Regulation of Reprogramming and Cellular Plasticity through Histone Exchange and Histone Variant Incorporation

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Early embryonic cells are totipotent and can generate a complete organism including embryonic and extraembryonic tissues. After division, cells lose their potency as they move toward a pluripotent state characterized by decreased cellular plasticity. During this transition, drastic changes in transcriptional programs occur in parallel with global chromatin reorganization. The epigenetic mechanisms governing the changes in chromatin signatures during the transitions of cellular plasticity states are starting to be understood. Among these mechanisms, recent studies highlight the importance of histone variant incorporation and/or eviction from chromatin in the regulation of the chromatin state that is linked to cellular potential. In this review, we discuss the role of histone variants during *in vivo* and *in vitro* reprogramming events. These results sustain the hypothesis that histone variants and histone exchange are key actors in the establishment of cellular plasticity programs.

Development starts with the fusion of two highly differentiated gametes (the spermatocyte and the oocyte) to form the zygote. The zygote is a totipotent cell that gives rise to all the embryonic and extraembryonic lineages. Generating a complete functional organism requires a precise order of molecular, cellular, and tissue-level events that combine cell division, reorganization, migration, differentiation, and cell death. These changes are driven—or at least supported—by a plethora of drastic spatiotemporal changes in transcriptional programs occurring in embryonic cells despite containing similar genetic information. Thus, epigenetic regulation plays a critical role in the control of transcriptional programs during development.

Epigenetic regulation includes DNA methylation, histone posttranslational modifications, chromatin remodeling, noncoding RNA, and histone variants. All of these elements can alter the properties of the chromatin and its interacting partners, thus controlling transcription. Among these epigenetic factors, histone variants are emerging as key regulators of transcription in embryonic stem (ES) cells and during development. Here, we put forward a hypothesis to consider histone replacement and chromatin assembly as a main driver of the chromatin configuration that sustains totipotency. We will first build on the known characteristics and mechanisms of histone variants with respect to their canonical counterparts in an exhaustive review and will subsequently elaborate our hypothesis that global histone exchange serves to consolidate totipotency *in vivo* and *in vitro*.

HISTONE VARIANTS: FACTS AND PROPERTIES

Canonical histones are encoded by several genes organized in clustered repeat arrays. They are transcribed dur-

ing S phase and their incorporation onto DNA only occurs during DNA synthesis. Histone variants differ from their canonical counterpart in amino acid sequence. In contrast to canonical histones, histone variants are typically encoded by a single copy gene whose transcription can occur outside of S phase leading to deposition of the variant independently of replication. Histone variant deposition onto chromatin is tightly regulated by specific histone chaperones and is thought to occur at specialized domains, although the mechanisms for specific targeting are unknown. In this section, we will introduce H2A and H3 histone variants and we will provide a brief description of their expression patterns during mouse preimplantation development.

H3 Variants

Mammalian cells possess two canonical H3 histones, H3.1 and H3.2, that differ only in a single residue (Cys to Ser substitution at position 96) (Hake and Allis 2006). Mammals also have additional H3 variants: H3.3, the H3 testis-specific variants H3.4 (previously referenced as H3t) (Witt et al. 1996) and H3.5 (Schenk et al. 2011), the centromeric variant cenH3 (also known as CENP-A), and the primate-specific variants H3.Y.1 and H3.Y.2 (Wiedemann et al. 2010). Mammalian H3.3 is encoded by two intron-containing genes (*h3f3a* and *h3f3b*) encoding identical proteins (Wellman et al. 1987) that differ from the canonical H3.1 and H3.2 by only four or five amino acids, respectively, including residue 31 in the amino-terminal tail (Ala in H3.1/H3.2 vs. Ser in H3.3). H3.3 incorporation into chromatin is performed by the chromatin remodeling complexes Chd1 (Konev et al. 2007) and ATRX/Daxx (Drané et al. 2010; Lewis et al. 2010) and the histone chaperone HIRA

(Tagami et al. 2004). H3.3 is associated with transcriptionally active regions (Schwartz and Ahmad 2005; Jin et al. 2009; Goldberg et al. 2010; Chen et al. 2013), and H3.3-containing nucleosomes are intrinsically less stable in vitro, especially when associated with the H2A variant H2A.Z (Jin and Felsenfeld 2007). In the mouse oocyte, H3.3 is present as a maternal factor and is incorporated before H3.1/H3.2 into the male pronucleus after fertilization (Torres-Padilla et al. 2006). The double H3.3 knockout (h3f3a and h3f3b) results in embryonic lethality in the mouse (Jang et al. 2015), but not in *Drosophila*, where deletion of H3.3 leads to sterility (Hödl and Basler 2009; Sakai et al. 2009).

