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Review Article

DNA methylation dynamics at transposable elements in mammals

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Transposable elements dominate the mammalian genome, but their contribution to genetic and epigenetic regulation has been largely overlooked. This was in part due to technical limitations, which made the study of repetitive sequences at single copy resolution difficult. The advancement of next-generation sequencing assays in the last decade has greatly enhanced our understanding of transposable element function. In some instances, specific transposable elements are thought to have been co-opted into regulatory roles during both mouse and human development, while in disease such regulatory potential can contribute to malignancy. DNA methylation is arguably the best characterised regulator of transposable element activity. DNA methylation is associated with transposable element repression, and acts to limit their genotoxic potential. In specific developmental contexts, erasure of DNA methylation is associated with a burst of transposable element expression. Developmental regulation of DNA methylation enables transposon activation, ensuring their survival and propagation throughout the host genome, and also allows the host access to regulatory sequences encoded within the elements. Here I discuss DNA methylation at transposable elements, describing its function and dynamic regulation throughout murine and human development.

Introduction

Mammalian genomes are replete with repetitive sequences, accounting for approximately half of our genetic material [1,2]. Repetitive sequences were once seen as non-functional, 'junk DNA' [3]. This view was supported by the low sequence conservation of the repetitive genome between closely related species, which is generally used to infer functional significance [4]. Furthermore, in most cellular and developmental contexts, repetitive sequences are constitutively heterochromatic and largely transcriptionally inert, and perturbing this state is deleterious to the organism [5–7]. Nevertheless, it has long been known that repetitive DNA harbours regulatory potential, and can influence expression from the coding genome [8,9]. A number of recent studies have begun to ascribe putative developmental functions to a subset of repetitive elements within the mammalian genome [10–16]. Such research has contributed to a shifting view of repetitive sequences from merely junk-DNA, to potentially serving the host by being co-opted into regulatory roles during development (comprehensively discussed in [17]). While such research is in its infancy, we are beginning to develop the appropriate genetic and molecular tools to address any functional roles that specific repetitive sequences may serve in mammalian development.

The genome comprises both tandem and interspersed repeats. Tandem repetitive units occur consecutively and adjacently. Whereas interspersed repeats are dispersed throughout the genome and are non-adjacent. Most interspersed repeats are derived from transposable elements. Transposable elements are thought to have diverse evolutionary origins, including exposure of the host species to exogenous retroviruses and horizontal transfer events. As such, transposable element content varies vastly between species [18–20]. Transposable elements can be broadly classified into two classes: retrotransposons (class

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I) and DNA transposons (class II). DNA transposons employ a self-encoded transposase to directly excerpt and re-insert themselves throughout the host genome, while retrotransposons utilise reverse transcriptase to transpose via an RNA intermediate (Figure 1). DNA transposon activity is largely extinct in Mammalia, whereas a number of evolutionarily young retrotransposons can still move within the host genome [21–24]. Over evolutionary time transposable elements have contributed considerably to genome diversity [25].

Retrotransposons can be classified into long terminal repeat (LTR) and non-LTR elements. LTR elements are characterised by two identical non-coding elements, the LTR, at their 5 and 3 prime ends (Figure 1). The LTR regulates expression of retroviral genes involved in retrotransposition. The dominant LTR in mammals are endogenous retroviruses (ERVs), which account for ∼8% of the human genome [1]. Non-LTR elements include long and short interspersed nuclear elements (LINEs and SINEs). LINEs are the dominant non-LTR found in eutherian mammals, accounting for ∼21% of the human genome [1,2,4]. LINEs contain a 5′ UTR that acts as a promoter, and a 3′ polyadenylation signal, book-ending one or two open reading frames encoding proteins involved in transposition (Figure 1). SINEs are non-autonomous and do not encode proteins, relying wholly on the co-option of LINE machinery for their transposition [26]. The diversity, abundance and activity of each transposable element family varies greatly between mammalian species (reviewed in [4]). For example, IAP ERV elements are murine specific and retain retrotransposition potential, whereas ERVs have been rendered all but immobile in humans [27–29].

Broadly speaking, transposable elements are transcriptionally silenced in committed cells. Notable exceptions are cells of the brain during neurogenesis, and the placenta. It has been suggested that active retrotransposition in the brain may function to generate neural diversity [30]. In the murine placenta, ERV elements are highly expressed, and based on histone and transcription factor occupancy, have been proposed to contribute to a tissue-specific enhancer network [12]. It is worth noting that functional annotation based solely on chromatin profiling can overestimate the regulatory function of transposable elements; recent work *in vitro* has shown that despite bearing the biochemical hallmarks of active enhancers, only a subset of such transposable elements contribute significantly to gene regulation in embryonic stem cells [31]. Transposable elements are most active in cells that undergo epigenetic reprogramming; namely germ cells and blastomeres of the pre-implantation embryo [10,32,33]. Timely activation of young LINE1 elements are necessary for pre-implantation development, raising the possibility that transposable element expression has regulatory functions during reprogramming [11,34,35]. Additionally, ERVL elements appear to act as alternative promoters that drive the coordinated expression of a gene network from the embryonic genome in the earliest stages of mouse and human development [10,36]. While such observations warrant functional investigation, for example by perturbing ERVL expression *in vivo* to assess the effects on zygotic genome activation, these studies raise the possibility that transposable elements have defined roles during pre-implantation development. In this light, it would be of interest to investigate whether transposable elements are subject to developmental regulation. Closer inspection has shown that transcriptional profiles vary between different transposable elements families, suggestive of specific regulation [32]. More recent work has found transcription of some ERVs is driven by specific binding of trans-activating proteins [37–40]. Ascertaining the scope of transposable element function and regulation during epigenetic reprogramming is an active area of research, made possible by the advancement of low input next-generation sequencing technologies allowing profiling in the pre-implantation embryo.

