Biochimica et Biophysica Acta 1819 (2012) 757–762

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagrm

Review

The ghosts in the machine: DNA methylation and the mystery of differentiation ☆

Victorino Briones, Kathrin Muegge *

Laboratory of Cancer Prevention, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD, USA

ARTICLE INFO

Article history:
Received 16 November 2011
Received in revised form 1 February 2012
Accepted 9 February 2012
Available online 22 February 2012

Keywords:
DNA methylation
SNF2
Embryogenesis

ABSTRACT

Methylation regulates DNA by altering chromatin and limiting accessibility of transcription factors and RNA polymerase. In this way, DNA methylation controls gene expression and plays a role in ES cell regulation, tissue differentiation and the development of the organism. In abnormal circumstances methylation can also induce diseases and promote cancer progression. Chromatin remodeling proteins such as the SNF2 family member Lsh regulates genome-wide cytosine methylation patterns during mammalian development. Lsh promotes methylation by targeting and repressing repeat sequences that are imbedded in heterochromatin. Lsh also regulates cytosine methylation at unique loci. Alterations in histone modifications (such as H3K4me3, histone acetylation, H3K27me3 and H2Aub) can be associated with DNA methylation changes making Lsh-mediated cytosine methylation part of a larger epigenetic network defining gene expression and cellular differentiation during development. This article is part of a Special Issue entitled: Chromatin in time and space.

Published by Elsevier B.V.

1. What is cytosine methylation?

Cytosine methylation involves a covalent modification at the carbon 5 position of the cytosine base [1,2]. In somatic mammalian cells cytosine methylation occurs preferentially in the CpG context while in ES cells, interestingly, it is also present at non-CpG sites [3]. Most eukaryotic genomes contain cytosine methylation but the distribution greatly varies between organisms [4,5]. For example, in honey bees cytosine methylation is almost exclusively concentrated at genes. In contrast, in mammalian cells genes as well as intergenic regions are highly methylated with the exception of so-called CpG islands, these are short CG rich regions often located around transcriptional start sites.

There are several important waves of global cytosine methylation changes in mammals [4,6,7]. First, genome-wide erasure of cytosine methylation occurs in primordial germ cells between days 10.5 and 13.5 during murine gestation. This is followed by re-methylation of the genome and establishment of a gender specific methylation pattern at imprinted sites (DMR = differentially methylated regions). A second wave of genome-wide reduction of methylation happens briefly after fertilization, and is based, in part, on an active process of de-methylation of the male genome. After implantation, re-methylation occurs and is associated with cellular differentiation suggesting that specific patterns are established in distinct tissues. In addition, large scale changes in cytosine methylation are observed after reprogramming and generation of iPS cells using overexpression of the four "Yamanaka" factors Oct4, Sox2, Klf4 and c-myc [8]. Thus methylation patterns in iPS cells resemble closely those in ES cells but differ from somatic tissues suggesting that specific cytosine methylation patterns mark pluripotency [3,8,9].

2. What are the functional consequences of DNA methylation?

DNA methylation plays a role in genomic imprinting (or parental allele specific expression), it regulates X inactivation and contributes to tissue specific gene expression patterns [1,2,7]. Deletions of the major DNA methyltransferases, Dnmt1 and Dnmt3b, lead to early lethality during embryogenesis [10,11]. In addition, enzymes that are involved in de-methylation, including iterative oxidation of methylated cytosine and subsequent base excision by repair enzymes, are crucial and their targeted deletion in mice also results in embryonic lethality [12,13]. This suggests that cytosine methylation plays an important role in development and the findings are consistent with a model of DNA methylation as part of the epigenetic memory.

However, several questions remain unresolved. Although tissue specific methylation is in part associated with gene expression, the cause and consequences of DNA methylation in the process of transcription remain undetermined. Although CG methylation is generally thought to result in gene silencing, particularly in cancer cells at tumor suppressor genes [14], methylation of the gene body is observed throughout most of the animal kingdom [4,5] and does not correlate with gene expression in somatic cells [3,8]. The loss of Dnmt1 in cultured cells results in both the up- and downregulation of many genes [15]. Genes that are directly targeted by Dnmt3a can

☆ This article is part of a Special Issue entitled: Chromatin in time and space.

* Corresponding author at: Laboratory of Cancer Prevention, National Cancer Institute, BLDG 560, Room 12-91, Frederick, MD 21701. USA. Tel.: +1 301 846 1386; fax: +1 301 846 7077.
E-mail address: Kathrin.Muegge@nih.gov (K. Muegge).

1874-9399/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.bbagrm.2012.02.013

be up- or down-regulated upon loss of Dnmt3a bringing cytosine methylation in a more complex transcriptional context [16]. Recent analysis in ES cells suggests a positive correlation between non-CG methylation and gene expression in ES cells [3]. Although, there is currently no hypothesis linking non-CpG methylation to gene expression, several mechanisms have been described that connect CG methylation to transcriptional repression [17]. Binding of transcription factors may be modulated by cytosine methylation and thus alter transcriptional initiation. Recognition of methyl-cytosine or unmethylated CG sites can result in histone modifications that modulate transcription. For example, interaction of DNA with methyl DNA binding proteins can lead to HDAC recruitment and hypoacetylated chromatin is associated with repression. Specific methyl–DNA binding proteins (MecP2 and CTCF) can also affect splicing and Pol II stalling, which then compromises Pol II elongation [18,19]. Finally, cytosine methylation may contribute to nucleosomal positioning [20] and ultimately to changes in chromatin structure and nuclear architecture.

