Commentary

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The third barrier to transgenerational inheritance in animals: somatic epigenetic resetting

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After early controversy, it is now increasingly clear that acquired responses to environmental factors may perpetuate across multiple generations—a phenomenon termed transgenerational epigenetic inheritance (TEI). Experiments with *Caenorhabditis elegans*, which exhibits robust heritable epigenetic effects, demonstrated small RNAs as key factors of TEI. Here, we discuss three major barriers to TEI in animals, two of which, the "Weismann barrier" and germline epigenetic reprogramming, have been known for decades. These are thought to effectively prevent TEI in mammals but not to the same extent in *C. elegans*. We argue that a third barrier—that we termed "somatic epigenetic resetting"—may further inhibit TEI and, unlike the other two, restricts TEI in *C. elegans* as well. While epigenetic information can overcome the Weismann barrier and transmit from the soma to the germline, it usually cannot "travel back" directly from the germline to the soma in subsequent generations. Nevertheless, heritable germline memory may still influence the animal's physiology by indirectly modifying gene expression in somatic tissues.

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Introduction

I'm taking down the barriers, and loving what I find.

Elton John (1981)

Conrad H. Waddington coined the term "epigenetics" in 1942 to describe the complex interactions between the genome and the environment that drive developmental processes. Today, this understanding of epigenetics has been expanded to include inheritance across cellular or organismal generations through mechanisms other than direct changes in the DNA sequence. The molecular agents behind this inheritance include regulatory RNAs, chromatin modifications, and self-templating structural complexes, such as prions. Still, the idea that environmentally induced epigenetic changes, functionally similar to genetic mutations, may generate phenotypic variation that natural selection may act upon and influence animals' evolutionary trajectory remains controversial. This is largely because we still lack a mechanistic understanding of how epigenetic changes might be transmitted and maintained.

Recent studies using the nematode *C. elegans* have provided extensive insights into the mechanisms of epigenetic inheritance involving small RNAs, as well as the limits to TEI in animals. In particular, two mechanisms, the Weismann barrier and germline epigenetic reprogramming, prevent TEI by blocking information flow from the soma to the germline and by erasing epigenetic memory during early embryonic development, respectively (Fig 1). These effectively inhibit TEI in mammals but evidence shows that, at least in *C. elegans*, RNA-mediated TEI can overcome both the Weismann barrier and epigenetic reprograming of the germline. Here, we propose a third barrier, "somatic epigenetic resetting," that limits TEI in *C. elegans* as well by disabling the transfer of epigenetic information from the germline into soma cells.

The first barrier: the Weismann barrier

In the late 1800s, August Weismann proposed that hereditary continuity occurs solely through the "germplasm," which resides in the nucleus of the germ cells, and that the soma, which becomes separated from the germline during embryonic development, does not influence heritable traits. This segregation of the germline and soma that blocks the long-term inheritance of somatic programs became known as the "Weismann barrier." Note that in plants, the soma-to-germ boundary is blurred as the germ cells (meiocytes) are derived from somatic cells that are exposed to environmental and developmental cues. Not surprisingly, TEI is relatively common in plants.

Weismann argued that this soma-germline barrier prevents the inheritance of acquired characteristics in animals, in contrast to Darwin's pangenesis hypothesis which posits that "gemmules"—tiny particles released from different body parts—travel to the gametes and "vote" on the constitution of the progeny, thereby transmitting life experiences across generations. He claimed that there is no need to assume that

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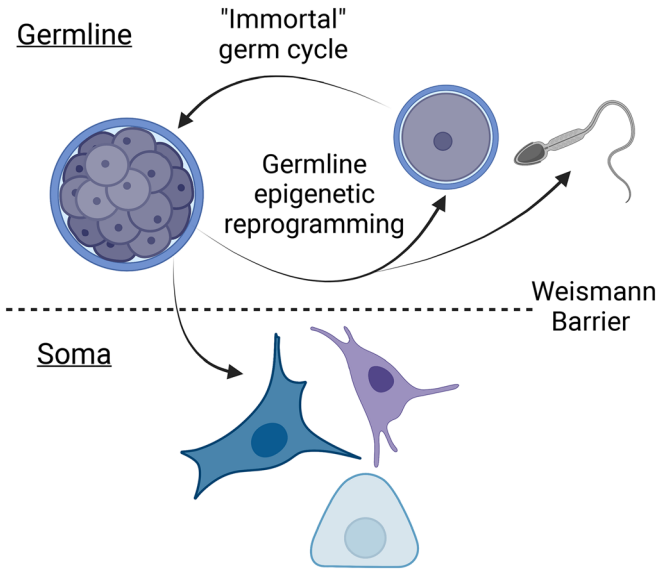

