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# Natural genetic engineering: a programmed chromosome/DNA elimination

Malgorzata Kloc<sup>\*1,2,3</sup>, Jacek Z Kubiak<sup>4,5</sup>, and Rafik M Ghobrial<sup>1,2</sup>

<sup>1</sup>The Houston Methodist Research Institute, Houston, TX, USA; <sup>2</sup>The Houston Methodist Hospital, Department of Surgery, Houston, TX, USA; <sup>3</sup>The University of Texas, MD Anderson Cancer Center, Department of Genetics Houston, TX, USA; <sup>4</sup>Laboratory of Molecular Oncology and Innovative Therapies, Department of Oncology, Military Institute of Medicine, 04-141 Warsaw, Poland; <sup>5</sup>Univ. Rennes, UMR 6290, CNRS, Institute of Genetics and Development of Rennes, Dynamics and Mechanics of Epithelia group, Faculty of Medicine, 35000 Rennes, France

Corresponding author: Malgorzata Kloc  
[mkloc@houstonmethodist.org](mailto:mkloc@houstonmethodist.org)

## Abstract

Typically, all cells of a given organism have the same set of chromosomes. However, there are exceptions to this rule, and in many organisms, the somatic cells and germ cells, various types of somatic cells or organs, or females and males, have different genomes. One of the sources of such differences is chromosome/DNA elimination/chromatin diminution that is a naturally programmed phenomenon. We describe chromosome/DNA elimination in various organisms and present the current hypotheses on its origin, mechanisms, significance, and consequences.

**Keywords:** Programmed DNA elimination; chromosome elimination, diminution, gene silencing, repetitive sequences, B chromosomes, super-Mendelian inheritance, chromosome drive.

## Introduction

The phenomenon of chromosome elimination (diminution) was discovered in 1887 by Theodor Boveri in a parasitic roundworm *Ascaris megalocephala* (*Parascaris equorum*) (Boveri, 1887). Over the years, chromosome/DNA elimination has been described in unicellular and multicellular (both invertebrate and vertebrate) organisms. At present, more than 100 species from different taxonomic groups, and 10% of all vertebrates, are known to undergo chromosome/DNA elimination, also called a programmed genome rearrangement (PGR) (Smith et al., 2021; Wang and Davis, 2014; Dedukh and Krasikova, 2021; Zagoskin and Wang, 2021). There are two main types of chromosome elimination. One creates the difference between the germline and soma, and another is related to sex determination and sex chromosome dosage compensation. Another category is the curious behavior (elimination or accumulation) of “parasitic” B chromosomes.

### 1. Elimination from the somatic cell line

One of the most common is a developmentally programmed elimination of chromosomes (E chromosomes) from the presumptive somatic cells. In contrast, only the presumptive germ cells retain the whole genome containing the somatic (S) and eliminated (E) chromosomes. In consequence, these organisms become chimeric. Another example of programmed elimination is the chromosome/DNA elimination from the somatic nucleus (macronucleus) during the post-conjugation development in *Ciliata* (Clay et al., 2019). Chromosome elimination is also common in plants where, during seedling development, the somatic cells of some organs undergo chromosome elimination.

### 2. Elimination in sex determination

In some organisms, the sex determination may occur through paternal genome elimination (PGE, parahaploidy, pseudoarrhenotoky; (Herrick and Seger 1999; Nelson-Rees, 1980; Sabelis and Nagelkerke, 1988). For example, in many species of insects, such as the booklice (*Psocodea*) species *Liposcelis*, the embryos destined to be males lose one paternal chromosome set and become haploid, while the females remain diploid (Beukeboom, 2017; Hodson et al., 2017). Another example is the sex chromosome dosage compensation, which occurs through sex chromosome elimination in some organisms.

### 3. Elimination or accumulation of B chromosomes

Around 15% of all eukaryotes and some plants contain two sets of chromosomes; a regular set of chromosomes (A chromosomes) and a supernumerary chromosome set (B chromosomes) (Jones

N and Houben A. B chromosomes in plants: escapees from the A chromosome genome? Trends in Plant Science, 8: 417-423, 2003; ISSN 1360-1385, [https://doi.org/10.1016/S1360-1385\(03\)00187-0](https://doi.org/10.1016/S1360-1385(03)00187-0); Camacho JPM, Chapter 4 - B Chromosomes, Editor(s): T. Ryan Gregory, The Evolution of the Genome, Academic Press, 2005, p. 223-286, ISBN 9780123014634, <https://doi.org/10.1016/B978-012301463-4/50006-1>; <https://phys.org/news/2019-06-chromosome-firstmechanisms-chromosomes-uncovered.html>). The B chromosomes are non-essential and regarded as genomic parasites. Although they are lost from some cells/organs, they can also transmit themselves in a super-frequency non-Mendelian fashion, called “the B chromosome drive” (Dalla Benetta et al., 2019; Ruban et al., 2020).

In general, chromosome/DNA elimination may involve entire chromosome sets, individual chromosomes, or fragments of chromosomes (sometimes referred to as chromatin diminution) and bestows rearrangement on the genome. Among many theories on the significance of chromosome/DNA elimination, one commonly accepted is that it represents gene silencing/inactivation of the redundant part of the genome. In the upcoming paragraphs, we describe the examples of chromosome/DNA elimination in some of unicellular and multicellular organisms and discuss the existing hypotheses on the roles of these phenomena.

## 1. Ciliates

Ciliates have dimorphic nuclei, which embody a separation of soma and germline in a single cell. Macronucleus (MAC), typically polyploid, contains a somatic genome transcribed/translated during vegetative growth. Micronucleus (MIC), an equivalent of the germline and typically diploid, has a transcriptionally silent genome that is inherited by the progeny. In *Oxytricha trifallax*, MAC contains ~ 100 chromosomes, and MIC has ~ 16,000 nanochromosomes. After sexual reproduction, MAC develops from a copy of MIC by eliminating over 90% of the MIC’s DNA (internal eliminated sequences (IESs) and rearranging, after de novo telomere addition, a remaining DNA into the nanochromosomes (Chalker and Yao, 2011; Chen et al. 2014; Clay et al., 2019). The IESs contain both coding and repetitive sequences, including transposons. Studies on *Paramecium* and *Tetrahymena* showed that the eliminated DNA is tagged for elimination by scan RNAs (scnRNAs), (Fig.1). The scnRNAs are small RNAs synthesized from the MIC genome. First, the scnRNAs derived from the MIC translocate to the old MAC, and the scnRNAs homologous to any old MAC sequences are removed. Next, the remaining scnRNAs (homologous to MIC-specific sequences) are

transported to the developing MAC where they recognize homologous sequences tagging them for elimination (Chalker and Yao, 2011; Clay et al., 2019; Feng and Guang, 2013; Mochizuki, 2020; Mochizuki et al., 2002; Mochizuki and Gorovsky, 2004; Schoeberl et al., 2012). In *Tetrahymena* the Dicer-like enzyme DCI1p processes the MIC transcripts into the double stranded scnRNAs (Mochizuki et al., 2002; Mochizuki and Gorovsky, 2004). Subsequently, the endoribonuclease (Slicer) activity of the Argonaute protein Twi1p removes one strand of ds scnRNA. Then, the Twi1p-scn RNA complexes scan and heterochromatize DNA fragments tagging them for elimination (Fig. 1), (Noto et al., 2010). The excision of the eliminated sequences from the developing MIC chromosomes is carried out by the transposase enzymes, either translated from the transposons or from the “domesticated” transposon that was integrated, as an individual gene, into the genome (Chalker and Yao, 2011). In *Tetrahymena*, the IESs are assembled into membrane-less heterochromatin bodies, which will be eliminated from the macronucleus. Kataoka and Mochizuki (2017) showed that the prion-like RNA-binding protein Jub6p, a component of heterochromatin, is essential for the formation of heterochromatin bodies (Fig. 1). In *Paramecium tetraurelia* the IES elimination depends on Nowa1p and Nowa2p, which are similar to the RNA-binding prion protein Prp27 (Gabus et al., 2001; Kataoka and Mochizuki, 2017; Nowacki et al., 2005). This indicates that the prion-like RNA-binding proteins may be a common component of chromatin elimination machinery in ciliates.