Sustained transposable element activation can threaten genome integrity [41–43]. Epigenetic reprogramming also occurs in transformed cells, and transposable element expression is a property of many tumours (Figure 2) [44,45]. Transposon activation can contribute to oncogenesis and result in genetic instability [46–51]. The burden of sustained transposable element activation is also apparent in oocytes. The reserve of oocytes undergoes extensive apoptosis after birth, resulting in an 80% reduction [52,53]. In mouse, this process known as foetal oocyte attrition has been associated with high levels of the LINE1-encoded ORF1p protein, and can be temporarily attenuated by treatment with reverse transcriptase inhibitors. A recent study has found that mutating a pathway for transducing information about DNA damage in combination with reverse transcriptase inhibition can rescue foetal oocyte attrition, suggesting that excessive LINE1 activity in oocytes is genotoxic [54].

A balance needs to be achieved between accessing the regulatory potential harboured in repetitive elements, while limiting the havoc that their sustained activation can wreak on the host genome. It follows that tunable, yet stable mechanisms have evolved to regulate transposable element expression throughout mammalian development. DNA methylation is a well-characterised chromatin modification that is central to transposable element control. It has been suggested that DNA methylation evolved precisely to silence transposable elements [55].

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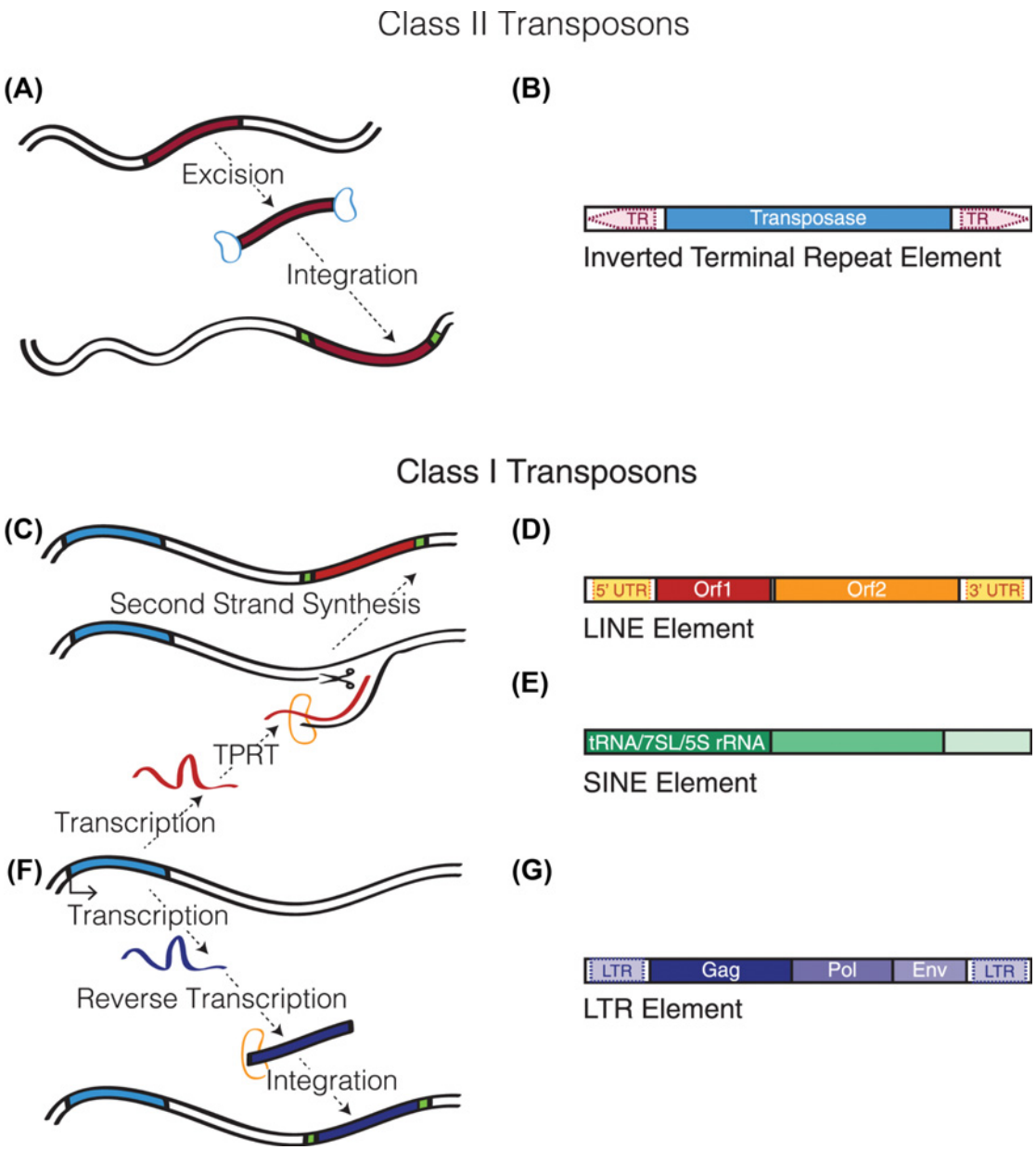

