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Transgenerational inheritance: how impacts to the epigenetic and genetic information of parents affect offspring health

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BACKGROUND: A defining feature of sexual reproduction is the transmission of genomic information from both parents to the offspring. There is now compelling evidence that the inheritance of such genetic information is accompanied by additional epigenetic marks, or stable heritable information that is not accounted for by variations in DNA sequence. The reversible nature of epigenetic marks coupled with multiple rounds of epigenetic reprogramming that erase the majority of existing patterns have made the investigation of this phenomenon challenging. However, continual advances in molecular methods are allowing closer examination of the dynamic alterations to histone composition and DNA methylation patterns that accompany development and, in particular, how these modifications can occur in an individual's germline and be transmitted to the following generation. While the underlying mechanisms that permit this form of transgenerational inheritance remain unclear, it is increasingly apparent that a combination of genetic and epigenetic modifications plays major roles in determining the phenotypes of individuals and their offspring.

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OBJECTIVE AND RATIONALE: Information pertaining to transgenerational inheritance was systematically reviewed focusing primarily on mammalian cells to the exclusion of inheritance in plants, due to inherent differences in the means by which information is transmitted between generations. The effects of environmental factors and biological processes on both epigenetic and genetic information were reviewed to determine their contribution to modulating inheritable phenotypes.

SEARCH METHODS: Articles indexed in PubMed were searched using keywords related to transgenerational inheritance, epigenetic modifications, paternal and maternal inheritable traits and environmental and biological factors influencing transgenerational modifications. We sought to clarify the role of epigenetic reprogramming events during the life cycle of mammals and provide a comprehensive review of how the genomic and epigenomic make-up of progenitors may determine the phenotype of its descendants.

OUTCOMES: We found strong evidence supporting the role of DNA methylation patterns, histone modifications and even non-protein-coding RNA in altering the epigenetic composition of individuals and producing stable epigenetic effects that were transmitted from parents to offspring, in both humans and rodent species. Multiple genomic domains and several histone modification sites were found to resist demethylation and endure genome-wide reprogramming events. Epigenetic modifications integrated into the genome of individuals were shown to modulate gene expression and activity at enhancer and promoter domains, while genetic mutations were shown to alter sequence availability for methylation and histone binding. Fundamentally, alterations to the nuclear composition of the germline in response to environmental factors, ageing, diet and toxicant exposure have the potential to become hereditably transmitted.

WIDER IMPLICATIONS: The environment influences the health and well-being of progeny by working through the germline to introduce spontaneous genetic mutations as well as a variety of epigenetic changes, including alterations in DNA methylation status and the post-translational modification of histones. In evolutionary terms, these changes create the phenotypic diversity that fuels the fires of natural selection. However, rather than being adaptive, such variation may also generate a plethora of pathological disease states ranging from dominant genetic disorders to neurological conditions, including spontaneous schizophrenia and autism.

Key words: epigenetic inheritance / epigenetic reprogramming / disease aetiology / fertilization / genomics / germline / human reproduction / neurological diseases / non-genetic inheritance / transgenerational inheritance

Introduction

The process of sexual reproduction involves the transmission of genetic information from both progenitors to the resulting offspring via gametes, giving rise to a fully functional multicellular eukaryotic organism from a single-celled zygote (Surani *et al.*, 2007). In multicellular eukaryotic organisms an individual is composed of two main cell types: the somatic cells, which comprise the majority of the organism but have no inheritable function and germ cells, specialized cells that differentiate into mature gametes and carry all inheritable information from one generation to the next. During fertilization the haploid gametes produced by each parent fuse and recombine their genetic information generating a diploid zygote that develops into an individual comprising the inherited information provided by its progenitors. Although widely acknowledged that genetic material inside the gametes carries information from parents to offspring, it has become increasingly evident that the DNA sequence alone is unlikely to convey the entirety of the inherited information. Instead, correlative evidence suggests that epigenetic information contained in molecular elements that regulate genome activity independently of the DNA sequence has the potential to contribute to the information transmitted from one generation to the next (Skinner *et al.*, 2010). As an important caveat however, an experimentally verified mechanism to account for this mode of inheritance in mammalian species has yet to be conclusively established (Skvortsova *et al.*, 2018).

Classical Mendelian genetics has for a long time been at the basis of our understanding of heredity, breeding and evolution. Mutations in the DNA sequence of single genes, or small clusters of genes, have been shown to generate particular biological phenotypes that are then transmitted to all subsequent generations, most often with a gene mutation that leads to the inheritance of specific disease phenotypes (Gasparini *et al.*, 2000; Antonarakis and Beckmann, 2006; Bell *et al.*, 2011). However, cases have been reported where individuals with identified disease causing genetic mutations fail to express most or all of the symptoms expected from the associated genetic disorder (Xue *et al.*, 2012; Cooper *et al.*, 2013). Similarly, environmental factors to which both parents and offspring can be directly exposed during their lifetime do not produce consistent alterations to the genetic code and thus also fail to explain the inheritance of altered phenotypes (Jirtle and Skinner, 2007; Skinner *et al.*, 2010; Guerrero-Bosagna *et al.*, 2012). Moreover, an increasing number of inherited disease phenotypes have been reported in response to environmental exposures, which cannot be explained by genetic mutations alone, given the absence of evidence linking the disease aetiology with alterations to the gene sequence or other genetic abnormalities (Nilsson *et al.*, 2018c). Such observations provide the impetus to interrogate the molecular mechanisms by which epigenetic information is transmitted between generations, and the importance of this information for offspring development (Perez and Lehner, 2019).

Conrad Waddington first coined the term 'epigenetics' to describe the processes by which an organism interacts with the environment to produce observable phenotypic traits (Waddington, 1942) as in the inherited wing patterns observed in *Drosophila* in response to heat shock (Waddington, 1953). The definition of epigenetics has since been modified to include all changes taking place in the genome that are not associated with the DNA sequence itself (Holliday, 1994; Akhtar and Cavalli, 2005). With the discovery of genomic imprinting in the late 1980s and clarification of the essential role that DNA methylation plays in the development of mammals (Hadchouel *et al.*, 1987; Sapienza *et al.*, 1987; Sutherland *et al.*, 2000), the molecular processes responsible for epigenetic modifications started to be identified. In the ensuing decades it has been shown that epigenetic transgenerational

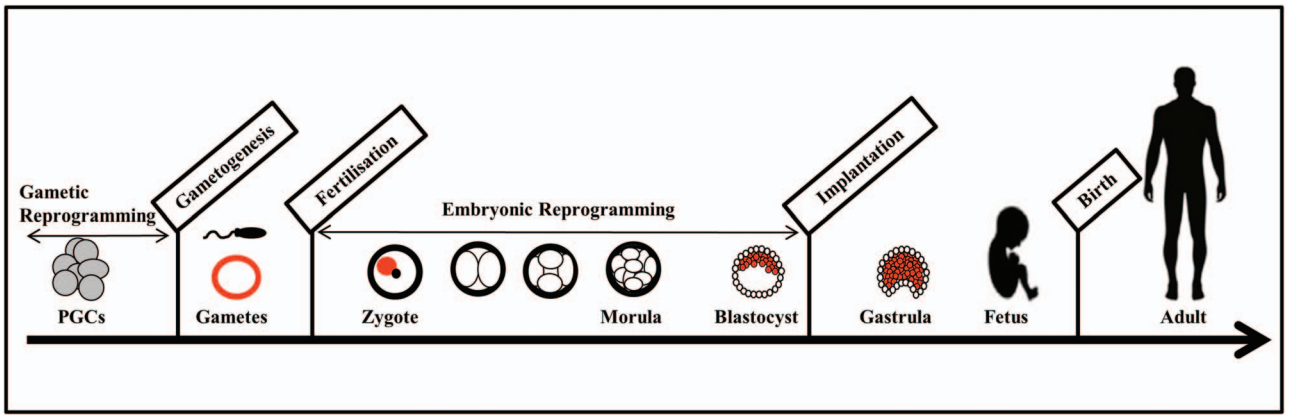

Figure 1 Epigenetic reprogramming cycles. During mammalian life, cells are submitted to two major genome-wide epigenetic reprogramming events. The Gametic Reprograming event takes place in PGCs of embryos during germline cell development, as PGCs migrate to the genital ridge. PGCs experience genome-wide DNA demethylation, removal and resetting of parental imprints, histone modifications and inactive-X-chromosome reactivation. The Embryonic Reprogramming event starts immediately after fertilization and lasts until the blastocyst stage of embryo development, when cells experience DNA demethylation, the removal and resetting of parental imprints and histone modifications.

inheritance of disease and phenotypic variation is relatively common in plants, where mitotically stable epigenetic changes can be transmitted through the germline to alter genome activity independently of DNA gene sequences in the offspring (Schmitz and Ecker, 2012; Weigel and Colot, 2012). In animals, and particularly in mammals, inheritance of epigenetic changes is a much rarer event. However, variations in DNA methylation levels have been reported to impact the expression of exogenous transgenes and endogenous alleles leading to phenotypic changes, such as variation in the coat colour of mice, from one generation to the next (Morgan *et al.*, 1999; Rakyan *et al.*, 2002). Furthermore, it has been shown that epigenetic inheritance is not necessarily limited to the DNA methylation status alone, but rather encompasses a range of alternative complex molecular processes (Champroux *et al.*, 2018). In this manner, progenitors are now acknowledged to contribute more than just their DNA to the offspring.

