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AGE-RELATED STEM CELL MODIFIERS (L KURIAN AND A PAPANTONIS, SECTION EDITORS)

Enhancer Remodeling During Early Mammalian Embryogenesis: Lessons for Somatic Reprogramming, Rejuvenation, and Aging

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Abstract Early during mammalian embryogenesis, epiblast cells undertake major cell fate decisions, becoming specified towards either the perishable soma or the immortal germline. Despite the importance of these developmental transitions, the transcriptional regulatory mechanisms orchestrating them have remained poorly characterized due to the transient nature and scarcity of the involved cell populations. However, our view of these processes is dramatically changing due to advances in mouse and human embryonic stem cell (ESC) differentiation models that faithfully recapitulate peri-implantation transitions. Recent studies using these models have uncovered enhancers as critical *cis*-regulators during the maintenance, extinction, or re-establishment of pluripotency. Here, we review the major transcriptional and epigenetic regulators controlling the remodeling of enhancer landscapes during mammalian peri-implantation development. Last but not least, we discuss how a global and mechanistic understanding of enhancer remodeling can provide important insights into somatic reprogramming, the molecular basis of aging, and the implementation of cellular rejuvenation strategies.

Keywords Enhancers · Pluripotency · Germline · Reprogramming · Aging · Stem cell

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Introduction

Following implantation of the mouse blastocyst, highly relevant and dynamic cellular transitions take place during a developmental time window that we will refer to as peri-implantation development (E4.5–E7.5) (Fig. 1). The pre-implantation epiblast (~E4.5) displays naïve or ground-state pluripotency, as it can give rise to all embryonic lineages, including the germline [1]. Immediately after implantation, the epiblast progresses to a transient state that has been recently referred to as formative pluripotency (~E5.5–6.5) and which is characterized by the dismantling of the naïve pluripotency expression program [2]. Subsequently and coinciding with the beginning of gastrulation, the epiblast cells acquire a primed pluripotent state (~E6.5–7.5) and start expressing somatic germ layer specifiers [2]. Remarkably, while both formative and primed pluripotent cells are able to differentiate into all somatic lineages, only formative cells are competent for primordial germ cell (PGC) induction and specification (~E6–6.5) [3]. Moreover, PGC specification involves and functionally requires the re-activation of naïve pluripotency genes, including several transcription factors (TFs) with master regulatory functions [4, 5•, 6]. Therefore, a pluripotency cycle maintained by a shared transcriptional regulatory network has been hypothesized to exist between the naïve epiblast and the germline [1].

As mammalian embryogenesis proceeds, the acquisition of novel cellular identities requires not only the establishment of new gene expression programs but also the extinction of previously existing ones. It is now widely accepted that enhancers play a preponderant role during these developmental events by integrating the regulatory activities of TFs, epigenetic regulators, and signaling pathways [7]. Consequently, developmental transitions are accompanied by dramatic changes in enhancer repertoires, involving not only the activation but also the silencing of a