## 2. Nematodes

Elimination of chromosome fragments (chromatin diminution) from the prospective somatic cells occurs in the development of many species of parasitic nematodes (order *Ascaridida*, the superfamilies *Ascaridoidea*, and *Cosmocercioidea*). In contrast, the free-living nematodes, such as *Caenorhabditis elegans*, do not eliminate DNA. The exceptions are nematodes from the genus *Strongyloides spp.* These nematodes, which besides the parasitic forms, also have facultative free-living males and females, use the elimination of entire chromosomes or chromosome fragments for sex determination (Mochizuki, 2020; Müller et al., 1996; Nemetschke et al., 2010; Streit et al., 2016; Gonzalez de la Rosa et al., 2021). Comparison of eliminated DNA between *Ascaris suum*, *Parascaris univalens*, *Ascaris lumbricoides*, and *Toxocara canis* showed that they eliminate 5%–10% of the germline expressed genes and different percentages of repetitive sequences. Thirty-five percent of the eliminated germline-expressed genes are evolutionary conserved among all studied nematodes. These genes encode proteins that have defined functions: bind ribonucleotides, 3' UTR of RNA, have kinase activity, or participate in the Wnt signaling. They are probably the components of synaptonemal complexes or germ cell

determinants such as germinal granules and germplasm. Some of these conserved genes are specifically expressed in the male germline, which suggests that one of the roles of DNA elimination is to remove genes involved in the development and functions of male germ cells (Wang et al., 2017).

Cytologic aspects of chromosome elimination were especially well studied in *Parascaris univalens* with only two chromosomes (Müller et al., 1996). After fertilization, during the first cleavage of the zygote (P0), these chromosomes segregate equally to two, P1 and S1, daughter cells (Fig. 2). During the second division, the P1 cell also equally distributes chromosomes to its P2 and S2 progeny. However, the dividing S1 cell breaks the central portion of two chromosomes into multiple small chromosomes and the heterochromatic ends. The small chromosomes are inherited by the S1a and S1b progeny, while the heterochromatic termini degrade in the cytoplasm. The elimination process is repeated during the next four rounds of cleavage divisions. Eventually, all cells with reduced chromatin (S1-S5 descendants) become pre-somatic cells, and the P5 becomes the primordial germ cell (Fig. 2), (Müller et al., 1996). Although other parasitic nematodes differ in the timing and details of chromatin diminution, the ultimate outcome remains the same, i.e., the somatic lineage has a reduced amount of chromatin, especially heterochromatin. For example, in *Ascaris suum*, chromatin elimination starts from the third cleavage. The 24 germline chromosomes split into 36 somatic chromosomes, and some internal and end fragments are eliminated (Müller et al., 1996; Tobler, 1986). Wang et al. (2020) mapped all 72 chromosome breaks in *Ascaris suum* (Fig. 3). They found that breaks occur within 3-6kb long chromosomal break regions (CBRs). Because the CBRs do not show any consensus sequences, it is unknown if the CBRs or the unidentified sequences in their vicinity induce breaks. It is also unknown what enzymes catalyze these double-stranded DNA breaks. The 48 breaks remove all telomeres and some genes in their vicinity. The removal of all germline telomeres and the novo formation of somatic telomeres suggest that replacing of germline telomeres with the somatic ones must be of great importance for the functioning of somatic cells. It is also unknown what (if any) is the importance of the removed genes located in the vicinity of the telomeres. Another 24 breaks eliminate 12 internal regions of the chromosomes, which account for 18% of the germline genome, and split the 24 germline chromosomes into the 36 somatic chromosomes (Fig. 3). Eventually, this process removes 1,000 genes, and all 120-bp satellite repeats. Because the eliminated chromosome fragments lose their kinetochores (we still do not know why and how) they cannot interact with the mitotic spindle and segregate during cell division. Eventually, they become surrounded by a double membrane into the micronuclei,

and undergo autophagocytosis (Mohizuki, 2020; Wang et al., 2020;). After the comparison of the isoform number of specific genes (for example, rpS19 and aleg-3) between parasitic (they have two isoforms of each gene) and free-living (they have one isoform) nematodes, Müller et al. (1996) concluded that chromatin diminution in parasitic nematodes eliminates one copy of those genes, which had been duplicated in their ancestors. However, more recent comparative genome analysis of programmed DNA elimination in nematodes suggests that eliminated genes are not necessarily duplicated in the parasitic nematodes (Wang et al. 2017).

In the nematode *Strongyloides papillosus*, which switches the life cycle between parasitic and free-living, chromatin elimination (diminution) plays a role in sex determination (Nemetschke et al., 2010). Females of this worm, who live in the hosts' intestines, parthenogenetically produce female and male larval progeny. Female larvae either develop into infective third-stage larvae (iL3) that infect a new host, or together with male larvae, develop into free-living worms that reproduce sexually. While *Strongyloides stercoralis* and *S. ratti* have XX/X0 sex determination pattern, the identity of sex chromosomes in *S. papillosus* remained for many years controversial. Females of *S. ratti* and *S. stercoralis* have two pairs of medium-sized autosomes and two X chromosomes (XX), while males have two pairs of medium-sized autosomes and one X chromosome (X0). In contrast, the *S. papillosus* females have one pair of long chromosomes and one pair of medium-sized chromosomes. The *S. papillosus* males have a single long chromosome, three medium chromosomes, and one short chromosome. Comparative studies of the *S. ratti* and *S. papillosus* chromosomes showed that during *S. papillosus* evolution the X chromosomes fused with the autosomes creating a pair of long chromosomes. The males of *S. papillosus* use sex-specific chromatin diminution to remove an internal fragment of one of the two long chromosomes (Fig. 4). The eliminated segment is homologous to the X chromosome of *S. ratti*, which indicates that the purpose of the elimination process in *S. papillosus* is to restore the X0 genotype in the male (Nemetschke et al., 2010). During spermatogenesis only the intact long chromosome and two original M chromosomes are included into sperm (Fig.4).

### 3. Copepods

Chromatin diminution that eliminates ~ 90% of the genome was described in Copepods already in the 1800ties - early 1900ties (Amma, 1911; Fischer, 1851; Wierzejski, 1892) and, subsequently, thoroughly studied in many copepods including *Cyclops kolensis* (Degtyarev et al., 2004; Grishanin, 1995; Grishanin and Zagoskin, 2018), *Cyclops furcifer* (Beermann and Meyer, 1980), *Mesocyclops edax*, and *Mesocyclops longisetus* (Clower et al., 2016; Drouin, 2006;

McKinnon and Drouin, 2013; Rasch and Wyngaard, 2008; Sun et al., 2014; Zotkevich et al., 2008; Schutt et al., 2021). In *C. kolensis*, chromatin diminution occurs during the 4th and 5th embryonic division. In the 4th division, 6 out of 8 cells eliminate chromatin. One of the remaining two cells eliminates chromatin during the 5th division, while another retains an entire genome (Grishanin and Zagoskin, 2018). All cells with eliminated chromatin are precursors of soma, and the cell with a complete genome is a precursor of the germline. The chromatin elimination does not affect the number of chromosomes in the prospective somatic cells but drastically reduces chromosome length from 11–20µm to 2.6–7µm, resulting in the loss of 94–98% of total DNA (Semeshin et al., 2011; Wyngaard et al., 2011). Studies of chromatin diminution of cyclopoid copepods showed that chromatin diminution during embryogenesis results in a somatic genome that is one half the size of the germline genome (Schutt et al., 2021). This led to the hypothesis that chromatin diminution constrains somatic nuclear DNA content in cyclopoid copepods (Schutt et al., 2021). Eliminated DNA (eDNA) is circular and enclosed within 500–600 DNA granules (per single nucleus) surrounded by the double membrane with temporary pores (Grishanin and Zagoskin, 2018). Subsequently, the granules fuse, pores disappear, and eDNA becomes degraded. The presence of pores in the granule membrane may facilitate the entry of degradation enzymes to the granules. Further studies suggested that eDNA circles derive from the looping, excision, and subsequent re-ligation of the chromosome fragments (Beermann and Meyer, 1980). Studies of chromatin elimination in *Mesocyclops edax* showed eliminated DNA in the form of Feulgen-positive droplets (Clower et al., 2016). Because this study was performed at the light microscopy level, it is unknown if these “DNA droplets” are surrounded by the membrane and how (if) they are related to the eDNA granules described in other copepods. Interestingly, chromatin diminution not only changes the size of chromosomes but affects the interphase nuclei architecture. They become like the heterochromatic nuclei of fully differentiated cells (Grishanin and Zagoskin, 2018). Molecular analysis showed that eDNA contains repetitive, noncoding, AT-rich sequences (Degtyarev et al., 2004). Further studies showed that, during evolution, the copepod genome undergoes, for an unknown reason, explosive replication of repetitive sequences. The fact that only a subset of these sequences is lost indicates that eliminating repetitive sequences is non-random and selective (Grishanin and Zagoskin, 2018; Zotkevich et al., 2008). Eliminated DNA contains evolutionary younger elements with a high frequency of DNA transposons and long interspersed nucleotide elements (LINEs). It seems that chromatin diminution in copepods not only eliminates the overload of the repetitive DNA but also removes rDNA, and protein-coding DNA sequences (McKinnon and



Drouin, 2013; Degtyarev et al., 2004; Zagoskin et al., 2010), which may be unnecessary or harmful for the function of somatic cells.