3. The significance of cytosine methylation

The enzymatic machinery that maintains cytosine methylation patterns at the replication fork consists of Dnmt1 and the hemi-methylation binding protein Uhrf1 [2]. Patterns of cytosine methylation appear stable, making it an attractive mechanism to participate in the epigenetic memory (although at specific genomic sites cyclic methylation and de-methylation have been reported [21]). Large scale cytosine methylation changes (up to several Mb in size) as well as site specific changes including the promoter region of pluripotency genes such as Oct4 or Nanog, accompany the transition from pluripotent cells to somatic cells or in the reverse process from somatic to iPS cells (refer to Fig. 1 for a general summary of transitional epigenetics events occurring in ES cells, somatic cells and iPS cells) [3,8,9,22]. This suggests a functional role for cytosine methylation during cellular differentiation, reprogramming and possibly regenerative biology. In addition, frequent observations of genomic hypomethylation and site specific hypermethylation at tumor suppressor genes in cancer suggest a role in tumorigenesis [14]. Moreover, the possibility of transgenerational epigenetic inheritance [23] suggests another avenue to explore aside from genetic inheritance of familial diseases. In this manner, identifying factors that play a critical role in cytosine methylation raise the prospect of controlled modulation of the epigenetic memory for therapeutic purposes.

4. The physiologic role of Lsh

Murine Lsh was first cloned using a degenerative PCR technique to amplify novel helicase super family members in T cell precursors [24]. The gene is a member of the SNF2 subfamily of helicases, which largely consist of chromatin remodeling proteins (Fig. 2). Because of the prominent expression profile of murine Lsh in proliferating T or B cells, it was termed Lsh (lymphoid specific helicase) [24–26], although, Lsh mRNA has been detected at low levels in many tissues. The human gene has been cloned from human leukemic cells and is also known as PASG (proliferation associated gene) [27], other names for Lsh are HELLS (helicase, lymphoid specific) or SMARCA6 (SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 6).

Lsh−/− mice are embryonic lethal [28,29], pointing to an important role during development which is not shared by all SNF2 homologues. For example, neither mice with a targeted deletion of the SNF2 homologues Brm or Rad54 are lethal [30–32]. Lsh−/− mice have multiple developmental defects, kidney necrosis, reduced embryonal growth, aberrant gene expression of various Hox genes, signs of premature aging, and early senescence of fibroblasts [28,29,33,34]. In addition, a defect in the generation of stem cells has been observed in multiple tissues: neither male nor female germ cells thrive [35,36]; there is a delay or incomplete differentiation of ES cell differentiation in *in vitro* cultures [37] and an impaired lymphoid development and defects in hematopoiesis [26,38]. In short, Lsh plays a unique role in murine development, and is a non-redundant SNF2 family member.

Members of the SNF2 family disrupt histone–DNA interactions and perform chromatin remodeling in part via nucleosomal sliding and by altering the accessibility to nucleosomal DNA. In line with this role as a SNF2 family member, Lsh is found exclusively in the nuclear compartment and associates with chromatin [39]. It localizes at heterochromatic regions and deletion of Lsh alters chromatin structure at heterochromatic sites [39,40]. In particular, Lsh controls cytosine methylation, and deletion of Lsh shows a 50% reduction of cytosine methylation as measured, for example, by HPLC [41–43]. This property of Lsh (to affect cytosine methylation) is shared with DDM1, the Lsh homologue in *A. thaliana*. Indeed, DDM1 has been identified based on the occurrence of DNA hypomethylation in mutants (decrease in DNA methylation 1) [44]. In addition, Lsh deletion alters H3K4me3 level [40], which is again a phenotype shared with DDM1 mutants in *A. thaliana* [45]. However, it is not yet known whether the increase in H3K4me3 is due to DNA hypomethylation, or if DNA hypomethylation follows H3K4me3 increases. For example, Cfp1 is a DNA binding protein recognizing unmethylated CpG islands and recruiting the H3K4me3 methyltransferase Setd, thus connecting hypomethylated DNA to a rise in H3K4me3 [46]. On the other hand, DNMTs associate preferentially with H3 histone tails devoid of H3K4me3 modification and thus linking a decrease of H3K4me with methylated DNA [47,48]. In addition to cytosine methylation, changes in H3K27me3 and H2AK116 ubiquitylation have been observed at specific loci in Lsh−/− cells [34]. This would suggest, at least in part, functional interaction of Lsh with other epigenetic pathways such as the Polycomb silencing pathway.

5. Maintenance of methylation versus *de novo* cytosine methylation

The distinction between both pathways is important since for therapeutic purposes one would like to interfere mostly with *de novo* methylation, e.g. by blocking aberrant *de novo* methylation at tumor suppressor genes or by controlling site specific *de novo* methylation during cellular differentiation for use in regenerative medicine. There may be a partial overlap of the two pathways. For example, Dnmt1 controls maintenance, since it resides at the replication fork, and it efficiently methylates hemi-methylated DNA and is supported by the hemi-methylation binding protein Uhrf1 [2,49]. On the other hand, it may not be exclusively involved in maintenance since many reports have shown recruitment of Dnmt1 to genomic sites via interaction with specific transcription factors suggesting a role in *de novo* methylation [50,51]. Conversely, the *de novo* methyltransferases Dnmt3a and Dnmt3b, may contribute to maintenance since deletion in cell lines results in a moderate loss of cytosine methylation at some repetitive sequences [52]. Several observations suggest that Lsh primarily aids in *de novo* methylation. For example, Lsh is required for *de novo* methylation of retroviral sequences introduced into cell lines and is not obligatory for maintenance of *in vitro* pre-methylated episomal DNA [53]. Also, Lsh does not co-localize with Dnmt1 or does not localize at sites of replication in early S-phase which would be expected for a role in maintenance [39]. Moreover, during *in vitro* culture Lsh has been shown to be required for complete establishment of cytosine methylation at pluripotency genes such as Oct4 or Nanog [37]. Partial depletion of Lsh in ES cell cultures compromises silencing of pluripotency genes and delays their expression during embryogenesis [37]. Furthermore, Lsh is not in general required for genomic imprints as would be expected for a functional role in maintenance [54]. Finally, global analysis of cytosine methylation in Lsh−/− MEF cell lines demonstrates discrete