Figure 1. Barriers to epigenetic inheritance in animals.
The segregation of the soma and the germline during development forms the Weismann barrier, which prevents soma-to-germline transfer of hereditary information. In mammals, reprogramming events in the zygote and in the primordial germ cells effectively reset nearly all chromatin marks, limiting transgenerational epigenetic inheritance. The germ cells are often referred to as immortal" as they contain the hereditary information that is transmitted from generation to generation.

inheritance of acquired traits exists in metazoans, since evolution can be explained without it. However, Weismann did concede that environmental factors may impart heritable effects if the germplasm is *directly* exposed to them. It is worth noting that Weismann restricted germplasm to the content of the nucleus. Today, we know that, in addition to nuclear factors, cytoplasmic substances, including germ granules, play a pivotal role in carrying nongenetic/epigenetic information across generations (see discussion below).

Weismann's wall stood for more than a century, but it has been crumbling in recent years with increasing evidence that the soma can indeed influence gene expression in the germline and that it can generate a heritable response independent of the DNA sequence. What are the mechanisms that direct heritable information flow from the soma to the germline?

Seminal work in *C. elegans* showed that the introduction of dsRNA to the soma may elicit systemic RNA interference (RNAi), including silencing of germline genes (Fire *et al*, 1998). This process is facilitated by SID-1, an RNA channel that shuttles dsRNAs into the cells (Winston *et al*, 2002; Fig 2A). In the germline, dsRNA transport is supported by the LDL-superfamily endocytosis receptor, RME-2 (Marré *et al*, 2016; Wang & Hunter, 2017).

Furthermore, targeting germline genes using dsRNA can generate long-term RNAi effects that last for tens or even hundreds of generations in clear violation of Weismann's principle. These transgenerational effects require RNA-dependent RNA polymerase (RdRP)-mediated small RNA amplification. Exo-siRNAs, as well as endogenous small RNAs, guide RdRP to the target mRNA to generate abundant secondary 22G siRNAs complementary to the mRNA template. This amplification system enables the maintenance and propagation of dsRNA signals across many generations without dilution (reviewed in Rechavi & Lev, 2017).

Soma-to-germline transmission was further demonstrated by the expression of hairpin-derived dsRNA in the neurons that induces robust systemic silencing of germline genes for at least 30 generations. While SID-1 is required for initiating this process, it is dispensable once the silenced state was established. The epigenetic memory is maintained in the germline by the germline nuclear argonaute HRDE-1 (Devanapally *et al*, 2015).

Subsequent work showed that endogenous small RNAs produced in neurons can similarly affect germline transcription. Strikingly, neuronal small RNAs may alter chemotaxis behavior transgenerationally by regulating the silencing of germline genes (Posner *et al*, 2019). Interestingly, this brain-to-germline communication has been shown to be largely independent of SID-1 (Posner *et al*, 2019). Indeed, numerous studies reported systemic RNAi effects in mutants devoid of SID-1. For example, in RNAi-sensitized genetic backgrounds, dsRNA exposure leads to the silencing of neuronal genes even though neurons do not express SID-1 (e.g., Lehner *et al*, 2006). It was therefore suggested that endogenous small RNAs synthesized in the nervous system could generate transgenerational effects in the germline via other RNA transporters and/or indirectly by regulating signaling molecules such as neuropeptides and neurotransmitters (Posner *et al*, 2019).