#### 4. Insects

Chromosome/chromatin elimination is prevalent among many groups of insects. It serves one of the two purposes: the differentiation between germline and soma or sex determination (Jazdowska-Zagrodzinska et al., 1992; Sánchez, 2014). Pioneering studies of chromosome diminution in the gall midges (gall gnats), such as *Miastor metraloas* and *Monathropalpus buxi*, belonging to the lower group of Diptera *Cecidomyiidae*, were initiated in 1935 by Polish cytologist Zygmunt Kraczkiewicz and continued after the World War II, by him (he passed away in 1971) and his research team at the Department of Cytology of Warsaw University in Poland, until early 1990 (Geyer-Duszyńska, 1951, 1961, 1966; Jazdowska-Zagrodzinska et al., 1992; Kraczkiewicz, 1935, 1938; Kraczkiewicz and Matuszewski, 1958; Matuszewski, 1982). The germ cells of gall midges have two sets of chromosomes (S and E). In an early embryo, all presumptive somatic nuclei eliminate the E set while the nuclei of germline cells retain a complete genome. The eliminated chromosomes are delayed in anaphase of mitotic division, and thus, not included in the reconstituted telophase nuclei. The hypothesis is that the nuclei of the presumptive germ cells are protected against elimination through contact with the germplasm (oosome) that produces some (still unidentified) inhibitor of chromosome elimination. Indeed, experimental removal of oosome caused all cells (germ cells and somatic cells) to eliminate the E chromosomes. In contrast, when the oosome was misplaced by centrifugation, all nuclei now in contact with the oosome, retained both chromosome sets.

Another group of insects eliminating chromosomes are fungus gnats (sciarid flies) from the *Sciaridae* (*Bradysia*) family of Diptera (Goday and Esteban, 2001). The zygote of these insects contains three X chromosomes of different origin (one maternal and two paternal) and two sets (maternal and paternal origin) of autosomes (A). Some species of *Sciaridae* also contain three or more L chromosomes. In early development, female embryos eliminate one paternal X chromosome, while male embryos eliminate two paternal X chromosomes from the presumptive somatic cells. In contrast, the presumptive germ cells eliminate one paternal X chromosome in both sexes and all but two L chromosomes (Goday and Esteban, 2001). Another chromosome elimination occurs during male spermatogenesis (Fig. 5). In the first meiotic division, the chromosomes do not pair. There is no distinct metaphase, and division proceeds from prometaphase directly to anaphase. The I meiotic spindle is unipolar. During anaphase I, the

maternal A and X chromosomes (and paternal and maternal L chromosomes) segregate to the spindle pole. In contrast, paternal A and X chromosomes move to the opposite pole, where they are included into the cytoplasmic bud subsequently excised from the primary spermatocyte. During spermatocyte meiosis II the spindle is bipolar. In metaphase, maternal A chromosomes and L chromosomes align in metaphase plate, and X chromosome attaches to the spindle microtubules at one pole. At anaphase, A and L chromatids segregate to opposite poles. The whole (containing unseparated chromatids) X chromosome segregates to one of the poles. After the division, a pole containing A, L, and X chromosomes becomes the spermatid nucleus, while the opposite pole containing only A, L chromosomes is excised in the cytoplasmic bud. Thus, the functional sperm has only maternally derived chromosomes (Fig. 5); (Amabis et al. 1979; Goday and Esteban, 2001; Rieffel and Crouse, 1966). Although very little is known about the molecular and genetic mechanisms of paternal genome elimination (PGE) it must be based on genome imprinting, which allows distinguishing between paternal and maternal chromosomes. This, in turn, may involve heterochromatinization and epigenetic changes, which tag the chromosome for elimination.

## 5. Jawless Fish

Chromosome elimination occurs during the germline-soma differentiation in the ancient jawless fish such as sea and river lamprey and several species of hagfish (Kojima et al., 2010; Kohno et al. 1998; Nakai et al., 1991; Smith et al., 2018; Yan et al., 2016). The analysis of DNA content and genetic diversity in a Korean river lamprey *Lampetra morii* showed that out of 1.618 pg (approximately 1.582 Gb) haploid nuclear DNA content present in germline cells, around 15% of DNA is lost in somatic cells (Yan et al., 2016). During embryonic development of sea lamprey *Petromyzon marinus*, (vampire fish that is a parasitic fish of freshwater/marine habitat), the presumptive somatic cells eliminate ~0.5 Gb of DNA out of a total ~2.3 Gb genome present in the presumptive germ cells. They also reduce chromosome numbers from ~198 to ~164 (Smith et al., 2010, 2018; Timoshevskiy et al., 2016). In *Petromyzon marinus*, DNA elimination occurs during several successive cell divisions starting at 7<sup>th</sup> cleavage and concluding in the early neurula stage embryo (~ 3 days postfertilization) (Smith et al., 2018; Timoshevskiy et al., 2016; Yamazaki et al., 2003). The eliminated material consists of entire chromosomes and large chromosome fragments, which lag in the anaphase. In telophase, eliminated DNA becomes packaged into compacted structures called the micronuclei (MNi). The lagging chromatin and MNi are enriched in repetitive sequences and contain repressive chromatin modifications

(H3K9me3 and 5meC), which indicate epigenetic silencing through DNA and histone methylation (Timoshevskiy et al., 2016). *In silico* analysis and mapping of the repetitive sequences showed that certain repetitive elements (Germ1, 2, and 6) are present in all 12 eliminated chromosomes (Timoshevskiy et al., 2019). Authors suggest that these sequences mark the chromosomes for elimination and may be responsible, through modifying interactions with the mitotic spindle, for their delayed movement in the anaphases (Timoshevskiy et al., 2019). Strange and Moore (2019) compared the Germ1 element, which contains somatically rare (SR) region and a fragment of 28S rDNA, of sea lamprey *Petromyzon marinus* with *Lampetra aepyptera*, *Lethenteron appendix*, and three species of *Ichthyomyzon*. They found that while the SR regions were related between different lampreys, the 28S-fragments were species/genus-specific. Thus, they concluded that these two regions of Germ1 element were assembled independently in each lamprey lineage. Timoshevskiy et al. (2017) developed a hybridization-based approach to compare chromosome elimination (programmed genome rearrangement, PGR) and resolve its ancestral origin in the lamprey lineage. They concluded that the presence of lagging chromatin and micronuclei in two studied lamprey species *Petromyzon marinus* and *Entosphenus tridentatus*, which diverged million years ago, indicate that that chromosome elimination in lampreys occurred for at least the last 40 million years.

The hagfish (slime eels) are marine fish with rudimentary vertebrae but lacking a spine. During development, they eliminate 20.9–74.5% of a total genome from the presumptive somatic cells (Kohno et al., 1998). In the germ cells, some chromosomes and the sub-termini or termini of other chromosomes are highly condensed (heterochromatic), and most of those are lost from the presumptive somatic cells. In the Taiwanese hagfish *Paramyxine sheni*, the eliminated heterochromatin consists of tandem highly repetitive DNA sequences (EEPs1–4) lacking open reading frames and any significant homology to known transposable elements (Kojima et al., 2010). It remains unknown why these specific sequences are eliminated and how the chromosomes that lost subterminal or terminal fragments rearrange and reconstruct.