Figure 1. Transposable element classification

(**A**) Mobilisation of Class II transposable elements is through the action of transposase (blue) which acts on the transposon (red) to excise and subsequently reintegrate the DNA element elsewhere in the host genome, in a so-called 'cut-and-paste' mechanism. Transposition results in a characteristic duplication of the target site (TSD) (green) into which it is integrated. (**B**) Inverted Terminal Repeat elements are DNA transposons found in mammalian genomes, encoding a transposase (blue) which mediates transposition throughout the host genome, flanked by terminal repeats (pink). (**C**) Class I transposable elements are transcribed and mobilised via an RNA intermediate. Long interspersed nuclear element (LINE) mRNA (red) is reverse-transcribed by a transposon-encoded reverse transcriptase (yellow) at the new insertion site, which has been cleaved by a LINE-encoded endonuclease. This process is known as target primed reverse transcription (TPRT). The mechanisms for second strand synthesis have not been identified. Integration into the host genome results in TSD (green). (**D**) LINE elements typically encode two proteins involved in retrotransposition, flanked by 3′ and 5′ UTRs encoding regulatory sequences. (**E**) Short interspersed nuclear elements (SINEs) are composite elements, and can be broken down into a 5′ head, a central body and a 3′ tail. SINEs can be classified by the origin of their 5′ head, which can be derived from tRNA, 7SL RNA or rRNA. (**F**) Unlike LINE elements, endogenous retrovirus (ERV) elements (dark blue) are not reverse-transcribed at their target site. Reverse transcription is mediated by a retrovirally encoded enzyme (yellow) and integration into the host genome results in TSD (green). (**G**) ERVs encode retroviral proteins Gag, Pol and Env, flanked by regulatory sequences in the long terminal repeat (LTR). The Env protein is by and large non-functional in most ERV elements.

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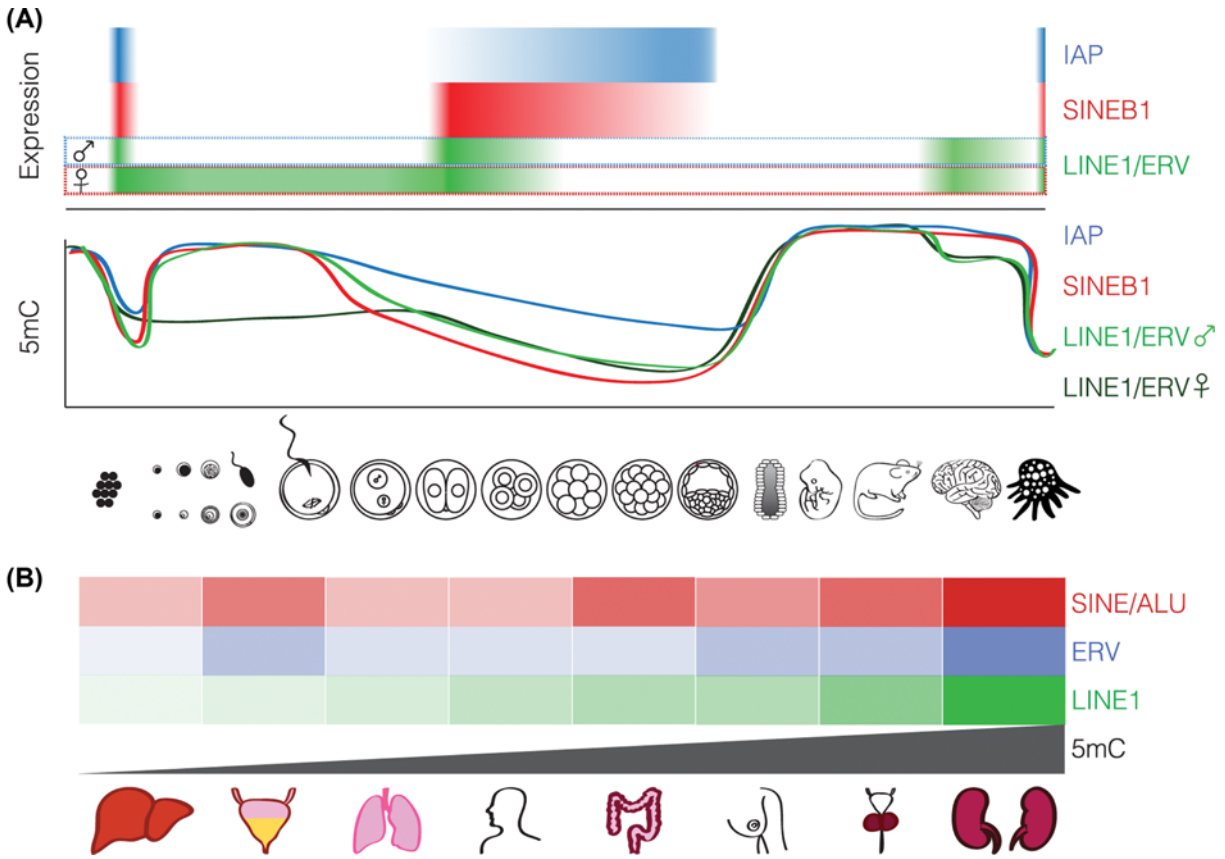