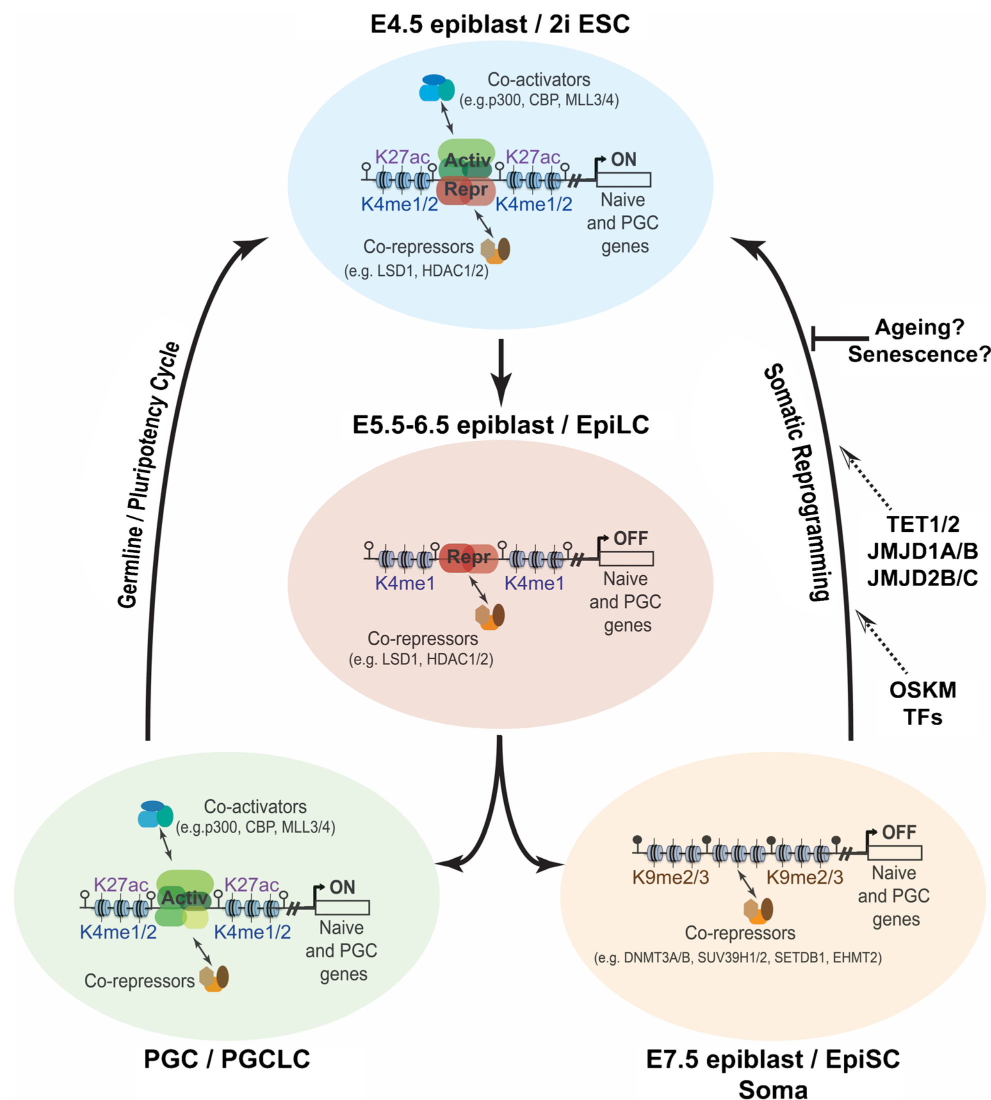

Fig. 1 Remodeling of enhancers associated with naïve pluripotency and early germline genes. During mouse peri-implantation transitions, enhancers controlling the expression of naïve pluripotency and early germline genes are active in naïve pluripotent cells (i.e., E4.5 epiblast/2i ESC), as reflected by the presence of H3K4me1/2 and H3K27ac and their overall hypomethylation (*empty lollipops*). These enhancers are bound by pluripotency TFs (e.g., OCT4, SOX2, NANOG) (activators: *green ovals*) that recruit co-activators and sustain their active state. Moreover, these enhancers are also bound by transcriptional repressors (e.g., FOXD3, TCF3) (*red ovals*) that recruit co-repressors and promote the decommissioning of these regulatory sequences. As a result, epiblast cells exit naïve pluripotency and transiently acquire a formative pluripotent state (i.e., E5.5–6.5/EpiLC), in which naïve pluripotency/germline enhancers become decommissioned as they loose H3K27ac and H3K4me2, while retaining H3K4me1 and remaining hypomethylated. Subsequently, these enhancers will undergo two alternative fates: (i) in the germline, these enhancers become re-activated through the binding of PGC and naïve pluripotency master regulators (*green ovals*), which reinstate the typical chromatin signature of active enhancers (i.e., H3K4me1/2, H3K27ac); (ii) in the primed pluripotent epiblast (E7.5/EpiSC) and in the soma, these enhancers get fully silenced, as they gain H3K9me2/3 and become hypermethylated (*filled lollipops*). The repressive chromatin environment established around naïve pluripotency/germline enhancers in the soma ensures their stable silencing and represents a major roadblock for somatic reprogramming

large number of enhancers [8]. The paucity and transient nature of the cell populations involved in peri-implantation development have precluded the molecular characterization of the transcriptional and epigenetic changes that accompany these cellular transitions. Recent advances in ESC culture conditions, together with newly developed differentiation protocol, allow a faithful recapitulation of early embryogenesis, both in mouse and in humans. Chiefly, these in vitro models are genetically tractable and can provide relevant peri-implantation cellular states in numbers compatible with genomic and biochemical approaches. In the next sections, we briefly describe these in vitro models and how they have been used to characterize enhancer dynamics during peri-implantation transitions, giving special emphasis to a core set of enhancers controlling the expression of naïve

pluripotency and early germline genes ("pluripotency/germline enhancers") (Fig. 1).