## 6. Birds

Among birds, chromosome elimination has been best studied in zebra finch *Taeniopygia guttata*, a model organism for vocal learning and neuroendocrinology research. Zebra finch germline cells contain a Germline-Restricted Chromosome (GRC). GRC is eliminated, as the micronucleus, during male meiosis, and thus it is maternally inherited through the egg (Biederman et al., 2018; Dos Santos et al., 2017; Pigozzi and Solari, 2005). The GRC eliminated

in meiosis is enriched in heterochromatin protein 1, and its histone H3 is methylated at lysine 9 and hypo-methylated at lysine 4, histone H4 is methylated at lysine 20 and (generally) hypo-acetylated. Meiotic elimination of GRC is probably related to the absence of the inner centromere protein INCENP and centromere inactivation (Ishii et al., 2016; Schoenmakers et al., 2010). In embryonic development, GRC is also eliminated from all somatic cells in both sexes. As GRC consists of over 120 million base pairs, it is the longest of all 80 (2n) finch chromosomes (Biederman et al., 2018; Dos Santos et al., 2017). It has been known that GRC contains repetitive and non-coding sequences, but a recent subtractive transcriptomic analysis of female and male germline tissues showed that GRC also has a single protein-coding gene, the  $\alpha$ -Soluble NSF Attachment Protein ( $\alpha$ -SNAP) that is involved in membrane fusion. So far, this is the only known coding gene located in the germline-restricted chromosomes. Surprisingly, the paralogs (somatologs) of  $\alpha$ -SNAP are also present in the somatic cell genome (Biederman et al., 2018). Although the exact role of this gene remains unknown, its retention both in the germline and soma suggests an essential biological function. However, the most recent phylogenetic studies and the studies of sequence content of GRCs in birds give some clues on the scope and significance of programmed DNA elimination (Asalone et al., 2021; Kinsella et al., 2019; Torgasheva et al., 2019; 2021). Kinsella et al. (2019) sequenced zebra finch GRC and showed that it contains copies of sequences present on a non-eliminated chromosome (A chromosome). They also showed that the zebra finch GRC is enriched in genes engaged in ovary development and contains >115 genes paralogous to single-copy genes on 18 autosomes and the sex chromosome (Z chromosome). Asalone et al. (2021) co-opted the existing computational methods designed to study differential gene expression to analyze zebra finch GRCs. Such comparative coverage analysis allowed them to identify new GRC genes, one encoding a hypothetical protein and another encoding an RNase H-like domain. The comparative genomics analyses indicated that through millions of years of evolution, genes had been added to the GRC. Some of these genes, such as *bicc1* encoding an RNA-binding protein that regulates gene expression during embryogenesis, and the E3 ubiquitin-protein ligase *trim71*, were already present in the songbird ancestor. In contrast, the other genes are evolutionarily new (Kinsella et al., 2019). Other comparative analyses of two species of estrildid finches, two species of martins, and great tit, showed that their GRCs are highly similar, although they evolved independently for tens of millions of years (Torgasheva et al., 2021). However, a comparison of birds from different taxonomic orders showed that 16 analyzed songbird species have GRCs of varying size and genetic content. In contrast, the GRCs are absent from 8 studies species belonging to other

avian orders. Authors suggest that about 35 million years ago, the GRC formed as a parasitic minichromosome in the songbird ancestor. However, its size and genetic content changed during evolution in the descendant bird lineages (Torgasheva et al, 2019).

## **Marsupials**

Among many Marsupials with chromosome elimination, the best studied are various species of the bandicoots, small, rat-like, nocturnal omnivores. In these animals, silencing of the sex chromosomes occurs through their elimination. In bandicoots, female embryo eliminates paternally derived X chromosome, and male embryo eliminates Y chromosome from the somatic cells, such as intestinal and corneal epithelium, liver, spleen, lymphocytes and hematopoietic cells (Hayman and Martin, 1974; Johnston et al., 2002). In contrast, for example in kangaroos, the paternal X is just inactivated by heterochromatinization into a Barr body (Sharman, 1971). In bandicoots, chromosome loss occurs during pouch development but differs in timing between different cell types, with the hematopoietic cells eliminating first and the corneal epithelium the last (Close, 1979, 1984; Johnston et al., 2002). In *Isoodon obesulus* the paternal X chromosome and the Y chromosome replicate DNA much later than other chromosomes. The late replication feature may tag them for silencing and elimination (Johnston et al., 2002). Thus, the elimination of sex chromosomes in Marsupials is the mechanism for sex chromosome dosage compensation. However, it is still unknown why these chromosomes are lost instead of being deactivated (like in kangaroos and other mammals).

## **8. Plants, Wasps, and B chromosomes**

The polyploidization of the genome during plant evolution is a prevalent phenomenon. It can occur either by multiplying a single genome (autopolyploidy) or combining nonidentical but related genomes (allopolyploidy) (Yang et al., 2019; Huang and Zhu, 2019). It is also widespread that the allopolyploids eliminate chromosomal DNA during development from the somatic cells in a tissue-specific manner. Studies on the hexaploid bread wheat, *Triticum aestivum*, that originated only 10,000 years ago from three diploid *Triticum* and *Aegilops* progenitors, showed that it eliminates both genome-specific (GSS) and chromosome-specific (CSS) non-coding DNA sequences either from leaves only or from both leaves and roots, but not from the germ cells (Khasdan et al., 2010). However, it remains unknown why the elimination occurs in the specific tissues and how/if it is adaptively significant.

Many plants, like 15% of all eukaryotic species, carry, in addition to standard chromosomes (A chromosomes), the supernumerary B chromosomes (Hanlon et al., 2018). Although B chromosomes are expandable, and without any known function, they are preferentially inherited at the super-Mendelian frequency through the “chromosome drive” mechanism (Dalla Benetta et al., 2019; Ruban et al., 2020). Recent studies on rye species showed that the sister chromatids of B chromosomes do not separate (non-disjunct) during the asymmetrical division that produces the germ cells (sperm of the pollen) and somatic (vegetative) cell, and preferentially segregate on the asymmetrical spindle to the generative pole. This process, rerepeated through several generations, leads to the accumulation of supernumerary B chromosomes in the germ cells (Dalla Benetta et al., 2019). Ruban et al. (2020) studied B chromosome behavior (elimination and drive) in developing roots and during the first mitosis of the pollen, respectively, in the goatgrass *Ae. speltoides*. In the goatgrass embryo, seven days after fertilization, the root progenitor cells eliminate B chromosomes. The chromatids of B chromosomes do not separate and, in anaphase, remain non-disjunct in the equator. During telophase, they are enclosed in the micronucleus and eliminated (Fig.6). However, in the same species, B chromosomes are preferentially included in the prospective germ cell during the first mitotic division of the pollen. The first mitosis of the pollen is asymmetrical. It will result in a large vegetative cell, which will be a source of nutrients for the growth of the pollen tube, and a small generative cell, which after another division, will form sperm cells. The division spindle of the first mitosis in the pollen is asymmetrical, and the lagging, non-disjunct B chromosomes segregate into the generative nucleus (Fig. 6).

