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Gene Silencing and Sex Determination by Programmed DNA Elimination in Parasitic Nematodes

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Abstract

Maintenance of genome integrity is essential. However, programmed DNA elimination removes specific DNA sequences from the genome during early development. DNA elimination occurs in unicellular ciliates and diverse metazoa ranging from nematodes to vertebrates. Two distinct groups of nematodes use DNA elimination to silence germline-expressed genes in the soma (ascarids) or for sex determination (*Strongyloides spp.*). Data suggest that DNA elimination likely evolved independently in these nematodes. Recent studies indicate that differential CENP-A deposition within chromosomes determines which sequences are retained and lost during *Ascaris* DNA elimination. Additional studies are needed to determine the distribution, functions, and mechanisms of DNA elimination in nematodes.

Keywords

Programmed DNA elimination; nematodes; chromatin diminution; CENP-A; holocentric chromosomes; *Ascaris*, *Parascaris*, *Strongyloides*

INTRODUCTION

Programmed DNA elimination (PDE) is a developmental process in which portions of the genome are reproducibly eliminated. This process occurs in diverse eukaryotes ranging from single-celled ciliates, nematodes, and crustaceans to birds and jawless fish (lampreys) [1]. DNA elimination may occur through the loss of entire chromosomes or only portions of chromosomes. In this review, we summarize recent studies on DNA elimination in parasitic nematodes. Additional information can be found in excellent earlier reviews from pioneers in the field [2–9].

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COMPETING INTERESTS

None declared

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Discovery

Programmed DNA elimination was first described in a parasitic nematode of horses, now called *Parascaris univalens* [10], by the great cell biologist Theodore Boveri. Boveri observed that in the precursor somatic cells during the first several divisions of the *P. univalens* embryo, the very large pair of chromosomes were broken, large portions lost, and many new small chromosomes formed and retained in the somatic cell lineages (Fig. 1A–B; see similar process in *Ascaris*, Fig. 1C–D). Notably, the large *Parascaris* chromosomes enabled Boveri [11,12] to describe the chromosome behaviors during a cell cycle and centrosomes which led to the chromosome theory of heredity and enabled van Beneden [13] to discover and describe the process of meiosis.

Nematodes with Programmed DNA elimination

Current data suggest that DNA elimination in nematodes is limited to two nematode orders (Order Ascaridida, the superfamilies Ascaridoidea and Cosmoceroidea, and the order Rhabditida, the family Strongyloididae). It does not appear to occur in the model nematode *Caenorhabditis elegans* [14,15]. DNA elimination likely evolved independently in these two nematode groups (see below). Nematodes are a broad phylum with many highly divergent genera. Do other groups of nematodes have DNA elimination? Current methods for identification of DNA elimination include cytological analysis of chromosomes during mitosis (insensitive if only small portions of the genome are eliminated) and genome sequencing and comparison at appropriate stages. The choice of stages is critical for the identification and analysis of DNA elimination. For example, older studies described DNA elimination in *Toxocara spp.* [16]. The *Toxocara* genome [17] was recently characterized but the material and approach used for the sequencing was not appropriate for the identification and analysis of DNA elimination.

DNA elimination in Ascaridia: *Parascaris* and *Ascaris*

Most ascarids that have been examined exhibit DNA elimination [2,5]. However, only two genera with DNA elimination have been analyzed in any detail: *Parascaris* and *Ascaris*, two related ascarids that infect horses and pigs/humans, respectively. In these nematodes, programmed DNA elimination occurs only in the somatic cells during the first several cell divisions (Figs. 1–2), where chromosomes break and new chromosomes are formed. The process is most in extreme in *Parascaris* where the entire genome is in a single diploid pair of large chromosomes ($1n = 1$; each ~ 2.5 Gb in size) that undergo DNA elimination, leading to the loss of ~90% of the genome (mostly short 5 and 10 bp repeats) and the generation of many new chromosomes [18] (Wang et al., unpublished). In comparison, DNA elimination in *Ascaris* leads to the loss of ~13% of the genome, largely 121 bp repetitive sequences, and the haploid chromosome number increases by 50%. Strikingly, ~700 (or 5%) genes are also lost in *Ascaris* [19]. These eliminated genes are primarily expressed in the germline and early embryo suggesting that DNA elimination in *Ascaris* is an extreme mechanism of gene regulation for silencing genes in the somatic cells through their elimination. Independent DNA elimination events occur in five pre-somatic cells in *Ascaris* leading to distinct cell lineages that differentiate into specific tissues (Fig. 2). Comparative sequence analysis of these different tissues as well as both sexes indicates that the chromosome regions

eliminated and retained are the same during the five elimination events and between males and females [19].