Enhancer Dynamics During the Transition from Naïve to Primed Pluripotency

Mouse ESC (mESC) have been traditionally grown under "LIF + serum" conditions, which results in heterogeneity and metastability as these cells reversibly switch between naïve and primed pluripotent states [9]. Importantly, these two states can now be independently maintained in culture, as represented by mESC grown under "2i + LIF" (2i ESC, 2i = MEK and GSK3 inhibitors) and epiblast stem cell (EpiSC), respectively [10, 11]. Moreover, the recently described epiblast-like cells (EpiLC) resemble the pre-gastrulating epiblast and display formative pluripotency, a transitional state between naïve and primed pluripotency [2, 6].

The transcriptional regulatory network that maintains the naïve pluripotent state has been extensively characterized. This network consists of a set of "core" (OCT4/POU5F11, SOX2) and "ancillary" (NANOG, ESRRB, KLF4, KLF2, TBX3, PRDM14) TFs that, together with the final effectors of key signaling pathways (LIF, WNT, BMP), sustain naïve pluripotency through cooperative binding and consequent activation of a broad set of enhancers [9, 12, 13]. This involves the recruitment of co-activators (e.g., p300, BRG1) and the establishment of a chromatin signature characteristic of active enhancers (nucleosomal depletion, H3K4me1/2, H3K27ac) [14, 15]. Moreover, at least one of these TFs, PRDM14, ensures naïve pluripotency by repressing, in a polycomb dependent manner, key signaling pathways (i.e., FGF), and epigenetic regulators (i.e., *Dnmt3A/B*) that promote the transition to primed pluripotency [16].

The establishment of EpiSC and the differentiation of 2i mESC into EpiLC has enabled the characterization of the transition from naïve into formative and then primed pluripotency. During these transitions, core pluripotency TFs (OCT4 and SOX2) remain highly expressed and partner with a different set of TFs, such as OTX2, leading to the cooperative activation of many novel enhancers and their target genes [17•, 18••]. Although some of these enhancers display a primed or poised state in 2i ESC, most of them lack any pre-marking (e.g., H3K4me1, DNAseI hypersensitivity) and are activated de novo in EpiLC [18••]. Overall, the gene expression programs of naïve, formative, and primed pluripotent cells are markedly different, yet they express some genes in common. Remarkably, genes active in both mESC and EpiSC are apparently regulated by different sets of enhancers in each cell type [19•], including a set of EpiSC-specific enhancers referred to as "seed" enhancers that are frequently used at subsequent developmental stages in somatic tissues [10]. Interestingly, it has been recently reported that differentiated somatic cells with the capacity to self-renew (e.g., macrophages) might be able to do so by activating an ESC-like self-renewal gene expression program [20••]. Once again, ESC and differentiated cells utilize distinct sets of enhancers to control such self-renewal program. It would be interesting to determine if "seed" enhancers are preferentially used by self-renewing somatic cells.