A different mechanism underlying the B chromosome drive operates in wasps where the B chromosomes accumulate by eliminating A chromosomes. The jewel wasp *Nasonia vitripennis* has a B chromosome called the PSR (Paternal Sex Ratio) that is paternally inherited through the sperm (Aldrich and Ferree, 2017; Dalla Benetta et al., 2019; Reed, 1993;). After fertilization, during the first cleavage, the PSR chromosome-produced proteins (or RNA) induce histone modifications, heterochromatinization, and elimination of the sperm chromosomes, thus creating a haploid embryo. Because in wasps’ the males develop from the unfertilized eggs (haploid) and females develop from fertilized eggs (diploid), the genome elimination induced by the B chromosome converts the diploid female embryos into haploid males, which carry the B chromosome (Dalla Benetta et al., 2019; Reed, 1995). Comparative analysis of B chromosomes from different animal and plant species showed that they contain both coding- and non-coding sequences. The coding sequences represent all functional categories of genes: microtubule and cell division apparatus, differentiation, proliferation, cell junctions, neuronal synapse,

recombination, DNA repair, transcriptional regulators, metabolism regulators, sex differentiation and determination, immune response, cell signaling, and others (Dalla Benetta et al., 2019). Most of the B-linked coding sequences are degenerate copies of genes present in the A chromosomes. The exception is the wasp B chromosome that contains the transposon-like sequences absent from the wasp A chromosomes but common between different wasp species. Besides the coding sequences, B chromosomes contain highly repetitive DNA sequences such as transposable elements, and simple and complex satellite-like repeats, which are often found in the A chromosomes in the vicinity of centromeres and in the heterochromatin [Dalla Benetta et al., 2019; Marques et al., 2018]. This suggests that first B chromosomes derived from a duplication of A chromosomes that lost the euchromatic regions but retained the centromeric and pericentromeric regions, which, later in evolution, underwent mutations, translocations, inversions, and deletions, making the B chromosomes non-functional and quasi parasitic (Dalla Benetta et al., 2019; Marques et al., 2018). Application of novel, ingenious techniques such as transcriptome analysis combined with the laser capture microdissection (LCM) allowed, recently, for detailed analysis of eliminated B chromosomes in the central meristematic region of *Aegilops speltoides* embryos (Boudichevskaia et al. 2020). By comparing RNA sequences of meristematic cells of embryos with and without B chromosomes, authors identified 14,578 transcript isoforms, i.e., 35% of 41,615 analyzed, differentially expressed during B chromosome elimination. Further analysis allowed identifying 341 transcript isoforms unique for B chromosomes in central meristematic cells and functional annotation of 70 of these transcripts. Among these transcripts, some were genes/proteins relevant to spindle checkpoint, kinetochore function, and chromosome segregation, for example, the kinetochore protein Nuf2, Sgo1 (involved in making the cohesion complex), kinetochore complex component, Mis12, and spindle and kinetochore associated complex subunit 2, Ska2 (Boudichevskaia et al. 2020). The identification of such B-specific transcripts is essential for understanding mechanisms and the importance of B chromosome function and elimination.

### **Unanswered questions and future directions**

The tremendous diversity of chromatin/chromosome elimination among taxa and closely related species strongly suggests that they evolved independently. The consensus is that DNA elimination is an evolutionarily very ancient (hundreds of millions of years old) phenomenon and plays a role in gene silencing/ sex chromosome dosage compensation and/or removing the overload of repetitive sequences and transposable elements. However, it remains a mystery why,

in the first place, some species have repetitive DNA/transposon overload, and some do not. Also, why do some organisms silence the X chromosome by heterochromatinization while others invent extremely complicated mitotic or meiotic events to remove it? In some species, DNA elimination encompasses protein-coding sequences. We still do not know why there is a need to eliminate the specific genes only in some but not other closely related species. Another unresolved issue is the origin and significance of B chromosomes and their elimination or accumulation. Why do some organisms need to eliminate B chromosomes, and some not only retain them but accumulate their supernumerary copies?

The large-scale comparative studies and genome sequencing projects, such as a recently established database of karyotypes and sex chromosomes in over 13 000 species (from 29 orders) of insects (Blackmon et al., 2017) or comparative analysis of genome elimination in nematodes (Wang et al. 2017), lampreys (Strange and Moore, 2019; Timoshevskiy et al. 2017; and birds (Asalone et al., 2021; Kinsella et al., 2019; Torgasheva et al., 2019; 2021 ) will allow analyzing phylogenetic patterns and mechanisms of chromosome elimination and create a comparative framework for testing various hypotheses.

### **Conflict of Interests**

None of the authors has any conflict of interests

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## Figure Legends

### Figure 1. DNA elimination in *Tetrahymena*

*Tetrahymena*, like other ciliates, had a germline micronucleus (MIC) and somatic macronucleus (MAC). The genome of MIC is inherited by the progeny. After sexual reproduction, a new MAC develops from the MIC after eliminating internal eliminated sequences (IESs) from the germline chromosomes. The chromosome fragments destined for elimination are tagged by scan RNAs (scnRNAs). The scnRNAs are small RNAs synthesized from the MIC genome. First, the scnRNAs derived from the MIC translocate to the old MAC, and the scnRNAs, which are homologous to any old MAC sequences are removed. Next, the remaining scnRNAs (homologous to MIC-specific sequences) are transported to the developing MAC where they recognize homologous sequences tagging them for elimination. The precursor ds RNAs are processed into ds scnRNAs by the Dicer-like enzyme DCI1p. Subsequently, the endoribonuclease (Slicer) Twi1p, slices out one of the strands in ds scnRNA. Then the Twi1p - scnRNA complex moves to the developing MAC, where they recognize homologous sequences and mark them by heterochromatinization for elimination. The IESs are excised by the transposase enzymes. In *Tetrahymena*, the IESs form the membrane-less heterochromatin bodies, which are subsequently degraded. The formation of heterochromatin bodies is facilitated by the prion-like RNA-binding protein Jub6p. The remaining chromosome fragments, after substituting the germline telomeres with the newly formed somatic telomeres, are restructured into the nanochromosomes of the new MAC.

**Figure 2. Chromatin elimination during germline and soma differentiation in *Parascaris univalens*.**

*Parascaris univalens* has only two chromosomes. After fertilization, during the first division of the zygote (P0), chromosomes segregate equally to P1 and S1 daughter cells. Subsequently, P1 divides into P2 and S2, P2 divides in P3 and S3, P3 divides in P4 and S4, and P4 divides in P5 and S5. All P1-5 cells keep intact chromosomes. However, the dividing S1-5 cells eliminate heterochromatic ends of chromosomes (red color) and break the central segment of chromosomes (green color) into multiple small chromosomes. The small chromosomes are inherited by the S progeny, while the heterochromatic termini degrade in the cytoplasm. Eventually, all cells with reduced chromatin (S lineage) become pre-somatic cells, and the P5 becomes the primordial germ cell that divides into the germ cells.

**Figure 3. Chromatin elimination in *Ascaris suum***

*Ascaris suum* has 24 germline chromosomes. The chromatin elimination starts from the third embryonic division. The 48 breaks remove all germline telomers (Tg), and 24 breaks within the 3-6kb long chromosomal break regions (CBRs) eliminate 12 internal regions of the chromosomes and split 24 germline chromosomes into 36 somatic chromosomes. The somatic chromosomes reconstitute new somatic telomers (Ts). Eliminated chromosome fragments lose the kinetochores and cannot interact with the mitotic spindle and segregate. Eventually, they become surrounded by a double membrane into the micronuclei and are degraded by autophagocytosis.

**Figure 4. Sex specific chromatin diminution in male *Strongyloides papillosus***

*S. papillosus* females have one pair of long chromosomes (2L) and one pair of medium chromosomes (2M). Homologous chromosomes are depicted in dark and light colors. The long chromosomes derived in evolution from the fusion the X chromosomes with the autosomes. Thus, the female has the XX phenotype. In contrast, *S. papillosus* males have a single long chromosome (1L), three medium chromosomes (3M), and one short chromosome (1S). The males of *S. papillosus* use sex-specific chromatin diminution to remove an internal fragment of one of the two long chromosomes. This elimination restores the X0 genotype in the male. During spermatogenesis only the intact long chromosome and two original M chromosomes are incorporated into sperm.

### **Figure 5. Chromosome elimination during *Sciara ocellaris* spermatogenesis**

The first meiotic division does not have a distinct metaphase, and division proceeds from the prometaphase directly to the anaphase. The I meiotic spindle is unipolar. In anaphase I, the maternal autosomes (mA) and the maternal X chromosome (mX) segregate to the spindle pole. In contrast, paternal autosomes (pA) and the paternal X chromosome (pX) move to the opposite pole, where they are included in the cytoplasmic bud. In meiosis II, the spindle is bipolar. In metaphase, maternal A chromatids align in metaphase plate, and X chromosome with unseparated chromatids attaches to the microtubules at the pole. At anaphase II, the maternal A chromatids segregate to opposite poles. In telophase II, the pole containing the maternal A and maternal X chromosomes becomes the spermatid nucleus. In contrast, the opposite pole containing a subset of maternal autosomes, paternal autosomes and paternal X chromosomes is excised in the cytoplasmic bud and degraded. Thus, the functional sperm has only maternally derived chromosomes (modified from Goday and Esteban, 2001). Some *Sciaridae* also have the L chromosomes, which for simplicity, are not depicted in the figure.

