View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Edinburgh Research Explorer

THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

Dynamics and Mechanisms of DNA Methylation Reprogramming

Citation for published version:
Pennings, S, Revuelta, A, Mclaughlin, KA, Abd Hadi, NA, Petchreing, P, Ottaviano, R & Meehan, RR 2019, Dynamics and Mechanisms of DNA Methylation Reprogramming. in *Epigenetics and Regeneration.* Elsevier, pp. 19-45. https://doi.org/10.1016/B978-0-12-814879-2.00002-9

Digital Object Identifier (DOI):
10.1016/B978-0-12-814879-2.00002-9

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Peer reviewed version

Published In:
Epigenetics and Regeneration

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

Download date: 12. Jun. 2020

DYNAMICS AND MECHANISMS OF DNA METHYLATION REPROGRAMMING

Sari Pennings*, Ailsa Revuelta[†], Katy A. McLaughlin[†], Nur Annies Abd Hadi*, Petchroi Petchreing*, Raffaele Ottaviano[†], Richard R. Meehan[†]
Centre for Cardiovascular Science, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, United Kingdom*
MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, United Kingdom[†]

ABSTRACT

The molecular profiling of DNA and histone modifications in multiple in vitro and in vivo systems has enabled unique explorations of potential epigenetic regulatory pathways. An important question is whether these epigenetic pathways drive development or are subservient to genetically encoded signaling pathways? Broad changes in epigenomic profiles are a crucial observation in many mammalian developmental stages and are thought to result from the action of key signaling/gene regulatory networks. In this review, we will introduce the epigenetic basic principles and focus on how the study of DNA modification dynamics has contributed to our understanding of early development and the hypothesis that epigenome dependency is exquisitely linked to cellular states.

Keywords: DNA methylation reprogramming, embryogenesis, stem cells, gene regulatory networks

CHAPTER OUTLINE

2.1 Introduction 1
2.2 DNA Methylation Machinery 3
2.3 DNA Demethylation 8
2.4 DNA Methylation in Early Development 9
2.5 How Necessary is DNA Methylation Reprogramming for Early Development? 12
2.6 DNA Methylation Trajectories During Reprogramming of Somatic Cells 14
2.7 6-Methyldeoxyadenosine in Mammalian DNA? 17
2.8 Future Perspectives 18
Acknowledgments 18
Chapter citation 18
References 18

2.1 INTRODUCTION

The potential relevance of epigenetics is now well appreciated in multiple biological contexts; embryo and germline development, tissue-specificity and cellular reprogramming, carcinogenesis and cancer genetics, neuronal and behavioral studies, nutrition and metabolism, disease studies and longevity, population and livestock studies and finally, plant development[1]. This is a late twentieth and early twenty-first century highpoint for a concept that was promulgated by Conrad Waddington to resolve the paradox of cellular differentiation in organisms: how can embryological cells with similar genetic material differentiate into multiple and distinct cell types[2, 3]? The fields of developmental and stem cell biology in plant and animal studies have led the way in demonstrating that signaling pathways, homeostasis mechanisms and gene regulatory networks organize the progression from a fertilized egg through embryogenesis and organogenesis, into a fertile adult.[4–8] On this fundamentally genetic basis of development and disease, a differential contribution by parental genomes in mammals ensures successful developmental outcomes. This was identified as being due to parentally imprinted loci by a combination of classical and molecular genetic methodologies and represents one of the early examples of epigenetic regulation in mammals.[9–12]
Conrad Waddington introduced the term “epigenetics” in 1942, combining the concepts of “epigenesis” (the development of an organism) and “genetics” (the study of heredity of trait variation). Defining epigenetics as “the whole complex of developmental processes” connecting genotype to phenotype, he further used the word “epigenotype” to describe the biological networks linking disturbances in genotype to observable abnormal phenotypes.[3] As an embryologist, Waddington’s definition of epigenetics was centered around the process of development and morphogenetic heritability. The definition of epigenetics has itself matured and been extended over time to give us a generally accepted modern molecular definition of “the study of stable changes (through cell division) in gene expression that are not explained by changes in DNA sequence”.[13] In other words, epigenetics is the scientific discipline studying how, in the absence of genetic changes, phenotypic traits in cell types and organisms can be set up and maintained. An impressive example of epigenetics in action can be found during mouse embryogenesis. Epigenetic modifications are dramatically remodelled to help orchestrate the eventual differentiation of a fertilized zygote into an organization of highly specialized cells that will eventually constitute the whole adult organism.[14, 15] This concept is illustrated in Waddington’s often-cited model of the “epigenetic landscape”, which depicts the apparently irreversible fate decisions that a cell is required to make during development. These decisions are underpinned by the complex networks of gene expression defining the pathways that the cell will utilize during its self-regulating progression through development.[16,17] Of course we now know that these differentiated states can be reprogrammed to earlier embryonic identities via other trajectories that are mediated either by nuclear transfer of somatic cells into enucleated oocytes, or the forced expression of pluripotency factors in a differentiated cell.[18, 19] This reversal is associated with characteristic changes in epigenetic states that imitate embryonic profiles.[20,21] Epigenetic processes are utilized in multiple organisms including plants, bacteria, insects and animals.[7, 22,23] Our focus here will be mainly on mammalian studies.
An array of molecular mechanisms are known to be linked to epigenetic regulation of the genome; impacting on gene expression and organization of higher order chromatin structures, maintenance of cellular identity, preservation of

genome integrity, metabolism, and response to environmental damage.[24] The best studied of these involve the direct covalent modification of DNA and of histones, the proteins around which DNA is wrapped, in the various levels of chromatin organization that organize and package genetic information in the cell.[25–27] Research into these two epigenetic mechanisms progressed separately throughout the 1970s and 1980s, with the term epigenetics only being adopted to describe the collective research of these chromatin modifications in the 1990s (with less stress on the inheritance of these modifications during cell division), and a rapid increase in research under this term ensuing from the year 2000.[28] In recent years, there has also been increasing evidence uncovering the importance of non-coding RNAs (ncRNAs) in epigenetic regulation of gene expression and maintenance of cell identity, through their interplay with chromatin.[29, 30]

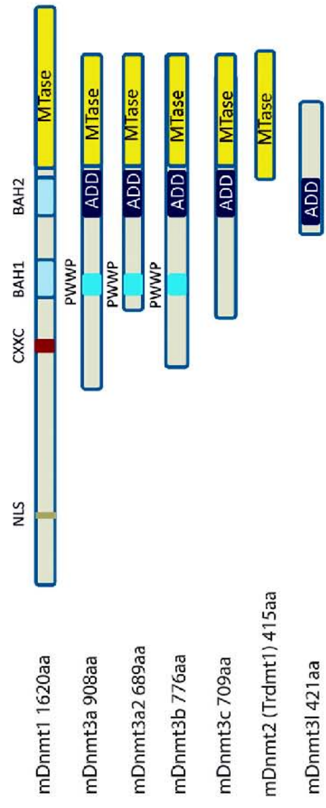
2.2 DNA METHYLATION MACHINERY

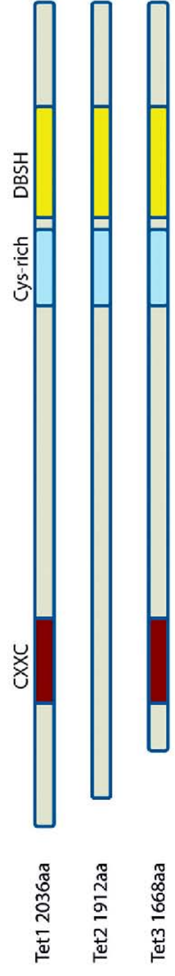
DNA methylation is an epigenetic modification involving the covalent addition of a methyl group from the methyl donor S-adenosyl methionine (SAM) to cytosine by DNA methyltransferases (DNMTs). 5-methylcytosine (5mC) is primarily found in the context of CpG dinucleotides that are symmetrically methylated on double stranded DNA in animal and plant genomes.[25, 26] In 1975 this modification was described for the first time as an epigenetic mark in mammalian model systems, which could be established de novo, or could be stably inherited through somatic cell divisions and potentially maintain gene expression patterns following mitosis.[31–33] The association with gene silencing made in these key papers was further established by studies showing that in vitro methylated DNA is transcriptionally inactive following transfer into Xenopus oocytes or mammalian cell lines, functionally linking DNA methylation to transcriptional repression.[34, 35] This contribution to gene silencing was found to be enacted partly through the formation of transcriptionally inactive chromatin, promoting stable maintenance of gene expression signatures.[36] These findings uphold the view that a fundamental role of DNA methylation involves the formation of a less accessible chromatin structure in concert with the heritable maintenance of this altered state. However, the relationship between DNA methylation and gene silencing is proving to be more complex and sometimes indirect.[37, 38]
The conversion of cytosine to 5mC results in the generation of a relatively stable mark that is present at similar levels across most adult tissues. This is in contrast to 5-hydroxymethylcytosine (5hmC), a derivative of 5mC with potential roles in DNA demethylation and regulation of gene expression.[39] However, immunohistochemical studies with 5mC specific antibodies indicated that in early mouse development and during germline specification, DNA methylation can be dynamically programmed.[40–42] This has been confirmed by high resolution sequencing analyses, which also found distinct DNA methylation patterns among different tissue types, between individuals, during ageing and disease states.[6, 43–51] Differential DNA methylation at enhancer elements, with concurrent changes in histone modifications and transcription factor binding, can occur at the level of cells, tissues and individuals.[43, 52, 53] In addition, altered methylation profiles at regulatory elements are a feature of many diverse cancer types.[54, 55] The potential molecular mechanisms underlying this dynamic regulation have been further elucidated in recent years with the discovery of a potential demethylation pathway involving 5mC derivatives that are generated by methylcytosine dioxygenases.[56–58]
The maintenance methyltransferase DNMT1 and its cofactors (e.g., UHRF1, PCNA) have been classically considered

responsible for the perpetuation of DNA methylation during cell divisions, whereas de novo DNA methylation is initially established in mouse development by a combination of the denovo methyltransferases, DNMT3A and DNMT3B, acting in concert with their non-catalytic cofactor DNMT3L (Fig. 2.1A).[59–62] DNMT3L itself is essential during germ cell development to ensure that endogenous retrotransposons are inactivated.[63] Recently a new isoform, DNMT3C, was identified in mice that is essential for retrotransposon methylation and repression in the mouse male germline.[64] Its inactivation affects mouse fertility, but it is not normally expressed in ES cells.[65] Somatic patterns of DNA methylation participate at multiple levels (locus specific, genome wide and indirectly) and in many developmental processes, including X-chromosome inactivation, genomic imprinting, retrotransposon silencing, gene repression and genome stability.[37, 47, 66-69] Both DNA strands are symmetrically methylated at CpGs during replication, as hemi-methylated DNA is a potent substrate for the maintenance DNA methyltransferase, DNMT1, responsible for perpetuating the parental pattern.[70] Genome-wide profiling demonstrates that methylated CpG (MeCpGs) are pervasive throughout mammalian genomes, with the exception of discrete GC rich non methylated CpG“islands” (CGI). CGIs are stretches of DNA approximately 1000bp in length that have an elevated G +C content, little CpG depletion, and are normally free from DNA methylation.[71–73] Up to 60% of human genes are associated with CGIs and predictions suggest that the human genome contains more than 25,000 CGIs with a slightly lower number (23,000) in the mouse genome. Most CGIs are associated with the 5’ ends of genes, including the promoters of housekeeping genes, as well as a proportion of tissue-restricted genes and developmental regulator genes (Fig.2.2).[72,74, 75] These regulatory landmarks are also associated with initiation sites of DNA replication.[76, 77] The protection mechanisms that prevents de novo methylation of promoter associated CGIs maybe DNA-encoded and evolutionarily conserved. The binding of transcription factors may render CGIs resistant to the DNMTs either sterically or by promoting a chromatin environment that is refractory to DNA modification.[78] For example, unlike active X counterparts, promoter CGIs located on the inactive X chromosome in females readily acquire DNA methylation.[79]