As stated above, developmental transitions require not only the induction of new gene expression programs but also the dismantling of previously existing ones. FOXD3 and TCF3/TCF7L1 are transcriptional repressors that promote the exit from naïve pluripotency by mediating the silencing of distinct sets of ground-state regulators (e.g., TCF3 targets: *Tfcp2l1*, *Klf2*, *Esrrb*, *Nanog*; FOXD3 targets: *Prdm14*, *Tbx3*, *Pramel6/7*) [21, 22••]. This suggests that these two repressors might be independently required to exit naïve pluripotency by dismantling discrete parts of the naïve expression program. FOXD3 executes its repressive function by mediating the decommissioning of a subset of naïve pluripotency enhancers [22••]. Mechanistically, this involves the displacement of pluripotency TFs and co-activators as well as the recruitment of co-repressors (e.g., LSD1, HDAC1/2, NuRD) [22••, 23]. It is currently unknown if TCF3 repressive function involves a similar enhancer decommissioning mechanism, but data from other cellular contexts suggest that this could be certainly the case and could then involve the recruitment of additional co-repressors (e.g., TLE/Groucho) [24]. The repressive activity of these two and most likely other, yet undiscovered, TFs leads to the decommissioning of a large set of enhancers during the transition from naïve (i.e., 2i ESC) to formative pluripotency (i.e., EpiLC,), which involves a progressive loss of H3K27ac and H3K4me2 and retention of H3K4me1 [18••, 22••] (Fig. 1). Interestingly, during this transition, these enhancers start gaining H3K9me2 as part of their silencing mechanism, although they remain hypomethylated despite the raising levels of de novo DNA methyltransferases (i.e., DNMT3A/B) [25•, 26, 27]. As primed pluripotency emerges (i.e., EpiSC), naïve pluripotency enhancers become fully decommissioned, completely loosing not only H3K27ac but also H3K4me1, while gaining even higher levels of H3K9me2 [19•, 25•]. Although not formally shown, since DNMTs display negative and positive crosstalk with H3K4me1/2 and H3K9me2/3, respectively [28, 29], it is likely that these enhancers will become accessible for DNMT3A/B and hypermethylated already in EpiSC. Upon somatic differentiation, the repressive chromatin environment around naïve pluripotency enhancers becomes consolidated, stable, and largely inaccessible [30] (Fig. 1).

The characterization of how enhancer landscapes are remodeled during the transition from naïve to primed pluripotency has been by and large performed using murine in vitro models. Traditional culture conditions for human ESC (hESC) results in a cellular state that, in many aspects, resembles mouse EpiSC and are thus considered to display primed pluripotency [10]. However, after extensive effort, the long-sought conditions to induce naïve pluripotency in hESC were

recently reported [31•, 32••]. Perhaps not surprisingly, these two hESC states manifest markedly different transcriptomes and epigenomes as well as enhancer repertoires [32••, 33•]. It remains to be elucidated if mouse and humans use the same or different TFs to control the major enhancer landscape remodeling that characterizes the transition from naïve to primed pluripotency.

Common Enhancers Participate in the Maintenance of Naïve Pluripotency and in Germline Specification

Soon after implantation, epiblast cells become primed towards somatic differentiation and start to express somatic lineage specifiers. However, in response to high levels of BMP4 and WNT3 emanating from the extraembryonic tissues adjacent to the proximo-posterior epiblast, a few epiblast cells revert somatic differentiation and initiate PGC specification [34]. This process is tightly regulated by a group of master regulators (BLIMP1/PRDM1, PRDM14, TFAP2C) that become sequentially activated in PGC precursors (~E6.25–6.75) [3, 35••, 36]. Importantly, these TFs are all expressed in naïve pluripotent cells, in which, at least PRDM14, is also functionally required to maintain the ground pluripotent state [16]. Likewise, NANOG, a key naïve pluripotency regulator, plays a major role during PGC specification [4, 5•]. The coordinated action of these TFs results in marked transcriptional changes that eventually lead to the establishment of a gene expression program remarkably similar to that of the naïve epiblast [1]. These transcriptional changes include (i) the repression of the incipient somatic program and of genes required for DNA methylation, (ii) the re-activation of the naïve pluripotency network, including highly relevant TFs (e.g., *Nanog*, *Oct4*, *Sox2*) (iii) the silencing of genes that are upregulated during the transition from naïve to formative pluripotency and that characterize the formative pluripotent state (e.g., *Otx2*, *Pou3f1*, *Foxd3*, *Tcf3*), and (iv) the induction of early germline genes (e.g., *Rhox5/6/9*, *Nanos3*) [6, 22••, 34]. As a result, a cluster of approximately 40 PGCs is formed (~E7.25), which then undergo major epigenetic reprogramming (i.e., global DNA demethylation, imprinting erasure, changes in histone modification profiles) as they proliferate and migrate towards the genital ridges [34].

