Chapter 8

Resetting Cell Fate by Epigenetic Reprogramming

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Additional information is available at the end of the chapter

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Abstract

Epigenetic modifications and their regulations govern the identity of every cell type in an organism. Cell differentiation involves a switch in gene expression profile that is accompanied by heritable changes of epigenetic signatures in the differentiated cell type. Differentiation is generally not reversible, thereby conferring cell fate decisions once an altered epigenetic pattern is set. Nevertheless, attempts have been made to reverse a differentiation cell fate to a pluripotent state by various experimental approaches, such as somatic cell nuclear transfer, cell fusion and ectopic expression of defined transcription factors. The fundamental basis of all these strategies is to mediate epigenetic reprogramming, which allows a permanent and completed conversion of cell fate. A comprehensive understanding of the dynamic of epigenetic changes during cell differentiation would provide a more precise and efficient way of reprogramming cell fate. Here we summarize the epigenetic aspects of different reprogramming strategies and discuss the possible mechanisms underlying these epigenetic reprogramming events.

Keywords: epigenetic, reprogramming, somatic cell nuclear transfer, cell fusion, transcription factors, pluripotency, differentiation, cell fate

1. Introduction

Development is a complex process that involves a series of cell differentiation pathways starting from totipotent embryonic cells. According to Waddington's concept of epigenetic landscape, a cell has to interact with surrounding stimuli and respond by giving a phenotype which defines its identity during development [1]. Each cell experiences different inter/intra-cellular signals and hence has its epigenetic signature of cell identity, which in turn directs its own specific gene expression pattern without alteration of DNA sequences (with the exception of the immunoglobulin genes in B and T cells). It is now clear that the diversity of cell type specific gene expression pattern is mediated by means of epigenetic mechanisms,

such as DNA methylation, covalent histone modifications and chromatin remodeling. Once a cell's identity is set, it is difficult to convert it to other unrelated lineages, thus leading to stable and irreversible differentiated cell states. Nevertheless, there are a few exceptions of cell fate conversion during embryo development and adult tissue/organ regeneration, e.g. vascular endothelium to smooth muscle cells [2], which involve changing a cell's epigenetic signature into another unrelated kind.

Apart from a few exceptions of natural cell fate conversion events, different strategies have been developed aiming to reprogram differentiated somatic cell fate to a pluripotent state (**Figure 1**). A historical strategy of reprogramming is by somatic cell nuclear transfer (SCNT) experiments. SCNT involves transplantation of a cell nucleus into an enucleated egg/oocyte in order to generate a "cloned" animal with an equivalent genetic composition as the donor individual. It is possible to derive embryonic stem (ES) cells from nuclear transplanted (NT) embryos (ntES cells), which shows indistinguishable pluripotent gene expression profiles