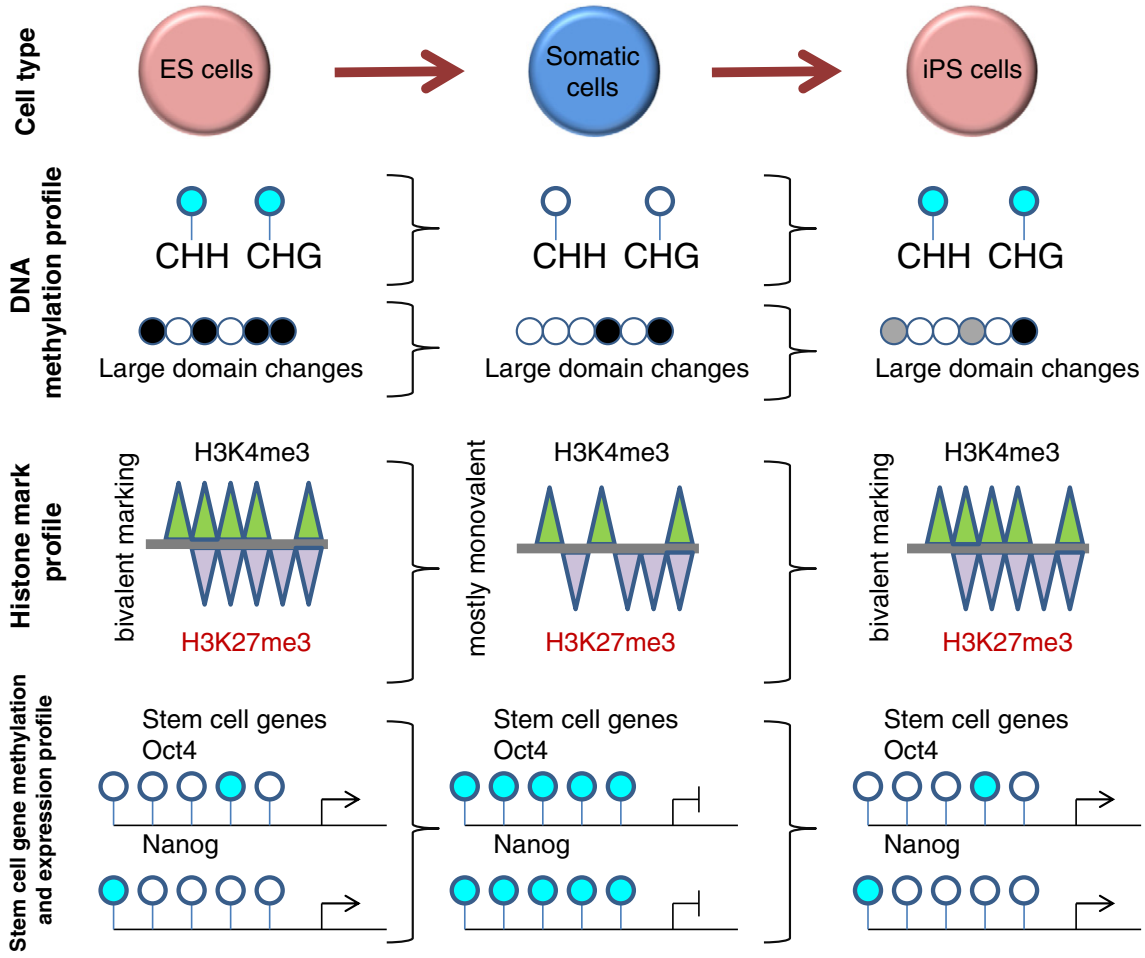

Fig. 1. Global cellular epigenetic profiles present during ES cells as it differentiates to somatic cells and then further to iPS cells. DNA methylation occurs in three different sequence context: CG, CHG or CHH methylation (H stands for C, A, or T) [3]. ES cells, and iPS cells show a higher frequency of non-CG methylation than somatic cells [8,9]. During transition from ES cell to somatic cells large regions alter DNA methylation, however, iPS cells often show an aberrant pattern [8]. Most bivalent marks (H3K4me3 and H3K27me3) in ES cells become monovalent in somatic cells and return to bivalent again in iPS cells [9,72,73]. Stem cell genes also show changes in the DNA methylation profile changing from significantly hypomethylated in ES and iPS cells to become hypermethylated in somatic cells. While upregulated in ES and iPS cells, stem cell genes remain silenced in somatic cells.

genomic sites that are affected by Lsh [42,43]. These findings contradict a general role of Lsh in maintenance of methylation, but instead suggest a primary role for Lsh in establishment of cytosine methylation at specific genomic loci.

6. How does Lsh affect cytosine methylation?

The precise molecular pathway for control of cytosine methylation needs to be further elucidated. Altering histone /DNA interactions may support binding of factors and, in particular, may promote access of Dnmts to the nucleosome. Use of an *in vitro* methylation assay and SssI methyltransferase revealed that nucleosomal DNA showed reduced accessibility unless SNF2 homologues were added to the assay [55]. Thus it may be hypothesized that complete methylation of nucleosomal DNA requires SNF2 factors. In support of this model, the presence of Lsh increases association of Dnmt3b (and methylation) at specific genomic sites [34,37,43]. Lsh and DDM1, the Lsh homologue in *A. thaliana*, belong to the SNF2 family and chromatin remodeling activity has been demonstrated for DDM1 [56]. Co-immunoprecipitation of Lsh with Dnmt3 has been reported [34,53,57]. This interaction may be based on their association with chromatin, or alternatively, due to interaction of Dnmt3b and Lsh as part of a larger complex or network. For example, both Dnmt3b and Lsh are interacting partners of an ES cell specific chromatin remodeling complex [58]. It remains to be shown if Lsh is part of multiple distinct complexes at different stages of development.

