nature cell biology

FOCUS | REVIEW ARTICLE
https://doi.org/10.1038/s41556-018-0242-9

Intergenerational and transgenerational epigenetic inheritance in animals

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Animals transmit not only DNA but also other molecules, such as RNA, proteins and metabolites, to their progeny via gametes. It is currently unclear to what extent these molecules convey information between generations and whether this information changes according to their physiological state and environment. Here, we review recent work on the molecular mechanisms by which 'epigenetic' information is transmitted between generations over different timescales, and the importance of this information for development and physiology.

DNA is a reliable information transfer system because of the accuracy of DNA replication. Humans, for example, copy 6 billion bits of information to their offspring with an error rate of approximately 2 bits per 100 million[1]. However, eggs and sperm contain more than DNA, and it has become increasingly apparent in recent years that other molecules beyond the genome sequence can also transfer information between generations. Moreover, this information can be altered following change in the physiological and environmental conditions of previous generations. Multiple mechanisms have been proposed to underlie non-DNA sequence-based inheritance and these can be either genome-associated (for example, covalent modifications of DNA and histones or transfer of small RNAs complementary to genomic sequences) or genome-independent (for example, microbiome transfer)[2]. Non-DNA sequence-based inheritance also varies in generational duration, with inheritance spanning one generation to a seemingly indefinite number.

The terms 'intergenerational' and 'transgenerational' are often used to describe such effects and require clarification. Transgenerational effects refer exclusively to phenomena that could not be ascribed to direct effects of a particular trigger on the affected organism. For instance, an environmental stimulus can directly affect a gestating embryo (and the already-formed oocytes within a female embryo in mammals[2,3]). As such, only altered phenotypes occurring in the second (in the case of male transmission) or third (in the case of female transmission) generation after a trigger can truly be described as transgenerational inheritance. Effects spanning shorter timescales are described as parental or intergenerational. Nonetheless, many described intergenerational effects share mechanisms with transgenerational effects. Another term that warrants discussion is epigenetic, whose once broader meanings[4] have narrowed in recent years, despite objections[5], to genome-associated mechanisms of non-DNA sequence-based inheritance—chiefly, DNA methylation, histone modifications and inherited RNAs[6].

Although DNA-based information transfer is extremely high fidelity, other mechanisms are far less robust, resulting in differences in the timescales of reliable information transfer[7]. One point of confusion concerns two separate distinctions that are often conflated: first, genetic (that is, DNA-based) versus epigenetic mechanisms of inheritance, and second, environmentally responsive versus unresponsive phenomena. Inheritance of environmentally acquired traits can also be mediated through genetic inheritance, as occurs in the CRISPR (clustered regularly interspaced short palindromic repeats) innate immunity system of prokaryotes[8]. Conversely, stable long-term transcriptional repression can be achieved by an inherited epigenetic memory, but one that is largely unresponsive to the environment and physiology[9–12]. It is the question of whether epigenetic mechanisms can provide a heritable (and potentially adaptive) memory of ancestral environmental exposure that has proven the most controversial[3].

Numerous examples of intergenerational and transgenerational effects in animals have been described, for instance, using model organisms such as *Caenorhabditis elegans* that reproduce quickly and allow simple control of genomic variation. However, we would contest that few well-established transgenerational effects are adaptive, in the sense of preparing future generations for enduring altered environmental conditions. Adaptive transgenerational effects, although conceivable for species such as *C. elegans* with lifecycles that are short with respect to environmental fluctuations, would be unlikely for long-lived animals such as humans. Here, we aim to give examples of non-DNA sequence-based inheritance, and review the mechanisms by which ancestral state can affect future generations and how these mechanisms change as we look to increasing timescales.

Parental effects

Examples of parental genotype or environment affecting progeny phenotype independent of inherited DNA ('parental effects') are numerous. However, with direct contact between the individuals exposed to a trigger and their immediate progeny (or their mate), many mechanisms can be involved. To confidently implicate specific mechanisms of inheritance, careful experimental design and interpretation are required[3]. Particular research effort has been directed at paternal effects[6], with the expectation that limiting a male's interactions with their partner and progeny to the act of mating alone will narrow potential mechanisms down to those transmitted via gametes. Even so, genome-independent mechanisms may still affect progeny phenotypes (Fig. 1). For example, microbiome transfer from father to mother can rescue the intergenerational effects of maternal antibiotic use in *Drosophila melanogaster*[13], and apparent paternal effects may in fact be cryptic maternal effects, when paternal conditions, such as depression-like states in mice[14], influences maternal investment or care.