Historically, uncovering the molecular basis of the transcriptional and epigenetic changes underlying PGC specification has been a daunting task due to the scarcity of these cells. However, the Saitou lab established an in vitro protocol whereby primordial germ cell-like cells (PGCLC) are induced from EpiLC, faithfully recapitulating murine PGC specification [6]. This novel protocol has dramatically improved our understanding of this process, allowing, among other things, to uncover the set of enhancers involved in germline specification [37••]. Analogously to the extensive changes in enhancer usage observed during the transition from naïve to primed pluripotency, PGC specification is also accompanied by a considerable remodeling of enhancer landscapes [37••]. These enhancer changes can be broadly divided into two major categories: (i) enhancers that become rapidly and transiently activated (day 2 PGCLC) and that most likely control the induction of mesodermal genes (e.g., *T*) directly involved in the earliest steps of PGC specification [38] and (ii) enhancers activated later during PGC specification (day 6 PGCLC) and that presumably promote the re-activation of naïve pluripotency and early germline genes. Notably, this second group of regulatory elements includes multiple enhancers that are originally active in naïve ESC and that become decommissioned in EpiLC [22••, 37••]. This supports the existence of a pluripotency/germline cycle in which a core set of pluripotency/germline enhancers might control the expression of naïve pluripotency and early germline genes in the pre-implantation epiblast and PGCs [1] (Fig. 1).

Intriguingly, upon exit from naïve pluripotency, the initial decommissioning of pluripotency/germline enhancers does not entail a full silencing of these key regulatory elements. Instead, in EpiLC/E6.5 epiblast, these regulatory sequences acquire a transient poised state in which they retain H3K4me1, remain hypomethylated, and are most likely bound by TFs (e.g., FOXD3, OCT4) [18••, 22••, 26, 27] (Fig. 1). The presence of H3K4me1 and TFs is known to be refractory for the enzymatic activity of DNMT3A/B, which could explain why pluripotency/germline enhancers remain hypomethylated in the face of the extensive de novo DNA methylation that occurs in EpiLC and the post-implantation epiblast [28, 39, 40]. We have previously hypothesized that such poised state, imposed at least partially by FOXD3, could then facilitate the re-activation of pluripotency/germline enhancers and their associated genes upon binding of PGC specifiers (e.g., PRDM14, TFAP2C) [22••, 35••, 36]. This could explain why naïve pluripotency and early germline genes are re-activated before the major wave of DNA demethylation that occurs at later stages of PGC specification and that might be critically required to induce genes involved in meiosis and late germline development [41, 42]. As stated above, the poised state of pluripotency/germline enhancers in EpiLC is transient, since these regulatory sequences become progressively silenced and heterochromatinized upon transition to primed pluripotency/EpiSC and subsequent somatic differentiation [19•, 25•, 30]. Like the poised state of pluripotency/germline enhancers, germline competence is also transient and short-lived as it is a unique property of the pre-gastrulating post-implantation epiblast in vivo and of EpiLC in vitro [3, 6]. Hence, it is tempting to speculate that germline competence might be at least partially dependent on the poised hypomethylated state of pluripotency/germline enhancers.

Similarly to the transition from naïve to primed pluripotency, much of what we know about enhancer dynamics during PGC specification has been described using murine in vitro models. However, the Surani and Saitou labs have

recently reported two similar protocols whereby hESC can be efficiently differentiated into PGCLC [43•, 44••]. These in vitro models have been already used to reveal the major similarities (e.g., BLIMP1, PRDM14, TFAP2C, NANOG) but also the notable differences (e.g., SOX17) among the TFs involved in human PGC specification with respect to the mouse [43•, 44••]. We anticipate that these in vitro models will be soon used to uncover how enhancer repertoires change during specification of the human germline.

