Feature Article

Epigenetic inheritance of acquired traits through DNA methylation

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Implications

- DNA methylation inheritance, including nuclear DNA methylation and mitochondrial DNA methylation, is a new and controversial issue since the mechanisms are still under heated debate.
- A better understanding of epigenetic inheritance mechanisms can lead to improvements in genetic evaluation.
- Exploring DNA methylation inheritance mechanisms could help us draw a better blueprint of how phenotypes can be shaped and provide us with a deeper understanding of evolutionary biology.

Key words: DNA methylation, epigenetic inheritance, mitochondrial DNA methylation, phenotype

Introduction

The DNA sequence can pass inheritable information to offspring by its precise replication during cell division. In mammals, DNA methylation mainly occurs in 5′-Cytosine-phosphate-Guanine-3′ (CpG) by adding a methyl group to the fifth carbon of the Cytosine (5mC). This mark is replicated at each cell division by the action of the catalytic enzyme named DNA methyltransferase 1 (DNMT1).

The earliest research regarding DNA methylation inheritance was documented in plants where DNA methylation was correlated to floral symmetry in *Linaria vulgaris*, proving that the Linaria Cycloidea (*Lcyc*) gene methylation mutation could be inherited for several generations (Cubas et al., 1999). When talking about DNA methylation inheritance, it is pivotal to distinguish between intergenerational and transgenerational inheritance. The former mainly indicates F0 to F1 transmission but with one exception: the germline (F2) of a fetus (F1) can respond to environmental factors while in utero. Under this condition, the F0, F1, and the future F2 (germline of the fetus) are all exposed to the altered environment, thereby constituting intergenerational inheritance. Transgenerational inheritance means that the altered epigenetic modification can be inherited even if the successive offspring are not directly exposed to the same environment.

This paper summarizes the recent progress of 5mC inheritance research in humans and animals, emphasizing dairy cows. The first section of this review summarizes the potential mechanisms involved in DNA methylation inheritance, while sections two and three discuss paternal and maternal 5mC inheritance. The remaining sections include a description of possible mechanisms of mitochondrial DNA methylation inheritance, and 5mC inheritance in different species. Finally, the challenges and limitations of research in DNA methylation inheritance are presented.

Why DNA methylation is inheritable?

Several prerequisites must be met to achieve DNA methylation inheritance between generations. Firstly, DNA methylation should be able to pass from somatic cells to their daughter cells as cells are dividing. Secondly, DNA methylation should be capable of being maintaining from the somatic cells to the germline. Last but not least, DNA methylation should resist the two DNA methylation reprogramming events that happen after fertilization and early gonadogenesis.

Soma to Soma. DNA methylation includes *de novo* methylation and maintenance methylation. *De novo* methylation requires DNMT3a, DNMT3b, and DNMT3L to establish methylation on unmodified DNA. In contrast, the maintenance of DNA methylation requires DNMT1, which catalyzes CpG methylation in a copy-paste manner, therefore causing symmetrically methylated CpG dinucleotides in both DNA strands. Although this process is stable, if DNMT1 and UHRF1 (ubiquitin-like with PHD and RING finger domains 1) are at a low level, DNA methylation will be diluted as cells divide (passive demethylation) (Harrison et al., 2016). As for active demethylation, it requires the TET (Ten-eleven translocation) protein family (TET1, TET2, TET3) to oxidize the 5mC to 5hmC,5fC, and finally to 5CaC and therefore restoring the non-methylated cytosine status.

Soma to germ-line. Current research points to three potential information carriers that could achieve soma to germline

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transmission: the small non-coding RNA (sncRNA), the chromatin state (histone modifications), and DNA methylation.

Chen et al., (2016) proposed that extracellular vesicles (EV) could potentially transfer sncRNA from somatic cell to the germline as the provided evidence that the concentration of tRNAs-derived small RNA (tsRNA) was higher in epididymis than in testis and epididymosomes can fuse with and transfer tsRNAs into sperm, which indicates that mature sperm could absorb sncRNA via EV transfer (Figure 1).

As for DNA methylation, the molecules that mediate the transmission of methylation status between soma to germline could be mobile RNAs, hormones, odorants, metabolites, transcription factors, and cytokines (Allis et al., 2015; Kazachenka et al., 2018). Cytokines are sensitive to environmental stimuli and can be distributed rapidly to the whole body. Besides, sperm have been reported to express hormone and olfactory receptors (O'Hara & Smith, 2015; Milardi et al., 2018), therefore, the male germline could respond to the hormone, cytokine, and odorant fluctuations in the blood (Yankulov, 2015).

