



Epigenetic reprogramming in plant and animal development

Suhua Feng¹, Steven E. Jacobsen^{1,*}, and Wolf Reik^{2,*}

¹Howard Hughes Medical Institute and Department of Molecular, Cell and Developmental Biology, University of California at Los Angeles, Los Angeles, CA 90095, USA.

²Laboratory of Developmental Genetics and Imprinting, The Babraham Institute, Cambridge CB22 3AT, UK, and Centre for Trophoblast Research, University of Cambridge, CB2 3EG, UK.

Abstract

Epigenetic modifications of the genome are generally stable in somatic cells of multicellular organisms. In germ cells and early embryos, however, epigenetic reprogramming occurs on a genome-wide scale, which includes demethylation of DNA and remodeling of histones and their modifications. Mechanisms of genome-wide erasure of DNA methylation are being unraveled, which involve modifications to 5-methylcytosine (5mC) and DNA repair. Epigenetic reprogramming has important roles in imprinting, the natural as well as experimental acquisition of totipotency and pluripotency, control of transposons, and epigenetic inheritance across generations. Small RNAs and inheritance of histone marks may also contribute to epigenetic inheritance and reprogramming. Reprogramming occurs in flowering plants and in mammals and the similarities and differences illuminate developmental and reproductive strategies.

Introduction

Epigenetic modifications of DNA and its associated chromatin proteins, while not altering the DNA's primary sequence, also contain inheritable information. Such modifications, including cytosine methylation, posttranslational modifications of histone tails and the histone core, and the positioning of nucleosomes (octamer of histone subunits), influence the transcriptional state and other functional aspects of chromatin. For example, methylation of DNA and certain residues on the histone H3 N-terminal tail (e.g. H3K9) are important for transcriptional gene silencing (TGS) and formation of heterochromatin. Such marks are essential for the silencing of non-genic sequences, including transposons, pseudogenes, repetitive sequences, and integrated viruses, which could become deleterious to cells if expressed and hence activated. Epigenetic gene silencing is also important in developmental phenomena such as imprinting in both plants and mammals, as well as in cell differentiation and reprogramming.

DNA methylation occurs in three different sequence contexts (CG, CHG, and CHH, where H=C, T, or A). In both mammals and plants, CG methylation is maintained by the maintenance DNA methyltransferase termed DNA (cytosine-5)-methyltransferase 1 (Dnmt1) in mammals and DNA METHYLTRANSFERASE 1 (MET1) in Arabidopsis, and a cofactor which recognizes hemimethylated DNA at replication foci called Ubiquitin-like containing PHD and RING finger domains 1 (UHRF1) in mammals and the VARIATION IN METHYLATION (VIM) family proteins in Arabidopsis (1). In addition, the mammalian *de novo* DNA methyltransferases Dnmt3a and Dnmt3b are also required for the maintenance of CG methylation at some loci (2). CHG methylation is common in Arabidopsis and other

*To whom correspondence should be addressed. jacobsen@ucla.edu (S.E.J.); wolf.reik@bbsrc.ac.uk (W.R.).