H2A Variants: H2A.X, H2A.Z, macroH2A, and H2A.B

H2A has the largest number of variants described, with biochemical and functional properties that clearly distinguish each of them.

H2A.X possesses a carboxy-terminal tail containing a serine–glutamine (SQ)-rich motif and its function during DNA repair has been studied extensively. After a DNA double-strand break, H2A.X is phosphorylated by the ATM, ATR, and DNA-PK kinases on S139 (γ H2A.X) (Rogakou et al. 1998) leading to the recruitment of several DNA repair proteins (Fernandez-Capetillo et al. 2004). Additional biological functions of H2A.X, non-related to the DNA damage response, have been described (for review, see Turinetto and Giachino 2015). In mice, after fertilization, H2A.X is detected by immunofluorescence in both pronuclei and remains present at the two- and four-cell stages; subsequently the intensity of the signal decreases from the morula stage (Ziegler-Birling et al. 2009; Nashun et al. 2010; Wossidlo et al. 2010). H2A.X^{-/-} mice are viable but display genome instability, a growth delay, and male infertility (Celeste et al. 2002).

H2A.Z is 60% identical to canonical H2A and is well-conserved during evolution, suggesting an important role in the organism. Vertebrates possess two H2A.Z genes (H2A.Z.1 and H2A.Z.2) that encode proteins that differ by three residues (Eirín-López et al. 2009). In yeast, the incorporation of H2A.Z (Htz1) into chromatin is mediated by the histone chaperone Chz1 (Luk et al. 2007) and the histone exchanging nucleosome remodeling complex SWR1 (Mizuguchi et al. 2004). Although no H2A.Z-specific factors involved in H2A.Z incorporation have been identified in metazoans, a recent article identified ANP32E displaying histone chaperone activity specific for the H2A.Z/H2B dimer eviction from chromatin (Mao et al. 2014; Obri et al. 2014). In mice, embryos lacking H2A.Z fail to develop beyond the blastocyst stage (Faast et al. 2001). By immunofluorescence, H2A.Z signal is lost shortly after fertilization and reappears at the late two-cell stage or late morula stage depending on the studies (Nashun et al. 2010; Bošković et al. 2012). At the blastocyst stage, H2A.Z is detected at higher level in the nuclei of trophectoderm cells compared with inner cell mass cells (Rangasamy et al. 2003; Nashun et al. 2010).

MacroH2A is a vertebrate-specific histone H2A variant. MacroH2A variants are atypical histone variants because of the presence of a large carboxy-terminal non-histone domain called the macro domain (>210 amino acids) (Pehrson and Fried 1992). Mammalian cells encode three macroH2A proteins: macroH2A.2 and the two spliced variants macroH2A.1.1 and macroH2A.1.2. MacroH2A is generally considered as transcriptionally repressive because of its association with silenced chromatin (Costanzi and Pehrson 1998). In vitro, macroH2A has inhibitory effects on transcription factor binding (Angelov et al. 2003) and transcription initiation (Doyen et al. 2006). MacroH2A dynamics have been studied by FRAP (fluorescence recovery after photobleaching), which reveals that the mobility of macroH2A is lower than that of H2A suggesting a more stable association to the chromatin (Gaume et al. 2011). Double knockout mice for macroH2A.1 and macroH2A.2 display impaired prenatal and postnatal growth despite a relatively normal early development (Pehrson et al. 2014). MacroH2A is present in mouse oocytes and then lost in the zygote shortly after fertilization (Chang et al. 2005; Nashun et al. 2010) and becomes detectable again at the morula stage and persists in the blastocyst in both trophectoderm and inner cell mass cells (Chang et al. 2005; Nashun et al. 2010).

H2A.B (previously referenced as H2Abbd for H2A Barr body-deficient) is a divergent H2A variant (only 48% sequence identity with H2A) found exclusively in mammals (Chadwick and Willard 2001). H2A.B is encoded by three copies of the h2afb gene. In vitro studies have demonstrated that H2A.B confers a lower stability to the nucleosome (Eirín-López et al. 2008) and is more mobile than H2A in cells (as assessed by FRAP) (Gautier et al. 2004) and that H2A.B-containing nucleosomes organize only 118 pb of DNA (Bao et al. 2004). All these points argue for a positive role of H2A.B in chromatin accessibility and transcription (Zhou et al. 2007). In agreement, H2A.B is found associated with actively transcribed genes in HeLa cells (Tolstorukov et al. 2012).

Additional H2A and H2B variants exist, which are specifically expressed in the germline, in particular in the testis, that include TH2A, TH2B, H2A.L.1, and H2A.L.2, which are thought to be involved in facilitating incorporation of transition proteins and removal of histones necessary for the formation of the mature sperm (Govin et al. 2007; Soboleva et al. 2012; Montellier et al. 2013).