Mechanisms for DNA elimination in *Parascaris* and *Ascaris*

Cytological analysis of the large chromosomes of *Parascaris* suggests that a break occurs at the junction of a central “euchromatin” region and the highly condensed and historically described “heterochromatin” tips of the chromosomes during DNA elimination (Fig 1A–B). The large heterochromatic tips are not segregated into daughter cells and lost, while the central region is broken into a large set of new chromosomes [18]. *Ascaris* germline chromosomes are considerably smaller and the cytological position of the breaks are not well-characterized, but several occur at the chromosome ends [18]. In addition, it is unclear whether all chromosomes in *Ascaris* undergo DNA elimination and whether any new chromosomes in *Parascaris* are derived from the heterochromatic tips.

A key question is what determines or defines where the breaks will occur and what is the mechanism of DNA breakage. The retained broken chromosomes are healed by addition of new telomeres [20,21] and 50 locations where new telomeres are added were identified in *Ascaris* [19]. While the fidelity of the location of the telomere addition is very highly conserved at the chromosomal level, the exact location can vary from 200–2,000 bp in different cell lineages and individuals. Telomere addition may involve resection of additional sequences and thus the exact sites of the breaks are not known [19]. Analysis of DNA sequences 5 kb on either side of the 50 sites of where telomeres are added did not identify any unique sequence motifs, structures, or other features that might define or designate where the breaks are to occur. One interpretation of these observations is that the sites are not identified through specific sequence motifs. Alternatives might include specific histone or other chromatin modifications and/or recognition by small RNAs, as occurs in ciliates [22–24], in the vicinity of the break sites. Endogenous DNA double-strand breaks are often associated with replication and transcription collisions and other forms of replication stress [25,26]. Higher order organization of the chromosomes and their interactions might also play a role in defining where the breaks occur. Another key question is how this overall process occurs only in somatic cells and not the germline cells of the embryo and how it is developmentally regulated.

A model for *Ascaris* DNA elimination is shown in Fig. 3A [18]. During DNA elimination mitoses, chromosomes align at metaphase and chromosomal breaks occur (Fig. 3A-1). Regions that will be retained or eliminated may be marked (epigenetically) prior to or at the time of breakage. During anaphase, chromosomal regions are selectively retained and eliminated by chromosomal segregation (Fig. 3A-2). Most organisms have monocentric chromosomes, that is, each chromosome has a single centromere/kinetochore region (Fig. 3B). However, those nematodes studied to date have holocentric chromosomes [6,27–30] (Fig. 3C). Holocentric chromosomes have multiple centromeric regions, kinetochores, and microtubule attachment sites punctuated along the length of the chromosome [6,31–34]. This raises a key question for what determines which portions of chromosomes are retained or eliminated following chromosome breakage. In most organisms, the histone H3 variant CENP-A is incorporated into nucleosomes that epigenetically mark genomic regions to

become functional centromeres [30,35–37]. Recent data [38] suggest that germline chromosomes in ascarids have CENP-A uniformly distributed along chromosomes in the mitotic region of the germline (Fig. 3E). During development, regions that will be lost during DNA elimination undergo significant reduction in CENP-A. These chromosome regions consequently do not have functional centromeres/kinetochores and thus are not segregated during DNA elimination (Fig. 3A and 3D). These data suggest that 1) CENP-A deposition and centromere/kinetochore formation is dynamic in these nematodes and 2) that deposition of CENP-A contributes to the identification of chromosomal regions to be retained and lost during DNA elimination. Thus, CENP-A deposition plays a regulatory role and provides a mechanism for DNA elimination.

