

## REVIEW

# Programmed DNA elimination in the parasitic nematode *Ascaris*

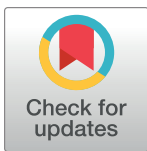
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## Abstract

In most organisms, the whole genome is maintained throughout the life span. However, exceptions occur in some species where the genome is reduced during development through a process known as programmed DNA elimination (PDE). In the human and pig parasite *Ascaris*, PDE occurs during the 4 to 16 cell stages of embryogenesis, when germline chromosomes are fragmented and specific DNA sequences are reproducibly lost in all somatic cells. PDE was identified in *Ascaris* over 120 years ago, but little was known about its molecular details until recently. Genome sequencing revealed that approximately 1,000 germline-expressed genes are eliminated in *Ascaris*, suggesting PDE is a gene silencing mechanism. All germline chromosome ends are removed and remodeled during PDE. In addition, PDE increases the number of chromosomes in the somatic genome by splitting many germline chromosomes. Comparative genomics indicates that these germline chromosomes arose from fusion events. PDE separates these chromosomes at the fusion sites. These observations indicate that PDE plays a role in chromosome karyotype and evolution. Furthermore, comparative analysis of PDE in other parasitic and free-living nematodes illustrates conserved features of PDE, suggesting it has important biological significance. We summarize what is known about PDE in *Ascaris* and its relatives. We also discuss other potential functions, mechanisms, and the evolution of PDE in these parasites of humans and animals of veterinary importance.



## OPEN ACCESS

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## Introduction

*Ascaris* is a parasitic nematode that lives in the small intestine of humans and pigs [1]. The human (*A. lumbricoides*) and pig (*A. suum*) parasites can cross-infect and have little difference in anatomy, physiology, and genome sequences [2–6]. For simplicity, we use *Ascaris* to refer to both species in this review. *Ascaris* was estimated to infect >700 million people worldwide in 2021 [7]. Most cases are found in impoverished children [8–10]. *Ascaris* infections (ascariasis) often have mild or no symptoms, but heavy infections can cause intestinal blockages and even death if left untreated [8,10]. Ascariasis contributes significantly to global disability-adjusted life years and perpetuates the cycle of poverty [9,11].

*Ascaris* is a member of the Ascarididae parasitic nematode family, known as ascarids, that infect a wide variety of vertebrates, including *Parascaris univalens* and *P. equorum* (horse), *Toxocara canis* (dog) and *T. catti* (cat), *Baylisascaris procyonis* (raccoon) and *B. schroederi* (panda), as well as *Ascaridia galli* (chicken). Deworming drugs are widely used to attempt to control Ascarididae and other parasitic infections [3,8,12]. However, recurrent drug treatment has led to drug resistance in nematodes of veterinary importance [8,12–14]. Thus, new drugs are needed to fight the increasing drug resistance.

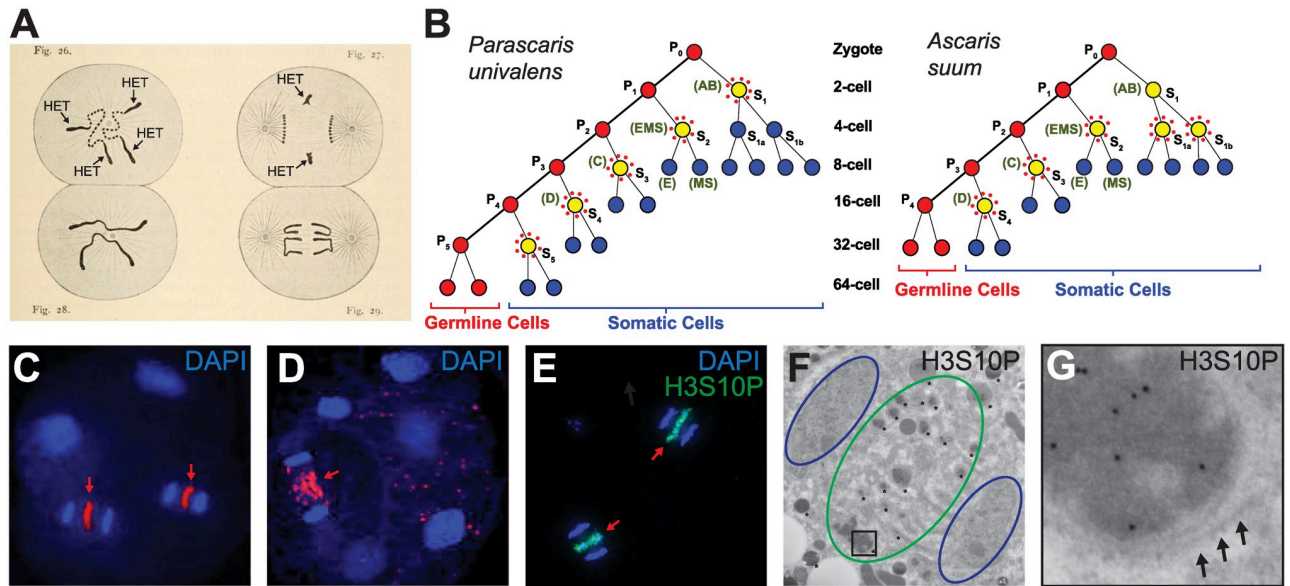
Ascarids have been used as models for both basic and biomedical research [1]. In addition to studies on the pathogenesis of *Ascaris* [15,16], ascarids have contributed to our understanding of meiosis [17,18], the chromosome theory of heredity, centrosome function in chromosome segregation [19,20], trans-splicing [21–24], neurobiology [25,26], metabolism [27–30], amoeboid sperm in nematodes [31–34], and programmed DNA elimination (PDE) [35–41]. This review will focus on *Ascaris* as a model for PDE, a form of genome alteration in which organisms reproducibly discard certain DNA sequences during early development. We summarize the current understanding of the PDE process in *Ascaris*, including the formation of DNA breaks, loss of sequences, and the potential molecular mechanisms, functions, and evolution of PDE in nematodes. We also discuss future directions and how PDE research can contribute to understanding the basic biology of these parasitic nematodes.

