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Epigenetic regulation of somatic cell reprogramming

Yixuan Wang, Yan Bi and Shaorong Gao

Pluripotent stem cells, having self-renewal capacities and multi-lineage differentiation abilities, offer great potential in disease modeling and therapeutic applications. The successful generation of induced pluripotent stem cells (iPSCs) by the Yamanaka group in 2006 is a milestone event in both reprogramming and stem cell research fields, which makes *in vitro* somatic cell reprogramming and personalized stem cell therapy feasible. During the past 10 years, several important progresses have been made in uncovering the molecular mechanisms involved in the reprogramming process, which shed light on improving the reprogramming efficiency and iPSC quality. Here, we briefly review the important progresses in the epigenetic regulation including histone and DNA modifications during somatic cell reprogramming.

Address
Clinical and Translational Research Center of Shanghai First Maternity and Infant Hospital, Shanghai Key Laboratory of Signaling and Disease Research, School of Life Sciences and Technology, Tongji University, Shanghai 200092, China

Corresponding authors: Wang, Yixuan (wangyixuan@tongji.edu.cn), Gao, Shaorong (gaoshaorong@tongji.edu.cn)

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Introduction

Somatic cell reprogramming, which involves erasing the somatic memories and obtaining pluripotent state similar to that of embryonic stem cells (ESCs), represents the research trend in the field of stem cell biology. iPSCs, generated by ectopic expression of key transcriptional factors, exhibit similar characteristics to ESCs with a remarkable developmental plasticity and the capacity of indefinite self-renewal, offering significant prospects for disease modeling and potential clinical therapy. More importantly, the forced expression of a set of transcriptional factors can not only achieve somatic cell reprogramming, but also fulfill the conversions among different types of cells or trans-differentiation. Although the Yamanaka factors (Oct4, Sox2, Klf4, and c-Myc, OSKM) alone are able to reprogram somatic cells into iPSCs, the mechanisms involved in the reprogramming process are still in the blackbox. Recent work from Plath's lab demonstrates that collaborative interactions exist among Yamanaka factors and with stage-specific transcription factors, directing both somatic-enhancer inactivation and pluripotency-enhancer selections to orchestrate reprogramming events [1•]. Several studies dissecting the reprogramming process have shown that the transcriptional factors need assistants from different numbers of epigenetic modifiers including histone post-translational modifying enzymes, nucleosome remodeling factors, histone chaperones and newly discovered DNA modifying enzymes at different stages during somatic cell reprogramming [2–5]. These epigenetic modifiers, mainly recruited by specific transcriptional factors, help the reprogramming cells to reset and rebuild the epigenetic information stored on chromatin, breaking the concrete barriers between various cell types.

Different from the prokaryotic cells in which the expression of genes only needs naked DNA as templates, transcription of genes, which is the most fundamental biological process responsible for cell identities, in eukaryotic cells is tightly correlated with the chromatin state. Massive changes in the chromatin state, at DNA and/or histone levels can significantly influence the transcriptome and ultimately result in cell fate conversions. Comparison of the somatic state with the pluripotent state reveals that the somatic cells show a dense chromatin state (heterochromatin) while most stem cells exhibit an open and loose chromatin state (euchromatin) more feasible to accommodate quick changes on transcriptome. The reprogramming process is very inefficient to overcome such barriers. Thus, understanding the roles of epigenetic factors during reprogramming will help to improve the efficiency of iPSC technique and generate more qualified iPSCs during reprogramming. This review will focus on the current understanding of epigenetic regulators involved in somatic cell reprogramming.

Histone modifications and responsible enzymes in reprogramming

Chromatin marks, modifiers and readers can serve as both facilitators and impediments during somatic reprogramming process. The idea that H3K9me3-marked heterochromatin in somatic cells are early barriers to reprogramming is supported by the findings that depletion of several H3K9 methyltransferases including SUV39H1/H2, EHMT1, EHMT2, and SETDB1 in fibroblast cells increases the efficiency of iPSC production [5–7]. Dissection of human reprogramming process from