Between generations

Two potential models for DNA methylation generational inheritance were proposed, namely, the "escapee model" and the "reconstruct model" (Figure 2).

Escapee model. After fertilization, the paternal genome undergoes rapid active demethylation, while slower passive demethylation happens to the maternal genome. *De novo* methylation then begins in blastocysts as tissues start to differentiate. Subsequently, a second more complete demethylation occurs during primordial germ cell (PGC) migration to the undifferentiated gonad. However, DNA methylation reprogramming does not eliminate all DNA methylation marks (Irmler et al., 2020), and some regions can resist this process and act as the so-called "escapee" marks (Figure 2).

Imprinted genes, for example, expressed only from one of the parental chromosomes, are regulated by methylation of imprinting control regions (ICRs). During the gametogenesis process, a non-erased imprint may lead to lethality or other specific diseases (Buiting et al., 2003). Besides, other regions such as repetitive elements, evolutionary young retrotransposons, and some single copy loci can escape the DNA methylation reprogramming process and, therefore, can be theoretically transmitted to future offspring (Table 1). For example, many loci were reported to act as escapees in humans, as they were at a minimum of 30% methylation level in the PGC development process (Tang et al., 2015). In this well-designed research, approximately 1,400 escapee regions were in repeat-free loci, mainly in promoter, enhancer, gene body, and CpG-island (CGI) regions. The Transforming Acidic Coiled-Coil Containing Protein 2 (TACC2) is an example of an androgen-responsive cell cycle regulator with an escapee