plant genomes and is maintained by a feed-forward loop that is formed by a plant-specific DNA methyltransferase, CHROMOMETHYLASE 3 (CMT3), and a histone methyltransferase, KRYPTONITE (KYP) (1,3–4). CHH methylation is also abundant in plants and is maintained by the RNA-directed DNA methylation (RdDM) pathway, which actively targets the DNA methyltransferase DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2; a homolog of Dnmt3) to DNA using 24-nt small interfering RNAs (siRNAs) (1) (Fig. 1). CHG and CHH methylation are also present at detectable levels in mammals, especially in stem cells, and this methylation is likely introduced by Dnmt3a and Dnmt3b (5–6). *De novo* methylation of DNA in all of these sequence contexts is generally established by the Dnmt3 (mammals) and DRM2 (Arabidopsis) methyltransferases. Mammals do not have an Arabidopsis-like RNA-directed DNA methylation pathway, but in germ cells PIWI-associated RNAs (piRNAs) are thought to guide Dnmt3 activity (7). Mammals have a non-catalytic paralogue of *de novo* methyltransferase, Dnmt3L, which interacts with Dnmt3a and unmethylated H3K4 (as does Dnmt3a and Dnmt3b) (8–10), implying a targeting mechanism of these methyltransferases to chromatin. Unmethylated CpG islands are specifically bound by CXXC finger protein 1 (Cfp1), which in turn recruits histone H3K4 methyltransferase, SET domain containing 1 (SETD1) (11), suggesting that H3K4 methylation and therefore exclusion of Dnmt3 from CpG islands could help explain how promoters remain unmethylated. Consistently, it has been shown that demethylation of H3K4 is important for acquisition of DNA methylation in imprinted genes in oocytes (12). Additionally, transcription can also help to establish *de novo* DNA methylation at imprinted regions (13). Most recently, it has been shown that the nucleosome landscape also influences the methylation patterning in both plant and animal genomes (14).

Some histone modifications are also thought to be actively maintained during DNA replication, in part facilitated by the association of the histone modification enzymes with the DNA replication machinery. For example, the mammalian histone H3K9 methyltransferases, G9A and SET domain bifurcated 1 (SETDB1), the mammalian H4K20 methyltransferase, SET domain containing 8 (SETD8), and the plant ARABIDOPSIS TRITHORAX-RELATED PROTEIN 5 (ATXR5) and ATXR6 histone H3K27 monomethyltransferases interact with the replication protein proliferating cell nuclear antigen (PCNA) (15–16) (see also Reinberg review in this issue). However, histone methylation can also be very dynamic, and is controlled by the combined action of both histone methyltransferases and histone demethylases, as well as by the proteins that read these histone marks (16).

Through the developmental regulation of these epigenetic mechanisms, both plants and animals undergo epigenetic reprogramming in various cell types and developmental stages, which either serve to transmit epigenetic information between cells or between sexual generations, or to reset epigenetic marks to reduce the risk of perpetuating dangerous epigenetic alleles.

DNA Methylation throughout the Arabidopsis life cycle

In order to maintain genome integrity from generation to generation, transposons and repetitive DNA elements must be kept under tight regulation in reproductive cells. One of the ways that plants achieve this is through the stable inheritance of DNA methylation. Plants frequently show meiotic inheritance of gene silencing (1). Furthermore, plants are not known to undergo genome-wide waves of demethylation in germ cells as occurs in animals. However, large scale reprogramming occurs in non-germ line reproductive cells, and this reprogramming may function to reinforce silencing of transposable elements in germ cells (see below).

One way to actively reprogram the epigenome is to remove methylated cytosines. The Arabidopsis genome encodes four bifunctional helix-hairpin-helix DNA glycosylases / AP lyases, REPRESSOR OF SILENCING 1 (ROS1), DEMETER (DME), DEMETER-LIKE 2

The male gametophyte of *Arabidopsis* (a pollen grain) contains two sperm cells, which fertilize the egg cell and central cell, respectively, and a vegetative nucleus (Fig. 1). Transposon expression is generally upregulated in pollen, and certain transposons even become mobile in pollen, contrasting with the situation in most other tissues (24). Reduction of transposon methylation and their reactivation appears to occur in the vegetative nucleus, which is supported by the fact that transposon reactivation and movement is not inherited by the next generation (24). It has been shown that several key RdDM pathway proteins (RDR2 and DCL3) and CHG methylation maintenance pathway proteins (CMT3 and KYP) have reduced expression levels in pollen, and in addition DECREASE IN DNA METHYLATION 1 (DDM1), an important chromatin remodeler required for DNA and histone methylation and transposon silencing, is exclusively localized in sperm cells but not in the vegetative nucleus (24). These results suggest a model in which hypomethylation of the vegetative cell may reactivate transposons that could serve to reinforce transposon silencing in the adjacent sperm cells (Fig. 1) (24).

