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The stem cell - chromatin connection

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Abstract

Stem cells self-renew and give rise to all differentiated cell types of the adult body. They are classified as toti-, pluri- or multi-potent based on the number of different cell types they can give rise to. Recently it has become apparent that chromatin regulation plays a critical role in determining the fate of stem cells and their descendants. In this review we will discuss the role of chromatin regulators in maintenance of stem cells and their ability to give rise to differentiating cells in both the animal and plant kingdom. We will highlight similarities and differences in chromatin-mediated control of stem cell fate in plants and animals. We will consider possible reasons why chromatin regulators play a central role in pluripotency in both kingdoms given that multicellularity evolved independently in each.

Keywords

Chromatin remodeling; Histone modification; Stem cells; Pluripotency; Differentiation

Introduction

In mammals, the fate of pluripotent embryonic stem cells (ESCs) is regulated by pluripotency transcription factors. These include the POU domain transcription factor Oct4, the divergent homeodomain protein Nanog, and the high mobility group (HMG)-box transcription factor Sox2 [1; see Table 1 for a list of pluripotency and chromatin regulators discussed in this review]. These pluripotency factors activate each other as well as other ESC-specific genes and repress expression of developmental regulators in ESCs [2]. Pluripotent cell fate can be induced in differentiating cells, such as fibroblasts, by expressing these and additional transcription factors to give rise to induced pluripotent stem cells [3].

Plant stem cell populations reside in the growing tips of the shoot and the root in meristems. As for adult stem cells in animals, maintenance of the two pluripotent plant stem cell populations is entirely dependent on the stem cell niche [4]. Pluripotency transcription factors also regulate maintenance of stem cells in plants; they exercise this role from within the shoot or root stem cell niche.

In *Arabidopsis thaliana*, the pluripotency regulators and homeodomain transcription factors WUSCHEL (WUS) and SHOOT MERISTEMLESS (STM) maintain the shoot apical meristem stem cells. Consistent with this role, ectopic *WUS* and *STM* expression induces

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formation of ectopic stem cell pools [5]. However, only a subset of the co-expressing cells is responsive and the ectopic stem cells are not properly maintained [5], suggesting a requirement for additional pluripotency regulators. In contrast to animal pluripotency transcription factors, *WUS* and *STM* cannot activate each other [6]. *WUS* expression levels are positively correlated with the size of the stem cell population and restricted via negative feedback by the CLAVATA (CLV) signaling pathway [7,8]. *STM* promotes cell division and inhibits differentiation of the stem cells in the shoot apical meristem [6,9,10] together with other KNOTTED1-LIKE HOMEODOMAIN (KNOX) transcription factors, such as KNOTTED in ARABIDOPSIS THALIANA1 (KNAT1), KNAT2 and KNAT6 [11]. During differentiation, expression of the KNOX genes is repressed by the MYB-domain transcription factor ASYMMETRIC LEAVES 1 (AS1) together with the LATERAL ORGAN BOUNDARIES (LOB) domain transcription factor AS2 [12-14].

Stem cell populations in the root meristem are controlled by the pluripotency regulators and double AP2 domain transcription factors PLETHORA1 (PLT1) and PLT2, together with the GRAS transcription factors SCARECROW (SCR) and SHORTROOT (SHR) [15-17]. A local maximum of the plant hormone auxin induces *PLT1* and *PLT2* expression [18], while SHR induces *SCR* expression in the stem cell niche [19]. In agreement with their role as pluripotency regulators, PLT and SCR can induce formation of ectopic root stem cell pools [15,18]. A WUS-like regulator, WOX5 has also been implicated as a root stem cell pluripotency regulator [20,21].

Recent investigations have shown that pluripotency transcription factors are regulated by and act together with chromatin regulators [1,22,23]. The basic unit of chromatin, the nucleosome, is formed by 147 base pairs of DNA wrapped around the histone octamer: two copies each of histone H2A, H2B, H3 and H4. Nucleosomes are separated by linker DNA associated with the linker histone H1. The initial positioning of nucleosomes on the DNA is partially genome encoded [24]. Nucleosomes are progressively folded into higher order chromatin [25], which is increasingly inhibitory to processes that require access to genomic DNA, such as transcription, replication, and DNA repair. However, DNA accessibility in the context of chromatin is regulated by the activities of several protein complexes including ATP-dependent chromatin remodeling enzymes, enzymes mediating covalent modifications of histones or the DNA, and chromatin assembly factors [22].