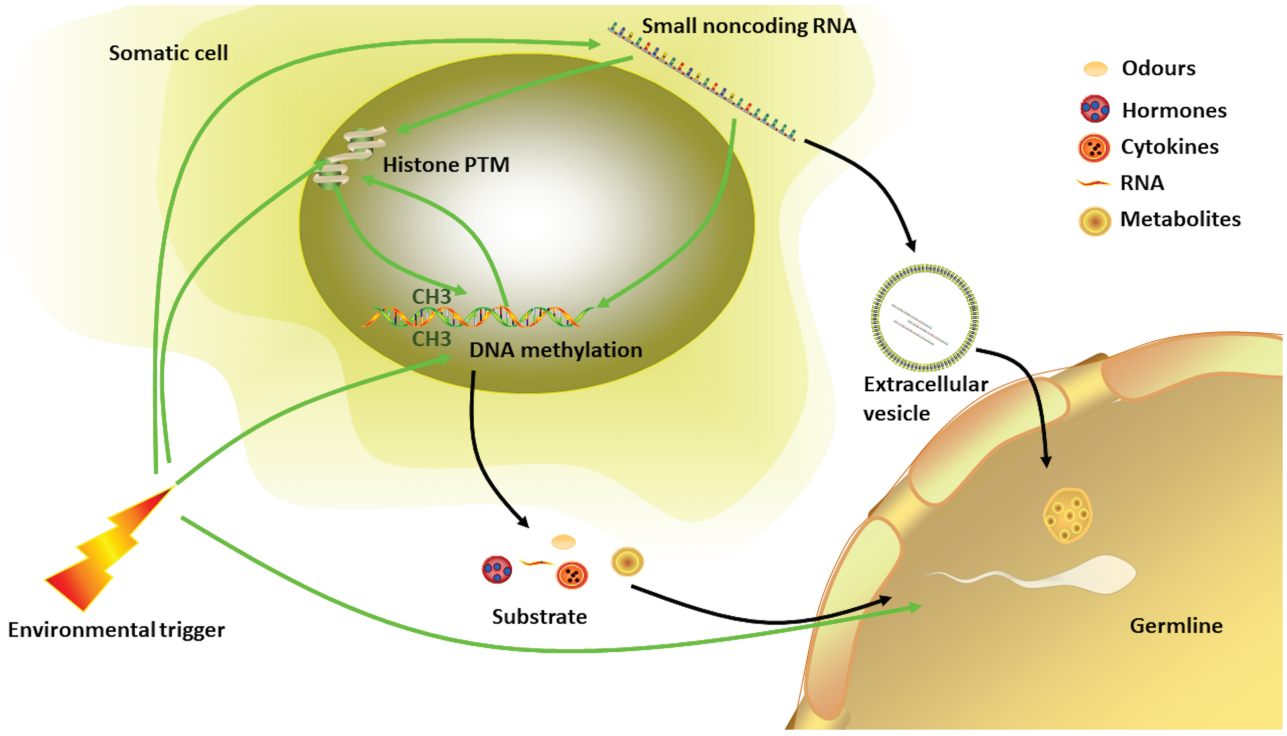


Figure 1. Potential pathways for epigenetic information flow. Environmental factors are possible to stimulate the somatic cell and germ cell response. In the somatic cells, epigenetic changes, including DNA methylation, histone PTM (Post-Translational Modification), and small non-coding RNA, could react accordingly and communicate with each other. Besides, the changed DNA methylation status in somatic cells may transmit to the germline via the intermediate of some substrate such as odors, hormones, cytokines, RNA, or metabolites. Furthermore, the altered small non-coding RNA is possible to be passed onto the germline via extracellular vesicles.

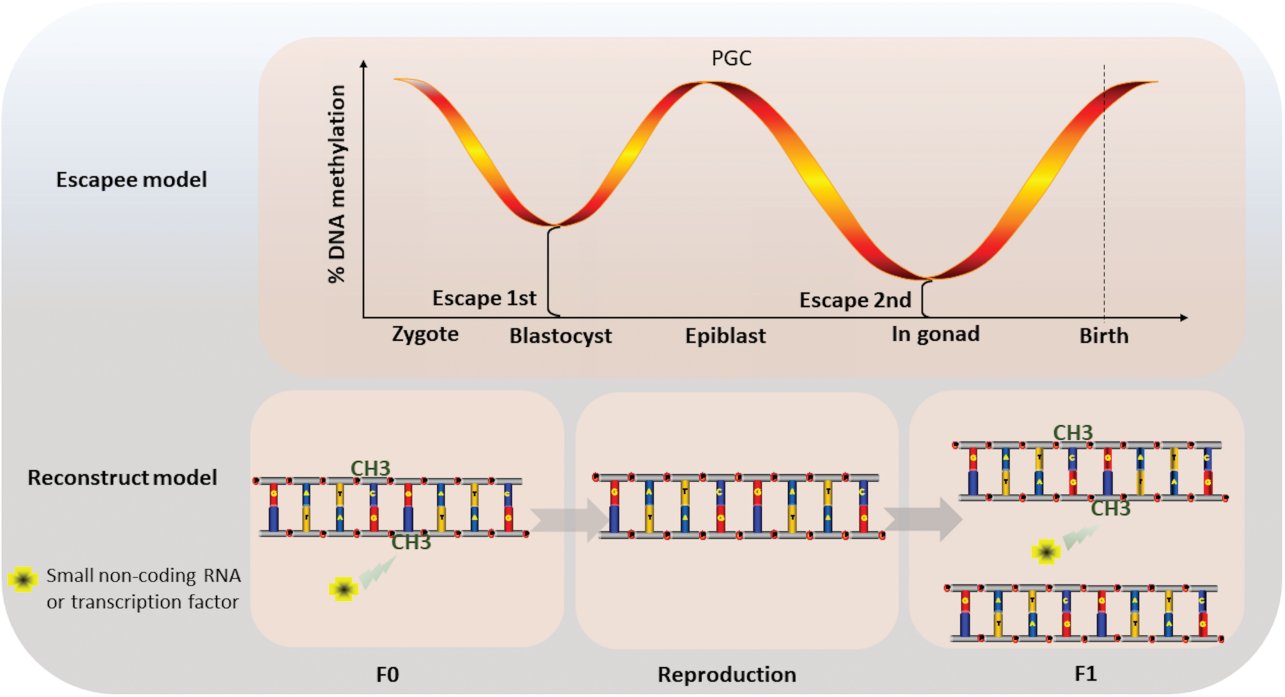


Figure 2. Potential mechanisms of DNA methylation inheritance across generations. Some regions could escape DNA methylation reprogramming after fertilization and the PGC development process in the escapee model. In the reconstruct model, after reproduction process, DNA methylation may re-establish with the help of some small non-coding RNAs or transcription factors.

region in its promoter. Many of these escapee-associated genes are expressed in the brain, with latent association to nerve and metabolic disorders (Tang et al., 2015).