The parental effects of diet and obesity are a well-studied paradigm[15], with obvious health relevance given the rise in obesity rates in Western countries in the past few decades. Intergenerational effects of parental nutrition have been suggested in humans[16,17] and demonstrated in rodents[18–25], *D. melanogaster*[26] and *C. elegans*[27,28]. In mammals, for example, undernutrition or overnutrition in either parent commonly affects offspring glucose metabolism[15]. Counterintuitively, the intergenerational effects of maternal and

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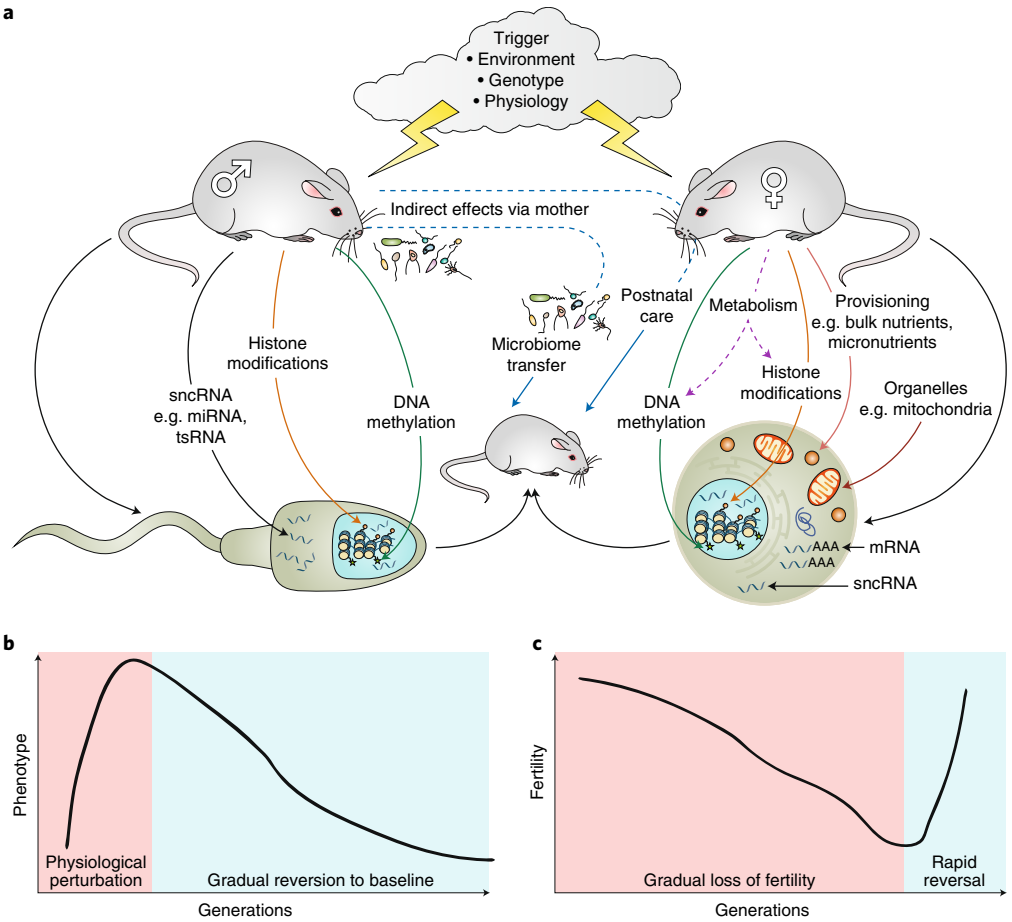

Fig. 1 | Mechanisms of transfer of information about ancestral environment or physiology over generations. a, Many mechanisms of transmission of information about environmental experience or physiological state can underlie inheritance over a single generation, from parents to progeny, both genome-associated (for example, covalent modifications of histones, sncRNAs, including tsRNAs and miRNAs, and DNA methylation, among others) and genome-independent (for example, microbiome transfer). Paternal effects are not always mediated by gametes but may act via the mother indirectly. **b**, Gradual changes in epigenetic marks might underlie transgenerational memory. A loss of gene repression caused by an environmental or physiological insult, for example, by perturbation of heterochromatin-mediated transcriptional repression, can reset gradually over generations, providing a transgenerational memory of ancestral experience. **c**, Mutations or natural variation in various epigenetic pathways can lead to Mrt phenotypes in *C. elegans*, in which fertility is lost gradually over generations but can be rapidly restored by changing conditions. The prevalence of this phenotype in mutants affecting chromatin modifications and small RNA pathways indicates the importance of epigenetic pathways in the maintenance of normal development and physiology.

paternal diet are often qualitatively and quantitatively similar[24,25,29,30]. However, such effects are often non-monotonic[26,27] and depend on the developmental stage when parental or grandparental exposure[16,18] occurs and on progeny sex[16–18,20] and diet[26]. For instance, both low-sugar and high-sugar paternal diets increased offspring adiposity in *D. melanogaster*, but only when the offspring were themselves challenged with a high-sugar diet[26].