In terms of alternative epigenetic processes, the cytoplasmic contents of the parental gametes can contribute bioactive molecules (e.g. non-coding RNAs, ncRNA; Hutcheon *et al.*, 2017), along with nutrients and hormones to the offspring, which have in turn been implicated in the regulation of their development during embryogenesis (Jodar *et al.*, 2013; Rodgers *et al.*, 2015; Conine *et al.*, 2018). Evidence from studies of humans and mice suggest that parental care during growth may also influence the development of the offspring by modulating environmental interactions (Stein and Lumey, 2000; Kaati *et al.*, 2002; Pembrey *et al.*, 2006; Mashoodh *et al.*, 2018). The epigenetic landscape of the nuclear genome may also be indirectly influenced by the germline organelles that pass through the parental lineage to the progeny. By way of example, maternally inherited mitochondria fulfill an essential role in the provision of the intermediary metabolites necessary to generate and modify epigenetic marks in the nucleus (Stimpfel *et al.*, 2018). The recent findings that human mitochondrial DNA is methylated (Ghosh *et al.*, 2014) and that a diversity of small ncRNAs are encoded by the mouse mitochondrial genome (Larriba *et al.*, 2018) also raise the interesting possibility of more direct epigenetic cross talk between the two genomes (Cheikhi *et al.*, 2019). Furthermore, transfer of epigenetic information involving chemical modifications is not restricted to DNA methylation but also encompasses the post-translational modification (PTM) of nuclear DNA associated proteins, with bound histones being particularly amenable to methylation, acetylation and phosphorylation (Raychaudhuri *et al.*, 2008; Godfrey *et al.*, 2011; Lavebratt *et al.*, 2012; Wang *et al.*, 2012).

The mutable nature of epigenetic marks coupled with the ability of environmental stimuli to influence epigenetic change (Waterland *et al.*, 2010; Talens *et al.*, 2012; Nilsson *et al.*, 2018c) has prevented us from fully understanding the flow of epigenetic information inheritance. In mammals, investigating transgenerational epigenetic inheritance is further hindered by two major epigenetic reprogramming events (Figs 1 and 2) that erase and replace the majority of existing epigenetic marks; one of which occurs prior to and during fetal gonadal sex determination (Lane *et al.*, 2003; Delaval *et al.*, 2007; Seisenberger *et al.*, 2012; Monk, 2015) and the second immediately after fertilization (DeBaun *et al.*, 2003; Feng *et al.*, 2010). Thus, unlike the well-documented phenomena in plant and invertebrate models, there remains active debate as to whether transgenerational epigenetic inheritance in mammals is more of an exception than the rule. Accordingly, for the purpose of this review, transgenerational methods of epigenetic inheritance in plants have been excluded due to inherent differences in the reproductive processes of both groups (Hauser *et al.*, 2011; Paszkowski and Grossniklaus, 2011; Becker and Weigel, 2012). Rather, we shall focus on the me progenitors may transmit genetic and epigenetic information to their offspring and influence their phenotypes. The interdependent relationship between genetic and epigenetic modifications will also be discussed, concentrating on how genetic alterations can affect the overall epigenetic profile of cells and epigenetic changes may, in turn, influence gene expression. Finally, we shall consider how factors such as age and oxidative stress in the germline can affect the flow of transgenerational inherited information from parent to offspring.

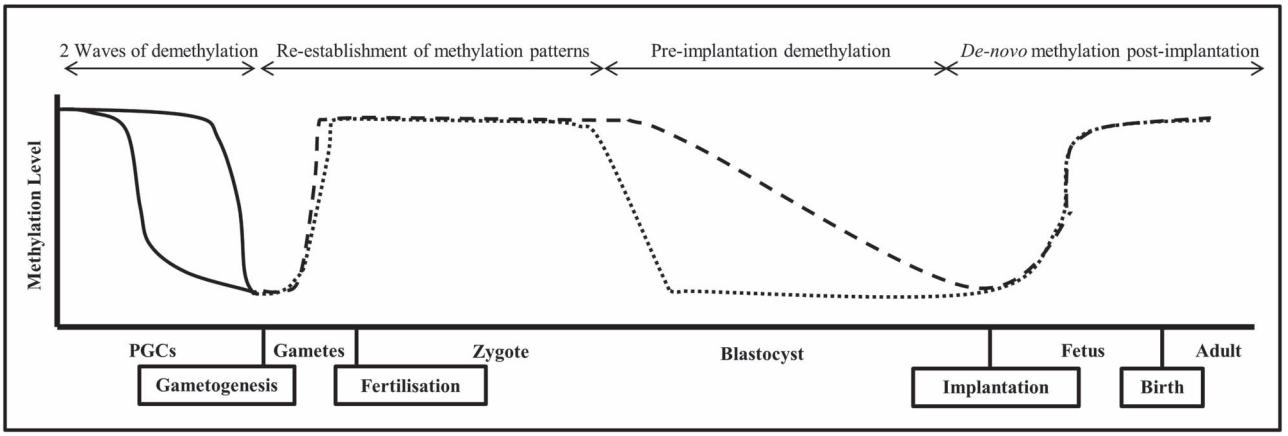

Figure 2 DNA methylation dynamics in human cells. Epigenetic reprogramming erases pre-existing DNA methylation patterns in PGCs in two consecutive waves of demethylation (black lines). During the final stages of gametogenesis, sex imprinting re-establishes sex-specific methylation patterns in spermatozoa (dotted line) and in oocytes (dashed line). After fertilization, paternal- and maternal-derived genomes undergo passive and active DNA methylation erasure, followed by formation of a highly demethylated blastocyst. *De-novo* DNA methylation patterns are established post-implantation and are unique to the resulting offspring.

Known forms of epigenetic information

In the mammalian genome, epigenetic information is predominantly captured in the patterns of DNA methylation, the spectrum of histone modifications and in the complement of ncRNA species (Table I). DNA methylation is the molecular process by which a methyl group is covalently attached to cytosines in the DNA sequence (Law and Jacobsen, 2010). DNA methylation has long been associated with regulation of gene expression owing to the imposition of steric hindrance. Specifically, the protrusion of the methyl group from the DNA structure interferes with the binding of transcription factors, thus inhibiting transcriptional activity and causing gene silencing (Fig. 3; Aravin *et al.*, 2007; Carmell *et al.*, 2007). In most cell types DNA methylation occurs predominantly at clusters of CpG dinucleotides, known as CpG islands, which are dispersed throughout the majority of the DNA (Jones and Liang, 2009; Illingworth *et al.*, 2010). In pluripotent cells, however, DNA methylation has also been found to occur abundantly outside of the CpG islands (Meissner *et al.*, 2008; Hawkins *et al.*, 2010). DNA methylation is strongly implicated in maintaining genomic stability via regulation of promoters, up to 50% of which are present in CpG islands, and repetitive DNA sequences. The repetitive DNA sequences comprise both long interspersed nuclear elements and short interspersed nuclear elements (Jones and Liang, 2009; De Carvalho *et al.*, 2012). In most cell types, a single methyltransferase family of enzymes is responsible for the maintenance and *de novo* methylation throughout the entire genome, with DNA methyltransferase 1 (DNMT1) being the enzyme most commonly employed for imposing these epigenetic marks (Law and Jacobsen, 2010; Lyko, 2018). In the course of DNA synthesis, methyltransferase enzymes replicate the methylation marks present in the template strand in the daughter strand, thus ensuring inheritance of the correct epigenetic pattern during both mitotic and meiotic cycles of cellular division (Cedar and Bergman, 2009; Probst *et al.*, 2009). Although DNA methylation is a relatively stable epigenetic process accurately replicated at each cellular division, there are situations in which this process has proven to be more dynamic, with active methylation and demethylation occurring in non-dividing cells participating in base excision–repair pathways to repair their damaged DNA (Wu and Zhang, 2010; Yamagata *et al.*, 2012). Furthermore, profound alterations to established DNA methylation patterns are known to occur in order to re-establish pluripotency in the germline and totipotency in embryonic stem cells (Sabour and Scholer, 2012; Ficz *et al.*, 2013; Habibi *et al.*, 2013; Takashima *et al.*, 2014).

Histones are the principal protein component conferring dynamism and fluidity to the chromatin structure, packaging the DNA and acting as an important mechanism for regulating gene expression by determining which DNA regions remain accessible to the cell's gene regulation and transcriptional machinery (Weintraub and Groudine, 1976; Narlikar *et al.*, 2002; Felsenfeld and Groudine, 2003). Histones also control the activity of regulatory elements within the DNA sequence and may influence the expression of specific cellular phenotypes (Schones and Zhao, 2008; Margueron and Reinberg, 2010). PTM of the primary histone structure can occur via methylation, acetylation, phosphorylation and sumoylation of specific residues (El Kennani *et al.*, 2018). Each of these modifications can, in turn, modulate chromatin folding (Shogren-Knaak *et al.*, 2006; Fierz *et al.*, 2011) and influence the binding of regulatory proteins (Patel and Wang, 2013; Zentner and Henikoff, 2013). Similarly, the exchange of histones with protamines during differentiation of the male germline has a profound effect on chromatin structure and gene expression (Zhou *et al.*, 2011). Histone epigenetic modifications go beyond the direct interaction between histones and the DNA sequence since heterochromatin proteins may bind to already repressively modified histones to indirectly constrict the chromatin structure and fully restrict access to large sections of the genome by transcription-activating proteins (Ebert *et al.*, 2006).

Table I Common epigenetic modifications and associated effects on the mammalian genome.

