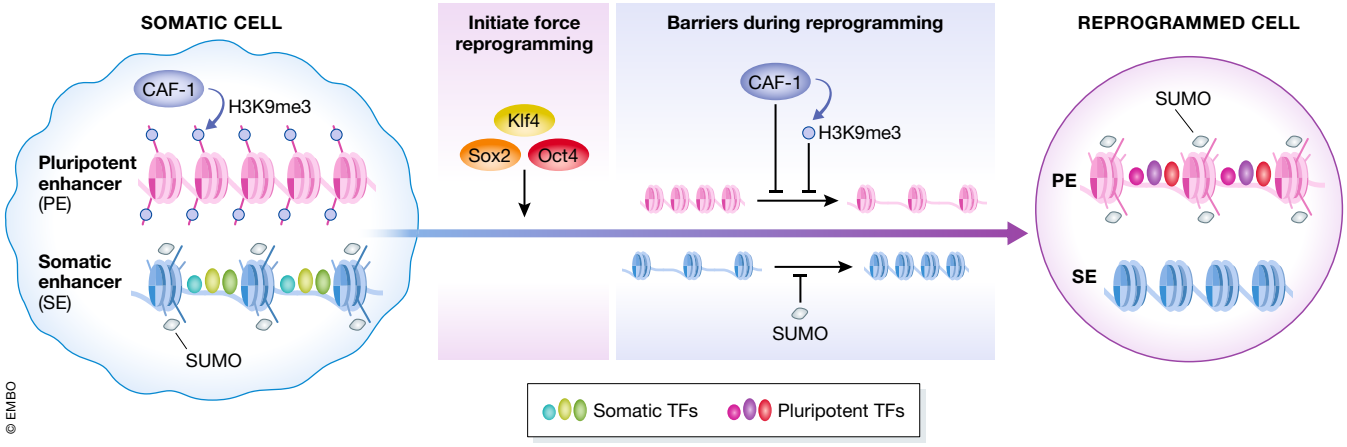

Figure 3. Interfaces between pluripotent and somatic cell states.
In each cell type, there are transcription factors, chromatin remodeling factors and epigenetic modifying enzymes that maintain a specific cell identity and resist reprogramming unless a specific group of genes is activated to alter cell fate.

fragments of open chromatin, is the most popular technique used in both cultured cells and clinical tissue samples (Table 1).

Recently, we systematically reanalyzed the dynamics of chromatin accessibility by ATAC-seq in Yamanaka factor-induced reprogramming of MEFs (Li *et al*, 2017). We found that shortly after the ectopic expression of OSK in MEFs during the early stage of reprogramming, the open somatic enhancers (O) are closed (C) quickly (hereafter OC). On the other hand, the closed pluripotency-related enhancers are gradually opened (hereafter CO) by OSK directly to establish the pluripotency network. Schwarz *et al* (2018) reported similar chromatin accessibility dynamics, i.e., the rapid closing of MEF-specific regions at day 3 in reprogramming-efficient (SSEA-1^+CD73^+Sca-1^-) cells followed by the opening of pluripotency-related loci at day 6 in reprogramming-efficient (SSEA-1^+EpCAM^+Sca-1^-) cells. We analyzed the transcription factor binding motifs in the OC/CO categories and found that pluripotency-associated TF motifs only appeared in CO peaks. OCT/SOX/KLF are the most enriched pluripotency factors at these loci which is in agreement with their reported pioneer function (Soufi *et al*, 2015). Other pluripotency factors such as TCF/TFCP2L1/ESRRB only appeared in later stages, suggesting their roles in establishing the mature iPSCs state (Fig 4). Esrrb has been reported to enhance reprogramming by recruiting the core pluripotency factors Oct4/Sox2 and Nanog to closed chromatin to activate ESC super-enhancers, indicating an important prerequisite for the co-binding of pioneer factors to specific enhancers (Adachi *et al*, 2018). A recent study shows that Oct4 loosens heterochromatin and facilitates Klf4 binding to epithelial genes to promote MET, as Oct4 deficiency blocks Klf4 binding and inhibits MET (Chen *et al*, 2020a). On the other hand, OC peaks are enriched in motifs of somatic TFs, such as members of the AP-1/TEAD/RUNX family. It indicates that OSK reprogramming factors are not responsible for the closing of somatic enhancers (Li *et al*, 2017; Zviran *et al*, 2019).

Do pluripotent TFs and somatic TFs bind to the same enhancers? Two studies with low efficiency (< 1%) reprogramming systems found that OSK bind to SUMO and H3K27ac modified somatic enhancers that are co-occupied by AP1/RUNX/TEAD (Fig 1) (Chronis *et al*, 2017; Cossec *et al*, 2018). However, studies in highly efficient reprogramming systems (> 50%) revealed that OSK do not bind to closed somatic enhancers at any stage during reprogramming (Fig 1) (Li *et al*, 2017; Zviran *et al*, 2019). We noted that enhancers that are open only in the intermediate stage, but closed at both the start and end of reprogramming, are enriched with both OSK and somatic AP-1 super family TFs during reprogramming of MEF to iPSCs (Fig 4) (Li *et al*, 2017).