Small RNAs may be involved in this communication between the vegetative cell and the sperm cells. A class of siRNAs that is 21 nucleotides in length and corresponds to *Athila* retrotransposons, the largest transposon family, is detected in sperm cells. Since *Athila* retrotransposons remain silenced in sperm cells but are activated in the vegetative nucleus, this supports the idea that the 21-nt siRNAs are produced in the vegetative nucleus and then travel to their site of action – sperm cells-- where they mediate the silencing of transposons through a yet unknown mechanism (Fig. 1) (24). A common theme is that both male and female gametophytes contain nurse cells in which massive epigenetic reprogramming may serve to reinforce transposon silencing in the germline (Fig. 1).

Another example of small RNAs silencing transposons at a distance occurs when the megaspore mother cell differentiates from somatic tissues (25). Mutations in ARGONAUTE 9 (AGO9), a member of the *Arabidopsis* Argonaute family of proteins, result in the reactivation of transposons in the ovule (including the egg cell), in which *AGO9* expression is not detected (Fig. 1). Remarkably, *AGO9* is not expressed in the reproductive cells themselves (megaspore mother cell, megaspore, or developing female gametophyte), but in the companion cells surrounding the female gametophyte. Notably, the transposon targets of AGO9 are similar to those reactivated in pollen and evidence suggests that AGO9-mediated transposon silencing utilizes components of known silencing pathways including the 24-nt RNA-directed DNA methylation pathway (25). Whether the AGO9-associated 24-nt siRNAs are the mobile signal remains to be tested.

Resetting of histone modifications in *Arabidopsis*

In addition to DNA methylation, plants also reprogram histones and their associated marks, and as opposed to DNA methylation which is typically inherited, some histone modifications are known to be reset in each generation. Since plants do not set aside a germ line early in development (germ cells are differentiated from adult somatic cells), some type of “reprogramming” process is likely needed to erase the effects of epigenetic marks caused by external stimuli (such as development or stress). For example, Polycomb group proteins (PCG) mediate silencing of *FLOWERING LOCUS C (FLC)* in *Arabidopsis*, which controls flowering time (26). In winter-annual accessions of *Arabidopsis*, *FLC* is expressed at high levels to repress the initiation of flowering. During vernalization (prolonged cold treatment such as during winter), *FLC* becomes modified by H3K27 trimethylation, which helps to turn off *FLC* expression epigenetically. When winter passes and temperatures becomes warmer, trimethylation and silencing of *FLC* persists, and therefore *Arabidopsis* can flower in response to environmental cues such as photoperiod. When gametes are formed through meiosis, H3K27 trimethylation marks on *FLC* are removed by an unknown mechanism and

in the seeds *FLC* becomes re-expressed. Thus, flowering is inhibited by *FLC* until the next-generation plants encounter cold weather.

Resetting of histone marks may involve, in part, global replacement of histones (27). The histone variant H3.3 can be incorporated in the absence of DNA replication, and thus is a candidate for the “replacement” histone H3 during reprogramming. HISTONE THREE RELATED 10 (HTR10) is exclusively expressed in male reproductive cells but after karyogamy of sperm and egg cell nuclei, the paternal HTR10 signal disappears within a matter of hours before S phase of the first zygote division (27). This suggests that HTR10 is

in DMRs of imprinted genes does depend on DNMT1 (46), so how DNMT1 might be targeted during this reprogramming phase to key regions in the genome such as imprinted genes will be important to understand (47).

Hence the current evidence for the initiation and regulation of genome-wide erasure of DNA methylation in PGCs and the zygote points to initiating events that modify 5mC (such as deamination and hydroxylation, but perhaps also others) which would trigger a BER response. It is of course still possible that bifunctional DNA glycosylases of the type that excise 5mC in plants also exist in animals (but none have been found so far); conversely homologues of APOBEC deaminases, and TET and ALKBH type hydroxylases have yet to be described in plants.