fibroblasts to iPSCs also discovers megabase-sized domains of the fibroblast genome refractory to OSKM binding with the dominant H3K9me3 chromatin feature during the 48 hours of reprogramming [6]. And most of these regions will become accessible in the minority of cells that become pluripotent [6]. In addition, this study also demonstrates that the initial binding pattern of the four factors is markedly different compared to that in pre-iPSCs, iPSCs and ESCs. OSK act as pioneer factors initially binding to the distal elements of genes required for reprogramming with closed chromatin features in lack of evident histone marks, while c-Myc enhances chromatin binding by OSK factors [6]. Interestingly, recent work analyzing mouse reprogramming process holds different views [1•]. Although emphasizing the pioneer activity of OSK and changes in binding pattern of the four factors during reprogramming, they have found that OSK predominantly bind active somatic enhancers with H3K4me1 and H3K27ac marks in initial stage of reprogramming and immediately initiate their inactivation by inducing somatic transcriptional factors redistribution [1•]. Furthermore, H3K9me3 is also a primary epigenetic determinant, and removal of this mark leads to fully reprogrammed iPSCs. Vitamin C can accelerate reprogramming and knockdown of Kdm3b in pre-iPSCs blocks vitamin C-induced further reprogramming, indicating the cooperative work between vitamin C and H3K9me3 demethylase to reduce the H3K9me3 level in pre-iPSCs [8,9]. While BMP contributes to build H3K9me3 blocks to stop reprogramming at pre-iPSC stage, and knockdown of the H3K9 methyltransferase SETDB1 rescues the inhibitory effect of BMP, suggesting that H3K9 methyltransferases as downstream targets of BMPs [9]. H3K9me3 and DNA methylation-specific reader proteins, including the Hp1 (Cbx3) [7] and Mbd3 [10] can also impede reprogramming. However, studies have found MBD3/NuRD complex function is required in induction of pluripotency from neural stem cells and reprogramming of epiblast-derived stem cells to naïve pluripotency, suggesting the positive role of MBD3/NuRD complex in reprogramming in a context-dependent manner [11].

In contrast, active chromatin marks, for example H3K4me2, are targeted to some pluripotency-related enhancers during early stage of reprogramming [12]. This process may cooperate with the H3K4me3 reader WDR5 and H3K27 demethylase UTX, which are two core components in mammalian trithrox complex that facilitate self-renewal of ESCs and somatic cell reprogramming [13,14]. Other histone modifying enzymes including the H3K36 demethylase KDM2A/B and chromatin remodeling complex BAF also help to improve the reprogramming efficiencies of iPSC [15–17]. Moreover, histone arginine deiminase PADI4 facilitates reprogramming by citrullination of histone H1 and replacing them from chromatin [18]. Taken together, it appears a highly orchestrated exchange of histone modifications through space and time underlies the transition of somatic cells toward a pluripotent state during the reprogramming process.

Histone variants in reprogramming

In mouse and human genome, canonical histone proteins are encoded by multiple copies of genes spreading all over the genome. These genes share the same protein sequences with little differences in DNA sequences. Although these genes are under highly purifying selection during evolution, a small group of non-canonical variants of histones emerged from canonical histones with one or a few amino acid differences. These histone variants are expressed at relatively low levels but have distinct biological functions by altering the conformation of nucleosomes. Massive replacements of canonical histones by non-canonical histone variants (or vice versa) can be observed during fertilization or germ cell generation, which are correlated with cell reprogramming and cell fate alteration.

Recent study in histone variant H2A.X function reveals it functions as a quality control marker to distinguish the developmental potentials of mouse ESCs or iPSCs [19•]. In ESCs, H2A.X is specifically targeted to negatively regulate extra-embryonic lineage gene expression, preventing trophectoderm lineage differentiation. The specific H2A.X deposition patterns in ESCs are faithfully recapitulated in iPSCs that support the development of 'all-iPSC' animals via tetraploid complementation, while aberrant deposition of H2A.X will result in failure to generate 'all-iPSC' embryonic development, up-regulation of extra-embryonic lineage genes, and a pre-disposition to extra-embryonic differentiation.

Two histone variants TH2A and TH2B, highly expressed in the mouse oocyte, play important roles in activation of paternal genome during fertilization and OSKM-induced somatic reprogramming [20•]. Combinational transduction of Oct4, Klf4 and Th2a/b can reprogram somatic cells into the pluripotent state efficiently. Notably, the roles of TH2A/B in reprogramming are very likely due to their deposition of chaperone NPM. Moreover, NPM can lead to global de-condensation of sperm chromatin during fertilization and of somatic cell nuclei during somatic cell nuclear transfer (SCNT), thus significantly increase the success rates of reprogramming, suggesting that the TH2A/B replacement is a general phenomena for both *in vitro* and *in vivo* reprogramming and genome reactivation. Recent studies have also shown that TH2A/B as well as NPM can reprogram human somatic cells and improve the quality of human iPSCs.

