RESEARCH HIGHLIGHT

Transgenerational inheritance of engineered cytosine methylation in mice

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The occurrence of transgenerational epigenetic inheritance has overwhelming evidence in plants and invertebrates; however, the substantial erasure of DNA and histone modifications during early embryogenesis has been considered an insurmountable barrier to the transmission of epigenetic information in mammals. In a recent issue of *Cell*, Takahashi et al. demonstrate that engineered DNA methylation states of promoter-associated CpG islands can be inherited for multiple generations in mice.

DNA methylation and histone modifications are important regulators of chromatin organization, DNA replication and repair, transposon activity and gene expression. The deposition and removal of these modifications is a tightly regulated process that allows cell differentiation and adaptation to changing environments.[1] In invertebrates, plants and fungi, chromatin states were shown to be inherited independently of changes to the DNA sequence across multiple generations,[2] an example of what is known as "transgenerational epigenetic inheritance".

Methylation of nucleic acids naturally occurs at cytosine or adenine residues: while methylated adenine (m6A) is usually found in eucaryotic or viral RNA,[3] the methylation of cytosine residues (5mC) is a frequent event in CpG sequence contexts of DNA.[4] During mitosis, CpG methylation patterns are faithfully re-established on the newly synthesized strand by DNA methyltransferases in somatic cells.[5] In contrast, the zygotic DNA faces a global loss of DNA methylation after fertilization and only gradually re-establishes DNA methylation of CpG motifs in a process called epigenetic reprogramming.[6] Moreover, primordial germ cells (PGCs) experience another wave of de-methylation during early embryonic development.[6] Owing to these resetting events, transgenerational inheritance of parental DNA methylation patterns has been considered highly unlikely in mammals for many decades.[7] It is important to note, however, that some repetitive elements which belong to a class of endogenous retroviral mobile elements, named intracisternal A particles (IAPs), escape the de-methylation machinery allowing the transmission of heritable methylation states in mice.[8]

Now, Takahashi et al. showed that the inheritance of acquired methylation states is not restricted to highly repetitive transposable elements only but can also occur in promoter-associated CpG islands (CGIs) of endogenous genes.[9] In this study, the authors induced DNA methylation of normally unmethylated CGIs in promoter regions of two metabolism-related genes, the *Ankyrin repeat domain 26* (*Ankrd26*) and the *low-density lipoprotein receptor* (*Ldlr*), by temporally integrating CpG-free DNA stretches into their CGIs in mouse embryonic stem cells (mESCs) (Fig. 1a). Typically, CGIs in promoters of active genes remain unmethylated; Takahashi et al. previously found that artificially integrating CpG-free DNA into promoters leads to methylation of adjacent sequences and shutdown of transcription even when the trigger (the CpG-free exogenous DNA) is removed.[10]

In their new study, they first demonstrated that the methylation state was stably maintained after the removal of the CpG-free sequence from the promoters of two metabolism-related genes: up to 95% (*Ankrd26*) and 35% (*Ldlr*) of CpGs within the respective CGIs were methylated in a fraction of clones leading to a strong silencing of gene expression. By injecting these modified mESCs into 8-cell embryos of mice, high-contribution chimeras were generated for both genes. Interestingly, the hypermethylation status of the *Ankrd26* and *Ldlr* promoters was maintained throughout development and resulted in a strong phenotype when both alleles were hypermethylated (*Ankrd26*). Compared to their wild-type control, *Ankrd26* chimeras showed significantly elevated body weight and serum leptin levels resembling the phenotype of homozygous *Ankrd26* knockout mice. Strikingly, when crossing these chimeras with wild-type mice, the methylation state of *Ankrd26* and *Ldlr* promoters was inherited to the next generations (up to F6) through both the maternal and paternal germlines (Fig. 1b). Moreover, when two heterozygous F1 mice were crossed, the resulting homozygous hypermethylated progeny showed greatly diminished *Ankrd26* and *Ldlr* mRNA and protein levels as well as strong metabolic disorders resembling the phenotype of the original chimeras. In addition, H3K9me3, a histone modification usually associated with gene silencing, was highly enriched at methylated promoter-associated CGIs.

Next, the authors investigated how the methylation state of promoter-associated CGIs was transmitted through the germline, given the well-established near-complete erasure of DNA methylation during epigenetic reprogramming in the early embryos and later again in PGCs. To this end, Takahashi et al. collected PGCs, gametes, and early embryos of *Ankrd26* or *Ldlr* hypermethylated mice and performed bisulfate sequencing. Intriguingly, methylation of highly methylated CGIs was only partially erased in PGCs and was maintained during gametogenesis (Fig. 1c, left panel). In contrast, sparsely methylated CGIs showed almost complete loss of methylation during gametogenesis (Fig. 1c, right panel).