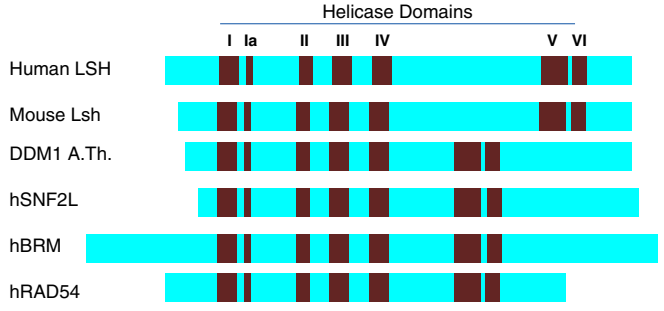

Fig. 2. Various members of the SNF2 family member including Lsh (mouse) demonstrate conserved homology of the seven helicase domains with 99% homology between mouse and human. Homology of various members also shows a range of similarity greater than 90%.

7. Which sites in the genome are affected by Lsh?

One of the most dramatic findings with respect to epigenetics is that knockdown of Lsh produces significant hypomethylation at several repeat elements, including major and minor satellite sequences, retroviral elements, Line1 and Sine repeats [29,41,59]. For comparison, Dnmt3a deletion has no effect on repeats and Dnmt3b deletion results in reduction of methylation only at major satellites [11]. The reduction of cytosine methylation at repetitive sequences in the

absence of Lsh has further functional consequences and is accompanied by increases in histone acetylation and H3K4me3 modifications resulting in elevated transcripts of repeat elements [59]. Elevated gene expression of repeat elements pose the threat of enhanced genome instability in Lsh−/− cells similarly reported for DDM1 mutants [33]. Until recently, it was thought that repeat elements (in particular transposable elements) play an evolutionary role in genome shuffling and the generation of novel regulatory sequences by moving in germ cells [60]. However, recent evidence suggests transposon mobility in neurons as participating in neurologic disorders such as the RETT syndrome [61]. Many human epithelial cancers express a surprisingly high level of repetitive sequences (up to 17% of total RNA) and aberrant gene expression and for human lung cancer it is hypothesized that DNA methylation changes cause high levels of Line 1 expression and Line 1 retrotransposition [62,63]. Also derepression of satellite sequences is found in BRCA1 mutant cells and ectopic expression of satellite sequences can induce genomic instability in normal cells [64]. Whether Lsh-mediated DNA hypomethylation can exacerbate satellite expression in cancer cells is currently unknown, but factors that repress repeat elements may help to prevent genomic instability in cancer cells.

8. What other genomic sites does Lsh target besides repeats?

Previous studies demonstrated cytosine hypomethylation at selected genomic targets [34,37,42,65]. For example, Lsh−/− MEFs show de-repression of HoxC6 and HoxC8 genes accompanied by reduced cytosine methylation [65]. Hox loci also show reduced association of Polycomb proteins such as Bmi1 and Ezh2 in Lsh−/− cells [34]. Consequently, reduced histone modifications mediated by polycomb proteins such as H3K27me3 or H2AK116ub are found in the absence of Lsh and this may result in increased gene expression at diverse Hox genes [34]. As another example, reduced Lsh protein levels result in cytosine hypomethylation at genes specifically expressed in pluripotent cells, such as Oct4 or Nanog [37,42]. Recent genome-wide studies identified multiple specific genomic sites, supporting the notion that Lsh has a specific effect on unique sites in the genome [42,43]. These reports confirmed reduction of cytosine methylation at pluripotency genes [42,43] and moreover found, surprisingly, also hypermethylation at a subset of CpG island promoters in Lsh−/− MEF cell lines [43]. Aberrant hypermethylation has been also reported for DDM1 mutants, the Lsh homologue in *A. thaliana* [66]. In mammals hypermethylation at CpG islands is a phenomenon that may be linked to prolonged *in vitro* culture [67] and a hallmark in cancer cells [14]. Whether hypermethylation at CpG islands can be modulated by Lsh in cancer cells is currently unknown. In breast cancer cell lines, however, selected upstream promoter regions show a cytosine methylation pattern that depends on Lsh, and Lsh interference by siRNA can inhibit modestly the growth of those cancer cells [68]. The transition of ES to somatic cells as well as reprogramming (transition from somatic cells to IPS) is associated with a re-organization of cytosine methylation at larger chromosomal domains ranging from 200,000 bp to several Mb [8,9]. Lsh−/− MEFs also show differentially methylated regions of 2–4 Mb in comparison to wild type MEFs [43]. It is hypothesized that the larger differentially methylated domains in ES cells compared to somatic cells may be part of the epigenetic memory, and factors involved in re-organization are likely to play a role in the epigenetic memory and identity of cells. Larger domains may play a role in the nuclear architecture as attachment of genomic loci to the nuclear envelope or in the organization of specialized nuclear regions such as ribosomal RNA transcription or transcription of non-coding RNA.

9. DNA methylation and transcription

Multiple mechanisms explain how DNA methylation can control gene expression such as interference with DNA binding factors, interaction of DNA methylation to other epigenetic silencing pathways, the relationship of DNA methylation to other histone modifications, and the effect on chromatin organization. In Lsh−/− MEF cell lines DNA methylation at Hox genes was found to affect Pol II elongation [65]. In wild type MEF cell lines HoxC8 was methylated and transcriptionally repressed, however, Pol II was found to be engaged at the transcriptional start site. In the absence of Lsh, the HoxC8 gene was hypomethylated and Pol II showed successful elongation (with increased Ser2 Pol II and H3K36me3 modification over the gene body) leading to mature transcripts. Importantly, a catalytically active Dnmt3b was required to mediate cytosine methylation and Pol II stalling at Hox loci, implicating a functional role for cytosine methylation in gene transcription at those sites [65]. In breast cancer cell lines several genes with cytosine hypermethylation upstream of the transcriptional start sites showed Pol II stalling with release of stalled Pol II upon demethylation by Azazytidine or siLSH interference [68]. In both examples, H3K27me3 and the polycomb protein EZH2 were associated with DNA methylation [65,68]. Polycomb proteins are thought to mediate gene silencing in part via Pol II stalling [69,70]. In addition, DNA binding factors such as MecP2 and CTCF which are controlled by methyl-cytosine have been implicated in the regulation of splicing at distinct loci [18,19] and splicing is closely involved in the regulation of successful Pol II elongation [71]. Further investigation is required to determine how cytosine methylation intimately regulates nucleosomal positioning and Pol II mediated transcription.

