

Evolution of Developmentally Regulated Genome Rearrangements in Eukaryotes

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ABSTRACT Developmentally regulated genome rearrangements (DRGR)—processes that alter genomes either in specific cells or during specific life cycle stages—are widespread throughout eukaryotes. This contrasts with the view that genome structure and content remain essentially constant throughout an organism's life cycle. Here we review three categories of developmentally regulated genome processing in eukaryotes: genome-wide rearrangements, targeted rearrangements, and a special case of amplification of ribosomal DNA genes. Mapping these types of DRGR onto eukaryotic phylogeny indicates that each type of processing is found in multiple independent lineages. We propose that such genome rearrangements were present within the last common ancestor of extant eukaryotes, and that future research will yield evidence of homologous epigenetic mechanisms underlying genome processing among diverse eukaryotes. *J. Exp. Zool. (Mol. Dev. Evol.)* 304B:448–455, 2005. © 2005 Wiley-Liss, Inc.

In the textbook scenario, eukaryotes have the same genomic content in every nucleus (as either haploid or diploid) at every stage of life. In contrast to this simplistic depiction, genomes are processed during development in many eukaryotic lineages, either in specific cells or life cycle phases. To elucidate the origin and evolution of genome processing, we examine the phylogenetic distribution and diversity of developmentally regulated genome rearrangements (DRGR) in the context of our current understanding of eukaryotic phylogeny. The organisms that undergo genome processing are widely distributed across the phylogeny of eukaryotes, including lineages as divergent as ciliates, animals, kinetoplastids, Foraminifera, and *Entamoebae* (Fig. 1). The presence of diverse forms of DRGR in multiple lineages in the eukaryotic tree of life suggests that some form of DRGR existed within the last common ancestor of extant eukaryotes.

Here we synthesize information on three types of DRGR in eukaryotes: (1) genome-wide rearrangements—the elimination of specific regions of chromosomes in organisms that sequester their germline nuclei; (2) targeted rearrangements—chromosomal rearrangements that occur only in a limited number of genes, including antigenic variation in kinetoplastids, vertebrate antibody diversity, and yeast mating-type switching; and (3) the special case of chromosomal rearrangements

involving rDNA processing in a broad diversity of organisms (Table 1). Although diverse in the target and extent of processing, these three types of DRGR are united in that they each generate diversity in genome architecture and are found across multiple lineages of eukaryotes, suggesting ancient origins. Because of our focus on early eukaryotic evolution, we do not discuss any processes known only in single lineages, such as paternal chromatin elimination in animals (e.g., Goday and Esteban, 2001) or examples of DRGR found in prokaryotes (e.g., Cerdano-Tarraga et al., 2005).

The goal of this synthesis is to provide a framework for studying the origins and diversification of DRGR in eukaryotes. Based on the examples of DRGR discussed here, we propose a hypothesis whereby (1) genome processing was present in the last common ancestor of extant eukaryotes and (2) the evolution of the diverse types of DRGR was enabled by epigenetic phenomena, such as RNA interference (RNAi). This

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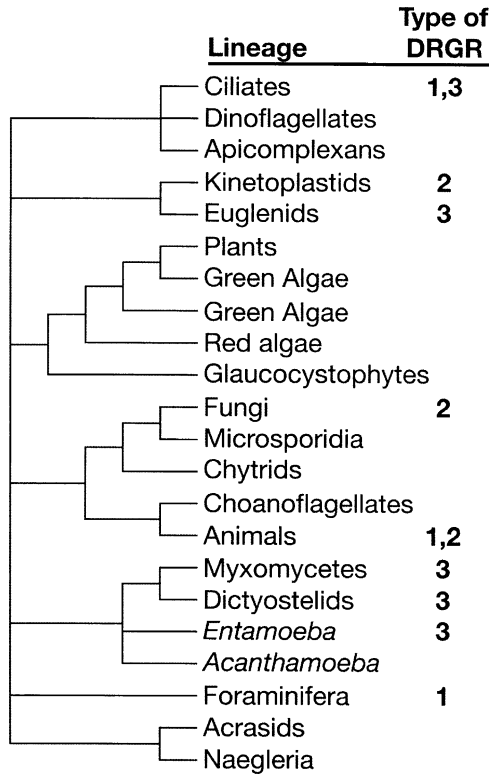


Fig. 1. Phylogenetic distribution of developmentally regulated genome rearrangements. Selected clades of eukaryotes shown on a phylogeny modified from Stechmann and Cavalier-Smith (2003) and Baldauf et al. (2000). Numbers indicate the type(s) of rearrangement(s) found within a lineage (see Table 1).