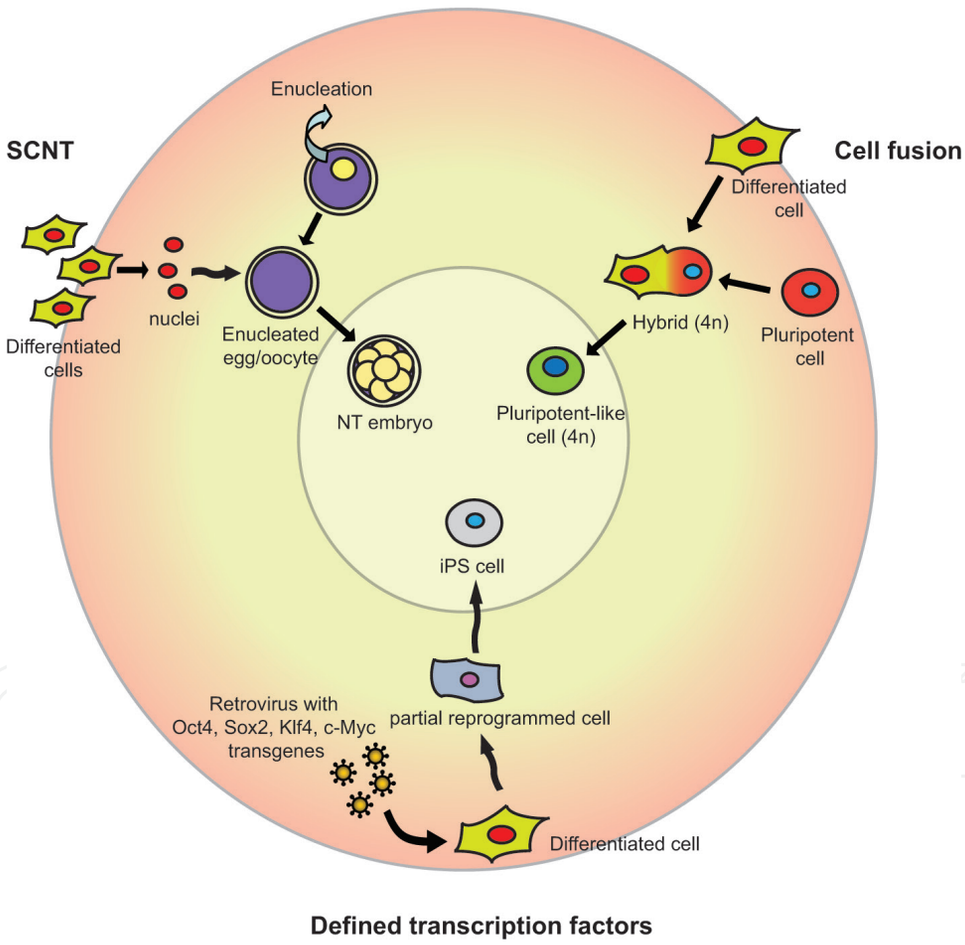

Figure 1. Strategies of reprogramming cell fate. Differentiated cells can be reprogrammed to pluripotent state by somatic cell nuclear transfer (SCNT), cell fusion, and ectopic expression of defined transcription factors. SCNT involves transplantation of a single differentiated cell nucleus into an enucleated egg/oocyte, which develops as a nuclear transplanted (NT) embryo. Cell fusion involves artificial fusion of a differentiated and a pluripotent cell to form a tetraploid pluripotent-like cell. Defined transcription factors (Oct4, Sox2, Klf4, c-Myc) can be ectopically expressed in differentiated cells and convert them to induced pluripotent stem (iPS) cells.

when compared to the normal ES cells derived from fertilized embryos. Another strategy of reprogramming is achieved by fusion of a differentiated cell with a pluripotent cell in order to generate a pluripotent-like tetraploid hybrid cell [3–5]. It has been proposed that cellular components, such as transcription factors, in the pluripotent cell are able to reprogram the differentiated cell nucleus. This idea aligns with the use of cell extracts from pluripotent cell types to revert differentiated cells into a pluripotent-like state [6, 7]. Presumably the cytoplasmic "reprogramming factors" from the pluripotent cells can be isolated and concentrated to achieve a higher reprogramming efficacy. A third strategy involves ectopic expression of defined transcription factors in somatic differentiated cells to generate induced pluripotent stem (iPS) cells. Delivery of the ectopic transcription factors can be achieved by viral approaches, such as the use of retrovirus, lentivirus, adeno-associated virus or Sendai virus, or by using episomal vesicles, or by direct mRNA or protein transfection. This technique has been successfully applied to reprogram a vast number of differentiated somatic cell types. Importantly, iPS cells can also be generated by using combinations of microRNAs (miRNAs) or small chemical molecules without the needs of ectopic expression of reprogramming factors [8]. The three reprogramming strategies show different reprogramming kinetics and efficiencies, which can be associated with the distinct epigenetic mechanisms in the erasure of somatic cell epigenetic signature and re-establishment of the pluripotent one. In this review, we focus on the dynamic changes of epigenetics mediated by different reprogramming strategies and how the modulation of epigenetic status improves the reprogramming efficiency.

2. Epigenetic reprogramming by SCNT

SCNT was first done by Briggs and Kings in 1952, who transplanted a blastula nucleus into an enucleated egg of the amphibian *Rana pipiens* [9]. Few years later, Gurdon *et al.* succeeded by using differentiated *Xenopus* intestinal epithelial donor nuclei for SCNT [10]. In 1997, the first cloned mammal, Dolly the sheep, was generated [11], and since then, more than 23 other mammalian species have now been successfully cloned [12]. Normal development of nuclear transplanted (NT) embryos requires recapitulation of the gene expression profile that supports the embryogenesis process by the differentiated donor nucleus. This involves re-activation of pluripotency genes, in particular *Oct4*, *Nanog* and *Sox2*, and repression of somatic lineage genes. In fact, the efficiency of reprogramming by SCNT is generally very low and less than 1% of NT embryos can develop into normal adults [13–15]. The cloned newborns often suffer from developmental abnormalities owing to incomplete reprogramming. It has been observed that *Oct4* was aberrantly expressed in cloned mouse blastocysts derived from cumulus donor nuclei [16, 17]. Besides, continuous expression of other somatic donor marker genes was demonstrated in some *Xenopus* NT embryos [18]. Some imprinted genes in donor cells were found to be aberrantly expressed in cloned embryos, presumably owing to the incomplete epigenetic reprogramming of the regulatory regions of imprinting loci [19–21]. Dysregulation of imprinted genes, such as *Igf2*, *Igf2r*, *H19*, and *Xist*, in cloned embryos can lead to both fetal and placental overgrowth and result in embryonic lethality or an abnormal growth condition called "large offspring syndrome", which is commonly found in cloned