10. Conclusion and outlook

Lsh is critical for establishment of DNA methylation patterns in mice. Since Lsh is a non-redundant SNF2 family member, Lsh−/− mice represent a unique tool to gain further insights into the molecular mechanism of DNA methylation. Several questions as to the functional consequences of DNA methylation in diverse biologic processes of differentiation, transformation and reprogramming deserve further scrutiny. For example, the relationship of DNA methylation and transcription is not fully understood. Further in-depth genome wide analysis to determine at single base pair resolution how Pol II initiation, elongation and splicing are affected and control developmental gene expression is needed. The Lsh model may give an opportunity to explore transcription by examining Pol II engagement in the same tissue type under different methylation pattern. Furthermore, new insights into the role of satellite sequences and genomic instability suggest that genomic hypomethylation in cancer de-represses those satellites, and identifying factors that control DNA methylation may prevent this pathway of tumorigenesis. Finally, transition from pluripotency to somatic cells as well as reprogramming is associated with large scale genome methylation changes. To examine which factors are involved in this process and how it affects chromatin organization may prove useful in improving and directing the cellular fate during cellular differentiation more efficiently.

Acknowledgments

We thank our colleagues Drs. Nancy Colburn and Jonathan Keller for critical reviewing of the manuscript. This project has been funded with federal funds from the National Cancer Institute, National Institutes of Health, under contract HHSN261200800001E. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products or organizations imply endorsement by the US government. This research was supported by the Intramural Research Program of NIH, National Cancer Institute, Center for Cancer Research.