Maternal provisioning and metabolism. Maternal provisioning to offspring may mediate effects of maternal diet[31,32] or other physiological factors. For example, increased provisioning of a lipoprotein yolk complex to offspring with advancing maternal age facilitates progeny growth and starvation resistance in *C. elegans*[33]. Offspring phenotypes may also be affected by provisioning of specific regulatory products, such as mRNAs[34,35], or essential micronutrients, such as zinc[36]. Physiological alterations in maternally supplied organelles, particularly mitochondria, could also underlie parental effects of diet, as a maternal high-fat diet impairs fetal mitochondrial function in mice[24]. Perturbation of maternal metabolism genetically[37] or by metabolite intake can influence epigenomic regulation in progeny and even further generations[38]. For instance, progeny DNA methylation can be influenced by maternal dietary intake of methyl donors in mice[39], with striking heritable effects on coat colour. Similar effects have also been suggested in humans, in which seasonal changes in dietary intake of methyl donors around conception in rural mothers correlate with alterations in DNA methylation in their children[40].

Microbiome transfer. Non-DNA-based inheritance may act via transfer of an altered parental microbiome[13]. Bacterial strains can be inherited maternally in humans[41], although the mechanisms—whether by breast milk, birth canal or even placental transfer—remain unclear[42]. In mice, diet-induced microbiome changes, specifically a progressive loss of taxonomic diversity due to a Western-style low-fibre diet, are cumulative over generations and are eventually irreversible via extinction of specific microbiotic subpopulations[43]. This finding suggests that multigenerational environmental exposure could cause a stable transgenerational alteration of progeny physiology via the microbiome.

DNA methylation in sperm. DNA methylation at cytosine residues has been suggested to mediate parental dietary effects in mammals[15]. Genomic imprinting, in which a gene's expression depends

on whether it is inherited paternally or maternally, is associated with differences in DNA methylation and demonstrates that DNA methylation states can be transmitted between generations in mammals[44]. The sperm methylome can be altered by interventions that produce intergenerational or transgenerational effects, such as in utero malnutrition[45], early-life overnutrition[46] and diabetes[47] in mice and by obesity in humans[48,49]. However, the mechanisms by which sperm methylation could be modified at specific sites are unclear. Moreover, methylation is largely erased upon fertilization[50] and it is not obvious how alterations could affect gene expression in progeny with high penetrance[15]. Reportedly, sperm methylation was unaffected by several diets that induce phenotypic effects in progeny[51].

Although cytosine methylation is absent from many organisms, such as *D. melanogaster*[52] and *C. elegans*[53], DNA methylation can occur at adenosine residues, although its functional importance and whether it carries information across generations[54] are unclear[55].

Small non-coding RNAs in sperm. Small non-coding RNAs (sncRNAs), particularly transfer RNA-derived small RNAs (tsRNAs) and microRNAs (miRNAs), are emerging as possible mediators of environmental information transmission through sperm in mammals[56]. Derived from precursor or mature tRNAs, tsRNAs are of diverse size and biogenesis mechanism[57] and have been implicated in various cellular processes, including repression of transposable elements[57–59]. Like miRNAs, tsRNAs can interact with small RNA-binding proteins of the Argonaute family to induce post-transcriptional gene silencing[57,60] via sequence complementarity to the 3′ untranslated regions of target mRNAs[60,61].

tsRNAs constitute most of the sncRNA pool in mature mammalian sperm[62], with miRNAs a distant second[58,63]. Sperm tsRNAs are reportedly altered by diet[63] or exposure to an endocrine disruptor[64] in rodents and by obesity in humans[49], whereas sperm miRNAs are altered by psychological stress in mice[65,66] and men[67] and by parental genotype[68], diet[69–71] and environmental deprivation[72] in mice, all conditions that are associated with paternally acquired disorders. Crucially, in several cases, zygotic injection of total sperm RNA[66,68,71,72], sncRNA fractions[63,71] or specific sncRNAs[58,68,70,73] could partially or fully recapitulate these paternally acquired phenotypes[15]. In mice, inheritance of sncRNA-mediated phenotypes relies on the activity of the RNA methyltransferase DNMT2[71,74], indicating that RNA modifications may constitute an additional layer of regulation important for transmission of acquired phenotypes through sperm[63]. In keeping with a role in repressing transposons, which often use conserved tRNAs as primers for replication[59], a specific sperm-borne tsRNA influenced by paternal diet was found to specifically regulate genes governed by the pluripotency-promoting endogenous retroviral element MERVL in the mouse zygote[58]. Remarkably, sperm tsRNAs do not originate from sperm tRNAs but are acquired via transfer of extracellular vesicles from the epididymis[58], offering a hint of soma-to-germline transmission of information. Recent results indicate that sperm miRNAs similarly acquired during epididymal transit are essential for embryonic development[75].