Function of DNA elimination in *Parascaris* and *Ascaris*

Strikingly, many of the chromosomal break sites [39] and the majority of the genes lost are conserved between *Ascaris* and *Parascaris* (Wang et al., unpublished). This further suggests that DNA elimination is a highly conserved, regulated process. The elimination of germline and early embryo expressed genes suggests an extreme mechanism of gene regulation by silencing genes in the somatic cells through their elimination. Notably, not all germline and early embryo expressed genes are eliminated [19,40]. The majority of DNA eliminated in both genera is repetitive satellite sequence. While repetitive sequences are important for recombination and genome evolution in the germline, it remains unclear why they need to be eliminated in somatic cells. Additional DNA in the somatic cells might be a burden during early embryo cell cycles, but the elimination does not seem like to be related to early embryo cell division, as it can be relatively slow (~20 hrs/division in *Ascaris*) or fast (~3 hrs/division in *Parascaris*) and the determinate cleavage is also present in *C. elegans* where DNA elimination does not occur.

The entire *Parascaris univalens* germline genome consists of one single pair of very large chromosomes. Following DNA elimination, the number of *Parascaris* somatic chromosomes increases to a haploid number of 35 (29 autosomes and 6 sex chromosomes) [18]. In *Ascaris*, the haploid germline chromosome number is 24 (19 autosomes and 5 sex chromosomes) whereas following DNA elimination the number increases to 36 (28 autosomes and 8 sex chromosomes) [18]. While the germline genomes sizes and chromosome numbers differ greatly between the two, the number of somatic chromosomes and the somatic genome sizes are very similar. Given the conservation of many break sites between the two nematodes, is DNA elimination a mechanism to generate a required specific set and organization of chromosomes to function effectively and/or properly in somatic cells?

DNA elimination in *Strongyloides* spp

Small intestinal parasites of the genus *Strongyloides* spp. use **programmed** DNA elimination (PDE) for sex determination. In addition to parasitic individuals these worms can also form facultative free-living generations consisting of males and females. The parasitic adults are all females but produce female and male progeny through mitotic parthenogenesis [41]. An increased host immune response results in more males being produced [42]. This environmentally influenced sex determination through PDE has

presumably evolved fairly recently from a genetic XX/XO sex determining system as it is still present today in the close relative *Parastrongyloides trichosuri* [43,44]. Two types of sex determining DNA elimination are known in *Strongyloides* spp. [43,44], 1) the elimination of an entire chromosome and 2) loss of only a portion of a chromosome (Fig. 4).

In the rat parasite *Strongyloides ratti*, males inherit only one of the two X chromosomes from their mothers along with the two pairs of autosomes (2n=6 in females and 2n=5 in males) [42]. The loss of the chromosome most likely occurs during the single mitotic oocyte maturation division [45]. Nothing is known about the mechanism of loss or how the environmental stimulus elicits the loss. In the sheep parasite *S. papillosus*, the X chromosome appears inserted into one of the autosomes [46,47]. Therefore, females have only two pairs of chromosomes (2n=4) [48,49] and the evolutionarily X chromosome derived chromatin is flanked by autosomal chromatin [46,47]. In males, the X derived portion of one of the two homologous chromosomes is eliminated [48,49]. Both autosomal ends are retained as independent chromosomes resulting in males with 5 chromosomes (2n=5) [48,49]. During the single mitotic maturation division in oocytes giving rise to males both homologous longer chromosomes undergo what appear to be structural and organizational changes. In one chromosome, breaks occur and there is loss of the central region. The other homolog does not break and is normally segregated. These data suggest that the identification of the chromosome region for elimination is a two-step process. First the sequences are identified and then only one copy is selected for elimination. The region that is lost does not attach to the division spindle and is not incorporated into the daughter nuclei [48]. Which one of the two homologous chromosomes undergoes diminution appears random [47].