References

[1] H. Cedar, Y. Bergman, Linking DNA methylation and histone modification: patterns and paradigms, Nat. Rev. Genet. 10 (2009) 295–304.
[2] S.K. Ooi, A.H. O'Donnell, T.H. Bestor, Mammalian cytosine methylation at a glance, J. Cell Sci. 122 (2009) 2787–2791.
[3] R. Lister, M. Pelizzola, R.H. Dowen, R.D. Hawkins, G. Hon, J. Tonti-Filippini, J.R. Nery, L. Lee, Z. Ye, Q.M. Ngo, L. Edsall, J. Antosiewicz-Bourget, R. Stewart, V. Ruotti, A.H. Millar, J.A. Thomson, B. Ren, J.R. Ecker, Human DNA methylomes at base resolution show widespread epigenomic differences, Nature 462 (2009) 315–322.
[4] S. Feng, S.J. Cokus, X. Zhang, P.Y. Chen, M. Bostick, M.G. Goll, J. Hetzel, J. Jain, S.H. Strauss, M.E. Halpern, C. Ukomadu, K.C. Sadler, S. Pradhan, M. Pellegrini, S.E. Jacobsen, Conservation and divergence of methylation patterning in plants and animals, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 8689–8694.
[5] A. Zemach, I.E. McDaniel, P. Silva, D. Zilberman, Genome-wide evolutionary analysis of eukaryotic DNA methylation, Science 328 (2010) 916–919.
[6] T.M. Geiman, K. Muegge, DNA methylation in early development, Mol. Reprod. Dev. 77 (2010) 105–113.
[7] W. Reik, Stability and flexibility of epigenetic gene regulation in mammalian development, Nature 447 (2007) 425–432.
[8] R. Lister, M. Pelizzola, Y.S. Kida, R.D. Hawkins, J.R. Nery, G. Hon, J. Antosiewicz-Bourget, R. O'Malley, R. Castanon, S. Klugman, M. Downes, R. Yu, R. Stewart, B. Ren, J.A. Thomson, R.M. Evans, J.R. Ecker, Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells, Nature 471 (2011) 68–73.
[9] R.D. Hawkins, G.C. Hon, L.K. Lee, Q. Ngo, R. Lister, M. Pelizzola, L.E. Edsall, S. Kuan, Y. Luu, S. Klugman, J. Antosiewicz-Bourget, Z. Ye, C. Espinoza, S. Agarwahl, L. Shen, V. Ruotti, W. Wang, R. Stewart, J.A. Thomson, J.R. Ecker, B. Ren, Distinct epigenomic landscapes of pluripotent and lineage-committed human cells, Cell Stem Cell 6 (2010) 479–491.
[10] E. Li, T.H. Bestor, R. Jaenisch, Targeted mutation of the DNA methyltransferase gene results in embryonic lethality, Cell 69 (1992) 915–926.
[11] M. Okano, D.W. Bell, D.A. Haber, E. Li, DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development, Cell 99 (1999) 247–257.
[12] D. Cortazar, C. Kunz, J. Selfridge, T. Lettieri, Y. Saito, E. MacDougall, A. Wirz, D. Schuermann, A.L. Jacobs, F. Siegrist, R. Steinacher, J. Jiricny, A. Bird, P. Schar, Embryonic lethal phenotype reveals a function of TDG in maintaining epigenetic stability, Nature 470 (2011) 419–423.
[13] S. Cortellino, J. Xu, M. Sannai, R. Moore, E. Caretti, A. Cigliano, M. Le Coz, K. Devarajan, A. Wessels, D. Soprano, L.K. Abramowitz, M.S. Bartolomei, F. Rambow, M.R. Bassi, T. Bruno, M. Fanciulli, C. Renner, A.J. Klein-Szanto, Y. Matsumoto, D. Kobi, I. Davidson, C. Alberti, L. Larue, A. Bellacosa, Thymine DNA glycosylase is essential for active DNA demethylation by linked deamination-base excision repair, Cell 146 (2011) 67–79.
[14] S.B. Baylin, P.A. Jones, A decade of exploring the cancer epigenome—biological and translational implications, Nat. Rev. Cancer 11 (2011) 726–734.
[15] L. Jackson-Grusby, C. Beard, R. Possemato, M. Tudor, D. Fambrough, G. Csankovszki, J. Dausman, P. Lee, C. Wilson, E. Lander, R. Jaenisch, Loss of genomic methylation causes p53-dependent apoptosis and epigenetic deregulation, Nat. Genet. 27 (2001) 31–39.
[16] H. Wu, V. Coskun, J. Tao, W. Xie, W. Ge, K. Yoshikawa, E. Li, Y. Zhang, Y.E. Sun, Dnmt3a-dependent nonpromoter DNA methylation facilitates transcription of neurogenic genes, Science 329 (2010) 444–448.
[17] R.J. Klose, A.P. Bird, Genomic DNA methylation: the mark and its mediators, Trends Biochem. Sci. 31 (2006) 89–97.
[18] S. Shukla, E. Kavak, M. Gregory, M. Imashimizu, B. Shutinoski, M. Kashlev, P. Oberdoerffer, R. Sandberg, S. Oberdoerffer, CTCF-promoted RNA polymerase II pausing links DNA methylation to splicing, Nature (2011).
[19] J.I. Young, E.P. Hong, J.C. Castle, J. Crespo-Barreto, A.B. Bowman, M.F. Rose, D. Kang, R. Richman, J.M. Johnson, S. Berget, H.Y. Zoghbi, Regulation of RNA splicing by the methylation-dependent transcriptional repressor methyl-CpG binding protein 2, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 17551–17558.
[20] J.C. Lin, S. Jeong, G. Liang, D. Takai, M. Fatemi, Y.C. Tsai, G. Egger, E.N. Gal-Yam, P.A. Jones, Role of nucleosomal occupancy in the epigenetic silencing of the MLH1 CpG island, Cancer Cell 12 (2007) 432–444.
[21] R. Metivier, R. Gallais, C. Tiffoche, C. Le Peron, R.Z. Jurkowska, R.P. Carmouche, D. Ibberson, P. Barath, F. Demay, G. Reid, V. Benes, A. Jeltsch, F. Gannon, G. Salbert, Cyclical DNA methylation of a transcriptionally active promoter, Nature 452 (2008) 45–50.
[22] S. Feng, S.E. Jacobsen, W. Reik, Epigenetic reprogramming in plant and animal development, Science 330 (2010) 622–627.
[23] E.L. Greer, T.J. Maures, D. Ucar, A.G. Hauswirth, E. Mancini, J.P. Lim, B.A. Benayoun, Y. Shi, A. Brunet, Transgenerational epigenetic inheritance of longevity in *Caenorhabditis elegans*, Nature 479 (2011) 365–371.
[24] C.D. Jarvis, T. Geiman, M.P. Vila-Storm, O. Osipovich, U. Akella, S. Candeias, I. Nathan, S.K. Durum, K. Muegge, A novel putative helicase produced in early murine lymphocytes, Gene 169 (1996) 203–207.
[25] T.M. Geiman, S.K. Durum, K. Muegge, Characterization of gene expression, genomic structure, and chromosomal localization of Hells (Lsh), Genomics 54 (1998) 477–483.
[26] T.M. Geiman, K. Muegge, Lsh, an SNF2/helicase family member, is required for proliferation of mature T lymphocytes, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 4772–4777.
[27] D.W. Lee, K. Zhang, Z.Q. Ning, E.H. Raabe, S. Tintner, R. Wieland, B.J. Wilkins, J.M. Kim, R.I. Blough, R.J. Arceci, Proliferation-associated SNF2-like gene (PASG): a SNF2 family member altered in leukemia, Cancer Res. 60 (2000) 3612–3622.
[28] T.M. Geiman, L. Tessarollo, M.R. Anver, J.B. Kopp, J.M. Ward, K. Muegge, Lsh, a SNF2 family member, is required for normal murine development, Biochim. Biophys. Acta 1526 (2001) 211–220.