Epigenetic modifications	Effect on genome function
DNA methylation	Methylation at promoter sites associated with gene silencing. Methylation in gene region associated with regulation of gene activity.
Histone methylation	Methylation of amino acid residues in histone associated with both transcriptional repression and activation, dependent on residue.
Histone acetylation	Acetylation increases access to DNA for transcription. Allows the genome-wide reprogramming in sperm protamination.
Histone phosphorylation	Phosphorylation of histones associated with chromatin compaction. Regulates chromatin structure and chromosome condensation during cell division.
Histone sumoylation	Small ubiquitin-related modifier (SUMO) proteins bind to histones. Associated with transcription activation and gene silencing.
Histone variants	Histone variants, e.g. H2A.Z, CENP-A, H2AX perform various specialized functions including DNA repair, gene regulation and centromere function.
Small non-coding RNAs	Micro RNAs and PIWI-interacting RNA (piRNAs) affect transcriptional repression and activation, and translational repression.
Long non-coding RNAs	Suggested to have high variety of functions, known to regulate large-scale transcriptional repression in imprinting.

In addition to DNA and histone modifications, several classes of RNA have been implicated in epigenetic inheritance in multiple organisms, including sperm-borne and maternal stores of mRNA and long ncRNA as well as siRNA, PIWI-interacting RNA (piRNA) and micro RNA (miRNA); all of which form part of the RNA interference (RNAi) pathway that regulates gene expression, translation and silencing (Taft *et al.*, 2010; Gapp and Bohacek, 2018; Trigg *et al.*, 2019). The epigenetic effects resulting from the inheritance of ncRNA remain to be fully resolved in mammals. However, recent studies have shown that ncRNAs can influence the phenotype of individuals in a similar manner to that of the more widely studied DNA and histone modifications, owing to their ability to promote activation or repression at transcription sites upon base-complementation pairing with the genetic sequence (Teixeira *et al.*, 2009; Heneghan *et al.*, 2010; Taft *et al.*, 2010). The epigenetic modification of the mouse *Kit* gene remains the best known example in mammals, where the wild-type progeny of *Kit* heterozygous parents display the modified *Kit* phenotype in the absence of the mutant allele for multiple consecutive generations (Rassoulzadegan *et al.*, 2006). The observed effect has been attributed to the inheritance of *Kit*-specific miRNAs (Rassoulzadegan *et al.*, 2006) and, although the precise molecular basis for this form of epigenetic transfer is still not fully understood, methyltransferase DNMT2 is known to be involved (Kiani *et al.*, 2013).

Epigenetic inheritance

The effects of the epigenetic information housed within the cells of an individual can exert influence throughout its entire lifespan, with effects ranging from provision of cellular identity and regulation of gene expression to promoting differentiation into different cell types and the manifestation of unique phenotypes. Although the cellular epigenetic profile remains relatively stable overtime, several regions of the genome actively respond to internal cellular processes and environmental forces, leading to alteration and adjustment of existing epigenetic marks (Peaston and Whitelaw, 2006; Zentner and Henikoff, 2013). The specific loci so affected are governed by nucleosome dynamics, reflecting a complex interplay of histone composition, histone PTMs and nucleosome occupancy and positioning within chromatin (Lai and Pugh, 2017). As with genetic mutations, most spontaneous epigenetic modifications have either a neutral or deleterious effect on the individual and may impair normal cellular processes before being transmitted to the next generation. The few potential adaptive epigenetic modifications that may respond advantageously to environmental factors would still need to be successfully transmitted via the gametes to the offspring to impact on future generations by enhancing reproductive success. Nevertheless, as we discuss below, the inheritance of phenotypes not explained by Mendelian genetics has been documented in mammals (Meyer *et al.*, 2009; Schmitz and Ecker, 2012; Weigel and Colot, 2012; Docherty *et al.*, 2014), suggesting that epigenetic marks have the potential to be transmitted from parents to offspring via the gametes (Sharma, 2012; Moore and Stanier, 2013). Consistent with this model, insights from recent studies suggest DNA methylation is not erased and re-established with equivalent efficiency across the genome, meaning a previously underappreciated portion of the mammalian genome may actually escape this form of reprogramming and thereby contribute to epigenetic inheritance (Skvortsova *et al.*, 2018).

Inheritance of methylation patterns

Pioneering studies investigating global DNA methylation patterns in mammals revealed that methylation levels were consistently lower in embryonic cells than in the mature gametes or the zygote prior to implantation (Monk *et al.*, 1987; Kafri *et al.*, 1993; Rougier *et al.*, 1998; Mayer *et al.*, 2000; Oswald *et al.*, 2000). Subsequent work revealed that after this key developmental phase, methylationrises to levels similar to those found in somatic cells, thus establishing a new methylation-pattern during embryogenesis that is essential for normal development (Moore and Reik, 1996; Rivera and Ross, 2013). These findings led to the proposal that epigenetic information could not be inheritably transmitted, because any marks carried by the gametes or early embryo

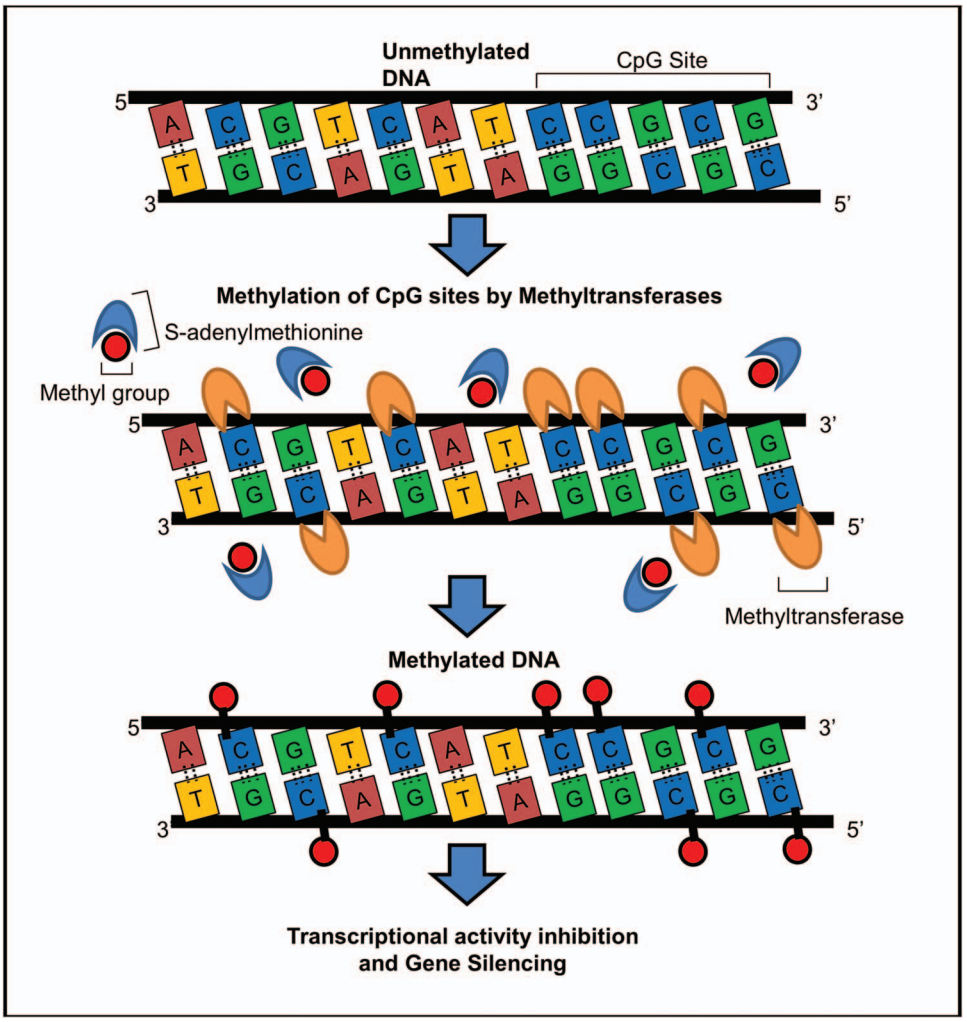

Figure 3 DNA methylation caused by DNA methyltransferases attaching methyl groups to cytosine nucleotides. Methylation at CpG sites alters the DNA structure and interferes with transcription factors, which leads to inhibition of transcriptional activity and gene silencing.

would be subject to erasure and replacement with a new methylation pattern following embryonic implantation. Additionally, epigenetic reprogramming and imprinting events were found to take place in the primordial germ cells (PGCs) of the developing embryo of multiple mammalian species (Lees-Murdock and Walsh, 2008), thus further restricting the potential for inheritance of parental DNA methylation patterns.

Notwithstanding these multiple reprogramming events, numerous studies have documented the influence of non-genetic factors on the phenotype of offspring due to inherited epigenetic modifications that resist erasure and replacement (Drake *et al.*, 2005; Goldberg *et al.*, 2007; Jirtle and Skinner, 2007; Sasaki and Matsui, 2008; Ding *et al.*, 2012; Fullston *et al.*, 2012; Fig. 2). Indeed, not only has DNA methylation been shown to be incompletely erased (Kearns *et al.*, 2000; Sutherland *et al.*, 2000), but also several genomic regions have been found that consistently resist demethylation in PGCs (Seisenberger *et al.*, 2012) and in embryonic cells (Weaver *et al.*, 2009; Rivera and Ross, 2013). On the weight of this evidence it is now apparent that reprogramming events are highly regulated nuclear processes that ensure that most epigenetic modifications accumulated during a parent's lifetime are detected and corrected to minimize detrimental effects to the offspring (Reik, 2007; Faulk and Dolinoy, 2011). Illustrating the importance of these events, it is known that alterations to established DNA methylation patterns, such as global hypomethylation, are commonly associated with the development of cancer due to abnormal gene expression, chromosomal instability, reactivation of retrotransposons and loss of imprinting (Wilson *et al.*, 2007; Fleming *et al.*, 2008). Similarly, hypermethylation at specific sites inducing tumour suppressor genes has also been correlated with tumour development (Fraga *et al.*, 2007).