Similar open and closed chromatin dynamics were observed in the inducible OSKM reprogramming system as well as in the B-cell reprograming system. Somatic enhancers are rapidly closed (OC processing) while pluripotent enhancers are activated in a stepwise manner (CO processing) (Cheloufi *et al*, 2015; Di Stefano *et al*, 2016; Knaupp *et al*, 2017). Furthermore, similar OC/CO chromatin dynamics were observed in chemically induced pluripotent stem cells (ciPSCs), BMP4-driven primed to naive transition (PNT) and human iPSCs formation (Cao *et al*, 2018; Liu *et al*, 2020; Yu *et al*, 2020). The open chromatin in the starting cell population was quickly closed after small chemical treatment and the pluripotent loci were opened step by step, accompanied by somatic TF inactivation and pluripotency TF activation. Interestingly, quick closing of the somatic transcription program of donor cells was also reported in SCNT (Djekidel *et al*, 2018). After injecting the somatic nucleus into the enucleated oocyte, donor chromatin rapidly changes from the open to the closed state, and the 3D chromatin architecture reorganizes (Djekidel *et al*, 2018; Miyamoto *et al*, 2018; Chen *et al*, 2020b). However, during normal fertilization, chromatin transitions to a more open state with less chromatin being closed during development from the zygote to the ICM stage, suggesting that a special chromatin remodeling mechanism controls the developmental path (Lu *et al*, 2016; Wu *et al*, 2016; Gao *et al*, 2018; Wu *et al*, 2018).

Based on the understanding of chromatin dynamics during somatic reprogramming, Wang *et al* (2019) successfully established a novel MEF reprogramming system with Jdp2-Jhdm1b-Mkk6-Glis1-Nanog-Esrrb-Sall4 7 non-Yamanaka factors (7F-iPSCs). In this reprogramming system, Sall4 regulates both chromatin opening and closing, Glis1 promotes chromatin opening and Jdp2 promotes

Table 1. The methods to study chromatin accessibility dynamics.

Method	Approach to fragment DNA	How the method works	Cell number	Number of articles published[a]	References
Dnase-seq	DNase digestion	Digestion of DNA that is not protected by proteins to identify accessible DNA	1–10 million	205	Boyle *et al* (2008), Hesselberth *et al* (2009)
FAIRE-seq	Sonication	Extracts unprotected DNA fragments with formaldehyde to obtain accessible DNA	> 10 million	69	Giresi *et al* (2007), Simon *et al* (2012)
Mnase-seq	MNase digestion	Digestion of linker DNA between two nucleosomes, obtains genome-wide nucleosome profile	1–10 million	99	Schones *et al* (2008)
ATAC-seq	Tn5 transposition	Inserts index DNA fragments into nucleosome and TF-free areas to identify both accessible DNA and partial nucleosome profiles	1–50,000	4292	Buenrostro *et al* (2013), Buenrostro *et al* (2015b), Cusanovich *et al* (2015)
NOMe-seq	Sonication	Extracts DNA after M.CviPI treatment and bisulfite conversion to obtain nucleosome profiles and DN A methylation information	1–1 million	20	Kelly *et al* (2012), Pott (2017), Clark *et al* (2018)

[a]Data are retrieved from the NCBI website as of 07/2020.