Another study in mice showed that 4,730 loci could escape the PGC methylation reprogramming. More than 95% of these loci were repetitive elements, and 233 single copy loci with a methylation level higher than 40% were observed. Interestingly, these escapees were always adjacent to telomeric or intracisternal A particle (IAP) elements (Hackett et al., 2013). The IAP is an endogenous retroviral sequence and constitutes a class of transposable elements that could induce genomic mutations. Regions and elements that can escape the DNA methylation reprogramming process are summarized in Table 1.

Reconstruct model. In 2013, Jablonka proposed that environmentally induced DNA methylation could be partially erased when transmitted to the next generation (Jablonka, 2013). Therefore, even if the observed phenotype may disappear in the F1 generation, the altered phenotype could be observed again in later generations following a minor environmental stimulation. Later, Miska & Ferguson-Smith (2016) proposed that sncRNA, transcription factors, and metabolic loops could regulate this reconstruct process (Figure 2). The reconstruct model was later partly confirmed by Kazachenka et al. (2018), as their study proved that the methylation of the variably methylated IAP transposons could be re-established between generations. These variably methylated IAP transposons are flanked by binding sites for CTCF (CCCTC binding factor), CTCF is known as a transcription factor, which can be considered a modulator of the methylation status. Therefore, the variably methylated IAPs could be inherited by offspring in a reconstructed way with the help of CTCF.

The interplay between epigenetic markers

The possible interplay between DNA methylation, small non-coding RNA, and histone PTM is summarized in Figure 1. The loss of H3K9 methylation in embryonic stem cells had been reported to cause CpG methylation to decrease in centromeric satellites, indicating that DNA methylation can be directly regulated by histone methylation such as H3K9 methylation (Lehnertz et al., 2003). Meanwhile, DNA methyltransferase (DNMT) and methylation CpG binding domain (MBD) could recruit complexes containing histone deacetylases (HDACs), therefore influencing histone acetylation (Bird, 2002). Thus, histone PTM and DNA methylation could regulate and influence each other.

As for sncRNA, they could also modulate DNA methylation and histone modification. Small non-coding RNAs usually function by silencing the target mRNA. This process recruits Argonaute (AGO) or PIWI (P-element Induced Wimpy testis) proteins, which lead to further recruitment of chromatin-modifying enzymes (CMEs) or DNA methyltransferase (DNMT), thereby regulating histone modification and DNA methylation.

Table 1. Reported regions and elements that have the escapee character

Regions/elements	Human	Mouse	References
Retrotransposon	Short interspersed nuclear element-variable number of tandem repeats-Alu (SVA)	Intra-cisternal A particle (IAP)	(Tracey et al., 2013; Tang et al., 2015; Morgan et al., 1999)
Sub telomeric regions		+	(Guibert et al., 2012)
Pericentromeric satellite repeats	+		(Tang et al., 2015)
Single copy loci		+ (Usually adjacent to IAP or telomeric regions)	(Guibert et al., 2012; Hackett et al., 2013)
Long Interspersed nuclear element-1 (LINE-1)	+		(Tang et al., 2015)
Repeat free regions (promoter, enhancer, exons, gene body)	+ (About 1426 repeat free escapees located in these regions)		(Tang et al., 2015).
L1 Homo sapiens-specific (L1HS) transposon	+		(Gkountela et al., 2015)
HERVK transposon	+		(Tang et al., 2016)
LTR–endogenous retrovirus sequence 1 (LTR-ERV1)		+	(Guibert et al., 2012)
DMR of imprinting loci (escape first erasure and resistance to second erasure)	+	+	(Monk, 2015)
Endogenous retrovirus K (ERVK)		+	(Popp et al., 2010)
Variably erased CpG island (VEC)		+	(Seisenberger et al., 2012)

Note. + indicates the regions or elements that had an escapee character had been reported.

Paternal DNA methylation inheritance

Only the germline has the potential to carry nongenetic information to the next generations. Many factors were reported to influence paternal DNA methylation inheritance. For example, nutrition has a significant impact on DNA methylation inheritance. In rodents, offspring of male rats fed a high-fat diet harbored specific DNA methylation patterns (Ng et al., 2010) and showed a reduced birth weight and decreased numbers of islet B cells (de Castro Barbosa et al., 2016), besides, after being fed with a high-fat diet, the sperm of the F0 and F1 rats showed similar DNA methylation and microRNA profile changes (de Castro Barbosa et al., 2016). In male mice, abnormal sperm DNA methylation triggered by prediabetes could also be transmitted and increased the risk of diabetes in the following two generations (Wei et al., 2014a).