Unlike transgenerational RNAi effects, which, in *C. elegans*, are mediated by RdRP-amplified small RNAs, short-lived intergenerational signals from the soma to the germline affecting only the immediate F1 and F2 progeny (see Box 1) may be transmitted by any number of biomolecules that do not self-amplify. Such molecules might exert a significant effect if sufficient active parental material remains in the children and grandchildren. For example, yolk secreted from the mother's intestine is loaded into developing embryos via receptor-mediated endocytosis and mediates stress adaptation and fitness in the offspring. Similarly, in response to various forms of mild stress, somatic insulin signaling may promote increased stress resistance and proteostasis in the progeny (reviewed in Perez & Lehner, 2019).

In mammals, the case of TEI across the Weisman barrier is not as well understood as in *C. elegans*. Many molecules and mechanisms, such as metabolites, RNA, DNA methylation and histone modifications, as well as changes to the microbiome, have been suggested to mediate intergenerational effects (Perez & Lehner, 2019). Circulating RNAs received special attention as good candidates for transmitting information from the soma to the germline as these molecules could potentially regulate the expression of specific target genes and as they are present in most bodily fluids, including in extracellular vesicles, which can be imported into germ cells.

Research in mice showed that different small RNA species, including tRNA fragments

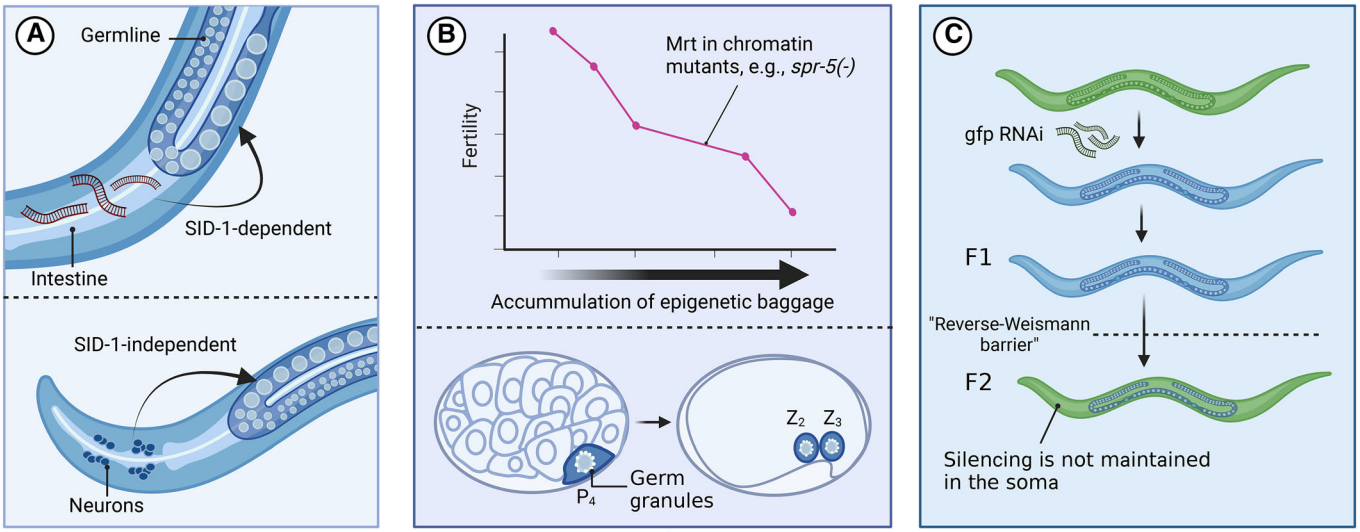

Figure 2. Three barriers to transgenerational epigenetic inheritance in *Caenorhabditis elegans*.

(A) The Weismann barrier limits information flow from the soma to the germline. Nevertheless, in *C. elegans*, exo- and endo-siRNA may reach the germline via SID-1-dependent and SID-1-independent mechanisms. (B) Germline epigenetic reprogramming resets the epigenome in every germ cycle. In many chromatin and RNAi inheritance mutants, incomplete epigenetic resetting causes transgenerational accumulation of epigenetic baggage and a mortal germline" or Mrt phenotype. In *C. elegans*, the germline blastomere P_4 is formed at the 16-24-cell stage. P_4 subsequently divides to generate two primordial germ cells Z_2 and Z_3. Germ granules that are partitioned into the primordial germ cells (highlighted in blue) may regulate endogenous small RNA production and relay epigenetic information across generations, bypassing reprogramming events in the germ cell nuclei. (C) Somatic epigenetic resetting prevents TEI in the soma. RNAi-mediated silencing triggers a robust heritable effect in the germline, but not in the soma. We dub this block in germline-to-soma transfer of epigenetic memory the reverse-Weismann barrier."