TABLE 1. Three types of developmentally regulated genome rearrangements and the lineages in which they occur

Category	Type of DRGR	Lineages
1	Genome-wide rearrangements	Animals: Nematodes, Copepods, Hagfish Foraminifera Ciliates
2	Targeted rearrangements	Vertebrate immune system Trypanosome antigens Yeast mating type
3	rDNA—a special case	Animals <i>Entamoeba</i> Euglenids Dictyostelids and myxomycetes Ciliates

hypothesis arises from the observation of DRGR in phylogenetically diverse eukaryotes coupled with emerging evidence for epigenetic phenomena underlying the architecture of eukaryotic genomes.

DRGR Type 1: Genome-wide rearrangements

We define genome-wide rearrangements as developmentally regulated changes that occur across the genome and differentiate somatic chromosomes from germline chromosomes (Fig. 2(1)). These genome-wide rearrangements occur in at least three, non-sister lineages of eukaryotes: animals, Foraminifera, and ciliates.

Chromatin diminution in animals

Chromatin diminution, the selective elimination of portions of chromosomes during animal development, is found in at least three disparate lineages: nematodes, copepods, and hagfish.

Nematodes—Chromatin diminution was first described by Boveri in the horse parasite *Parascaris univalens* (Boveri, 1887) and has since been described in 10 different nematodes, most in the parasitic family Ascarididae (Muller and Tobler, 2000). Chromatin diminution in nematodes takes place during germline and soma segregation and involves two processes: chromosome fragmentation and loss of chromosomal material from presomatic cells. During this process nearly all heterochromatin is eliminated such that somatic cells contain almost exclusively euchromatin. For example, in *P. univalens* 85% of total DNA is eliminated, and in *Ascaris suum* 25% is eliminated (Moritz and Roth, '76). The eliminated heterochromatin is rich in highly repetitive sequences and also contains middle-repetitive sequences, including transposable elements (Muller and Tobler, 2000).

Studies of the molecular mechanisms of chromosomal breakage from *A. suum* reveal that breakage occurs within specific chromosomal regions (approximately 6 kb long), but can occur at different sites within these regions. After breakage, telomeres are added to ends of eliminated and retained chromosomes (Muller and Tobler, 2000).

Boveri (1887) suggested that germline limited chromatin is essential for the germinal quality of a blastomere. This idea is supported by the recent finding that at least three single-copy genes are eliminated from somatic cells, but appear to be essential to the germline (Muller and Tobler, 2000). Further, Muller and Tobler (2000) argue that diminution serves as a gene silencing mechanism in somatic cells.

Copepods—Chromatin diminution has been observed in at least eight species of copepods,

primarily in *Cyclops* (Kloc and Zagrodzinska, 2001). As in nematodes, chromatin diminution in copepods occurs during early embryonic cleavage divisions, at the time of germline and soma differentiation (Beermann, '77). This diminution of heterochromatin results in elimination of 35–90% of the germline genome (Rasch and Wyngaard, '99; Wyngaard and Gregory, 2001). In contrast to nematodes, copepod chromosomes are not fragmented during diminution; rather, heterochromatin is either removed by breakage and healing of chromosomal ends or excision of internal sequences (Beermann, '77).