Enhancer Remodeling During Somatic Reprogramming

Ectopic expression of a limited set of transcription factors (OCT4, SOX2, KLF4, C-MYC (OSKM)) is sufficient to reprogram somatic cells towards pluripotency [45, 46]. Both mouse and human induced pluripotent stem cells (iPSC) display remarkable transcriptional, epigenetic, and functional similarities to ESCs, which, not surprisingly, includes highly similar enhancer repertoires [47, 48]. In addition to its obvious and far-reaching clinical applications, the generation of iPSC offers a tractable system to investigate the transcriptional and epigenetic mechanisms controlling developmental transitions [49, 50]. Unfortunately, somatic reprogramming is typically inefficient and great efforts have been devoted to uncovering the major facilitators and roadblocks of this process [30, 51•, 52–55, 56••, 57]. Somatic cells are frequently arrested in a partially reprogrammed state (i.e., pre-iPSC), in which the re-activation of core pluripotency regulators is a major rate-limiting step towards the acquisition of full pluripotency [30, 49, 51•, 52, 54]. Remarkably, the set of genes that are especially refractory to re-activation during reprogramming includes not only pluripotency regulators but also early germline genes, overall coinciding with genes that dynamically change their expression during peri-implantation transitions, as described in previous sections [30, 52, 54]. We will now review evidence suggesting that, similarly to peri-implantation transitions, the re-activation of these pluripotency/germline genes during reprogramming depends on the epigenetic status and accessibility of their associated enhancers (Fig. 1).

Seminal work from the Zaret lab demonstrated that exogenous OSKM TFs were able to bind to a large set of distal sequences already at the initial steps of somatic reprogramming [30]. Importantly, these sequences could be broadly divided into two groups: (i) sites with accessible chromatin in somatic cells, as represented by DNAseI hypersensitivity and presence of H3K4me1/2 [57] and (ii) sites with closed chromatin in which OSK acted as pioneer TFs and facilitated C-MYC binding. Importantly, the second group included important enhancers that in mESC are active and occupied by endogenous pluripotency TFs. The binding of OSKM TFs to these enhancers leads to their subsequent activation, which, interestingly, precedes the induction of their target genes [30, 58]. These results suggest that, despite the major epigenetic barriers that are believed to exist between different cellular states and despite the refractory effect of silent chromatin on TF binding, ectopic OSKM TFs can readily access a remarkable fraction of their endogenous binding sites in ESCs. Importantly though, the reprogramming TFs initially fail to bind a core set of enhancers associated with relevant pluripotency/germline genes, which remain silent and inaccessible, thus impeding the re-activation of their critical target genes [30]. Overall, these observations illustrate the importance of pluripotency/germline enhancers for the acquisition of pluripotency and also raise major questions regarding the inaccessibility of these sequences and the chromatin features that make them so resistant to TF binding. Epigenomic analysis in MEFs and pre-iPSC indicates that these enhancers are hypermethylated and enriched in H3K9me2/3, creating a heterochromatic environment particularly impenetrable for TFs [30, 51•, 52, 58] (Fig. 1). In full agreement with this, enzymes that methylate (e.g., DNMT1, DNMT3A) or demethylate (TET1, TET2, AID) 5mC have been shown to act as inhibitors and facilitators of reprogramming, respectively [52, 53, 59, 60]. Likewise, H3K9 methyltransferases (HMTs) (e.g., SUV39H1, SUV39H2, SEDB1, EHMT2) act as reprogramming barriers, while H3K9me2/3 demethylases (e.g., JMJD1A, JMJD1B, JMJD2B, JMJD2C) increase reprogramming efficiency [30, 51•, 55]. DNA methylation has been traditionally investigated in the context of promoter regions and repetitive elements, but a major role in controlling enhancer activity, mostly by interfering with TF binding, has been recently appreciated [40, 61, 62•]. On the other hand, reprogramming experiments suggest that H3K9me3/me2 might have an equally important yet largely unappreciated role in enhancer silencing [30, 51•, 55]. The importance of DNA methylation and H3K9me2/3 as epigenetic reprogramming barriers that prevent the re-activation of pluripotency/germline enhancers is in perfect agreement with the epigenetic changes displayed by these regulatory elements during embryogenesis [17•, 18••, 19•, 22••, 25•, 26, 27, 37••, 63, 64] (Fig. 1). In addition to DNA hypermethylation and gain of H3K9me2/3, the chromatin changes observed at pluripotency/early germline enhancers during peri-implantation transitions suggest that complete loss of H3K27ac and H3K4me1/2 can also contribute to the full silencing of these regulatory elements [17•, 18••, 19•, 22••, 25•, 37••]. Accordingly, enzymes and protein complexes mediating histone deacetylation (e.g., HDAC1, MBD3, NuRD) and H3K4 demethylation (e.g., LSD1) at enhancer elements can act as somatic reprogramming barriers [56••, 65, 66].