Different from the positive roles of TH2A/B and their chaperone in somatic reprogramming and paternal genome activation, some other histone variants hinder

efficient reprogramming. MacroH2A is one of the repressive histone variants associated with differentiation and involved in maintenance of gene silencing and X-chromosome inactivation. After fertilization, the maternal macroH2As progressively disappear until just before zygotic genome activation (ZGA) at late 2-cell stage, while the expression of embryonic macroH2A begins only after the 8-cell stage, which suggests a negative role of this histone variant during reprogramming [21]. Nuclear transfer experiments show macroH2As provide the inactive X-chromosome and pluripotency genes resistance to reprogramming in donor nuclei [22]. Also, the removal of macroH2As increases iPSC formation frequency up to 25-fold [23]. Chromatin immunoprecipitation shows macroH2As occupy pluripotency gene promoters and overlap with the repressive histone mark H3K27me3 [24], which is later replaced by the active histone mark H3K4me3 during reprogramming [25]. Thus, while some histone variants are important for the activation of quiescent genomes, some play important roles in maintaining the somatic cell epigenome at the repressive state.

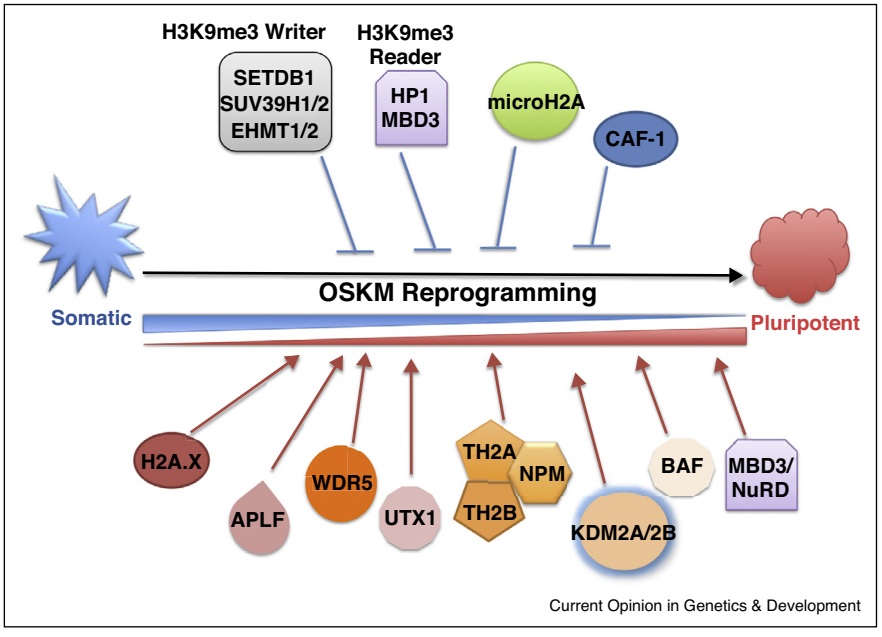
Similar to histone variants, some histone chaperones also participate in somatic cell reprogramming. Aprataxin PNK-like factor (APLF), a histone H3/H4 tetramer chaperone, can promote the assembly and activity of non-homologous end joining (NHEJ) protein complexes [26]. During reprogramming, APLF can accelerate the process of cellular reprogramming and increase the efficiency of iPSC generation by augmenting the expression of E-cadherin (Cdh1) and its implicated mesenchymal-to-epithelial transition (MET) [27].

CAF-1 is the most important replication dependent histone chaperone responsible for re-assembling nucleosome right after DNA replication [28]. With the help of CAF-1, dissociated old histones and newly synthesized histones are randomly incorporated into two sister chromosomes. In a recent study from Hochedlinger's lab [29••], they used two comprehensive RNAi screen to identify important chromatin factors during reprogramming from mouse fibroblasts to iPSCs, and found that the subunits of the CAF-1 complex emerged as the most prominent hits. Mechanistic studies revealed that the suppression of CAF-1 could lead to a more accessible chromatin structure at enhancer elements early during reprogramming, indicating its important role as a regulator of somatic identity during the transcription factor-induced cell fate transitions and cellular plasticity modulation. Overall, the recently discovered histone modification-related factors functioning in somatic cell reprogramming were summarized in Figure 1 and Table 1.