Hagfish—At least eight different species of hagfish also undergo chromatin diminution. Similar to nematodes and copepods, the chromatin elimination in hagfish occurs during early embryogenesis and the eliminated chromatin is largely heterochromatic (Nakai et al., '91; Kubota et al., '97). Unlike what is known in nematodes and copepods, hagfish eliminate not only regions of chromosomes, but also entire chromosomes. Eliminated chromatin accounts for 21–55% of the total genome (Nakai et al., '91). Little is known about the mechanism of elimination; however, sequence analysis of eliminated DNA reveals regions of repetitive DNA that are highly conserved across species (Kubota et al., '97).

Genome-wide rearrangements in microbial eukaryotes

To date, genome-wide rearrangements are known from two groups of microbial eukaryotes: Foraminifera (the so-called shelled amoebae) and ciliates. The rearrangements in these groups are similar to chromatin diminution in animals except that the rearrangements occur in differentiated nuclei within a single cell.

Foraminifera—DRGR differentiates germline and somatic genomes in heterokaryotic Foraminifera including several genera within the order Rotaliida (Lee et al., '91). As in ciliates, the somatic nucleus contains more DNA than the germline nucleus, and is the site of mRNA synthesis (Grell, '73; Lee et al., '91). As few molecular data exist for this group, it is difficult to draw conclusions about patterns of DRGR in Foraminifera with respect to other eukaryotes.

Ciliates—Ciliates, a lineage where DRGR are relatively well studied, present an extreme example of genome processing (Fig. 2(1)). Rearrangements in this lineage involve a multitude of processes including deletion of specific chromoso-

mal regions and amplification of remaining chromosomes. Within every single-celled ciliate, the two distinct nuclei are the micronucleus, analogous to a germline nucleus, and the transcriptionally active macronucleus. During development of the macronucleus, genomes sometimes experience massive loss of chromosomal regions and rearrangements in the genome, and can yield up to 25,000,000 gene size chromosomes in a single macronucleus (reviewed in Katz, 2001; Jahn and Klobutcher, 2002; Yao et al., 2002).

Site-specific fragmentation and amplification of chromosomes occurs during development of the macronucleus in all ciliates studied to date (e.g., see the discussion of rDNAs below). The extent of fragmentation at other loci, however, varies dramatically among ciliates. For example, ciliates in the class Oligohymenophorea, including *Paramecium* and *Tetrahymena*, fragment at approximately 200 positions in their chromosomes generating macronuclear chromosomes that are 100–1,500 kbp long (Jahn and Klobutcher, 2002; Yao et al., 2002). In contrast, in at least three classes, Spirotrichea, Phyllopharyngea, and Armophorea, fragmentation results in as many as 25,000 unique chromosomes, many of which contain only a single gene (Prescott, '94; Riley and Katz, 2001). In addition, each of these chromosomes can be replicated up to 15,000 times (Prescott, '94; Klobutcher and Herrick, '97). Data on specific mechanisms of chromosome breakage exist for a few genera, including *Tetrahymena thermophila* and *Euplotes crassus*, which have conserved sequences at or near the site of fragmentation (Klobutcher et al., '98; Yao et al., 2002). In *Paramecium teraurelia*, there are no similarly conserved chromosome breakage motifs (Klobutcher et al., '98; Yao et al., 2002).

Another form of DRGR that is found in all ciliates whose micronuclei have been studied is the removal of internal eliminated sequences (IESs), intervening DNA segments analogous to introns except that they are excised as DNA during macronuclear development. The structure and content of IESs is highly variable among species, and IESs range in size from less than 100 bp to greater than 5 kbp (Jahn and Klobutcher, 2002; Yao et al., 2002; Katz et al., 2003). The shorter IESs typically have short direct repeats at their ends, while the longer IESs have open reading frames and appear similar to transposable elements (Jahn and Klobutcher, 2002; Yao et al., 2002).