Comparison of DNA elimination in nematodes

DNA elimination in *Strongyloides* spp. differs from that in ascarids. 1) It creates a DNA difference between the sexes and not between the soma and the germ line. 2) Only one DNA copy is eliminated resulting in a reduction of gene dosage but not complete silencing of the gene. Thus, the process does not serve as a mechanism for complete gene elimination as in ascarids. 3) In ascarids (as well as many other metazoa [1]), DNA elimination leads to the removal of repetitive sequences. The genomes of *Strongyloides* spp. appear to have little repetitive sequence [46]. 4) DNA elimination in ascarids is constitutive occurring invariably in all individuals whereas in *Strongyloides* spp. it is induced by environmental cues and only in individuals destined to become males. 5) In ascarids, DNA elimination occurs in multiple cells in an individual whereas in *Strongyloides* spp. it occurs only at the one cell stage. 6) Lastly, in ascarids a soma vs. germline distinction is created in individuals whereas in *Strongyloides* spp. it does not. Overall, these differences and the large phylogenetic distance between these nematodes, strongly suggests that DNA elimination almost certainly evolved independently in these two nematode groups. This further supports the suggestion that DNA elimination may have evolved independently in different groups of metazoa [1]. Are the overall cellular and molecular DNA elimination mechanisms the same or different in ascarids vs *Strongyloides* spp.? Given that we currently have little information on how breaks occur, a comparison is currently not possible. However, the regulated loss of CENP-

A deposition and functional centromeres/kinetochores in *Ascaris* may also explain how a portion of a chromosome or an entire chromosome is lost in *Strongyloides* spp..

CONCLUDING REMARKS

Recent technological advances and their application to the study of programmed DNA elimination have led to new insights and a renewed interest in this fascinating phenomenon first described in the late 19th century. In nematodes, DNA elimination likely evolved independently in at least two groups and serves either 1) in gene silencing and distinguishing between the soma vs germline (ascarids) or 2) in sex determination (*Strongyloides* spp.). It remains to be determined whether other nematode groups use DNA elimination and what functions it might serve. While some progress has recently been made in determining how sequences are defined for retention and elimination in ascarids, many aspects of the mechanism(s) of DNA elimination remain unknown. Future studies are likely to provide further insights into the mechanism(s) and functions of this interesting process that defies the fundamental need for genome stability in most organisms.

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HIGHLIGHTS

- DNA elimination likely evolved independently in at least two groups of nematodes
- Elimination process is precise, highly regulated, and conserved in ascarid nematodes
- Ascarids eliminate repeats and ~700 single copy genes to form the somatic genome
- Ascarid CENP-A chromosome deposition defines DNA sequences for retention or elimination
- *Strongyloides* removes one copy of the sex chromosome in males for sex determination

Box**Outstanding questions for DNA elimination in nematodes**

- Does programmed DNA elimination exist in other groups of nematodes? Is DNA elimination only associated with parasitic nematodes? Are there additional functions for DNA elimination in nematodes?
- How are the DNA breakpoints generated during nematode DNA elimination? Does DNA replication stress, transcription, or the higher order/spatial organization of the chromosomes play a role in breakpoint generation?
- Do chromatin organization and epigenetic marks play a role in retaining or eliminating DNA or the DNA breakpoints during DNA elimination?
- In ascarids, why are repetitive sequences eliminated in somatic cells and do these sequences play a role in the mechanism of DNA elimination?
- In *Strongyloides* spp., how are environmental cues read to induce DNA elimination? What mechanism ensures that only one copy of a chromosome or chromosome region is eliminated? Are the breakpoints precisely defined as in ascarids? Are the retained chromosomal fragments healed by telomeres or protected from degradation by an alternative mechanism?
- Are the molecular mechanisms for DNA elimination in *S. ratti* and *S. papillosus* similar or different?

Figure 1. Programmed DNA elimination during early embryo mitoses in *Parascaris* and *Ascaris*
A,B. *P. univalens* embryos. **A.** Two-cell embryo showing one cell with the single pair of germline chromosomes. The big arrows indicate the heterochromatic arms of the chromosomes (H) and the small arrows point to the euchromatic regions of the genome (Eu).
B. Somatic cell undergoing programmed DNA elimination from a two-cell embryo. The retained portions of the germline chromosomes (Eu) are fragmented into $\sim 2n = 70$ chromosomes. The heterochromatic arms that will be eliminated (H, big arrows) remain visible. Adapted from Muller & Tobler [5]. **C,D.** *Ascaris* embryos. **C.** Four-cell embryo with two cells undergoing DNA elimination. **D.** Six-cell embryo with one cell undergoing

chromatin diminution. Note that DNA to be eliminated is present as fragments (artificially colored in red) between chromosomes segregating in early anaphase (**C**); DNA fragments (red) derived from a previous cell diminution can be seen in the cytoplasm of cells to the right (**D**). Adapted from Wang & Davis [1].