Box 1. Definition of inter- and transgenerational epigenetic inheritance

Intergenerational inheritance refers to heritable responses that only persist for one or two generations. As these heritable effects can be attributed to the *direct* exposure of the progeny and developing gametes to environmental stimuli, the Weismann barrier remains intact. It has been proposed that these short-term intergenerational effects may be important for adaption to a fluctuating environment.

Effects that last for at least two generations through the male germlines or three generations through female germline are referred to as transgenerational. These long-lasting effects persist in the progeny for generations in the absence of the original trigger.

(tRFs) and microRNAs, are delivered from somatic epididymis cells to maturing sperm via extracellular vesicles. Many studies suggested that these small RNAs are not only important for embryo implantation but may also mediate intergenerational effects in response to environmental triggers such as dietary and psychological stress (reviewed in Sharma, 2019). Crucially, using a low-bias RNA cloning technique termed Ordered Two-Template Relay and a method called PANDORA-seq which minimizes the influence of nucleobase modifications on sequencing, two recent studies found that the small RNA composition of mature mouse sperm is in fact very different from what was previously suggested (Shi *et al*, 2021; preprint: Gustafsson *et al*, 2022). Moreover, the recent finding of cell-free DNA contamination in sperm preparation further complicates the interpretation of studies regarding sperm DNA methylation (Galan *et al*, 2021). Together, these reports force a reappraisal of the heritable agent(s) in the mammalian sperm.

Nevertheless, it is clear that alteration of the gamete epigenome could propagate to the F1 progeny. Unlike intergenerational effects, however, transgenerational effects are much more controversial in mammals. This is in part because such inheritance must involve the amplification of the heritable signal, such as RNA molecules, and such amplification mechanisms have not yet been shown to exist in mammals. In particular, RdRPs have not been found in the mouse genome.

In sum, it is clear that, at least in *C. elegans*, one of the two known barriers to epigenetic inheritance, the "Weismann barrier," is breached. This allows somatic responses to environmental changes to reach the germline via distinct routes.

The second barrier: epigenetic reprogramming in the germline

Epigenetic reprogramming is thought to be important for ensuring the totipotency of the zygote and the subsequent execution of species-typical developmental program. In mammals, two waves of reprogramming in the zygote and in the primordial germ cells (PGCs) effectively reset nearly all epigenetic marks, including DNA methylation and histone modifications (Hajkova *et al*, 2008; Guibert *et al*, 2012). Upon fertilization, the maternal and paternal genomes undergo differential cytosine demethylation via both active and passive mechanisms. This is followed by rapid remethylation by the *de novo* DNA methyltransferases DNMT3A and DNMT3B, which drive somatic differentiation postimplantation. In the PGCs, which are derived from the primitive ectoderm, the epigenome is once again reprogrammed in part to remove genomic imprints and somatic epigenetic marks established during the postimplantation development. These

two dramatic resetting events impose a significant barrier to epigenetic inheritance in mammals where the evidence for TEI remains scarce.

It is crucial to note that *C. elegans* and many other invertebrates lack cytosine methylation (5mC). Even in non-mammalian vertebrates that deploy DNA methylation to control gene expression, their methylomes do not appear to undergo drastic reprogramming during embryogenesis (Ortega-Recalde *et al*, 2019; Xu *et al*, 2019). Hence, it has been proposed that DNA methylation reprogramming evolved to facilitate genomic imprinting in mammals, rather than resetting epigenetic memory *per se* (Sarkies, 2022).

