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Programmed DNA Elimination in Multicellular Organisms

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Abstract

Genetic information typically remains constant in all cells throughout the life cycle of most organisms. However, there are exceptions where DNA elimination is an integral, developmental program for some organisms, associated with generating distinct germline vs. somatic genomes. Programmed DNA elimination occurs in unicellular ciliates and diverse metazoa ranging from nematodes to vertebrates. DNA elimination can occur through chromosome breakage and selective loss of chromosome regions or the elimination of individual chromosomes. Recent studies provide compelling evidence that DNA elimination is a novel form of gene silencing, dosage compensation, and sex determination. Further identification of the eliminated sequences, genome changes, and in depth characterization of this phenomenon in diverse metazoan is needed to shed new light on the functions and mechanisms of this regulated process.

Introduction

In multicellular organisms, germ cells maintain the genetic information and ensure its integrity for the next generation, while somatic cells undergo differentiation and specialization. The genetic makeup of the germline and somatic cells is typically the same throughout the organism's life cycle. However, there are exceptions to the general genome constancy observed in most organisms. During the development of some organisms, major genome changes can occur in various cell types [1,2]. One well-known example is the recombination events in the vertebrate immune system that generates diversity in antibodies and receptors in B and T cells, respectively [3]. Another major developmental genome change is programmed DNA elimination where specific DNA sequences, up to ~90% of the genome in some cases, are eliminated from somatic lineages. Since its discovery in 1887 [4], programmed DNA elimination in animals has been the subject of much interest and speculation [5–7]. The best-studied examples of programmed DNA elimination in eukaryotes are those present in the single-cell ciliates (see recent reviews [8–10]). Recently, high-throughput sequencing has been used in multicellular organisms to comprehensively

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examine genome changes that occur during programmed DNA elimination. Here, we review the broad range of organisms that demonstrate this phenomenon, and what is known regarding the function(s) and molecular mechanism(s) of programmed DNA elimination in metazoa.

Distribution and identification of programmed DNA elimination

Programmed DNA elimination has been described in single-cell ciliates and a diversity of multicellular animals including more than 100 species from nine major taxonomic groups (Fig. 1 and Table 1). In most cases, programmed DNA elimination is associated with either differentiation of somatic cells or sex determination [1,6]. Two types of programmed DNA elimination, chromatin diminution and chromosome elimination, have been described (see Table 1). In chromatin diminution, chromosomes break and regions of the chromosomes are lost. Diminution occurs in ciliates and some parasitic nematodes, copepods, spotted ratfish, hagfish, and lampreys. In chromosome elimination, entire chromosomes are lost. This elimination occurs in some nematodes, insects, mites, finches, and bandicoots, as well as in some hagfish [11]. Given its wide phylogenetic distribution, programmed DNA elimination likely has arisen independently in these different lineages [6]. Outstanding questions remain including what the selective pressure for this process is, whether this pressure is the same in different organisms, and whether elimination serves the same function in diverse organisms?

Programmed DNA elimination typically has been identified through careful cytological studies of chromosome behavior during development. Theodor Boveri first discovered the diminution process by studying the chromosome segregation behavior in the horse parasitic nematode, *Parascaris univalens* [4]. Boveri's analysis contributed to the establishment of chromosome theory of heredity and the first nematode cell lineages [12,13]. The single, large germline chromosome pair, a large increase in somatic chromosome number, and elimination of over 85% of the germline genome in somatic cells enabled Boveri to readily observe and describe chromatin diminution (Fig. 2). Soon thereafter, DNA elimination was described in several other nematodes including the related nematode *Ascaris suum* in 1895 (see Fig. 2), and then in insects and other organisms (Fig. 1 and Table 1, see review [6]). In the most recent discovery of chromatin diminution, Smith et al. followed a repetitive germline-specific DNA marker, germ1, in the germline and somatic tissue of lamprey to find that germ1 is eliminated in somatic tissues [14].

The historical identification of DNA elimination using cytological methods has been serendipitous, and only large-scale genome changes are likely to be discovered by these approaches. The current broad use of high-throughput sequencing in diverse organisms, such as the Genome 10K Project [15], and single-cell sequencing may lead to the identification of additional examples of DNA elimination. These studies will likely contribute to our understanding of the breadth and frequency of DNA elimination in different metazoa, as well as whether genome differences might be present within different cells in mammals.