The re-activation of pluripotency/germline enhancers and their associated genes seems to be an important and early step during PGC specification [5•, 22••, 37••]. Interestingly, enhancer re-activation seems to occur effortlessly in PGCs, which is in stark contrast to the refractory nature of this process during

somatic reprogramming. As mentioned earlier, germline competence might require a silent (loss of enhancer RNAs (eRNAs) and H3K27ac) but poised chromatin state (i.e., H3K4me1, bound by TFs such as FOXD3) at pluripotency/early germline enhancers that protects them from DNA hypermethylation [22••, 26–28, 39] (Fig. 1). Like germline competence itself, this poised chromatin state is also transient, progressively transitioning to a fully silent and heterochromatic state as gastrulation and somatic differentiation begins [19•, 25•, 63, 64]. So, why do these regulatory elements demand such a resilient and impenetrable chromatin environment in the soma? From a functional standpoint, spurious re-activation of these enhancers could interfere with proper execution of somatic differentiation programs and/or the maintenance of somatic identity, potentially leading to tumorigenesis [49]. However, mechanistically, it remains unclear how this unique set of regulatory elements are specifically targeted by DNMTs and H3K9 HMTs. One possibility is that this is a rather passive process, in which the absence of positive regulatory inputs (e.g., TFs, co-activators) and transcriptional activity allows the spreading of a default repressive chromatin environment in a rather unspecific and progressive manner. In agreement with this model, most gene promoters are contained within CpG islands that protect them from DNA methylation through the establishment of antagonistic chromatin signatures (e.g., H3K4me2/3, H3K27me3) [28, 67, 68]. In contrast, enhancers typically display low CpG contents that could in principle render them susceptible to DNMTs activity. Nonetheless, this fails to explain why many of the active enhancers in ESC, once silent in somatic cells, do not gain H3K9me2/3 and/or become hypermethylated and are thus easily bound and re-activated by OSKM TFs upon reprogramming [30]. In fact, many enhancers active during embryogenesis remain hypomethylated once silenced in adult somatic tissues in a so-called vestigial state [69]. Alternatively, more direct and targeted regulatory mechanisms might be in place to ensure an efficient silencing of pluripotency/germline enhancers. For example, enhancers associated with core pluripotency genes (e.g., *Sox2*, *Oct4*) become silenced and hypermethylated in a targeted manner upon differentiation [63, 64]. In the case of *Oct4*, the transcriptional repressor GCNF might be involved in this process through the recruitment of DNMT3A/B [70]. Although a general targeting mechanism directing DNA methylation and H3K9me2/3 to pluripotency/germline enhancers has not been described, such a mechanism seems to exit for the promoter regions of genes involved in late germline development and gametogenesis. In this case, the transcription factor MAX and the E2F6 complex recruit H3K9 HMTs (e.g., G9a, GLP) to these promoters in order to ensure stable silencing of the associated genes in ESC and somatic tissues [71•, 72]. Future studies should aim at elucidating the mechanisms involved in the silencing of pluripotency/germline enhancers during embryogenesis, as this might illuminate novel strategies to improve the efficiency of somatic reprogramming.