Figure 2. Transposable element expression and methylation dynamics

Representative transposable element expression and 5mC profiles throughout murine development. Peak transposable element expression coincides with, but does not directly trace 5mC reprogramming in primordial germ cells and the pre-implantation embryo. IAP (blue), SINE (red), and LINE and ERV (green) expression peak at approximately E13.5 in primordial germ cells, when somatic 5mC is erased. In the male germline, 5mC is rapidly re-established and is associated with transposable element repression. In the female germline, expression of LINE1 and some ERV family members persists. DNA methylation of LINE1 and ERV elements in the female germline is established more slowly and at a lower level than in males (dark green line) [68,78]. Following fertilisation, transposable element expression peaks in the two-cell stage embryo. 5mC continues to decline until the blastocyst stage, and upon implantation, somatic DNA methylation is achieved [79]. Transposable element repression is largely maintained in somatic tissues, with the exception of the brain, in which transposable element mobilisation is associated with reduced 5mC in specific neuronal populations [92]. Cancer is associated with reduced 5mC at transposable elements, and an increase in their expression. (**B**) Transposable element hypomethylation in a range of human cancers. Schematic representation of methylation data from [121]. From left to right, in order lowest 5mC levels relative to healthy tissue, to highest 5mC at transposons: Liver Hepatocellular Carcinoma; Bladder Urothelial Carcinoma; Lung squamous cell carcinoma; Head-Neck Squamous Cell Carcinoma; Colon Adenocarcinoma; Breast Invasive Carcinoma; Prostate Adenocarcinoma; and Kidney renal papillary cell carcinoma.

DNA methylation contributes to transposable element repression

DNA can be covalently modified by a methyl group at cytosine and adenosine. In mammals, the most abundant DNA modification is a methyl group attached to the fifth carbon position of cytosine (5mC) in the context of a CpG dinucleotide [5,6]. 5mC serves as a binding platform for proteins with a methyl binding domain, which can further modify the chromatin to regulate transcription. 5mC in CpG-rich contexts is generally associated with transcriptional repression. Much of the 5mC content in somatic cells can be attributed to methylation of transposable elements. 5mC is one of the predominant means for transposable element repression in somatic cells.

In mammals, 5mC is catalysed by DNA methyltransferase enzymes (DNMTs). DNMT1 recognises hemimethylated DNA through the chaperone UHRF1, and catalyses 5mC following DNA replication. This provides a robust

mechanism for 5mC maintenance. *De novo* methylation of cytosine is catalysed by DNMT3 enzymes. DNMT3A and DNMT3B are important for the *de novo* methylation of transposable elements. In mouse, DNMT3L is a non-catalytic homologue of the DNMTs, and is a cofactor for directing DNMT3A and DNMT3B to transposable elements in male germ cells [56]. DNMT3C has been described in mouse to *de novo* methylate promoters of evolutionarily young transposable elements in the male germline [57]. Much of what we know about 5mC function at transposable elements has been learned by disrupting these enzymes. Generally speaking, depletion of 5mC is associated with marked transposable element up-regulation and genome instability [7,56,57]. Perturbing DNA methylation has varying effects on transposable element expression, depending on the class, the cellular and developmental context, and the evolutionary age of the transposable elements [58–62]. This is in part due to an interplay between DNA methylation and other silencing pathways that have evolved to ensure robust transposable element repression.

While DNA methylation can acutely silence transposable elements by regulating the chromatin, 5mC can also induce mutations in the underlying transposable element sequences. 5mC is prone to spontaneous deamination, yielding a thymine in place of a cytosine [63]. Over time, accumulation of such mutations can restrict transposable element expression permanently [64]. In this way DNA methylation may contribute to the perpetual immobilisation of evolutionarily older transposable elements.