Even in mammals, a number of rare loci, including some transposable elements, evade reprogramming and may potentially carry epigenetic memory across generations (Guibert *et al*, 2012). For example, the demethylation of intracisternal A particle (IAP) retrotransposons causes ectopic expression of the downstream agouti gene, leading to yellow fur and obesity. Interestingly, the coat color of the progeny of agouti mutants changes with methyl-supplemented diets. This effect is propagated for two generations but is lost by the third (reviewed in Heard & Martienssen, 2014).

In *C. elegans*, as in mammals, the chromatin undergoes global, though incomplete, reprogramming after fertilization, accompanied by a dramatic loss of histone H3 modifications in the germ lineage. Failure of this epigenetic reprogramming leads to a mortal germline (Mrt) phenotype whereby the animals become progressively sterile over several generations—which is indicative of TEI. For example, loss of the LSD1 ortholog SPR-5 causes transgenerational accumulation of H3K4me2, which leads to dysregulation of spermatogenesis genes and sterility (Katz *et al*, 2009). Many mutations in genes acting in the RNAi inheritance pathway, such as argonautes *hrde-1* and *wago-4*, nuclear RNAi factors *nrde-1/2/4*, and methyltransferase *met-2* and *set-32*, similarly result in a Mrt phenotype, which is exacerbated by heat stress. These findings suggest that the germline RNAi pathway is involved in preventing the inheritance of aberrant epigenetic baggage across generations. Indeed, the Mrt phenotype in mutants lacking H3K9me1/2 can be rescued by eliminating HRDE-1, which carries heritable small RNAs, suggesting that accumulation of ectopic small RNA pool causes germline mortality (Lev *et al*, 2017; Fig 2B). Interestingly, SPR-5 and MET-2 act synergistically in reprogramming the epigenetic landscape to avoid the inheritance of aberrant epigenetic marks. Animals lacking both *spr-5* and *met-2* showed severe developmental delay and ectopic expression of germline genes in somatic cells (Carpenter *et al*, 2021).

As discussed previously, exogenous dsRNA leads to the amplification of sequence-complementary siRNAs and deposition of suppressive histone modifications on the targeted locus, resulting in robust TEI in the germline. Likewise, endogenous small RNAs may induce silencing of germline genes transgenerationally. In the absence of environmental challenges and selective pressure, the small RNA pool and chromatin state undergo frequent heritable changes. However, most, but not all, of these "epimutations" are short-lived, lasting for 2–3 generations on average, perhaps due to passive dilution or active reprogramming mechanisms (Beltran *et al*, 2020; preprint: Wilson *et al*, 2022). On the contrary, environmental stress, such as starvation and heat, may cause epimutations to persist for more than 10 generations in some instances (e.g., Ewe *et al*, 2020).

Small RNA-mediated epigenetic memory is amplified and maintained by the RdRPs RRF-1 and EGO-1 in M granules and P granules, respectively. Germ granules are part of the perinuclear "nuage" and play a major role in regulating small RNA homeostasis and coordinating TEI. Moreover, the penetrance of TEI is controlled, at least partly, by the heat-shock factor HSF-1 in the germline (Houri-Zeevi *et al*, 2020), while the duration of TEI is modulated by a set of so-called *motek* (modified transgenerational epigenetic kinetics) genes (Houri-Ze'evi *et al*, 2016). Similarly, *heri-1* limits TEI perhaps by resetting the epigenetic marks deposited by the germline nuclear RNAi pathway downstream of HRDE-1 (Perales *et al*, 2018).

The unequivocal evidence for TEI in worms thus reveals that the second barrier to epigenetic inheritance, "germline epigenetic reprogramming" is also breached, raising important questions about the limits that prevent parental responses to stress and changes in the environment from affecting their descendants.

The third barrier: somatic epigenetic resetting

As mentioned previously, dramatic reprogramming in the mammalian preimplantation embryo erases most epigenetic modifications in all cells, except the marks deposited on imprinted loci and some transposons. This process is repeated in the germline precursors; however, in these cells, imprints are removed and re-established in a sex-specific manner. These dramatic reprogramming events are thought to leave very little room for TEI. In contrast, in *C. elegans*, RNAi-induced silencing of germline genes may persist across multiple generations despite substantial chromatin remodeling during embryogenesis owing to the RdRP-mediated siRNA amplification cycle.