Chromatin regulation of pluripotency and differentiation

Chromatin remodeling

SWI2/SNF2 chromatin remodeling ATPases in both mammals and plants directly control expression of pluripotency transcription factors. These chromatin remodeling complexes utilize the energy released by ATP hydrolysis to disrupt contacts between histones and DNA, resulting in changes in nucleosome conformation, position, composition and occupancy [26]. Those changes have been shown to alter DNA accessibility, regulating access of gene-specific or general transcription factors to the DNA to activate or repress gene expression [27]. In mammalian ESCs, a unique SWI2/SNF2 complex called esBAF has been described, which contains the chromatin remodeling ATPase BRAHMA related gene 1 (Brg1; also called Smarca 4) as enzymatic subunit [28]. esBAF contains a unique subset of associated regulatory factors (BAFs) critical for its function in ESCs [28]. Brg1 regulates maintenance of pluripotency and initiation of differentiation [22]; loss of Brg1 leads to loss of self-renewal in ESCs [28,29]. Brg1 binds to the regulatory regions of the pluripotency genes *Oct4*, *Sox2*, and *Nanog* as well as to their direct targets [30]. In addition, Brg1 physically interacts with the Oct4 and Sox2 transcription factors [28]. Surprisingly, Brg1 not only represses expression of developmental regulator genes in ESCs but also that of pluripotency genes [30]. It has been proposed that downregulation of pluripotency gene

expression by Brg1 may fine-tune their expression level to facilitate exit from self-renewal during differentiation [30].

Mutations in the homologous *Arabidopsis* SWI2/SNF2 chromatin remodeling ATPases SPLOYED (SYD) and BRAHMA (BRM) cause defects in the maintenance of the stem cell population in the shoot apical meristem [31-33]. The molecular mechanism that underlies shoot meristem phenotype of *brm* mutants is currently not understood. SYD is required to maintain expression of the pluripotency regulator *WUS* in the shoot apical meristem during reproductive development and occupies *WUS* regulatory regions [33]. *Arabidopsis* BRCA-1 ASSOCIATED RING DOMAIN 1 (BARD1) represses *WUS* expression outside of the stem cell niche [34]. Since BARD1 associates with SYD it was proposed that BARD1 may regulate *WUS* expression by inhibiting SYD complex activity [34]. Both SYD and BRM also play a role in embryonic shoot apical meristem formation by controlling organ boundary gene expression [35]. The composition of SYD/BRM complex in stem cells or differentiating cells is not yet known, although many SWI2/SNF2 BAFs are conserved between plants and animal [36].

A different type of chromatin remodeling ATPase, the chromodomain-containing remodeling factor PICKLE/GYMNOS (PKL) controls differentiation in *Arabidopsis* [37]. *PKL* mutations enhance *as1* and *as2* phenotypes and cause ectopic meristem formation due to de-repression of *KNOX* gene expression [10].

Histone acetylation

Histone acetylation plays a role in pluripotency regulator gene expression in both mammals and plants. In addition, histone acetyl transferases (KATs) and histone deacetylases (HDACs) are important cofactors for pluripotency regulators. KATs add acetyl groups to lysines in the amino-terminal tails or in the core of histones. This modification generally promotes activation of transcription by reducing strength of the interaction between histones and DNA [22]. In ESCs, the p300 KAT acts as a transcriptional coactivator of pluripotency transcription factors [38] and directly activates *Nanog* expression [39]. In addition, recruitment of p300 to its target genes depends on the presence of one or more of the pluripotency factors, Nanog, Oct4 and Sox2 [38]. Another KAT, Tip60 is also required to maintain stem cell identity and regulates similar target genes as Nanog [40]. The KAT activity of the Tip60-p400 complex appears to be required to repress developmental regulator gene expression in ESCs. One possible explanation for the unexpected repressive role of Tip60-p400 is that Tip60-p400 activates a repressor of developmental regulator genes [40].

In *Arabidopsis*, the AtGCN5 KAT restricts *WUS* expression to the stem cell niche; in *atgcn5* mutants *WUS* is ectopically expressed [41]. As for Tip-60 in mammals (above), it has been proposed that AtGCN5 activates a negative regulator of *WUS* expression [41]. AtGCN5 has an apparent opposite role in root stem cells, where it is required for root stem cell maintenance and activates the pluripotency regulator *PLT* [42,43]. KATs have not yet been implicated as pluripotency transcription factor coactivators in *Arabidopsis*.

HDACs, by contrast, create a closed chromatin conformation that represses transcription. The Nucleosome Remodeling Deacetylase (NuRD) complex contains both ATP-dependent chromatin remodeling and deacetylase activities [44]. Mbd3, a core subunit of NuRD complex is required for pluripotency. ESCs lacking Mbd3 are slow growing and have differentiation defects [45]. An ESC-specific co-repressor complex containing many NuRD subunits called NODE interacts with Nanog and Oct4 and represses developmental regulators in ESCs [46].