The occurrence of CGI promoter methylation can impair transcription factor binding and result in stable silencing of gene expression.[72] Profiling experiments in mice indicate that a high proportion of autosomal methylated CGI genes correspond to genes normally expressed in early development and the germline, where they can have posttranscriptional roles in suppressing transposon activity during periods of DNA hypomethylation.[47, 66, 67, 80] The phenomenon of aberrant promoter CGI hypermethylation is recognized as a hallmark of cancer, which in some cases, but not all, may lead to transcriptional inactivation of potential tumor suppressor genes in response to signaling cues.[54] How DNA methylation deposition at regulatory sequences interferes with transcription networks is complex but different pathways have been proposed. One pathway involves the recruitment of methyl CpG-binding proteins (MeCPs) that can target chromatin modifying activities that promote gene silencing.[37, 81] However, inactivation of various MeCPs (including in a triple combination) has not been linked with global changes in gene expression or with regulation of well-characterized methylation-dependent genes such as Dazl and Tex19.1[67, 82]. A more plausible model is the direct interference of transcription factor activator binding by 5mC modified bases. When tested by high-throughput screening, the binding of 1453 factors, out of approximately 1500 tested, was affected by methylated

s





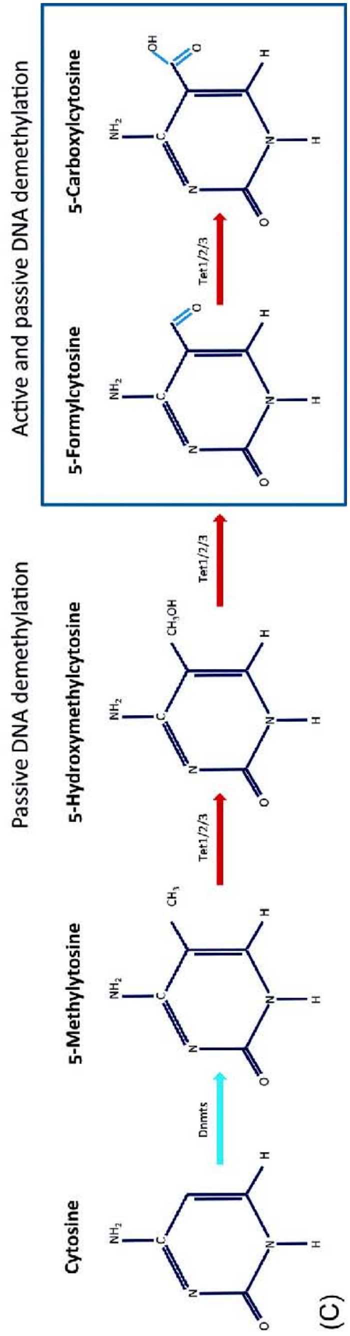


FIG. 2.1

(A) Domain structure of DNA methyltransferases. The C-terminal catalytic methyltransferase domains (MTASE) are shown (Yellow). The NLS (nuclear localization signal), the CxxC domain (which interacts with unmethylated CpGs) and two Bromo-Adjacent Homology (BAH) domains are shown for DNMT1. The DNMT3 family consists of DNMT3A, DNMT3A2, DNMT3B, DNMT3C and DNMT3L. The N-terminal regulatory domains of DNMT3A, DNMT3A2 and 3B contain the Pro-Trp-Trp-Pro (PWWP) domain important for DNA binding as well as the ADD, a PHD-like zinc finger domain important in targeting. DNMT3C is germline specific and does not contain a PWWP domain, but the catalytic methyltransferase domain is conserved. DNMT2, tRNA methyltransferase (Trdmt1) lacks regulatory domains and does not significantly methylate mammalian DNA in vivo. The cofactor DNMT3L lacks the PWWP domain as well as an active catalytic domain. (B) Schematic representation of TET proteins. All TET proteins contain at the C-terminal a DSBH domain consisting of eight β-strands forming two four-stranded antiparallel β-sheets typical of the 2-oxoglutarate (2OG)-and Fe(II)-dependent oxygenase superfamily. A cysteine-rich region (Cys-rich region) is also present upstream of the DBSH domain in all TET proteins. Together, the DSBH and Cys-rich domains represent the catalytic domain of TET proteins. A CXXC zinc finger domain is present at the N-terminal of both TET1 and TET3 proteins whereas TET2 lacks this potential DNA targeting domain.C. Cytosine (C) is converted to 5-methylcytosine (5-mC) by DNMT proteins. 5-mC is converted to 5hydroxymethylcytosine (5-hmC) by TET proteins, which can be relatively stable in the genome. 5hmC can be reiteratively converted to 5-formylcytosine (5-fC) and 5-carboxycytosine (5-caC) by TET proteins. 5-hmC, 5-fC and 5-caC are hypothesised to function in replication-dependent passive demethylation pathways, whereas 5fc and 5caC may participate in active demethylation pathways that incorporate DNA repair pathways to replace modified bases with cytosine (C).

CpGs in their cognate binding sites.[83] However, 47 factors could also bind to methylated target sequences and some TFs exhibit specific binding activity to methylated and unmethylated DNA motifs of distinct sequences. In addition, it is possible that depending on the cellular context, inhibitory effects of DNA methylation can be overridden without demethylation of target promoters suggesting that certain activating factors can overcome the inhibitory effects of DNA methylation.[84, 85] This could shift primary control of transcriptional states towards gene-regulatory networks (GRNs), with epigenetic mechanisms acting downstream to reinforce initial GRN-mediated decisions.[5, 86] A third possible mechanism is that DNA methylation may influence nucleosome positioning at promoter regions to affect transcription initiation.[87, 88]

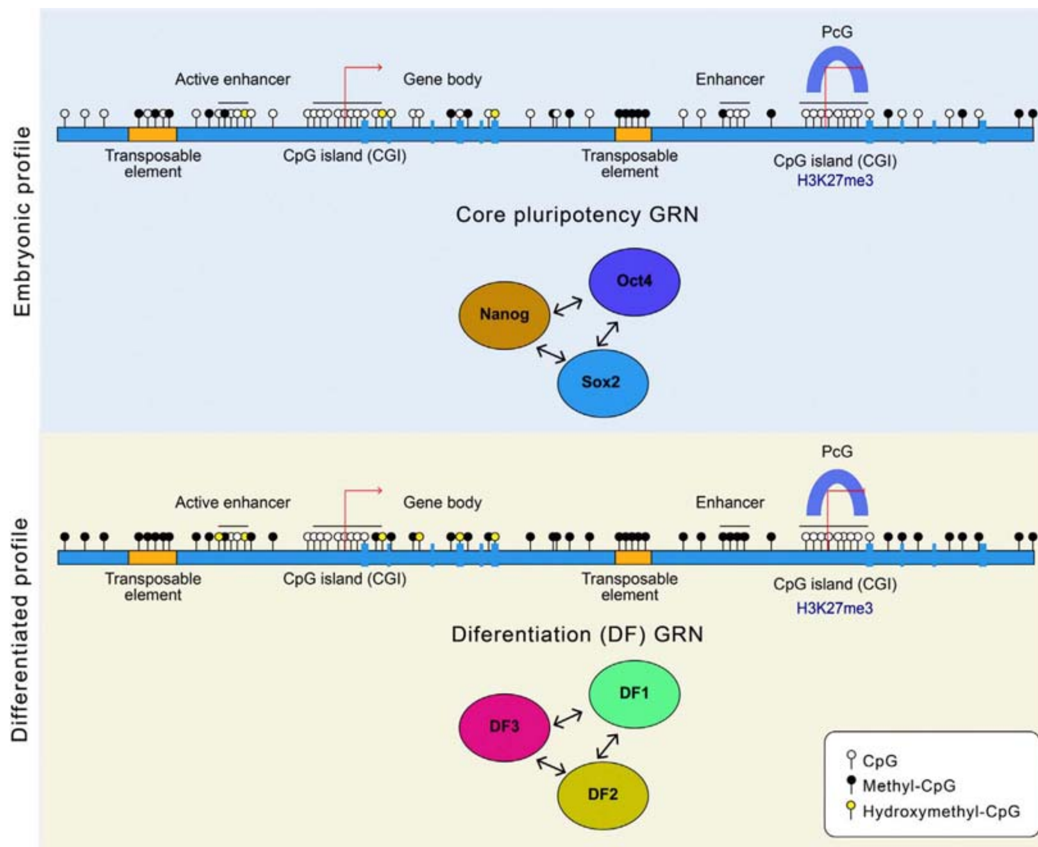


FIG. 2.2

Depiction of a generalised DNA methylation landscape in early embryonic and differentiated cells. In embryonic cells, the pluripotency gene regulation network (GRN) drives self-renewal and development with a reduced to absent dependency on DNA methylation. Active and inactive CpG island genes can be distinguished by the absence or presence of the Polycomb Regulated Complex (PRC) 2-directed trimethylation at lysine 27 on histone H3 (H3K27me3). In differentiated cells, DNA methylation is necessary to repress transposon activation, reinforce tissues states and direct differentiation factors (DFs) forming unique gene regulatory networks (GRNs). In differentiated cells, 5-hydoxymethylcytosine can mark active enhancers and is enriched at the gene bodies of expressed genes.

It should be noted that DNA methylation profiling has been used extensively in reprogramming and disease studies to chart changes in cell state, and can additionally be linked to physiological processes, such as ageing and metabolism.[20, 44, 51, 53, 89–95] Differentially methylated promoters associated with gene inactivation in different tissue types have been identified, but these appear to correspond to a remarkably small number of genes that are normally expressed in the germline.[66, 67] On the other hand, most silent nonmethylated CGI genes are associated with a histone-repressive modification profile that is dependent on Polycomb Repressive Complexes 1 and 2, which are responsible for adding a ubiquityl moiety to histone H2A at Lys119 (H2AK119ub1; PRC1) and the addition of one to three methyl groups to histone H3 at Lys27 leading to H3K27me3 (PRC2), respectively, at silenced promoters.[71, 96]

2.3 DNA DEMETHYLATION

DNA methylation had long been considered to be a relatively stable epigenetic modification until evidence demonstrated that this modification may be more dynamic and complex than previously thought. In theory, loss of DNA methylation, or DNA demethylation can occur through passive or active processes. Two basic mechanisms leading to DNA demethylation have been proposed: (A) a passive mechanism through which remethylation of hemi-methylated substrates during DNA replication is prevented, thus leading to progressive loss of 5mC in concert with cellular proliferation, and (B) active processes that remove the modification or modified bases from DNA without necessarily requiring DNA replication.[97–102]

The discovery of abundant amounts of 5hmC in mammalian DNA together with the methylcytosine oxygenase enzymes responsible for its generation had a dramatic impact on the DNA methylation field—it required a revision of many cherished hypotheses and provided a multitude of new possibilities for the impact of DNA modifications on genome regulation, organization and integrity.[56, 57, 97, 103] By using thin layer chromatography (TLC), high pressure liquid chromatography (HPLC) and mass spectrometry (MS), Kriaucionis and Heintz detected the presence of 5hmC in Purkinje cells and granule cells comprising approximately 0.6% and 0.2% of total nucleotides, respectively.[56] Coinciding with this observation, Rao and coworkers discovered a key player responsible for active DNA demethylation, the Ten-Eleven-Translocation (TET)gene, Tet1. Theyfound that TET1 can catalyze the conversion of 5mC to 5hmC in a 2-oxoglutarate (2OG) and Fe(II)-dependent manner.[57] Soon after, two other TET family members, TET2 and TET3, were also identified as being able to catalyze a similar reaction (Fig. 2.1B).[104] 5hmC can be generated from 5mC and subsequently further oxidized to 5-formylcytosine (fC) and 5-carboxylcytosine (caC) through the action of the TET enzymes (Fig. 2.1C).[58, 104] This pathway is implicated in enzymatic DNA demethylation processes during development and in disease states including response to environmental exposure.[58, 105–108] TET family proteins (TET1, TET2 and TET3) share a conserved C-terminal catalytic domain of Cys-rich and DSBH regions with homology to the dioxygenase superfamily.[58] Their potential redundancy adds an additional degree of complexity to unravelling the role of TET proteins and 5hmC in epigenetic regulation during development. Several possible DNA replication-independent active DNA demethylation pathways were inferred from in vitro studies; TET-catalysed 5hmC conversion into 5fC and 5caC can be efficiently excised from DNA by the DNA repair enzyme thymine-DNA glycosylase (TDG).[109] Subsequent repair of the resulting abasic site via base excision repair (BER) can generate an unmodified cytosine residue, thus completing the process of DNA demethylation.[109, 110] TDG possesses robust excision activity towards 5fC-and 5caC-containing DNA, but not 5hmC-and 5mCcontaining DNA.[109, 111]

Another possibility is that 5fC and 5caC can be directly deformylated or decarboxylated by a putative DNA deformylase or DNA decarboxylase enzyme, which eventually leads to the restoration of unmodified cytosine.[112] Intriguingly, 5caC decarboxylase activity has been detected in mouse embryonic stem cell (mESC) lysates, but no putative decarboxylase has yet been identified.[113] The first in vitro evidence for direct enzymatic decarboxylation of 5caC to unmodified cytosine demonstrated that IDCases (isoorotate decarboxylases) possess decarboxylase function albeit with weak activity.[114] Another study found that C5-MTases (cytosine-5-S-adenosylmethionine-dependent DNA

methyltransferases) can convert 5caC directly to unmodified cytosine in vitro but are lacking this ability toward 5fC.[115] The existence of 5fC DNA deformylase and 5caC DNA decarboxylase is uncertain. Alternatively, 5mC or 5hmC can be deaminated by cytidine deaminases, leading to replacement of the residue with an unmodified cytosine residue and resulting in active DNA demethylation. The activation-induced cytidine deaminase(AID)/apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) family of cytidine deaminases are well characterized for their ability to deaminate 5mC or 5hmC to generate thymidine (T) or 5-hydroxymethyluracil(5hmU), respectively. As these are present in mismatched T:G and 5hmU:G base pairs, they have been suggested to be excised by TDG or single-strand-selective monofunctional uracil DNAglycosylase1(SMUG1), another DNA glycosylase, leaving intact abasic sites in DNA. The resulting abasic sites are repaired by BER to restore unmodified cytosines.[116]
On the other hand, a recent study presented in vitro evidence that the mammalian de novo DNA methyltransferases DNMT3A and -3B, in addition to their well-known methyltransferase activity, could also function as reduction-oxidation (redox) state-dependent DNA dehydroxymethylases that can convert 5hmC directly to unmodified cytosine, although the exact pathways are yet to be better defined and the physiological relevance has yet to be demonstrated.[117, 118]
Mapping of the relatively stable 5hmC mark indicated it could have additional functions in active transcription and genome stability.[119–122] Delineating the precise roles of TET proteins in development is still ongoing, for example Drosophila melanogaster has a reduced repertoire of DNA methyltransferases (only DNMT2)and barely detectable amounts of 5mC present in its genome, yet it contains a highly conserved TET homologue (dTet) that regulates 5hmC levels in mRNA.[123] The dTet homologue is essential for fly development, perhaps by mediating mRNA translation efficiency of selected transcripts in different tissue contexts.[124] In an ES cell model, it has been recently shown that TET2 catalyses the 5hmC modification of MERVL retrotransposon-derived RNAs resulting in their destabilization; suggesting that potentially transcriptionally active endogenous retrovirus RNAs can be modulated by resident TET enzymes.[125]

2.4 DNA METHYLATION IN EARLY DEVELOPMENT

In early embryo development and during primordial germ cell (PGC) establishment, DNA methylation undergoes extensive reprogramming. Blastocyst epigenetic programming is under the control of signal induction pathways; this can be mimicked to a certain extent in mouse ES cell models grown in precise media conditions (Fig. 2.3) [47,49, 50,93, 126, 127].

