Unravelling the complex mechanisms of transgenerational epigenetic inheritance

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Abstract

There are numerous benefits to elucidating how our environment affects our health: from a greater understanding of adaptation to disease prevention. Evidence shows that stressors we are exposed to during our lifetime might cause disease in our descendants. Transgenerational epigenetic inheritance involves the transmission of 'information' over multiple generations via the gametes independent of the DNA base sequence. Despite extensive research, the epigenetic mechanisms remain unclear. Analysis of model organisms exposed to environmental insults (e.g., diet manipulation, stress, toxin exposure) or carrying mutations in the epigenetic regulatory machinery indicates that inheritance of altered DNA methylation, histone modifications, or non-coding RNAs are key mechanisms. Tracking inherited epigenetic information and its effects for multiple generations is a significant challenge to overcome.

Highlights

- Epigenetic information can be altered by environmental stressors.

- The mechanisms of epigenetic inheritance are complex and unclear.

- DNA and histone modifications, and non-coding RNAs are mechanistic candidates.

- Heritable epigenetic marks at transposable and repeat elements may be key.

- This phenomenon has broad implications from evolution to disease prevention.

Abbreviations

F0, parental generation; F1, first filial generation; F2, second filial generation; F3, third filial generation; IAP, intracisternal A particles; DMR, differentially methylated regions; miRNA, microRNA; ncRNA, non-coding RNA; piRNA, piwi-interacting RNA; RNAi, RNA interference; TEI, transgenerational epigenetic inheritance

Introduction

In recent years, the concept that epigenetic factors are inherited has rapidly developed. As more studies show that environmental stressors (e.g., poor diet, toxins, or psychological stress [1-4]) influence the epigenome, it is becoming clear that the environment experienced during our lifetime may impact the health of our descendants. How commonplace epigenetic inheritance is and the underlying mechanisms remain uncertain, though substantial research over the last few years have improved our understanding of this phenomenon.

We define transgenerational epigenetic inheritance (TEI) as the transmission of non-DNA base sequence information between generations via the germline [5,6]. Epigenetic changes in the first generation (F0) occurring after exposure to an environmental insult increases risk for specific phenotypes in subsequent generations (F1, F2, F3, etc.) even when they are not exposed to the insult themselves. To be transgenerationally inherited, the phenotype must persist beyond the F2 generation when inherited via the paternal lineage and F3 generation via the maternal lineage [5,6] (Figure 1). Both sperm and oocytes [7-9] transmit epigenetic information to the next generation, but paternal inheritance is typically studied for experimental tractability and lack of confounding influences (e.g., the uterine environment). A multigenerational search for inherited epigenetic factors, such as DNA and histone methylation, and non-coding (nc) RNAs, has ensued.

TEI in human populations is becoming evident [10], though it is difficult to study due to long generation times, genetic diversity and variable environmental conditions wherein we live. Plant and animal models of TEI, in

which genetic and environmental conditions are meticulously controlled, are key for mechanistic exploration and for overcoming the challenges associated with tracking epigenetic information over multiple generations. A greater understanding of TEI will have important implications for disease risk prediction and prevention.

DNA Methylation: an important mechanistic candidate

Methylation of single DNA residues is well studied in the context of TEI. In mammals, 5-methylcytosine (5mC) is the predominant form of methylated DNA. In organisms (e.g., bacteria, fungi, *Caenorhabditis elegans* and *Drosophila melanogaster*) that lack or have low levels of 5mC, other forms of methylated DNA, such as the recently identified N6-methyladenine (6mA), are widespread [11]. 5mC is generally associated with gene repression [12] whereas 6mA is thought to promote activity [13]. The reality may be far more complex; linking methylation status to a specific gene expression profile and phenotype is challenging. For DNA methylation to be a heritable epigenetic mark, it should be mitotically and meiotically stable [5,6] and escape epigenetic reprogramming that normally occurs in primordial germ cells and post-fertilization embryos [14-16] (Figure 2). This epigenetic 'erasure' generates a totipotent state required to form the next generation [16]. Remarkably, 5mC within specific genomic regions including repeat sequences (e.g., intracisternal A particles [IAPs]) and rare regulatory elements (e.g., promoters next to IAPs) is resistant to reprogramming [14,15] (Figure 2). Presumably, this occurs to maintain genomic stability during widespread erasure [17]. Abnormal DNA methylation patterns caused by environmental

stressors would have to generate resistance to reprogramming to appear and cause phenotypes in subsequent generations.