Identification of eliminated sequences provides insights into the function of DNA elimination

A key to understanding DNA elimination is defining the organization of chromosomes and their eliminated sequences. Early studies using DNA reassociation kinetics demonstrated that significant amounts of repetitive DNA were eliminated from the parasitic nematode *A. suum*; subsequent studies demonstrated that the major eliminated repeat was a 121 bp tandemly repeated satellite [16]. Later, seminal studies demonstrated that some transposon elements [17] and three single-copy genes were eliminated in *A. suum* [18–21]. Furthermore, by comparing the genomic sequences around chromosomal breakage regions, Muller et al. demonstrated that new telomeres were added at the DNA breaks and several break sites were conserved between the nematodes *P. univalens* and *A. suum* [22,23].

More recently, a comprehensive genomic approach was used to compare the genome differences between the germline (spermatids) and somatic cells (intestine) of a single male *A. suum* [24]. Wang et al. sequenced, *de novo* assembled, and compared the germline and somatic genomes from a male *A. suum* and found that ~43 Mb (~13%) of DNA was eliminated from the intestinal genome. Seventy percent of the eliminated DNA was repetitive sequences consisting predominantly of the previously described 121 bp tandem repeat. Surprisingly, the other eliminated sequences (~12.7 Mb) were single-copy sequences corresponding to ~700 protein-coding genes that are exclusively expressed in the germline and early embryos. A major group of the eliminated genes is associated with translation, demonstrating that the translation machinery may be very different between the germline and soma, supporting and extending earlier observations made by Muller et al. [18,19]. Notably, ~50 eliminated genes are orthologs to well-characterized genes in *C. elegans* whose loss is associated with clear phenotypes in germline formation, gametogenesis, and early embryogenesis. This large-scale elimination of germline genes suggests that DNA elimination may be an extreme and permanent mechanism for germline gene regulation in *A. suum*, deleting rather than repressing their expression in somatic cells. Wang et al. also identified ~50 breakpoints where chromosome regions were lost and telomere addition occurred on the retained chromosomes in the somatic cells, but no DNA fusions or rearrangements were observed. This genomic study significantly extends our understanding of the eliminated sequences and DNA breakpoints in *A. suum*, however, the current genome assemblies do not enable large-scale characterization of changes at the chromosomal level. Improved genome assemblies and additional studies are now needed to provide an overall view of the organization of the chromosomes and their alterations during diminution. High-throughput analysis of chromatin diminution in the related nematode *P. univalens* demonstrated that many of the break sites are conserved between the two nematodes as previously suggested [23], and also indicates that the genes eliminated are similar to those observed in *A. suum* (Wang, J. and Davis, R.E., unpublished data). This further supports the idea that diminution is a highly regulated and conserved process in these related parasitic nematodes.

Recent studies identified chromatin diminution in the sea lamprey and also demonstrated the elimination of both repetitive and single-copy sequences. Smith et al. used flow cytometry to

measure the DNA content in lamprey testes and blood and found that ~20% (~500 Mb) of the lamprey germline genome is eliminated in somatic cells [14]. Further comparisons between sperm and liver DNA using array comparative genomic hybridization and genome survey sequences indicated that the eliminated DNA consists not only of repetitive sequences, but strikingly, also a few thousand genes [25]. The eliminated genes include homologs of vertebrate genes that function in either the development or maintenance of the germline. Given that a large number of germline-associated genes are eliminated in these divergent organisms, nematodes and lampreys, this suggests a possible common function of chromatin diminution. It will be interesting to see if loss of single-copy germline-associated genes is a common feature within other metazoa that undergo diminution such as copepods, where ribosomal RNA gene copy number can be regulated by diminution [26].