DNA methylation reprogramming also occurs in somatic cell contexts, which are linked to cellula rdifferentiation and cellular transformation processes.[106,120,128] Developmental DNA reprogramming depends on a combination of DNA demethylation and remethylation pathways.[47, 100, 126, 129, 130] TET enzyme conversion of 5-methyl cytosine may be one pathway of demethylation and its iron and α-ketoglutarate (αKG) dependent mechanism can be enhanced by the

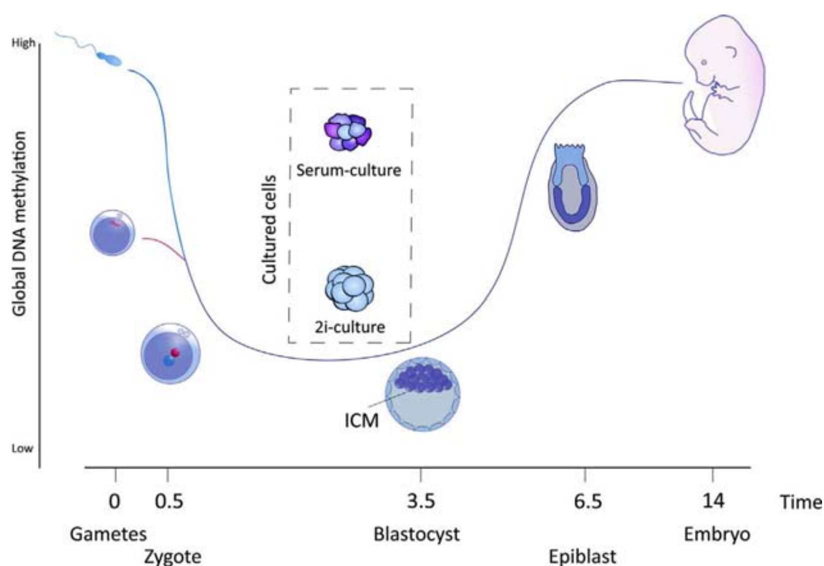


FIG. 2.3

Depiction of dynamic DNA methylation reprogramming during early mouse development, and ES cell culture models for signal-induced DNA methylation changes between metastable primed ES cells grown in serum/LIF and ground state ES cells grown in "2i" conditions, which are relatively hypomethylated.

addition of vitamin C.[57,58,92,131] TET-mediated oxidation of 5mC can instigate both active and passive demethylation pathways. As oxidative derivatives are not well recognized by the methylation maintenance machinery, this may cause passive demethylation through DNMT1 enzyme inhibition after DNA replication.[132] The presence of 5caC, or 5fC can also potentially trigger active erasure by the action of DNA glycosylases such as TDG, followed by BER.[29, 58, 109, 133] However, the contribution of TET-mediated demethylation to DNA methylation reprogramming pathways is context dependent. In mammals, active demethylation of sperm pronuclear DNA shortly after fertilization is thought to be important for subsequent zygotic gene activation and embryonic development.[40, 134] The detection of 5hmC and its derivatives in zygotes suggested they may contribute to this process and TET3 was originally thought to be a possible mediator of zygotic demethylation.[46, 99, 135–137] However, subsequent investigation demonstrated that the disappearance of 5mC and the appearance of 5hmC in the paternal genome may not be linked.[100]
It is believed that this epigenetic reprogramming is an essential process that helps restore the totipotency in both zygotes and PGCs cells, thus permitting the cells to preserve their developmental potency and allowing the subsequent capability to differentiate into different cell lineages.[138] The first epigenetic reprogramming occurs in the early zygote stage, since both oocyte and spermatozoa are differentiated cells which need to be reprogrammed to restore the totipotency.[139] In mice, the DNA methylation levels in the male pronuclei rapidly diminish within 4 hours after fertilization, before the first cell division. The female pronuclei undergo a passive demethylation process due to the lack of a DNMT1 enzyme in the zygote, resulting in the failure of DNA methylation maintenance from parental strands to daughter strands during DNA replication.[14, 15] In addition, despite the genome-wide erasure of DNA methylation, some regions of the genome are still methylated, including paternal imprinted genes, IAP

retrotransposons and some parts of heterochromatin that are situated around the centromere of the chromosome. These conserved methylated sequences are thought to help with the parental imprinted gene maintenance, IAP transposition repression, and maintaining chromosomal stability.[140]

The discovery of the TET enzyme mechanism of oxidation of 5mC to 5hmC suggested a strong candidate for the active demethylation process in zygotes, as both TETs and 5hmC are found enriched especially in male pronuclei when compared with female pronuclei. In addition, the TET3 enzyme — not TET1, 2 — is highly expressed in oocytes and zygotes with the level later sharply reduced in the 2cell stage and gradually decreased during cleavage stages.[141] 5mC in male pronuclei could therefore be actively demethylated by the TET3 enzyme and converted into 5hmC. An experiment with TET3 deficient zygotes derived from conditional knockout mice indicated that paternal-genome conversion of 5mC into 5hmC fails to occur and the level of 5mC remains persistent.[142] While active demethylation by the TET3 enzyme occurs in mouse male pronuclei, DNA demethylation in female pronuclei remains constant and seems to escape this active demethylation. One potential mechanism for this protection from DNA methylation is through the proposed inhibition of the TET3 enzyme by PGC7 (or Dppa3 or Stella) protein.[143] Stella, which is found in female pronuclei through its association with H3K9me2 reduces the affinity of TET3 to these chromosomes resulting in low enzyme activity. Moreover, it is also believed that the little expression of Stella that is found in the male pronuclei is associated with the protection of the imprinted geneand chromosomal stability.[144] However, the hypothesis of 5mC conversion to 5hmC is not the only model for the DNA demethylation pathway in zygotes. Other reports propose that 5hmC is not merely the intermediate of the demethylation process, but instead has its own independent epigenetic role in development. Petra Hajkova's group revealed that in zygotes, the overall 5hmC expression is delayed and not synchronized with genome-wide 5mC demethylation.[100] The increase in 5hmC levels was only detected in PN4 stage zygotes after the major drop of5mC level in the early PN3 stage, leaving a gap between PN2 to early PN3 stage where no 5mC nor 5hmC level was detected in male pronuclei. In addition, the group proposed that the expression of 5hmC is derived from de novo methylation by DNMT3 enzymes. The hypothesis was investigated by incubating a methyltransferase inhibitor with IVF zygotes, which resulted in a significant decline of 5hmC levels in the male pronuclei, whereas there was no significant difference in 5mC level in control and treated groups. This suggested that 5hmC deposition was not dependent on genome-wide 5mC demethylation.[100] Moreover, Iqbal et al., 2011 proposed that the demethylation of 5mC to 5hmC is not followed by the base excision oxidation pathway, since the 5hmC remains present after mitotic activity and through all cleavage stage embryos.[141, 145] Collectively, these findings suggest that 5hmC is a stable marker and may possibly have its own role during early development.

The methylation level of the cleavage stages embryos is progressively reduced over the course of development until the blastocyst stage. The demethylation process during this stage occurs by passive demethylation. The DNMT1 oocyte variant (DNMT1o), which is inherited from the female and found in oocytes, disappears during cell division in zygotes.[146, 147] This lack of DNMT1 is thought to account for the demethylation during cleavage stages.[148] In contrast, the DNA demethylation in PGCs shows a highly dynamic process. After their migration to the genital ridges, DNA methylation is rapidly erased at E13.5 despite the DNMT1 enzyme being available in the cells. The short time scale of the process in the presence of DNMT1 enzyme was suggested to be due to active demethylation.[41] The demethylation

process in PGCs is thought to be responsible for erasing the parentally inherited imprinted genes, which were protected during the genome-wide demethylation in early development.[149–151] It is also noteworthy that inactivation of TDG preferentially affects late stage embryonic development, which implies that it has no role in paternal demethylation.[152] In early development, the bulk of 5mC, 5hmC, 5fC and 5caC appear to be lost by replication-dependent dilution. However,it is clear in many other developmental and disease contexts that inhibition of TET activities can be associated with 5mC accumulation by loss of either passive or active demethylation pathways, which may or may not have functional consequences[153–157]. It should also be borne in mind that additional functions for TET enzymes have been identified that have yet to be fully explored.[123, 125, 156, 158–161] Combinatorial analysis has shown that the patterns of DNA modifications vary greatly between tissue and cell types — to the extent that 5hmC profiling itself can be used as an exquisite identifier of cell state or tissue type as it is preferentially present at the gene bodies of expressed genes.[20, 39, 162–164] A consensus view is that 5hmC-modified DNA modification patterns can thus act as identifiers of cell state. Proximal enrichment of 5hmC at enhancers upstream of annotated transcriptional start sites (TSS) suggests a role for these regions in the regulation of gene expression in development.[119, 120] Not surprisingly, the enhancer related marks, H3K4me1/H3K27ac, are associated with 5hmC at upstream regions flanking transcription start sites (TSS).[120, 165]

2.5 HOW NECESSARY IS DNA METHYLATION REPROGRAMMING FOR EARLY DEVELOPMENT?

Early studies demonstrating that the maintenance (DNMT1) and de novo methyltransferases (DNMT3A and DNMT3B) are required for mouse development to differing extents strongly implied that DNA methylation is essential to complete this process; the inactivation of DNMTs could be associated with some misexpression, especially of endogenous retrotransposons in post-blastocyst E9.5 embryos (Table 2.1)[60, 61, 176].

