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A Chimeric Chromosome in the Ciliate *Oxytricha* Resulting from Duplication

Yi Zhou, **Helmae Wubneh**, **Clayton Schwarz**, and **Laura F. Landweber**
Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ 08544, USA

Laura F. Landweber: lfl@princeton.edu

Abstract

In a process similar to exon splicing, ciliates use DNA splicing to produce a new somatic macronuclear genome from their germline micronuclear genome after sexual reproduction. This extra layer of DNA rearrangement permits novel mechanisms to create genetic complexity during both evolution and development. Here we describe a chimeric macronuclear chromosome in *Oxytricha trifallax* constructed from two smaller macronuclear chromosomes. To determine how the chimera was generated, we cloned and sequenced the corresponding germline loci. The chimera derives from a novel locus in the micronucleus that arose by partial duplication of the loci for the two smaller chromosomes. This suggests that an exon shuffling-like process, which we call MDS shuffling, enables ciliates to generate novel genetic material and gene products using different combinations of genomic DNA segments.

Keywords

Ciliate; Duplication; Gene shuffling; Chimeric chromosome; Micronucleus; *Oxytricha*

Ciliates possess two types of nuclei, a coding-rich somatic macronucleus (MAC), responsible for most active transcription, and a germline micronucleus (MIC) that is transcriptionally silent during vegetative growth (Prescott 1994). In stichotrichous ciliates, such as *Oxytricha trifallax* (also called *Sterkiella histriomuscorum*), the MAC genome is composed of tens of thousands of chromosomes that typically contain one gene with very limited non-coding sequences. During sexual reproduction, a new MAC develops from a copy of the MIC through a process that eliminates part (>95% in *Oxytricha*) of the MIC genome, including transposable elements, and internal-eliminated sequences (IES). Furthermore, hundreds of thousands of the remaining macronuclear-destined sequences (MDS), flanked by directed repeats called pointers, undergo DNA splicing. These events sometimes involve segment reordering (unscrambling) to assemble the MDS into functional MAC chromosomes, and can also introduce alternative sites of telomere addition (Williams et al. 2002), IES deletion (Prescott and DuBois 1996), or chromosome fragmentation (Seegmiller et al. 1997) to produce somatic variation. Furthermore, duplications have contributed significantly to ciliate divergence and evolution via gene dosage constraints over

Correspondence to: Laura F. Landweber, lfl@princeton.edu.

Yi Zhou and Helmae Wubneh have contributed equally to this work.

Genbank entries MAC A: FJ346571; MAC B: FJ346573; MAC C haplotype 1: FJ346575; MAC C haplotype 2: FJ346577; MIC A: FJ346570; MIC B: FJ346572; MIC C haplotype 1: FJ346574; MIC C haplotype 2: FJ346576; MIC D: FJ349559.

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time (Eisen et al. 2006). As an evolutionary pathway, events of MDS duplication may encourage the appearance of new somatic rearrangements and gene products.

Here we report a novel chimeric MAC chromosome in *O. trifallax* that arose via germline duplication events that brought together MDS segments from two different MAC chromosomes, a process reminiscent of exon shuffling in eukaryotes (Gilbert 1978). The 3.1 kb chimeric MAC chromosome (labeled MAC B in Fig. 1) in *O. trifallax* strain JRB310 is composed of two parts, each of which shares significant identity (>95%) at the nucleotide level with two smaller MAC chromosomes (MAC A and MAC C; Fig. 1). Both MAC A and MAC C contain regions of high similarity to a 3′–5′ exonuclease and a CCCH-type zinc-finger protein from another ciliate, *Tetrahymena*.

Despite the high sequence similarity, each MAC chromosome contains distinct point mutations; therefore, it is unlikely that the chimeric chromosome results from alternative DNA splicing events that use the same MDS segments during MAC development. To investigate the origin of the chimeric chromosome, we cloned the corresponding MIC germline loci of the three MAC chromosomes using a combination of inverse (Ochman et al. 1988) and conventional PCR. We recovered four MIC loci (labeled A, B, C, and D). MIC loci A, B, and C are the corresponding loci for MAC chromosomes A, B, and C, respectively (Fig. 1). MIC loci A and B are homozygous and MIC locus C is heterozygous in *O. trifallax* strain JRB310. The MDS sequences from the respective MIC and MAC loci are identical, including their segregating allelic information for chromosome C. In other words, each MIC allele gives rise to precisely one MAC allele, supporting the conclusion that these MIC sequences are the authentic germline loci for the corresponding MAC chromosomes and not paralogous locations.