Histone modifications. There is some[24,26,28], but little, evidence for covalent modifications of histones mediating parental effects. However, histone modifications at some loci are certainly transmitted between generations in mammals[76], fish[77] and worms[78]. Thus, it is plausible that they could also underlie some parental effects. In *C. elegans*, an epigenetic memory of germline transcription, mediated histone H3K36 trimethylation (H3K36me3) on active genes[79–81] and H3K27me3 on repressed genes[78,81], is passed from each generation to the next and is essential for germline viability[79–81], representing an example of non-environmentally responsive epigenetic inheritance that is critical for normal development and physiology.

Multigeneration epigenetic inheritance

Examples of true transgenerational epigenetic inheritance (TEI) induced by parental genotype, physiology or environment have been increasingly numerous in model animals. However, in most cases, the effects described have a limited duration, for example, typically spanning 3–4 generations in *C. elegans*[82–85]. Characterized mechanisms commonly involve inheritance via gametes of genome-associated epigenetic information, such as histone modifications and small RNAs. Likely reasons for the limited duration of many transgenerational effects might be passive and active mechanisms that regulate small RNA populations and histone modifications across generations[86].

Inheritance of RNA interference in *C. elegans*. Although occurring in artificial laboratory conditions, the inheritance of gene silencing induced by ancestral RNA interference (RNAi) in *C. elegans*[87] has provided the most incontrovertible demonstration of TEI and has been invaluable in dissecting the mechanisms involved. Worms supplied with exogenous double-stranded RNA (dsRNA), usually by feeding, employ an amplification machinery that results in systemic silencing of complementary genes in almost all tissues, including the germline. dsRNA is processed by Dicer and accessory proteins to form primary short interfering RNAs (siRNAs). Primary siRNAs bind to a member of the Argonaute protein family and guide them to complementary mRNA transcripts. RNA-dependent RNA polymerases (RdRPs) are then recruited to produce abundant secondary siRNAs (otherwise known as 22G RNAs for their length and 5′ guanosine bias). RdRP-associated silencing is found in diverse taxa, although not in vertebrates[88]. In turn, these 22G RNAs engage various Argonautes to destroy complementary mRNAs, inhibit transcription[89] and deposit the repressive chromatin marks H3K9me3 and H3K27me3 at the target locus[89–91].

Gene silencing induced by dsRNA can be inherited[87,92], typically for up to three generations[83] but sometimes for longer when selecting for the resulting phenotype[92]. The nuclear Argonaute heritable RNAi defective 1 (HRDE-1) is dispensable for gene silencing in exposed worms but is necessary for its inheritance in subsequent generations[93], demonstrating that *C. elegans* possess machinery dedicated to intergenerational information transmission. The nuclear RNAi pathway, which shuttles 22G RNAs into the nucleus[94], is required for RNAi inheritance[95]. The limited duration of the silencing response may be due to dilution of siRNAs over generations[90]. Unlike primary siRNAs, secondary siRNAs rarely serve as templates for further amplification, and the duration of the gene silencing response induced by dsRNA is therefore limited[96,97]. The repressive H3K9me3 and H3K27me3 footprints triggered by secondary siRNAs also persist in the absence of the dsRNA trigger for at least two generations[90,91], although H3K9me3 deposition is dispensable for heritable silencing at some loci[98,99]. Interestingly, the H3K9 methylase gene *met-2*, which is responsible for H3K9me1/2, limits the generational duration of some dsRNA-induced silencing by suppressing siRNA inheritance machinery[100]. Application of an additional dsRNA trigger unrelated to the original target in subsequent generations can extend the duration of inherited silencing, possibly due to disruption of negative feedback that may limit heritable response duration by supressing the RNAi machinery[101].

Why did *C. elegans* evolve the ability to respond to dsRNA with systemic-targeted silencing? The RNAi machinery is required for some antiviral responses in *C. elegans*[102–104], and inheritance of parental antiviral small RNAs has been suggested to block the transmission of virus infection between generations[84,105]. However, a heritable response was not observed for the only known natural virus of *C. elegans*[106].

Small RNAs and histone modifications in TEI. The importance of small RNAs for RNAi inheritance in *C. elegans* underscores mobile

RNAs as an attractive candidate for mediating transgenerational inheritance in multiple species[107]. dsRNA produced in somatic tissues, including neurons, can be inherited in *C. elegans*[108], and reports indicate the transfer of somatic RNAs to gametes in mice[58,75,109]. The RNAi pathway in *C. elegans* was also found to target endogenous genes, utilizing a similar amplification mechanism as exogenous RNAi[110,111]. Indeed, endogenous RNAi is necessary for transgenerational inheritance of gene regulatory and physiological changes in response to ancestral starvation[112] and heat stress[85].