DNA methylation/demethylation in reprogramming

Deposition of methylation on CpG dinucleotides in chromatin results in transcriptional silencing, heterochromatin formation, imprinting formation and genomic stability. DNA methyltransferases (DNMTs) are one type of epigenetic modifiers that specifically catalyze CpG dinucleotide methylation (mCpG). So far, there are three DNMTs

Figure 1



Histone modifiers and variants in the OSKM-mediated reprogramming of somatic cells to pluripotent state. The above ones such as SETDB1 and microH2A can inhibit reprogramming, while the ones below including H2A.X, WDR5, etc. can facilitate reprogramming.

Table 1

Epigenetic modifications and modifiers in OSKM-induced somatic reprogramming.

Epigenetic modifications/ modifiers	Biochemical function	Role for reprogramming	Reference
SUV39H1/H2	H3K9 methyltransferase	Suppress reprogramming	Onder TT, Kara N, Cherry A, Sinha AU, Zhu N, Bernt KM, Cahan P, Mancarci BO, Unternaehrer J, Gupta PB, *et al.*: **Chromatin-modifying enzymes as modulators of reprogramming**. *Nature* 2012, **483**:598-U119
EHMT1, EHMT2	H3K9 methyltransferases	Associated with transcriptional repression	Onder TT, Kara N, Cherry A, Sinha AU, Zhu N, Bernt KM, Cahan P, Mancarci BO, Unternaehrer J, Gupta PB, *et al.*: **Chromatin-modifying enzymes as modulators of reprogramming**. *Nature* 2012, **483**:598-U119
SETDB1	H3K9 methyltransferases	Suppresses reprogramming	Sridharan R, Gonzales-Cope M, Chronis C, Bonora G, McKee R, Huang C, Patel S, Lopez D, Mishra N, Pellegrini M, *et al.*: **Proteomic and genomic approaches reveal critical functions of H3K9 methylation and heterochromatin protein-1gamma in reprogramming to pluripotency**. *Nat Cell Biol* 2013, **15**:872-882.
MBD3	H3K9me3 and DNA methylation-specific reader protein	Content-dependent manner	Rais Y, Zviran A, Geula S, Gafni O, Chomsky E, Viukov S, Mansour AA, Caspi I, Krupalnik V, Zerbib M, *et al.*: **Deterministic direct reprogramming of somatic cells to pluripotency**. *Nature* 2013, **502**:65-70. dos Santos RL, Tosti L, Radzisheuskaya A, Caballero IM, Kaji K, Hendrich B, Silva JC: **MBD3/NuRD facilitates induction of pluripotency in a context-dependent manner**. *Cell Stem Cell* 2014, **15**:102-110.
HP1	H3K9me3 and DNA methylation-specific reader protein	Binds specifically to methylated histone H3K9 via their chromodomain critical barriers of reprogramming	Sridharan R, Gonzales-Cope M, Chronis C, Bonora G, McKee R, Huang C, Patel S, Lopez D, Mishra N, Pellegrini M, *et al.*: **Proteomic and genomic approaches reveal critical functions of H3K9 methylation and heterochromatin protein-1gamma in reprogramming to pluripotency**. *Nat Cell Biol* 2013, **15**:872-882.
WDR5	H3K4me3 reader	Interacts with the pluripotency transcription factor Oct4	Ang YS, Tsai SY, Lee DF, Monk J, Su J, Ratnakumar K, Ding J, Ge Y, Darr H, Chang B, *et al.*: **Wdr5 mediates self-renewal and reprogramming via the embryonic stem cell core transcriptional network**. *Cell* 2011, **145**:183-197.
UTX	H3K27 demethylase	Directly partners with OSK reprogramming factors and uses its histone demethylase catalytic activity to facilitate iPSC formation	Mansour AA, Gafni O, Weinberger L, Zviran A, Ayyash M, Rais Y, Krupalnik V, Zerbib M, Amann-Zalcenstein D, Maza I, *et al.*: **The H3K27 demethylase Utx regulates somatic and germ cell epigenetic reprogramming**. *Nature* 2012, **488**:409-413.
KDM2A/B	H3K36 demethylase	Contributes to gene activation by binding to and demethylating the gene promoters to promote iPSC generation	Liang G, He J, Zhang Y: **Kdm2b promotes induced pluripotent stem cell generation by facilitating gene activation early in reprogramming**. *Nat Cell Biol* 2012, **14**:457-466.
BAF	Histone remodeling complex	Increases reprogramming efficiency by facilitating enhanced Oct4 binding to target promoters	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.
PADI4	Histone arginine deiminase	Facilitates reprogramming by citrullination of histone H1 and replacing them from chromatin	Christophorou MA, Castelo-Branco G, Halley-Stott RP, Oliveira CS, Loos R, Radzisheuskaya A, Mowen KA, Bertone P, Silva JC, Zernicka-Goetz M, *et al.*: **Citrullination regulates pluripotency and histone H1 binding to chromatin**. *Nature* 2014, **507**:104-108.
H2A.X	Histone variant	A quality control marker to distinguish the developmental potentials of mouse ESCs or iPSCs	Wu T, Liu Y, Wen D, Tseng Z, Tahmasian M, Zhong M, Rafii S, Stadtfeld M, Hochedlinger K, Xiao A: **Histone variant H2A.X deposition pattern serves as a functional epigenetic mark for distinguishing the developmental potentials of iPSCs**. *Cell Stem Cell* 2014, **15**:281-294.