## Overview of PDE

PDE was discovered in 1887 by cell biologist Theodor Boveri in the horse parasite *P. univalens* [42]. The *P. univalens* germline contains a single, large chromosome with 2 heterochromatic (HET) chromosome arms that are mainly composed of repeats (see below on the nature of eliminated DNA). Boveri observed that this single chromosome was fragmented during early embryogenesis in pre-somatic cells (Fig 1A). The resulting chromosome fragments were passed to the daughter nuclei, while the HET arms were eliminated from the reduced somatic genome [42] (Fig 1B). Since the discovery of PDE, single-cell ciliates and over 100 metazoan species from phylogenetically diverse groups, including nematodes, insects, mites, copepods, ratfishes, hagfishes, lamprey, songbirds, and bandicoots, have been observed to eliminate DNA [36,43]. However, within each phylogenetic group of metazoans, only a small percentage of organisms undergo PDE, suggesting PDE evolved independently among these groups [36,44].

Not surprisingly, the PDE processes are mechanistically diverse. In metazoans, there are 2 major types of PDE: (1) loss of an entire chromosome(s); and (2) fragmentation of chromosomes resulting in the loss of genetic material, coincident with germline-to-soma differentiation. The second form of PDE is particularly intriguing as it requires the generation of double-stranded DNA breaks (DSBs). Nematodes and ciliates are known to introduce DSBs during PDE [45]; based on cytological studies, other animals such as hagfish and copepods also likely break their chromosomes during PDE [46,47]. In single-cell ciliates, domesticated transposases are responsible for many of the PDE DNA breaks [48]. However, the mechanisms and machinery associated with the DNA breaks in metazoans remain largely unknown. More general descriptions and discussions on PDE in diverse organisms have been summarized in recent reviews [40,43,45,49–52].

*Ascaris* remains one of the most extensively studied models for PDE in metazoans. Early work in *Ascaris* provided robust cytological and molecular data that revealed critical features of *Ascaris* PDE [37,44,53]. Recent genomics studies have expanded our understanding of *Ascaris* PDE to the whole genome at the chromosomal level [41]. These studies are in



**Fig 1. PDE occurs during nematode early embryogenesis.** (A) Theodor Boveri's drawing of *P. univalens* DNA elimination in a two-cell embryo. Left: interphase, chromosomes in the pre-somatic cell (top) are fragmented from PDE, while the chromosomes from the germ cell (bottom) are intact. Right: anaphase, the pre-somatic cell sequesters the chromosome fragments and leaves the HET arms at the metaphase plate. Image from Wikimedia commons. (B) *P. univalens* and *Ascaris* early cell lineage during early development. Germ cells are red, pre-somatic cells are yellow and surrounded by red dots, and somatic cells are blue. PDE occurs during successive divisions in early embryogenesis. Figure adapted from [37,38]. (C) A 4-cell *Ascaris* embryo with 2 cells undergoing PDE. DAPI staining showing eliminated DNA (artificially colored in red for emphasis) is left at the metaphase plate (red arrow) but retained DNA is segregated to the daughter nuclei. (D) A 6-cell *Ascaris* embryo with 1 cell undergoing PDE. Eliminated DNA begins to degrade during late anaphase (red arrow). Eliminated DNA fragments from previous PDE events are seen scattered across the embryo. Images in (C) and (D) are from [36]. (E) DAPI and H3S10P immuno-staining of *Ascaris* PDE. Eliminated DNA at the metaphase plate (red arrow) is stained with an antibody against H3S10P. (F) H3S10P Immuno-EM shows double-membrane bound H3S10P-positive structures between daughter nuclei during telophase of *Ascaris* PDE. Daughter nuclei are circled in blue. The region containing eliminated DNA is circled in green (for comparison with (E)). Double-membrane structures positive for H3S10P are marked with an asterisk (\*). (G) Inset from (F). Arrows point to double-membrane structure. (E) and (F) are adapted from [41].

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part facilitated by the large size of *Ascaris* and its enormous reproductive capacity [1] with large numbers of zygotes available from the uteri. These zygotes undergo synchronous development under laboratory conditions [1]. Their prolonged cell cycle (approximately 15 to 20 hours) makes it possible to isolate embryos at precise stages of development [54], including before, during, and after PDE. Although the parasitic lifestyle of *Ascaris* makes maintenance and genetic manipulation difficult, decades of molecular work in *Ascaris* have provided one of the most comprehensive understandings of PDE in a metazoan [6,45,49,50].

Recently, studies in the free-living nematode *Oscheius tipulae* have shown that it undergoes PDE [55,56]. In addition, cytological studies in the *Mesorhabditis* family of nematodes indicate that they also eliminate DNA during embryogenesis [57]. With more complete genomes, rigorous cytological studies, and comparative analysis on the differences in genome coverage between germline and somatic tissues, we predict more organisms with PDE will be identified in the near future [58]. The recent identification of PDE in free-living nematode species prompts questions about how frequently PDE occurs among metazoans (especially nematodes) and the selective advantage(s) it may provide. Free-living nematodes with PDE also present new opportunities to study PDE in genetically tractable systems [56]. These studies will complement work in *Ascaris* and provide comparative and evolutionary perspectives on PDE in metazoans.