Histone modifications are important in RNAi inheritance in *C. elegans* and various histone modifications have been implicated in other cases of transgenerational inheritance, including methylation of H3K4 in mice[113] and *C. elegans*[54,82,114,115], H3K9 in *C. elegans*[9,12,54,116,117] and H3K27 in *C. elegans* and *D. melanogaster*[116,118,119]. Stress-induced perturbations to histone modifications may revert slowly over generations[117] in *C. elegans*, leaving a gradually fading transgenerational memory. In some cases of histone-associated transgenerational memories, global levels of histone modifications remain modified in later generations[117,118], whereas in others, global levels are unchanged[28,82], implying differential regulation of histone marks at specific loci[120]. In *C. elegans*, transgenerational inheritance of longevity phenotypes caused by ancestral mutations in the conserved COMPASS H3K4 methylases is dependent on the corresponding demethylase[82], demonstrating that alterations in the antagonistic activity of chromatin-modifying enzymes over generations can induce transgenerational phenotypes[54].

TEI to pre-adapt progeny to environmental conditions. Despite increasing popularity, the evidence for adaptive, environmentally responsive TEI remains scant. Most documented cases of inheritance of environmental experience occur in artificial contexts[114,117], even when those experiments attempt to mimic naturally occurring challenges[84], and the relationship of ancestral environment to alterations in progeny gene regulation or physiology in terms of fitness is often far from clear[84,85,112,118]. Nonetheless, a few reports suggest such a possibility. Exposure of *C. elegans* to heavy metals leads to increased resistance to the same stresses in future generations, termed transgenerational hormesis[115]. Likewise, ancestral starvation in *C. elegans* induces transgenerational resistance to starvation[121,122]. Although TEI effects were mostly described in *C. elegans*, a striking case of potentially adaptive TEI involving soma-to-germline communication was found in mice, in which a conditioned fear response to a specific odour in male mice was inherited over two generations[123]. In this case, the effect was associated with enlargement of neuroanatomical structures in progeny and with hypomethylation of the corresponding odour receptor locus in the sperm of exposed males (although not their sons). Still, at present, it seems that adaptive, environmentally responsive TEI, if it exists, is the exception rather than the rule. Nonetheless, epigenetic mechanisms can transfer information about ancestral state between generations, and although the extent of this transfer is typically limited to a few generations, some specific cases—arising from a loss of gene repression—can lead to longer-lasting memories.

Long-lasting TEI

Despite the meagre evidence for adaptive memory of environmental conditions, an adaptive transgenerational memory exists to distinguish 'self' genetic elements from that of potentially harmful 'foreign' sequences. Repetitive genomic regions, such as transposons, are constitutively repressed by heterochromatin. Rather than becoming re-established de novo each generation, the heterochromatic state of repetitive regions is often inherited. Environmental insults disrupting this repression can quantitatively modulate expression from heterochromatic regions, taking many generations to restore.

For instance, growth at an elevated temperature[117] or impaired DNA replication during embryogenesis[124] can impair heterochromatic suppression of transgene arrays in *C. elegans* that can take more than 10 generations to fully re-establish (Fig. 1b). Expression of a subset of endogenous repetitive elements repressed by H3K9me3 also heritably increased at an elevated temperature, albeit for fewer generations[117]. Heat can also derepress pericentromeric heterochromatin in *D. melanogaster*[125], leading to a long transgenerational epigenetic memory of ancestral environment. In both *C. elegans* and *D. melanogaster*, multiple generations of heat exposure and consequent derepression were required to maximize inheritance duration[117,125]. These results are consistent with the gradual restoration of heterochromatic regions perturbed by stress, the 'healing' of an 'epigenetic wound'[86]. Thus, this memory may result from a limited capacity to restore disturbed heterochromatin within a single generation, although it is unclear why this would be so and whether it has ever been co-opted for an adaptive purpose.

The mortal germline of *C. elegans*. A reciprocal phenomenon to this slow recovery following chromatin perturbation is the mortal germline (Mrt) phenotype of *C. elegans* mutants (and some naturally occurring strains[126]). These animals display a progressive reduction in fertility, which is often temperature sensitive, that accumulates over generations and ultimately results in sterility (Fig. 1c). Whereas some Mrt cases result from genetic changes such as telomere loss[127,128], many mutated genes that cause the Mrt phenotype are involved in histone modifications[54,93,100,129–132] or small RNA pathways[93,131,133,134], and the phenotype can be rapidly reverted by returning animals to the permissive temperature[120,126,131], altering diet[135], re-introducing functional gene copies[129] or introducing downstream mutations[100], demonstrating the epigenetic nature of these transgenerational phenotypes. Interestingly, the Mrt phenotype of *C. elegans* Piwi mutants results not from a loss of germline totipotency but rather from the aberrant (and reversible) induction of reproductive quiescence, normally induced under stress, as a consequence of transcriptional dysregulation in the germline[135]. If this finding is generally applicable, it explains why the Mrt phenotypes can be reverted so rapidly.