Gene unscrambling, an additional ciliate DRGR, has been found exclusively in the class Spirotri-

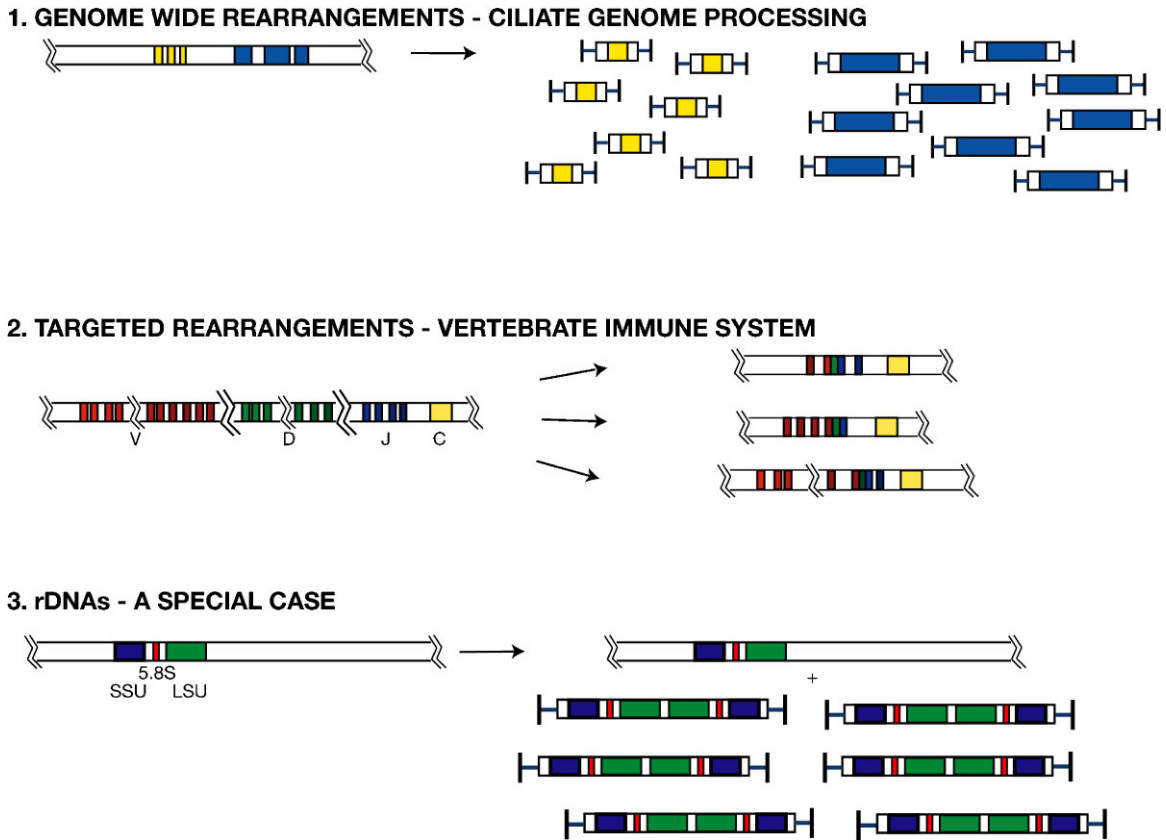


Fig. 2. Examples of the three types of DRGR. (1) Genome-wide rearrangements in extensively processing ciliates involve chromosome breakage, elimination of DNA, and amplification of processed chromosomes. Colored rectangles represent coding regions and white regions are non-coding; “T” bars at the end of chromosomes represent telomeres. (2) Targeted rearrangements are exemplified here by V(D)J processing in vertebrate immune systems. In this case, a single locus is processed to produce a diversity of antibody genes by joining various V, D, and J regions. (3) rDNAs are amplified in a variety of eukaryotes. Shown here is a representation of rDNA processing in *Tetrahymena thermophila*, a ciliate in which multiple palindromic copies of the rDNA locus are generated on small macronuclear chromosomes from a single micronuclear locus.

chea. In some members of this class, the coding domains of the micronuclear copies of genes are interrupted by IESs and are out of order (scrambled) with respect to the macronuclear copy (Prescott, '94). Although little is known about the mechanism of unscrambling, it appears to involve recombination between short repeated sequence elements shared by the MAC destined sequences that get joined together during unscrambling (Landweber et al., 2000; Prescott, 2000; Jahn and Klobutcher, 2002).