## *Ascaris* PDE breaks chromosomes and eliminates DNA

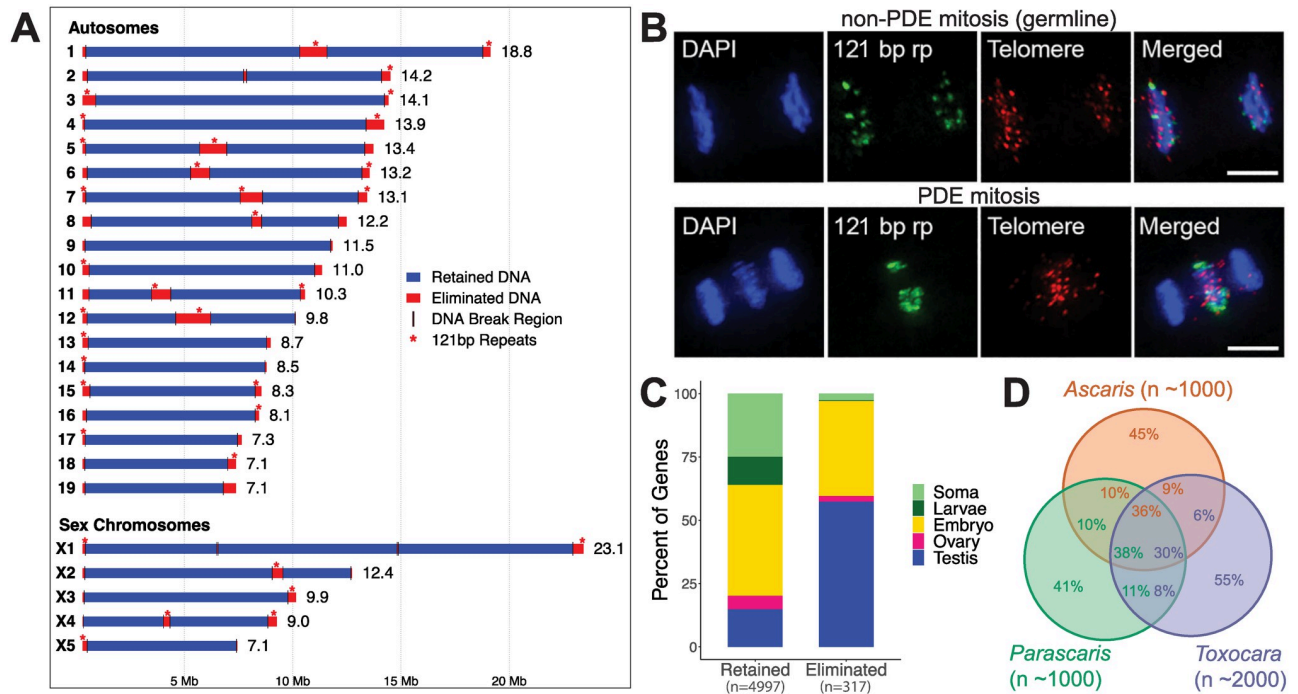
In most multicellular organisms, PDE events occur during early development during the germ-to-soma transition [45,49]. However, the exact timing of PDE varies among species. Cytological data suggest that *P. univalens* PDE transpires during the 2 to 32 cell stages, while *Ascaris* PDE occurs during the 4 to 16 cell stages. In both cases, PDE takes place in consecutive early embryo divisions in 5 independent pre-somatic cells (Fig 1B and 1C) [37]. In contrast, PDE in several other systems occurs in many cells at a single embryo division, such as the free-living nematode *O. tipulae* (fourth division) [56], the copepod *Mesocyclops edax* (fifth division), and the sea lamprey *Petromyzon marinus* (sixth division) [49]. It is unclear why organisms undergo PDE at different stages of embryogenesis and whether the timing of PDE is strictly associated with the transition from germline to somatic cells.

Cytological studies have revealed elements of chromosome behavior during *Ascaris* elimination mitoses. At metaphase, retained and eliminated DNA are both aligned at the metaphase plate. In early anaphase, the retained DNA segregates to the daughter cells while the eliminated DNA remains at the metaphase plate (Fig 1C). In late anaphase, eliminated DNA breaks down into smaller pieces (Fig 1D). The eliminated DNA stains with an antibody against H3S10P, a histone modification mark associated with condensed chromosomes during mitosis (Fig 1E–1G). Immunogold labeling of H3S10P for electron microscopy (EM) showed that the eliminated DNA is sequestered into double membrane-bound structures during telophase that resemble mammalian micronuclei [59]. The DNA likely undergoes autophagy and degradation in the subsequent cell divisions (Fig 1F and 1G) [41].

What sequences are eliminated during PDE? Early molecular cloning and cytological work showed that a large amount of DNA eliminated in *P. univalens* is associated with the HET arms (Fig 1A) that consist of 5- and 10-mer repeats [60]. Likewise, eliminated DNA in *Ascaris* is mainly composed of 121-bp tandem satellite repeats [41,61]. These early observations lead to the assumption that PDE mainly eliminates repeats. However, seminal work indicated that at least 3 single-copy genes are eliminated in *Ascaris* [62–64]. Over the last decade, the *Ascaris* genome was sequenced and improved with multiple iterations using state-of-the-art technologies [38,40,41,65,66]. The latest version was built with long reads (PacBio) and 3D genome conformation data (Hi-C), resulting in 24 fully assembled germline chromosomes [41]. A comparison between the germline and the somatic genome enabled comprehensive identification of eliminated sequences, DNA breaks, and formation of new telomeres during *Ascaris* PDE [41].