Although DNA elimination events are often associated with germ-soma differentiation, others are associated with sex determination. In sciarid flies, the elimination of one or two paternal X chromosomes in the pre-somatic cells determines the sex of the embryo (see reviews [27,28]). In a recent study on chromatin diminution in the parasitic nematode of sheep, *Strongyloides papillosus* [29], Nemetschke et al. used genetic crosses to determine that one of the two copies of a whole section of a chromosome undergoes DNA elimination by chromatin diminution. The region eliminated corresponds to a sex chromosome that is entirely eliminated in the closely related parasitic nematode of rats, *S. ratti*. This demonstrated that diminution provides a means to restore the sex chromosome ratio in males, and thus functions in the sex-determination system in this organism. Additional analyses suggest that chromatin diminution in *S. papillosus* is a derived state in *Strongyloides* species, evolved as a consequence of an X chromosome and autosome fusion that requires chromatin diminution to generate *S. papillosus* males [30].

A common theme in organisms that exhibit DNA elimination is the elimination of large amounts of repetitive sequences (see Table 2). In chromatin diminution, the eliminated repeats are typically tandem repeats that vary from 2 – 172 bp. Recent observations in zebra finch show that repetitive sequences are eliminated during chromosome elimination [31]. The conserved elimination of repetitive sequences in somatic cells raises the key question: why is it that the eliminated sequences are primarily repetitive? Clearly, repetitive sequences play key roles in genome evolution, recombination, and meiosis. They may also play additional roles in germline development and maintenance. A recent study in copepods suggests chromatin diminution in somatic cells may be necessary to reduce the ongoing repeat expansion and load in the germline [32]. A difficult but important goal will be to determine the location and organization of the repeats on chromosomes undergoing diminution. Such studies might provide important insights into the function of simple repeats in the germline, as well as perhaps their potential role in contributing to the process of diminution.

A variety of theories/hypotheses have been proposed to explain the biological significance of programmed DNA elimination [1,6,7,27,33–35] including mechanisms for 1) gene silencing, 2) dosage compensation, 3) sex determination, 4) position-effects for gene expression, 5) germline development and meiosis, and 6) germline and soma differentiation. The recent studies on *Ascaris* and the lamprey, where significant numbers of germline and

early embryonic genes are eliminated and thus silenced in the somatic cells, provides strong support for a role in gene silencing. Recent studies also suggest it is a mechanism for dosage compensation in *Ascaris*, where many eliminated genes have undergone duplication, and sex determination in *S. papillosus* [29], flies [27], and birds [36]. The association of DNA elimination with germ-soma differentiation also poses the interesting question of whether somatic DNA elimination contributes to the differentiation of specific cell lineages. Wang et al. [24] compared DNA elimination in different cell lineages in *A. suum*, that exhibit deterministic cleavage similar to that observed in *C. elegans*, and found that the overall genomic content and the breakpoints are the same in all five precursor somatic cells undergoing diminution. In the sea lamprey, flow cytometry data indicate there might be subtle variation in the somatic genome size in different cells, although all markers assayed thus far exhibit uniform loss across different somatic tissues [14,25]. Thus, while current data suggest that the sequences lost from diminution are overall the same in all cells, it remains to be determined whether variations in diminution or the resulting chromosomal position effects might have functional significance that contributes to the differentiation of various cell lineages.

Molecular mechanisms of DNA elimination

Early mechanistic studies in *Parascaris* and *Ascaris* focused on the role of cytoplasmic determinants and the germ plasm in diminution (see reviews [1,5,6]). Using a variety of methods including doubly fertilized eggs, centrifugation, ultraviolet irradiation, and chemical induction [37], these studies suggested that cytoplasmic factors play a key role in chromatin diminution and may be segregated between the germline and soma. No specific factors have yet been identified that contribute to diminution [38–40]. Studies in ciliates have shown that small RNAs (piRNAs) and domesticated transposons are involved in programmed DNA rearrangement and elimination [10]. The piRNAs target sequences for retention or elimination in different types of ciliates [8,41] whereas the transposons lead to DNA breaks [42]. A recent study used high-throughput sequencing to examine total small RNA profiles during *A. suum* diminution; however, no correlation between small RNAs and diminution was observed [24,43]. Additional studies are required to determine whether specific Argonaute proteins and small RNAs contribute to DNA elimination in metazoa.