Other environmental triggers such as chemical molecules were also reported to induce paternal DNA methylation inheritance. The exposure of gestating rats to high doses of endocrine disrupters such as insecticides and fungicides resulted in altered sperm DNA methylation in male offspring, lasting at least four generations (Anway et al., 2005). Similarly, the F1 male offspring of mice exposed to endocrine disrupters during pregnancy showed spermatogenic disorders accompanied by methylation alterations. Interestingly, there were no significant effects in female offspring in this study (Anway et al., 2005). Besides, other endocrine disruptors such as bisphenol A had also been reported to alter sperm DNA methylation, causing hypomethylation of Long Interspersed Element-1 (LINE-1) (Miao et al., 2014). Weyrich et al. (2016) demonstrated that exposure of male wild guinea pigs to high temperatures decreased DNA methylation levels in the liver and testis of F0 and F1 (Weyrich et al., 2016).

In zebrafish, maternal DNA methylation profiles are only preserved until the 16-cell stage after fertilization, and then they are erased and reprogrammed. At the blastocyst stage, maternal DNA methylation mimics the sperm methylation profile, indicating that the father plays a more significant role in DNA methylation inheritance in zebrafish (Jiang et al., 2013).

Maternal DNA methylation inheritance

Imprinted genes, heterochromatin in centromeric-pericentromeric regions, and repeat elements had been reported to escape DNA methylation reprogramming on the maternal side (Ge & Sun, 2019). The most persuasive evidence of maternal DNA methylation inheritance is the Agouti viable yellow (Avy) loci in mice. Although genetically identical, the coat color of the mice may range from entirely brown to yellow, the methylation of transposable elements could explain this quantifiable color transition phenomenon. The mothers of yellow agouti mice tend always to bear offspring of identical coat color, which can be observed through multiple generations (Cropley et al., 2006). In rodents, transposable elements make up approximately 40% of the whole genome, and most transposons are usually methylated to maintain genome integrity. Only 1% of them could be variably methylated, which means that they could respond to external factors and be inherited by future generations (Kazachenka et al., 2018).

Another well-documented example is the Axin-fused (AxinFu) mouse model. In this model, IAP methylation regulates the tail kink phenotype within AxinFu, and DNA methylation can be maternally and paternally inherited in this model (Rakyan et al., 2003). The two cases mentioned above are both transposon methylation inheritance.

Considering the factors that affect maternal DNA methylation inheritance, nutritional intake (zinc, vitamin B12, folic acid), maternal obesity, and diabetes can all influence DNA methylation in oocytes, indicating a latent maternal intergenerational DNA methylation inheritance. Similarly, chemicals can also alter oocyte DNA methylation. The exposure of female mice to chemicals such as cyclophosphamide (CPM) before conception resulted in methylation alterations in F1 oocytes, which can be further passed on to the F2 generation (Giovanna et al., 2019).

Is mitochondrial DNA methylation also inheritable in a maternal-dependent way?

The mitochondrion has a unique structure and is maternally inherited, since sperm mitochondria DNA is in the middle piece of the sperm body and will be lost during fertilization. Abnormal mitochondria DNA mutations can be maternally inherited for several generations (Ge & Sun, 2019). By possessing its DNA and machinery, this organelle can perform DNA replication, transcription, and translation. There are no introns or intergenic regions in the mitochondrial genome, and the D loop structure controls gene regulation and replication. Moreover, gene expression regulation in mitochondria is associated with mtDNA methylation, which might exist in different forms, including 6mA, CpG methylation, CHH methylation, and CHG methylation. The enzymes DNMT1, DNMT3a, DNMT3b, TET1, and TET2, were detected in mitochondria, proving the existence of CpG methylation in this organelle (Lopes, 2020).

Moreover, previous research indicated that mtDNA 5mC is maternally inherited, especially in D-loop regions. However, a later study from Koh et al. (2018) found that the 6mA content was 8000 fold higher in the mitochondrial genome than the nuclear genome. Hao et al. (2020) further validated 6mA enrichment in human mitochondria and found that 6mA affected the replication and the transcription processes of mtDNA. These findings suggest that 6mA might act as the dominant DNA methylation in human mitochondria. Our team was the first to study the dynamics and distribution of mtDNA 5mC in dairy cows (Sirard, 2019). We suggested that mtDNA methylation could be inherited from oocytes to early embryos since mtDNA methylation patterns were more conserved between oocytes and blastocysts than granulosa cells. Another recently published article from our team also indicated that the 5mC frequency is biased and not symmetrically distributed on both strands of bovine mitochondrial DNA in oocytes and embryos (de Lima & Sirard, 2020). Mitochondrial DNA methylation may also respond to different environmental stressors such as metals, smoking, food supplements, drugs, and pollutants (Sharma et al., 2019). Together, the correlation between mtDNA methylation, maternal inheritance, and phenotype is still a new and attractive field of study that requires much more work and validation.