Several significant findings emerged from these *Ascaris* genome analyses. First, all germline chromosome ends have a DSB in the subtelomeric region, resulting in the removal and remodeling of each chromosome end (Fig 2A and 2B) [41]. In addition, internal DSBs lead to an increased number of chromosomes from 24 in the germline to 36 in the somatic cells (Fig 2A). No genome rearrangements occur during *Ascaris* PDE [41]. Second, approximately 1,000 (5% of the total) genes are present in the eliminated DNA. These genes are expressed in the germline, primarily during spermatogenesis (Fig 2C) [40,41]. Their elimination from the somatic cells suggests that PDE in *Ascaris* is an extreme gene silencing mechanism [38,40]. Third, telomeres are added to the broken DNA ends. The telomere addition sites occur within a 3 to 6 kb region called chromosomal breakage region (CBR). The CBRs appear to have no common motif or sequence features (see below), suggesting a sequence-independent mechanism of break site recognition [40]. Last, the CBRs and eliminated sequences are consistent among the 5 independent pre-somatic cells, between male and female worms, and between the human (*A. lumbricoides*) and pig (*A. suum*) parasites [40].

Most ascarid species undergo PDE [37]. How conserved is PDE among ascarids? A comparative genomic study identified approximately 1,000 eliminated genes in the horse parasite



**Fig 2. Genetic material lost during PDE.** (A) A linear presentation of *Ascaris* germline chromosomes. All chromosome ends and 12 internal regions are eliminated (shown in red). Many larger chromosomes have internally eliminated sequences which split them into smaller chromosomes in the soma. Eliminated regions containing 121-bp repeats are marked with a red \*. Figure adapted from [6]. (B) FISH of 121-bp repeats and telomeric sequences. Top: anaphase in a germline embryo undergoing non-PDE mitosis, with telomeres and 121-bp repeats passing to daughter nuclei. Bottom: anaphase of a PDE mitosis showing 121-bp repeats and telomeres are left at the metaphase plate. (C) Eliminated genes are highly enriched with testis and embryo-expressed genes. Shown are changes of the proportion (in percentage, y-axis) of tissue-specific genes in retained and eliminated DNA (x-axis). (D) Conservation of eliminated genes among *Ascaris*, *Parascaris*, and *Toxocara*. About 36% of genes eliminated in *Ascaris* have their orthologous genes eliminated in *Parascaris* and *Toxocara*. These genes tend to have enriched expression in the testis. Figure adapted from [6].

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*P. univalens* and approximately 2,000 in the dog parasite *T. canis* [40]. About one-third of the eliminated genes are conserved among these 2 species and *Ascaris* (Fig 2D). The conserved, eliminated genes in all 3 species are primarily expressed during spermatogenesis, suggesting a common mechanism for silencing testis genes in the somatic cells. Interestingly, the amount and composition of eliminated repetitive sequences varies drastically among the 3 species. *Ascaris* eliminates 121-bp repeats (10% of the genome) (Fig 2A and 2B), while *P. univalens* removes 5-bp and 10-bp repeats (89% of the genome), and *T. canis* throws away 49-bp satellite sequences (9% of the genome) [40]. The differences in tandem repeat composition may reflect their different origins and/or species-specific adaptations during nematode evolution.

In summary, *Ascaris* undergoes PDE in all 5 pre-somatic cells during the 4 to 16 cell stages. During PDE, 72 DSBs are introduced at specific sites (Fig 2A). The DSBs remove all chromosome ends and split germline chromosomes into smaller somatic chromosomes, thus increasing the number of chromosomes in the soma. During this process, approximately 18% of the germline genome is lost. The eliminated DNA is composed of satellite repeats and contains approximately 1,000 genes. Following DSB formation, all broken ends, including the ends of eliminated sequences, are healed with de novo telomere addition.

### Molecular mechanisms of *Ascaris* PDE

What mechanisms are involved in PDE and how is the process regulated? Boveri noted that after centrifugation of *P. equorum* embryos, a “ball” of cytoplasmic factors would form at the