Owing to their resistance to reprogramming, the methylation status of repetitive elements is a mechanistic candidate of TEI [6,15]. A classic mouse model of TEI involving an IAP element is the agouti viable yellow (A^{vy}) epiallele [18]. Hypomethylation of a cryptic promoter in the IAP element upstream of the agouti gene drives its expression leading to a yellow coat colour, obesity and diabetes [19]. This hypomethylated status is inherited over several generations through the maternal line [18] and can be manipulated by environmental factors [20,21]. For example, providing a methyl-rich diet to A^{vy} females decreases the frequency of yellow coats in their offspring [20,21]. It is unclear whether DNA methylation at the IAP element is normalised [21] or if an indirect effect is responsible [20].

Beyond the A^{vy} model, it has been difficult to identify differentially methylated regions (DMRs) in the genome that are stable over multiple generations and that correlate with a phenotype. This is even when unbiased approaches to assess the germline methylome are implemented. One successful example is in a pre-diabetic mouse model characterized by insulin resistance and impaired fasting glucose [22]. F0 males transmit a similar pre-diabetic phenotype to the F1 and F2 generations [22]. Whole 5mC methylome analysis of sperm from F0 males revealed altered DNA methylation patterns compared to controls [22]. However, only a few of these abnormal patterns persisted in pancreatic islets of the male F1 and F2 offspring [22]. Conversely, unbiased methylome analysis of sperm from mice (F1) exposed to severe undernutrition while *in utero*, revealed altered DNA methylation that coincided

with reduced birth weight and a robust metabolic phenotype [2,23]. Over 100

DMRs concentrated in CpG islands and intergenic regions were identified [2].

However, the subset of DMRs that were assessed in F2 somatic tissues were

not maintained, even though neighbouring genes showed altered expression

and the metabolic phenotype was observed [2]. This suggests a parallel

epigenetic mechanism may be involved. Future methylome-wide analysis of

the F2 generation and beyond will more thoroughly determine whether DMRs

are inherited.

Reproducibility of TEI data is another challenge. An example of this is

the rodent vinclozolin model [24]. Males (F1) exposed *in utero* to the

endocrine disruptor vinclozolin transmit several adult onset diseases up to the

F4 generation [4]. Analysis of promoter regions revealed widespread

alteration of 5mC in mature sperm of the F3 generation following ancestral

vinclozolin exposure [25]. However, others showed that altered DNA

methylation patterns in purified prospermatogonia of the F1 offspring were not

apparent in the F2 generation [26]. The discrepancy between studies may

come down to technical differences, including the sperm population assessed

and method of methylation analysis used, or it may reflect the natural

epigenetic variability that exists between individuals [27].

Dysregulation of methylation machinery may initiate TEI

The machinery vital for the establishment and maintenance of DNA

methylation may be an important initiator of TEI. In *Arabidopsis thaliana*, a

mutation in the *DNA METHYLTRANSFERASE 1* (*MET1*) gene leads to

heritable hypomethylation at a repetitive region near the transcriptional start

site of the *FLOWERING WAGENINGEN* (*FWA*) gene [28]. This hypomethylation leads to ectopic *FWA* expression causing a late flowering phenotype for several wildtype generations [29]. Similarly, mutations in the mouse homolog of *MET1,* DNA methyltransferase 1 (*Dnmt1*), cause an analogous effect. Wildtype offspring derived from males mutant for *Dnmt1* showed a greater frequency than expected of DNA hypomethylation at the agouti locus and a yellow coat [30]. Importantly, whether DNA methyltransferases contribute to the mechanism of TEI beyond these epialleles requires further exploration.

Alternatively, in *Drosophila*, the DNA 6mA demethylase (DMAD) suppresses transposon expression in the ovary by ensuring low 6mA levels at these sites [13]. Although it is unclear whether dysregulation of DMAD and 6mA at transposable elements causes a transgenerational effect, it may play a yet-to-be determined mechanism in the *Drosophila* TEI model whereby females are fed a high calorie diet results in obesity in the F2 generation [31].

Remarkably, limiting the substrate for DNA methyltransferases leads to transgenerational effects on development [3]. A mutation in the mouse methionine synthase reductase *(Mtrr)* gene, which is necessary for the transmission of one-carbon methyl groups [32], results in epigenetic instability and the inheritance of congenital abnormalities at least up to four wildtype generations [3]. Even though these transgenerational effects occur through the maternal lineage, embryo transfer experiments demonstrated that the consequences were via the germline and independent of the uterine environment [3]. Specific germline-inherited epimutations have not yet been

identified in the *Mtrr* model nor is it clear whether the regulation of DNA methylation machinery is affected.

Is there a role for histone modifications in TEI?