Similarly, WUS may recruit HDAC activities to repress expression of genes that control differentiation. This is thought to occur via interaction with two WUS-interacting proteins called WSIP1 and WSIP2 that bind to the conserved EAR motif in the C-terminal domain of WUS [47]. WSIP1, also called TOPLESS, and WSIP2 are similar to Gro/TLE co-repressors, which recruit HDACs to target genes to repress transcription [48]. Hence both animal and plant HDACs repress developmental regulator gene expression in stem cells.

Histone Methylation

Histone lysine methyltransferases (KMTs) exhibit high substrate specificity with respect to the lysine residue modified and the type of methylation added (mono-, di- or tri-methylation). Methylation of two lysines of histone H3 (lysine 4 and lysine 27) plays an important role in regulation of pluripotency. H3K27 KMTs are components of the Polycomb Repressive complex 2 (PRC2), while H3K4 KMTs are Trithorax Group (TrxG) proteins [49,50]. Trimethylation of H3K27 (H3K27me3) by PRC2 and subsequent activity of the larger PRC1 complex represses expression of differentiation genes in ESCs and pluripotency gene expression in differentiating cells in mammals. Thus far direct evidence is only available for the latter role in plants.

The mammalian PRC2 has three core subunits, the EZH2 KMT, SUZ12, and EED (Table1). Loss of activity in any of these causes similar but not identical defects including upregulation of developmental genes in ESCs, as well as failure to repress pluripotency regulators and to activate developmental regulators in differentiating cells [51]. The EZH1 KMT acts together with EZH2 to repress expression of developmental regulators in ESCs [52]. The PRC1 complex, composed of ca. 10 subunits including POLYCOMB, RING1A and RING1B, also plays a role in maintaining stem cell identity and is required for proper differentiation [53]. PRC1 promotes higher order chromatin compaction to further repress the expression of target genes [49]. Consistently, ESCs depleted of RING1B showed de-repression of developmental regulator genes and abnormal differentiation [54,55].

The *Arabidopsis* PRC2 complex prevents pluripotency gene transcription during differentiation. A homolog of the EZH2 H3K27 KMT, CURLY LEAF (CLF), directly binds to the *STM* promoter and deposits H3K27me3 modifications at this locus [56]. Both *STM* and *KNAT2* expression is de-repressed in *clf* mutants [57]. A second EZH2 homolog, SWINGER (SWN), acts partly redundantly with CLF in inhibiting *STM* expression [58]. Other PRC2 complex components also play roles in repressing KNOX transcription; silencing of the *Arabidopsis* PRC2 core subunit and EED homolog FERTILIZATION INDEPENDENT ENDOSPERM (FIE) causes ectopic *STM*, *KNAT2*, and *KNAT6* expression [57]. Interestingly, the moss *Physcomitrella patens* FIE (PpFIE) is required to maintain pluripotency of the apical daughter cells [59]. Although it is not yet clear what the direct targets of PpFIE are, the observed phenotype is consistent with a possible role for PRC2 in repression of differentiation gene expression in pluripotent cells in plants. The plant PRC1 complex is divergent from its metazoan counterpart and apparently contains TFL2/LHP1, a protein similar to metazoan HETEROCHROMATIN PROTEIN1 (HP1), in lieu of POLYCOMB [60,61]. The *Arabidopsis* genome does not contain a POLYCOMB ortholog. Notably, like POLYCOMB and unlike HP1, LHP1 binds to H3K27me3, the histone modification generated by PRC2 [62]. Recently, a double mutant in the *Arabidopsis* PRC1 complex components *atring1a* and *atring1b* was shown to cause ectopic shoot meristem formation and de-repression of KNOX gene expression [61], suggesting that PRC2 and PRC1 jointly repress pluripotency gene expression in differentiating cells in *Arabidopsis*.

Methylation of H3K4 by TrxG KMTs is an activating histone modification [63]. In human ESCs, many genes marked with H3K4me3 encode proteins involved in proliferation [64]

including Oct4, Sox2 and Nanog [64-67]. Hence TrxG proteins activate pluripotency regulator gene expression. The *Arabidopsis* TrxG proteins ATX1 and ATX2 are responsible for H3K4me3 and H3K4me2 methylation, respectively [68]. Thus far no direct role in stem cell maintenance has been described for either protein, perhaps because of functional redundancy with related H3K4 KMTs [69].