In this capacity it is required primarily for subsequent heritable transcriptional repression of retrotransposons, maintenance of monoallelic expression of imprinted genes and for the maintenance of X chromosome inactivation in female cells.[25] However, charting the dynamics of DNA methylation suggests that mouse blastocysts are significantly hypo-methylated, implying that DNA methylation is also required for postblastocyst embryos as differentiation occurs, perhaps to ensure stable memory of differentiated expression states (Fig.2.4).[15,47,80,177] Pluripotent embryonic stem (ES) cells that are derived from blastocysts adopt an alternative epigenetic constitution in culture, but these primed ES cells can be reprogrammed to a more naı¨ve epigenetic state reminiscent of the hypomethylated inner cell mass (ICM) when cultured with inhibitors of two kinases (Mek and GSK3), known as “2i”.[93, 178, 179] However, it should be noted that ES cells lacking maintenance and de novo methyltransferases (TKOcells) grown in standard medium can self-renew and are indistinguishable from their wild-type counterparts as primed stem cells; it is only upon differentiation that they lose out in competition with their wild-type counterparts.[180] Sakaue and colleagues subsequently showed by nuclear transfer experiments that hypomethylated nuclei from TKO ES cells could support mouse preimplantation development until the blastocyst stage in an equivalent fashion to WT ES nuclei.[166] Subsequently few TKO cells were observed in the embryo proper (which is normally methylated), but they did contribute to normally hypomethylated extra-embryonic tissues. This was validated by in vitro studies in which TKO ES underwent apoptosis during their differentiation into

Table 2.1 Phenotypes of Mutated Epigenetic Modifiers in ES Cells and Mice

Dnmts	**KO ES Cells**	**Homozygous KO Embryo**	**Ref.**
Dnmt1	Self-renew	Growth retarded after E6.5, lethal before E12.5	60
Dnmt3a	Self-renew	Lethal postnatally	61
Dnmt3b	Self-renew	Lethal post E9.5	61
Dnmt3a/Dnmt3b DKO	Self-renew	Lethal before E9.5	61
Dnmt1/Dnmt3a/ Dnmt3b TKO	Self-renew	Nuclear transfer experiments show development supported up to blastocyst stage	166
Dnmt3L	Self-renew	Altered germ cell development	63
Dnmt3C	Not expressed	Impaired male germ cell development	65
Tets			
Tet1	Self- renew	Viable, fertile and grossly normal but some mutant mice are born with a slightly smaller body size	167
Tet2	Self- renew	Predisposition to myeloid leukemia	168
Tet3	Self- renew	Maternal KO causes neonatal sublethality	169
Tet1/Tet2 DKO	Self- renew	Some DKO embryos exhibit mid-gestation abnormalities with perinatal lethality. Viable and overtly normal DKO mice are also observed	154
Tet1/Tet2/Tet3 TKO	Self- renew	TKO embryos have gastrulation phenotypes, including primitive streak patterning defects; they mimic phenotypes in embryos with gain-of-function Nodal signaling	170
Other factors			
Tdg	Self-renew	Tdg-null embryos isolated up to embryonic day E10.5 appear alive and normal, but lethal before E12.5	171
Uhrf1 (NP95)	Self- renew	Homozygous mutants exhibit abnormal development	172, 173
Hells (Lsh)	Self- renew	Growth retardation and premature aging phenotypes in mice with Hells disruption	174, 175

epiblast lineages, but not extraembryonic lineages.[166] These observations suggest that extraembryonic lineage cells, like ES cells and embryos during preimplantation development, can survive and proliferate in the absence of DNA methyltransferases and that the impact of global hypomethylation is cell-type dependent. Mutation of other factors associated with the DNA methylation/demethylation pathways tends not to affect the capacity of ES cells to self-renew and results in later stage developmental defects in mice (Table 2.1). Remarkably, hypomethylated (TKO) trophoblast stem (TS) cells can misexpress genes normally repressed by DNA methylation, but this does not compromise their cellular identity.[166] The lack of dependence of ES cells and early embryos on DNA methylation and other repressive epigenetic mechanisms is an example of what has been described as an “epigenetic paradox”, in which early cell fate is determined primarily by pluripotency networks. Epigenetic mechanisms only Come on line and become necessary

during differentiation, when specialized cellular functions are incompatible with pluripotent states (Figs.2.3 and 2.4) [181]. Lsh/Hells is a putative helicase required to maintain methylation and silencing at interspersed endogenous retro viruses (ERVs).[182] Lsh-/- mutants die either at birth or later from a premature aging phenotype associated with cellular senescence; significantly, their genomes are globally DNA hypomethylated, indicating that significant global loss of DNA methylation is not a strict impediment to postblastocyst development even though many ERVs are misexpressed[174, 183, 184]. Epigenetic regulation thus serves to reinforce cell fate decisions and maintain the genome integrity of committed cells over time; subsequent ongoing environmental exposure and age-associated changes can lead to cellular breakdown and malfunctioning homeostasis resulting in pathophysiologic states that are incompatible with life[51, 163].

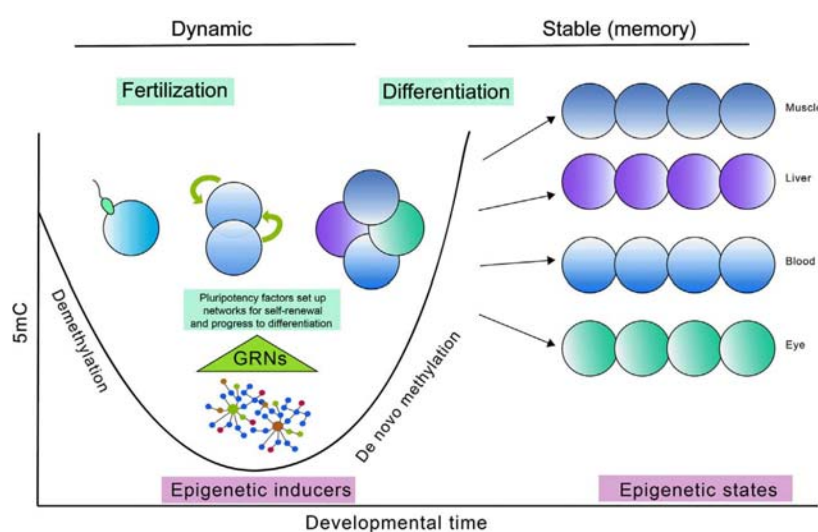


FIG. 2.4

General model illustrating that dynamic pluripotent states in low DNA methylation conditions in early development direct the formation of gene regulatory networks (GRN) that will specify and maintain stable differentiated states, which are reinforced by epigenetic pathways so that stable cell memory or identity is maintained during cell division.

2.6 DNA METHYLATION TRAJECTORIES DURING REPROGRAMMING OF SOMATIC CELLS

As differentiation proceeds, epigenetic barriers are set up to preserve tissue-specific demarcation states in somatic cells. Reversion to earlier pluripotency or trans-differentiation is incompatible with organ specific function but it is now recognized that tissue regeneration via stem cellactivation can be forced under extreme physiological stress such as liver injury.[185] A combination of diet-induced liver injury along with inhibition of hepatocyte proliferation can result in significant regeneration of functional hepatocytes from biliary cells in a mouse model.[186] In addition, it is reported that laterally confined growth of fibroblasts on micropatterned substrates can induce nuclear reprogramming to stem-like cells with high efficiency in the absence of any exogenous reprogramming factors.[187] This process may replicate tissue niches that maintain stem cells for potential utilization in various organs. Expression analysis confirmed there

was a systematic progression from a mesenchymal to a stem cell-like transcriptome during this progression to a stem-like state over ten days.[187] In mammals, the first demonstration of reversion of a somatic cell to pluripotency was via somatic cell nuclear transfer experiments in sheep, which could give rise to cloned lambs derived from the nuclei of differentiated foetal and adult cells.[18, 188] Subsequent immunostaining experiments suggested that sheep fibroblast somatic nuclei were partially demethylated upon transfer to recipient sheep oocytes and underwent a stepwise passive loss of DNA methylation during early development that is not as extreme as observed in early mouse embryogenesis.[189, 190] Between the eight-celland blastocyst stages, DNA methylation levels in sheep nuclear transfer embryos are comparable with those derived in vivo, but the distribution of methylated DNA is abnormal in a high proportion, probably as a consequence of failed nuclear reorganization of somatic heterochromatin.[189] Both remodeling of DNA and epigenetic reprogramming appear therefore critical for development of both fertilized and nuclear transfer embryos. Cloned mice could also be achieved by somatic cell nuclear transfer with success rates partially determined by the nuclear donor tissue type.[191, 192] Successful reprogramming to generate live animals correlated with activation of pluripotency networks and normal DNA methylation dynamics.[191, 193] The development of in vitro reprogramming methodologies to transform somatic cells to a state resembling pluripotency revolutionized the regenerative medicine field and prompted the development of direct somatic cell state conversion methodologies to other differentiated cell types.[19, 194, 195]

Takahashi and Yamanaka first demonstrated that induced pluripotent stem cells (iPSCs) could be generated over time via the ectopic expression of only four transcription factors (TFs):OCT4, SOX2, KLF4 and c-MYC..[19, 195] This so-called OSKM cocktail can convert embryonic and adult fibroblasts to iPSCs that can subsequently support mouse development. Many laboratories used the original protocol as the basis for generating iPSCs from diverse species and cell types and subsequently used these as models for early development and in vitro differentiation.[194–196] Essentially these experiments illustrated that epigenetic barriers are removable, as reprogramming by TFs converts somatic cells with a distinct somatic epigenetic profile to one resembling a pluripotent state in iPSCs.[20, 164, 197]

In standard OSKM conversion protocols, iPSC-like colonies can appear 7–10 days after OSKM activation and a number of studies have generated molecular profiles (expression and epigenetic states) of transition states from the originating somatic cell to the iPSC cells that can support mouse development. [21, 197–199] These studies have identified unique trajectories that facilitate conversion of somatic cells to iPSCs.[91, 198, 200–203] For example global levels of 5-methylcytosine fluctuate during conversion, which would be predicted to direct effects on chromatin organization and enhancer usage (Fig. 2.5).[91, 203, 204] This may occur independently of transcriptional activation of pluripotency markers and specific changes at regulatory regions (Fig.2.5).[91] Expression analysis suggests there are surgesin the upregulation of pluripotency genes, and transient bursts of upregulation of genes associated with alternative tissue types.[198] This implies that OSKM reprogramming may take advantage of several energetically or biologically favorable routes to pluripotency that do not necessarily represent a simple reversal of developmental processes, which by their nature are species specific. Not surprisingly, early transcriptional events orchestrated by the OSKM network correlate with a general relaxationof somatic chromatin states.[204] This probably facilitates passage to a combined chromatin environment and gene

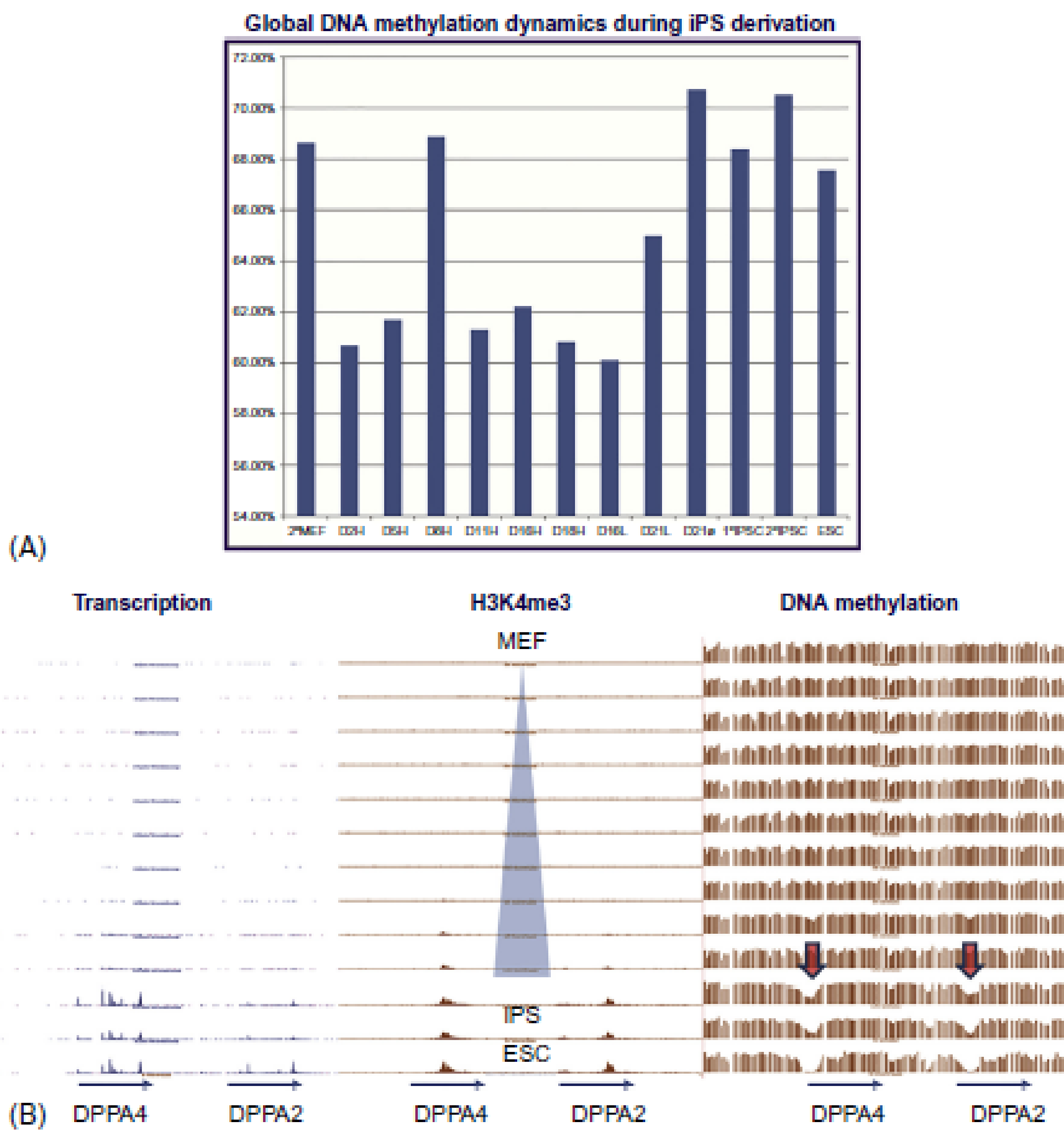


FIG. 2.5

(A) The global changes in 5mC levels are plotted during secondary reprogramming of mouse embryonic fibroblast to induced pluripotent stem cells (IPSCs); various transition states were captured in the study by Nagy and colleagues[91]. (B) Transcriptional activation of the developmental pluripotency associated factors DPPA4 and DPPA2 occurs late in OSKM reprogramming of fibroblasts and is coincident with the occurrence of the activating mark, H3K4me3, and hypomethylation at their promoters (red arrows). The order of the samples is the same as the 5mC analysis in (A). The genome browser data is derived from the Project Grandiose portal at http://www. stemformatics.org.

regulatory network that opens up a route to stem cell fates. It would thus be not surprising if epigenetic signatures could potentially impact on reprogramming. Loss of function studies suggested that conditional deletion of the de novo methyltransferase's, Dnmt3a and Dnmt3b,in fibroblasts did not impact on iPSC generation, however,in this study the effects on global methylation levels and chromatin organization were not examined.[205] In contrast, ablation of the

methylcytsosine dioxygenases Tet1-3,in embryonic fibroblasts inhibits their reprogramming to iPSCs because of a block in the mesenchymal to-epithelial transition (MET) step.[29] In embryonic fibroblasts (MEFS), TET-dependent reactivation of a set of miRNAs (miR-200 family) is essential for reprogramming; in contrast, neonatal keratinocytes can be reprogrammed efficiently upon conditional deletion of the only functional Tet3 allele, while neural progenitor cells (NPC) lacking TET enzymes can also be efficiently reprogrammed.[29] This suggests that tissue-specific epigenetic profiles influence reprogramming trajectories. In addition, Tet1 is highly expressed in ESCs and can replace multiple transcription factors during somatic cell reprogramming to generate high-quality mouse iPSCs in concert with the OCT4TF.[206, 207] It will be interesting to know in future if hypomethylated embryonic fibroblasts have altered reprogramming trajectories to an IPSC state.