How cells define the breakpoints and what cellular machinery acts on them is likely to provide important insights into the mechanism of chromatin diminution. The sites for chromosomal breakage are conserved in each generation in parasitic nematodes and the sea lamprey. In the sea lamprey, distinct short palindromic sequences at three independent breakpoint regions were observed, suggesting site-specific recombination might facilitate DNA elimination [25]. Analysis of the 50 breakpoints identified in *Ascaris* demonstrated high fidelity of the break sites at the chromosomal level. However, the break sites can be heterogeneous (ranging over 200–2000 bp at a site) [22,24], and no conserved sequence motifs or other characteristics were identified 5 kb on either side of the DNA breakpoint regions [24]. It also remains to be determined whether DNA destined for elimination or retention in *Ascaris* and lampreys undergoes large-scale chromatin reorganization that could be involved in the mechanisms of diminution. In the sea lamprey, recent observations identified extra-nuclear aggregations of repressive chromatin (Herdy, J.R. III and Smith, J.J.,

personal communication), similar to those observed during elimination in ciliates and finch [44–46], suggesting an interrelationship between epigenetic silencing and loss. Studies on chromosome elimination in insects and finches indicate that a number of epigenetic modifications are associated with elimination of chromosomes including changes in histone H3/H4 acetylation, H3S10 phosphorylation, and DNA methylation ([44,46–50] and see recent review [28]).

A key question in DNA elimination is how chromosomes or portions of chromosomes are selectively lost and thus not segregated during cell division (Fig. 2). Loss or alterations in centromeres, kinetochore assembly, microtubule attachment, or chromosome segregation could lead to DNA elimination. Studies on chromosome elimination in insects suggest that chromosome loss is most likely a function of a segregation defect in the metaphase/anaphase transition [48,51]. In sciarid flies, reduction in the dephosphorylation of H3S10P is associated with a failure or retardation in sister chromatid separation [48]. In contrast, chromosome elimination in finches may be associated with a defect in kinetochore–microtubule interactions [47]. In chromatin diminution, once DNA breaks occur in monocentric chromosomes, regions that retain the centromeres would likely be properly segregated, whereas those regions that lack them would not and thus be eliminated. Genomic regions without centromeres could also fuse with other chromosome regions that retain their centromeres and thus be faithfully segregated as observed in copepods [52]. Nematodes such as *Ascaris* and *Parascaris* have holocentric chromosomes; kinetochore activity and microtubule attachment sites extend along the length of holocentric chromosomes. The location of centromeres is typically constant on most chromosomes. However, recent data in *C. elegans* suggest that centromere deposition can be dynamic [53]. In addition, unpublished studies in *Ascaris* indicate that the centromeric histone H3 variant Cenp-A marks chromosomes that will be retained, but is greatly reduced or absent on chromosomes that will be lost in diminution mitoses (Wang, J. and Davis, R.E., unpublished data). This is consistent with data from *Parascaris* that a kinetochore plate is absent in chromosome regions that will be lost [54] and suggests that centromere deposition may play an important role in determining chromosomal regions that will be retained or lost.

Perspective

Programmed DNA elimination occurs in ciliates and diverse multicellular organisms. Recently, chromatin diminution was described in the sea lamprey, a jawless vertebrate, extending the distribution of diminution into vertebrate lineages. New findings indicate that in addition to the loss of repetitive sequences, many protein-coding genes are lost in chromatin diminution, suggesting that diminution serves as a mechanism for gene regulation and silencing. Programmed DNA elimination is a complex biological process that requires the identification of sequences to be eliminated and a mechanism for their elimination. Additional studies are needed to define the mechanism(s) for selective loss of chromosomes or chromosome regions, breakage of chromosomes, and chromatin organizational changes associated with DNA elimination. Analysis of DNA elimination in different systems is likely to give new insight into the permanent gene silencing, the genome dynamics, the evolution of genomes, the role of repetitive sequences, and perhaps also information on genome alterations in cancer and other diseases.