mammals [22–24]. Since the SCNT process does not increase the frequency of genetic alterations, it is suggested that the variable phenotypes observed in cloned embryos are associated with the reprogrammed epigenetic status of the donor nuclei [25]. This is supported by the findings that the developmental defects in cloned animals were not transmittable to the next offspring generation, indicating the presence of aberrant epigenetic reprogramming [26]. Aberrant DNA methylation patterns were indeed observed in NT embryos [27]. It was also demonstrated that the bovine NT blastocysts lack asymmetric patterns of both H3K9 methylation and acetylation between the inner cell mass and trophectoderm [28], which may account for abnormal cloned embryo development.

Although the rate of successful SCNT is very low, the reprogramming ability of factors in the egg/oocyte is highly efficient as the transplanted nuclei take less than 1 day to initiate cell division and trigger the "normal" developmental program. The donor cell epigenetic status has to be reprogrammed in order to support the embryonic program of development. In fact, genome-wide demethylation was observed in the cloned blastocysts [29]. It has been shown that the *Oct4* promoter of somatic cells undergoes DNA demethylation after nuclear transplantation into the germinal vesicle of *Xenopus* oocytes [30]. This demethylation of the *Oct4* promoter was found to be mediated by Tet3 in a mouse SCNT study [31], which is essential for the reactivation of *Oct4* expression for successful SCNT. In addition, chromatin remodeling factors, such as ISWI and BRG1, are documented in facilitating the reprogramming of cell fate. It has been shown that ISWI, which is a chromatin remodeling ATPase, is able to dissociate TATA binding protein in somatic nuclei after incubation in the *Xenopus* egg extract, suggesting that ISWI-containing complexes are facilitating epigenetic reprogramming in an egg environment [32]. Besides, *Xenopus* egg extract depleted of BRG1 protein showed an abolishment of the reprogramming ability and hence failed to induce *Oct4* expression in the somatic nuclei [33]. Therefore, chromatin structure remodeling is believed to be one of the reprogramming mechanisms. In addition, the maternal-derived histone H3 variant H3.3 in the enucleated egg was found to replace the canonical histone H3 in the donor nuclei after SCNT, leading to the reactivation of key pluripotent genes that are originally associated with repressive histone marks [34]. Histone H2A variant, macroH2A, also plays an important role in the reactivation of female donor cell's inactive X chromosome during reprogramming. It was shown that knockdown of macroH2A facilitates X-reactivation and the expression of pluripotent genes in cloned *Xenopus* embryos [35]. More recently, it has been demonstrated that the H3K9 tri-methylation (H3K9me3) of the donor cell genome is a major epigenetic barrier to SCNT. Ectopic expression of H3K9 demethylases *Kdm4b* or *Kdm4d* in the mouse donor cells de-repressed the genomic regions that are resistant to reprogramming and thus significantly improving SCNT efficiency [36, 37]. Similarly, removal of H3K9me3 by ectopic expression of other H3K9 demethylases also demonstrated improved reprogramming efficiency in human and bovine SCNT experiments [38, 39].

Interestingly, it has been shown that the epigenetic state of a differentiated cell is directly correlated with its reprogrammability [40]. SCNT with ES cell nuclei demonstrated a much higher efficiency of generation of NT blastocysts than using other somatic cell types [41]. This could be associated with a more relaxed chromatin configuration in ES cells that may render their epigenome more susceptible for reprogramming [42]. Alternatively, it has been demonstrated that the cloning efficiency can be significantly improved by pre-treating the more

differentiated and condensed chromatin state of somatic nuclei with epigenetic modifying agents, e.g. 5-aza-deoxycytidine and trichostatin A (TSA), that facilitate chromatin relaxation [43–45]. Interestingly, the effect of TSA treatment in improving SCNT is associated with the reactivation of a subset of genes that are repressed by H3K9me3 in the somatic cells [46], presumably through introducing histone hyper-acetylation at their promoters. Altogether, SCNT provides a quick route of epigenetic reprogramming for a differentiated cell to a pluripotent state. Identification of the responsible reprogramming factors in the egg and oocyte cytoplasm will be one of the key future directions to improve the efficiency of SCNT and therapeutic cloning.