Table 1 (*Continued*)

Epigenetic modifications/ modifiers	Biochemical function	Role for reprogramming	Reference
TH2A/2B	Histone variant	Play important roles in activation of paternal genome during fertilization and OSKM-induced somatic reprogramming	Shinagawa T, Takagi T, Tsukamoto D, Tomaru C, Huynh LM, Sivaraman P, Kumarevel T, Inoue K, Nakato R, Katou Y, *et al.*: **Histone variants enriched in oocytes enhance reprogramming to induced pluripotent stem cells**. *Cell Stem Cell* 2014, **14**:217-227.
NPM	Nucleoplasmin	Leads to global decondensation of somatic cell nuclei and thus facilitate somatic cell nuclear transfer (SCNT)	Tamada H, Van T N, Reed P, *et al*.: **Chromatin decondensation and nuclear reprogramming by nucleoplasmin**. *Mol Cell Biol* 2006, **26**:1259-1271.
MacroH2A	Histone variant	Provide the inactive X-chromosome and pluripotency genes resistance to reprogramming in donor nuclei	Pasque V, Gillich A, Garrett N, Gurdon JB: **Histone variant macroH2A confers resistance to nuclear reprogramming**. *EMBO J* 2011, **30**:2373-2387.
Aprataxin PNK-like factor (APLF)	Histone H3/H4 tetramer chaperone	Accelerate the process of cellular reprogramming and increase the efficiency of iPSC generation	Syed KM, Joseph S, Mukherjee A, Majumder A, Teixeira JM, Dutta D, Pillai MR: **Histone chaperone APLF regulates induction of pluripotency in murine fibroblasts**. *J Cell Sci* 2016, **129**:4576-4591.
CAF-1	Histone chaperone	Lead to a more accessible chromatin structure at enhancer elements early during reprogramming	Cheloufi S, Elling U, Hopfgartner B, Jung YL, Murn J, Ninova M, Hubmann M, Badeaux AI, Euong Ang C, Tenen D, *et al.*: **The histone chaperone CAF-1 safeguards somatic cell identity**. *Nature* 2015, **528**:218-224.
TET1	DNA dioxygenase	Content-dependent manner to promote/suppress reprogramming; capable of replacing essential reprogramming factors during reprogramming	Costa Y, Ding J, Theunissen TW, Faiola F, Hore TA, Shliaha PV, Fidalgo M, Saunders A, Lawrence M, Dietmann S, *et al.*: **NANOG-dependent function of TET1 and TET2 in establishment of pluripotency**. *Nature* 2013, **495**:370-374. Hu X, Zhang L, Mao SQ, Li Z, Chen J, Zhang RR, Wu HP, Gao J, Guo F, Liu W, *et al.*: **Tet and TDG mediate DNA demethylation essential for mesenchymal-to-epithelial transition in somatic cell reprogramming**. *Cell Stem Cell* 2014, **14**:512-522. Gao Y, Chen J, Li K, Wu T, Huang B, Liu W, Kou X, Zhang Y, Huang H, Jiang Y, *et al.*: **Replacement of Oct4 by Tet1 during iPSC induction reveals an important role of DNA methylation and hydroxymethylation in reprogramming**. *Cell Stem Cell* 2013, **12**:453-469. Chen J, Gao Y, Huang H, Xu K, Chen X, Jiang Y, Li H, Gao S, Tao Y, Wang H, *et al.*: **The combination of Tet1 with Oct4 generates high-quality mouse-induced pluripotent stem cells**. *Stem Cells* 2015, **33**:686-698.
TET2	DNA dioxygenase	Recruited to Nanog and Esrrb loci, essential for transcriptional activation at the pluripotency genes; interact with NANOG to increase reprogramming efficiency to fully qualified iPSCs	Doege CA, Inoue K, Yamashita T, Rhee DB, Travis S, Fujita R, Guarnieri P, Bhagat G, Vanti WB, Shih A, *et al.*: **Early-stage epigenetic modification during somatic cell reprogramming by Parp1 and Tet2**. *Nature* 2012, **488**:652-655. Costa Y, Ding J, Theunissen TW, Faiola F, Hore TA, Shliaha PV, Fidalgo M, Saunders A, Lawrence M, Dietmann S, *et al.*: **NANOG-dependent function of TET1 and TET2 in establishment of pluripotency**. *Nature* 2013, **495**:370-374.
METTL3	m^6A writer	Positive influence for reprogramming	Chen T, Hao YJ, Zhang Y, Li MM, Wang M, Han W, Wu Y, Lv Y, Hao J, Wang L, *et al.*: **m(6)A RNA methylation is regulated by microRNAs and promotes reprogramming to pluripotency**. *Cell Stem Cell* 2015, **16**:289-301.