### **Figure 6. Elimination and drive of Chromosome B in plants**

The upper panel of the diagram shows B chromosome elimination during root development in the goatgrass *Ac. speltoides*. During development, seven days after fertilization, the cells destined for the root eliminate B chromosomes. The metaphase spindle is symmetrical, and the A (green color) and B (red color) chromosomes align in the metaphase plate. In anaphase, the chromatids of A chromosomes separate to the opposite poles. In contrast, the chromatids of the B chromosome remain non-disjunct in the equator. In telophase, B chromosomes are enclosed in the micronucleus, eliminated, and later degraded. The cells containing nuclei with A chromosomes only will develop into the root cells.

The lower panel shows preferential segregation of B chromosomes (chromosome drive) into the prospective germ cell during the first mitotic division of *Ac. Speltoides* pollen. The first mitosis of the pollen and the mitotic spindle are asymmetrical. The dividing cell has a generative and vegetative pole. The metaphase plate with aligned A and B chromosomes is shifted toward the generative pole. In anaphase, the non-disjunct B chromosomes and one set of A chromatids segregate to the generative pole. Telophase results in two different nuclei: a generative nucleus with B and A chromosomes and a large vegetative nucleus with A chromosomes only. After the

division, the vegetive cell will be a source of nutrients for the growing pollen grain tube, and a small generative cell, after another mitotic division, will form sperm cells.