Stable TEI of gene silencing

Despite their long duration, the TEI effects described above can be eventually reverted upon encountering permissive conditions. Nevertheless, stable and irreversible TEI of gene silencing has been found in invertebrates.

Small RNA-triggered stable silencing. The inherited repression of transposons and foreign DNA is essential for maintaining lineage fitness. Single-copy germline-expressed green fluorescent protein transgenes in *C. elegans*, a clear example of 'foreign' DNA, can undergo spontaneous silencing, resulting in fully penetrant, stably inherited silencing for more than 20 generations with no evidence of reversion[9–12]. This indefinite silencing is triggered by endogenous small RNAs called Piwi-interacting RNAs (piRNAs) and so was termed RNA-induced epigenetic silencing. piRNAs are sncRNAs expressed from genomic clusters ranging from tens to thousands of individual piRNA sequences[136]. Although their length and biochemical characteristics vary across species, piRNAs interact with widely conserved Piwi proteins, which are part of the Argonaute family, to effect silencing[137]. Genomically encoded primary piRNAs guide Piwi proteins to complementary transcripts and initiate amplification to form secondary small RNAs, resulting in gene silencing. In zebrafish, mice and *D. melanogaster*, the destruction of transposon transcripts guided by Piwi-bound primary piRNAs can be coupled to the production of secondary piRNAs from the targeted transcript, leading to a feed-forward amplification response termed the ping-pong cycle[137]. In *C. elegans*, transcript targeting by piRNAs instead leads to RdRP-catalysed production of 22G RNAs, which effect heritable silencing through the nuclear RNAi pathway

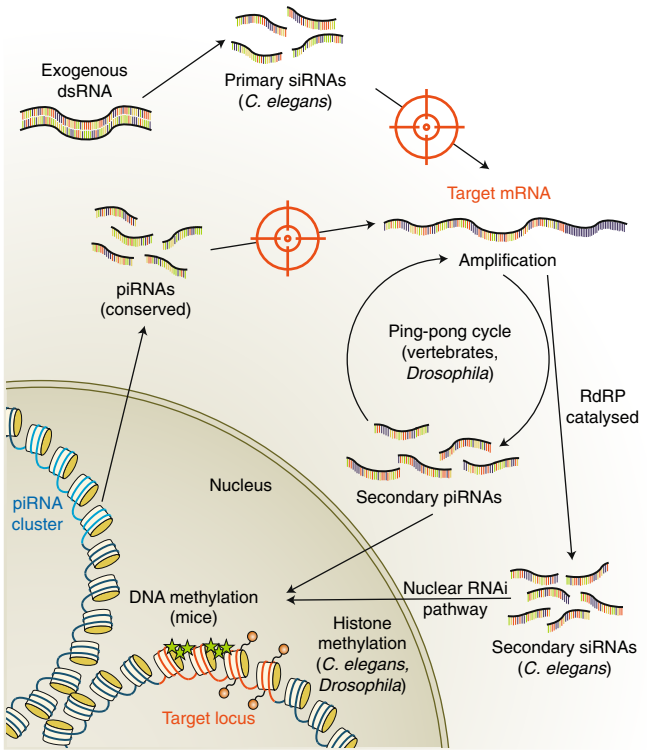

Fig. 2 | Small RNA pathways can direct histone methylation and DNA methylation to repress specific loci. Small RNAs, for example, siRNAs and piRNAs, guide proteins of the Argonaute family to destroy target mRNA transcripts, coupled with amplification processes, including the ping-pong cycle in vertebrates and *Drosophila* to generate secondary piRNAs and RdRP-mediated amplification in *C. elegans* to generate secondary siRNAs. These secondary small RNAs would be transported into the nucleus to deposit repressive marks, such as histone methylation and DNA methylation, on corresponding genomic loci. These marks are often heritable and the crosstalk between small RNA and chromatin pathways may be essential for stable gene silencing. The red targets indicate the process of specific targeting of transcripts for destruction.

in conjunction with *hrde-1* (refs [9–12]), a machinery shared with heritable dsRNA-induced silencing. piRNA-mediated silencing not only represses transposons but also targets many endogenous transcripts, which can potentially be subject to transgenerational epigenetic memory[85].