H1 Isoforms

The linker histone H1 plays critical roles in the compaction of chromatin and the arrangement of higher-order chromatin structure, as well as in the recruitment of numerous proteins that modify chromatin fiber accessibility or conformation (McBryant et al. 2010). Eleven different H1 variants have been identified in mammals so far, including somatic (H1.0 to H1.5 and H1.10) or germ cell–specific variants (testis-specific H1.6 [previously referenced as H1t], H1.7, oocyte-specific H1.8

[previously referenced as H1 α)] and H1.9 (for review, see Happel and Doenecke 2009). Although none of the H1 isoforms are essential for mouse development, presumably because of compensation (Sirotkin et al. 1995; Lin et al. 2000; Rabini et al. 2000; Fan et al. 2001), they are important for the fine regulation of gene expression (Alami et al. 2003; Happel and Doenecke 2009), acting both as activators and repressors of transcription (Clau-sell et al. 2009; Kamieniarz et al. 2012).

Because of their specific features and diversity, histone variants confer additional regulatory levels to tightly control gene expression, DNA repair, and chromosome compaction and recombination. In this review, we discuss recent findings involving histone variants in the regulation of transcription during mammalian development. Most of the work presented below concerns the mouse embryo as a model system.

HETEROCHROMATIN ESTABLISHMENT AND MAINTENANCE BY HISTONE VARIANTS: REGULATION OF REPETITIVE ELEMENTS

After fertilization, genome-wide epigenetic reprogramming includes *de novo* establishment of chromatin domains such as heterochromatin. Heterochromatin is a condensed chromatin state resulting in transcriptional silencing of the corresponding genomic regions. Heterochromatin can be classified into constitutive versus facultative. Contrary to facultative heterochromatin, constitutive heterochromatin corresponds to chromosomal regions that are never converted into euchromatin, such as centromeres, telomeres, and repetitive elements. An example of facultative heterochromatin is the inactive X chromosome in female mammals that can be either packaged into heterochromatin and silenced or organized into euchromatin and expressed. At the molecular level, heterochromatin is characterized by specific epigenetic marks including DNA methylation, repressive histone posttranslational modifications, the presence of characteristic heterochromatin proteins, and the incorporation of specific histone variants in the nucleosome core particle.

Eukaryotic genomes contain a large proportion of repetitive sequences organized in clusters (rDNA, centromeres, and telomeres) or interspersed in the genome (transposable elements). Repetitive elements constitute a permanent threat for genomic stability. Thus, heterochromatinization of these loci is crucial to silence them and prevent, for example, recombination or retrotransposition. The specific presence of histone variants on repetitive sequences suggests an important role in the formation and/or maintenance of heterochromatin on these sequences.

Genome-wide localization studies of H3.3 have demonstrated that in addition to its association with active histone modifications, H3.3 is also enriched in (TTAGGG) $_n$ repeats, hallmark of vertebrate telomeres (Goldberg et al. 2010). H3.3 localization at telomeres was confirmed by immunofluorescence in mouse ES cells (Wong et al. 2009). H3.3 is incorporated into telo-

meres during S phase, and a phosphorylated form of H3.3 (H3.3S31P) is detected on telomeres during mitosis (Wong et al. 2009). Interestingly, upon differentiation, the telomeric signal of H3.3S31P decreases, concomitantly with an increased association of heterochromatin marks (H3K9me3 and H4K20me3) at telomeres (Wong et al. 2009). The H3.3 histone chaperone ATRX colocalizes with H3.3 on telomeres (Wong et al. 2010) and is responsible for H3.3 deposition at these regions (Goldberg et al. 2010; Lewis et al. 2010). To support these observations, chromatin immunoprecipitation (ChIP) analysis has also shown that ATRX is physically associated with telomeres (Goldberg et al. 2010). Interestingly, ATRX depletion leads to the up-regulation of the telomeric repeat containing RNA (TERRA) suggesting that H3.3 is important for proper silencing of telomeric repeats (Goldberg et al. 2010), at least partially by recruiting HP1 α (Wong et al. 2010).

H3.3 has also been associated with endogenous retroviral elements (Classes I and II endogenous retroviruses [ERVs]) (Elsässer et al. 2015). As is the case on telomeres, H3.3 deposition on ERVs is performed by ATRX/Daxx and not by HIRA (Elsässer et al. 2015). In this study, the authors demonstrated that ATRX/Daxx-dependent deposition of H3.3 on ERVs is important for their repression through the recruitment of the histone methyltransferase ESET, which in turn establishes H3K9me3 on ERVs (Elsässer et al. 2015).