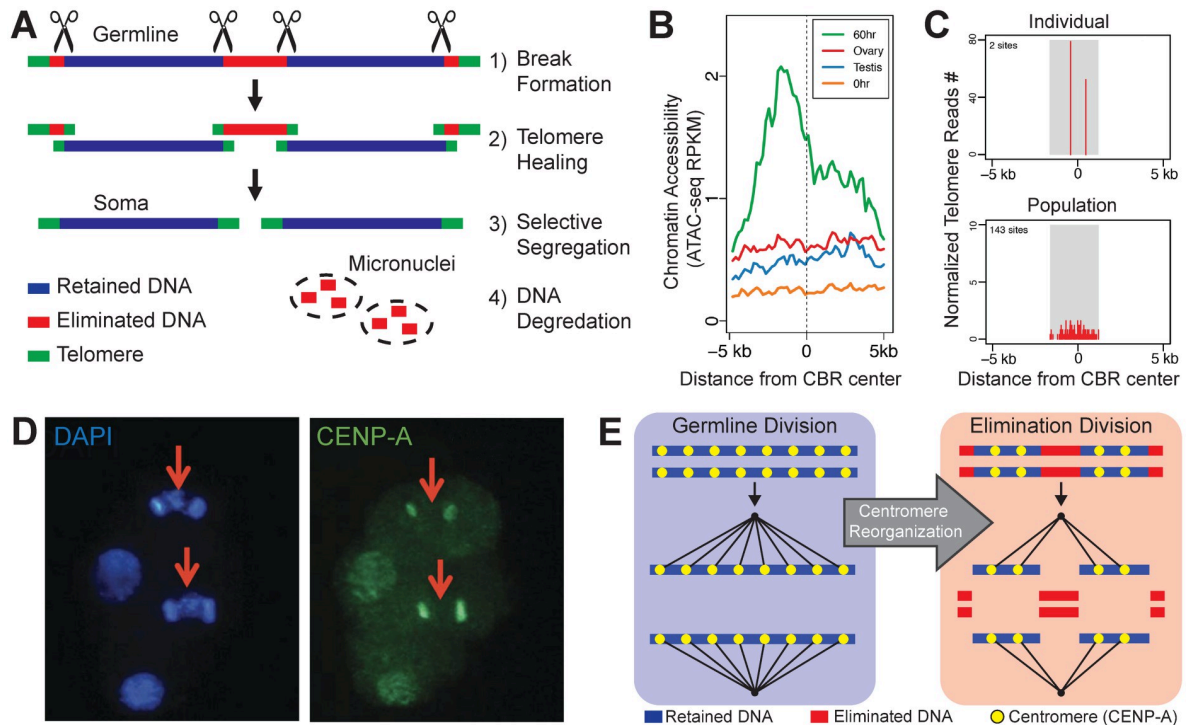
animal pole. The 2 blastomeres proximal to this ball would undergo PDE, while the 2 blastomeres distal to the ball would not. This led to the hypothesis of a cytoplasmic factor that induces PDE [44]. Subsequent studies by Moritz showed that UV irradiation of *Parascaris* embryos resulted in all cells undergoing PDE [44,67]. This suggests that a UV-sensitive factor is responsible for inhibiting PDE in germline cells. Experiments by Oliver and Shen, using dinucleated *Ascaris* embryos obtained by treatment with cytochalasin D (to prevent cytokinesis), further demonstrated that 1 blastomere always undergoes PDE while the other never does [68]. Together, these early experiments suggest a complex regulatory system where PDE-promoting factors associate with pre-somatic cells and PDE-inhibiting factors associate with germ cells [68]. These regulatory factors have yet to be identified.

At the molecular level, the process of PDE in *Ascaris* can be summarized in 4 major steps: (1) recognition of the sites for DNA breakage and generation of chromosome breaks; (2) healing broken DNA ends by telomere addition; (3) selective segregation of retained DNA; and (4) degradation of eliminated DNA (Fig 3A). It's not clear if these steps are coupled or if they are independent, sequential events. Overall, little is known about the molecular details of *Ascaris* PDE. Below, we summarize what is known and discuss possible molecular mechanisms involved in these steps.

*How are break sites recognized and generated?* Analysis of break regions for the presence of specific DNA sequences, structural motifs, palindromes, Z-DNA, and hairpins have revealed no apparent features that define or target CBRs for PDE break formation [41]. Additionally, no small RNAs or long non-coding RNAs appear to directly mark the break sites or contribute to PDE [66,69]. A recent analysis suggested that G-quadruplex DNA is enriched in extended (10 to 20 kb) regions covering 40 *Ascaris* CBRs [70]. However, G-quadruplexes are also enriched in other regions of the *Ascaris* genome, and many CBRs have no G-quadruplexes [70], suggesting G-quadruplex DNA is probably not a component of the CBRs.

One consistent feature of *Ascaris* CBRs is the increased chromatin accessibility before and during PDE, as measured by ATAC-seq (Fig 3B) [40,41]. This accessibility may play a significant role in *Ascaris* PDE. In many systems, replication stress, transcription, and R-loop formation facilitate chromatin opening [71–73]. In addition, these factors are a source of genome instability and could lead to DSB formation [74]. Therefore, it is tempting to speculate that replication and transcription may be responsible for chromatin opening and DSB formation at *Ascaris* CBRs. The open chromatin may allow an endonuclease to access the CBR to generate the DSBs. In the free-living nematode *O. tipulae*, a conserved motif is necessary for the DNA break and telomere addition required for PDE [56]. In contrast, *Ascaris* CBRs have no common motif, thus a sequence-independent mechanism, such as 3D genome organization [75–77], may be needed to confine break formation to the CBRs.

*How are the ends processed after a DNA break and how are telomeres added?* Interestingly, genome sequencing indicates that 2 independent telomere addition sites are often observed in a single PDE event; the 2 sites are presumably from the 2 homologous chromosomes (Fig 3C) [40,78,79]. In a population of *Ascaris* worms, telomere addition sites are randomly distributed within a constrained 3- to 6-kb region, which defines the CBRs (Fig 3C). This suggests the telomere addition sites are sequence-independent and random within the restricted genomic regions. Remarkably, telomere addition occurs not only to retained DNA but also to eliminated DNA [40,78,79]. This indicates that telomere addition in *Ascaris* could be non-selective; it may heal all available PDE DNA breaks. This universal healing likely protects the broken DNA ends from undergoing fusions and genome rearrangements [36]. Analysis of these telomere addition sites has revealed that a single nucleotide of homology is sufficient for telomere addition in vivo [40]. Since the *Ascaris* telomeric repeat (TTAGGC) contains all 4 bases, the requirement of a single nucleotide means telomere addition can occur at any site within the



**Fig 3. PDE mechanisms.** (A) A model of PDE process in *Ascaris*. See text for detailed description. (B) ATAC-seq of *Ascaris* shows CBRs have open chromatin during PDE (60 hours, 4-cell). (C) *Ascaris* telomere addition forms in 3–6-kb regions of the genome. Top: telomere addition from a single worm’s intestine (derived from 1 PDE event) shows 2 telomere addition sites. Bottom: telomere addition from a population of worms shows telomeres are randomly distributed across the CBR. (B) and (C) adapted from [40]. (D) CENP-A staining of a 4-cell embryo with 2 cells undergoing PDE. Eliminated DNA (red arrows) is not stained with CENP-A, suggesting the loss of centromeres in the eliminated DNA. Adapted from [39]. (E) A model of centromere reorganization for PDE. Left: germline division with centromeres distributed across the entire chromosome. Right: PDE mitosis with centromeres absent on eliminated regions of the genome. Adapted from [39].