identified in mouse and human genome, DNMT1 for maintenance of methylation and DNMT3A/B for de novo methylation. All of the three DNMTs are enriched in ESCs, suggesting their important roles in supporting pluripotency of ESCs. Addition of their inhibitor 5-aza-cytidine (5-AZA) during reprogramming greatly improves the iPSC induction efficiency more than 30 folds [30], indicating that DNA methylation may serve as a barrier for somatic reprogramming.

Most recently, a group of mCpG derivatives has been discovered as well as the catalyzing enzymes, the TET (Ten-Eleven-Translocation) family dioxygenases, which can convert 5-methylcytosine (5mC) to 5-hydroxymethyl-cytosine (5hmC) [31,32]. The 5-hydroxymenthylcytosine can be further oxidized to 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) by TET proteins. Both 5fC and 5caC can be specifically recognized and replacement by thymine DNA glycosylase (TDG), and then converted back to unmodified cytosine through DNA base-excision repair (BER) and nucleotide excision repair pathways to achieve active DNA demethylation [33].

TET3 is the first enzyme identified in converting methyl-cytosine by active DNA demethylation pathway during zygotic activation. During zygotic activation, TET3 is predominantly enriched in male pronucleus, and knock-out of Tet3 in zygote can imped the active DNA demeth-ylation of paternal genome and delay the subsequent activation of paternal pluripotency-related genes during early embryo development [34]. However, both Tet1 and Tet3 knockout mice are partially lethal, which suggest that redundant functions may exist in Tet family proteins [34,35]. Tet family protein-dependent DNA modifica-tions are also important for reprogramming. Both Tet2 and Parp1 are recruited to *Nanog* and *Esrrb* loci for establishment of early epigenetic modifications, which is essential for transcriptional activation at the pluripo-tency genes to complete the reprogramming process [36]. Nanog can interact with Tet1 and Tet2, increasing the reprogramming efficiency to fully qualified iPSCs [37]. Interestingly, Tet1 can work as a double-edged sword for somatic cell reprogramming regulation. It can promote or suppress the reprogramming depending on the absence or presence of vitamin C [8]. In the absence of vitamin C, Tet1 can boost somatic cell reprogramming independent of MET; whereas addition of vitamin C during the reprogramming process, or knockout of Tet1 can enhance reprogramming. However, another study also showed that depletion of Tet1 or its downstream TDG impaired reprogramming by blocking MET, suggesting that Tet1 may serve as a booster for mid-stage or late-stage of reprogramming [38•]. Furthermore, Tet1 (T) is capable of replacing essential reprogramming factors during reprogramming. It can replace Oct4 and fulfill the somatic cell reprogramming in combination with Sox2 (S), Klf4 (K) and c-Myc (M) [39]. Analyzing the efficient TSKM secondary reprogramming system reveals that both 5mC and 5hmC modifications increase at an intermediate stage of the reprogramming process, correlating with a transi-tion in the transcriptional profile. 5hmC enrichment is involved in the demethylation and reactivation of genes and regulatory regions that are important for pluripo-tency, indicating that changes in DNA methylation and hydroxymethylation play important roles in genome-wide epigenetic remodeling during reprogramming. Impor-tantly, the combination of Tet1 with Oct4 is enough to reprogram the cells toward a high-quality pluripotent state with normal 5hmC levels. These OT (Oct4-Tet1)-iPSCs can also efficiently generate 'all-iPSC' mice with a normal life span and no obvious tumorigenicity was observed [40], compared to the OSKM-derived 'all-iPSC' mice. In summary, Tet1 can replace multiple Yamanaka factors to achieve reprogramming, further elucidating the important roles of epigenetic modifiers during this process.