Despite their opposing roles, H3K27me3 and H3K4me3 co-occupy many regulatory elements in ESCs in mammals [70]. It has been proposed that these 'bivalent histone modifications' keep developmental genes poised for activation in ESCs to resolve to either H3K27me3 (repressed state) or H3K4me3 (activated state) upon differentiation [63,70; Fig. 1]. By contrast, recent genome-wide analyses suggest that the H3K4me2 and not H3K4me3 is associated with H3K27me3 in bivalent domains in *Arabidopsis* [71]. Furthermore, the H3K27 and H3K4 methylation status is not only controlled by KMTs, but also by histone lysine demethylases (KDMs), which like the KMTs have high substrate specificity. An H3K4me3 KDM represses developmental gene expression in ESC together with the PRC2 complex [72]. Conversely, an H3K27me3 KDM is required for differentiation [73] and this type of KDM is frequently associated with TrxG complexes. These combined activities are well suited to coordinately regulate the balance between H3K4me3 and H3K27me3 [51].

Thus, two opposing histone methyl marks on H3K4 and on H3K27 play an important role in stem cell maintenance and differentiation in mammals by controlling expression of proliferation genes and developmental regulator genes. While H3K27me3 is required for repression of pluripotency gene expression in differentiating plant cells, it remains to be determined what role methylation of H3K4 or H3K27 plays in plant stem cell maintenance.

Histone chaperones

Histone chaperones have important roles in chromatin assembly and disassembly both during and outside of replication [74]. In both mammals and plants, histone chaperones are required to maintain repressed chromatin states. For example, components of the replication-coupled assembly factor CAF-1 complex are required for chromatin condensation and maintenance of repressive histone modifications in ESCs [75]. Mutations in the replication-independent histone chaperone HirA cause accelerated differentiation in ESCs [76], suggesting a role for this histone chaperone in stem cell maintenance.

In *Arabidopsis*, histone chaperones are also required to maintain condensed chromatin, in addition they repress pluripotency regulator gene expression in differentiating cells. CAF-1 subunit loss-of-function *fasciata (fas)* mutants exhibit ectopic foci of *WUS* and *SCR* expression, suggesting that CAF-1 is critical for restricting stem cell fate to the shoot and root meristem [77]. The ectopic *SCR* expression pattern in *fas* mutant roots varies among individual plants and among neighboring cells suggesting a stochastic collapse of transcriptionally repressed chromatin states [77,78]. *Arabidopsis RETINOBLASTOMA-RELATED* acts synergistically with CAF-1 to inhibit root stem cell proliferation by repressing expression of pluripotency transcription factors [79,80]. Similarly, loss of function of the NAP1 histone chaperone homolog *NAP1-RELATED PROTEIN1 (NRP1)* and *NRP2* causes increased expression of the root pluripotency regulator *PLT2* and NRP1 binds to the *PLT2* locus [81]. In the shoot, AS1 recruits HIRA to the *KNAT1* and *KNAT2* loci [82]. Reduced HIRA activity causes *KNAT1* and *KNAT2* de-repression, suggesting that the AS1-AS2/HIRA complex maintains repressive chromatin at the *KNAT1* and *KNAT2* loci during differentiation [82].

DNA methylation

DNA methylation in mammals occurs on cytosine residues in CpG dinucleotides and represses transcription [83]. The promoters of *Oct4* and *Nanog* as well as those of other ESC-specific genes are free of methylation in ESCs, yet are heavily methylated in differentiating cells [1,83]. Conversely, during induction of pluripotency, DNA methylation at *Oct4* and *Nanog* promoters diminishes, suggesting a causal role for DNA demethylation in reprogramming [83]. DNA methylation has not yet been implicated in repression of pluripotency genes in differentiating cells in *Arabidopsis*.

Perspective

In summary, despite some of the differences described above, chromatin regulators clearly play an important role in stem cell self-renewal and in the ability of stem cells to give rise to differentiating cells in both animals and plants. One explanation for the evolutionary conserved role of chromatin regulators in pluripotency may be that stem cell chromatin is fundamentally different from that of differentiating cells and that maintenance of this unique chromatin state requires the activity of chromatin regulators.

Indeed, ESCs are characterized by highly accessible chromatin, hyperdynamic chromatin association of architectural proteins such as HP1 and histone H1, and a preponderance of activating histone modifications relative to differentiating cells [Fig. 1; 76,83,84]. In addition, ESCs are more transcriptionally active, both in genic and intergenic regions than differentiating cells, and expression of genes encoding for chromatin remodeling complex components is elevated [84]. The open chromatin and stochastic transcription may allow ESCs and their immediate descendants to readily assume one of many lineages-specific transcription programs [83,84]. It remains to be seen whether adult stem cells in animals or pluripotent plant stem cells likewise have highly accessible chromatin and increased transcription.