DRGR Type 2: Targeted rearrangements

Targeted rearrangements are DRGR that affect only specific loci within a genome (Fig. 2(2)). The details of the type of loci vary among lineages, and there are surely many more examples of targeted DRGR yet to be discovered.

Antibody diversity in vertebrates

The generation of antibody diversity in vertebrate immune response is perhaps the most well-known example of a DRGR. Jawed vertebrates are capable of producing a tremendous diversity of antibodies due to the rearrangement of chromosomal regions in immune cells. During development of lymphocytes from stem cells, the regions of DNA encoding antibodies are assembled from previously non-adjacent segments of DNA. This rearrangement is performed by a site-specific recombinase including proteins encoded by the genes RAG1 and RAG2 (Gellert, '97; Oettinger, '99). For example, during assembly of genes encoding light chains of antibodies in humans, one of approximately 100 possible variable (V) segments is joined to one of five joining (J) segments and then to the one constant (C) segment. Similar processes are used

in forming genes that encode the heavy chain of antibodies (Lewis, '94). In both cases, an additional level of variability is introduced by the loss of a small, variable number of nucleotides where the gene segments are joined (Lodish et al., 2000; Alberts et al., 2002).

While V(D)J rearrangements mediated by RAG genes have not been found in earlier diverging animals, recent evidence indicates that lampreys, jawless vertebrates, experience a different form of DRGR in their immune system. The generation of diversity in the adaptive immune response in lampreys involves DNA rearrangements of leucine-rich repeat cassettes to produce mature variable lymphocyte receptors (Flajnik, 2004; Pancer et al., 2004). This finding suggests that there likely exist a variety of DRGR in animals to generate diversity in the immune response.

Antigenic variation in kinetoplastids

A system analogous to rearrangements in vertebrate immunity is the use of chromosome rearrangements in pathogens to produce diversity at antigen loci. Such DRGR are found in Trypanosomes, parasitic microbial eukaryotes that cause diseases such as African sleeping sickness in humans (*Trypanosoma brucei*) and nagana in livestock (*T. congolense* and *T. vivax*) (Donelson, 2003). The surface antigens of trypanosomes, variant surface glycoproteins (VSGs), are changed at a rate of 10^{-2} – 10^{-7} new VSGs per cell division (Turner and Barry, '89; Turner, '97). The rapid turnover of VSG allows the parasite to escape detection by antibodies produced by the host to recognize the "old" VSG.

Variation in VSG expression is determined in part by genomic processing. In the genome of *T. brucei* there are approximately 1,000 VSG genes present; however, only one of these genes is expressed at any given time. In order for a VSG to be actively expressed, it must be present at a VSG expression site. There are about 20 expression sites found linked to the telomeres of different chromosomes and only one of these expression sites is active at a time. VSG genes are found scattered throughout the genome; thus in order for a VSG gene to be expressed it must be moved to the expression site that is currently active (Donelson, 2003).

There are several mechanisms of genome rearrangements that allow VSGs to be moved into and out of the active expression site. Duplicative transposition, likely the most common mechanism

of changing VSG genes, occurs when the VSG in the active expression site is replaced with a duplicated copy of a different VSG from another chromosomal location. Another example is telomere exchange, the reciprocal exchange of two telomeres and their associated VSG genes activating one VSG and inactivating the other (Donelson, '95, 2003).

Yeast mating type

DRGR at the mating-type locus (MAT) underlie mating-type determination in yeast and other fungi. *Saccharomyces cerevisiae* haploid cells exist as one of two mating types determined by the allele, a or α , present at the MAT. Each mating-type allele produces a mating factor and a receptor that enable recognition and fusion with its opposite mating type. Haploid cells change from one mating type to the other via gene rearrangements. According to the cassette model (Hicks et al., '77), two donor loci contain an unexpressed copy of the a or α allele, which is transposed to the MAT locus where it can be expressed. This directional gene conversion is catalyzed by HO endonucleases, and the sequences at the donor loci remain unchanged during this rearrangement (Haber, '98; Alberts et al., 2002).