For unknown reasons, however, silencing of somatic genes is not inherited past the F1 generation with a few exceptions (see below). While it is reasonable to suggest that the target gene's transcript must be present in the germline to serve as a template for RdRP-mediated amplification, this is not the whole story. Even when gene expression is knocked down in animals that express the target in both the germline and the soma, a robust transgenerational silencing response is observed only in the progeny's germline, but not in the soma. These results suggest that the soma has a reduced capacity to carry epigenetic memory perhaps due to differential epigenetic dynamics. Moreover, we argue that "somatic epigenetic resetting" creates the "reverse-Weismann barrier" that prevents epigenetic information flow from the germline to the soma (Fig 2C). It raises the question whether it is possible that epigenetic information is carried across generations by factors which are present only in the germline, akin to the germplasm theory. That is, the differential developmental trajectory of germ cells and somatic cells may give rise to the reverse-Weisman barrier.

During early embryogenesis in *C. elegans*, maternal factors are segregated unevenly into the blastomeres, and each cell lineage acquires a distinct epigenetic landscape that defines its differentiation states. The germline precursor P_4 blastomere acquires two unique regulatory systems from the mother: the CCCH zinc-finger protein PIE-1 and the P granules (protein-RNA condensates) both of which are required for proper germline development as they repress the acquisition of somatic fates. Additionally, PIE-1 initiates the PGC developmental program by regulating germline transcripts, including the nanos homolog *nos-2*. Loss of maternal *pie-1* leads to the misspecification of germline into intestine (Wang & Seydoux, 2013).

While P granules are not essential for early specification of germ cell fate, they are crucial for inhibiting somatic fate and therefore required for robust differentiation of germ cells into functioning gametes in the postembryonic animal (Updike *et al*, 2014). Many RNAi factors, including proteins which are required specifically for RNAi inheritance, reside in germ granules. Indeed, as we will discuss below, germ granules, including P granules and the related Z and M granules found in the maternal germline, as well as the sperm-specific PEI granules, have recently been implicated in small RNA inheritance (reviewed in Lev & Rechavi, 2020). It is important to note that, while germ granules in *C. elegans* are maternally provided ("preformation"—a derived mode of germ cell specification), germ granules in mammals are not found in the oocyte and early embryos but are instead formed *de novo* in every germ cycle ("induction" or "epigenesis"—an ancestral mode; Extavour & Akam, 2003). It is reasonable to speculate that the frequency of TEI in different organisms may be, at least partly, explained by these differences.

Disrupting P granules by removing PPTR-1 or MEG-3/4, which stabilize and localize them, causes dysregulation of endo-siRNA and modulates the potency of dsRNA-induced RNAi inheritance (Lev *et al*, 2019). Moreover, an aberrant small RNA pool in *meg-3/4* double mutants leads to heritable silencing of RNAi genes and reduced RNAi competence in wild-type descendants (Dodson & Kennedy, 2019; Ouyang *et al*, 2019). ZNFX-1, a core component of the Z granules, and the argonaute WAGO-4 function in parallel with HRDE-1 to mediate RNAi-induced TEI (Ouyang *et al*, 2022). Similarly, MUT-16, which initiates the assembly of M granules, is important for producing RdRP-dependent secondary siRNAs required for robust TEI (Phillips *et al*, 2012). It is therefore possible that RNAi-induced silencing of somatic genes does not propagate across generations because the soma lacks certain factors in the small RNA pathway that carry parental epigenetic memory, including those that reside in germ granules (Fig 3). In other words, we propose that the loss of germline identity prevents the persistence of epigenetic memory in the somatic lineages.

Nevertheless, in rare cases, heritable epigenetic information in *C. elegans* may escape "somatic resetting." Repetitive transgenic arrays are often silenced in the germline by the piRNA pathway; epigenetic regulation of somatically expressed transgenes, on the contrary, is much less prevalent. Still, unlike endogenous genes and low-copy or single-copy integrated transgenes, some highly repetitive transgenes may be heritably silenced in the soma. For example, a repetitive transgene expressing viral genes is transgenerationally silenced, an effect which depends on the RdRP RRF-1 (Rechavi *et al*, 2011).