In humans, hypermethylation of the promoter regions regulating two tumour suppressor mismatch repair genes, *MLH1* and *MSH2*, has been associated with hereditary non-polyposis colorectal cancer (Chan *et al.*, 2006; Hitchins *et al.*, 2007). The abnormal methylation status at these sites, particularly in the promoter region of *MLH1*, has been found to reduce or impair gene expression and, in time,

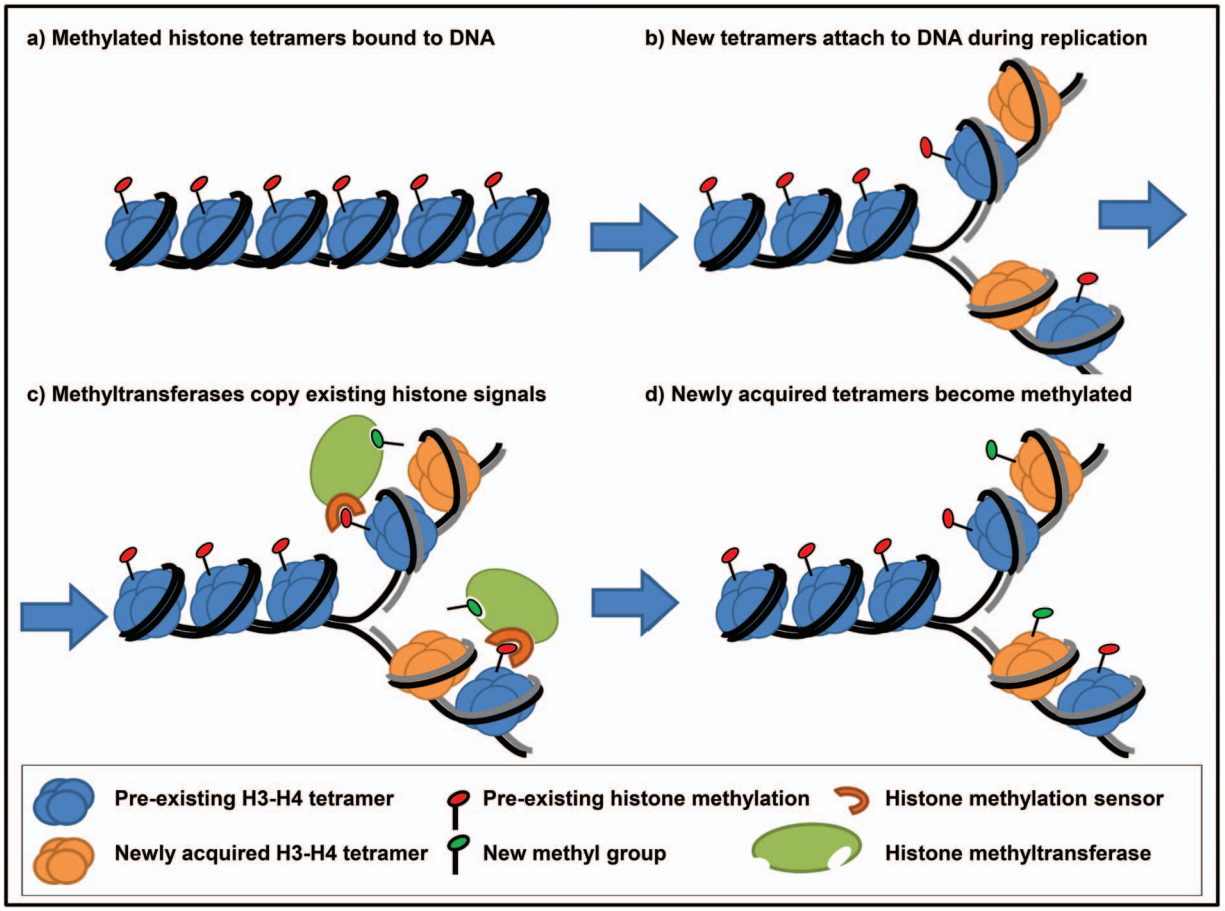

Figure 4 Model of histone methylation inheritance. Diagram displaying conservative histone segregation and histone methylation-copying during DNA replication. Inherited H3-H4 tetramers (only half the nucleosome octamer is depicted, histones H2A and H2B are not shown for clarity purposes) are transferred to daughter DNA strands where methylation sensors recognize histone methylation patterns in neighbouring histones, and coupled methyltransferases copy the methylation signal onto newly acquired tetramers.

trigger microsatellite instability in the germline of individuals who possess a single functional allele of these genes (Suter *et al.*, 2004; Hitchins *et al.*, 2007). *MLH1* hypermethylation has been found to be established predominantly during oogenesis and therefore inherited via the maternal line (Fleming *et al.*, 2008). Inheritance of altered DNA methylation levels and gene expression has also been found with less severe consequences in other mammalian species. In isogenic agouti viable yellow (A^{vy}) and axin-fused ($Axin^{Fu}$) mice, small variations in DNA methylation induce a variety of phenotypes despite their similar genetic identity (Rakyan *et al.*, 2002). Changes to the methylation status of the intra-cisternal A particle long terminal repeat, a retrotransposon located upstream of the coding sequence of the A^{vy} gene and within intron 6 of $Axin^{Fu}$, cause the mice to display yellow fur, early onset obesity, diabetes, increased tumour susceptibility and a kinked tail phenotype due to altered protein expression (Duhl *et al.*, 1994; Morgan *et al.*, 1999; Rakyan *et al.*, 2003). In this situation, different phenotypes are transmitted via the paternal gametes of the A^{vy} mice and from both the paternal and maternal lineage for $Axin^{Fu}$ (Morgan *et al.*, 1999; Rakyan *et al.*, 2003; Blewitt *et al.*, 2006).

Inheritance of histone modifications

Transmissible epigenetic modifications to histones were first detected in *Caenorhabditis elegans*, where alterations to the methylation status of histone H3 lysine 4 (H3K4) in the parents were passed down to the offspring to affect changes in their fertility and longevity (Katz *et al.*, 2009; Greer *et al.*, 2011). In humans and mice, methylation changes to H3K4 and to histone H3 lysine 27 (H3K27) in the paternal germline have been found to impact the overall chromatin structure in the gametes and affect promoter regions of genes essential for embryonic development (Hammoud *et al.*, 2009; Brykczynska *et al.*, 2010). Histone modifications also encompass the replacement of core histones with histone variants, such as H3.3, which can be subjected to unique epigenetic modification and both direct the remodelling of sperm chromatin and influence patterns of gene expression in the embryo. Illustrative of this phenomenon, abnormal histone replacement in the sperm cells of mice has been linked to paternal chromosome loss and increased risk of early embryonic death arising from genome instability

that could not be countered by the fertilized oocyte (Chong *et al.*, 2007).

As with DNA methylation, histone modifications are subjected to multiple epigenetic reprogramming events that erase histone methylation marks and exchange the histones attached to the DNA (Hajkova *et al.*, 2008). Initially, the occurrence of chromatin repackaging in maturing spermatozoa was thought to remove all histones and subsequently replace them with protamines (Ward and Coffey, 1991). However, it has since been shown that approximately 1–2% and 4–15% of the nuclear genome in the spermatozoa of mice and humans, respectively, is not repackaged in this manner and that several histones and histone methylation marks endure the reprogramming events (Hammoud *et al.*, 2009; Brykczynska *et al.*, 2010). For instance, histone methylation marks, such as H3K27me3 (trimethylation of H3K4), have been found to withstand reprogramming in mature human spermatozoa and influence genes capable of histone binding at the transcriptional start sites, thereby repressing gene expression during gametogenesis and early embryogenesis (Hansen *et al.*, 2008; Brykczynska *et al.*, 2010). Such findings suggest that a few epigenetic modifications may enable transcriptional state memory across generations (Lim and Brunet, 2013).

Despite the gathering evidence supporting the heritability of histone modifications, as a whole, the process of transmitting histone changes from one generation to the next is poorly understood (Skvortsova *et al.*, 2018). The system for transmitting epigenetic information via histones is imprecise and tolerates a certain degree of variation in histone structure (Xu *et al.*, 2012; Huang *et al.*, 2013). Not only is histone methylation not required to exist in a symmetrical manner within the nucleosome but also the corresponding residues on the two copies of the same histone within the nucleosome can be differentially methylated, functioning as 'silent' modifications (Chen *et al.*, 2011). Furthermore, the observed conservative segregation of H3-H4 tetramers indicate that epigenetic inheritance does not require strict methylation-copying events when coupled with copying of epigenetic patterns from neighbouring pre-existing histones (Fig. 4; Hansen *et al.*, 2008; Margueron and Reinberg, 2010; Xu *et al.*, 2010). Nevertheless, the histone methyltransferases can amplify pre-existing histone modifications and provide a measure of epigenetic control over the assembly of heterochromatin (Nakayama and Takami, 2001; Margueron and Reinberg, 2010) that can potentially be inherited. Notably, in oocytes, histone modifications and DNA methylation act in opposition, such that DNA methylation prevents histone methylation (Eckersley-Maslin *et al.*, 2018). Despite this phenomenon, large histone H3K4me3 domains have been detected in mouse oocytes, where they are implicated in modulating the maternal-to-zygotic transition (Dahl *et al.*, 2016; Zhang *et al.*, 2016; Hanna *et al.*, 2018).