DRGR Type 3: Ribosomal DNAs—a special case

Developmentally processed rDNAs are present in diverse taxa across the eukaryotic tree of life (Fig. 2(3)). Although many eukaryotes have clusters of tandemly arrayed rDNA genes, rDNAs are subject to developmentally regulated rearrangements and amplifications in Euglenids (Ravel-chapuis et al., '85; Ravel-chapuis, '88; Zaitseva et al., '95), *Entamoebae* (Bhattacharya et al., '98; Bagchi et al., '99; Paul et al., 2002), slime molds (both dictyostelids and myxomycetes; (Ferris et al., '83; Simon and Olins, '94), animals (Mais et al., 2002), and fungi (Huber and Rustchenko, 2001).

The developmental amplification of rDNAs in diverse eukaryotes likely evolved as a mechanism for maintaining large pools of homogenous rRNAs in response to increases in requirements for translation. For example, rDNAs in the unicellular alga *Euglena gracilis* can be extrachromosomal and increase in number during the exponential phase of growth of the cells (Ravel-chapuis et al., '85). Similarly, amplified rDNAs are found during growth in developing frog embryos (Mais et al.,

2002). Ribosomal DNAs are also processed in all ciliates examined to date, yielding ~10–15 kb chromosomes containing a single or pair of rDNA loci (Prescott, '94) (Fig. 2(3)). In an extreme case, the rDNA genes of *Entamoeba histolytica* are located only on circular extrachromosomal molecules and the number of these molecules varies throughout the life cycle (Bagchi et al., '99).

A MODEL: EPIGENETICS DRIVES THE EVOLUTION OF DRGR

We hypothesize that epigenetic phenomena underlie the diversification of DRGR in eukaryotes. Consistent with this hypothesis is the demonstrated role of epigenetics in two of the three categories of DRGR. In the case of genome-wide rearrangements, RNAi is involved in both chromosome fragmentation and IES excision in ciliates (Preer, 2000; Mochizuki et al., 2002). For example, in *T. thermophila*, both dicer homologs and small RNAs regulate the development of the macronuclear genome (Mochizuki et al., 2002). It is likely that a similar process plays a role in chromatin diminution in animals, as RNAi can mark heterochromatin (Hall et al., 2002; Volpe et al., 2002), enabling its elimination.

Epigenetic phenomena play a role in targeted DRGR, the second category presented above. In vertebrate immune systems, immunoglobulin loci move within the nuclei of B cells and one allele is packaged into active chromatin (Goldmit et al., 2005). In *Trypanosoma brucei*, epigenetic silencing of VSGs is regulated by chromatin remodeling proteins (DiPaolo et al., 2005). In addition, the mating-type locus in *Schizosaccharomyces pombe* uses an epigenetically organized chromatin structure to control recombination (Hall et al., 2002; Volpe et al., 2002; Petrie et al., 2005). As for our third category of DRGR, we speculate that epigenetic phenomena structure chromatin to regulate the amplification of rDNAs in diverse eukaryotes.

Reliance on epigenetic phenomena may have allowed for the observed diversification of DRGR in eukaryotes by decoupling genome processing and DNA sequence evolution. By its very nature, epigenetics enables rapid evolution of chromosomal features, such as the cis-acting sequences that direct rearrangements. Alternative hypotheses to explain the diversity of DRGR found in eukaryotes include multiple origins of each type of DRGR and/or involvement of other molecular mechanisms. These hypotheses must explain two features: the broad phylogenetic distribution of similar types of

rearrangements and the diversity of specific molecular signals underlying these types.

SUMMARY

In sum, we argue that developmentally regulated genome processing is widespread, ancient, and represents an often-overlooked mechanism that affects the evolution of genome architecture in eukaryotes. We have provided a framework for further analysis by defining different types of rearrangements, proposing an origin of DRGR before the last common ancestor of extant eukaryotes, and hypothesizing that epigenetic phenomena enabled the diversification of DRGR. Based on our hypothesis, further illumination of DRGR across the eukaryotic tree of life should reveal homologous epigenetic phenomena underlying genome processing.

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