Recent work in *C. elegans* has elucidated how primary piRNAs provide surveillance over germline transcription[138–141]. Whereas piRNAs in mammals and *D. melanogaster* exhibit near-perfect complementary base pairing with targets[137], *C. elegans* piRNAs, like miRNAs, tolerate mismatches outside of a 5′ seed region[141]. In this way, thousands of piRNAs can engage the entire germline mRNA transcriptome[138]. At least three mechanisms have been proposed to explain how genes necessary for germline function escape this promiscuous silencing. Sequence elements called periodic An/Tn clusters, which are largely intronic, are associated with germline-expressed genes[142] and can protect foreign sequences from becoming silenced via an unknown mechanism[141,143]. Another mechanism may involve as-yet-uncharacterized features intrinsic to the coding sequence that prevent silencing[139]. A third mechanism involves the Argonaute CSR-1 (refs [144,145]). 22G RNAs associated with CSR-1 are complementary to almost all germline-expressed genes[146] and they together have been proposed to licence gene expression[144,145] by protecting mRNAs from piRNA targeting and subsequent siRNA generation[138]. Interestingly, both CSR-1 and the *C. elegans* Piwi orthologue PRG-1, along with other TEI-related proteins[147,148], reside in perinuclear phase-separated liquid-like granules[146,149] with a defined spatial organization[147], suggesting that the temporal order of transit through this system of granules of mRNAs exiting the nucleus may be important for RNA-directed silencing and licencing mechanisms[147,148]. However, this hypothesis awaits experimental verification.

Mechanisms of stable silencing. In *C. elegans*, once piRNAs initiate silencing, the targeted repression can persist for many generations even in the absence of the triggering piRNA–Piwi complex[9,12,150]. However, in some cases, Piwi may still act to maintain silencing[139]. The maternal transmission of tertiary 22G RNAs, downstream of secondary 22G RNAs and the germline nuclear RNAi pathway including *hrde-1*, is sufficient for this inheritance, indicating that an amplification loop maintains high levels of siRNAs in the absence of both the trigger and the initially silenced locus[97]. Mutually reinforcing feedback between RNAi pathways and repressive chromatin, such as those demonstrated in *Schizosaccharomyces pombe* and *Arabidopsis thaliana*[151], would explain the extraordinary stability of this silencing[86]. An analogous mechanism has been proposed in *D. melanogaster*[152], although to date, such a feedback has not been convincingly demonstrated in animals. Nonetheless, it is clear that stable gene silencing generally involves multiple epigenetic pathways. In *C. elegans*, the multigenerational stability of piRNA-initiated silencing requires both the RNAi pathway and chromatin modifiers, especially H3K9 methyltransferases[9,11]. Secondary piRNAs also guide DNA methylation in mice[153,154] and heterochromatin formation in *D. melanogaster*[155–158] (Fig. 2).

Conclusions and outlook

Non-DNA sequence-based inheritance of information occurs in multiple species and is important for development and physiology. One major purpose of epigenetic inheritance is to maintain the repression of repetitive elements. In addition, it transmits information about gene expression programmes, for instance, the germline programme in *C. elegans*, to offspring. What is more controversial is the extent to which transmitted epigenetic information is modulated by the environment and physiology and whether this process is ever adaptive.

Non-DNA sequence-based inheritance of acquired information can occur over different timescales (Table 1), with the set of mechanisms changing and narrowing as we look to further generations. Parental effects over a single generation can act via many mechanisms with phenotypic consequences. However, little evidence exists to date for multigenerational memory of physiological alterations following environmental changes, even though the potential for longer-lasting memories has been demonstrated with the underlying mechanisms dissected. Epigenetic inheritance of transcriptional repression can, for example, sometimes be perturbed by environmental insults, with gradual restoration over generations leading to a transgenerational transfer of information about ancestral environmental experience. Similarly, on shorter timescales, inheritance of small RNAs can occur. However, evidence is still lacking for either of these capacities for information transfer ever being employed to alter progeny physiology adaptively in the light of ancestral experience. Owing to the long duration of a single human generation, adaptive epigenetic inheritance seems unlikely over any generational timescale, although intergenerational inheritance of environmental insult-triggered disorders, as demonstrated in rodents, could have a medically relevant effect on individual physiology.