The inheritance of histone modifications is not as well studied when considering TEI mechanisms. Most histones in mouse (99%) and human (85%) sperm are removed and replaced by protamines to enable compact packaging of DNA during sperm maturation [33]. Recently, protamine modifications were identified [34], yet whether or not the 'protamine code' passes on epigenetic information between generations is uncertain. Histone retention in sperm tends to be at the promoters of housekeeping and developmentally-regulated genes [35] while histones are retained throughout the genome in the oocyte [36]. Whether abnormal histone modifications in either germ cell influence the phenotype of the offspring is under investigation.

Recent evidence suggests that histone modifications and their regulatory enzymes convey epigenetic memory across generations. In *C. elegans*, histone 3 lysine 27 trimethylation (H3K27me3) regulated by the polycomb repressive complex 2 (PRC2) transmits memory of X-chromosome repression transgenerationally [37]. In another example, even though deficiencies in the H3K4me3 regulatory complex in *C. elegans* lead to increased longevity that persists transgenerationally, global H3K4me3 levels appear normal in the offspring [38]. Likewise, ectopic expression of *KDM1a*, a human H3K4 demethylase, during mouse spermatogenesis causes developmental abnormalities for three wildtype generations [39]. Regardless, wildtype sperm of the F1 generation displayed normal epigenome-wide

H3K4me2 profiles as well as normal DNA methylation patterns [39].

Therefore, while disruption of the histone methylation machinery may initiate

transgenerational inheritance of a phenotype, a second epigenetic factor may

be involved.

Interconnection of epigenetic mechanisms are exemplified in worms

with a mutation in a *KDM1a* ortholog (*spr-5*). The *spr-5* mutants have a

progressive transgenerational decline in fertility and an accumulation of

H3K4me2 [40]. Correspondingly, 6mA levels also increase transgenerationally

in these mutants [41] indicating another epigenetic mechanism is present.

When a 6mA DNA methyltransferase was knocked down in *spr-5* mutant

worms, the transgenerational loss of fertility phenotype was partially

suppressed [41]. Cross-talk between these two epigenetic pathways is

evident [41], but further experiments to determine the nature of these

interactions are required.

Non-coding RNAs: linking soma to germline

A mechanistic role of ncRNAs is currently at the forefront of TEI research.

Small ncRNAs act as sequence guides directing DNA or histone methylation,

and by post-transcriptionally regulating mRNA [42]. RNA inheritance is best

studied in *C. elegans* [43]. Starvation-induced expression of small RNAs or

exogenous RNA interference (RNAi) results in heritable gene silencing that

persists for several generations [44,45]. Although the mechanism is complex,

it is hypothesized that piwi-interacting RNA (piRNA), which typically silences

transposons in the germline, and exogenous RNAi may converge into a

common pathway requiring secondary small RNAs and chromatin regulatory complexes to ultimately bring about stable TEI [45].

RNA inheritance also occurs in mammals. ncRNAs from mouse sperm exposed to an environmental stressor are sufficient to cause phenotypes [1,9,46]. For example, traumatic stress in mice (F1) due to maternal separation in early postnatal life is associated with behavioural phenotypes in the F2 male offspring [1]. Deep sequencing of F1 sperm revealed upregulation of several microRNAs (miRNAs), which when microinjected into fertilized oocytes led to similar behavioural phenotypes in the resulting offspring [1]. This technique demonstrates a causal relationship between germline RNA and phenotype. Similarly, mice fed either a high fat [9] or low protein diet [8] have increased levels of fragmented tRNA species in sperm and offspring with metabolic disease [8,9]. Fragmented tRNAs can repress genes associated with the endogenous retroelement, MERVL, and might influence feto-placental development [8]. Synthetic versions of high fat diet-induced fragmented tRNAs in sperm were insufficient to cause metabolic disease [9]. This might be because the synthetic tRNAs lacked necessary modifications. Indeed, RNA methylation mediated by the methyltransferase *Dnmt2* is required for the transmission of phenotype in the *Kit* paramutant model [47]. These studies indicate that ncRNA may be a mechanism for TEI, although whether this method of inheritance is sustained in subsequent generations is yet-to-be determined. Remarkably, sperm tRNA fragments may originate in the epididymis and transported extracellularly into sperm by exosomes [8]. Thus, exosomes derived from the male genital tract may

communicate the environmental conditions experienced by the paternal generation to his mature sperm [8,48,49].

Challenges

Identifying the heritable epigenetic information transmitted across multiple generations is difficult even in models with definitive phenotypic inheritance. The following reasons contribute to this challenge.