Epigenetic Changes During Aging and Rejuvenation

Aging and age-associated cellular processes, such as senescence, represent major impediments for somatic reprogramming [73–75••]. Nevertheless, hiPSC have been successfully established from geriatric donors as well as from samples of progeria patients displaying accelerated aging, thus demonstrating that age-related barriers can be overcome and that cells can be rejuvenated [76–78]. As a result, there is growing interest in using somatic reprogramming as a model to molecularly and mechanistically understand age-associated barriers, as this can provide valuable insights into future rejuvenation strategies [79]. Although evidence is still limited, preliminary observations indicate that age-associated epigenetic alterations might represent major reprogramming roadblocks. A work from the Lopez-Otin laboratory has demonstrated that aging-associated NF-KB activation lead to a strong induction of DOT1L and consequently increased levels of H3K79me2 [75••]. DOT1L inhibition greatly facilitated somatic reprogramming and corrected age-associated alterations, thus representing a promising anti-aging target [75••]. Mechanistically, DOT1L increased expression lead to higher H3K79me2 levels at fibroblast-specific genes linked to epithelial-to-mesenchymal transition (EMT), presumably reinforcing their active state and preventing their silencing, which is a critical and early step during fibroblast reprogramming [53, 75••]. Based on this data, it would be interesting to evaluate if the rejuvenating effects of DOT1L inhibition are limited to mesenchymal cell types or instead, more general and effective in epithelial tissues as well. On the other hand, it has been conclusively demonstrated that aging results in hypermethylation of polycomb target genes, an epigenetic phenomenon that has also been observed in cancer [80–83]. Presumably, this hypermethylation locks already silent genes in an even more stable repressive chromatin state. A recent integrative analysis of human reprogramming has revealed an orchestrated re-activation of broad developmental regulators that recapitulate, in an inverse manner, the events occurring during normal embryogenesis [56••]. Since many of these developmental regulators are bona-fide polycomb targets, we speculate that their age-associated hypermethylation might block their re-activation and thus could represent a major reprogramming barrier. These and other examples emphasize that epigenetic alterations are a hallmark of aging, which, chiefly, can be pharmacologically corrected [75••, 84].

A systematic evaluation of how enhancer landscapes and their associated chromatin features are affected by aging and age-associated processes has not been performed yet. However, since aging leads to a loss in cellular homeostasis and considering the major role of enhancers in conferring cellular identity, it is likely that epigenetic alteration of these regulatory sequences will be involved in the aging process [84, 85]. Hence, it would be interesting to evaluate how the

chromatin environment of pluripotency/germline enhancers evolves upon aging, as the re-activation of these regulatory elements represents a milestone during somatic reprogramming (Fig. 1). It is conceivable that the levels of silencing epigenetic marks (DNA methylation, H3K9me2/3) will further increase at these enhancers or even extend to other pluripotency regulatory sequences that normally remain hypomethylated in somatic tissues. On the other hand, it has been recently shown that both oncogene-induced senescence and accelerated aging (as represented by progeroid syndromes) dramatically alter the three-dimensional architecture of the human genome, preferentially affecting lamina-associated heterochromatin, also known as lamina-associated domains (LADs) [86–88]. Interestingly, the association of these heterochromatin domains with the nuclear lamina is linked to transcriptional repression and G9a-dependent deposition of H3K9me2 [89, 90]. Since, as stated above, pluripotency/germline enhancers and their associated loci gain H3K9me2/3 and become silenced upon somatic differentiation [25•, 30, 63, 64], it is likely that they will frequently reside within LADs in somatic cells. Moreover, it has been recently shown that pluripotency enhancers frequently contact each other, forming spatially organized genomic clusters that have been proposed to confer robustness to the pluripotent state [91]. We postulate that during aging, pluripotency/germline loci might not only retain heterochromatic marks but also lose their endogenous chromatin topology, which might further complicate the three-dimensional re-organization of the pluripotency network upon reprogramming. Thus, it is tempting to speculate that topological disruption of LADs during senescence and/or aging might exacerbate the re-activation of pluripotency loci during reprogramming.

Conclusion

The last few years have witnessed a dramatic change in our view of the transcriptional and epigenetic regulatory mechanisms involved in the control of early mammalian embryogenesis. The combination of ESC-based differentiation protocols together with next-generation sequencing approaches have resulted in a more global and mechanistic understanding of how pluripotent states are established, extinguished, or maintained. Recent advances on single-cell sequencing approaches suggest that the possibility of similarly characterizing these regulatory processes in vivo is within reach [89, 92, 93]. We anticipate that, as the comprehension of pluripotency keeps increasing, this will undoubtedly help improving the efficiency of iPSC generation. Last but not least, since somatic reprogramming can be seen as a cellular rejuvenation process, uncovering the major facilitators and barriers of somatic reprogramming can have far-reaching implications for the molecular understanding of aging and for the therapeutical treatment of age-related disorders.

Compliance with Ethical Standards

Conflict of Interest Patricia Respuela and Alvaro Rada-Iglesias declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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