H3.3, together with ATRX/Daxx, is also found on pericentric DNA repeats in mouse embryo fibroblasts (MEFs) (Drané et al. 2010). The absence of any of these partners leads to a decrease in pericentric transcripts (Drané et al. 2010). Similarly, in mouse embryos, H3.3 localizes to the paternal pericentric chromatin during the first S phase and is important for pericentric heterochromatin establishment and integrity (Santenard et al. 2010). In this context, the authors propose the following model: H3.3 promotes the transcription of major satellite RNA, which in turn leads to an efficient silencing of pericentromeric domains (Santenard et al. 2010). In neurons, a recent study has shown that ATRX is specifically recruited to centromeric repeats by the combinatorial histone modifications (H3K9me3 and H3K9S10Ph) (Noh et al. 2015) suggesting a link between specific histone modifications and histone variant deposition.

Although the role of H3.3 in the transcription and the establishment of heterochromatin on repetitive elements (ERVs, telomeres, pericentromeres) is not fully understood (Fig. 1), H3.3-dependent establishment of heterochromatin is important for the maintenance of genome integrity of cells in culture (Jang et al. 2015). Heterochromatin establishment may involve unknown additional factors depending on the genetic environment or the cellular context. It is tempting to assume that H3.3, in agreement with its association with open chromatin structure and transcription, plays a role in the first step of the establishment of heterochromatin by promoting the transcription of small noncoding RNA (Santenard et al. 2010). When established, heterochromatin is then stabilized by the recruitment of additional factors such as heterochromatin

Figure 1. H3.3 is required to establish and maintain heterochromatin on repetitive elements. (A) H3.3 is associated with endogenous retroviral elements. ATRX/Daxx-dependent deposition of H3.3 on ERVs is important for their silencing through the recruitment of the histone methyltransferase ESET that establishes H3K9me3. (B) H3.3 and ATRX/Daxx are found on pericentric DNA repeats. They are involved in the transcription of major satellite dsRNA, which are then required to recruit HP1β and to establish heterochromatin. (C) ATRX/Daxx deposits H3.3 on telomeric repeats. In this context, H3.3 is important for their silencing through the recruitment of HP1α.

proteins 1 (HP1) (Santenard et al. 2010; Wong et al. 2010) and histone methyltransferases (Elsässer et al. 2015).

Pericentric heterochromatin also contains H2A.Z (Rangasamy et al. 2003). Although the role of H2A.Z on pericentromeres is unknown, the specific interaction of HP1α with H2A.Z-containing nucleosomes (Fan et al. 2004) suggests that H2A.Z might enhance the recruitment of factors involved in heterochromatin formation and maintenance including HP1α. During spermiogenesis, testis-specific histone H2A variants are expressed. Among them, H2A.L.1 and H2A.L.2 are specifically incorporated in pericentric regions (Govin et al. 2007). Although their function is also still unknown, they might guide epigenetic reprogramming of paternal pericentric heterochromatin after fertilization (Govin et al. 2007).

Apart from the repetitive elements of the genome, during developmental progression there are at least two additional paradigms of heterochromatin formation and silencing: X inactivation and meiotic sex chromosome inactivation (MSCI). Although the former occurs after fertilization, and prior to implantation, the latter takes place during male germline formation. We will not treat these two models in this review and will just mention that histone variants have also been implicated in silencing during MSCI, as is the case of H3.3 (van der Heijden et al. 2007), and in X chromosome inactivation, as is the case of macroH2A (Pasque et al. 2011; Minajigi et al. 2015).

Heterochromatin establishment and maintenance is a complex process that is under constant investigation. Specific histone variants are present in heterochromatin, but their role in the establishment or maintenance of heterochromatin is still unclear. Their presence at these regions may act at multiple levels: (1) by altering the properties of the nucleosomal core particle, making the chromatin less accessible for transcription factors, (2) by allowing transcription of specific RNA potentially involved in heterochromatin formation, and (3) by recruiting specific effectors of heterochromatin formation. Most importantly, their involvement in key developmental epigenetic

phenomena makes them a candidate to regulate genome reprogramming and cellular plasticity.

EPIGENETIC REPROGRAMMING: RESETTING CELLULAR PLASTICITY

Reprogramming is the reversal of an epigenetic state resulting in changes in cellular identity. In mammals, the early embryo undergoes a genome-wide reprogramming event that parallels, and most likely underlies, the acquisition of totipotency. Large-scale reprogramming of differentiated cells can also be obtained *in vitro* by at least two approaches, somatic cell nuclear transfer (SCNT) and induced pluripotent stem cells (iPSCs). SCNT reprograms to totipotency, whereas iPSC formation entails reprogramming toward pluripotency, both thus resulting in the acquisition of increased cellular plasticity.