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Figure 2. *Ascaris* early embryo development, cell lineage, and DNA elimination

Primordial germ cells (P) are in red, cells undergoing DNA elimination are represented by yellow filled circles surrounded by dots, and blue cells (S) are precursor somatic cells and lineages. The primordial germ cells numbers correspond to their division state. P₀ is the zygote, whereas P₁ through P₄ represent the primordial germ cell derived from each subsequent cleavage of the germ cells as illustrated. S₁–S₄ cells are successive precursor somatic cells derived from each division of a germ cell Adapted from Boveri [50,51] and Muller & Tobler [4,5].

Figure 3. Model for DNA elimination and mechanism for the loss of chromosomal regions from holocentric chromosomes

A. Model of DNA elimination in *Ascaris* [18]. Following alignment, chromosomes in somatic cells undergo chromosome breaks producing fragments of chromosomes (1). Chromosome fragments that are retained (blue) have centromeric sites for microtubule attachment to facilitate chromosome segregation, whereas chromosomal fragments that will be eliminated (red) remain at the metaphase plate, are not segregated, and are lost (2). **B.** Monocentric chromosomes have a single centromere (blue box) where spindle microtubule attachment occurs. Fragmentation of a monocentric chromosome would lead to a loss of acentric chromosomal regions during chromosome segregation. **C.** Holocentric chromosomes are generally thought to have multiple centromeric regions distributed along the chromosome length that serve as sites for microtubule attachment. This distribution of microtubule attachment sites would predict no loss of chromosomal fragments following chromosome breakage during DNA elimination. **D.** CENP-A is reduced in genomic regions that remain at the metaphase plate and will be lost during *Ascaris* DNA elimination. Immunohistochemical staining of CENP-A on a 4-cell *Ascaris* embryo with two cells undergoing DNA elimination mitoses (anaphase) indicates the DNA to be eliminated (red arrows) has much less CENP-A than the DNA that will be segregated and retained. **E.** CENP-A and centromeres/kinetochores in germline *Ascaris* chromosomes are distributed along the length of the chromosomes. During development, CENP-A deposition is lost on regions of chromosomes that will be lost during DNA elimination. Thus, dynamic CENP-A deposition defines and regulates which portions of chromosomes will be lost and retained during DNA elimination.

Figure 4. DNA elimination in *Strongyloides* spp

Chromosomal configuration in females (left) and males (right) in *S. ratti* (top) and *S. papillosus* (bottom). Chromosomes and chromosomal regions present in two copies in both sexes are in blue; chromosomes and regions present in two copies in females but only one copy in males are in red. Note in *S. ratti*, one whole chromosome is lost in males, while in *S. papillosus*, only part of a chromosome is lost during programmed DNA elimination.

Summary Table

The Who, What, When, How, and Why of DNA elimination in nematodes.

	Who	What	When	How	Why
Ascarids	All somatic precursor cells in all individuals eliminate DNA	Multiple terminal and internal fragments of many chromosomes are lost Repetitive sequences and germline specific genes are lost All copies are lost	Elimination during early embryonic cleavage divisions	Chromosomes break at defined positions Eliminated regions are lost due to lack of kinetochore activity from loss of CENP-A deposition Mechanism for sequence recognition and double-strand breaks unknown	Elimination of repetitive sequences Silence a subset of germline expressed genes
<i>S. ratti</i>	Elimination occurs only in oocytes giving rise to males	Entire X chromosome is lost Only one copy of X is lost	Elimination during mitotic oocyte maturation division	Unknown	Male sex determination in response to environmental stimulus
<i>S. papillosus</i>	Elimination occurs only in oocytes giving rise to males	One internal portion of a chromosome is lost Only one copy of these sequences is lost	Elimination during mitotic oocyte maturation division	Eliminated regions lack kinetochore activity Mechanism for sequence recognition and double-strand breaks unknown	Male sex determination in response to environmental stimulus