2.7 6-METHYLDEOXYADENOSINE IN MAMMALIAN DNA?

A potential new addition to the DNA modification profile of mammals is 6-methyldeoxyadenosine (6mA), which has been intensively studied in bacteria and was more recently characterized in invertebrates. In bacteria, 6mA was identified as a component of restriction-modification (R-M) systems that methylate target recognition sites to make these refractory to digestion by restriction enzymes.[23] This is part of a bacterial host-defense mechanism against infection/invasion by non-modified phages and plasmids. In 2015 6mA was identified at various levels of abundance in Chlamydomonas, C. elegans, and Drosophila, with the suggestion that it has a gene regulatory function.[208–211] Despite ongoing research efforts to detect its potential presence and function in mammals and other vertebrates, the existence of 6mA in mammals remains controversial.[212–214] A recent ultrasensitive LC-MS2-based approach, using an isotopically labelled m6dA standard, was unable to confirm the presence of m6dA in vertebrates.[215] This impacts on the potential for detecting 6mA by sequencing methodologies, including real-time sequencing where the less abundant a modification the greater the coverage is needed to detect its signal above background, especially when using target sequence enrichment as part of the analysis pipeline.[216] DNA immunoprecipitation with modification-specific antibodies followed by sequencing (DIP-seq) was recently shown to have an intrinsic background due to the affinity of IgG for short unmodified DNA repeats, which becomes even more problematic if the modification being assayed has very low abundance.[217] Genome-wide 6mA DIP-seq in mice can generate profiles nearly identical to those observed with random IgG antibodies on DNA samples lacking the target modifications.[217] Matched input and DIP-IgG sequencing controls can overcome this background effect and enable improved data interpretation, especially if complementary non-antibody-based techniques are also used to validate DIP-based findings. Further investigation is needed of the relevance of 6mA in mammalian DNA. In contrast, the contribution of 6-methyladenosine to RNA biology (stability, splicing and targeting) seems robust.[218]

2.8 FUTURE PERSPECTIVES

Future studies should concentrate on dissecting the cause-consequence relationships of epigenetic systems in development, and the role of epigenetic plasticity in driving cellular transitions and in maintaining cell identity. Finally, the availability of new gene-editing tools and single-cell sequencing studies may enable exquisite investigation of the functional importance of developmental epigenetic profiles, especially when allied with novel in vitro proxies for in vivo development.[219–223]

ACKNOWLEDGMENTS

Work in RRM's lab is supported by the Medical Research Council (ref: MC_PC_U127574433 and MC_UU_00007/17). KM and AR were supported by MRC studentships. Research in SP's lab is supported by the BBSRC and BHF; NAAH was supported by a MARA scholarship and PP by a Thai Government scholarship.

CHAPTER CITATION

Pennings, S., Revuelta, A., McLaughlin, K.A., Abd Hadi, N.A., Petchreing, P., Ottaviano, R., Meehan, R.R. Dynamics and mechanisms of DNA methylation reprogramming. In 'Epigenetics and Regeneration', D. Palacios, Ed. (2019) Vol 11, pp19-45; Volume 11 of 'Translational Epigenetics', T. O. Tollefsbol, Series Editor, Academic Press (Elsevier). https://doi.org/10.1016/B978-0-12-814879-2.00002-9

REFERENCES

1. Nicoglou A, Merlin F. Epigenetics: a way to bridge the gap between biological fields. Stud Hist Phil Biol Biomed Sci. 2017;66:73–82.
2. Waddington CH. The Strategy of the Genes: A Discussion of Some Aspects of Theoretical Biology. London: Ruskin House/George Allen and Unwin Ltd; 1957.
3. Waddington CH. The epigenotype. 1942. Int J Epidemiol. 2012;41(1):10–13.
4. Ptashne M. Epigenetics: core misconcept. Proc Natl Acad Sci U S A. 2013;110(18):7101–7103.
5. Peter IS, Davidson EH. Implications of developmental gene regulatory networks inside and outside developmental biology. Curr Top Dev Biol. 2016;117:237–251.
6. Lander ES. Initial impact of the sequencing of the human genome. Nature. 2011;470(7333):187–197.
7. Provart NJ, Alonso J, Assmann SM, Bergmann D, Brady SM, Brkljacic J, et al. 50 years of Arabidopsis research: highlights and future directions. New Phytol. 2016;209(3):921–944.
8. Wang G, Kohler C. Epigenetic processes in flowering plant reproduction. J Exp Bot. 2017;68(4):797–807.
9. Barton SC, Surani MA, Norris ML. Role of paternal and maternal genomes in mouse development. Nature. 1984;311(5984):374–376.
10. McGrath J, Solter D. Completion of mouse embryogenesis requires both the maternal and paternal genomes. Cell. 1984;37(1):179–183.
11. Barlow DP, Stoger R, Herrmann BG, Saito K, Schweifer N. The mouse insulin-like growth factor type-2 receptor is imprinted and closely linked to the Tme locus. Nature. 1991;349(6304):84–87.
12. Stoger R, Kubicka P, Liu CG, Kafri T, Razin A, Cedar H, et al. Maternal-specific methylation of the imprinted mouse Igf2r locus identifies the expressed locus as carrying the imprinting signal. Cell. 1993;73(1):61–71.
13. Bird A. Perceptions of epigenetics. Nature. 2007;447(7143):396–398.
14. Seisenberger S, Peat JR, Hore TA, Santos F, Dean W, Reik W. Reprogramming DNA methylation in the mammalian life cycle: building and breaking epigenetic barriers. Philos Trans R Soc Lond Ser B Biol Sci. 2013;368(1609):20110330.
15. Seisenberger S, Peat JR, Reik W. Conceptual links between DNA methylation reprogramming in the early embryo and primordial germ cells. Curr Opin Cell Biol. 2013;25(3):281–288.

16. Nissen SB, Perera M, Gonzalez JM, Morgani SM, Jensen MH, Sneppen K, et al. Four simple rules that are sufficient to generate the mammalian blastocyst. PLoS Biol. 2017;15(7):e2000737.
17. WennekampS, MeseckeS, NedelecF, HiiragiT.A self-organization framework for symmetry breakingin the mammalian embryo. Nat Rev Mol Cell Biol. 2013;14(7):452–459.
18. Campbell KH, McWhir J, Ritchie WA, Wilmut I. Sheep cloned by nuclear transfer from a cultured cell line. Nature. 1996;380(6569):64–66.
19. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006;126(4):663–676.
20. Wang T, Wu H, Li Y, Szulwach KE, Lin L, Li X, et al. Subtelomeric hotspots of aberrant 5-hydroxymethylcytosine-mediated epigenetic modifications during reprogramming to pluripotency. Nat Cell Biol. 2013; 15(6):700–711.
21. Apostolou E, Hochedlinger K. Chromatin dynamics during cellular reprogramming. Nature. 2013;502 (7472):462–471.
22. Zhong X. Comparative epigenomics: a powerful tool to understand the evolution of DNA methylation. New Phytol. 2016;210(1):76–80.
23. LoenenWA,DrydenDT,RaleighEA,WilsonGG,MurrayNE. HighlightsoftheDNA cutters:ashorthistory of the restriction enzymes. Nucleic Acids Res. 2014;42(1):3–19.
24. Liu H, Li S, Wang X, Zhu J, Wei Y, Wang Y, et al. DNA methylation dynamics: identification and functional annotation. Brief Funct Genomics. 2016;15(6):470–484.
25. Edwards JR, Yarychkivska O, Boulard M, Bestor TH. DNA methylation and DNA methyltransferases. Epigenetics Chromatin. 2017;10:23.
26. Goll MG, Bestor TH. Eukaryotic cytosine methyltransferases. Annu Rev Biochem. 2005;74:481–514.
27. Khorasanizadeh S. The nucleosome: from genomic organization to genomic regulation. Cell. 2004;116 (2):259–272.
28. Deichmann U. Epigenetics: the origins and evolution of a fashionable topic. Dev Biol. 2016;416(1):249–254.
29. Hu X, Zhang L, Mao SQ, Li Z, Chen J, Zhang RR, et al. Tet and TDG mediate DNA demethylation essential for mesenchymal-to-epithelial transition in somatic cell reprogramming. Cell Stem Cell. 2014;14 (4):512–522.
30. Cruz-Santos MC, Aragon-Raygoza A, Espinal-Centeno A, Arteaga-Vazquez M, Cruz-Hernandez A, Bako L, et al. The role of microRNAs in animal cell reprogramming. Stem Cells Dev. 2016;25(14):1035–1049.
31. Holliday R, Pugh JE. DNA modification mechanisms and gene activity during development. Science. 1975;187(4173):226–232.
32. Riggs AD. X inactivation, differentiation, and DNA methylation. Cytogenet Cell Genet. 1975;14(1):9–25.
33. Riggs AD, Pfeifer GP. X-chromosome inactivation and cell memory. Trends Genet. 1992;8(5):169–174.
34. Stein R, Razin A, Cedar H. In vitro methylation of the hamster adenine phosphoribosyltransferase gene inhibits its expression in mouse L cells. Proc Natl Acad Sci U S A. 1982;79(11):3418–3422.
35. Vardimon L, Kressmann A, Cedar H, Maechler M, Doerfler W. Expression of a cloned adenovirus gene is inhibited by in vitro methylation. Proc Natl Acad Sci U S A. 1982;79(4):1073–1077.
36. Wolffe AP, Matzke MA. Epigenetics: regulation through repression. Science. 1999;286(5439):481–486.
37. Reddington JP, Pennings S, Meehan RR. Non-canonical functions of the DNA methylome in gene regulation. Biochem J. 2013;451(1):13–23.
38. Reddington JP, Sproul D, Meehan RR. DNA methylation reprogramming in cancer: does it act by re-configuring the binding landscape of Polycomb repressive complexes? BioEssays. 2014;36(2):134–140.
39. Globisch D, Munzel M, Muller M, Michalakis S, Wagner M, Koch S, et al. Tissue distribution of 5-hydroxymethylcytosine and search for active demethylation intermediates. PLoS ONE. 2010;5(12):e15367.
40. Mayer W, Niveleau A, Walter J, Fundele R, Haaf T. Demethylation of the zygotic paternal genome. Nature. 2000;403(6769):501–502.
41. Hajkova P, Erhardt S, Lane N, Haaf T, El-Maarri O, Reik W, et al. Epigenetic reprogramming in mouse primordial germ cells. Mech Dev. 2002;117(1–2):15–23.