3. Epigenetic reprogramming by cell fusion

Cell fusion is a natural event that is crucial for fertilization and in various organs such as placenta, skeletal muscles and bones [47]. It has been proposed that the fusion of bone marrow-derived stem cells and tissue cells, e.g. hepatocytes, is one of the mechanisms of tissue repair [48, 49]. Cell fusion experiments using pluripotent cells, e.g. an ES or embryonic germ (EG) cell, were shown to be able to reprogram a differentiated cell type [3–5]. Both ES and EG cells possess reprogramming ability and are able to reactivate pluripotent genes and silence differentiation genes in the somatic cell nucleus within a tetraploid hybrid cell after cell fusion. It is indeed the case that the new transcription profile of a hybrid cell is partly contributed by the reprogrammed somatic nucleus to a pluripotent-like state. Moreover, injection of hybrid cells into normal diploid blastocysts demonstrated their contribution to all three germ layers in the chimeras [4, 50], indicating the pluripotent nature of hybrid cells. Similar to the SCNT, different somatic cell types show different kinetics of reprogramming by the cell fusion approach, which could be associated with the somatic chromatin accessibility status [51, 52].

Cell fusion with a pluripotent cell can trigger extensive epigenetic reprogramming in the differentiated cell nucleus. It has been shown that reactivation of *Oct4* from the somatic nucleus occurs before DNA replication after cell fusion [53], suggesting the involvement of active demethylation process [54]. This was further supported by the functional roles of Tet1 and Tet2 in the demethylation of *Oct4* and imprinted control regions by fusing somatic cells with EG cells [55]. Besides, in a cell fusion experiment using thymocytes and ES cells from two different mouse strains, it was observed that the epigenetic profile of the somatic cell nucleus was reprogrammed to a similar pattern to that of the ES cell. Global histone H3 and H4 acetylation and H3K4 di- and tri-methylation were increased in the hybrid cell to a level comparable to ES cells, whereas these modifications are weak in the parental somatic thymocytes. Examination of gene specific loci showed that the *Oct4* promoter was enriched with H3 acetylation and the promoter of the thymocyte marker *Thy-1* was enriched with H3K27 tri-methylation in both ES cell and hybrid cell chromatin, whereas these epigenetic modifications are missing in the thymocyte [56]. Hence, the somatic genome has undergone epigenetic reprogramming triggered by fusion with the ES cell, suggesting that the process of cell fusion mediates a transcription activation-permissive chromatin state in the hybrid genome. In addition, silencing the somatic differentiation genes was shown to be associated with polycomb repressive complexes in the cell fusion experiment using ES cells [57].

Reprogramming to pluripotency by cell fusion approach requires lengthy selection of the successfully reprogrammed hybrid cells. The reprogramming efficiency of cell fusion is usually less than 0.001%, depending on the somatic cell types [50, 58]. The low reprogramming efficiency in hybrid cells can be largely enhanced by manipulation of key pluripotency-associated genes like *Nanog* [58, 59] and *Sall4* [60], or by activation of the Wnt signaling pathway [61], emphasizing the importance of these factors in cell fusion reprogramming. Overexpression of *Nanog* or *Sall4* in ES cells demonstrated a several hundred-fold increase in reprogramming efficiency after cell fusion. Similarly, treatment of ES cells with Wnt3a for 24–48 hours enhanced the reprogramming of somatic cells by 20-fold. However, owing to the low reprogramming efficiency and the tetraploid genome of the resulting hybrid cells, reprogramming by the cell fusion approach becomes less promising in regenerative medicine.