Developmental Reprogramming after Fertilization

The oocyte and the sperm cells are two highly specialized cells. Upon fertilization, they undergo a drastic reprogramming sequence to produce the totipotent zygote. The two parental genomes remain physically segregated 24 h after fertilization and exhibit different chromatin organizations. The first reprogramming event of the male pronucleus requires the replacement of sperm protamines with maternal histones. Indeed, although some histones remain at specific loci in the sperm nucleus (Hammoud et al. 2009; Brykczynska et al. 2010), most of the paternal genome is unpacked and new histones are incorporated. This exchange occurs rapidly, long before the first mitosis and zygotic genome activation (ZGA). Newly incorporated histones are hyperacetylated and hypomethylated in agreement with the open chromatin structure observed specifically in the paternal pronucleus (for review, see Burton and Torres-Padilla 2014). Histone variants are also incorporated into the male pronucleus

during this reprogramming event (Fig. 2A) leading to a remarkable asymmetry between the male and female pronuclei. In the mouse oocyte, gamete-specific histone variants are expressed and are subsequently incorporated into the male pronucleus upon fertilization, including H1.8, TH2A, and TH2B. H1.8 expression starts in the oocyte and ends at the two-cell stage, coincident with major ZGA (Tanaka et al. 2001). H1.8 is quickly incorporated into paternal pronucleus after fertilization (Gao et al. 2004). When ectopically expressed in ES cells, H1.8 selectively binds hypomethylated loci and promotes their decondensation (Hayakawa et al. 2012) suggesting that specific incorporation of H1.8 into the male pronucleus may lead to the establishment of an open chromatin structure.

TH2A and TH2B were originally identified as testis-specific H2A and H2B histone variants, respectively (Shires et al. 1976; Trostle-weige et al. 1982). They are encoded by unique genes (*Hist1h2aa* and *Histh2ba*) localized at the end of a large histone gene cluster and have 15- and 16-amino-acid differences compared with H2A

and H2B, respectively. TH2A and TH2B have been recently found to be expressed in oocytes and in the zygote, where they are both incorporated into the paternal pronucleus after fertilization (Shinagawa et al. 2014). When WT male mice are crossed with double *Th2a* and *Th2b*^{-/-} knockout females, the authors observed delayed embryonic development compared with controls, underscoring the importance of these two maternally inherited histone variants (Shinagawa et al. 2014). Furthermore, a significant reduction of transcription is observed in two-cell-stage embryos, arguing for a role of TH2A and TH2B in ZGA (Shinagawa et al. 2014). The authors demonstrate that TH2A and TH2B are specifically involved in the activation of the paternal genome. In the absence of maternal TH2A and TH2B, the level of the active mark H3K4me3 in the paternal pronucleus is lower whereas the level of repressive marks is higher (H3K27me3 and DNA methylation) (Shinagawa et al. 2014). These differences have functional consequences on the transcription programs of later developmental

Figure 2. Histone variants control epigenetic reprogramming during cellular plasticity transitions. (A) After fertilization, the H3.3, H1.8, γ H2A.X, TH2A, and TH2B histone variants are incorporated into the male pronucleus after protamine removal. They are important to trigger totipotency, to form heterochromatin at specific genomic regions, and to properly regulate ZGA. (B) During somatic cell nuclear transfer (SCNT), a differentiated nucleus acquires a totipotent capacity. H3.3 is important for efficient reprogramming upon SCNT, whereas macroH2A acts as an epigenetic barrier. After implantation in the enucleated oocyte, H3.3 and H1.8 are incorporated in the donor nucleus, whereas macroH2A is quickly removed. (C) During iPS, histone variant composition is dramatically modified to sustain the acquisition of a pluripotent chromatin. H2A.Z, TH2A, and TH2B histone variants favor reprogramming efficiency whereas H1.8 and macroH2A inhibit it.

stages, because transcription of *Nanog* is inhibited from the eight-cell-stage embryo specifically on the paternal allele (Shinagawa et al. 2014). Altogether these observations support a role for the maternal histone variants TH2A and TH2B in the formation of an open chromatin structure on the male genome.

The large genome-wide assembly process taking place soon after fertilization also involves H3.3 because the paternal pronucleus incorporates H3.3 in a replication- and transcription-independent manner, mediated largely by HIRA (van der Heijden et al. 2005; Torres-Padilla et al. 2006; Inoue and Zhang 2014; Lin et al. 2014). The absence of maternal HIRA prevents core histone deposition on the paternal pronucleus and compromises ZGA of ribosomal genes suggesting an additional role of H3.3 in the activation of specific genes from the embryonic genome (Lin et al. 2014). As we have discussed above, H3.3 deposition during early development is also important for the establishment of heterochromatin domains (Santenard et al. 2010). However, it remains unclear whether H3.3 deposition in the paternal pronucleus plays specific roles in facilitating an open chromatin structure or instead the incorporation of H3.3 represents only an intermediate step prior to the first phase of replication-dependent incorporation of the canonical histone H3.1/H3.2, which occurs a few hours later.