Epigenetic reprogramming

To mitigate the adverse effects that can arise from epigenetic modifications, two reprogramming regulatory events occur during gametogenesis and immediately after fertilization during the early stages of embryogenesis (Fig. 1). These combined processes ensure that most epigenetic alterations accumulated during the adult life of the parents are detected and corrected in order to minimize detrimental effects in the offspring (Reik, 2007; Faulk and Dolinoy, 2011). In somatic cells, mitosis gives rise to more somatic cells containing identical genetic information and a stable epigenetic configuration to ensure that a correct pattern of gene expression is maintained in daughter cells. Nevertheless, the epigenetic marks in these cells have no relevance to the pattern of information inherited by the offspring. Only the epigenetic patterns retained in the mature gametes have the potential to be passed down to the offspring and influence their phenotype (Daxinger and Whitelaw, 2012). However, the heritability of epigenetic modifications is highly restricted by the two major epigenetic reprogramming events in the mammalian life cycle (Gold *et al.*, 2018).

The role of epigenetic changes in the inheritance of non-genetic information is further complicated by the concepts of 'intergenerational' and 'transgenerational' inheritance (Perez and Lehner, 2019). Intergenerational epigenetic inheritance is a term used to define epigenetic modifications that are found in the adult progenitor, the first or the second generation of offspring in response to direct exposure to environmental factors inducing changes in the epigenetic profile of the adult, the fetus or PGCs. Conversely, the term transgenerational inheritance is only used to describe epigenetic modifications that are able to persist into the third, or later generations, in the absence of direct exposure to the factor that initiated the change (Daxinger and Whitelaw, 2012; Heard and Martienssen, 2014; Martos *et al.*, 2015; Skinner *et al.*, 2015).

To fully understand the role of epigenetic inheritance in mammals, the processes involved in the resetting of epigenetic marks need to be further explored. The two major epigenetic reprogramming events characterizing the mammalian life cycle have already been summarized in Fig. 1 and comprise, first, the reprogramming of PGCs to achieve a pluripotent state and, second, the post-fertilization reprogramming of the embryo to ensure a pattern of gene expression supportive of normal cell differentiation during development (Reik, 2007; Lange and Schneider, 2010).

Gametic epigenetic reprogramming

The genome-wide reprogramming event that occurs in the PGC pool proceeds through multiple stages in response to the appropriate cellular signals (Cowley and Oakey, 2012; Hackett and Surani, 2013; Heard and Martienssen, 2014; Martos *et al.*, 2015; Hill *et al.*, 2018). During their first phase of development, germ cells must undergo specification in order to separate them from the surrounding soma. The PGCs then migrate to the genital ridge whereupon epigenetic reprogramming and sex-specific differentiation occurs to return cells to a pluripotent state before they undergo their first meiotic division (Fig. 2). In mice, the timing of the epigenetic reprogramming occurs at slightly different stages due to specificities inherent in the differentiation pathways of oocytes and spermatozoa (Smallwood *et al.*, 2011; Cowley and Oakey, 2012). Nevertheless, the reprogramming of both cell types involves extensive DNA demethylation. During this process, the majority of the commonly methylated sites are erased from all chromosomes, particularly those sites silencing the X chromosome. However, a small number of specific genomic areas, including the sub-telomeric and retrotransposon regions, resist demethylation and thus retain their epigenetic markers (Hajkova *et al.*, 2002; Franklin *et al.*, 2010; Popp

et al., 2010; Schmitz et al., 2011; Guibert et al., 2012). It has been proposed that the resistance to demethylation that exists in the sub-telomeric regions is associated with the maintenance of appropriate telomere length and function while PGCs regain pluripotency (Jezek and Green, 2019). Accordingly, hypomethylation at these regions has been correlated with dysregulation of telomerase activity and cancerous phenotypes (Yehezkel et al., 2011; Wang et al., 2013; Zhang et al., 2014). Similarly, demethylation of retrotransposon regions that are normally subjected to stringent DNA-methylation-mediated repression has been linked to increased transcriptional activation, higher rates of retrotransposon insertion and a rise in recombination events among different unmethylated repeat regions, each of which exerts negative impacts on normal genomic activity (Moazed, 2011; Guibert et al., 2012). This initial round of epigenetic reprogramming terminates with DNA methylation in PGCs returning to similar patterns, and overall levels, to those found in somatic cells (Sasaki and Matsui, 2008).

This PGC genome-wide DNA demethylation event has been associated with triggering complementary epigenetic reprogramming events, including the demethylation of epigenetic marks on histones and the exchange of histones with non-canonical histone variants (Hajkova et al., 2002; Lee et al., 2002). These forms of epigenetic reprogramming, combined with those occurring in the DNA, are thought to facilitate the return of the PGC chromatin signature to a state of pluripotency and thus permit specific gene expression to occur during germ cell development. Concurrently, such changes enable the correction of epigenetic errors that may have accumulated during an organism's lifetime (Hajkova et al., 2008). In maturing male gametes, an additional epigenetic reprogramming event occurs in late spermatogenesis that involves the replacement of the majority of histones previously bound to DNA with protamines and other histone variant proteins. This major remodelling event serves to impose further condensation of the chromatin structure leading to tight packaging of the paternal genome held inside the nucleus of maturing spermatozoa (Ward and Coffey, 1991; Balhorn et al., 2000; Braun, 2001; Hajkova et al., 2008). Protamines, and most other male-inherited histone variants, in themselves carry no heritable epigenetic information since most are replaced by oocyte-specific histone variants immediately after fertilization (Dworkin-Rastl et al., 1994; Teranishi et al., 2004). However, a small number of male-inherited histones are retained in the zygote where they may be capable of transmitting epigenetic information to the developing embryo (Balhorn, 2007; Gaucher et al., 2010; Kota and Feil, 2010).

Embryonic epigenetic reprogramming

The second major phase of genome-wide reprogramming occurs during early embryonic development, commencing immediately after fertilization and persisting until blastocyst formation (Kono et al., 2004; Hirasawa et al., 2008; Kobayashi et al., 2012; Smith et al., 2012). During this key developmental window, the embryonic cells undergo global DNA demethylation and histone replacement (Labosky et al., 1994; Tada et al., 1998; Reik, 2007; Shin et al., 2010). These reprogramming events are essential for the acquisition of totipotency and generating a population of cells that are capable of indefinite proliferation and self-renewal during this early stage of development (Matsui et al., 1992; Durcova-Hills et al., 2001).

The global DNA demethylation in embryos differs significantly from that of the PGC demethylation process described above, with embryos having to initially process the two unique genomes derived from the male and the female gametes, each of which possesses a different chromatin structure and organization. Furthermore, embryonic reprogramming is not as comprehensive as in PGCs, since it allows retention of DNA methylation at imprinted loci that propagate a few specific maternally derived promoters and transposable elements (Borgel et al., 2010). Immediately after zygote formation, the highly methylated paternal genome is subjected to rapid and full demethylation before the first mitotic division to ensure proper chromosome pairing (Mayer et al., 2000; Okae et al., 2014; Smith et al., 2014). This is an active process involving a number of key events, including but not limited to the ten–eleven translocation methylcytosine dioxygenase (TET3) catalysed oxidation of 5-methyl-cytosine (Eckersley-Maslin et al., 2018). During the restructuring of the paternal genome, protamines and paternally derived histones are replaced by histone variants arising from the maternal stores within the oocyte (Yang et al., 2015), thus returning the genome to a less tightly bound and compacted chromatin configuration (Dworkin-Rastl et al., 1994; Teranishi et al., 2004). In contrast, the chromatin structure of the maternal genome remains relatively stable with minimal modifications occurring to the histones bound to the maternal DNA (Santos et al., 2002; van der Heijden et al., 2005). Indeed, the less methylated maternal genome only undergoes passive demethylation as a result of DNA replication, a process that leaves imprinted loci intact (Santos et al., 2002; Borgel et al., 2010). This phenomenon is at least partly attributed to the protection of the maternal genome from active demethylation by the presence of the developmental pluripotency-associated 3 protein (Santos et al., 2005; Nakamura et al., 2012; Peat et al., 2014).

These combined reprogramming events culminate in a globally demethylated, fused genome inside the cells of the pre-implantation embryo (Reik, 2007). In pre-implantation embryonic cells, the reprogramming event continues, rapidly remodelling the heterochromatin structure with modification of epigenetic marks in histones being necessary to accommodate the changes in gene regulation and expression that characterize this highly dynamic developmental period (Burton and Torres-Padilla, 2010). Following implantation, genome-wide *de novo* methylation takes place to establish the methylation pattern of the developing embryo (Smith et al., 2012). The maintenance of genomic imprints through the reprogramming phase has been proposed to underpin the differential behaviour of the paternal and maternal genomes post-fertilization (Reik and Walter, 2001; Hackett and Surani, 2013).