Evidence of inter and transgenerational inheritance of DNA methylation in different species

Humans. In humans, epidemiological studies indicated that parental food shortage or undernutrition made offspring more vulnerable to developing metabolic or cardiovascular diseases. Another epidemiological study indicated that the DNA methylation level on gene FK506 binding protein 5 (FKBP5) was higher in holocaust survivors compared to control groups, however, it decreased in offspring of holocaust survivors (Yehuda et al., 2016). FKBP5 is an essential regulator of glucocorticoid receptor sensitivity, which had been reported to be correlated with intergenerational effects (Lehrner et al., 2014). There is also evidence that is not epidemiologically based. For example, seasonal variations in methyl donor consumption during conception influenced the methylation status of 13 relevant plasma biomarkers, and these methylation changes could also be detected in infants' lymphocytes and hair follicles (Dominguez-Salas et al., 2014). Besides, cold weather exposure before mating had also been reported to lead to a higher CpG methylation level in sperm, accompanied by a change in the metabolic status in offspring (Skinner et al., 2018).

In general, human research showed that an altered parental environment could lead to changes in DNA methylation of offspring. However, few studies provided precise mechanisms by which specific loci could escape or re-establish methylation during (or after) methylation reprogramming and how the changes could be inheritable.

Rodents. A large number of studies were conducted to evaluate the impact of environmental toxicant exposure in mice, and alterations of DNA methylation in F3 were observed in several of these studies. Vinclozolin was the first chemical molecule to be associated with DNA methylation transgenerational inheritance. When pregnant rats were exposed to this fungicide, the F1 fetus and their germline (future F2) were also exposed at the same time, DNA methylation of the sperm was found altered in each generation with the direct exposure F1 and F2 generations being distinct from the F3 generation DNA methylation alteration (Ben Maamar et al., 2018). Furthermore, gestational nutrition conditions had also been proved to influence the F1 methylome alteration in a loci-specific way (Radford et al., 2014).

Other factors such as age, specific odors, and maternal separation could also impact the DNA methylation status in offspring. For example, Xie et al. (2018) showed that aging fathers and their offspring shared a similar DNA methylation alteration profile. Interestingly, another study indicated that acetophenone (odor) exposure to F0 mice could activate Olfr151, which is a known odor receptor, sperm DNA methylation from conditioned F0 males and F1 offspring showed hypomethylation CpG in the Olfr151 gene (Dias & Ressler, 2014). Besides, maternal separation may lead to offspring depression, and this phenomenon was correlated with the hypermethylation of the methyl CpG binding protein 2 (MeCP2) and the hypomethylation of

corticotropin-releasing factor receptor 2 (CRFR2) in F1 and F2 males (Liberman et al., 2019).

Dairy cows. Environmental factors may induce inheritable phenotypes in a nongenetic way in dairy cattle (Wu & Sirard, 2020). A study published by our team showed that specific DNA methylation changes in sperm DNA were associated with bull age (early-, peri- and post-puberty) (Lambert et al., 2018). A subsequent study showed that the age of the bull correlated with DNA methylation and transcriptome alterations in blastocysts, indicating a potential effect of age on offspring development (Wu et al., 2020). Additionally, metabolic stress in cows resulted in alterations of the DNA methylation profile of embryos. The affected pathways were mainly enriched in metabolic and mitochondrial dysfunction signalings (Chaput & Sirard, 2020), when these embryos were further transferred to non-metabolically stressed mothers, the newborn daughters displayed a specific blood DNA methylation signature as characterized by Whole Genome Bisulfite Sequencing (WGBS). A total of 1,861 Differentially Methylated Regions (DMRs) and 944 Differentially Methylated Cytosines (DMCs) were identified. Most DMRs were in intronic and intergenic regions, and DMR in promoter regions was mainly hypermethylated. Differentially Methylated Genes (DMGs) with methylation differences higher than 20% were mainly enriched in metabolism-related pathways. These results suggest that metabolism-related pathways in newborn calves were altered, with 64 DMGs clustered in metabolic signaling by Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis (Zhang et al., 2021).