42. Rougier N, Bourc'his D, Gomes DM, Niveleau A, Plachot M, Paldi A, et al. Chromosome methylation patterns during mammalian preimplantation development. Genes Dev. 1998;12(14):2108–2113.
43. Zhang B, Zhou Y, Lin N, Lowdon RF, Hong C, Nagarajan RP, et al. Functional DNA methylation differences between tissues, cell types, and across individuals discovered using the M&M algorithm. Genome Res. 2013;23(9):1522–1540.
44. Cruickshanks HA, McBryan T, Nelson DM, Vanderkraats ND, Shah PP, van Tuyn J, et al. Senescent cells harbour features of the cancer epigenome. Nat Cell Biol. 2013;15(12):1495–1506.
45. Feinberg AP, Koldobskiy MA, Gondor A. Epigenetic modulators, modifiers and mediators in cancer aetiology and progression. Nat Rev Genet. 2016;17(5):284–299.
46. Peat JR, Dean W, Clark SJ, Krueger F, Smallwood SA, Ficz G, et al. Genome-wide bisulfite sequencing in zygotes identifies demethylation targets and maps the contribution of TET3 oxidation. Cell Rep. 2014; 9(6):1990–2000.
47. Borgel J, Guibert S, Li Y, Chiba H, Schubeler D, Sasaki H, et al. Targets and dynamics of promoter DNA methylation during early mouse development. Nat Genet. 2010;42(12):1093–1100.
48. Meissner A, Mikkelsen TS, Gu H, Wernig M, Hanna J, Sivachenko A, et al. Genome-scale DNA methylation maps of pluripotent and differentiated cells. Nature. 2008;454(7205):766–770.
49. Smith ZD, Chan MM, Humm KC, Karnik R, Mekhoubad S, Regev A, et al. DNA methylation dynamics of the human preimplantation embryo. Nature. 2014;511(7511):611–615.
50. Smith ZD, Chan MM, Mikkelsen TS, Gu H, Gnirke A, Regev A, et al. A unique regulatory phase of DNA methylation in the early mammalian embryo. Nature. 2012;484(7394):339–344.
51. Cole JJ, Robertson NA, Rather MI, Thomson JP, McBryan T, Sproul D, et al. Diverse interventions that extend mouse lifespan suppress shared age-associated epigenetic changes at critical gene regulatory regions. Genome Biol. 2017;18(1):58.
52. Consortium EP. An integrated encyclopedia of DNA elements in the human genome. Nature. 2012; 489(7414):57–74.
53. Kundaje A, Meuleman W, Ernst J, Bilenky M, Yen A, Heravi-Moussavi A, et al. Integrative analysis of 111 reference human epigenomes. Nature. 2015;518(7539):317–330.
54. Sproul D, Meehan RR. Genomic insights into cancer-associated aberrant CpG island hypermethylation. Brief Funct Genomics. 2013;12(3):174–190.
55. Timp W, Feinberg AP. Cancer as a dysregulated epigenome allowing cellular growth advantage at the expense of the host. Nat Rev Cancer. 2013;13(7):497–510.
56. Kriaucionis S, Heintz N. The nuclear DNA base 5-hydroxymethylcytosine is present in Purkinje neurons and the brain. Science. 2009;324(5929):929–930.
57. Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y, et al. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science. 2009;324(5929):930–935.
58. Wu X, Zhang Y. TET-mediated active DNA demethylation: mechanism, function and beyond. Nat Rev Genet. 2017;18(9):517–534.
59. Hata K, Okano M, Lei H, Li E. Dnmt3L cooperates with the Dnmt3 family of de novo DNA methyltransferases to establish maternal imprints in mice. Development. 2002;129(8):1983–1993.
60. Li E, Bestor TH, Jaenisch R. Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. Cell. 1992;69(6):915–926.
61. Okano M, Bell DW, Haber DA, Li E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell. 1999;99(3):247–257.
62. QinW, LeonhardtH, PichlerG. Regulationof DNA methyltransferase1byinteractions and modifications. Nucleus. 2011;2(5):392–402.
63. Bourc'his D, Bestor TH. Meiotic catastrophe and retrotransposon reactivation in male germ cells lacking Dnmt3L. Nature. 2004;431(7004):96–99.

64. JainD,MeydanC,LangeJ,Claeys BouuaertC,LaillerN,MasonCE,etal.rahuisamutantalleleofDnmt3c, encoding a DNA methyltransferase homolog required for meiosis and transposon repression in the mouse male germline. PLoS Genet. 2017;13(8):e1006964.
65. Barau J, Teissandier A, Zamudio N, Roy S, Nalesso V, Herault Y, et al. The DNA methyltransferase DNMT3C protects male germ cells from transposon activity. Science. 2016;354(6314):909–912.
66. Crichton JH, Dunican DS, Maclennan M, Meehan RR, Adams IR. Defending the genome from the enemy within: mechanisms of retrotransposon suppression in the mouse germline. Cell Mol Life Sci. 2014; 71(9):1581–1605.
67. Hackett JA, Reddington JP, Nestor CE, Dunican DS, Branco MR, Reichmann J, et al. Promoter DNA methylation couples genome-defence mechanisms to epigenetic reprogramming in the mouse germline. Development. 2012;139(19):3623–3632.
68. Kelsey G, Feil R. New insights into establishment and maintenance of DNA methylation imprints in mammals. Philos Trans R Soc Lond Ser B Biol Sci. 2013;368(1609):20110336.
69. Reddington JP, Perricone SM, Nestor CE, Reichmann J, Youngson NA, Suzuki M, et al. Redistribution of H3K27me3 upon DNA hypomethylation results in de-repression of Polycomb target genes. Genome Biol. 2013;14(3):2013–2014.
70. Jeltsch A, Jurkowska RZ. Allosteric control of mammalian DNA methyltransferases—a new regulatory paradigm. Nucleic Acids Res. 2016;44(18):8556–8575.
71. Blackledge NP, Rose NR, Klose RJ. Targeting Polycomb systems to regulate gene expression: modifications to a complex story. Nat Rev Mol Cell Biol. 2015;16(11):643–649.
72. Deaton AM, Bird A. CpG islands and the regulation of transcription. Genes Dev. 2011;25(10):1010–1022.
73. BlackledgeNP,KloseR.CpGislandchromatin:a platformforgeneregulation. Epigenetics. 2011;6(2):147–152.
74. Illingworth RS, Gruenewald-Schneider U, Webb S, Kerr AR, James KD, Turner DJ, et al. Orphan CpG islands identify numerous conserved promoters in the mammalian genome. PLoS Genet. 2010;6(9): e1001134.
75. Bird AP. CpG-rich islands and the function of DNA methylation. Nature. 1986;321(6067):209–213.
76. Antequera F, Bird A. CpG islands as genomic footprints of promoters that are associated with replication origins. Curr Biol. 1999;9(17):R661–R667.
77. Delgado S, Gomez M, Bird A, Antequera F. Initiation of DNA replication at CpG islands in mammalian chromosomes. EMBO J. 1998;17(8):2426–2435.
78. Long HK, King HW, Patient RK, Odom DT, Klose RJ. Protection of CpG islands from DNA methylation is DNA-encoded and evolutionarily conserved. Nucleic Acids Res. 2016;44(14):6693–6706.
79. Gendrel AV, Tang YA, Suzuki M, Godwin J, Nesterova TB, Greally JM, et al. Epigenetic functions of smchd1 repress gene clusters on the inactive X chromosome and on autosomes. Mol Cell Biol. 2013;33 (16):3150–3165.
80. Auclair G, Guibert S, Bender A, Weber M. Ontogeny of CpG island methylation and specificity of DNMT3 methyltransferases during embryonic development in the mouse. Genome Biol. 2014;15(12):545.
81. Shimbo T, Wade PA. Proteins that read DNA methylation. Adv Exp Med Biol. 2016;945:303–320.
82. Joulie M, Miotto B, Defossez PA. Mammalian methyl-binding proteins: what might they do? BioEssays. 2010;32(12):1025–1032.
83. Hu S, Wan J, Su Y, Song Q, Zeng Y, Nguyen HN, et al. DNA methylation presents distinct binding sites for human transcription factors. elife. 2013;2:e00726.
84. Li X, Shang E, Dong Q, Li Y, Zhang J, Xu S, et al. Small molecules capable of activating DNA methylation-repressed genes targeted by the p38 mitogen-activated protein kinase pathway. J Biol Chem. 2018;293 (12):7423–7436.
85. Raynal NJ, Si J, Taby RF, Gharibyan V, Ahmed S, Jelinek J, et al. DNA methylation does not stably lock gene expression but instead serves as a molecular mark for gene silencing memory. Cancer Res. 2012;72 (5):1170–1181.

86. Peter IS, Davidson EH. Assessing regulatory information in developmental gene regulatory networks. Proc NatlAcad SciUS A. 2017;114(23):5862–5869.
87. Davey CS, Pennings S, Reilly C, Meehan RR, Allan J. A determining influence for CpG dinucleotides on nucleosome positioning in vitro. Nucleic Acids Res. 2004;32(14):4322–4331.
88. Pennings S, Allan J, Davey CS. DNA methylation, nucleosome formation and positioning. Brief Funct Genomic Proteomic. 2005;3(4):351–361.
89. Cacchiarelli D, Trapnell C, Ziller MJ, Soumillon M, Cesana M, Karnik R, et al. Integrative analyses of human reprogramming reveal dynamic nature of induced pluripotency. Cell. 2015;162(2):412–424.
90. Horvath S, Gurven M, Levine ME, Trumble BC, Kaplan H, Allayee H, et al. An epigenetic clock analysis of race/ethnicity, sex, and coronary heart disease. Genome Biol. 2016;17(1):171.
91. Lee DS, Shin JY, Tonge PD, Puri MC, Lee S, Park H, et al. An epigenomic roadmap to induced pluripotency reveals DNA methylation as a reprogramming modulator. Nat Commun. 2014;5:5619.
92. NestorCE,OttavianoR,ReinhardtD,CruickshanksHA,MjosengHK, McPhersonRC,etal.Rapid reprogramming of epigenetic and transcriptional profiles in mammalian culture systems. Genome Biol. 2015;16:11.
93. Marks H, Kalkan T, Menafra R, Denissov S, Jones K, Hofemeister H, et al. The transcriptional and epigenomic foundations of ground state pluripotency. Cell. 2012;149(3):590–604.
94. Veillard AC, Marks H, Bernardo AS, Jouneau L, Laloe D, Boulanger L, et al. Stable methylation at promoters distinguishes epiblast stem cells from embryonic stem cells and the in vivo epiblasts. Stem Cells Dev. 2014; 23(17):2014–2029.
95. Bogdanovic O, Lister R. DNA methylation and the preservation of cell identity. Curr Opin Genet Dev. 2017;46:9–14.
96. Simon JA, Kingston RE. Occupying chromatin: Polycomb mechanisms for getting to genomic targets, stopping transcriptional traffic, and staying put. Mol Cell. 2013;49(5):808–824.
97. Huang Y, Rao A. Connections between TET proteins and aberrant DNA modification in cancer. Trends Genet. 2014;30(10):464–474.
98. Inoue A, Shen L, Dai Q, He C, Zhang Y. Generation and replication-dependent dilution of 5fC and 5caC during mouse preimplantation development. Cell Res. 2011;21(12):1670–1676.
99. Inoue A, Zhang Y. Replication-dependent loss of 5-hydroxymethylcytosine in mouse preimplantation embryos. Science. 2011;334(6053):194.
100. Amouroux R, Nashun B, Shirane K, Nakagawa S, Hill PW, D'Souza Z, et al. De novo DNA methylation drives 5hmC accumulation in mouse zygotes. Nat Cell Biol. 2016;18(2):225–233. https://doi.org/10.1038/ ncb3296. Epub 2016 Jan 11.
101. Cortellino S, Xu J, Sannai M, Moore R, Caretti E, Cigliano A, et al. Thymine DNA glycosylase is essential for active DNA demethylation by linked deamination-base excision repair. Cell. 2011;146(1):67–79.
102. Shen L, Wu H, Diep D, Yamaguchi S, D'Alessio AC, Fung HL, et al. Genome-wide analysis reveals TET-and TDG-dependent 5-methylcytosine oxidation dynamics. Cell. 2013;153(3):692–706.
103. Koh KP, Rao A. DNA methylation and methylcytosine oxidation in cell fate decisions. Curr Opin Cell Biol. 2013;25(2):152–161.
104. Ito S, Shen L, Dai Q, Wu SC, Collins LB, Swenberg JA, et al. Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. Science. 2011;333(6047):1300–1303.
105. Thomson JP, Meehan RR. The application of genome-wide 5-hydroxymethylcytosine studies in cancer research. Epigenomics. 2017;9(1):77–91.
106. Thomson JP, Meehan RR. DNA methylation changes in cancer. In: Kaneda A, Tsukada Y, eds. DNA and Histone Methylation as Cancer Targets Cancer Drug Discovery and Development. New York: Humana Press; 2017:75–96. 1.
107. Meehan RR, Thomson JP, Lentini A, Nestor CE, Pennings S. DNA methylation as a genomic marker of exposure to chemical and environmental agents. Curr Opin Chem Biol. 2018;45:48–56.