4. Epigenetic reprogramming by defined transcription factors

In a groundbreaking discovery, Yamanaka *et al.* demonstrated that somatic cell state can be reprogrammed to a pluripotent state by the introduction of only four transcription factors; Oct4, Sox2, Klf4 and c-Myc, which are now also known as the Yamanaka factors. The first generation of iPS cells was obtained using a Fbx15-driven selection construct (Fbx15-iPS) and displayed a gene expression pattern very similar to that of normal pluripotent ES cells. However, the somatic epigenetic signature was only partially reprogrammed; the *Oct4* promoter, for example, retained some DNA methylation and no germline transmission was observed for these cells in chimeric mice. Hence these first-generation iPS cells were not fully pluripotent in nature [62]. Given the potential reprogramming capacity of these four factors, the second generation of iPS cells was generated by selection with a Nanog reporter construct (Nanog-iPS) [63, 64]. During the reprogramming process, the virally delivered transgenes were silenced, but on the contrary, the endogenous *Oct4* and *Sox2* loci were re-activated for the maintenance of pluripotency in iPS cells. In contrast to the Fbx15-iPS cells, these Nanog-iPS cells were able to undergo germline transmission in chimeric mice, and thus share this crucial feature of pluripotency with normal ES cells. These landmark studies that pioneered the derivation of mouse iPS cells led to the possibility of using the same strategy to generate human iPS cells. An initial study was performed by Thomas *et al.* in which a different combination of factors, OCT4, SOX2, NANOG and LIN28, was used to reprogram human fibroblasts into iPS cells [65]. Thereafter, Yamanaka and other groups succeeded in generating human iPS cells by using the same 4 Yamanaka factors as in the mouse iPS systems [66, 67]. To date, a number of different somatic cell types have been successfully reprogrammed into iPS cells, e.g. neural stem cells, keratinocytes, hepatocytes, gastric epithelia cells, pancreatic β cells, terminally differentiated B and T cells [8].

By using the defined transcription factor approach to reprogram cell fate, about 0.1–3% of the somatic starting cell population can be converted into iPS cells in around 2–3 weeks. The reprogramming efficiency is believed to be correlated with the differentiation state of the starting somatic cells. It has been shown that hematopoietic stem and progenitor cells can be reprogrammed to iPS cells 300 times more efficient than the terminally differentiated B and T cells [68]. Interestingly, partially de-differentiating mature B cells by either knockdown of

Pax5 or forced expression of *C/EBPα* resulted in efficient reprogramming by the Yamanaka factors [69], suggesting that the epigenetic status of differentiated cells is crucial in successful reprogramming. The dynamics of transcription profile and epigenetic patterns during the reprogramming process from somatic to iPS cells were studied in details. The reprogramming of somatic cell fate is a sequential stochastic event which involves a gradual silencing of the somatic lineage genes and the viral transgenes, and a sequential expression of alkaline phosphatase and SSEA1 in partially reprogrammed cells, whereas endogenous *Oct4* and *Nanog* are only activated in fully reprogrammed iPS cells. Induction of the four Yamanaka factors results in an immediate cellular response of inactivation of thousands of somatic lineage distal enhancers and, to a smaller extent, the H3K4me3-enriched somatic gene promoters [70, 71], leading to down-regulation of somatic identity genes. This initial phase of reprogramming is also accompanied by a global reduction of H3K27me3 resulting in loss of heterochromatin [72]. Meanwhile, mesenchymal transcription factors, such as *Snail1/2*, *Zeb1/2*, are repressed [70, 73, 74], whereas epithelial transcription factors, such as *Cdh1*, *Epcam*, are activated [75, 76], resulting in mesenchymal-epithelial transition (MET). This is associated with an increase in H3K4me2 at epithelial genes, but a decrease in H3K4me2 and H3K79me2 at mesenchymal genes [71, 77]. Despite an increase in global H3K36me2/3 level, loss of H3K36me2/3 was observed at the *Ink4-Arf* locus, leading to enhanced cell proliferation during reprogramming [78]. The following phase of reprogramming is marked by upregulation of endogenous pluripotency genes to establish transcriptional program, which is independent of transgene expression [73, 79]. The final phase of reprogramming involves elongation of telomeres, X-reactivation in female iPS cells, and upregulation of DNA methylation genes [79]. This coincides with loss of DNA methylation and downregulation of *Xist* expression in the somatic inactive X chromosome [80, 81].

A number of epigenetic remodeling factors are involved in the reprogramming events. Both polycomb (PcG) and trithorax (TrxG) group proteins were found to be crucial in the derivation of iPS cell colonies. Upon knockdown of *Wdr5*, which is a core component of TrxG protein complex, cells failed to establish H3K4me3 at the pluripotent genes, like *Oct4* and *Nanog*, for their reactivation [82]; whereas inhibition of the core components of the polycomb repressive complex 1 and 2 reduced reprogramming efficiency [83], partly because of the dysregulation of genes involved in the MET process [84]. This is similar to the findings that inhibition of H3K79 methyltransferase DOT1L facilitates the loss of H3K79me2 mark at the mesenchymal genes to promote MET during reprogramming [83]. The H3K27 demethylase Kdm6a (also known as Utx) can directly interact with Oct4/Sox2/Klf4 to remove the repressive H3K27me3 mark from the early pluripotent genes in somatic cells for their reactivation [85]. This is in agreement with the findings that depletion of histone H2A variant, macroH2A, enhances reprogramming, owing to its co-occupancy with H3K27me3 to repress pluripotent genes [86, 87]. Besides, the H3K36 demethylase Kdm2b (also known as Jhdm1b) enhances the activation of early responsive genes (*Cdh1*, *Epcam*, *Dsg2*, *Dsp*, *Irf6*) during reprogramming through the removal of H3K36me2 at their promoters [88]. Interestingly, H3K9me3 was also found to be one of the major epigenetic barriers in the generation of iPS cells [89], similar to the findings in SCNT experiments. Depletion of the H3K9 methyltransferases SUV39H1/H2, Ehmt1/2 and Setdb1 or inhibition of Cbx3, a protein that recognizes H3K9 methylation, enhances reprogramming by de-repressing *Nanog* and abolishing the cellular responses to BMP signaling [89–91]. Although