Firstly, only selected epigenetic loci are assessed in some studies attempting to show TEI. As a result, the full scope of epigenetic changes in each generation is not appreciated. In fact, a spectrum of epigenetic information (i.e., DNA methylation, histone modifications, and RNA expression) may act in concert to initiate and perpetuate the inheritance of phenotypes [39,41]. Ideally, we need to perform unbiased, large-scale studies incorporating epigenome-, genome-, and transcriptome-wide approaches over several generations in key models of TEI. This type of comprehensive analysis is costly, and likely will require collaboration between groups.

Secondly, an environmental insult may stochastically affect the epigenome in each germ cell, as evidenced by phenotypic variability within a single model [3], in addition to naturally-occurring epivariation between individuals [27]. Consequently, resolving specific epimutations is difficult when germ cells are pooled for analysis. The emergence of single cell methylome and transcriptome technologies will permit us to better understand germ cell heterogeneity [50].

Thirdly, different 'epimutations' may be established in each generation caused by epigenetic instability in the previous generation (Figure 3). In this

case, the search for stable epimutations transmitted over multiple generations may be fruitless. Support for this hypothesis comes from the observation that phenotypes frequently persist over more generations than identified epigenetic changes [1,2].

Fourthly, epigenetic instability might promote genetic instability. Indeed, genetic background (e.g., inbred versus outbred mice) can alter the susceptibility of an individual to transgenerational epigenetic effects [25]. Alternatively, the activation of transposable elements in the germline by DNA hypomethylation might lead to heritable genetic mutations [51]. Furthermore, analysis of the F3 generation following vinclozolin exposure in rats revealed changes in DNA methylation patterns associated with a significant increase in repeat element copy number variations [52]. It is also possible that epigenetic and genetic mechanisms might interact in TEI through telomere regulation. Telomeres are heterochromatic tandem repeats rich in repressive histone marks [53] that normally protect chromosome ends from degradation [54]. Telomere shortening is associated with aging-related diseases [54] and can occur in response to diet manipulation. For example, feeding female rats a low protein diet results in an intergenerational reduction in telomere length associated with premature reproductive aging in the F2 female offspring [55], though the F3 generation was not assessed to confirm a transgenerational effect. Exploring the epigenetic status and stability of telomeres in transgenerational models may open up a new line of questioning.

Lastly, attributing phenotype to particular epigenetic changes can be problematic. Utilizing epigenetic strategies, such as TALE-TET1-fusions [56] and CRISPR-Cas9 based acetyltransferases [57], will enable us to target and

alter epigenetic marks in vivo to determine the specific effects on gene expression and phenotype. These technologies are in their infancy and are currently limited by off-target effects. However, they provide many exciting possibilities for site-directed epimutagenesis.

Conclusions

Lamark's once discredited hypothesis that phenotypes acquired during a lifetime are passed on to offspring has been injected with new vitality, fuelling fresh perspectives on rapid adaption to a changing environment [58]. Human populations are likely affected by TEI as demonstrated by the Dutch Hunger Winter and Överkalix famine studies [10,59,60]. Fundamentally, a greater mechanistic understanding of TEI will impact our approach to disease prevention and prediction, the effects of which will hopefully have a lasting impact.

Figure Legends

Figure 1. *Comparing transgenerational epigenetic inheritance (TEI) between the paternal and maternal lineages.*

Epigenetic alterations and phenotypes induced by environmental insults in the F0 generation may be inherited via the germline over several generations (F1, F2, F3, etc.). In the paternal lineage: TEI occurs if direct exposure of an F0 male and his germ cells to an environmental insult causes a phenotype (star) and/or alters epigenetic patterns beyond the F1 generation. The F2 offspring is the first generation that was not directly exposed to the insult. In the maternal lineage: if environmental exposure occurs while a female is pregnant, the mother, the foetus (F1 generation) and its primordial germ cells (F2 generation) are all directly exposed. Thus, the persistence of phenotypes/epigenetic changes in the F3 generation and beyond is considered TEI [5,6]. Intergenerational inheritance is the term given to phenotypes/epigenetic effects that persist to only the F1 offspring via the paternal lineage and the F2 offspring via the maternal lineage. F0, parental generation; F1, first filial generation; F2, second filial generation; F3, third filial generation.