5mC can be oxidised by ten-eleven translocation (TET) enzymes, to produce 5-hydroxymethylcytosine (5hmC) [65]. It was initially suggested that TET enzymes mediate the erasure of 5mC during epigenetic reprogramming [66]. Indeed, following fertilisation, 5hmC accumulates on the paternal genome in the zygote concomitant with decreasing 5mC [67]. However in germ cells, loss of 5mC is not accompanied by a reciprocal increase in 5hmC, suggesting other mechanisms for DNA demethylation are at play [68]. In both germ cells and the pre-implantation embryo, it has now been proposed that TET proteins act to limit spurious *de novo* methylation events, as opposed to global 5mC erasure [68,69]. Although limiting TET activity results in 5mC retention at transposable elements, their transcriptional up-regulation ensues [70]. This highlights that 5mC alone is not sufficient for transposable element silencing, and that trans-activating factors are able to drive their expression in the presence of 5mC. The function of 5hmC itself at transposable elements is unclear. While 5hmC seems permissive for transposable element expression, the TET enzymes themselves can recruit repressive chromatin modifiers to 5hmC marked transposable elements to limit their expression, highlighting a dual role for TET enzymes in transposon regulation [71]. Thus, while knockout and *in vitro* studies have revealed much about 5mC and 5hmC function at transposable elements, it is important to remember that their function is dependent on both developmental context and the local chromatin environment.

Adenosine can be methylated at the nitrogen-6 position. N6-methyladenosine (m6A) is the most prevalent DNA modification in prokaryotic genomes, but has only recently been detected in mouse and human [72,73]. m6A accounts for <1% of adenosine in mammalian genomes. In eukaryotes, m6A is associated with both transcriptional repression and activation [74,75]. With regards to transposable elements, m6A deposition is associated with repression of evolutionarily young LINE1 elements in murine embryonic stem cell cultures [72]. *In vivo*, an increase in m6A has been associated with LINE1 silencing in the mouse brain [76]. Putative m6A events have also been reported in the promoters of young LINE1 elements in a human lymphoblastoid cell line [77]. It will be interesting to investigate whether m6A may play a broader role in the establishment of transposable element silencing following epigenetic reprogramming.

DNA methylation dynamics at transposable elements

DNA methylation dynamics are some of the best characterised throughout mammalian development. Due to the robust maintenance of DNA methylation by DNMT1, once catalysed, cell type-specific 5mC is stably maintained through mitosis in somatic cells. In order to give rise to the next generation, the somatic epigenome must be reprogrammed to generate highly specialised germ cells and to restore totipotency in the embryo. It follows that in mammalian development there are two major periods of epigenetic reprogramming in which somatic 5mC is almost completely erased: during primordial germ cell migration and in the pre-implantation embryo [78–82]. DNA demthylation can be passive, through the abolition of DNMT1 action such that 5mC is diluted through successive cell divisions, or active, through the oxidation of 5mC to 5hmC by TET family enzymes. Genome-wide DNA methylation dynamics have been reviewed extensively elsewhere [83–86], so I will focus on 5mC dynamics at transposable elements.

Murine primordial germ cells emerge ∼E6.5, at which time their epigenome resembles the somatic cells of epiblast. Erasure of 5mC initiates ∼E10.5, and DNA methylation is largely erased by E13.5. 5mC levels are reduced at LINE1-Tf and various ERV elements, while 5mC is somewhat retained at LINE1A and IAP ERV elements in both male and female PGCs [82]. Loss of 5mC is accompanied by a subtle increase in 5hmC, that is partially dependent on TET1.

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Reactivation of evolutionarily young LINE1 and IAPs occur concomitantly with the erasure of 5mC [68]. Dynamics for global 5mC re-establishment differ in parental genomes, and these differences extend to transposable elements [82]. In males, methylation is established quickly at evolutionarily young IAP ERV and LINE1 elements following reprogramming, and is maintained throughout spermatogenesis (Figure 2). The establishment of 5mC at transposable elements in male germ cells is critical; perturbation of DNA methylation pathways by DNMT3L or DNMT3C deletion results in genome instability that leads to apoptosis [56,57]. In female germ cells, methylation is re-established much more slowly, during the oocyte growth phase following birth. Consistent with reduced 5mC deposition during oogenesis, deletion of DNMT3L does not have as profound an effect as in male cells, and does not result in apoptosis in the female germ line [87]. Indeed, the female germline may have a higher tolerance for transposable element expression, in that it is comparable with that of the pre-implantation embryo, accounting for ∼20% of the transcriptome [32]. However, this may be dependent on transposable element family; LTR elements are highly expressed in the oocyte, with MALR elements accounting for the majority of transcripts. Whereas LINE1 expression is relatively low, likely because uncontrolled LINE1 expression is associated with apoptosis in oocytes [52].

The second wave of epigenetic reprogramming occurs following fertilisation. In the mouse and human zygote, the paternal genome is actively demethylated and differences in 5mC levels at transposable elements between parental genomes are largely resolved before the first cleavage division [79–81]. Throughout pre-implantation development, 5mC levels at LTR, LINEs and SINEs steadily decrease, whereas globally, IAPs are somewhat more resistant to DNA demethylation [88]. In the pre-implantation embryo DNA demethylation dynamics do not fully trace transposable element expression dynamics; young LINE1, IAP and ERV-L elements peak in expression at the two-cell stage, yet 5mC levels at these elements continue to decline until the blastocyst stage (Figure 2). This highlights that 5mC alone cannot account for transposable element expression dynamics during pre-implantation development [89]. Upon implantation, almost all transposon activity ceases, concurrent with acquisition of somatic 5mC levels. While DNA methylation of retrotransposons is well-characterised in the pre-implantation embryo, expression and chromatin dynamics at DNA transposons remain relatively underexplored.