External factors influencing epigenetic inheritance

The reprogramming events that take place in PGCs and early embryonic cells comprise the most intense period of epigenetic change experienced by the (epi)genome during the mammalian life cycle (Skvortsova et al., 2018). Any epigenetic modifications that endure both reprogramming events become integrated into the epigenetic pattern and thus persist throughout the life of the individual and may

be passed down to future generations (Heijmans *et al.*, 2008; Ng *et al.*, 2010). Thus, errors that arise during replication or in response to external environmental factors that fail to be corrected by normal epigenetic maintenance processes or reprogramming events can lead to long-term consequences affecting the phenotype and survival of an individual (Hitchins, 2010).

Multiple environmental factors have been found to influence the formation and maintenance of specific epigenetic patterns in both somatic and germline cells (Nilsson *et al.*, 2018a,b,c). The detrimental effects of environmental factors, such as diet composition or exposure to trace elements, have been shown to alter the native DNA methylation patterns and inflict histone modifications during gamete production and early embryonic development in mice (Waalkes *et al.*, 2004; Delage and Dashwood, 2008). However, exposure to environmental factors occurs primarily in somatic cells, which are not capable of transmitting the altered epigenetic pattern. Only modifications induced at an early developmental stage or in PGCs have the potential to become inheritable. These environmentally induced modifications have severe consequences, often precipitating the early onset of disease in affected individuals (Godfrey *et al.*, 2007).

In mammals, the diet of progenitors has been found to impact the inherited epigenetic information transmitted to the next generation. For instance, in rat models held under controlled dietary regimens, the offspring of males fed a chronic high-fat diet suffered from an early onset of impaired insulin secretion and glucose tolerance (Ng *et al.*, 2010). While the genomic composition of these rats was identical to the offspring conceived from control fathers not subjected to the high-fat diet, the overall gene expression profile proved to be significantly different. Indeed, among a large number of differentially expressed genes, interleukin 13 (*Il13ra2*) was the most dramatically affected, being characterized by hypomethylation in both the fathers consuming the high-fat diet fathers as well as their offspring. Since the offspring themselves were fed a control diet, such changes appear indicative of an epigenetic form of inheritance (Ng *et al.*, 2010). Similar alterations to insulin and glucose metabolism were reported in mouse models exposed to dietary interventions. Indeed, both of these metabolic parameters were negatively impacted in the offspring of fathers fed more frequently than controls, a phenotype that persisted in the two subsequent generations, again suggestive of transgenerational epigenetic inheritance transmitted via the male germline (Pentinat *et al.*, 2010). In humans, the transgenerational effect of diet on the epigenome has been linked to poor nutrition and reduced food availability.

In a classic example, the babies of Dutch women subjected to severe food restriction during pregnancy at the time of the Second World War were reported as having lower than normal weight at birth, a phenomenon that persisted in the following generation despite no dietary restrictions being imposed during conception or fetal growth (Roseboom *et al.*, 2006). Regrettably, the mechanisms underpinning these observations could not be fully investigated at the time owing to a paucity of techniques to analyse the epigenome. Nevertheless, more recent studies in humans have linked prenatal famine to alterations in the offspring, particularly to changes in the DNA methylation of imprinted genes, [such as insulin-like growth factor 2 (*Igf2*), insulin (*Ins*), guanine nucleotide binding protein α-stimulating (*Gnas*)] and in loci involved in growth and metabolic processes, including *Il10*, leptin (*Lep*) and ATP binding cassette A1 (*Abca1*; Heijmans *et al.*, 2008; Tobi *et al.*, 2009). Conversely, other studies have reported minimal alterations to the DNA methylation pattern of offspring of mothers under caloric restriction diet or fathers on low-protein diets (Jimenez-Chillaron *et al.*, 2009; Carone *et al.*, 2010). Similarly, there are reports of exposure to famine during gestation not eliciting significant alterations in DNA methylation profiles (Heijmans *et al.*, 2008). The transgenerational effects of diet have also been linked to the nutritional habits and diet composition of individuals. Human populations have been shown to be unintentionally exposing themselves to toxicants such as by ingesting food products containing acrylamide, a by-product in many carbohydrate-rich foods prepared at high temperatures (Dybing and Sanner, 2003; Katen and Roman, 2015). Acrylamide's principal metabolic product, glycidamide, induces the most severe genotoxic effects (Butterworth *et al.*, 1992) and results in the alkylation of protamines, DNA strand breaks via adduct formation and chromosomal aberrations (Von Tungeln *et al.*, 2009, 2012; Hansen *et al.*, 2010). The male germline of rodents has been shown to be particularly susceptible to acrylamide, with multiple studies reporting both an increase in glycidamide adducts and DNA damage in spermatocytes (Shelby *et al.*, 1986; Sega and Generoso, 1990; Bjorge *et al.*, 1996; Nixon *et al.*, 2012; Katen *et al.*, 2016). Furthermore, breeding experiments have revealed a link between reproductive toxicity and male exposure to high doses of acrylamide (Sakamoto and Hashimoto, 1986; Shelby *et al.*, 1986; Zenick *et al.*, 1986), as well as demonstrating that lower doses produce heritable translocations, reduced fertility and increased germline DNA damage (Katen *et al.*, 2016) and mutational load in their offspring (Russel *et al.*, 1991; Ehling and Neuhäuser-Klaus, 1992). These data suggest that environmental exposure must affect both the parental germline and endure the pre-implantation embryonic reprogramming event in order to be transgenerationally inherited.

Aside from diet, a variety of additional environmental factors have been found to impact epigenetic inheritance in mammals. For instance, exposure of rats to the anti-androgen endocrine disrupter vinclozolin was initially shown to trigger alterations to the DNA methylation pattern in the testes of adult males leading to the subsequent disruption of DNA methylation profiles in spermatozoa for the next two generations of unexposed males (Anway *et al.*, 2006a,b). Furthermore, compromised gonad development and spermatogenesis in the offspring of exposed fathers or grandfathers was correlated with the inheritance of alterations to the DNA methylation status at several promoter sites in spermatozoa (Guerrero-Bosagna *et al.*, 2010). However, despite multiple reports describing the persistence of the compromised phenotype for four generations in male rats (Anway *et al.*, 2006a,b) and three generations in female rats (Nilsson *et al.*, 2008) after initial exposure to vinclozolin, the existence of transgenerational epigenetic inheritance in mammals has been contested by studies that reported vinclozolin failed to induce any transgenerational abnormalities in the DNA methylation profile of exposed individuals or of subsequent generations (Schneider *et al.*, 2008; Inawaka *et al.*, 2009). Nevertheless, exposure to alternative toxic compounds, such as bisphenol A, during the early stages of embryonic development has been shown to provoke widespread alterations to the epigenome of both somatic and germline cells of exposed individuals (Chianese *et al.*, 2018), epigenetic changes that can be integrated and perpetuated via epigenetic transgenerational inheritance (Salian *et al.*, 2009; Skinner *et al.*, 2010).

Although there is now mounting evidence supporting transgenerational epigenetic inheritance in mammals in response to environmental

factors, such as diet and exposure to toxic chemicals, the extent to which such modifications of an individual's epigenetic profile impacts the phenotype of their offspring is still poorly understood. The impetus to improve our understanding of the mechanisms regulating the germline epigenome and its impact on inherited traits and disease susceptibility is given further credence by the contemporary practice of administering pharmaceuticals that directly target epigenetic modifying proteins. Notwithstanding the considerable promise of these novel therapeutic interventions to combat diseases such as cancer, their potential to elicit off-target effects on the epigenetic information contained in a patients' gametes remains poorly understood, as does the possibility that they could contribute to lasting effects on subsequent offspring (Jarred *et al.*, 2018; Western, 2018).

Inheritance of genomic information contained in telomeres

The information contained in coding genes is generally considered the main form of heritable information. However, as described above, epigenetic information contained in DNA methylation, histone PTMs and ncRNA are also able to modulate and influence the inherited phenotype of the offspring. Furthermore, it is now known that genomic information may be carried in the non-protein-coding repeat regions of the genome that form telomeres, promoters and/or enhancer regions (Table II). Mammalian telomeres are composed of non-coding DNA repeats that form nucleoprotein complexes and are located at the extremities of chromosomes. These structures serve the dual purpose of protecting chromosome ends from degradation as well as preventing end-to-end fusions (Blackburn, 2005; Palm and de Lange, 2008). In humans, telomere length at the extremities of specific paternal chromosomes has been shown to strongly influence the length of the equivalent telomeric regions in their offspring's chromosomes (Graakjaer *et al.*, 2006). In accounting for these findings, paternal and X chromosome-linked inheritance have been proposed as the main mechanism(s) influencing telomere length in offspring (Nordfjall *et al.*, 2005; Njajou *et al.*, 2007); although genetic variation at specific genomic loci has also been found to strongly determine the size of telomeres (Vasa-Nicotera *et al.*, 2005; Andrew *et al.*, 2006). Furthermore, the inheritance of this genomic information via the paternal side has been linked to increased activity of telomerase in the testes, where the enzyme functions to extend the telomere length of chromosomes carried by developing spermatozoa (Zalenskaya and Zalensky, 2002; Baird *et al.*, 2006). In contrast, no evidence has yet been reported supporting an inherited maternal effect on telomere length, which is consistent with the fact that telomere length remains constant in oocytes (Kimura *et al.*, 2008; Arbeev *et al.*, 2011).