Fig. 1

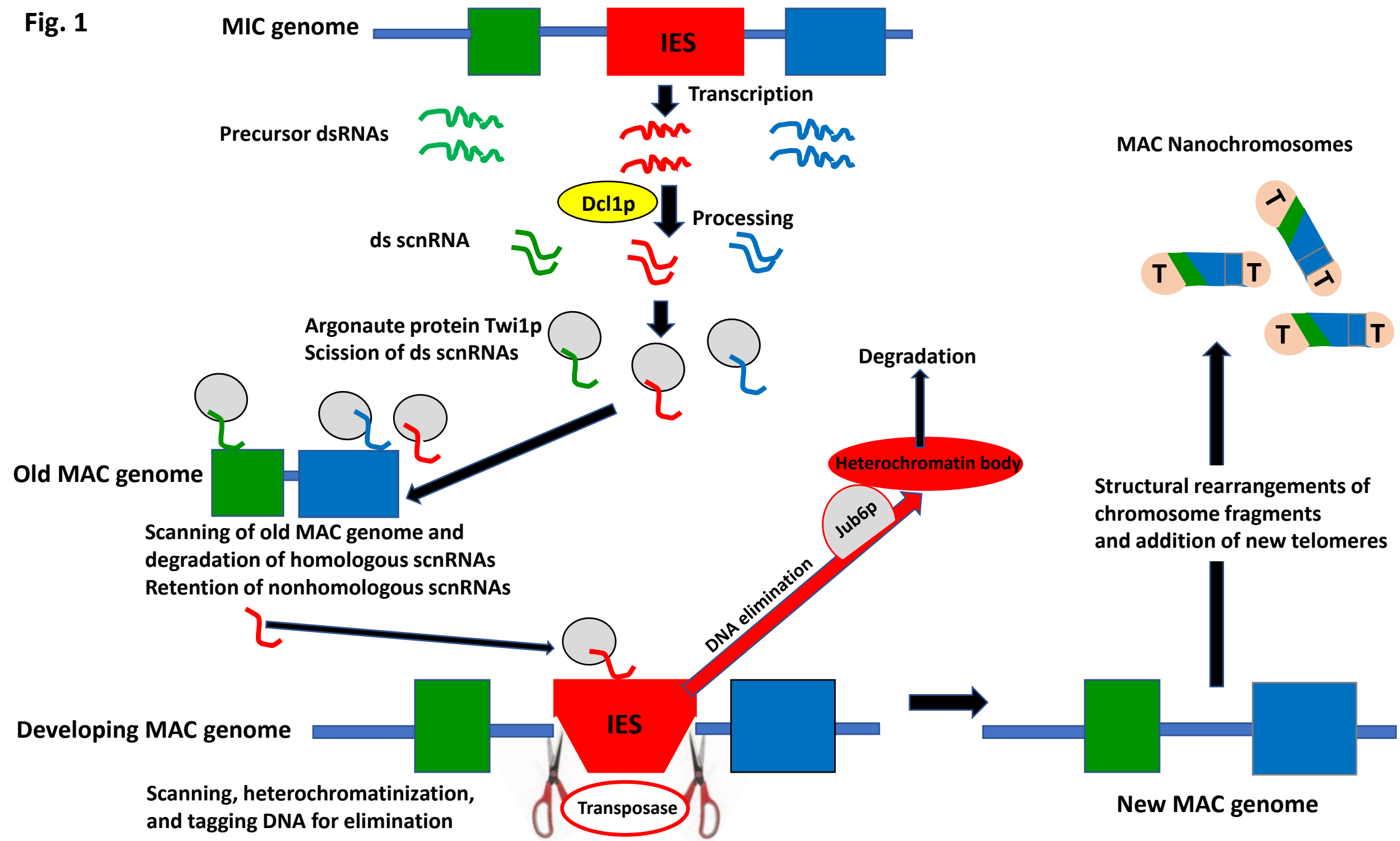


Fig. 2

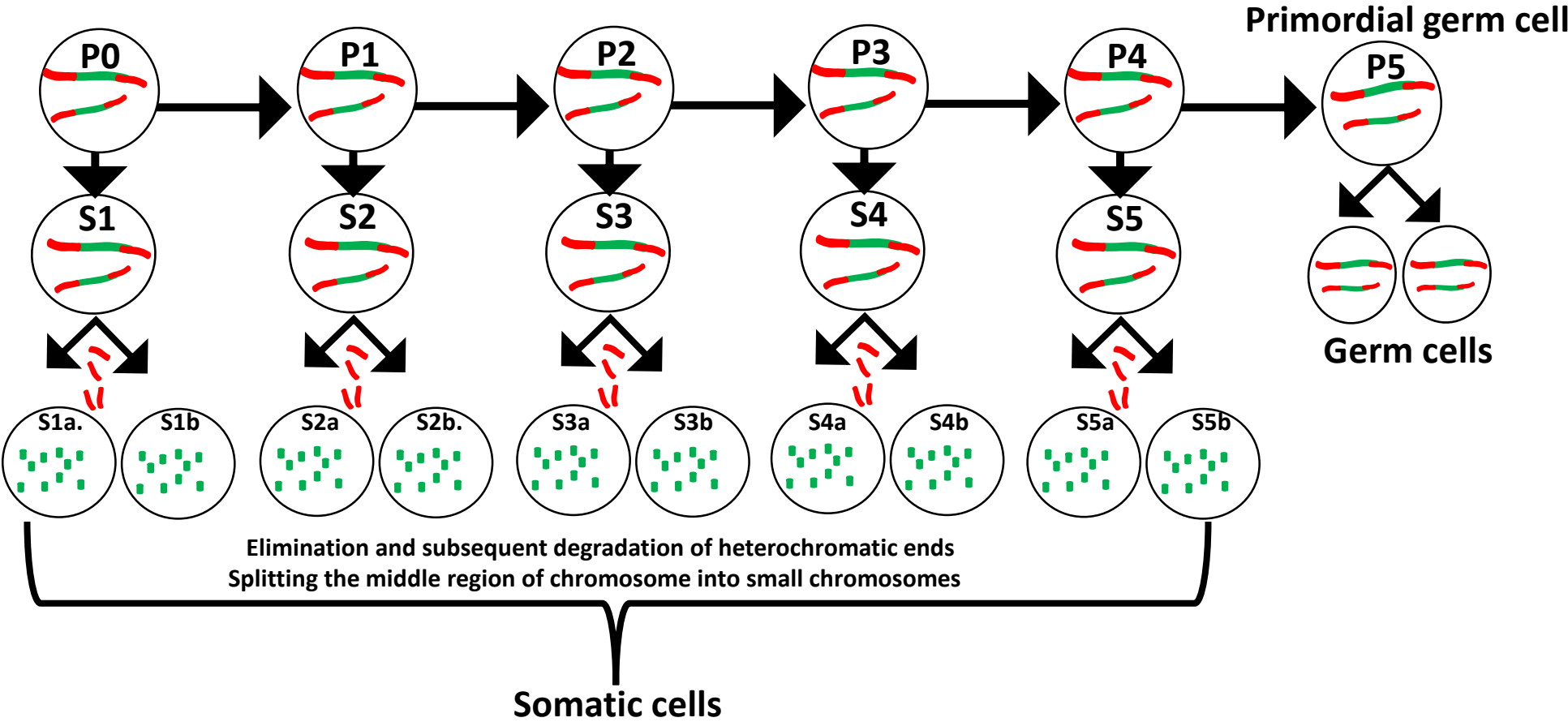
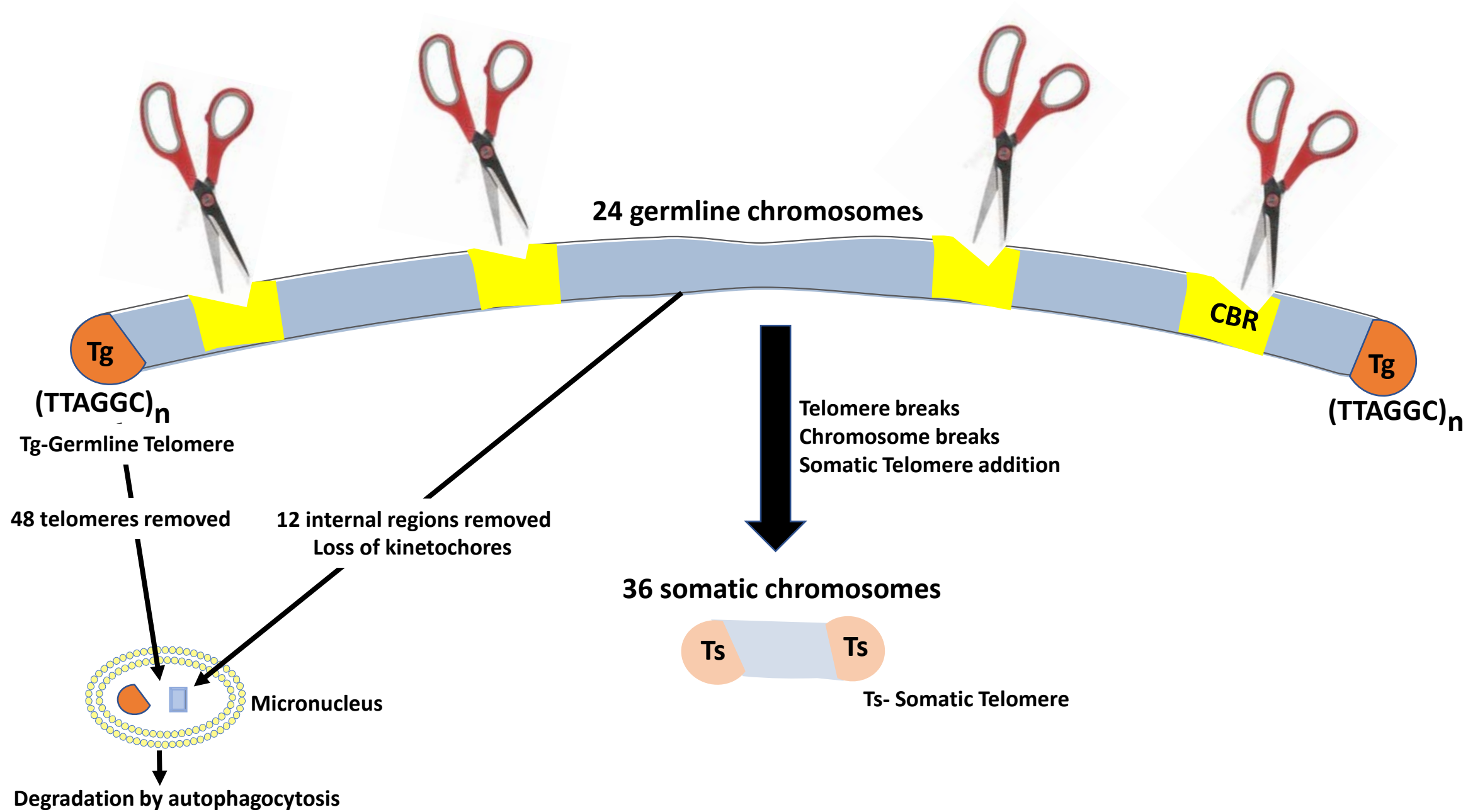


Fig. 3



**Fig. 4**

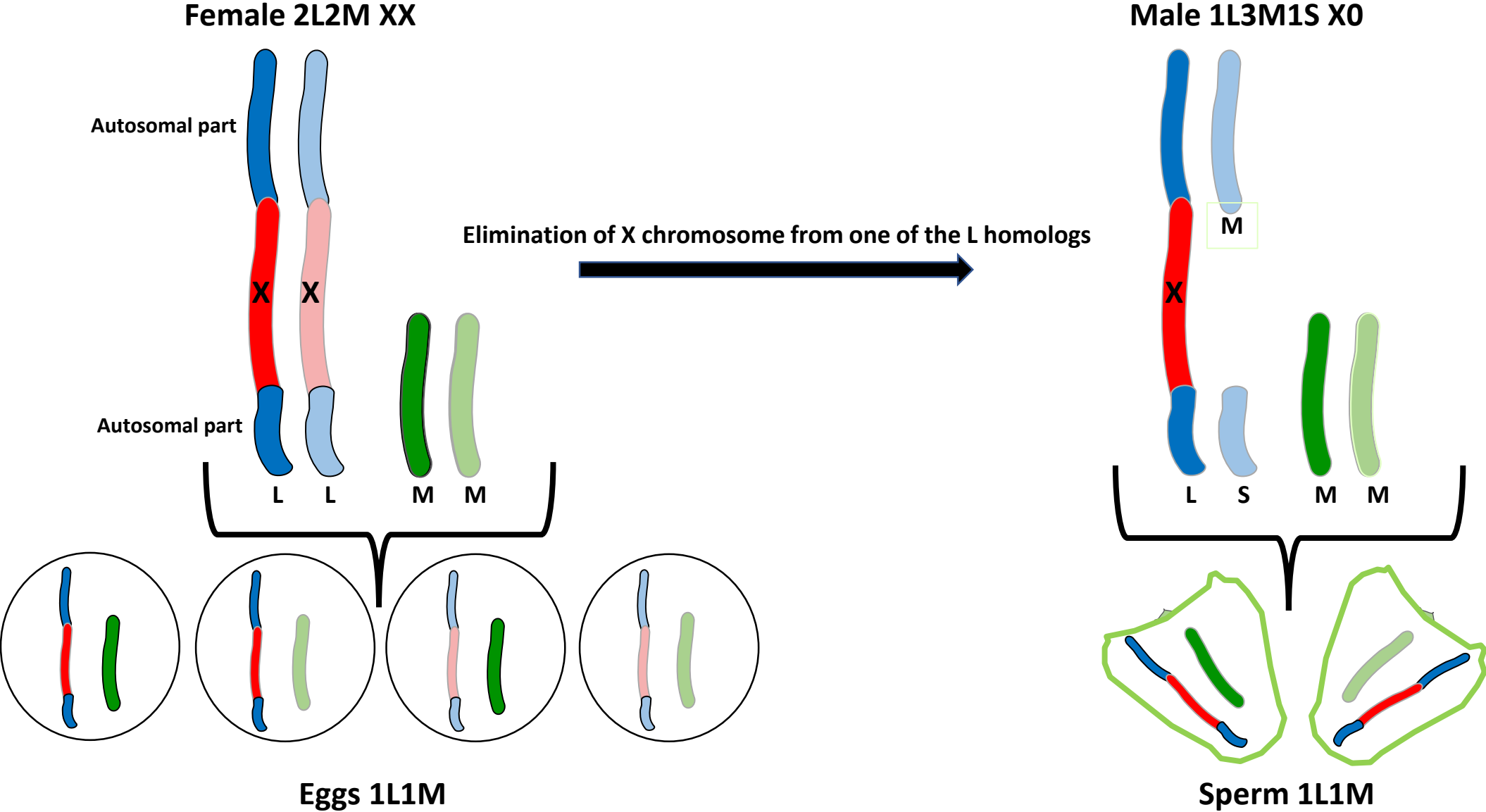
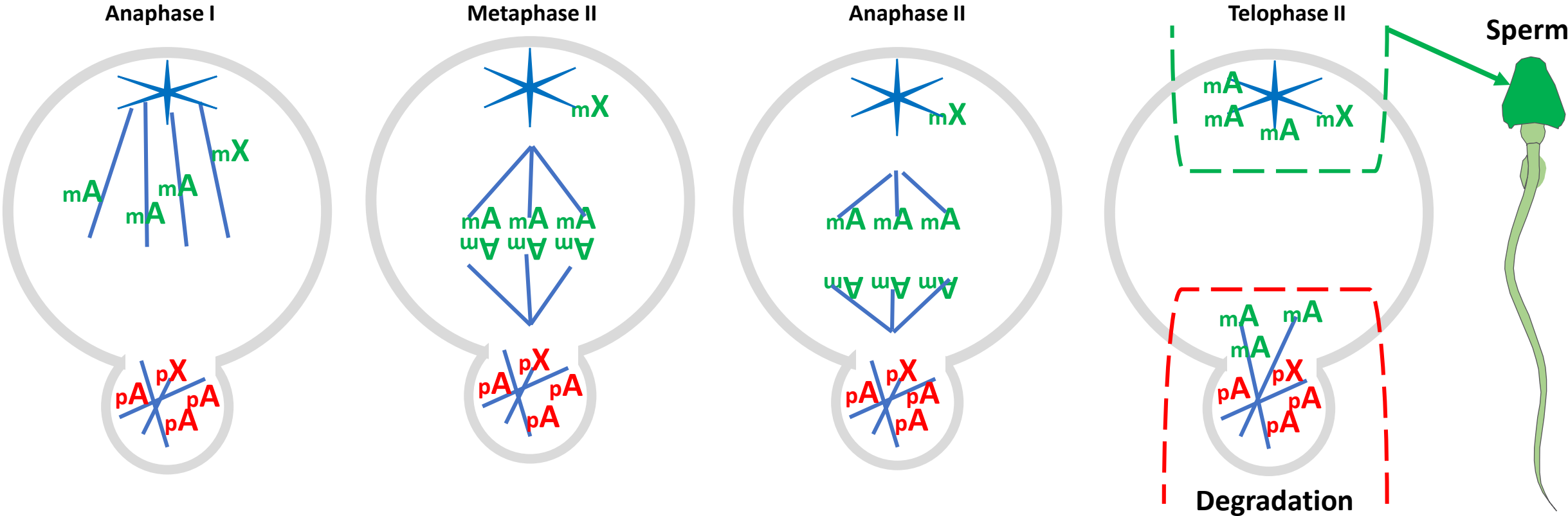