108. Thienpont B, Steinbacher J, Zhao H, D'Anna F, Kuchnio A, Ploumakis A, et al. Tumour hypoxia causes DNA hypermethylation by reducing TET activity. Nature. 2016;537(7618):63–68.
109. He YF, Li BZ, Li Z, Liu P, Wang Y, Tang Q, et al. Tet-mediated formation of 5-carboxylcytosine and its excision by TDG in mammalian DNA. Science. 2011;333(6047):1303–1307.
110. Weber AR, Krawczyk C, Robertson AB, Kusnierczyk A, Vagbo CB, Schuermann D, et al. Biochemical reconstitution of TET1-TDG-BER-dependent active DNA demethylation reveals a highly coordinated mechanism. Nat Commun. 2016;710806.
111. Maiti A, Drohat AC. Thymine DNA glycosylase can rapidly excise 5-formylcytosine and 5-carboxylcytosine: potential implications for active demethylation of CpG sites. J Biol Chem. 2011;286(41):35334–35338.
112. Branco MR, Ficz G, Reik W. Uncovering the role of 5-hydroxymethylcytosine in the epigenome. Nat Rev Genet. 2011;13(1):7–13.
113. Schiesser S, Hackner B, Pfaffeneder T, Muller M, Hagemeier C, Truss M, et al. Mechanism and stem-cell activity of 5-carboxycytosine decarboxylation determined by isotope tracing. Angew Chem Int Ed Engl. 2012;51(26):6516–6520.
114. Xu S, Li W, Zhu J, Wang R, Li Z, Xu GL, et al. Crystal structures of isoorotate decarboxylases reveal a novel catalytic mechanism of 5-carboxyl-uracil decarboxylation and shed light on the search for DNA decarboxylase. Cell Res. 2013;23(11):1296–1309.
115. Liutkeviciute Z, Kriukiene E, Licyte J, Rudyte M, Urbanaviciute G, Klimasauskas S. Direct decarboxylation of 5-carboxylcytosine by DNA C5-methyltransferases. J Am Chem Soc. 2014;136(16):5884–5887.
116. Nabel CS, Jia H, Ye Y, Shen L, Goldschmidt HL, Stivers JT, et al. AID/APOBEC deaminases disfavor modified cytosines implicated in DNA demethylation. Nat Chem Biol. 2012;8(9):751–758.
117. Chen CC, Wang KY, Shen CK. The mammalian de novo DNA methyltransferases DNMT3A and DNMT3B are also DNA 5-hydroxymethylcytosine dehydroxymethylases. J Biol Chem. 2012;287(40):33116–33121.
118. Carell T, Kurz MQ, Muller M, Rossa M, Spada F. Non-canonical Bases in the Genome: The Regulatory Information Layer in DNA. Angew Chem Int Ed Engl. 2018;57(16):4296–4312.
119. Bogdanovic O, Smits AH, de la Calle Mustienes E, Tena JJ, Ford E, Williams R, et al. Active DNA demethylation at enhancers during the vertebrate phylotypic period. Nat Genet. 2016;48(4):417–426.
120. Nestor CE, Lentini A, Hagg Nilsson C, Gawel DR, Gustafsson M, Mattson L, et al. 5- Hydroxymethylcytosine Remodeling Precedes Lineage Specification during Differentiation of Human CD4(+) T Cells. Cell Rep. 2016;16(2):559–570.
121. Kafer GR, Li X, Horii T, Suetake I, Tajima S, Hatada I, et al. 5-Hydroxymethylcytosine marks sites of DNA damage and promotes genome stability. Cell Rep. 2016;14(6):1283–1292.
122. Stroud H, Feng S, Morey Kinney S, Pradhan S, Jacobsen SE. 5-Hydroxymethylcytosine is associated with enhancers and gene bodies in human embryonic stem cells. Genome Biol. 2011;12(6):R54.
123. Delatte B, Wang F, Ngoc LV, Collignon E, Bonvin E, Deplus R, et al. RNA biochemistry. Transcriptomewide distribution and function of RNA hydroxymethylcytosine. Science. 2016;351(6270):282–285.
124. Wang F, Minakhina S, Tran H, Changela N, Kramer J, Steward R. Tet protein function during Drosophila development. PLoS ONE. 2018;13(1):e0190367.
125. Guallar D, Bi X, Pardavila JA, Huang X, Saenz C, Shi X, et al. RNA-dependent chromatin targeting of TET2 for endogenous retrovirus control in pluripotent stem cells. Nat Genet. 2018;50(3):443–451.
126. Nashun B, Hill PW, Hajkova P. Reprogramming of cell fate: epigenetic memory and the erasure of memories past. EMBO J. 2015;34(10):1296–1308.
127. Tang WW, Kobayashi T, Irie N, Dietmann S, Surani MA. Specification and epigenetic programming of the human germ line. Nat Rev Genet. 2016;17(10):585–600.
128. Hodges E, Molaro A, Dos Santos CO, Thekkat P, Song Q, Uren PJ, et al. Directional DNA methylation changes and complex intermediate states accompany lineage specificity in the adult hematopoietic compartment. Mol Cell. 2011;44(1):17–28.
129. Scourzic L, Mouly E, Bernard OA. TET proteins and the control of cytosine demethylation in cancer. Genome Med. 2015;7(1):9.

130. Baylin SB, Jones PA. Epigenetic determinants of cancer. Cold Spring Harb Perspect Biol. 2016;8(9).
131. Blaschke K, Ebata KT, Karimi MM, Zepeda-Martinez JA, Goyal P, Mahapatra S, et al. VitaminC induces Tet-dependent DNA demethylation and a blastocyst-like state in ES cells. Nature. 2013;500(7461):222–226.
132. Valinluck V, Sowers LC. Endogenous cytosine damage products alter the site selectivity of human DNA maintenance methyltransferase DNMT1. Cancer Res. 2007;67(3):946–950.
133. Zhang H, Zhu JK. Active DNA demethylation in plants and animals. Cold Spring Harb Symp Quant Biol. 2012;77:161–173.
134. Beaujean N, Hartshorne G, Cavilla J, Taylor J, Gardner J, Wilmut I, et al. Non-conservation of mammalian preimplantation methylation dynamics. Curr Biol. 2004;14(7):R266–R267.
135. Guo F, Li X, Liang D, Li T, Zhu P, Guo H, et al. Active and passive demethylation of male and female pronuclear DNA in the mammalian zygote. Cell Stem Cell. 2014;15(4):447–459.
136. Shen L, Inoue A, He J, Liu Y, Lu F, Zhang Y. Tet3 and DNA replication mediate demethylation of both the maternal and paternal genomes in mouse zygotes. Cell Stem Cell. 2014;15(4):459–471.
137. Wang L, Zhang J, Duan J, Gao X, Zhu W, Lu X, et al. Programming and inheritance of parental DNA methylomes in mammals. Cell. 2014;157(4):979–991.
138. Santos F, Hendrich B, Reik W, Dean W. Dynamic reprogramming of DNA methylation in the early mouse embryo. Dev Biol. 2002;241(1):172–182.
139. Habibi E, Stunnenberg HG. Transcriptional and epigenetic control in mouse pluripotency: lessons from in vivo and in vitro studies. Curr Opin Genet Dev. 2017;46:114–122.
140. Canovas S, Ross PJ. Epigenetics in preimplantation mammalian development. Theriogenology. 2016;86 (1):69–79.
141. Iqbal K, Jin SG, Pfeifer GP, Szabo PE. Reprogramming of the paternal genome upon fertilization involves genome-wide oxidation of 5-methylcytosine. Proc Natl Acad Sci U S A. 2011;108(9):3642–3647.
142. Gu TP, Guo F, Yang H, Wu HP, Xu GF, Liu W, et al. The role of Tet3 DNA dioxygenase in epigenetic reprogramming by oocytes. Nature. 2011;477(7366):606–610.
143. Nakamura T, Liu YJ, Nakashima H, Umehara H, Inoue K, Matoba S, et al. PGC7 binds histone H3K9me2 to protect against conversion of 5mC to 5hmC in early embryos. Nature. 2012;486(7403):415–419.
144. Szabo PE, Pfeifer GP. H3K9me2 attracts PGC7 in the zygote to prevent Tet3-mediated oxidation of 5-methylcytosine. J Mol Cell Biol. 2012;4(6):427–429.
145. Wossidlo M, Nakamura T, Lepikhov K, Marques CJ, Zakhartchenko V, Boiani M, et al. 5Hydroxymethylcytosine in the mammalian zygote is linked with epigenetic reprogramming. Nat Commun. 2011;2(241)https://doi.org/10.1038/ncomms1240.
146. Howell CY, Bestor TH, Ding F, Latham KE, Mertineit C, Trasler JM, et al. Genomic imprinting disrupted by a maternal effect mutation in the Dnmt1 gene. Cell. 2001;104(6):829–838.
147. Ratnam S, Mertineit C, Ding F, Howell CY, Clarke HJ, Bestor TH, et al. Dynamics of Dnmt1 methyltransferase expression and intracellular localization during oogenesis and preimplantation development. Dev Biol. 2002;245(2):304–314.
148. Meehan RR, Dunican DS, Ruzov A, Pennings S. Epigenetic silencing in embryogenesis. Exp Cell Res. 2005;309(2):241–249.
149. Rose CM, van den Driesche S, Meehan RR, Drake AJ. Epigenetic reprogramming: preparing the epigenome for the next generation. Biochem Soc Trans. 2013;41(3):809–814.
150. Rose CM, van den Driesche S, Sharpe RM, Meehan RR, Drake AJ. Dynamic changes in DNA modification states during late gestation male germ line development in the rat. Epigenetics Chromatin. 2014;7:19.
151. Yamaguchi S, Hong K, Liu R, Inoue A, Shen L, Zhang K, et al. Dynamics of 5-methylcytosine and 5-hydroxymethylcytosine during germ cell reprogramming. Cell Res. 2013;23(3):329–339.
152. Schuermann D, Weber AR, Schar P. Active DNA demethylation by DNA repair: facts and uncertainties. DNA Repair (Amst). 2016;44:92–102.
153. Dawlaty MM, Breiling A, Le T, Barrasa MI, Raddatz G, Gao Q, et al. Loss of Tet enzymes compromises proper differentiation of embryonic stem cells. Dev Cell. 2014;29(1):102–111.

154. DawlatyMM, Breiling A, Le T, RaddatzG, BarrasaMI, ChengAW, et al. Combined deficiency of Tet1 and Tet2 causes epigenetic abnormalities but is compatible with postnatal development. Dev Cell. 2013;24(3):310–323.
155. Wiehle L, Raddatz G, Musch T, Dawlaty MM, Jaenisch R, Lyko F, et al. Tet1 and Tet2 protect DNA methylation canyons against hypermethylation. Mol Cell Biol. 2016;36(3):452–461.
156. Verma N, Pan H, Dore LC, Shukla A, Li QV, Pelham-Webb B, et al. TET proteins safeguard bivalent promoters from de novo methylation in human embryonic stem cells. Nat Genet. 2018;50(1):83–95.
157. IchimuraN, ShinjoK,An B, ShimizuY,YamaoK,Ohka F, et al.Aberrant TET1MethylationCloselyAssociated with CpG Island Methylator Phenotype in Colorectal Cancer. Cancer Prev Res (Phila). 2015;8(8):702–711.
158. Pfaffeneder T, Spada F, Wagner M, Brandmayr C, Laube SK, Eisen D, et al. Tet oxidizes thymine to 5-hydroxymethyluracil in mouse embryonic stem cell DNA. Nat Chem Biol. 2014;10(7):574–581.
159. Fong KS, Hufnagel RB, Khadka VS, Corley MJ, Maunakea AK, Fogelgren B, et al. A mutation in the tuft mouse disrupts TET1 activity and alters the expression of genes that are crucial for neural tube closure. Dis Model Mech. 2016;9(5):585–596.
160. Khoueiry R, Sohni A, Thienpont B, Luo X, Velde JV, Bartoccetti M, et al. Lineage-specific functions of TET1 in the postimplantation mouse embryo. Nat Genet. 2017;49(7):1061–1072.
161. Li X, Yue X, Pastor WA, Lin L, Georges R, Chavez L, et al. Tet proteins influence the balance between neuroectodermal and mesodermal fate choice by inhibiting Wnt signaling. Proc Natl Acad Sci U S A. 2016;113(51):E8267–E8276.
162. Nestor CE, Ottaviano R, Reddington J, Sproul D, Reinhardt D, Dunican D, et al. Tissue type is a major modifier of the 5-hydroxymethylcytosine content of human genes. Genome Res. 2012;22(3):467–477.
163. Thomson JP, Lempiainen H, Hackett JA, Nestor CE, Muller A, Bolognani F, et al. Non-genotoxic carcinogen exposure induces defined changes in the 5-hydroxymethylome. Genome Biol. 2012;13(10):R93.
164. Thomson JP, Fawkes A, Ottaviano R, Hunter JM, Shukla R, Mjoseng HK, et al. DNA immunoprecipitation semiconductor sequencing (DIP-SC-seq) as a rapid method to generate genome wide epigenetic signatures. Sci Rep. 2015;5:9778.
165. Li X, Liu Y, Salz T, Hansen KD, Feinberg A. Whole-genome analysis of the methylome and hydroxymethylome in normal and malignant lung and liver. Genome Res. 2016;26(12):1730–1741.
166. Sakaue M, Ohta H, Kumaki Y, Oda M, Sakaide Y, Matsuoka C, et al. DNA methylation is dispensable for the growth and survival of the extraembryonic lineages. Curr Biol. 2010;20(16):1452–1457.
167. Dawlaty MM, Ganz K, Powell BE, Hu YC, Markoulaki S, Cheng AW, et al. Tet1 is dispensable for maintaining pluripotency and its loss is compatible with embryonic and postnatal development. Cell Stem Cell. 2011;9(2):166–175.
168. Li Z, Cai X, Cai CL, Wang J, Zhang W, Petersen BE, et al. Deletion of Tet2 in mice leads to dysregulated hematopoietic stem cells and subsequent development of myeloid malignancies. Blood. 2011; 118(17):4509–4518.
169. Inoue A, Shen L, Matoba S, Zhang Y. Haploinsufficiency, but not defective paternal 5mC oxidation, accounts for the developmental defects of maternal Tet3 knockouts. Cell Rep. 2015;10(4):463–470.
170. Dai HQ, Wang BA, Yang L, Chen JJ, Zhu GC, Sun ML, et al. TET-mediated DNA demethylation controls gastrulation by regulating Lefty-Nodal signalling. Nature. 2016;538(7626):528–532.
171. Cortazar D, Kunz C, Selfridge J, Lettieri T, Saito Y, MacDougall E, et al. Embryonic lethal phenotype reveals a function of TDG in maintaining epigenetic stability. Nature. 2011;470(7334):419–423.
172. Daxinger L, Harten SK, Oey H, Epp T, Isbel L, Huang E, et al. An ENU mutagenesis screen identifies novel and known genes involved in epigenetic processes in the mouse. Genome Biol. 2013;14(9):R96.
173. Muto M, Kanari Y, Kubo E, Takabe T, Kurihara T, Fujimori A, et al. Targeted disruption of Np95 gene renders murine embryonic stem cells hypersensitive to DNA damaging agents and DNA replication blocks. J Biol Chem. 2002;277(37):34549–34555.
174. Sun LQ, Lee DW, Zhang Q, Xiao W, Raabe EH, Meeker A, et al. Growth retardation and premature aging phenotypes in mice with disruption of the SNF2-like gene, PASG. Genes Dev. 2004;18(9):1035–1046.