Figure 2. *In mammals, inherited epigenetic information must escape multiple epigenetic reprogramming events in germ cells and the early embryo.* Reprogramming involves dynamic changes in the epigenetic patterns within the DNA of the germ cells and pre-implantation embryo between each generation to re-establish pluripotency. This excludes some repetitive elements (e.g., IAPs) and rare non-repeat loci, which remain highly methylated [15]. The graph (bottom right) indicates DNA methylation dynamics of germ cells [6]. In cases of transgenerational inheritance, abnormal epigenetic marks caused by an environmental insult must escape

multiple rounds of these reprogramming events. How these marks are stably transmitted between generations is the focus of much research. H3K27me3, histone 3 lysine 27 trimethylation; H3K4me3, histone 3 lysine 4 trimethylation; H3K9me2, histone 3 lysine 9 dimethylation; ncRNAs, non-coding RNAs; IAP, intracisternal A particle; E, embryonic day; F0, parental generation; F1, first filial generation; F2, second filial generation; F3, third filial generation; F4, fourth filial generation.

Figure 3. *Hypothesis: New epimutations may be generated in each generation.*

Some models of TEI reveal that phenotypes caused by an environmental insult persist over more generations than identified epigenetic abnormalities. This might be because epimutations inherited through the germline lead to more extensive epigenetic instability in the F1 offspring. This may result in an abnormal physiological or molecular milieu that causes new epimutations in the germ cells (i.e., F2 generation). For transgenerational inheritance to occur, epigenetically instability would be recreated in each subsequent generation. This hypothesis suggests that a different epigenetic profile would be expected in each individual of each generation rather than finding single stable epimutations that are consistently inherited. Red arrow, germline epigenetic inheritance; Red star, germ cell with one or more epimutation. F0, parental generation; F1, first filial generation; F2, second filial generation; F3, third filial generation; F4, fourth filial generation.

References

1. Gapp K, Jawaid A, Sarkies P, Bohacek J, Pelczar P, Prados J, Farinelli L, Miska E, Mansuy IM: **Implication of sperm RNAs in transgenerational inheritance of the effects of early trauma in mice**. *Nat Neurosci* 2014, **17**:667-669.
** By microinjecting sperm RNA into fertilized oocytes, this is one of the first studies to demonstrate a direct causal link between miRNAs in sperm and phenotype in the next generation.

2. Radford EJ, Ito M, Shi H, Corish JA, Yamazawa K, Isganaitis E, Seisenberger S, Hore TA, Reik W, Erkek S, et al.: **In utero undernourishment perturbs the adult sperm methylome and intergenerational metabolism**. *Science* 2014, **345**:785-793.
**Important for using unbiased methods, this study shows over 100 loci with altered DNA methylation in sperm of mice that were undernourished in utero. However, these epimutations did not persist to the F2 generation even though metabolic disease and gene misexpression were observed.

3. Padmanabhan N, Jia D, Geary-Joo C, Wu X, Ferguson-Smith AC, Fung E, Bieda MC, Snyder FF, Gravel RA, Cross JC, et al.: **Mutation in folate metabolism causes epigenetic instability and transgenerational effects on development**. *Cell* 2013, **155**:81-93.

4. Anway MD, Leathers C, Skinner MK: **Endocrine disruptor vinclozolin induced epigenetic transgenerational adult-onset disease**. *Endocrinology* 2006, **147**:5515-5523.

5. Daxinger L, Whitelaw E: **Understanding transgenerational epigenetic inheritance via the gametes in mammals**. *Nat Rev Genet* 2012, **13**:153-162.

6. Heard E, Martienssen RA: **Transgenerational epigenetic inheritance: myths and mechanisms**. *Cell* 2014, **157**:95-109.

7. Huypens P, Sass S, Wu M, Dyckhoff D, Tschop M, Theis F, Marschall S, de Angelis MH, Beckers J: **Epigenetic germline inheritance of diet-induced obesity and insulin resistance**. *Nat Genet* 2016, 10.1038/ng.3527.

8. Sharma U, Conine CC, Shea JM, Boskovic A, Derr AG, Bing XY, Belleannee C, Kucukural A, Serra RW, Sun F, et al.: **Biogenesis and function of tRNA fragments during sperm maturation and fertilization in mammals**. *Science* 2015, 10.1126/science.aad6780.
* The authors suggest that sperm obtain tRNA fragments by fusion with exosomes extruded by epididymal cells, thus linking somatic and germ cells. These tRNA fragments may act by repressing endogenous retroelements in the subsequent embryo.

9. Chen Q, Yan M, Cao Z, Li X, Zhang Y, Shi J, Feng GH, Peng H, Zhang X, Zhang Y, et al.: **Sperm tsRNAs contribute to intergenerational inheritance of an acquired metabolic disorder**. *Science* 2015, 10.1126/science.aad7977.
** The authors identify the significance of sperm small tRNAs in transmitting metabolic disease to the next generation. Furthermore, synthetic tRNAs are unable to mediate this process suggesting that RNA modification is important in TEI models.