Regardless of the species, parental experiences are more likely to predict environmental conditions than those of more distant ancestors. As such, adaptive effects seem more plausible in the context of intergenerational, rather than transgenerational, paradigms. Thus, the numerous and often more tractable cases of inheritance over a single generation offer fertile ground for researchers who

Table 1 | Examples of intergenerational or transgenerational inheritance over different timescales

Duration	Trigger	Species	Effects on progeny	Proposed mechanism of inheritance	Ref.
1 generation	Paternal high-sugar diet	*D. melanogaster*	High triglyceride levels (on a high-sugar diet)	Chromatin modifications in sperm (H3K9me3 and H3K27me3)	26
1 generation	Young mother	*C. elegans*	Slow development, reduced resistance to starvation and reduced fecundity	Reduced maternal provisioning of yolk to embryos (for starvation resistance and development)	33
1 generation	Paternal low-protein or high-fat diet	*Mus musculus* and *Rattus norvegicus*	Differential gene regulation during embryogenesis and metabolic disorders	Somatic tsRNAs acquired by sperm during epididymal transit	21,23,58,63
1 generation	Maternal antibiotic exposure	*D. melanogaster*	Delayed development	Heritable depletion of riboflavin-producing commensal bacteria	13
1–2 generations	Ancestral high-glucose diet	*C. elegans*	Reduced fecundity and resistance to oxidative stress	COMPASS H3K4 methylases are required for inheritance of stress resistance	28
2 generations	Maternal dietary supplementation with methyl donors	*M. musculus*	Alterations in coat colour	Increased DNA methylation at the *agouti* locus caused by retrotransposon insertion	39
2 generations	Undernourishment during pregnancy	*M. musculus*	Metabolic alterations	Hypomethylation of specific loci in F1 males	20,46
2 generations	Paternal odour-conditioned fear response	*M. musculus*	Inherited fear response to a specific odour	Neuroanatomical changes in progeny and locus-specific hypomethylation in sperm	123
2–3 generations	Exposure to various mild stresses	*C. elegans*	Increased stress resistance and proteostasis	Somatic insulin signalling and COMPASS H3K4 methylases in germline	115
3 generations	Ancestral mutation in COMPASS H3K4 methyltransferases	*C. elegans*	Increased longevity	Altered histone methylation and longevity phenotypes due to a possible alteration in lipid metabolism	82,159
3 generations	Overexpression of H3K4 demethylase in sperm	*M. musculus*	Reduced survival and developmental abnormalities	Alterations in sperm-borne RNA	113
3 generations	Ancestral development at an elevated temperature	*C. elegans*	Alterations in gene expression	Disruption of piRNA-initiated repression of endogenous transcripts by the RNAi pathway	85
Up to 3–4 generations (typically)	RNAi triggered by exogenous dsRNA	*C. elegans*	Inherited gene repression	Secondary siRNAs; histone methylation	83,87,92,93
3 generations	Ancestral starvation during the larval stage in wild-type worms	*C. elegans*	Alterations in gene expression and plasticity; increased stress resistance and lifespan	Inheritance of siRNAs bound to the nuclear Argonaute HRDE-1 (for expression differences)	112,121,122
3 generations	Heat shock during embryogenesis	*D. melanogaster*	Alterations in eye colour	Disruption of heterochromatin by phosphorylation of ATF-2	125
3–9 generations	Ancestral starvation during the larval stage in AMPK mutants	*C. elegans*	Reduced fecundity	Abnormal methylation of H3K4 by COMPASS histone methylases	114
14 generations	Growth at an elevated temperature	*C. elegans*	Increased expression from a repetitive transgene array	Loss of H3K9me3-mediated repression	117
Indefinite	Spontaneous transgene silencing in the germline	*C. elegans*	Stable gene silencing with no reversion	piRNA-targeting induced nuclear RNAi guided by secondary siRNAs; histone methylation	9–12

Here, we provide illustrative examples of some of the more compelling and better-characterized reports of intergenerational and transgenerational inheritance. These examples provide a diversity of mechanisms and demonstrate which mechanisms are more typical over different generational timescales. Many other examples are discussed in the main text.

wish to probe the mechanisms and adaptive importance of environmentally responsive non-DNA sequence-based inheritance. For example, the details of how soma-to-germline information transfer occurs are still elusive and may be better understood by studying experimentally tractable intergenerational systems. Indeed, research effort could be better directed at confirming and expanding the often-scant mechanistic details of previously described cases of intergenerational and transgenerational inheritance rather than

seeking out novel phenomena. Much work remains to establish how epigenetic information survives and is propagated between tissues and across generations, how widespread intergenerational and transgenerational phenomena are in natural contexts and what the physiological relevance of naturally occurring intergenerational and transgenerational inheritance could be.

Received: 6 August 2018; Accepted: 29 October 2018;
Published online: 02 January 2019

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Acknowledgements

This work was supported by a European Research Council (ERC) consolidator grant (616434), the Spanish Ministry of Economy and Competitiveness (BFU2017-89488-P and SEV-2012-0208), the AXA Research Fund, the Bettencourt Schueller Foundation, Agencia de Gestio d'Ajuts Universitaris i de Recerca (AGAUR; SGR-831), the EMBL-CRG Systems Biology Program and the CERCA Program/Generalitat de Catalunya.

Author contributions

M.F.P. and B.L. wrote the manuscript.

Competing interests

The authors declare no competing interests.

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