Based on sequence similarity, the germline region of the chimeric chromosome (MIC B) paralogous to MIC C most likely originated from one allele (haplotype 2). The duplication seems to have occurred quite recently since most of the duplicated IES regions still show significant similarity (≥75%). Overall, the duplicated sequences at MIC locus B retain the same MDS and IES architecture as MIC loci A and C, with a fusion that created a novel IES flanked by 3 bp pointers. Deletion of this IES creates the chimeric MAC B chromosome during development. Curiously, another paralogous site, MIC locus D, is similar to MIC locus B, but appears truncated, based on PCR. MIC D shares a short region in common with MIC C though absent from MIC B, suggesting that a deletion occurred in MIC B (white rectangle in Fig. 1). PCR and Southern hybridization failed to detect any MAC chromosome derived from MIC D. Therefore, we conclude that it is non-functional.

To examine the functionality of the chimeric chromosome MAC B, we compared the relative DNA copy number of MAC B versus the two shorter chromosomes A and C using Southern hybridization. The relative enrichment of MAC B versus A and C is very similar in different strains of *O. trifallax* and remains stable after sexual reproduction in individual clonal exconjugant cultures (Fig. 2). However, we cannot detect mRNA expression from chromosome B or C using either RT-PCR or Northern hybridization, possibly due to low expression levels under the surveyed physiological conditions. There are no frame-shifts or premature stop codons in the chimeric MAC B chromosome, compared to the MAC A and C coding regions; however, there is also no evidence of purifying selection (Ka/Ks ≈ 1 in homologous exon regions), probably due to the temporary relaxation of purifying selection shortly after a duplication event (Lynch and Conery 2000). Although we cannot determine if the chimeric chromosome produces viable transcripts and is functional, the observed maintenance of DNA copy number levels suggests that it is genetically stable. Therefore, it may be functional or at least reflect the evolutionary mechanisms that generate novelty among ciliate macro-nuclear chromosomes.

An analogy exists between DNA splicing events during ciliate MAC development and exon–intron splicing events during mRNA processing in eukaryotes. Both involve splicing and reassembly of nucleic acid segments to generate functional gene regions, and both display alternative processing (Edwalds-Gilbertet al. 1997; Williams et al. 2002; Kim et al. 2008; Katz and Kovner 2010). Similarly, duplication events of exons or MDSs may occur via unequal or non-homologous recombination, yielding duplicated, and/or chimeric segments, potentially producing new genes and proteins (Thompson and Schild 2001; Zhang 2003). The origin of a novel chimeric somatic chromosome in *Oxytricha* via duplication of MDSs from two different germline MIC chromosomes suggests that ciliates use a mechanism of MDS shuffling, reminiscent of exon shuffling (Gilbert 1978; Katz and Kovner 2010), to “mix and match” MDSs from different sources, thereby increasing the diversity of genetic information available to the organism, and contributing to their relatively high rates of protein evolution (Katz et al. 2004; Zufall et al. 2006). The exact role that MDS shuffling may play in the context of complex gene expression patterns and development is unknown. On one hand, MDS shuffling may create opportunity for the evolution of new genes. One the other hand, it may also provide functional redundancy by gene duplication events.

Methods

Cells and DNA

Vegetative and conjugating cell cultures of *O. trifallax* JRB310 and JRB510 were as described in (Nowacki et al. 2008). Whole cell DNA was extracted using AquaPure Genomic DNA Isolation Kit (BioRad). Micronuclear DNA was further purified from whole cell DNA by low melting point agarose gel electrophoresis.

PCR

Inverse PCR was used to amplify MIC regions containing sequences from the three MAC chromosomes. Conventional PCR was used to determine the self-ligation site of the inverse PCR product and to further extend the MIC sequences. A list of all the primers used and a detailed protocol for PCR are available in the supplementary methods.

Southern Hybridization

Southern hybridizations of isolated whole cell DNA were carried out as described in (Nowacki et al. 2008). The intensities of the bands were quantified using ImageJ (Girish and Vijayalakshmi 2004).