10. Pembrey M, Saffery R, Bygren LO: **Human transgenerational responses to early-life experience: potential impact on development, health and biomedical research**. *J Med Genet* 2014, **51**:563-572.

11. Sun Q, Huang S, Wang X, Zhu Y, Chen Z, Chen D: **N6-methyladenine functions as a potential epigenetic mark in eukaryotes**. *Bioessays* 2015, **37**:1155-1162.

12. Deaton AM, Bird A: **CpG islands and the regulation of transcription**. *Genes Dev* 2011, **25**:1010-1022.

13. Zhang G, Huang H, Liu D, Cheng Y, Liu X, Zhang W, Yin R, Zhang D, Zhang P, Liu J, et al.: **N6-methyladenine DNA modification in Drosophila**. *Cell* 2015, **161**:893-906.

14. Smallwood SA, Tomizawa S, Krueger F, Ruf N, Carli N, Segonds-Pichon A, Sato S, Hata K, Andrews SR, Kelsey G: **Dynamic CpG island methylation landscape in oocytes and preimplantation embryos**. *Nat Genet* 2011, **43**:811-814.

15. Hackett JA, Sengupta R, Zylicz JJ, Murakami K, Lee C, Down TA, Surani MA: **Germline DNA demethylation dynamics and imprint erasure through 5-hydroxymethylcytosine**. *Science* 2013, **339**:448-452.

16. Reik W, Surani MA: **Germline and Pluripotent Stem Cells**. *Cold Spring Harb Perspect Biol* 2015, **7**.

17. Lane N, Dean W, Erhardt S, Hajkova P, Surani A, Walter J, Reik W: **Resistance of IAPs to methylation reprogramming may provide a mechanism for epigenetic inheritance in the mouse**. *Genesis* 2003, **35**:88-93.

18. Morgan HD, Sutherland HGE, Martin DIK, Whitelaw E: **Epigenetic inheritance at the agouti locus in the mouse**. *Nature Genetics* 1999, **23**:314-318.

19. Miltenberger RJ, Mynatt RL, Wilkinson JE, Woychik RP: **The role of the agouti gene in the yellow obese syndrome**. *J Nutr* 1997, **127**:1902S-1907S.

20. Cropley JE, Suter CM, Beckman KB, Martin DI: **CpG methylation of a silent controlling element in the murine Avy allele is incomplete and unresponsive to methyl donor supplementation**. *PLoS One* 2010, **5**:e9055.

21. Dolinoy DC, Huang D, Jirtle RL: **Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development**. *Proc Natl Acad Sci U S A* 2007, **104**:13056-13061.

22. Wei Y, Yang CR, Wei YP, Zhao ZA, Hou Y, Schatten H, Sun QY: **Paternally induced transgenerational inheritance of susceptibility to diabetes in mammals**. *Proc Natl Acad Sci U S A* 2014, **111**:1873-1878.

23. Jimenez-Chillaron JC, Isganaitis E, Charalambous M, Gesta S, Pentinat-Pelegrin T, Faucette RR, Otis JP, Chow A, Diaz R, Ferguson-Smith A, et al.: **Intergenerational Transmission of Glucose Intolerance and Obesity by In Utero Undernutrition in Mice**. *Diabetes* 2009, **58**:460-468.

24. Nadeau JH: **The nature of evidence for and against epigenetic inheritance**. *Genome Biology* 2015, **16**:137.

25. Guerrero-Bosagna C, Covert TR, Haque MM, Settles M, Nilsson EE, Anway MD, Skinner MK: **Epigenetic transgenerational inheritance of vinclozolin induced mouse adult onset disease and associated sperm epigenome biomarkers**. *Reprod Toxicol* 2012, **34**:694-707.

26. Iqbal K, Tran DA, Li AX, Warden C, Bai AY, Singh P, Wu X, Pfeifer GP, Szabo PE: **Deleterious effects of endocrine disruptors are corrected in the mammalian germline by epigenome reprogramming**. *Genome Biol* 2015, **16**:59.

27. Shea JM, Serra RW, Carone BR, Shulha HP, Kucukural A, Ziller MJ, Vallaster MP, Gu H, Tapper AR, Gardner PD, et al.: **Genetic and Epigenetic Variation, but Not Diet, Shape the Sperm Methylome**. *Dev Cell* 2015, **35**:750-758.