Silenced repetitive transgenes can become re-expressed in the soma by exposing the animals to heat stress (Klosin *et al*, 2017). It was suggested that heat stress depletes H3K9me3 from the *hsp-90* (*daf-21*) transgene and the chromatin state persists in the early embryo, including the somatic lineages (Klosin *et al*, 2017). In a different study, an extrachromosomal transgenic array containing the *sid-1* promoter and 5′ untranslated region sequence was found to trigger epigenetic silencing of the *sid-1* endogenous locus. The silenced state was maintained by HRDE-1 in progeny that had lost the extrachromosomal array for 13 generations in the germline and four generations in the soma, thus breaking the reverse-Weismann barrier (Minkina & Hunter, 2017). We note that such unusual heritable "silencing in *trans*" in the soma might uniquely affect RNAi genes. Indeed, two recent reports showed that disrupting germ granules leads to silencing of RNAi genes, including *sid-1*, by the piRNA/HRDE-1 pathway (Dodson & Kennedy, 2019; Ouyang *et al*, 2019), suggesting that RNAi genes may be subjected to additional epigenetic regulation which may fine-tune TEI.

Although somatic epigenetic resetting generally prevents TEI in the soma, epigenetic memory in the germline may influence gene expression in somatic tissues indirectly, that is, non-cell-autonomously. For instance, mutations in the COMPASS complex lead to heritable longevity, partly by regulating lipid metabolism in the intestine, and this effect requires a functional germline (Greer *et al*, 2011). Chronic heat stress causes defects in spermatogenesis in hermaphrodites, leading to a heritable increase in the secretion of volatile sex pheromones (likely synthesized in the intestine) and enhanced male attraction that lasts for four generations in an HRDE-1-dependent manner after returning to normal cultivation temperature (Toker *et al*, 2022).

Additionally, as mentioned previously, neuronal small RNAs have been shown to trigger heritable changes in chemotaxis behavior by downregulating the expression of *saeg-2* in the germline, which may subsequently modulate behavior cell non-autonomously. Indeed, animals lacking the

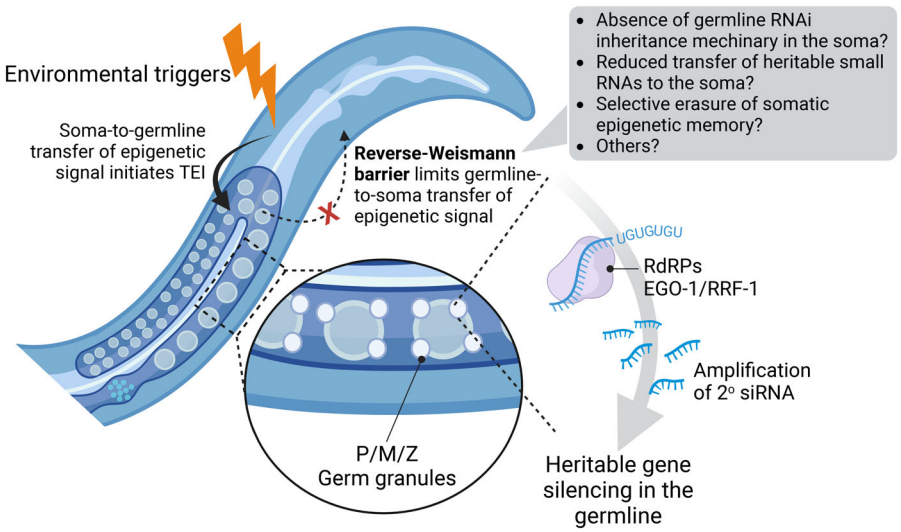

Figure 3. Reverse-Weismann barrier.