Ka/Ks Calculation

Sequence alignments are generated via tBLASTx. Values of Ka and Ks are calculated using PAML (Yang 2007) with the basic codon substitution models.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

MAC	Macronucleus
MIC	Micronucleus
MDS	Macronuclear-destined sequence
IES	Internal-eliminated sequence

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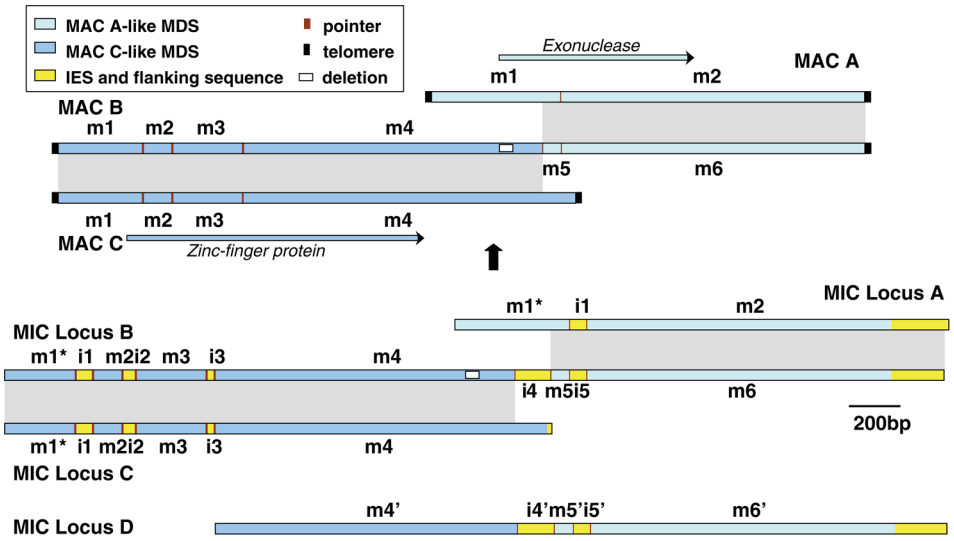

Fig. 1.

Schematic representation of the structures of the macronuclear chromosomes (MAC A, B, and C) and the corresponding micronu-clear loci (MIC Loci A, B, C, and D). Sequence regions sharing high similarity (>90%) are connected by *gray* areas. MDSs are labeled *m*; IESs are labeled *i*. The *arrows* next to MAC A and MAC C indicate the direction and approximate locations of the encoded genes. *Asterisks* indicate partial MDS on the available MIC sequence. *Single quotes* indicate paralogous sites. More details are provided in the supplementary tables

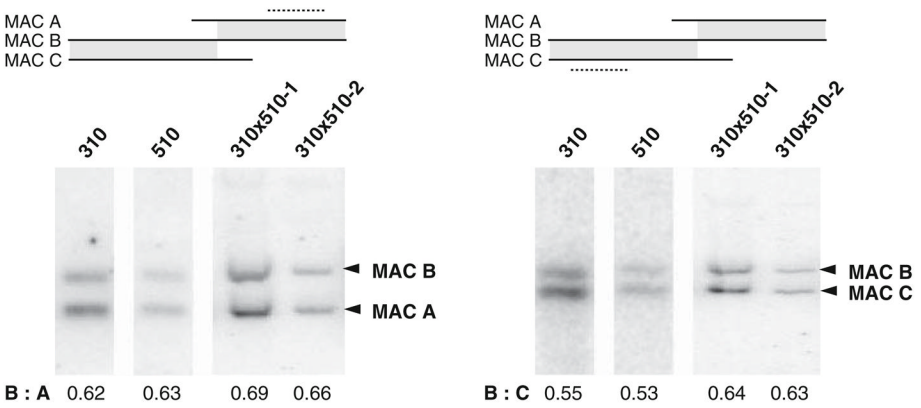

Fig. 2.

Southern hybridization of whole cell DNA from different *O. trifallax* clonal strains. DNA from JRB310, JRB510 and two clonal exconjugant cultures from mating those two strains (310 × 510-1 and 310 × 510-2) were hybridized to probes derived from the shared (>95% identity) regions between MAC A and B (*left panel*) and between MAC B and C (*right panel*). The relative positions of the probes are shown as *dotted lines* in the schematic representation on top of each panel. The relative intensities of the chimeric MAC B versus MAC A and C are shown under the blots