Epigenetic modifications and iPSC qualities

Epigenetic modifications and modifiers not only play central roles during reprogramming from somatic cells to iPSCs, but also contribute significantly to iPSC quali-ties, such as H2A.X and Tet1 as mentioned above [19•,40].

Moreover, abnormal epigenetic modifications accumu-lated in the iPSCs can ultimately affect iPSC pluripo-tency or tumorigenicity. Besides the histone variant H2A. X and DNA hydroxylase Tet1 mentioned above, DNA imprinting also influences iPSC qualities. By comparing genetically identical mouse ESCs and iPSCs, studies from Hochedlinger's lab has demonstrated that the expression state of imprinted *Dlk1-Dio3* gene cluster on chromosome 12qF1 can distinguish iPSCs with different extents of pluripotency and allow for the prospective identification of iPSC lines that have the full develop-ment potential of ESCs [41]. However, studies from Jaenisch group argued that loss of imprinting at this locus does not strictly correlate with reduced pluripotency of iPSCs. They believe that the level and stoichiometry of reprogramming factors during the reprogramming process play key roles in the resulting pluripotency of iPSCs. For example, higher expression of OK combined with lower expression of SM can produce iPSCs that efficiently support generation of 'all-iPSC mice' by tetraploid (4n) complementation [42]. In addition, deep comparison between '4n-ON' and the corresponding '4n-OFF' iPSC lines with the same genetic background and proviral integration sites strongly indicates that methylation status of the imprinted gene *Zrsr1* also contributes importantly to iPSC qualities [43].

Conclusions

The development of iPSC reprogramming technique provides a reliable platform for stem cell research and

regenerative medicine studies. While the forced expression of transcriptional factor combinations can successfully reprogram somatic cells to pluripotent state, recent Mechanistic studies reveal that the epigenetic modification and remodeling play key roles during the reprogramming process. Besides the epigenetic modifications discussed above, the recently identified N^6-methyladenosine (m^6A), a conserved epi-transcriptomic modification of eukaryotic mRNAs, also indicates has attracted extensive attention. By comparing the mRNA transcriptomes of four cell types with different degrees of pluripotency, studies from Zhou lab have reported that miRNAs regulates m^6A modification levels through modulating the m^6A writer METTL3 binding to mRNAs. They also indicates that m^6A has a positive influence on reprogramming to pluripotency [44]. Studies in zebrafish development have showed that Ythdf2, the m^6A eraser, plays important role in maternal mRNA clearance during the maternal-to-zygotic transition [45]. Although several important progresses have been made about this new identified modification, the crosstalk between RNA methylation and DNA methylation during reprogramming is still largely unknown. Deep investigation of these modifiers and remodeling factors will shed light on the enhancement of both reprogramming efficiency and improvements of iPSC qualities.

Conflict of interest statement

Nothing declared.

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Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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The authors mapped OSKM-binding, stage-specific TFs and chromatin states in discrete reprogramming stages. They found OSK predominantly bind active somatic enhancers early in reprogramming to inactivate them by several mechanisms. Pluripotency enhancer selection is a stepwise process which is dependent on collaborative TF binding. Loss-of and gain-of function experiments demonstrated that stage-specific TFs modulate reprogramming efficiency by influencing OSK occupancy and enhancer selection.

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