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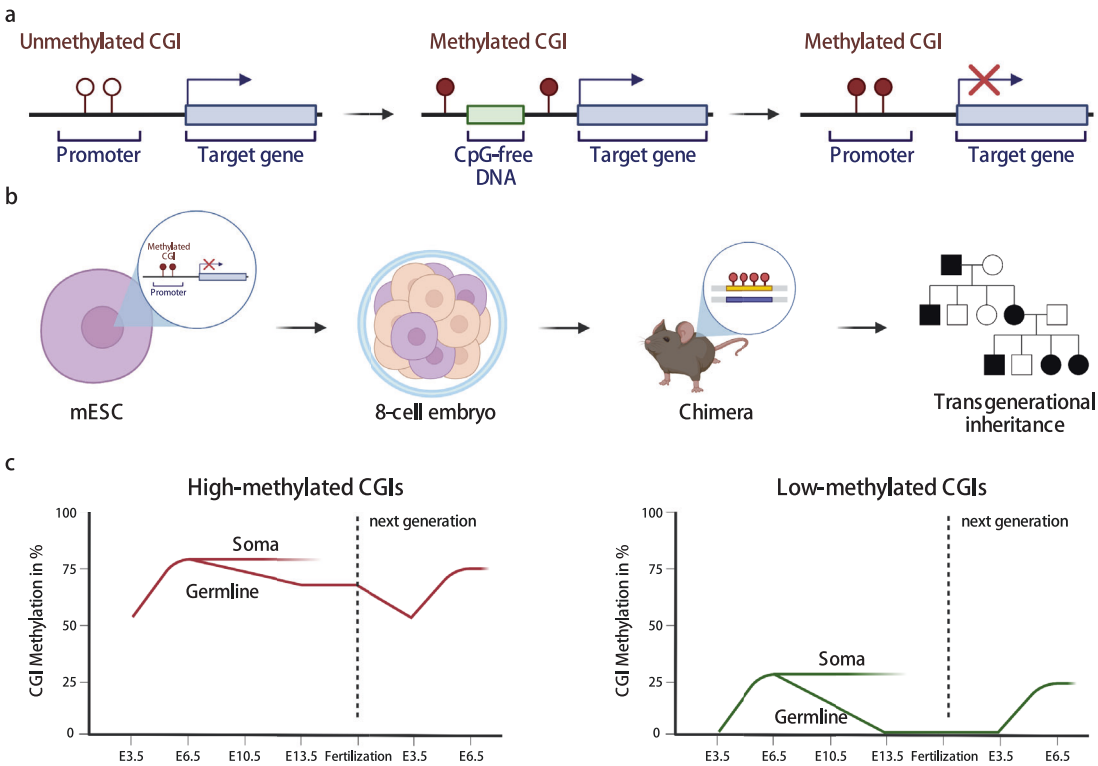

Fig. 1 Engineered cytosine methylation is inherited in mice. a In vitro methylation of CGIs by insertion and subsequent removal of a CpG-free stretch of DNA. **b** Generation and breeding scheme of mice harboring methylated CGIs in promoter regions. **c** Methylation dynamics in high- and low-methylated CGIs throughout development.

However, in both cases, these CGIs regained their methylation during epiblast formation (Fig. 1c).

This indicates that even when the methylation is removed completely from CGIs, the memory of methylation persists in some other form, and is inherited to the next generation. In other words, epigenetic modifications, such as CpG methylation, do not need to escape the global erasure of chromatin modifications, but can be re-established based on a so-far enigmatic molecular memory. The authors gathered compelling evidence that this memory is independent of any sequence context, i.e., the repetitiveness of transposable elements which was assumed to be required for the inheritance of methylation patterns of IAPs. Notably, the authors reported (although this data is not shown) that a simpler method for DNA methylation of CGIs, for example by the means of dCAS9 fused to a DNA methyltransferase, did not yield heritable phenotypes and methylation patterns. This supports the idea that the inherited memory of methylation patterns is not stored in the methyl group itself, but rather in additionally acquired chromatin signatures, such as histone marks, 3D folding, or perhaps in non-coding RNAs.

Taken together, Takahashi and colleagues greatly expanded our understanding of transgenerational inheritance of acquired traits in mammals. Their results suggest that transmission of epigenetic information might not be restricted to highly repetitive transposable elements but can also occur in promoter regions of endogenous genes. This is in line with the strong evidence for transgenerational inheritance in non-mammalian model organisms, such as *Arabidopsis*, *Drosophila* and *C. elegans*.[2] It remains to be seen to what degree these results could be generalized to other promoters (the authors reported similar observations in other loci that were not examined further in this study), and whether the effects are observed only upon artificial induction of CGI methylation through the reversible integration of long CpG-free DNA sequences into CGIs. While the results of this study await further experimental validation, their implications for most fields of biology and even human health could be enormous.

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ADDITIONAL INFORMATION

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