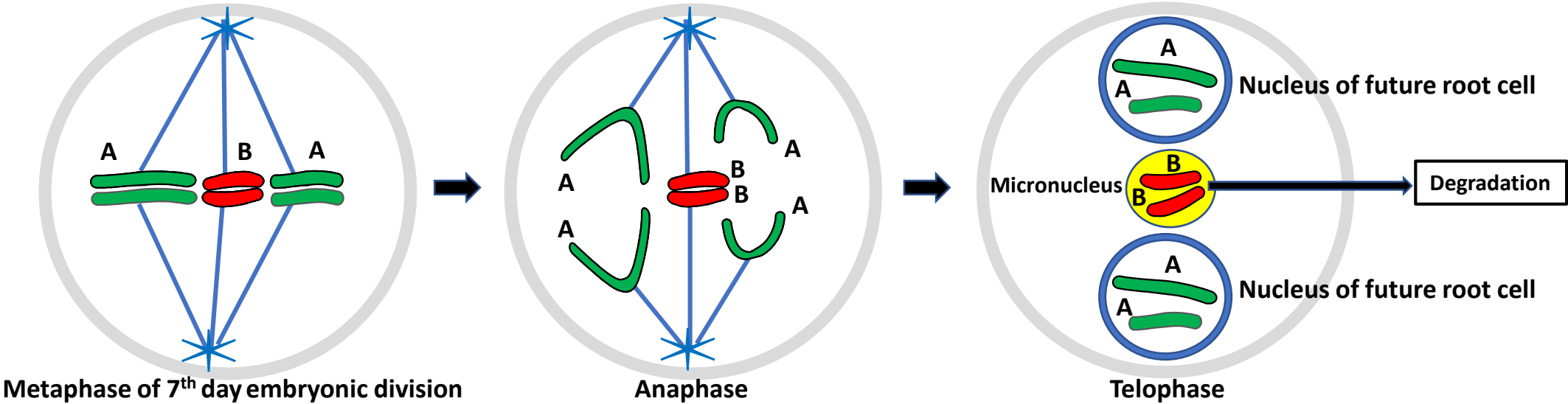


Fig. 5

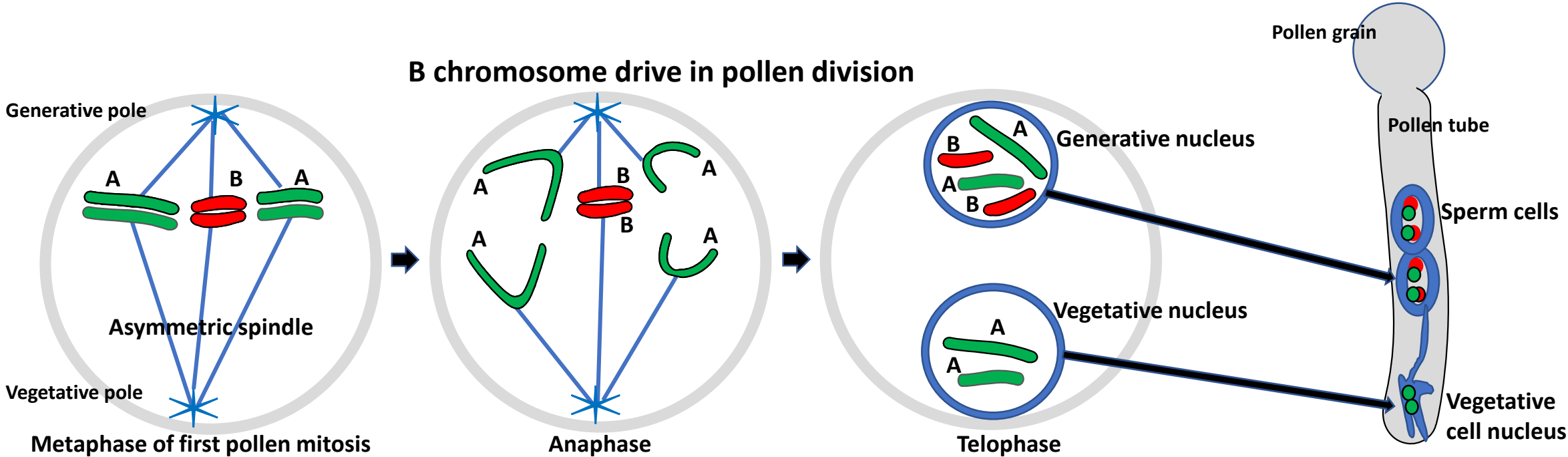


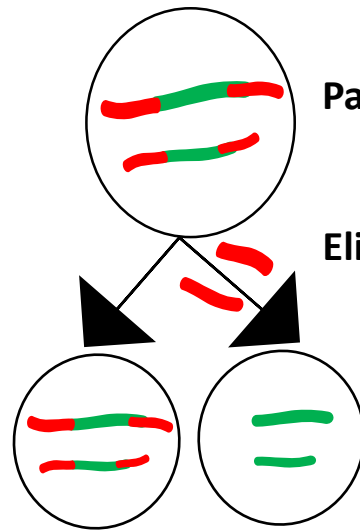
**Fig. 6**

**B chromosome elimination in prospective root cells**



**B chromosome drive in pollen division**





**Parental cell or cell nucleus with the full chromosome/chromatin set**

**Elimination of chromosomes or chromosome fragments**

**Resulting cells or cell nuclei with different chromosome/chromatin sets and different fate**