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Box

Outstanding questions in programmed DNA elimination

- Does chromatin diminution serve the same function in diverse organisms?
- How are the sites for DNA breaks in chromatin diminution identified, made, and processed? Is this process the same in the divergent organisms that undergo diminution?
- What are the molecular mechanisms that alter normal chromosome segregation leading to the elimination of portions of chromosomes or whole chromosomes?
- Does retained versus eliminated DNA undergo specific chromatin and chromosomal organization changes that contribute to DNA retention or elimination?
- Why are the majority of the sequences eliminated in chromatin diminution repetitive? What is the function of eliminating germline repetitive sequences in somatic cells? Do repetitive sequences contribute to the elimination process?
- Does chromatin diminution contribute to cell lineage determination or differentiation?
- Do small RNAs play a role in programmed DNA elimination as observed in ciliates?
- Does programmed DNA elimination contribute to some genomic mosaicism in vertebrates?
- Are processes associated with DNA elimination involved in pathological conditions such as cancer, disease, or other developmental abnormalities?

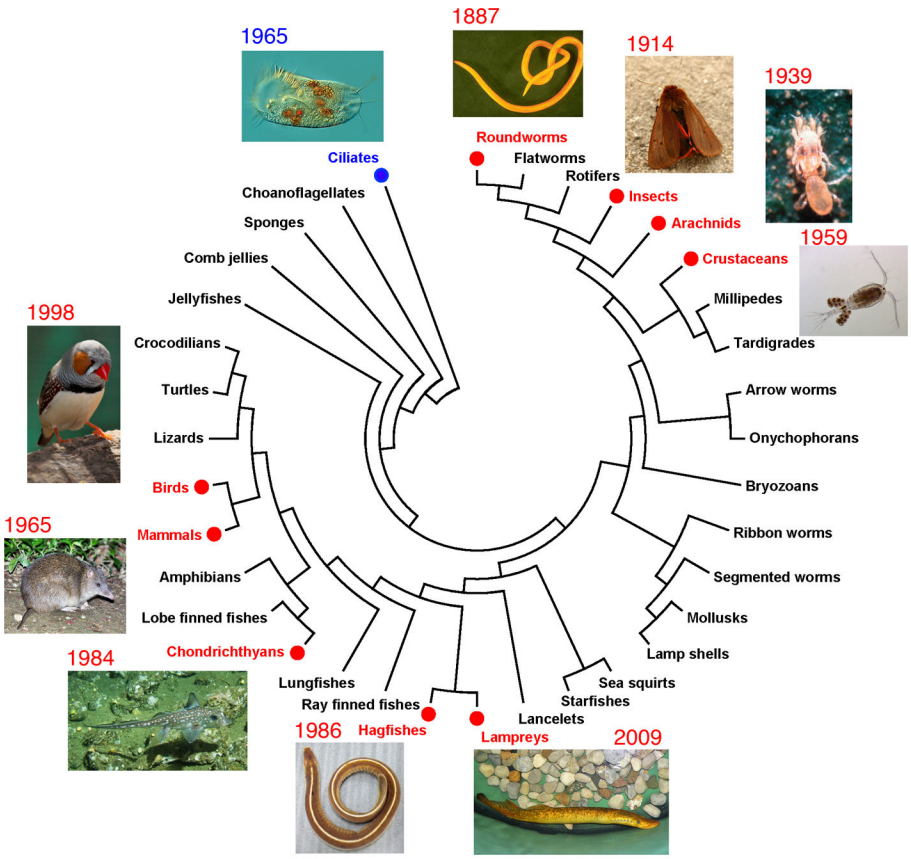

Fig. 1. Programmed DNA elimination in multicellular organisms

Organisms known to undergo DNA elimination are illustrated on a phylogenetic tree. The tree was constructed from 18S ribosomal RNA sequences using MEGA (v5.22) [55]. Common names are used for the groups. The tree is rooted on ciliates. Photo credits: Antonio Guillen from Water Project, Spain (ciliate *S. mytilus*), Colin Johnstone (nematode *P. univalens*), Entomart (moth *P. fuliginosa*), wiley library (mite *M. occidentalis*), James Haney (copepod *M. edax*), Jeremiah Smith (Sea lamprey *P. marinus*), Kinya G. Ota and Shigeru Kuratani (hagfish *E. burgeri*), wikipedia.org (Spotted ratfish *H. colliei* and Zebra finch *T. guttata*), and Joseph McKenna (bandicoot *I. macrourus*). The year that DNA elimination was discovered in each group of organisms is noted.