[29] L.Q. Sun, D.W. Lee, Q. Zhang, W. Xiao, E.H. Raabe, A. Meeker, D. Miao, D.L. Huso, R.J. Arceci, Growth retardation and premature aging phenotypes in mice with disruption of the SNF2-like gene, PASG, Genes Dev. 18 (2004) 1035–1046.
[30] J. Essers, R.W. Hendriks, S.M. Swagemakers, C. Troelstra, J. de Wit, D. Bootsma, J.H. Hoeijmakers, R. Kanaar, Disruption of mouse RAD54 reduces ionizing radiation resistance and homologous recombination, Cell 89 (1997) 195–204.
[31] J.C. Reyes, J. Barra, C. Muchardt, A. Camus, C. Babinet, M. Yaniv, Altered control of cellular proliferation in the absence of mammalian brahma (SNF2alpha), EMBO J. 17 (1998) 6979–6991.
[32] J. Wesoly, S. Agarwal, S. Sigurdsson, W. Bussen, S. Van Komen, J. Qin, H. van Steeg, J. van Benthem, E. Wassenaar, W.M. Baarends, M. Ghazvini, A.A. Tafel, H. Heath, N. Galjart, J. Essers, J.A. Grootegoed, N. Arnheim, O. Bezzubova, J.M. Buerstedde, P. Sung, R. Kanaar, Differential contributions of mammalian Rad54 paralogs to recombination, DNA damage repair, and meiosis, Mol. Cell. Biol. 26 (2006) 976–989.
[33] T. Fan, Q. Yan, J. Huang, S. Austin, E. Cho, D. Ferris, K. Muegge, Lsh-deficient murine embryonal fibroblasts show reduced proliferation with signs of abnormal mitosis, Cancer Res. 63 (2003) 4677–4683.
[34] S. Xi, H. Zhu, H. Xu, A. Schmidtmann, T.M. Geiman, K. Muegge, Lsh controls Hox gene silencing during development, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 14366–14371.
[35] R. De La Fuente, C. Baumann, T. Fan, A. Schmidtmann, I. Dobrinski, K. Muegge, Lsh is required for meiotic chromosome synapsis and retrotransposon silencing in female germ cells, Nat. Cell Biol. 8 (2006) 1448–1454.
[36] W. Zeng, C. Baumann, A. Schmidtmann, A. Honaramooz, L. Tang, A. Bondareva, C. Dores, T. Fan, S. Xi, T. Geiman, R. Rathi, D. de Rooij, R. De La Fuente, K. Muegge, I. Dobrinski, Lymphoid-specific helicase (HELLS) is essential for meiotic progression in mouse spermatocytes, Biol. Reprod. 84 (2011) 1235–1241.
[37] S. Xi, T.M. Geiman, V. Briones, Y. Guang Tao, H. Xu, K. Muegge, Lsh participates in DNA methylation and silencing of stem cell genes, Stem Cells 27 (2009) 2691–2702.
[38] T. Fan, A. Schmidtmann, S. Xi, V. Briones, H. Zhu, H.C. Suh, J. Gooya, J.R. Keller, H. Xu, J. Roayaei, M. Anver, S. Ruscetti, K. Muegge, DNA hypomethylation caused by Lsh deletion promotes erythroleukemia development, Epigenetics 3 (2008) 134–142.
[39] Q. Yan, E. Cho, S. Lockett, K. Muegge, Association of Lsh, a regulator of DNA methylation, with pericentromeric heterochromatin is dependent on intact heterochromatin, Mol. Cell. Biol. 23 (2003) 8416–8428.
[40] Q. Yan, J. Huang, T. Fan, H. Zhu, K. Muegge, Lsh, a modulator of CpG methylation, is crucial for normal histone methylation, EMBO J. 22 (2003) 5154–5162.
[41] K. Dennis, T. Fan, T. Geiman, Q. Yan, K. Muegge, Lsh, a member of the SNF2 family, is required for genome-wide methylation, Genes Dev. 15 (2001) 2940–2944.
[42] K. Myant, A. Termanis, A.Y. Sundaram, T. Boe, C. Li, C. Merusi, J. Burrage, J.I. de Las Heras, I. Stancheva, LSH and G9a/GLP complex are required for developmentally programmed DNA methylation, Genome Res. 21 (2011) 83–94.
[43] Y. Tao, S. Xi, J. Shan, A. Maunakea, A. Che, V. Briones, E.Y. Lee, T. Geiman, J. Huang, R. Stephens, R.M. Leighty, K. Zhao, K. Muegge, Lsh, chromatin remodeling family member, modulates genome-wide cytosine methylation patterns at nonrepeat sequences, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 5626–5631.
[44] J.A. Jeddeloh, T.L. Stokes, E.J. Richards, Maintenance of genomic methylation requires a SWI2/SNF2-like protein, Nat. Genet. 22 (1999) 94–97.
[45] A.V. Gendrel, Z. Lippman, C. Yordan, V. Colot, R.A. Martienssen, Dependence of heterochromatic histone H3 methylation patterns on the *Arabidopsis* gene DDM1, Science 297 (2002) 1871–1873.
[46] J.P. Thomson, P.J. Skene, J. Selfridge, T. Clouaire, J. Guy, S. Webb, A.R. Kerr, A. Deaton, R. Andrews, K.D. James, D.J. Turner, R. Illingworth, A. Bird, CpG islands influence chromatin structure via the CpG-binding protein Cfp1, Nature 464 (2010) 1082–1086.
[47] D. Jia, R.Z. Jurkowska, X. Zhang, A. Jeltsch, X. Cheng, Structure of Dnmt3a bound to Dnmt3L suggests a model for de novo DNA methylation, Nature 449 (2007) 248–251.
[48] S.K. Ooi, C. Qiu, E. Bernstein, K. Li, D. Jia, Z. Yang, H. Erdjument-Bromage, P. Tempst, S.P. Lin, C.D. Allis, X. Cheng, T.H. Bestor, DNMT3L connects unmethylated lysine 4 of histone H3 to de novo methylation of DNA, Nature 448 (2007) 714–717.
[49] K. Arita, M. Ariyoshi, H. Tochio, Y. Nakamura, M. Shirakawa, Recognition of hemimethylated DNA by the SRA protein UHRF1 by a base-flipping mechanism, Nature 455 (2008) 818–821.
[50] P.O. Esteve, H.G. Chin, S. Pradhan, Human maintenance DNA (cytosine-5)-methyltransferase and p53 modulate expression of p53-repressed promoters, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 1000–1005.
[51] K.D. Robertson, S. Ait-Si-Ali, T. Yokochi, P.A. Wade, P.L. Jones, A.P. Wolffe, DNMT1 forms a complex with Rb, E2F1 and HDAC1 and represses transcription from E2F-responsive promoters, Nat. Genet. 25 (2000) 338–342.
[52] T. Chen, Y. Ueda, J.E. Dodge, Z. Wang, E. Li, Establishment and maintenance of genomic methylation patterns in mouse embryonic stem cells by Dnmt3a and Dnmt3b, Mol. Cell. Biol. 23 (2003) 5594–5605.
[53] H. Zhu, T.M. Geiman, S. Xi, Q. Jiang, A. Schmidtmann, T. Chen, E. Li, K. Muegge, Lsh is involved in de novo methylation of DNA, EMBO J. 25 (2006) 335–345.
[54] T. Fan, J.P. Hagan, S.V. Kozlov, C.L. Stewart, K. Muegge, Lsh controls silencing of the imprinted Cdkn1c gene, Development 132 (2005) 635–644.
[55] K. Bouazoune, T.B. Miranda, P.A. Jones, R.E. Kingston, Analysis of individual remodeled nucleosomes reveals decreased histone–DNA contacts created by hSWI/SNF, Nucleic Acids Res. 37 (2009) 5279–5294.
[56] J. Brzeski, A. Jerzmanowski, Deficient in DNA methylation 1 (DDM1) defines a novel family of chromatin-remodeling factors, J. Biol. Chem. 278 (2003) 823–828.
[57] K. Myant, I. Stancheva, LSH cooperates with DNA methyltransferases to repress transcription, Mol. Cell. Biol. 28 (2008) 215–226.