28. Kankel MW, Ramsey DE, Stokes TL, Flowers SK, Haag JR, Jeddeloh JA, Riddle NC, Verbsky ML, Richards EJ: **Arabidopsis MET1 cytosine methyltransferase mutants**. *Genetics* 2003, **163**:1109-1122.

29. Soppe WJJ, Jacobsen SE, Alonso-Blanco C, Jackson JP, Kakutani T, Koornneef M, Peeters AJM: **The Late Flowering Phenotype of fwa Mutants Is Caused by Gain-of-Function Epigenetic Alleles of a Homeodomain Gene**. *Molecular Cell* 2000, **6**:791-802.

30. Chong S, Vickaryous N, Ashe A, Zamudio N, Youngson N, Hemley S, Stopka T, Skoultchi A, Matthews J, Scott HS, et al.: **Modifiers of epigenetic reprogramming show paternal effects in the mouse**. *Nature genetics* 2007, **39**:614-622.

31. Buescher JL, Musselman LP, Wilson CA, Lang T, Keleher M, Baranski TJ, Duncan JG: **Evidence for transgenerational metabolic programming in Drosophila**. *Dis Model Mech* 2013, **6**:1123-1132.

32. Elmore CL, Wu X, Leclerc D, Watson ED, Bottiglieri T, Krupenko NI, Krupenko SA, Cross JC, Rozen R, Gravel RA, et al.: **Metabolic derangement of methionine and folate metabolism in mice deficient in methionine synthase reductase**. *Mol Genet Metab* 2007, **91**:85-97.

33. Casas E, Vavouri T: **Sperm epigenomics: challenges and opportunities**. *Front Genet* 2014, **5**:330.

34. Brunner AM, Nanni P, Mansuy IM: **Epigenetic marking of sperm by post-translational modification of histones and protamines**. *Epigenetics Chromatin* 2014, **7**:2.

35. Erkek S, Hisano M, Liang CY, Gill M, Murr R, Dieker J, Schubeler D, van der Vlag J, Stadler MB, Peters AH: **Molecular determinants of nucleosome retention at CpG-rich sequences in mouse spermatozoa**. *Nat Struct Mol Biol* 2013, **20**:868-875.

36. Gu L, Wang Q, Sun QY: **Histone modifications during mammalian oocyte maturation: dynamics, regulation and functions**. *Cell Cycle* 2010, **9**:1942-1950.

37. Gaydos LJ, Wang W, Strome S: **Gene repression. H3K27me and PRC2 transmit a memory of repression across generations and during development**. *Science* 2014, **345**:1515-1518.

38. Greer EL, Maures TJ, Ucar D, Hauswirth AG, Mancini E, Lim JP, Benayoun BA, Shi Y, Brunet A: **Transgenerational epigenetic inheritance of longevity in Caenorhabditis elegans**. *Nature* 2011, **479**:365-371.

39. Siklenka K, Erkek S, Godmann M, Lambrot R, McGraw S, Lafleur C, Cohen T, Xia J, Suderman M, Hallett M, et al.: **Disruption of histone methylation in developing sperm impairs offspring health transgenerationally**. *Science* 2015, 10.1126/science.aab2006.
* This is the first study to show TEI downstream of altered histone methylation in mice. It also examines the broader epigenome including DNA and histone methylation, and the transcriptome.

40. Greer EL, Beese-Sims SE, Brookes E, Spadafora R, Zhu Y, Rothbart SB, Aristizabal-Corrales D, Chen S, Badeaux AI, Jin Q, et al.: **A histone methylation network regulates transgenerational epigenetic memory in C. elegans**. *Cell Rep* 2014, **7**:113-126.

41. Greer EL, Blanco MA, Gu L, Sendinc E, Liu J, Aristizabal-Corrales D, Hsu CH, Aravind L, He C, Shi Y: **DNA Methylation on N6-Adenine in C. elegans**. *Cell* 2015, **161**:868-878.
** This study indicates crosstalk between 6mA, the newly identified methylated DNA base, and histone modifications in a transgenerational *C. elegans* model displaying progressive decline in fertility

42. Yan W: **Potential roles of noncoding RNAs in environmental epigenetic transgenerational inheritance**. *Mol Cell Endocrinol* 2014, **398**:24-30.

43. Feng X, Guang S: **Small RNAs, RNAi and the inheritance of gene silencing in Caenorhabditis elegans**. *J Genet Genomics* 2013, **40**:153-160.

44. Rechavi O, Houri-Ze'evi L, Anava S, Goh WS, Kerk SY, Hannon GJ, Hobert O: **Starvation-induced transgenerational inheritance of small RNAs in C. elegans**. *Cell* 2014, **158**:277-287.