175. Ren J, Briones V, Barbour S, Yu W, Han Y, Terashima M, et al. The ATP binding site of the chromatin remodeling homolog Lsh is required for nucleosome density and de novo DNA methylation at repeat sequences. Nucleic Acids Res. 2015;43(3):1444–1455.
176. Walsh CP, Bestor TH. Cytosine methylation and mammalian development. Genes Dev. 1999;13(1):26–34.
177. Monk M, Boubelik M, Lehnert S. Temporal and regional changes in DNA methylation in the embryonic, extraembryonic and germ cell lineages during mouse embryo development. Development. 1987;99(3): 371–382.
178. Wongtawan T, Taylor JE, Lawson KA, Wilmut I, Pennings S. Histone H4K20me3 and HP1alpha are late heterochromatin markers in development, but present in undifferentiated embryonic stem cells. J Cell Sci. 2011;124(Pt 11):1878–1890.
179. Leitch HG, McEwen KR, Turp A, Encheva V, Carroll T, Grabole N, et al. Naive pluripotency is associated with global DNA hypomethylation. Nat Struct Mol Biol. 2013;20(3):311–316.
180. Tsumura A, Hayakawa T, Kumaki Y, Takebayashi S, Sakaue M, Matsuoka C, et al. Maintenance of self-renewal ability of mouse embryonic stem cells in the absence of DNA methyltransferases Dnmt1, Dnmt3a and Dnmt3b. Genes Cells. 2006;11(7):805–814.
181. Festuccia N, Gonzalez I, Navarro P. The epigenetic paradox of pluripotent ES cells. J Mol Biol. 2017; 429(10):1476–1503.
182. Dunican DS, Cruickshanks HA, Suzuki M, Semple CA, Davey T, Arceci RJ, et al. Lsh regulates LTR retrotransposon repression independently of Dnmt3b function. Genome Biol. 2013;14(12):R146.
183. Dennis K, Fan T, Geiman T, Yan Q, Muegge K. Lsh, a member of the SNF2 family, is required for genome-wide methylation. Genes Dev. 2001;15(22):2940–2944.
184. Dunican DS, Pennings S, Meehan RR. Lsh Is essential for maintaining global DNA methylation levels in Amphibia and fish and interacts directly with Dnmt1. Biomed Res Int. 2015;2015:740637.
185. Iismaa SE, Kaidonis X, Nicks AM, Bogush N, Kikuchi K, Naqvi N, et al. Comparative regenerative mechanisms across different mammalian tissues. NPJ Regen Med. 2018;3:6.
186. Raven A, Lu WY, Man TY, Ferreira-Gonzalez S, O'Duibhir E, Dwyer BJ, et al. Cholangiocytes act as facultative liver stem cells during impaired hepatocyte regeneration. Nature. 2017;547(7663):350–354.
187. Roy B, Venkatachalapathy S, Ratna P, Wang Y, Jokhun DS, Nagarajan M, et al. Laterally confined growth of cells induces nuclear reprogramming in the absence of exogenous biochemical factors. Proc Natl Acad Sci U SA. 2018;115(21):E4741–E4750.
188. Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KH. Viable offspring derived from fetal and adult mammalian cells. Nature. 1997;385(6619):810–813.
189. Beaujean N, Taylor J, Gardner J, Wilmut I, Meehan R, Young L. Effect of limited DNA methylation reprogramming in the normal sheep embryo on somatic cell nuclear transfer. Biol Reprod. 2004;71(1):185–193.
190. Young LE, Beaujean N. DNA methylation in the preimplantation embryo: the differing stories of the mouse and sheep. Anim Reprod Sci. 2004;82–83:61–78.
191. Hochedlinger K, Jaenisch R. Induced pluripotency and epigenetic reprogramming. Cold Spring Harb Perspect Biol. 2015;7(12):a019448.
192. Rideout 3rd WM, Wakayama T, Wutz A, Eggan K, Jackson-Grusby L, Dausman J, et al. Generation of mice from wild-type and targeted ES cells by nuclear cloning. Nat Genet. 2000;24(2):109–110.
193. BortvinA,EgganK,SkaletskyH,AkutsuH,BerryDL,YanagimachiR,etal.Incompletereactivationof Oct4-related genes in mouse embryos cloned from somatic nuclei. Development. 2003;130(8): 1673–1680.
194. Sindhu C, Samavarchi-Tehrani P, Meissner A. Transcription factor-mediated epigenetic reprogramming. J Biol Chem. 2012;287(37):30922–30931.
195. ShiY, InoueH,WuJC, YamanakaS. Induced pluripotent stemcell technology:a decadeof progress. Nat Rev Drug Discov. 2017;16(2):115–130.

196. Otani T, Marchetto MC, Gage FH, Simons BD, Livesey FJ. 2D and 3D stem cell models of primate cortical development identify species-specific differences in progenitor behavior contributing to brain size. Cell Stem Cell. 2016;18(4):467–480.
197. Maherali N, Sridharan R, Xie W, Utikal J, Eminli S, Arnold K, et al. Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. Cell Stem Cell. 2007;1(1):55–70.
198. Schwarz BA, Cetinbas M, Clement K, Walsh RM, Cheloufi S, Gu H, et al. Prospective isolation of poised iPSC intermediates reveals principles of cellular reprogramming. Cell Stem Cell. 2018.
199. O'Malley J, Skylaki S, Iwabuchi KA, Chantzoura E, Ruetz T, Johnsson A, et al. High-resolution analysis with novel cell-surface markers identifies routes to iPS cells. Nature. 2013;499(7456):88–91.
200. Benevento M, Tonge PD, Puri MC, Hussein SM, Cloonan N, Wood DL, et al. Proteome adaptation in cell reprogramming proceeds via distinct transcriptional networks. Nat Commun. 2014;5:5613.
201. Benevento M, Tonge PD, Puri MC, Nagy A, Heck AJ, Munoz J. Fluctuations in histone H4 isoforms during cellular reprogramming monitored by middle-down proteomics. Proteomics. 2015;15(18):3219–3231.
202. Clancy JL, Patel HR, Hussein SM, Tonge PD, Cloonan N, Corso AJ, et al. Small RNA changes en route to distinct cellular states of induced pluripotency. Nat Commun. 2014;5:5522.
203. Tonge PD, Corso AJ, Monetti C, Hussein SM, Puri MC, Michael IP, et al. Divergent reprogramming routes lead to alternative stem-cell states. Nature. 2014;516(7530):192–197.
204. Hussein SM, Puri MC, Tonge PD, Benevento M, Corso AJ, Clancy JL, et al. Genome-wide characterization of the routes to pluripotency. Nature. 2014;516(7530):198–206.
205. Pawlak M, Jaenisch R. De novo DNA methylation by Dnmt3a and Dnmt3b is dispensable for nuclear reprogramming of somatic cells to a pluripotent state. Genes Dev. 2011;25(10):1035–1040.
206. Chen J, Gao Y, Huang H, Xu K, Chen X, Jiang Y, et al. The combination of Tet1 with Oct4 generates high-quality mouse-induced pluripotent stem cells. Stem Cells. 2015;33(3):686–698.
207. Gao Y, Chen J, Li K, Wu T, Huang B, Liu W, et al. Replacement of Oct4 by Tet1 during iPSC induction reveals an important role of DNA methylation and hydroxymethylation in reprogramming. Cell Stem Cell. 2013;12(4):453–469.
208. Fu Y, Luo GZ, Chen K, Deng X, Yu M, Han D, et al. N6-methyldeoxyadenosine marks active transcription start sites in Chlamydomonas. Cell. 2015;161(4):879–892.
209. Greer EL, Blanco MA, Gu L, Sendinc E, Liu J, Aristizabal-Corrales D, et al. DNA methylation on N6adenine in C. elegans. Cell. 2015;161(4):868–878.
210. Heyn H, Esteller M. An Adenine code for DNA: a second life for N6-methyladenine. Cell. 2015;161(4): 710–713.
211. Zhang G, Huang H, Liu D, Cheng Y, Liu X, Zhang W, et al. N6-methyladenine DNA modification in Drosophila. Cell. 2015;161(4):893–906.
212. Koziol MJ, Bradshaw CR, Allen GE, Costa ASH, Frezza C, Gurdon JB. Identification of methylated deoxyadenosines in vertebrates reveals diversity in DNA modifications. Nat Struct Mol Biol. 2016;23(1):24–30.
213. Liu J, Zhu Y, Luo GZ, Wang X, Yue Y, Zong X, et al. Abundant DNA 6mA methylation during early embryogenesis of zebrafish and pig. Nat Commun. 2016;713052.
214. Wu TP, Wang T, Seetin MG, Lai Y, Zhu S, Lin K, et al. DNA methylation on N(6)-adenine in mammalian embryonic stem cells. Nature. 2016;532(7599):329–333.
215. Schiffers S, Ebert C, Rahimoff R, Kosmatchev O, Steinbacher J, Bohne AV, et al. Quantitative LC-MS Provides No Evidence for m(6) dA or m(4) dC in the Genome of Mouse Embryonic Stem Cells and Tissues. Angew Chem Int Ed Engl. 2017;56(37):11268–11271.
216. Zhu S, Beaulaurier J, Deikus G, Wu TP, Strahl M, Hao Z, et al. Mapping and characterizing N6methyladenine in eukaryotic genomes using single-molecule real-time sequencing. Genome Res. 2018; 28(7):1067–1078.
217. Lentini A, Lagerwall C, Vikingsson S, Mjoseng HK, Douvlataniotis K, Vogt H, et al. A reassessment of DNA-immunoprecipitation-based genomic profiling. Nat Methods. 2018;15(7):499–504.
218. Yue Y, Liu J, He C. RNA N6-methyladenosine methylation in post-transcriptional gene expression regulation. Genes Dev. 2015;29(13):1343–1355.
219. Amabile A, Migliara A, Capasso P, Biffi M, Cittaro D, Naldini L, et al. Inheritable silencing of endogenous genes by hit-and-run targeted epigenetic editing. Cell. 2016;167(1):219–32 e14.
220. Mizuguchi Y, Saiki Y, Horii A, Fukushige S. Targeted TET oxidase activity through methyl-CpG-binding domain extensively suppresses cancer cell proliferation. Cancer Med. 2016;5(9):2522–2533.
221. Rivron NC, Frias-Aldeguer J, Vrij EJ, Boisset JC, Korving J, Vivie J, et al. Blastocyst-like structures generated solely from stem cells. Nature. 2018;557(7703):106–111.
222. Harrison SE, Sozen B, Christodoulou N, Kyprianou C, Zernicka-Goetz M. Assembly of embryonic and extraembryonic stem cells to mimic embryogenesis in vitro. Science. 2017;356(6334):eaal1810.
223. Pijuan-Sala B, Guibentif C, Gottgens B. Single-cell transcriptional profiling: a window into embryonic cell-type specification. Nat Rev Mol Cell Biol. 2018.