It has been proposed that in differentiating cells, by contrast, chromatin primarily maintains lineage fidelity [Fig. 1A; 85]. Indeed, chromatin regulators play an important role in transcriptional memory to maintain heritable alternate states of gene activity, the classical definition of epigenetic regulation. Consistently, certain chromatin modifications and chromatin architectural proteins are mitotically heritable including DNA methylation and presence of PRC1 [86,87].

However, all known chromatin alterations are reversible, even those closely linked to transcriptional memory [88,89]. Furthermore, differentiating cells in both animals and plants have and can establish bivalent chromatin domains, thought to mark genes that are poised for activation or repression [Fig. 1B; 22,90]. In both kingdoms some lineage specific developmental regulator genes are only activated in late in morphogenesis and this activation requires a switch to activating chromatin configuration [Fig. 1B; 83,91]. Thus, in differentiating cells chromatin regulators are also required for transcriptional reprogramming [83].

Hence, the reason chromatin regulators have similar roles in pluripotency and differentiation in the plant and the animal kingdoms is perhaps not simply to maintain the highly accessible chromatin of stem cells. Instead we propose that the observed conserved role of chromatin regulators in pluripotency and differentiation is likely due to their unique ability to provide both stability and plasticity to transcriptional programs, key challenges for stem cells and their descendants as well as for differentiating cells in developing organisms [83]. In all of these cell types, chromatin regulators ensure faithful inheritance of transcriptional programs from mother to daughter cells, while being able to direct large scale transcriptional

reprogramming in response to critical developmental and environmental cues. The challenge for the future is to elucidate the regulatory events that lead from perception of endogenous or exogenous cues to alteration of chromatin regulator activity from memory to reprogramming at relevant loci.

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Figure 1. Dynamic chromatin in stem cells and differentiating cells

Center: Stem cell chromatin is highly accessible as indicated by the low nucleosome density on three developmental regulator genes and one pluripotency gene. In differentiating cells the existing chromatin modifications and chromatin structure at each of the four loci is inherited or copied after replication for transcriptional memory and lineage fidelity (A). In addition to transcriptional memory, chromatin regulators direct transcriptional reprogramming of specific loci in differentiating cells via alteration of histone modifications and nucleosome density in response to developmental or environmental cues (B).

DG: developmental regulator gene, PG: pluripotency gene, pro: promoter, Diff. Cell: differentiating cell, ESC: embryonic stem cell.

Table 1

Stem cell regulators in animals and plants

Category	Mammals	Arabidopsis	Activity	Role in stem cells
Pluripotency regulators	Sox2, Oct4, Nanog	WUS, KNOX (STM, KNAT1, KNAT2, KNAT6); PLT1, PLT2; SHR, SCR, WOX5	Transcription factors	Activate pluripotency genes, proliferation genes, repress developmental regulator genes
SWI2/SNF2 remodeling ATPases	Brg1	SYD, BRM	Alteration of nucleosome conformation, presence, and position	Mammals: repress developmental regulator genes, fine-tune (downregulate) pluripotency genes Plants: maintain expression of pluripotency genes
KAT	p300 Tip60	AtGCN5	Histone lysine acetylation	Both: activate expression of some pluripotency genes in stem cells
Co-repressor complex/HDACs	Gro/TLE	TPL (WSIP1), WSIP2	Histone lysine deacetylation	Both: repress developmental regulator genes in stem cells
PRC2 polycomb complex	Ezh2, Ezh1; Suz12; Eed	CLF, SWN; EMF2; FIE	H3K27methylation	Mammals: repress developmental regulator genes in ESCs, activate developmental regulator genes during differentiation Both: repress pluripotency genes during differentiation
PRC1 polycomb complex	Polycomb; Ring1A/B; etc.	LHP1; AtRING1a/b	Chromatin compaction	Mammals: repress developmental genes in ESCs Plants: repress pluripotency genes during differentiation
Trithorax group (TrxG) proteins	MLL	ATX1, ATX2	H3K4 methylation	Mammals: activate pluripotency genes Plants: unknown
Histone chaperones	CAF-1; NAP1; HirA	FAS1, FAS2; NRP1, NRP2; HirA	Chromatin assembly/disassembly	Both: required for condensed chromatin in ESCs Plants: repress pluripotency genes during differentiation

Histone lysine demethylases (KDMs), and enzymes controlling DNA methylation were not included here, despite their critical roles in pluripotency and differentiation. Sufficient information for across kingdom comparisons is not yet available for these chromatin regulators.