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CBRs. Telomerase activity from *Ascaris* cell-free extracts increases 10-fold from the one-cell to four-cell stage and remains elevated past the final PDE event [40,80]. This is consistent with RNA-seq data [81] and suggests telomerase is likely responsible for the synthesis of de novo telomeres during *Ascaris* PDE.

The telomere addition site is not necessarily the site where the initial DSB occurs. DSBs could occur at multiple sites within eliminated regions, adjacent to the CBRs followed by end resection, and/or at the site of telomere addition. Estrem and colleagues used END-seq, a technique that captures DSBs and end resections [82,83], to identify the position and timing of DNA breaks on synchronized embryos from before and during PDE stages [Estrem, Davis, and Wang, personal communications]. END-seq data identified that extensive resection occurs to generate 3'-overhangs necessary for telomere addition. This end resection is also conserved in *O. tipulae* PDE [56]. In addition, the timing of initial breaks detected by END-seq suggests *Ascaris* chromosomes are broken prior to the onset of mitosis, consistent with EM observations [41]. This indicates that cellular mechanisms are required to congress eliminated chromosome fragments to the metaphase plate during DNA elimination mitosis (see Fig 1).

*How is retained DNA recognized and selectively segregated after DSBs?* Immunohistochemical (IHC) analysis of histone marks (H3K4me1/3, H3K9me2/3, H3K36me2/3, H4K16ac, and H3K27me3) revealed no differences between retained and eliminated DNA in anaphase during PDE [41]. However, H3S10P is enriched on eliminated DNA (Fig 1E) and H2AK119ub is

associated with retained DNA [41]. In addition, 2 worm-specific Argonaute proteins (WAGOs) associated with endogenous small RNA (siRNA) pathways are differentially enriched in retained (WAGO-2) and eliminated (WAGO-3) DNA during PDE mitosis [Zago-skin, Wang, and Davis, personal communications]. It is unclear if these histone modifications or the WAGO argonautes are actively marking sequences for their retention/elimination or if they are an indirect consequence of PDE. For example, H3S10P on the eliminated DNA is likely due to a failure to dephosphorylate H3S10P as the eliminated DNA fragments are compartmentalized into micronuclei and may not progress through the normal metaphase to anaphase transition [41] (Fig 1F and 1G).

After the fragmentation of the chromosomes, how does the cell decide which chromosome pieces to keep and discard? Like the model nematode *Caenorhabditis elegans*, the chromosomes in *Ascaris* and *P. univalens* are holocentric, with multiple centromeres/kinetochores distributed along the length of the chromosomes, a feature that in principle makes DNA breakage more tolerable [39,84,85]. Staining data demonstrated that during PDE, the histone H3 variant CENP-A, the epigenetic marker of centromeres, is absent in the DNA to be eliminated (Fig 3D). This lack of centromeres in the eliminated DNA provides a mechanism for its subsequent loss after the DNA breaks, as no microtubules can attach to the DNA to facilitate its proper segregation during mitosis. ChIP-seq showed that CENP-A is evenly distributed across the genome in *Ascaris* male and female germlines [39]. These data suggest a dynamic reorganization of centromere deposition that enables *Ascaris* to selectively segregate the retained DNA into daughter nuclei while excluding the eliminated DNA (Fig 3D and 3E) [39]. The regulation of centromere reorganization has not been elucidated in *Ascaris*. However, transcription, histone modifications, and small RNAs have been linked to dynamic centromere deposition in fission yeast and nematodes [86–88].

*How is eliminated DNA degraded?* The enrichment of H3S10P on eliminated DNA provided a mechanism to track this chromatin following mitosis. H3S10P co-localizes with LGG1, an autophagosome marker [41]. Additionally, immuno-electron microscopy of H3S10P revealed many double membrane-bound structures are positive for H3S10P during the telophase following PDE (Fig 1F and 1G) [41]. These results suggest that, following mitosis, eliminated DNA is autophagocytosed into micronuclei-like structures. Large heterochromatic regions may take a few cell cycles to completely turn over, as remnants of the eliminated DNA from previous cell cycles are often seen in subsequent developmental stages (Fig 1D). The engulfment of the eliminated DNA into micronuclei may block the DNA from unnecessary repair and/or recombination events. Recent work has shown that micronuclei are prone to rupture, resulting in DNA fragmentation [89]. The mechanism of micronuclei fragmentation is not well understood, but aberrant replication or exposure to nucleases in the cytoplasm may drive this process [89–91]. Nematodes with PDE may provide a new model to study DNA fragmentation in micronuclei. For example, it will be interesting to determine if the DNA to be eliminated in *Ascaris* is turned over through an orderly or random process.