In somatic cells, DNA methylation of transposable elements is generally stably maintained, and is proposed to be the major mode for transposon silencing [84]. Accordingly, transposable element expression is limited in most somatic tissues. Nonetheless, LINE1 expression and retrotransposition have been observed in the mouse and human brain [30,90]. Globally, 5mC levels in the brain are similar to other somatic tissues, however 5hmC levels are higher, indicative of active demethylation by TET enzymes [91]. Cell type-specific profiling has revealed GABAergic neurons display reduced 5mC [92]. *In vivo*, higher rates of LINE1 transposition in the human brain have been associated with reduced 5mC levels [93]. In mouse, neural progenitor cells also display reduced 5mC at ERVs relative to other somatic tissues, and upon perturbation of histone 3 lysine 9 trimethylation (H3K9me3) by deletion of KAP1, ERV expression increased significantly, suggesting that reduced 5mC levetals may create a more permissive environment for transposable element expression [94]. In human neural progenitor cells, DNMT1 deletion results in LINE1 activation *in vitro*, which in turn act as alternative promoters to drive expression of a neuronal network [95]. Together these studies suggest a possible role for LINE1 activation in mammalian neurobiology, regulated in part by reduced 5mC levels at transposable elements in neurons.

Somatic reprogramming is observed in cancer, and DNA demethylation has been suggested to contribute to oncogenesis [96]. Accordingly, the transposable element burden is high in many cancers. DNA methylation, transposon expression and transposition rates vary depending on the tissue from which the tumour originates, suggesting that while transposable element expression is a common feature of tumours, their function and contribution to malignancy varies depending on the cellular context (Figure 2) [49,97]. Active transposable elements can insert themselves into oncogenes and tumour suppressors, perturbing their expression [49,98]. Transposable elements have been shown to act as regulatory elements and drive the expression of oncogenes [47]. Additionally, cancer-specific chimeric transcripts have been detected in tumours, originating from transposable element promoters [99]. Furthermore, chromatin relaxation at transposons is permissive for non-homologous recombination [50,51]. Despite the deleterious effect that transposable element activation has in transformed cells, recent work has shown that ERV activation in a number of cancer models can elicit an anti-tumour adaptive immune response that sensitises tumours to immunotherapy [100,101]. Therefore, somewhat counterintuitively, a more permissive chromatin environment that enables transposable element up-regulation may be exploited therapeutically in cancer.

Finally, reduced DNA methylation is associated with aging (reviewed in [102]). Global decreases in 5mC levels have been observed, as well as locus-specific changes that impact gene expression. In humans, aging-associated hypomethylation is seen at both Alu SINE and LINE1 elements [103,104]. Transposable elements are reported to be

transcriptionally activated and more mobile in aging tissues [105]. It will be important to determine whether transposable element activation associated with aging contributes to degenerative disorders, and whether attenuating DNA methylation may be of therapeutic benefit in such cases.

Targeting transposable elements for DNA methylation

A highly conserved pathway for targeting 5mC to transposable elements is the PIWI-interacting RNA (piRNA) pathway (reviewed in [106]). In mammals, the piRNA pathway is predominantly active in male germ cells. piRNAs are generated from both long non-coding RNAs transcribed from piRNA clusters containing transposon remnants, and from the mRNA of transposable elements themselves [107,108]. The primary piRNA molecules are processed in the cytoplasm by MILI in mouse, and are transported back into the nucleus where they interact with MIWI2 to pair with complementary nascent mRNAs (Figure 3). *Mili* and *Miwi2* mutant mice both show aberrant expression of LINE1, SINEB1 and IAP elements, associated with DNA hypomethylation [109,110]. The mechanism by which piRNAs direct DNA methylation of transposable elements in the male germline is unknown; however, a direct interaction between PIWI proteins and DNMT3A or 3B could not be detected, and the pathway has been placed upstream of DNMT3L [107]. The piRNA pathway is also active in oocytes, however oocytes lacking the PIWI proteins do not show such marked up-regulation of transposable elements, and only IAP expression significantly increases [111]. Given the female germline is less sensitive to loss of DNMT3L, and that global 5mC levels are much lower than in the male germline, it is not surprising that the piRNA pathway functions differently between sexes. In the oocyte a subset of transposons appears to be regulated by DICER-dependent endogenous siRNA (endosiRNA). Similarly, *in vitro*, acute depletion of 5mC in embryonic stem cells results in the production of DICER-dependent endosiRNAs that act to limit transposon expression [61]; however, it has not been investigated whether there is a link with endosiRNA pathways and *de novo* DNA methylation [112].