High levels of γ H2A.X are detected in the zygote, with the paternal pronucleus displaying augmented levels compared with the maternal one (Ziegler-Birling et al. 2009). This enrichment in the phosphorylated form of H2A.X seems to be independent of DNA damage as γ H2A.X does not colocalize with known DNA damage response proteins (Ziegler-Birling et al. 2009). It is possible that the phosphorylation of H2A.X after fertilization accompanies the DNA demethylation process occurring on the parental chromatin, which has been suggested to occur partially through the base excision repair pathway (Hajkova et al. 2010; Wossidlo et al. 2010).

The zygote is an excellent *in vivo* model for studying genome-wide reprogramming events using cell biology approaches. During this reprogramming phase, the contribution of histone variants, especially those that are gamete-specific, is beginning to be unveiled. Histone variants are involved in the formation of heterochromatic structures and the regulation of ZGA. It is also possible that the global histone exchange from a chromatin structure characterized primarily by incorporation of H3.3 toward a more “typical” chromatin configuration with the canonical histones H3.1 and H3.2, plays a role in promoting cellular plasticity.

SCNT

SCNT consists in transplanting a somatic nucleus into an enucleated metaphase II oocyte, leading to the creation of a viable embryo. So far, SCNT is the only experimental procedure to reprogram a differentiated nucleus into a totipotent state, thus demonstrating that the potential to regain totipotency is not lost in most adult cells (Gurdon and Melton 2008). Upon introduction into the oocyte, the donor nucleus undergoes a series of reprogramming

events controlled by the maternal factors present in the oocyte. This reprogramming toward a totipotent state results in global changes in chromatin composition to reset the epigenetic landscape of the somatic nucleus and leads to a switch in gene expression profile, from a somatic program to an embryonic one. During this process, several maternal factors including histone variants like H3.3 and H2A.X are incorporated into the donor nucleus (Chang et al. 2010; Nashun et al. 2011; Jullien et al. 2012). However, the oocyte can also fail to reprogram somatic nuclei, suggesting that the donor nucleus contains epigenetic barriers to reprogramming.

Deletion of H3.3 in the mouse oocyte compromises the efficiency of SCNT, preventing the reactivation of a subset of pluripotent genes (Wen et al. 2014). Maternal H3.3 depletion before nuclear transfer results in embryos arresting at the two- or four-cell stage (Wen et al. 2014). The authors show that maternal H3.3 and not H3.3 from the donor nucleus is crucial for SCNT and cannot be rescued by H3.2 (Wen et al. 2014). In agreement with this role of H3.3 in SCNT, Jullien et al. (2012) demonstrated that after nuclear transfer in *Xenopus*, maternal H3.3 is incorporated on major satellite repeats and the regulatory region of *Oct4* in an HIRA-dependent manner. Accordingly, H3.3 was also shown to be necessary for reprogramming.

Linker histone variants have also been involved in reprogramming during SCNT. After transplantation, the oocyte H1.8 is rapidly incorporated in the donor nucleus and persists until the two-cell stage, coincident with ZGA (Gao et al. 2004; Teranishi et al. 2004). In MEFs, H1.8 mobility as assessed by FRAP is higher compared with H1.2 (Teranishi et al. 2004), which would explain the increase in linker histone mobility after SCNT (Jullien et al. 2010). In *Xenopus*, the linker histone B4 (homologue of H1.8) is involved in reactivation of pluripotency-associated genes such as *Sox2*, *Nanog*, and *Oct4* after SCNT (Jullien et al. 2010). Taken together, these data suggest that during SCNT, the linker histone isoforms might be involved in nuclear remodeling and ZGA.

Apart from the roles of H3.3 and some H1 isoforms in promoting reprogramming upon SCNT, some studies have addressed the role of other histone variants in SCNT. For instance, macroH2A is quickly removed from the somatic nucleus after transplantation (Chang et al. 2010). The pattern of expression of macroH2A at the protein level is therefore similar to that of naturally produced embryos, as macroH2A is found in the nuclei from the morula stage only, but undetectable at earlier stages (Chang et al. 2005, 2010). Using *Xenopus* oocytes as an acceptor for a somatic nucleus, Pasque et al. (2011) showed that macroH2A is retained on the inactive X chromosome of differentiated mouse cells and suggested that this retention renders them resistant to reprogramming. Furthermore, macroH2A depletion in donor MEF nuclei improves reprogramming (Pasque et al. 2011). Altogether, these observations highlight an effect of macroH2A in the resistance to reprogramming during SCNT. Thus, while maternal H3.3 and linker histone variants are important for proper reprogramming upon SCNT, macroH2A interferes with reprogramming (Fig. 2B).