Poultry and pig. Chicken is an important farm animal that could produce about 300 eggs per year and showing a high economic value. To our knowledge, transgenerational research that was performed on chicken mainly focused more on the inherited phenotype instead of the detailed mechanism. For example, stress could induce the transgenerational modification of the chicken behavior, and the effects differ in the age at stress (Ericsson et al., 2017); however, no detailed mechanism had been documented. Besides, maternal whole blood serotonin, plasma -corticosterone, and feather damage are correlated with their offspring's feather pecking behavior (Ericsson et al., 2017). As for the pigs, one study showed that BPA exposure during pregnancy could decrease the DNA methylation of jejunum Pept1 in offspring (Liu et al., 2017). Another study on pigs showed that diet affects the future offspring epigenome; the differences in DNA methylation between F2 individuals could be induced by the differential feeding of F0 pig (Braunschweig et al., 2012). Moreover, methyl donor supplementation during gestation is also associated with the DNA methylation alteration in the IGF-1 gene promoter of the offspring (Jin et al., 2018).

The interplay between genetics and DNA methylation

Previous research indicated that most of the similarities in DNA methylation transgenerational research were attributed to genetic factors (McRae et al., 2014). 4.7 million cis and 630 thousand trans-meQTL had been identified in a previously published human blood research (Huan et al., 2019). Saadi et al. (2017) found that identical twin bulls produced offspring with divergent phenotypes and sperm with different methylation patterns (Saadi et al., 2017). They postulated that the different performances of daughters of identical twin bulls could be attributed to epigenomic differences. However, another study on twins suggested that identical twins' DNA methylation was surprisingly similar compared to dizygotic twins because of their similar genetic background (Hannon et al., 2018). This finding indicates that genetic factors strongly influence DNA methylation, requiring scientists to better control genetic background when performing DNA methylation inheritance research (Hannon et al., 2018). Besides, another mathematical model also showed that epigenetic modifications were genetically selected (Feinberg & Irizarry, 2009). It is also noteworthy that DNA methylation can be regulated by both *cis* and *trans* components (Orozco et al., 2015), when using genome-wide association study (GWAS) to map all the CpG to the single nucleotide polymorphisms (SNPs), 52% of hypervariable CpG were tightly associated with gene regulatory regions, and over half of the correlations were in *cis*. Meanwhile, DNA methylation could also be regulated by *trans* elements. For instance, the transcription factor FOXA1 could bind to CpG sites and block methyltransferase activity, leading to hypomethylation of CpG sites (Zhang et al., 2016).

The extreme case could be a genetic mutation that occurs at a CpG site. For example, spontaneous deamination of methylated Cytosine leads to Cytosine to Thymine transformation (You & Jones, 2012). In general, any mutation that replaces a Cytosine may influence methylation at that site, and single-site methylation can still cause a phenotype change, although the possibility is low. Johnson et al. found that sequence variations exist upstream or downstream of differentially methylated CpG sites (Johnson et al., 2014). It is also noteworthy that sometimes a genetic mutation will lead to an epimutation which could be named as the secondary epimutation and leading to transgenerational inheritance. Therefore, an accurate DNA methylation transgenerational inheritance should be caused by primary epimutation without altering the genetic component rather than the secondary epimutation (Horsthemke, 2018).

Challenges and perspectives

The scientific community has long overlooked Lamarck's theory that organisms can transmit acquired traits to their offspring. Although some detailed mechanism of the new phenotype acquisition due to altered DNA methylation inheritance have been elucidated, much more work is still needed to further solidify the mechanism of how epigenetic information flows from somatic cells to germline then be delivered to zygotes and finally be inherited to subsequent generations.

Research on inter or transgenerational inheritance of DNA methylation often includes confounding factors that are difficult to exclude (Figure 3). One of the critical confounding factors is DNA sequence differences, which play a more direct and