Dnmt3a/b were found to be dispensable [92], DNA demethylation of key pluripotency loci mediated by Tet proteins is required for efficient reprogramming [93–95].

Previous studies demonstrated that the partially reprogrammed iPS cells contained significantly fewer genes marked by the bivalent chromatin signature (co-existence of both H3K4 and H3K27 methylation) and an enrichment of DNA hyper-methylated loci when compared to the wild-type ES cells and the fully reprogrammed iPS cells [70]. Therefore, it is proposed that completion of the epigenetic reprogramming process is pre-requisite for the acquisition of pluripotency. This is supported by the observation that treatment of partially reprogrammed iPS cells with the DNA methyltransferase inhibitor 5-aza-cytidine was able to promote their transition into the fully reprogrammed pluripotent state [70]. Besides, inhibition of H3K27 methyltransferase Ezh2 by small molecule, GSK-126, reduced reprogramming efficiency [84], whereas inhibition of DOT1L by small molecule, EPZ004777, showed enhancement of reprogramming [83]. Various histone deacetylase inhibitors (HDACi) were also shown to improve reprogramming [96–100]. In combination with HDACi valproic acid, human iPS cells can be generated only with Oct4 and Sox2 with a comparable reprogramming efficiency by the four Yamanaka factors [101]. Interestingly, it was found that vitamin C can increase reprogramming efficiency by promoting the transition of pre-iPS cells to fully reprogrammed cells [102], potentially through acting as a cofactor of Kdm2b to induce H3K36me2/3 demethylation [78], activation of H3K9 demethylases (Kdm3a, 3b, 4c and 4d) to remove H3K9me3 [89], and promoting Tet-mediated DNA demethylation [103]. With the aid of small chemical molecules, the iPS cell reprogramming efficiency and duration could be further improved.

5. "Epigenetic memory" in reprogrammed cells

"Epigenetic memory" refers to the persistent expression of parental genes in the daughter cells through the inheritance of distinctive epigenetic marks. Consequently, the epigenetic profile of a parent cell is faithfully passed on to its daughter cells such that the gene expression pattern is memorized and maintained throughout cell generations. In the situation of reprogramming cell fate, the persistent somatic cell epigenetic signature and the expression of lineage genes in the reprogrammed cells is thus regarded as an example of epigenetic memory.

Even though it has been shown that prolonged *in vitro* culture of mammalian embryos can lead to aberrant expression of imprinted and non-imprinted genes owing to associated epigenetic 'errors' [104, 105]. It has been shown that many cloned embryos demonstrate different degrees of resemblance with donor cell gene expression patterns. The aberrant epigenetic pattern in cloned embryos is thought to be the result of persistence of the epigenetic memory of the donor cells. Indeed, the resemblance of DNA and histone modification patterns of NT embryos to those of the donor cell types supports this conclusion [45–47]. For example, both global and gene-specific patterns of DNA methylation in cloned bovine and mouse embryos were shown to be similar to those of their respective donor somatic cell types [28, 106, 107]. The phenomenon of epigenetic memory is also highlighted by the X inactivation pattern in cloned embryos. In normal fertilized embryos, the paternal X chromosome is preferentially silenced in the trophectoderm and extraembryonic endoderm lineages, whereas random X inactivation occurs in the inner cell mass. However, in NT embryos generated from female donor nuclei, the

inactive X chromosome of the donor cell is preferentially chosen for inactivation in the trophectoderm, which is in contrast to the random X inactivation in the embryo proper, demonstrating a certain extent of memory of the inactive X chromosome status [48]. Random X inactivation in the placenta was found in deceased cloned bovine embryos, which suggests that the persistence of this inactive X chromosome memory in the placenta may be crucial for fetal survival [49].