Following reprogramming in the zygote, 5mC is maintained at a subset of IAP elements, and needs to be restored at transposable elements globally by implantation. Deletion of the E3 ubiquitin ligase, *Uhrf1* in mouse oocytes lead to reduced 5mC at IAPs in the pre-implantation embryo [113]. UHRF1 is able to bind both hemimethylated DNA or H3K9me2/3 at the replication fork and recruit DNMT1 to ensure the maintenance of 5mC following mitosis [114] (Figure 3). Indeed, transposable elements resistant to DNA demethylation are enriched for H3K9me3 in the pre-implantation embryo, indicative of cross-talk between histone methylation and DNA methylation pathways for the maintenance of 5mC [115]. This cross-talk is further exemplified by the interaction between the KRAB-ZNF-KAP1 and DNMT pathways in the early embryo. Zinc finger proteins (ZNF) with a KRAB domain bind transposable elements specifically and recruit KAP1 to the chromatin. KAP1 in turn recruits the lysine methyltransferase SETDB1 to catalyse H3K9me2/3, which is followed by the deposition of 5mC (Figure 3) (reviewed in [116]). *In vitro*, the HUSH complex can recruit SETDB1 and 5mC to exogenous retroviruses [117,118]. As components of the HUSH complex localise to ERV and LINE1 elements, and repress evolutionarily young LINE1 elements in mouse embryonic stem cells, it would be interesting to investigate a possible cross-talk with KRAB-ZNF-KAP1 and DNMT pathways in development [118]. While the intersection of histone and DNA methylation pathways exists, it is worth highlighting that evolutionarily older transposable elements are targets for KRAB-ZNF-KAP1-mediated H3K9me3 deposition, whereas evolutionarily younger transposons tend to show a greater dependency on DNA methylation [119].

While H3K9me3 and 5mC can cooperate to silence transposable elements, trimethylation of histone 3 on lysine residue 27 (H3K27me3) does not occupy transposable elements marked by 5mC. Upon 5mC depletion *in vitro*, H3K27me3 redistributes over the repetitive genome and operates as an alternative silencing pathway to DNA methylation [59]. No such H3K27me3 compensatory pathways have been observed *in vivo*, although it would be interesting to investigate following sustained 5mC depletion, such as in the female germline or tumour cells.

In somatic cells, recent work has identified the transcription factor Ying Yang 1 (YY1) in the establishment of 5mC in the promoter of young LINE1 elements in the human brain [93]. Truncations and mutations in the YY1 binding motif embedded in the LINE1 promoter reduce YY1 occupancy, and are associated with DNA hypomethylation, transcriptional up-regulation and increased LINE1 mobilisation. The mechanisms underlying YY1-mediated 5mC deposition remain to be investigated. Conversely, *in vitro*, it has been shown that YY1 can activate antisense LINE1 expression [120]. An activating function for YY1 at LINE1 elements has not yet been identified *in vivo*, however precedent for dual activating and repressive functions is set by the TET family proteins. Further investigation will be necessary to better understand whether a dual role for YY1 in LINE1 regulation may exist in different developmental contexts.

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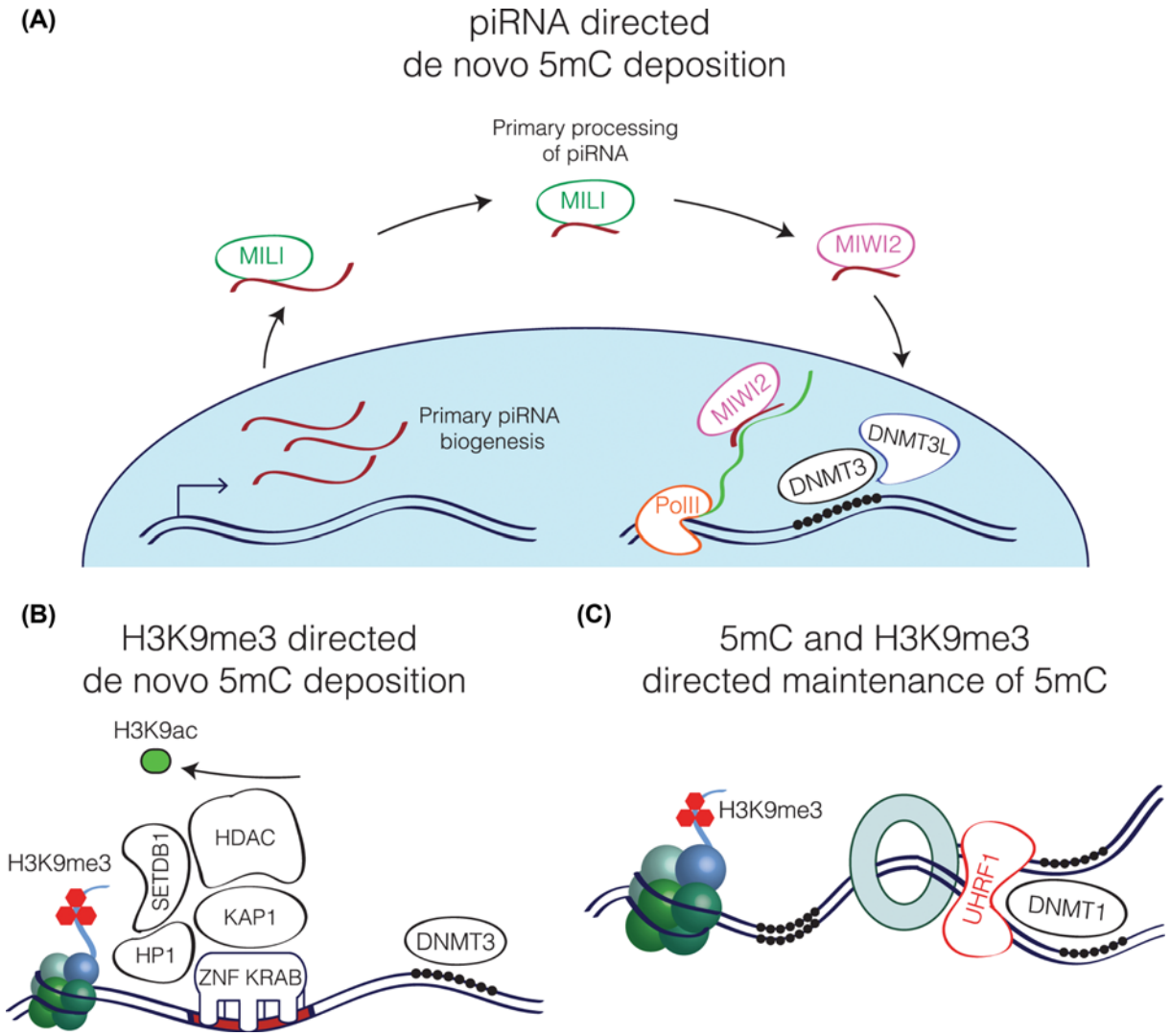