## Functions of *Ascaris* PDE

The presence of PDE in phylogenetically diverse organisms suggests PDE may provide some selective advantage [43,45,49]. One consequence of PDE is the permanent removal of specific repetitive elements in all somatic cells, a common feature among all known species that undergo PDE [40,55,56]. In *Ascaris*, 54% of the eliminated DNA consists of 121 bp tandem repeats. In addition, almost all satellite repeats (>99%) are eliminated from *Ascaris*, *P. univalens*, and *T. canis* [40]. These repeats likely have germline functions that are not needed (or are harmful) in the somatic cells. These functions could be related to meiotic chromosome pairing



and recombination, spacing and scaffolding of 3D genome organization in the germline, and evolution of novel genes and/or chromosomes [41,45,92–95]. Another benefit of removing repeats, particularly large amounts of satellites such as the 89% (2.2 Gb) eliminated from *P. univalens*, could be to downsize the genome, alleviating the burden of DNA replication and genome maintenance in somatic cells.

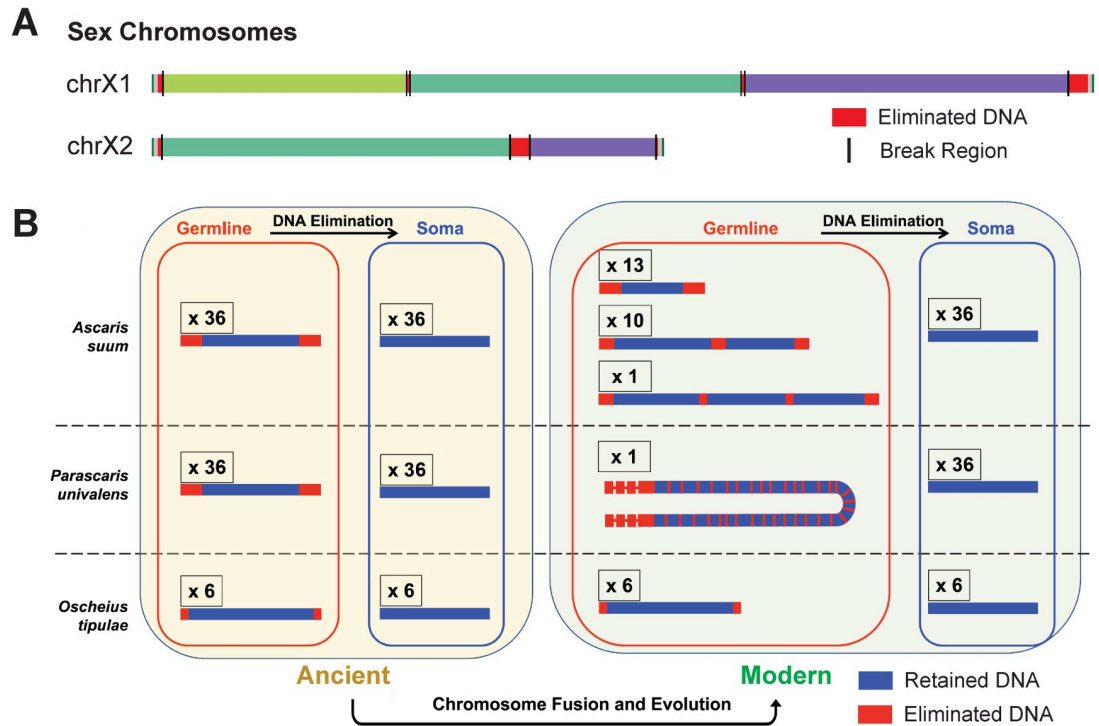
Another advantage of PDE could be the removal of germline-specific genes. Typically, germline genes in nematodes are silenced by histone modifications and small RNAs [96–98]. In *Ascaris*, most of the approximately 1,000 eliminated genes are expressed in the testis, ovary, or during early embryogenesis (Fig 2C) [38,40]. Thus, PDE provides a permanent means to silence these genes in the somatic cells. The removal of testis-expressed genes is conserved in the ascarids *P. univalens* and *T. canis* [6,40], further supporting the role of silencing germline-expressed genes for PDE in ascarids. This gene silencing appears to be a common theme for PDE as germline-specific genes are identified in phylogenetically diverse species that undergo PDE [40,43,45,49]. In *Ascaris*, many of the eliminated genes have paralogs in the genome, including a germline-specific ribosomal protein [62,99] and translation initiation factors that are involved in mRNA translation [38,99]. Duplication of genes provides an alternative means of gene regulation. This is consistent with the model that an ancient genome duplication in the *Ascaris* lineage was balanced by PDE to compensate for gene dosage or to retain specific genes and thus their function after gene duplication [38].

What is the benefit of chromosome end removal and remodeling? Repetitive DNA and novel genes are often enriched at the ends of the nematode chromosomes, while more conserved and housekeeping genes are concentrated in the middle of the chromosomes [100]. All 13 *Ascaris* germline chromosomes with no internal PDE breaks have this sequence organization [41]. The other 11 germline chromosomes are believed to be potential fusions of multiple ancestral chromosomes (see below section on the evolution of PDE). Removal of repeats and novel genes from chromosome ends may protect the soma from potentially harmful sequences while allowing their existence in the germline. This may provide an evolutionary advantage for the germline to sample novel genes or harbor repeats. An alternative hypothesis is chromosome ends act as pairing centers that are needed in the germline but deleterious to the somatic cells. Consistent with this notion, proteins involved in *C. elegans* meiotic pairing [92–95] appear absent from *Ascaris*'s genome [Wang, personal communications]. A recent study in the free-living nematode *O. tipulae* demonstrated a fail-safe mechanism is in place to ensure the removal of chromosome ends during PDE [56], emphasizing the importance of end removal for the biology of these species. Future studies are warranted to determine the functions of the eliminated DNA in the germline and the benefit of its removal in the somatic cells.