Reprogramming to Pluripotency upon iPSC Formation

Differentiated cells can be reprogrammed into a pluripotent state by the introduction of four transcription factors: Oct3/4, Sox2, Klf4, and cMyc (collectively called OSKM factors) (Takahashi and Yamanaka 2006). The resulting iPSCs provide a remarkable system to understand the molecular mechanisms involved in this change in cell plasticity *in vitro* (for review, see Apostolou and Hochdinger 2013). During reprogramming to pluripotency, fibroblasts first down-regulate genes associated with differentiation and subsequently reactivate endogenous pluripotency genes including *Oct4*, *Sox2*, and *Nanog* to sustain a stable pluripotent state and self-renewal. This reprogramming event involves resetting somatic epigenetic marks and the acquisition of a “pluripotent” chromatin structure. However, the acquisition of induced pluripotency is an inefficient process, suggesting the presence of intrinsic epigenetic barriers to reprogramming in the somatic cells. In this context, histone variants are key actors in favoring or inhibiting reprogramming (Fig. 2C).

Citrullination has recently been characterized on histone H1 isoforms H1.2, H1.3, and H1.4 (Christophorou et al. 2014). Citrullination promotes H1 eviction from chromatin, thus leading to a more open chromatin structure (Christophorou et al. 2014). Interestingly, when the activity of PADI4—the enzyme responsible for citrullination—is inhibited, the efficiency for iPSC reprogramming is reduced (Christophorou et al. 2014). Thus, it was proposed that during iPSC derivation, citrullination leads to the eviction of H1 isoforms from the chromatin, leading to the efficient expression of pluripotent genes such as *Nanog* (Christophorou et al. 2014). On the contrary, overexpression of H1.8 reduces iPSC generation, suggesting a role of this isoform as a barrier of reprogramming (Shinagawa et al. 2014). During reprogramming of human keratinocytes to iPSC, linker histone variant composition undergoes drastic changes. The expression levels of H1.0 and H1.10 decrease during reprogramming, whereas those of H1.1, H1.3, and H1.5 increase (Terme et al. 2011). During iPSC formation, acetylation of H1.4 on K34 increases and is thought to stimulate transcription by increasing H1 mobility (Kamieniarz et al. 2012). These observations suggest a specific role for H1 isoforms in the changes of chromatin condensation and accessibility during iPSC reprogramming. The lack of specific antibodies for all H1 isoforms has nevertheless hindered the study of H1 in these biological processes.

The biological role, if any, of γ H2A.X during iPSC formation remains elusive, although γ H2A.X levels are high during the reprogramming process itself (Marion et al. 2009; González et al. 2013). In a recent study, Wu et al. (2014) demonstrate the importance of H2A.X relocalization during iPSC reprogramming for efficient repression of extraembryonic genes and successful tetraploid complementation, suggesting that H2A.X may be an epigenetic mark for the developmental potential of iPSCs. H2A.Z is also important for iPSC formation, as its absence prevents reprogramming of somatic cells, where-

as its overexpression increases reprogramming efficiency by OSKM factors (Wang et al. 2015). As the TH2A and TH2B variants play an important role in male nucleus reprogramming after fertilization (see above) the authors also investigated their contribution during iPSC derivation (Shinagawa et al. 2014). The combination of TH2A and TH2B overexpression in MEFs enhances iPSC formation ninefold (Shinagawa et al. 2014). Interestingly, coexpression of a phosphomimetic form of their specific chaperone nucleoplasmin (NPM) increases OSKM-induced iPSC generation even further, 18-fold (Shinagawa et al. 2014). TH2A/TH2B and NPM-P overexpression enhance DNA demethylation of the regulatory sequences of *Nanog* (Shinagawa et al. 2014). Furthermore, TH2A and TH2B specifically associate with the X chromosome during iPSC formation but the effect of these two variants, if any, on the changes in chromatin accessibility of the X chromosome remains unclear (Shinagawa et al. 2014).

Reprogramming to iPSCs also entails the removal of macroH2A.1 and macroH2A.2 from the chromatin (Gaspar-Maia et al. 2013). The same remains true in human keratinocytes compared with the corresponding iPSCs (Barrero et al. 2013). Using several reprogramming protocols, Gaspar-Maia et al. (2013) showed that reprogramming is more efficient in double macroH2A knockout fibroblasts compared with WT cells. This effect is independent of the action of macroH2A on the inactive X chromosome (in contrast to its function in SCNT [Pasque et al. 2011]) as both male and female cells display a similar reprogramming rate (Gaspar-Maia et al. 2013). In human cells, only macroH2A.1 depletion—but not macroH2A.2 depletion—has an enhancing effect on reprogramming into iPSCs (Barrero et al. 2013). Interestingly, macroH2A is associated with ~25% of the genes that are bound by the OSKM factors (Gaspar-Maia et al. 2013), and its absence promotes the reacquisition of H3K4me2 at promoters of pluripotency genes during reprogramming (Barrero et al. 2013).