Figure 3. Targeting DNA methylation to transposable elements

DNA methylation is *de novo* targeted to transposable elements by non-coding RNAs (**A**) or histone modifications (**B**). (**A**) In male germ cells, primary piRNA molecules are transcribed from transposable elements themselves, or piRNA clusters, exported to the cytoplasm and processed by MILI. MIWI2 binds the processed piRNAs, returns to the nucleus and targets nascent transcription of transposable elements for DNA methylation. (**B**) KRAB-ZNF (zinc finger) proteins have evolved to recognise specific transposable element families. KRAB-ZNF proteins recruit KAP1 followed by SETDB1, which methylates H3K9. The transposable element subsequently acquired *de novo* DNA methylation. (**C**) DNA methylation is maintained by UHRF1. UHRF1 recognises hemimethylated DNA and chromatin marked by H3K9me2/3 at the replication fork, and recruits DNMT1 to maintain DNA methylation at a subset of transposable elements during pre-implantation development.

Conclusion

DNA methylation is an integral feature of transposable element control in mammals. Genome-wide mapping of 5mC throughout development has revealed a complex interplay between transposable elements, DNA methylation and other chromatin modifying pathways, which act in concert to ensure timely activation and repression of transposable elements during defined developmental windows. While differences between DNA methylation dynamics can often be explained by evolutionary age, or genetic sequence, in many cases it is unclear as to why transposable elements are differentially methylated or have differing sensitivities to loss of 5mC. Our understanding of the developmental function of transposable elements is in its infancy. The elucidation of specific transposable element family functions will likely help to make sense of why different classes are subject to differential regulation by 5mC, and may even reveal

novel roles for 5mC in genome regulation. Exploration of transposable element activation in diseases may also present novel therapeutic avenues, as has recently been demonstrated by attenuating DNA methylation in cancer cells. In both development and disease, the study of transposable elements is shifting from merely descriptive to functional, and regulatory pathways that target transposable elements will undeniably be entwined in enhancing our understanding of transposable element biology.

Summary

- Transposable elements are a source of structural and regulatory sequences, and are subject to epigenetic regulation.
- DNA methylation is employed to limit the expression and mobilisation of transposable elements in the host genome.
- DNA methylation at transposable elements is relatively stable in most somatic cells, but is reprogrammed in germ cells and the pre-implantation embryo concomitant with the highest transposable element activity.
- DNA methylation acts in concert with other epigenetic complexes and chromatin modifications to enable robust silencing of transposable elements.

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Competing Interests

The author declares that there are no competing interests associated with the manuscript.

Abbreviations

DNMT, DNA methyltransferase; endosiRNA, endogenous siRNA; ERV, endogenous retrovirus; H3K9me3, Histone 3 lysine 9 trimethylation; H3K27me3HH, Histone 3 lysine 27 trimethylation; IAP, Intracisternal A-type particle element; LINE, long interspersed nuclear element; LTR, long terminal repeat; m6A, N6-methyladenosine; PGC, Primordial germ cells; piRNA, PIWI-interacting RNA; SINE, short interspersed nuclear element; TET, Ten-eleven translocation; YY1, Ying Yang 1; ZNF, Zinc finger protein; 5hmC, 5-hydroxymethylcytosine.

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