PDE clearly poses advantages in *Ascaris* and other nematodes. It is a mechanism to silence germline sequences and repetitive elements in the somatic genome. End removal and remodeling appear conserved among nematodes. In addition, PDE can alter the somatic chromosome karyotype, possibly leading to changes in the 3D genome organization and gene expression in the soma. These functions may provide an additional benefit to the survival and fitness of the organisms.

## Evolution of PDE in nematodes

Comparative analysis of the *Ascaris* genome has revealed that 4 out of 5 sex chromosomes have undergone recent fusion events. These sex chromosomes are chimeric in their composition of Nigon elements (Fig 4A) [41,55]. Interestingly, the internally eliminated DNA represents the junction of presumptive fusion events on these sex chromosomes [41,55]. PDE splits these fused germline chromosomes into separate chromosomes in the somatic cells. Analysis



**Fig 4. An evolution model of PDE in nematode chromosomes.** (A) Fusions in *Ascaris* sex chromosomes. Two representative sex chromosomes with distinct ancestral regions (Nigon elements; each Nigon group is shown in a different color). Eliminated DNA is at the junction of different Nigon elements. (B) Fusion and evolution of chromosomes in nematodes with PDE. Left: hypothetical ancient chromosome karyotypes before and after chromosome end remodeling and DNA elimination in *Ascaris*, *P. univalens*, and *O. tipulae*. Right: modern karyotypes before and after DNA elimination showing internal breaks and end-remodeling. For simplicity, a representative chromosome is shown for all chromosomes within a group that have the same pattern of elimination (terminal and/or internal). The number of chromosomes for each group is indicated above the representative chromosome. (A) and (B) are adapted from [45].

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of the fully assembled *P. univalens* germline and somatic genomes reveals that it has the same number (36) of somatic chromosomes as *Ascaris* [Simmons and Wang, personal communications]. The internally eliminated regions in both *Ascaris* and *Parascaris* have sequence features that are common to the ends of nematode chromosomes [85], including their enrichment of repeats, genes encoding hypothetical proteins, and genes specifically expressed in the testis. This suggests that these internal eliminated regions were likely originated from the ends of ancestral chromosomes. Thus, the current single germline chromosome of *Parascaris* and the 24 *Ascaris* germline chromosomes can be viewed as fusion products of the 36 ancient germline chromosomes. These ancestral germline chromosomes also likely underwent PDE with end remodeling (Fig 4B).

PDE in *Ascaris* and *Parascaris* provides a way to resolve these fused germline chromosomes to form the 36 somatic chromosomes. The reduced number of chromosomes in the germline should decrease the probability of segregation errors during meiosis. In comparison, the smaller somatic chromosomes may be more adapted to the conventional gene expression regulation in nematode, as the organization of the genes and repeats in these chromosomes resembles a typical nematode chromosome [85]. Thus, both fused germline chromosomes and split somatic chromosomes may pose advantages. Recent data from some free-living nematodes indicates internal breaks also occur [85]. This finding does not preclude PDE as a mechanism to resolve chromosome fusions in ascarids. Instead, it suggests that de novo introduction of

DNA breaks in the middle of the chromosomes is possible, likely enabled by the holocentric nature of nematode chromosomes [85].

In nematodes, PDE has been discovered in 3 distinct clades, including many *Oscheius* species [55,56], *Caenorhabditis monodelphis*, and *Mesorhabditis* [57] from Clade V, ascarids (*Ascaris*, *Parascaris Toxocara*, and *Baylisascaris*) from Clade III [37], and *Strongyloides* [35,101,102] from Clade IV. A common theme among *Ascaris*, *P. univalens*, and *O. tipulae* PDE is the removal of all chromosome ends, followed by new telomere addition. This leads to the question whether PDE is an ancient trait, or if it has convergently evolved in multiple nematode clades. It is known that the model organism *C. elegans* (Clade V) does not undergo PDE [103,104]. But how common is PDE among nematodes? Other nematodes may also remodel their chromosome ends with PDE, but the removal of only small amounts of DNA may have eluded detection due to technical limitations such as the sensitivity of cytological approaches and/or the requirement of telomere-to-telomere genome assemblies [55]. PDE may be more common or favored in nematodes because the holocentric nature of their chromosomes that allows fusions and fissions to be tolerated [105,106]. However, holocentromeres are not required for PDE as it is seen in several phyla with monocentric chromosomes [36,45,49]. Nevertheless, end-remodeling appears to be a form of PDE that is often missed, suggesting PDE may be much more frequent than previously thought. Future identification of additional species with PDE will further elucidate the evolutionary relationship of PDE in various organisms.

## Perspective

PDE violates the general view of genome constancy and integrity. However, PDE is a natural process found in many branches of animal phyla, suggesting its common presence and biological significance. The number of species described with PDE has increased with the use of genome sequencing and modern cytology. However, mechanistic insights into PDE are mostly limited to single-cell ciliates. For metazoans, the nematode group has been a leading model to study PDE since its discovery over 130 years ago. In particular, the large size of *Ascaris*, the ability to dissect specific germline and somatic tissues, and its slow, synchronous embryo development have enabled robust biochemistry, molecular biology, and genomics to be carried out. Other parasitic ascarids, including *Parascaris* and *Toxocara*, have also been used as comparative models to study PDE. Many features of PDE, including DNA breaks, telomere addition, and chromosome end removal and remodeling, are conserved in these nematodes [6,40]. The newly established PDE model in the free-living nematode *O. tipulae* now adds genetic and functional approaches to study PDE [56]. Overall, these studies will unveil the functions and mechanisms of PDE in nematodes, including the human parasite *Ascaris*.

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