Interaction between genetic and epigenetic modifications

The inheritance of modified genetic/epigenetic information, such as altered DNA methylation patterns, histone modifications, coding gene mutations and/or shorter telomere lengths, is commonly associated with disease states and metabolic syndromes (Godfrey *et al.*, 2007; Ng *et al.*, 2010; Pentinat *et al.*, 2010; Willeit *et al.*, 2010; Xue *et al.*, 2012). Although epigenetic and genetic alterations were traditionally considered as two distinct mechanisms, more recent work has provided evidence that epigenetic modifications are capable of influencing, and in extreme cases promoting, DNA sequence mutations (Lutsenko and Bhagwat, 1999; Schuster-Bockler and Lehner, 2012; Tang *et al.*, 2012). Similarly, variation in the genome sequence can affect methylation marks at regulatory regions and condition histone binding (Kilpinen *et al.*, 2013; McVicker *et al.*, 2013), thus regulating overall genome stability (Skinner, 2011; You and Jones, 2012; Kasowski *et al.*, 2013). Illustrative of this phenomenon, short telomere lengths have been implicated in instigating cell senescence as well as genetic and epigenetic instability that increased the risk of cancer (Willeit *et al.*, 2010). Such changes may also influence the genetic and epigenetic information transmitted to future generations if incorporated into the genome of their gametes (Hao *et al.*, 2005; Roberts *et al.*, 2013).

Accordingly, environmental factors and biological processes influencing either epigenetic or genetic modifications have an attendant risk of impacting, either directly or indirectly, both forms of information. In extending the example described above whereby vinclozolin promotes DNA methylation changes and transgenerational inheritance of modified epigenetic patterns via the male gametes (Anway *et al.*, 2006a,b; Nilsson *et al.*, 2008; Guerrero-Bosagna *et al.*, 2010), subsequent investigations have shown that differential methylation in non-coding regions are correlated with a significant increase in genetic copy number variation mutations in offspring. Moreover these mutations are able to persist up to the third generation (Manikkam *et al.*, 2012; Skinner and Guerrero-Bosagna, 2014; Skinner *et al.*, 2015).

The impact of ageing on the inheritance of transgenerational information

The biological process of ageing involves widespread molecular mechanisms that influence the genetic and epigenetic composition of an individual's cells and is often associated with increased susceptibility to diseases and abnormal syndromes. Additionally, the age of parents at the time of conception has been shown to correlate with an increase in the number of genetic mutations carried by their children (Kong *et al.*, 2012). In humans, the offspring of ageing mothers experience an increased risk of non-disjunction anomalies giving rise to genetic disorders such as Down syndrome (Hassold and Hunt, 2009). In the case of fathers, the ageing process has been associated with an increase in the number of mutational errors in their genome and an attendant rise in the incidence of congenital anomalies, different forms of cancer and neurological abnormalities in their offspring (Malaspina, 2001; Murray *et al.*, 2002; Choi *et al.*, 2005; Grether *et al.*, 2009; Aitken and De Iuliis, 2010; Green *et al.*, 2010; Aitken, 2013). While the mechanisms linking increased disease risk and advanced paternal age have not yet been elucidated (Chen *et al.*, 2008; Petersen *et al.*, 2011), the circumstantial evidence linking paternal age with an increased level of DNA damage in sperm cells, a rise in the rate of mutations in their offspring and a variety of disease states (including complex neurological conditions such as autism and spontaneous schizophrenia) is extremely strong (Schmid *et al.*, 2007; Aitken and De Iuliis, 2010; Aitken *et al.*, 2012, 2013; Goriely and Wilkie, 2012; Kong *et al.*, 2012). In general,

Table II Common genetic modifications and associated effects on the mammalian genome.

Genetic modifications	Effect on genome function
Single nucleotide polymorphisms	Exchange of a single nucleotide in the DNA sequence. Predominantly neutral or deleterious effect on phenotype of individuals. Rarely evolutionary advantageous.
Copy number variants	Duplication or deletion of repeat elements in defined genomic regions affect genomic structure and regulate gene expression.
De novo transposable elements insertions	Insertion of mobile DNA elements into new genomic positions, commonly Alu sequence. May interrupt or modify gene function if transferred into the sequence of an extant gene.
Telomere length	Non-coding repeat DNA elements at the extremities of all chromosomes. Protect chromosomal degradation and end-to-end fusion. Telomere shortening known to provoke replicative senescence.

paternal age can have an impact on the well-being of children via three fundamental mechanisms: genetic mutations, telomere length and epigenetic changes to both the DNA and associated protein PTMs.

The accumulation of mutations in the germ cells of ageing mammalian fathers is commonly attributed to an increased incidence of replication errors in the PGCs that sees them transmitted to all developing sperm cells (Crow, 2000; Goriely *et al.*, 2009). However, in mice at least, the DNA proofreading and repair mechanisms presiding over the male germline have been shown to be extremely efficient in repairing errors. This may help explain the seemingly low rate of spontaneous mutations arising in the spermatozoa of ageing mice compared to that of their somatic cells (Hill *et al.*, 2005). Thus when age-related dominant mutations do occur in the male germline they are rare and frequently influenced by other factors such as enhanced stem cell fitness in spermatogonial stem cells, as reflected by the fibroblast growth factor receptor 2 (*Fgfr2*) mutation associated with Apert syndrome (Martin *et al.*, 2014). Indeed, mutations that enhance stem cell proliferation are known to give rise to 'hotspots' in the testes of spermatogonia carrying mutated genes (Maher *et al.*, 2018). Given the excellent DNA editing and repair capacity of the male germline, it is conceivable that the linear increase in *de novo* genetic mutations seen in children as a consequence of paternal ageing (Goldmann *et al.*, 2016; Goriely, 2016) may involve non-replication dependent mechanism(s), such as those discussed below, which are enacted after gamete production has occurred.

The length of telomeres is known to be consistently shortened after each chromosomal replication event (Blackburn, 2005) and in most proliferating tissues occurs concurrently with ageing (Ishii *et al.*, 2006; Kimura *et al.*, 2008). Thus, telomere length can be considered to function as a mitotic clock that regulates cell proliferation and, upon reaching a critical length, stalls cell division and initiates apoptosis. Several proliferating cell types, including stem and cancer cells, avoid cellular senescence by increasing telomerase activity and thus lengthening their telomeric regions (Blackburn, 2005). In humans, inheritance of telomere length has been proposed to occur via the paternal side (Nordfjall *et al.*, 2005; Njajou *et al.*, 2007), with paternal age influencing the length of telomeres in offspring for several generations (De Meyer *et al.*, 2007; Eisenberg *et al.*, 2012). The production of spermatozoa with longer telomeres in older men has been attributed to increased telomerase activity in the testes (Baird *et al.*, 2006; Kimura *et al.*, 2008). Interestingly, this process of inheritance regulating offspring telomere length is further compounded by the age of the paternal grandfathers. Thus, individuals conceived from a legacy of both grandfathers and fathers that were old at the time of conception possess telomeric regions that are longer than might otherwise be expected (Eisenberg *et al.*, 2012).

In addition to impacting genomic integrity, the ageing process has also been shown to alter both the methylation status of sperm DNA (Jenkins *et al.*, 2014, 2018a; Milekic *et al.*, 2015; Ciccarone *et al.*, 2018) and the composition of histones (Jenkins and Carrell, 2012). In the work by Jenkins *et al.* (2014), changes to sperm DNA methylation patterns of fertile donors were evaluated in two samples collected at intervals of 9–19 years. This strategy identified numerous age-related changes in the sperm DNA methylome, including an enrichment at genes previously associated with schizophrenia and bipolar disorder. While such data do not establish a causative relationship, they do raise the prospect that altered sperm DNA methylation profiles could contribute to an increased incidence of neuropsychiatric and other disorders in the offspring of older males (Jenkins *et al.*, 2014). Similarly, experiments in rodent models have provided initial evidence that paternal age can influence behavioural traits and exacerbate aeging-related pathologies in offspring (Jenkins *et al.*, 2018b), with recent genome-wide epigenetic analyses linking such changes to differential methylation of promoters for genes involved in the regulation of evolutionarily conserved longevity pathways (Xie *et al.*, 2018). In the context of histone modifications, it is known these DNA binding proteins continually acquire new methyl groups after each cell division (Wang *et al.*, 2018). Thus, older histones tend to accumulate and exhibit higher methylation levels compared to that of newly synthesized histones (Gonzalo, 2010; Xu *et al.*, 2012). In several mammalian cell types, the methylation status at specific sites in histones H3 and H4 has been shown to vary with age (Sarg *et al.*, 2002; Fraga and Esteller, 2007; Wang *et al.*, 2010). However, histone modifications within the male germline have not been studied in detail as a function of paternal age. Environmental and lifestyle factors are thought to alter histone retention by the male germline as well as histone alkylation status but the detailed nature of these changes and the developmental consequences for the offspring remain unknown. Given the reported changes to histone PTMs during somatic cell ageing (Agherbi *et al.*, 2009; Gonzalo, 2010), it is plausible that histone modifications may influence the state of the heritable information transmitted to the offspring of ageing parents. Similarly, the impact of ageing on the ncRNA species generated or acquired by the germline during gametogenesis or epididymal maturation (Nixon *et al.*, 2015) has not been clearly resolved but is likely to play an important role in the determination of offspring health (Yuan *et al.*, 2016).