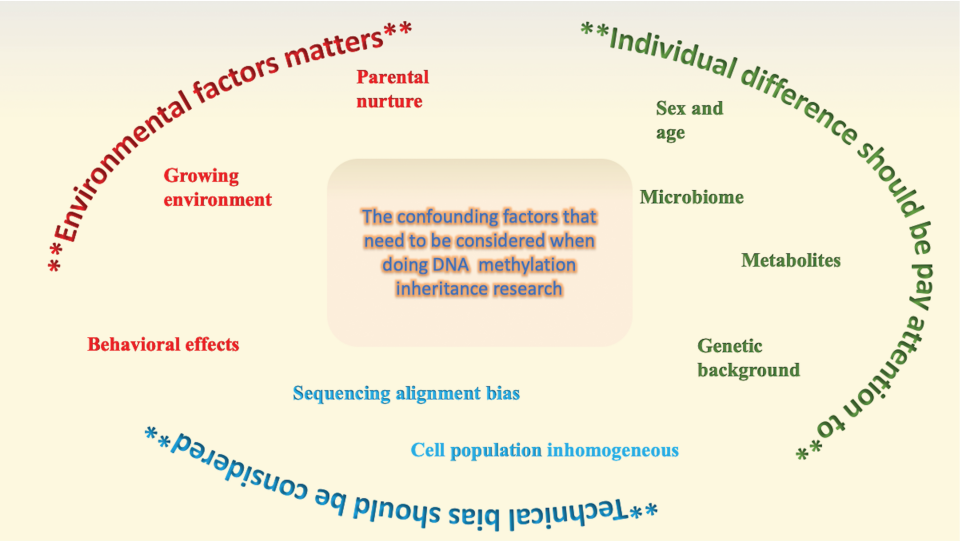
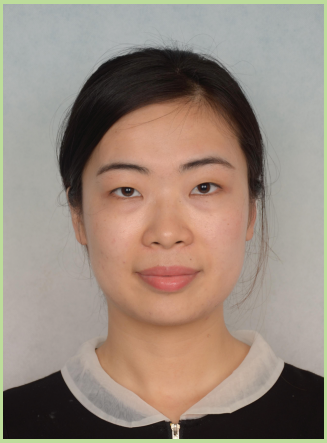


Figure 3. Confounding factors that should be considered in DNA methylation inheritance research. Environmental factors, individual difference and technique bias could all be the possible confounding factors which should be considered at the initial of the study design.

About the Authors

Marc-André Sirard is a full professor in the Department of Animal Sciences of the Faculty of Agriculture and Food Sciences at Laval University. He also holds the Canada Research Chair in functional genomics applied to animal reproduction since 2000. His work on oocytes and follicles in the bovine model has led to a better understanding of oocyte competence and generated technologies to improve oocyte quality both in domestic animals and in humans. With the goal of improving oocyte health, his laboratory began exploring the epigenetic contribution of male and female gametes as well as the in vitro influence on the early embryos, especially in the in vitro context. **Corresponding author:** marc-andre.sirard@fsaa.ulaval.ca



Ying Zhang currently is a Ph.D. candidate in the Department of Animal Sciences of the Faculty of Agriculture and Food Sciences at Laval University. Nowadays, she focused on screening epigenetic biomarkers that correlated with bull fertility, expecting combining the epigenetic and genetic tools together, providing a better breeding tool for the dairy farmers to improve their dairy cattle fertility. She received her MS degree from Northwest A&F University, specializing in male germline stem cell and small chemical molecules induced cell reprogramming. She also holds a BS in veterinary medicine from Northwest A&F university.

vital role in shaping the observed phenotype than DNA methylation differences. Additionally, parental nurture, behavioral effects, microbiome, and metabolites should all be considered as confounding factors in such research.

Genome-Wide Association Study (GWAS) was adopted to perform association analysis to link single nucleotide polymorphisms (SNPs) or Quantitative trait loci (QTL) to a specific phenotype. Correspondingly, Epigenome Wide Association Study (EWAS) could be adopted to link phenotype with DNA methylation. However, this type of study usually requires a large sample number, higher statistical power, and stringent environmental control to exclude confounding factors as much as possible. From a statistical perspective, the correlation between the phenotype and the altered methylation region will benefit from whole-genome methylation sequencing to identify specific functional epimutations. Another confounding factor we need to consider is the sequencing alignment bias caused by repetitive elements (Treangen & Salzberg, 2011). Moreover, methylation analysis of pooled cells could also mask individual cell differences and introduce a bias.

Conclusion

All in all, genetic knowledge cannot fully explain all the observed phenotypes. Studies on plants and identical twins further proved and complemented this. Therefore, exploring non-Mendelian and non-DNA-based inheritance mechanisms, especially the DNA methylation inheritance mechanism, could help us draw a more detailed blueprint of how phenotypes can be shaped, thus providing us a better understanding of evolutionary biology.

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