Experimental reprogramming in mammals

Experimental reprogramming to a pluripotent state can be achieved albeit inefficiently by fusion of somatic with pluripotent cells, by cloning, and by direct reprogramming using core transcription factors (48). With all three methods there is evidence that epigenetic reprogramming is a central component of achieving the goal of an embryonic or ES cell like (iPS) state. In cell fusion experiments between somatic cells and ES cells, key pluripotency genes such as *Oct4* and *Nanog* need to be demethylated; AID also seems to be important for demethylation in this system (49). Generation of iPS cells from somatic cells by transduction of core transcription factors (such as Oct4, Sox2, Klf4, c-myc) probably requires multiple epigenetic reprogramming steps while the cells that undergo reprogramming divide (50). DNA demethylation is clearly critical since incompletely reprogrammed iPS cells can become completely reprogrammed by treatment with the methylation inhibitor azacytidine (48). Inhibitors of histone deacetylases and histone methyltransferases are also beneficial, showing in general that repressive epigenetic modifications acquired during differentiation and somatic development need to be reversed to achieve the pluripotent state (48). Notably, reprogramming by cloning apparently results in better resetting of the epigenome than direct reprogramming with transcription factors, indicating perhaps that true totipotency requires passage through germ cells or zygotes (51). Unraveling the role of AID, hydroxymethylation and the TETs, and of the base excision repair pathway as well the methyltransferases in this process, and how the reprogramming network is connected with the pluripotency network, will not only be fascinating biology but will also have direct applications to regenerative medicine.

Comparative biology of epigenetic reprogramming

Whether genome scale epigenetic reprogramming has a unified purpose is not clear; some aspects of reprogramming are clearly conserved (or have been reinvented) in animals and plants with their contrasting (though sometimes surprisingly similar) reproductive and biological strategies. In mammals, zygotic reprogramming is broadly conserved though there may be some differences in timing or extent; *Xenopus* by contrast does not appear to show demethylation of the paternal genome (52). Hypomethylation of PGCs is also seen in human and pig fetal development, but has not been studied in non-mammalian organisms. As far as global DNA demethylation in PGCs and paternal demethylation in the zygote is concerned, it is therefore possible that this occurs primarily in mammals (and in the central cell in seed plants) which also have imprinting whose mechanism is based on DNA methylation. Clearly, demethylation in PGCs is necessary for erasure of imprints so that new imprints can be established properly later on according to the sex of the germ line (Fig. 2). Plants do not seem to erase imprints, instead they establish them by demethylation of the maternal genome in the endosperm after fertilization (with the endosperm being comparable to the placenta) (Fig. 1). Perhaps there are as yet undiscovered imprinted genes, which

acquire parent specific methylation patterns by (paternal) zygotic demethylation, in analogy to plants.

A second group of genes where demethylation in PGCs seems important are the germ line specific genes (eg *Dazl*, *Vasa*) which have specialized functions, for example in meiosis and germ cell differentiation. These genes are generally demethylated and expressed in germ cells, but in early PGCs they are methylated and silenced. Notably, genes that are demethylated in PGCs include those with a role in transposon control; *Tex19.1*, for example, silences members of the ERVK transposon family (53). Hence, global demethylation which in principle would lead to transcriptional activation and potentially transposition of active transposon families, at the same time activates defense mechanisms against transposons which are not needed in somatic cells where transposons are methylated. An extreme view of this scenario is the possibility that demethylated transposons produce small RNAs which in turn lead to *de novo* methylation and renewed silencing of transposons (Fig. 1) (24). Although it may sound paradoxical, reprogramming may have an important role in resetting the permanent silencing program for transposons across generations. Also, the fact that AID has a role in the erasure of methylation in PGCs is interesting in connection with roles of APOBEC deaminases in innate immunity and transposon control, establishing another potential link between the two.