The epigenetic memory in NT embryos that maintains the donor expression gene states can be explained by the inheritance of DNA methylation patterns. Heritability of DNA methylation is mediated by the activity of the maintenance DNA methyltransferase Dnmt1 which preferentially targets hemi-methylated DNA. Thus, Dnmt1 restores the parental methylation status on the newly synthesized daughter DNA strand, thereby maintaining a silent gene state after cell division [108]. Methylated donor genes, such as pluripotency genes, remain inactivated after SCNT, apparently owing to the persistent donor-specific methylation pattern in the cloned embryos, possibility mediated by the residual somatic form of Dnmt in the donor nucleus. DNA methylation therefore provides a plausible mechanism for the propagation of a silent memory state in SCNT [109]. On the other hand, an active gene memory of the donor differentiation state is also observed in NT embryos. For example, both the donor endoderm and neurectoderm markers, *Edd* and *Ncam*, were found to be aberrantly expressed in *Xenopus* NT embryos derived from the respective donor cell types [18]. This active gene memory was further demonstrated to be associated with the incorporation of a histone H3 variant H3.3 at the active gene loci in *Xenopus* NT embryos [110]. Histone variant H3.3 is enriched in the regulatory region of active genes and is preferentially marked by modifications associated with an active chromatin state, such as H3K4 methylation, H3K9 acetylation and H3K79 methylation [111, 112]. Experiments using a mutant form of H3.3 demonstrated that the K4 residue on H3.3 plays a key role in the inheritance of active epigenetic memory [110], proposing a model in which H3.3 K4 methylation creates an "active histone environment" for the recovery of active chromatin configuration in daughter cells after chromosomal replication [113].

Early studies of iPS cells demonstrated that the Nanog-iPS cells displayed not only a highly similar transcriptome to wild-type ES cells, but also an ES cell histone modification profile. Genome-wide comparison of histone modifications (H3K4 and H3K27 trimethylation) between ES cells, MEFs and MEF-derived iPS cells demonstrated that more than 94% of the ES- or MEF-signature genes in iPS cells have identical histone methylation marks as in ES cells. Only 0.7% of these signature genes retain the histone methylation status of the original MEFs [63]. However, other gene expression profile studies of iPS cells showed that a significant number of differentiation genes have a similar expression pattern to that in the somatic cell of origin, but not in ES cells [114–117]. This transcriptional memory in iPS cells was found to be correlated with biased differentiation towards the original cell lineage, and with less competence in differentiation to other unrelated somatic lineages [115, 118–121]. Importantly, the persistent expression of somatic genes in iPS cells was associated with the somatic DNA methylation pattern [117, 122–124], highlighting the crucial role of epigenetic regulation in the retention of memory. This is in fact similar to the observation that an incomplete removal of donor cell DNA methylation pattern was observed in some aberrantly developed NT embryos [125]. Strikingly, the addition of epigenetic modifying agents, such as DNA methyltransferase inhibitor 5-aza-2'-deoxycytidine (5-aza-dC), can enhance the iPS cell reprogramming efficiency [70]

and improve the differentiation competency to other unrelated somatic lineages [120]. Interestingly, it has been demonstrated that continuous passaging of iPS cells abrogates somatic DNA methylation patterns [115], which suggests a passive replication-dependent mechanism in loss of the parental memory in iPS cells. Nevertheless, a study showed that the epigenetic memory in some iPS cell lines cannot be removed even after extended passages [124]. Apart from DNA methylation, microRNA expression pattern was also shown to have a role in the retention of somatic memory in iPS cells derived from hematopoietic progenitors [126]. However, it should be emphasized that other profiling studies of iPS cells failed to find the gene expression and epigenetic differences when compared to ES cells [127, 128]. It thus proposes that the "somatic memory" in iPS cells could be an artifact of incomplete reprogramming resulting in variation between iPS cell lines [129]. It is also possible that there are individual iPS cell lines expressing gene signatures owing to culture conditions and laboratory practices [130], similar to the scenario that some ES cell lines exhibit preferential differentiation towards specific lineages [131–133]. In summary, the epigenetic memory in iPS cells remains a contentious issue.