In *Caenorhabditis elegans*, environmental triggers may induce changes in the expression of germline genes that persist for many generations owing to the RdRP-mediated siRNA amplification cycle operating in the perinuclear germ granules (three subclasses—P, M, and Z) in the germline. The reverse-Weismann barrier limits the transfer of heritable epigenetic information from the germline back to the soma (see text for details).

dsRNA-binding protein RDE-4, which is involved in the biogenesis of endo-siRNA, exhibit chemosensory defects under mild heat stress, which are rescued by knocking out *saeg-2* (Posner *et al*, 2019). Therefore, we argue that epigenetic memory is propagated in the germline and affects somatic function secondarily, as opposed to direct transfer of epigenetic information—for instance, in the form of small RNAs—from the germline to the target somatic tissue. Hence, it appears that while the original Weismann barrier (soma-to-germline) may be breached, the "reverse-Weismann barrier" effectively limits the transfer of heritable epigenetic information from the germline back to the soma.

There is, however, one notable exception. Kaletsky *et al* (2020) reported that *C. elegans* exposed to pathogenic *P. aeruginosa* (PA14) or, remarkably, one specific *Pseudomonas* short bacterial RNA called "P11," learn to avoid the bacterium. This learned behavior is transgenerationally inherited for four generations and requires the TGF-β ligand DAF-7 and Macoilin MACO-1 in the ASI sensory neurons and the Piwi protein PRG-1 in the germline. While the authors claimed that SID-1 is required for the TEI, SID-1 also appears to be required for learning (Kaletsky *et al*, 2020). In fact, SID-1's exact function is unclear. Specifically, it is not known which tissues it connects by dsRNA-mediated communication and which small RNAs does it shuttle—SID-1 was previously shown by Craig Hunter's laboratory to be dsRNA-specific (Shih & Hunter, 2011). It was further shown that SID-2, an apical intestinal membrane protein required for the uptake of environmental RNAi, is essential for P11-induced learning but is dispensable for avoidance induced by bacterial exposure. Surprisingly, knocking out *sid-2* blocks the transgenerational inheritance of the avoidance behavior, revealing an unexpected role of the intestine in maintaining epigenetic memory across generations.

Interestingly, *Cer1*, a retrotransposon in the Gypsy/Ty3 family, is also required for the heritable learned *Pseudomonas* avoidance by transmitting epigenetic information from the germline to neurons in the progeny. Exposure to Cer1 virus-like particles (VLPs) isolated from P14-trained animals was shown to be sufficient to induce avoidance behavior, as well as TEI. Moreover, while the loss of *Cer1* does not prevent learning, it impairs the inheritance of avoidance behavior. Based on RNAi-mediated knocking down of *Cer1* in the F1 progeny of PA14-trained animals and observing that the avoidance memory was fully recovered in F2 animals that were not exposed to *Cer1* RNAi, the authors suggest that Cer1 is specifically required for executing the inherited learned behavior (Moore *et al*, 2021). These results suggest that Cer1 may communicate a germline epigenetic state to a somatic tissue, in this case, the brain. Curiously, the effect of *Cer1* RNAi was not transmitted to the next generation.

Overall, this study implies that heritable epigenetic information, perhaps unique to bacteria and unconventional bacterial short RNAs, which are different from worm small RNAs, carried by Cer1 VLPs, travels from the germline to the soma and directly alters neuronal responses. This would violate the "somatic resetting barrier" or reverse-Weismann barrier. It is important to note that Cer1 was never detected outside the germline. Hence, the jury is still out on the question of whether germline Cer1 VLPs carry small RNA cargo to neurons to modulate behavioral responses *in vivo*.

Conclusion

Studies in *C. elegans* and other model systems during the past decades have provided detailed insights into the molecular mechanisms underlying TEI, as well as the barriers that prevent the persistence of epigenetic memory. Yet, many questions remain. Can histone modification and DNA methylation be inherited independent of small RNAs? To what degree do heritable epigenetic mechanisms contribute to evolutionary adaptation? Of particular relevance, what are the mechanisms that block the inheritance of somatic epigenetic signals (Fig 3)? Evolution certainly has gone to great lengths to halt the carry-over of epigenetic baggage across generations. Future studies will need to shed light on the specific genes that become the targets of heritable endogenous small RNAs and the molecular machinery that maintains the reverse-Weismann barrier.

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Author contributions

Chee Kiang Ewe: Writing – original draft; writing – review and editing. **Oded Rechavi:** Writing – original draft; writing – review and editing.

Disclosure and competing interests statement

The authors declare that they have no conflict of interest.

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