The extent of methylation reprogramming in PGCs is substantial and this limits the potential in mammals for epigenetic transgenerational inheritance. By contrast in plants where epigenetic reprogramming may not occur to such an extent in the germ line, examples of stable inheritance of epialleles over multiple generations are more common (54). In *C elegans*, histone demethylation in the germ line is needed in order to prevent accumulation over generations of aberrant epigenetic marks that interfere with normal physiology and limit lifespan (55). By analogy, epigenetic reprogramming in mammalian PGCs or early embryos may be important in order to prevent the accumulation of potentially detrimental epialleles which could otherwise cause chronic diseases and limit lifespan in human populations. The inheritance of histone marks and of small RNAs potentially through both oocyte and sperm might also contribute to epigenetic inheritance and to reprogramming across generations in animals and in plants (24–25,56–59).

Finally, epigenetic reprogramming is linked to regaining pluripotency and following that, lineage commitment. Early PGCs and cells in the early embryo are pluripotent and these cells as well as the stem cell lines that can be isolated from them (ES, EG cells) have unique epigenetic signatures, which are at least in part the outcomes of reprogramming. For example, some of the key pluripotency transcription factors (such as *Nanog*) are methylated in sperm but not in ICM or ES cells, so their demethylation is important in order to acquire pluripotency (43); in the absence of the highly expressed TET1 gene (which hydroxylates 5mC) the *Nanog* gene is repressed and its promoter becomes methylated (60).

There is one final epigenetic parallel between mammals and plants that is worth highlighting. Following demethylation in the early mammalian embryo, selective *de novo* methylation occurs in ICM cells and their descendants which is important for the identity and stability of embryonic lineages (28), while the placenta remains hypomethylated at the genome-wide level (31). Similarly, genome-wide demethylation in the plant endosperm but not the embryo (20–21) indicates that epigenetic regulation between the two primary lineages (embryonic, extraembryonic) is fundamentally different with this difference apparently being conserved (or reinvented) in plants and animals.

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Fig. 1.

Model of epigenetic silencing dynamics during *Arabidopsis* life cycle. In somatic cells, three different mechanisms are responsible for repressing transcription from transposable element (TE), DNA methylation (in all three sequence contexts), histone H3K9 dimethylation (H3K9me₂), and histone H3K27 monomethylation (H3K27me₁). Methyltransferases and proteins regulating these epigenetic marks are shown in the diagram. See text for details. In the female gametophyte, the central cell is demethylated by DME, which leads to TE activation and upregulation of RdDM. The siRNAs produced from TEs not only direct non-CG methylation in the central cell, but also might travel to egg cell and enhance the silencing of TEs there. In addition, AGO9-associated siRNAs produced in somatic companion cells also contribute to the silencing of TEs in the egg cell. In the male gametophyte, the vegetative nucleus does not express DDM1 and has reduced RdDM, which leads to TE activation and mobilization. A new class of 21-nt siRNAs is produced from TEs in the vegetative nucleus that travels to sperm cells to reinforcing TE silencing. After double fertilization, maternal TEs in the endosperm stay activated and produce PolIV-dependent siRNAs, which could function to silence the paternal TEs in the endosperm. The methylation levels in the embryo are elevated, possibly due to the siRNA signals transmitted from the endosperm. Different shadings indicate the level of DNA methylation, with black being the high, gray being medium, and white being low.

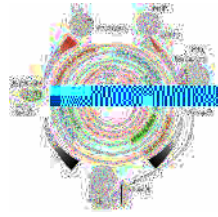


Fig. 2.

The two major phases of genome-wide erasure of DNA methylation in the early embryo and in primordial germ cells (PGCs) of the mouse are shown. Thickness of the outer arrows indicates levels of DNA methylation. Red, maternal genome; blue, paternal genome. After fertilization the paternal genome is more rapidly demethylated than the maternal one. During gametogenesis *de novo* methylation in spermatogenesis occurs earlier than in oogenesis. The inner circle shows factors or candidate factors that are implicated in *de novo* methylation, the maintenance of methylation, or demethylation, respectively. Solid arrows in the inner circle show at what developmental time these epigenetic regulators are thought to act.