The impact of oxidative stress on the inheritance of transgenerational information

The future direction of research on transgenerational information inheritance will now inevitably focus on the underlying mechanisms with a view to understanding how epigenetic changes in the germline can either be inherited in their own right or become fixed in the genome as genetic mutations in the offspring that will be transmitted to future generations. The proximal drivers for such epigenetic change include ageing as well as a variety of lifestyle and environmental factors such as cigarette smoking, alcohol consumption and exposure to chemical toxicants (Fullston *et al.*, 2017). We hypothesize that all such factors induce epigenetic modifications in the germline that have the potential to become converted into mutations in the offspring as a result of aberrant or inefficient repair. Since the female germline spends most of its life in a state of repose, we further suggest that this process of epigenetic change in gametes leading to genetic mutations in the offspring is largely focused on the male, as is demonstrable in the case of ageing, smoking and obesity (Kong *et al.*, 2012; Fullston *et al.*, 2017; Gunes *et al.*, 2018). In this context, there is clearly a variety of possible mechanisms that might underpin epigenetic changes in the male germline with potential impacts on offspring health (DNA methylation, histone/protamine PTMs, alterations to the complement of sperm-borne small ncRNAs, increased sperm DNA damage, changes to the sperm centrosome, etc.). However, the only one of these epigenetic changes that might readily precipitate a mutation in the offspring is chemical modification or damage to the DNA itself. We propose that a key element in this process is oxidative stress and that the major agent of change is 8-hydroxy-2′-deoxyguanosine (8OHdG) formation within the male germline.

Oxidative stress is known to be a major feature of ageing (Balaban *et al.*, 2005; Haigis and Yankner, 2010) and has been often associated with an increased production of reactive oxygen species (ROS) in spermatozoa (Aitken and Clarkson, 1987; De Iuliis *et al.*, 2009; Aitken *et al.*, 2010; Aitken and Curry, 2011). Increased ROS production has also been linked to epigenetic modifications in cancerous cells, where alterations to established DNA methylation patterns have been found to occur in response to elevated levels of ROS (Donkena *et al.*, 2010; Ziech *et al.*, 2010) that, in turn, interfere with methyltransferases activity, thereby reducing methylation patterns on both a local and global scale (Franco *et al.*, 2008). Among other known consequences of elevated ROS production, oxidative stress can promote gene silencing via hypermethylation of tumour suppressor gene promoter regions, thus promoting the expression of cancerous phenotypes (Campos *et al.*, 2007; Ziech *et al.*, 2011). The progressive accumulation of oxidative damage in mammalian cells has also been found to trigger telomere shortening and replicative senescence (Passos and von Zglinicki, 2005). However, one of the most profound changes induced by oxidative stress, and one of the most significant in terms of transgenerational information inheritance, is chemical modification of guanine bases to produce the highly mutagenic base adduct, 8OHdG.

Spermatozoa are particularly vulnerable to oxidative attack as a consequence of their limited capacity for DNA repair (Smith *et al.*, 2013a,b) and the minimal availability and restricted distribution of cytoplasmic space in which to house the antioxidant enzymes that protect somatic cells from oxidative attack. The presence of 8OHdG adducts in sperm chromatin is therefore relatively common, possibly due to the powerful oxidizing post-testicular environment presented by the male reproductive tract (Esteves *et al.*, 2017). Indeed, if the major antioxidant in this region of the male reproductive tract, glutathione peroxidase 5 (*Gpx5*), is functionally deleted then the spermatozoa exhibit high levels of 8OHdG formation and there is an increase in the incidence of birth defects and miscarriages above control levels as the males age (Chabory *et al.*, 2009).

When spermatozoa experience oxidative DNA damage as a consequence of ageing, lifestyle, environmental factors or simply the long and perilous journey to the site of fertilization, it is then the responsibility of the oocyte to repair this DNA damage prior to the initiation of S phase of the first mitotic division. If the oocyte conducts inadequate or aberrant repair of this DNA damage, it opens the opportunity for mutations to occur that will affect every cell in the body. Since 8OHdG lesions are the most common kind of DNA damage in spermatozoa, attention has focused on the base excision repair (BER) pathway responsible for repairing oxidative DNA damage, the first enzyme of which, 8-oxoguanine DNA glycosylase (OGG-1), is clearly present in spermatozoa (Smith *et al.*, 2013b).

OGG-1 cleaves the oxidized base out of the DNA duplex to generate a corresponding abasic site thereby destabilizing the ribose-phosphate backbone, leading to a β-elimination or a ring opening reaction of the ribose unit and a potential strand break. Because spermatozoa do not possess the next components of the BER pathway, namely apurinic endonuclease 1 and X-ray-repair-complementing-defective-repair-in-Chinese-hamster-cells 1, the abasic sites created by OGG-1 persist in the male genome until fertilization occurs. At this point, the oocyte, which contains these factors in abundance, continues the BER pathway in preparation for S phase of the first mitotic division (Smith *et al.*, 2013b). The major flaw in this otherwise laudable example of inter-gender co-operation is that the oocyte expresses OGG-1 at a relatively low level (Lord and Aitken, 2015). As a consequence, if the spermatozoon carries into the oocyte unresolved oxidized base lesions, the oocyte has a limited capacity to affect their removal. This is a significant biological problem because mammalian spermatozoa frequently express high levels of 8OHdG as a consequence of factors such as ageing (Selvaratnam *et al.*, 2015). The persistence of these highly mutagenic lesions into S phase of the first mitotic division may therefore explain why mutation frequencies rise as a linear function of paternal age, even though mutation rates in the germline itself are relatively low (Tiemann-Boege *et al.*, 2002; Aitken and Curry, 2011; Smith *et al.*, 2013a,b). When high levels 8OHdG are artificially created in spermatozoa by removing antioxidant protection via functional inactivation of the BER pathway, the result is a high mutational load carried by the offspring, an increased incidence of miscarriage and, for those embryos that do progress to term, birth defects and morbidity in the progeny, including cancer, resulting in a significant shortening of lifespan (Ohno *et al.*, 2014). Similarly, when 8OHdG adducts are created in spermatozoa as a result of heavy paternal smoking, one of the major consequences of this activity is a significant increase in childhood cancer rates (Lee *et al.*, 2009).

Given this background, it will now be important to determine which areas of the paternal genome are damaged under conditions of oxidative stress and determine how such damage can generate mutations leading to morbidity in the offspring. Sperm DNA is

condensed to a point close to its physical limits of compaction, forming an almost crystalline structure (Johnson *et al.*, 2011; Smith *et al.*, 2013a; Casas and Vavouri, 2014). In its fully compacted state, sperm DNA becomes extremely difficult to damage (Aitken *et al.*, 2003; Miller *et al.*, 2010). Regardless, small portions of the remodelled chromatin structure remain susceptible to oxidative damage, particularly genomic regions between protamine-bound DNA structures, histone-bound regions and domains attached to the nuclear membrane (Noblanc *et al.*, 2013; Kocer *et al.*, 2015).

Of course, not all of the genetic damage created following ROS exposure is confined to 8OHdG formation. Modifications of DNA methylation and histone chemistry can occur in response to ROS (Franco *et al.*, 2008; Donkena *et al.*, 2010; Ziech *et al.*, 2010). Moreover, such changes have the potential to alter the effectiveness of epigenetic erasure, the binding capacity of DNA domains and the successful removal of histones and replacement with protamines. Thus the oxidative stress that pervades the germline of sub-fertile patients has the potential to create a heavy burden of damage to both the genome and the epigenome of the embryo with clear implications for the health trajectory of the offspring. In light of the wide range of conditions associated with oxidative stress in the male germline that are traditionally treated by ART (age, cryostorage, varicocele, infection, obesity, smoking and exposure to toxicants), there are particular implications for the normality of offspring generated by such technology, which deserve our scrutiny (Zini *et al.*, 2008; Aitken, 2009; Aitken *et al.*, 2009; Kobayashi *et al.*, 2009).

Conclusion

In conclusion, epigenetic modifications to histones, telomeres, ncRNAs, DNA and subcellular structures (mitochondria and centrosome) inherited through the germline may all have important consequences for the phenotype of the offspring. However, there are a variety of mechanisms operating to wipe the epigenetic slate clean between generations, so that short-term adaptive changes to the epigenome are not transmitted to the progeny. Nevertheless, the ability of epigenetic changes to the DNA, particularly 8OHdG formation, to generate mutations (such as deletions and transversions) following aberrant or inefficient repair by the oocyte, can have a lasting impact on offspring health and be readily transmitted across the generations. As a result, any of the many environmental or lifestyle factors known to be capable of causing oxidative stress in the germline can influence the genetic variability in the offspring. This may have important evolutionary significance, with environmental change creating a state of oxidative stress in the testes that then increases genetic variation in the offspring, thereby facilitating the process of natural selection. However, at an individual level, such oxidatively induced genetic variation may be pathological and responsible for a wide variety of paternally determined conditions, from complex neurological diseases such as spontaneous schizophrenia and autism that are correlated with the oxidative stress associated with paternal age (Aitken *et al.*, 2013), to the childhood cancers associated with paternal smoking (Lee *et al.*, 2009). In this context we might regard the testes as either the engine of evolution or an agent of affliction depending on scale and adaptive significance. This proposed mechanism is therefore central to the origin of *de novo* mutations carried by children as a consequence of factors influencing the integrity of their parents' germline and disrupting the accurate flow of genetic and epigenetic information from one generation to the next.

Authors' roles

M.J.X. designed the study, identified the articles, drafted and revised the manuscript. R.J.A. designed the study and revised the manuscript. B.N. and. S.D.R. edited and revised the manuscript. All authors approved the final version of the manuscript.

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Conflict of interest

None of the authors has any conflict of interest related to this publication.

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