6. Conclusions and perspectives

Although the term "epigenetic landscape" was first introduced by Waddington in 1942 [1], our understanding of how the epigenome of a cell type is maintained and altered during differentiation is still far from complete. The reversal of the differentiated state of a cell has important implications for our understanding of normal development and for regenerative medicine. Epigenetic reprogramming provides heritable changes of cell identity, and thus is a key event for the complete and permanent conversion of cell fate (**Figure 2**). Although reprogramming of cell fate can be achieved by different strategies, the rate (reprogramming time) and efficiency (number of reprogrammed cells) are far from comparable to the natural event during fertilization/de-differentiation. Achieving a complete epigenetic reversion to generate reprogrammed cells or iPS cells with a comparable potency state of early embryos would imply that these cells can respond correctly to differentiation-promoting signals, and more importantly, decrease the tumorigenic potential owing to pre-disposing epimutations. Notably, the status of epigenetic memory in iPS cells can be regarded as a state of incomplete reprogramming. The biased differentiation owing to the persistent somatic epigenetic memory in iPS cells might be useful in efficient differentiation to the desired cell type of origin, which usually results in a heterogeneous cell population by using un-optimized differentiation protocols. On the contrary, it has been shown that *in vitro* culture condition can alter the epigenetic status of iPS cells [134]. With an optimized culture condition, a more homogeneous population of iPS cells can be obtained, which corresponds to the naïve state of pluripotency, and hence, further abrogate the somatic "epigenetic memory". A more recent approach in reprogramming involves the use of a combination of small chemical molecules and epigenetic modifying agents, without any ectopic expression of transcription factors [135, 136]. This approach seems to induce pluripotent reprogramming process different from the transcription factor-mediated approach. Therefore, unlocking the secrets

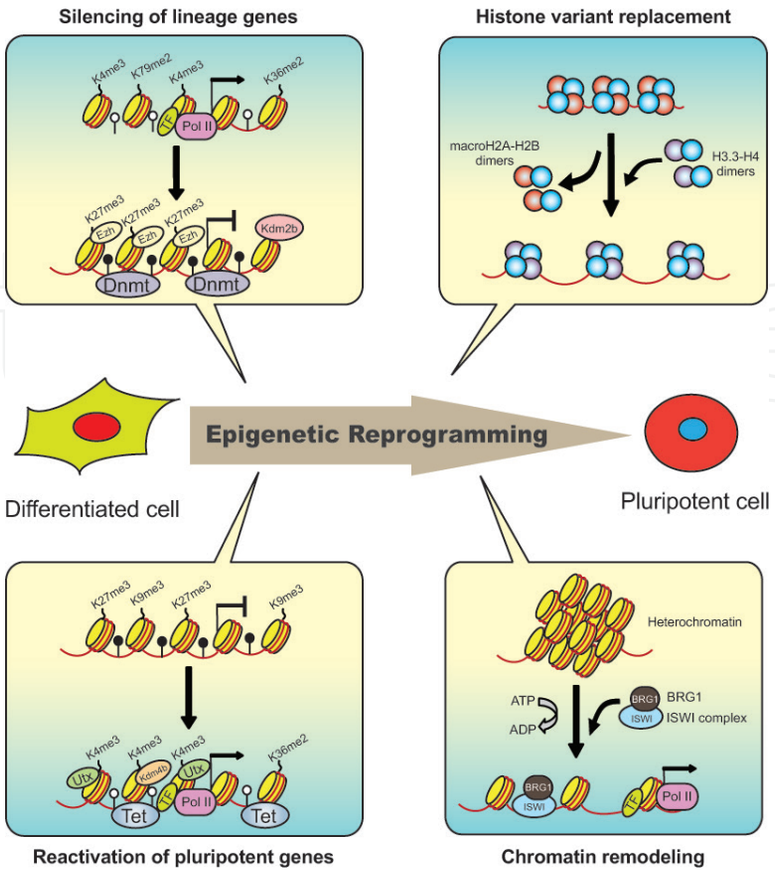

Figure 2. Mechanisms of epigenetic reprogramming. Epigenetic patterns in differentiated cells need to be reprogrammed to those of pluripotent cells, which results in silencing of differentiated genes and reactivation of pluripotency genes. Epigenetic reprogramming can be achieved by modulation of DNA methylation and histone modifications by various epigenetic modifying enzymes, such as Dnmt, Tet, Kdm2b, Kdm4b, Utx, Ezh2 (PcG), histone variant replacement, and chromatin remodeling enzymes. Other epigenetic mechanisms may also be involved. It is believed that a collaborative contribution of different epigenetic mechanisms is required for complete reversal of the differentiated cell state.

of epigenetic resetting mechanisms during cell differentiation can shed light on the development of more efficient and complete reprogramming approaches to further advance regenerative medicine.

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Conflict of interest

The authors declare that they have no conflict of interest.

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