45. Ashe A, Sapetschnig A, Weick EM, Mitchell J, Bagijn MP, Cording AC, Doebley AL, Goldstein LD, Lehrbach NJ, Le Pen J, et al.: **piRNAs can trigger a multigenerational epigenetic memory in the germline of C. elegans**. *Cell* 2012, **150**:88-99.

46. Grandjean V, Fourre S, De Abreu DA, Derieppe MA, Remy JJ, Rassoulzadegan M: **RNA-mediated paternal heredity of diet-induced obesity and metabolic disorders**. *Sci Rep* 2015, **5**:18193.

47. Kiani J, Grandjean V, Liebers R, Tuorto F, Ghanbarian H, Lyko F, Cuzin F, Rassoulzadegan M: **RNA-mediated epigenetic heredity requires the cytosine methyltransferase Dnmt2**. *PLoS Genet* 2013, **9**:e1003498.

48. Vojtech L, Woo S, Hughes S, Levy C, Ballweber L, Sauteraud RP, Strobl J, Westerberg K, Gottardo R, Tewari M, et al.: **Exosomes in human semen carry a distinctive repertoire of small non-coding RNAs with potential regulatory functions**. *Nucleic Acids Res* 2014, **42**:7290-7304.

49. Cossetti C, Lugini L, Astrologo L, Saggio I, Fais S, Spadafora C: **Soma-to-germline transmission of RNA in mice xenografted with human tumour cells: possible transport by exosomes**. *PLoS One* 2014, **9**:e101629.

50. Smallwood SA, Lee HJ, Angermueller C, Krueger F, Saadeh H, Peat J, Andrews SR, Stegle O, Reik W, Kelsey G: **Single-cell genome-wide bisulfite sequencing for assessing epigenetic heterogeneity**. *Nat Methods* 2014, **11**:817-820.

51. Kim S, Gunesdogan U, Zylicz JJ, Hackett JA, Cougot D, Bao S, Lee C, Dietmann S, Allen GE, Sengupta R, et al.: **PRMT5 protects genomic integrity during global DNA demethylation in primordial germ cells and preimplantation embryos**. *Mol Cell* 2014, **56**:564-579.

52. Skinner MK, Guerrero-Bosagna C, Haque MM: **Environmentally induced epigenetic transgenerational inheritance of sperm epimutations promote genetic mutations**. *Epigenetics* 2015, **10**:762-771.
* This paper highlights the effect of epigenetic instability on genetic stability in the vinclozolin model of TEI. The authors demonstrate an increase of copy number variants associated with altered DNA methylation in the F3 generation.

53. Dan J, Yang J, Liu Y, Xiao A, Liu L: **Roles for Histone Acetylation in Regulation of Telomere Elongation and Two-cell State in Mouse ES Cells**. *J Cell Physiol* 2015, **230**:2337-2344.

54. Calado RT, Dumitriu B: **Telomere dynamics in mice and humans**. *Seminars in hematology* 2013, **50**:165-174.

55. Aiken CE, Tarry-Adkins JL, Ozanne SE: **Transgenerational Developmental Programming of Ovarian Reserve**. *Sci Rep* 2015, **5**:16175.

56. Maeder ML, Angstman JF, Richardson ME, Linder SJ, Cascio VM, Tsai SQ, Ho QH, Sander JD, Reyon D, Bernstein BE, et al.: **Targeted DNA demethylation and activation of endogenous genes using programmable TALE-TET1 fusion proteins**. *Nat Biotechnol* 2013, **31**:1137-1142.

57. Hilton IB, D'Ippolito AM, Vockley CM, Thakore PI, Crawford GE, Reddy TE, Gersbach CA: **Epigenome editing by a CRISPR-Cas9-based acetyltransferase activates genes from promoters and enhancers**. *Nat Biotechnol* 2015, **33**:510-517.

58. Whitelaw E: **Disputing Lamarckian epigenetic inheritance in mammals**. *Genome Biol* 2015, **16**:60.

59. Bygren LO, Tinghog P, Carstensen J, Edvinsson S, Kaati G, Pembrey ME, Sjostrom M: **Change in paternal grandmothers' early food supply influenced cardiovascular mortality of the female grandchildren**. *BMC Genet* 2014, **15**:12.

60. Veenendaal MV, Painter RC, de Rooij SR, Bossuyt PM, van der Post JA, Gluckman PD, Hanson MA, Roseboom TJ: **Transgenerational effects of prenatal exposure to the 1944-45 Dutch famine**. *Bjog* 2013, **120**:548-553.