IN VIVO RELEVANCE: ROLE OF HISTONE VARIANTS AND HISTONE EXCHANGE IN ESTABLISHING PLASTICITY PROGRAMS

Because of their distinct biochemical properties, histone variants, when incorporated into nucleosomes, confer specific features to the chromatin. From the above discussion, it is clear that modifying global chromatin properties through variations in histone composition is a key requirement for reprogramming. The examples discussed above illustrate that this is not only the case for *in vivo* reprogramming (Fig. 2A) but also in SCNT (Fig. 2B) and during transcription factor–mediated reprogramming to pluripotency (Fig. 2C). This implies that the histone chaperones and assembly factors themselves are expected to participate in establishing cellular plasticity.

After protamine removal, the male pronucleus contains an unusually high proportion of the H3.3 variant (Torres-Padilla et al. 2006; Lin et al. 2014). Subsequently, during the following cell divisions H3.3 is “diluted” by the replication-dependent incorporation of canonical H3.1 and

H3.2 mediated by CAF1. Thus, CAF1-dependent histone deposition onto the chromatin in the embryo and in ES cells is anticorrelated with the plasticity state of the cells. Developmentally, totipotent cells are predisposed to generate pluripotent cells. We postulate that the transition from totipotency to pluripotency is characterized by a switch from a H3.3-driven chromatin architecture toward a more H3.1/H3.2-enriched chromatin (Fig. 3). CAF-1 is therefore essential for this process.

In vivo, the epigenetic state of the male chromatin is reset into a totipotent state through histone and protamine removal, either during spermiogenesis or at fertilization, respectively. In analogy to this resetting event, recent work has shown that down-regulation of chromatin assembly activity in ES cells induces the formation of two-cell-like cells, characterized by the acquisition of molecular features of totipotent cells (Ishiuchi et al. 2015). These findings suggest that a global reduction in nucleosomal occupancy promotes a cellular plasticity state that resembles that of the early embryo. Both together advocate for a very simple model whereby “erasing” information through removal of the basic unit of the chromatin itself promotes cellular plasticity (Fig. 3).

While these observations pertain histone H3, we expect that other histone variants might also contribute to main-

tain a more accessible chromatin state. An open chromatin structure is presumably important to establish totipotency features including high chromatin mobility (Bošković et al. 2014), the absence of chromocenters (Ishiuchi et al. 2015), and activation of endogenous retroelements (Macfarlan et al. 2012). How these features trigger totipotency in the physiological sense, or whether they are rather a consequence of the totipotency state, is unknown. Furthermore, the possible stabilization of the totipotent state has not been addressed. This stabilization may involve specific histone (canonical or variant) post-translational modifications, linker histone isoforms, and/or the incorporation of other histone variants. Importantly, studying histone dynamics and replacement in the early embryo and in two-cell-like cells might answer these questions and help in understanding how global changes in chromatin composition regulate totipotency.

CONCLUSION

The transitions between totipotency and pluripotency are accompanied by drastic changes in chromatin signatures. In particular, the incorporation and eviction of histone variants from the chromatin are key actors in the

Figure 3. Role of histone variants in establishing totipotent chromatin. Totipotent chromatin is characterized by reduced histone occupancy and a high proportion of the H3.3 histone variant. During developmental progression, replication-dependent canonical histone incorporation in the chromatin is associated with a dilution of H3.3 histone variant and a decreased cellular plasticity toward pluripotency. In vitro, totipotent-like features are obtained by inhibiting replication-dependent histone incorporation. The resulting two-cell-like cells possess similar chromatin characteristics compared with the two-cell-stage embryo. Whether this totipotent chromatin state can be stabilized is unknown. Such stabilization may involve specific histone (canonical or variant) posttranslational modifications, specific linker histone isoforms, and/or the incorporation of other histone variants. ES, embryonic stem.

establishment of plasticity programs. Although the role of H3.3 and its specific chaperones in totipotent versus pluripotent chromatin is starting to be understood, the interplay between histone variants during this process should be the subject of future investigations. How the specific combination of histone variants can trigger the establishment of a totipotent chromatin is unknown. Furthermore, we anticipate that the role of specific histone exchange factors is essential during the transitions of cell potency states and the acquisition of totipotency.

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