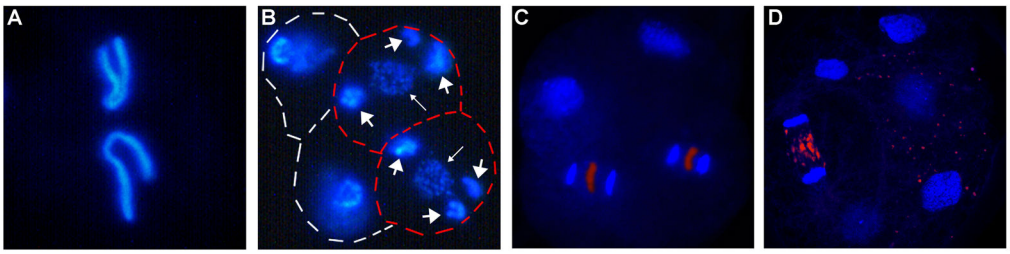

Fig. 2. Chromatin diminution in *Parascaris* and *Ascaris*

A and B. *P. univalens* embryos. **A.** 1-cell embryo showing the single pair of germline chromosomes. **B.** 4-cell embryo with two cells (outlined in red) undergoing diminution. The retained portions of the germline chromosomes are fragmented into many smaller chromosomes (small arrows). The heterochromatic arms that will be eliminated (big arrows) remain visible. **C and D**. *A. suum* embryos. **C.** 4-cell embryo with two cells undergoing chromatin diminution. **D.** 6-cell embryo with one cell undergoing chromatin diminution. Note that DNA to be eliminated is present as fragments (artificially colored 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**).

Table 1

Organisms with programmed DNA elimination.

Organism	First discovered (Year; Organism)	Common name	Other representative organisms	Known species*	D/E ***	References
Nematodes	1887; *Parascaris univalens*	Roundworm	*Ascaris suum; Strongyloides papillosus*	11	D/E	[4,35]
Insects	1914; *Phragmatobia fuliginosa*	Moth	*Sciara ocellaris; S. coprophila*	65	E	[27,56]
Arachnids	1939; *Pediculopis graminum*	Grass mite	*Metaseiulus occidentalis*	2	E	[57,58]
Crustaceans	1959; *Cyclops strenuus*	Copepod	*Cyclops kolensis; Mesocyclops edax*	17	D	[59,60]
Ciliates	1965; *Stylonychia mytilus*	Ciliate	*Tetrahymena thermophila; Oxytricha trifallax; Paramecium tetraurelia*	4,500**	D	[8,61]
Mammals	1965; *Isoodon macrourus*	Bandicoot	*Perameles nasuta*	10	E	[62,63]
Chondrichthyans	1984; *Hydrolagus colliei*	Spotted ratfish	*Chimaera monstrosa*	4	D	[64]
Hagfishes	1986; *Eptatretus burgeri*	Inshore hagfish	*Myxine glutinosa*	10	D/E	[11,65]
Birds	1998; *Taeniopygia guttata*	Zebra finch	*Lonchura domestica*	2	E	[36,46]
Lampreys	2009; *Petromyzon marinus*	Sea lamprey	-	1	D	[14]

* Minimum number of known species

** Ciliates exhibit nuclear dimorphism

*** D: Chromatin Diminution; E: Chromosome Elimination

Table 2

DNA elimination removes primarily repetitive sequences.

Organism	% Genome eliminated	% Repeat in eliminated sequence	Eliminated repetitive sequence	References
Nematode				
Ascaris suum	13	70	121 bp tandem repeats	[16,24]
Parascaris univalens	88	98	5- and 10-bp tandem repeats	[66,67]
Lamprey and hagfish				
Petromyzon marinus	20	~35% are Germ1	Germ1, 200bp tandem repeats, others.	[14,25,68]
Eptatretus cirrhatus	35	Majority*	4 tandem repeats, from 54 to 172 bp	[69]
Copepod				
Cyclops kolensis	94	Majority*	Tandem repeat with 10–30 bp motifs	[70]
Mesocyclops edax	90	Majority*	2-, 8-, or 9-bp tandem repeats and other	[32,71,72]

*All known sequences are repeats