[58] L. Ho, J.L. Ronan, J. Wu, B.T. Staahl, L. Chen, A. Kuo, J. Lessard, A.I. Nesvizhskii, J. Ranish, G.R. Crabtree, An embryonic stem cell chromatin remodeling complex, esBAF, is essential for embryonic stem cell self-renewal and pluripotency, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 5181–5186.
[59] J. Huang, T. Fan, Q. Yan, H. Zhu, S. Fox, H.J. Issaq, L. Best, L. Gangi, D. Munroe, K. Muegge, Lsh, an epigenetic guardian of repetitive elements, Nucleic Acids Res. 32 (2004) 5019–5028.
[60] W. Makalowski, Genomics. Not junk after all, Science 300 (2003) 1246–1247.
[61] A.R. Muotri, M.C. Marchetto, N.G. Coufal, R. Oefner, G. Yeo, K. Nakashima, F.H. Gage, L1 retrotransposition in neurons is modulated by MeCP2, Nature 468 (2010) 443–446.
[62] R.C. Iskow, M.T. McCabe, R.E. Mills, S. Torene, W.S. Pittard, A.F. Neuwald, E.G. Van Meir, P.M. Vertino, S.E. Devine, Natural mutagenesis of human genomes by endogenous retrotransposons, Cell 141 (2010) 1253–1261.
[63] D.T. Ting, D. Lipson, S. Paul, B.W. Brannigan, S. Akhavanfard, E.J. Coffman, G. Contino, V. Deshpande, A.J. Iafrate, S. Letovsky, M.N. Rivera, N. Bardeesy, S. Maheswaran, D.A. Haber, Aberrant overexpression of satellite repeats in pancreatic and other epithelial cancers, Science 331 (2011) 593–596.
[64] Q. Zhu, G.M. Pao, A.M. Huynh, H. Suh, N. Tonnu, P.M. Nederlof, F.H. Gage, I.M. Verma, BRCA1 tumour suppression occurs via heterochromatin-mediated silencing, Nature 477 (2011) 179–184.
[65] Y. Tao, S. Xi, V. Briones, K. Muegge, Lsh mediated RNA polymerase II stalling at HoxC6 and HoxC8 involves DNA methylation, PLoS One 5 (2010) e9163.
[66] H. Saze, T. Kakutani, Heritable epigenetic mutation of a transposon-flanked *Arabidopsis* gene due to lack of the chromatin-remodeling factor DDM1, EMBO J. 26 (2007) 3641–3652.
[67] A. Meissner, T.S. Mikkelsen, H. Gu, M. Wernig, J. Hanna, A. Sivachenko, X. Zhang, B.E. Bernstein, C. Nusbaum, D.B. Jaffe, A. Gnirke, R. Jaenisch, E.S. Lander, Genome-scale DNA methylation maps of pluripotent and differentiated cells, Nature 454 (2008) 766–770.
[68] Y. Tao, S. Liu, V. Briones, T.M. Geiman, K. Muegge, Treatment of breast cancer cells with DNA demethylating agents leads to a release of Pol II stalling at genes with DNA-hypermethylated regions upstream of TSS, Nucleic Acids Res. 39 (2011) 9508–9520.
[69] V.S. Chopra, J.W. Hong, M. Levine, Regulation of Hox gene activity by transcriptional elongation in *Drosophila*, Curr. Biol. 19 (2009) 688–693.
[70] J.K. Stock, S. Giadrossi, M. Casanova, E. Brookes, M. Vidal, H. Koseki, N. Brockdorff, A.G. Fisher, A. Pombo, Ring1-mediated ubiquitination of H2A restrains poised RNA polymerase II at bivalent genes in mouse ES cells, Nat. Cell Biol. 9 (2007) 1428–1435.
[71] S. Lin, G. Coutinho-Mansfield, D. Wang, S. Pandit, X.D. Fu, The splicing factor SC35 has an active role in transcriptional elongation, Nat. Struct. Mol. Biol. 15 (2008) 819–826.
[72] B.E. Bernstein, T.S. Mikkelsen, X. Xie, M. Kamal, D.J. Huebert, J. Cuff, B. Fry, A. Meissner, M. Wernig, K. Plath, R. Jaenisch, A. Wagschal, R. Feil, S.L. Schreiber, E.S. Lander, A bivalent chromatin structure marks key developmental genes in embryonic stem cells, Cell 125 (2006) 315–326.
[73] T.S. Mikkelsen, M. Ku, D.B. Jaffe, B. Issac, E. Lieberman, G. Giannoukos, P. Alvarez, W. Brockman, T.K. Kim, R.P. Koche, W. Lee, E. Mendenhall, A. O'Donovan, A. Presser, C. Russ, X. Xie, A. Meissner, M. Wernig, R. Jaenisch, C. Nusbaum, E.S. Lander, B.E. Bernstein, Genome-wide maps of chromatin state in pluripotent and lineage-committed cells, Nature 448 (2007) 553–560.