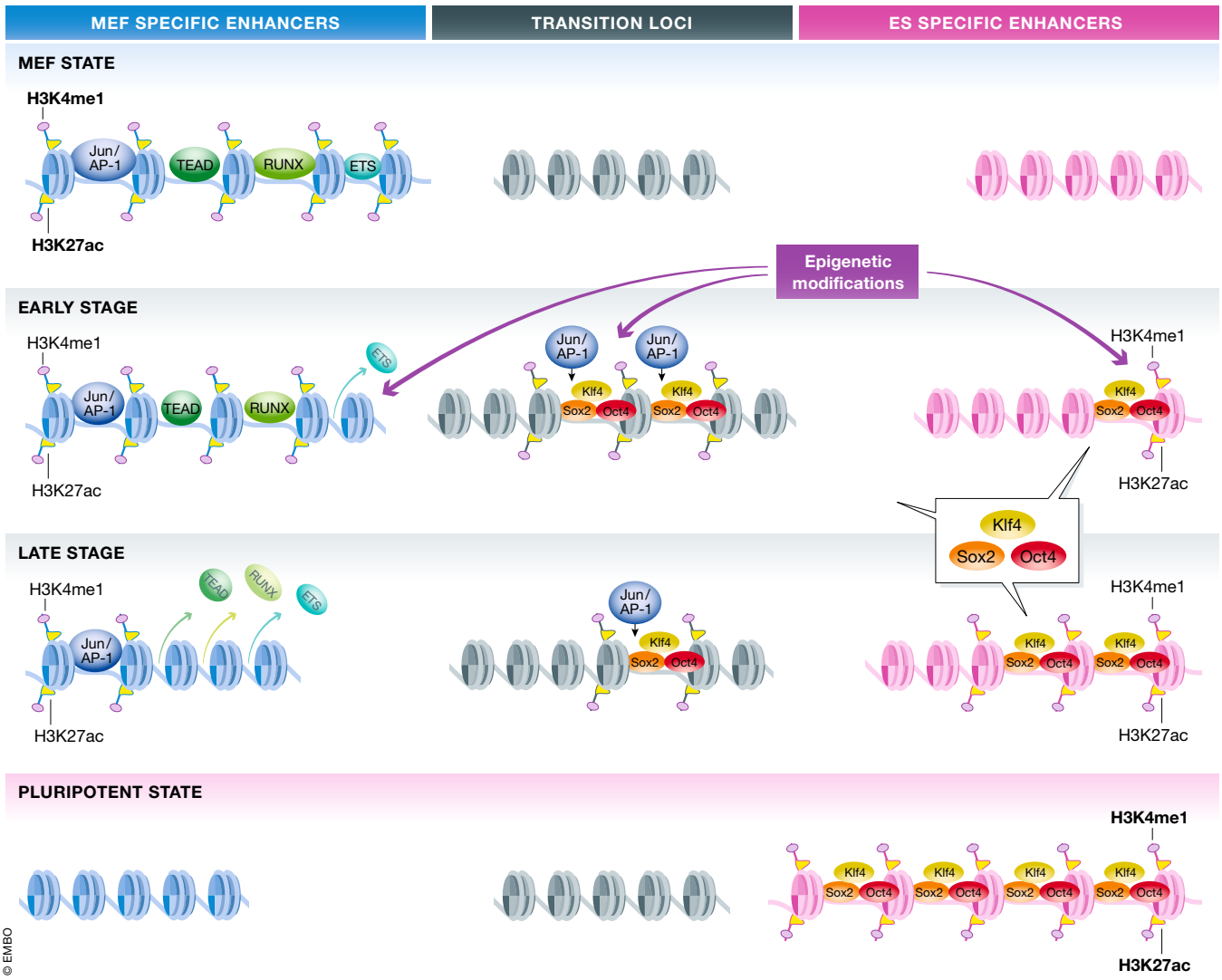

Figure 4. TFs and epigenetic modifiers drive chromatin dynamics.

During reprogramming, OSK first bind to closed ES-specific enhancers and induce chromatin opening (close to open, CO) to activate epigenetic modifiers such as Sap30, which promote the shutdown of the open chromatin (open to close, OC) bound by somatic factors. Thus, CO/OC dynamics control the reprogramming path until full iPSCs formation.

chromatin closing. Furthermore, Esrrb/Jdp2/Glis1/Sall4/Nanog function cooperatively to activate pluripotency genes. Thus, conserved chromatin accessibility dynamics are observed during cell fate transitions in both somatic reprogramming and SCNT.

Future perspectives

Cell type-specific chromatin conformation is stable and inheritable. In this review, we discussed TFs and epigenetic modifiers driving chromatin dynamics (CO/OC) in somatic cell reprogramming. The Yamanaka factor-induced somatic reprogramming provides a valuable system to investigate chromatin dynamics during cell fate conversions. It is established that Yamanaka factors function as pioneer factors that bind to closed chromatin and expel the nucleosome to create an open DNA sequence for other factors to bind (Soufi *et al*, 2012; Soufi *et al*, 2015). Somatic cell-specific loci are closed quickly while pluripotency loci are gradually activated. Meanwhile, higher order chromatin structure is established stepwise during the process while the underlying mechanism remains to be investigated (Stadhouders *et al*, 2018; Stadhouders *et al*, 2019).

ATAC-seq allows to measure open and closed chromatin directly. Studies carried out in early embryonic development, lineage cell differentiation, or somatic cell reprogramming indicate that chromatin structure is very flexible and reprogrammable. During differentiation, lineage specific factors function to shut down previously accessible loci and open new chromatin loci to establish a lineage specific gene regulatory network. The reprogramming of somatic cells to pluripotent stem cells is an artificially induced cell fate conversion. Our analysis of CO/OC changes during reprogramming reveals that CO loci are highly enriched with Oct4, Sox2, Klf4, the core

pluripotency transcription factors identified by Yamanaka. Yamanaka factors have distinct roles in chromatin remodeling, for example, Sox2 and Klf4 function to open pluripotency loci in early stages, whereas Oct4 fully establishes the pluripotency state. The Yamanaka factors also induce secondary response factors such as Sap30 that function to overcome intermediate states between somatic and pluripotent identities. With the conceptual framework of mixed somatic/pluripotent interfaces, as well as the mechanistic insight from the binary CO/OC model, we envision further development of concepts and models that may help to explain cell fate control.

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Author contributions

DP initiated and supervised the project. DL, XS, PZ and DP wrote the manuscript together. PZ and DP provided support throughout the project. and DP approved the final version.

Conflict of interest

The authors declare that they have no conflict of interest.

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