Organization of the Micronuclear Genome of Oxytricha nova

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ABSTRACT

In the hypotrichous ciliated protozoan Oxytricha nova, approximately 95% of the micronuclear genome, including all of the repetitive DNA and most of the unique sequence DNA, is eliminated during the formation of the macronuclear genome. We have examined the interspersion patterns of repetitive and unique and eliminated and retained sequences in the micronuclear genome by characterizing randomly selected clones of micronuclear DNA. Three major classes of clones have been defined: (1) those containing primarily unique, retained sequences; (2) those containing only unique, eliminated sequences; and (3) those containing only repetitive, eliminated sequences. Clones of type one and three document two aspects of organization observed previously: clustering of macronuclear destined sequences and the presence of a prevalent repetitive element. Clones of the second type demonstrate for the first time that eliminated unique sequence DNA occurs in long stretches uninterrupted by repetitive sequences. To further examine repetitive sequence interspersion, we characterized the repetitive sequence family that is present in 50% of the clones (class three above). A consensus map of this element was obtained by mapping approximately 80 phage clones and by hybridization to digests of micronuclear DNA. The repeat element is extremely large (approximately 24 kb) and is interspersed with both macronuclear destined sequences and eliminated unique sequences.

In ciliated protozoa, “germline” and “somatic” distinctions are made at the level of nuclei within a single cell. During vegetative growth of the organism, the macronucleus (the “somatic” nucleus) is responsible for all or most of the transcriptional activity while the micronucleus (the “germline” nucleus) is transcriptionally inert (RAIKOV 1982). During the sexual phase of the life cycle, the micronucleus participates in meiosis and sexual exchange while the macronucleus is destroyed and replaced by a new copy derived from the newly formed diploid micronucleus. The difference in structure between the macronuclear and micronuclear genomes is greatest in hypotrichous ciliated protozoa such as Oxytricha, Stylonychia and Euplotes (KLOBUTCHER and PRESCOTT 1987). The micronuclei in these species contain typical eukaryotic chromosomes (reviewed in RAIKOV 1982), while their micronuclear genomes consist of small, “gene-sized” linear DNA molecules (SWANTON, GRESLIN and PRESCOTT 1980; SWANTON, HEUMANN and PRESCOTT 1980; Kaine and SPEAR 1982; HELFTENBEIN 1985). Massive DNA rearrangement and elimination occurs during the formation of a macronucleus from a micronucleus (AMMERMANN et al. 1974; LAUTH et al. 1976; KLOBUTCHER, JAHN and PRESCOTT 1984; RIBAS-APARICIO et al. 1987). Reassociation kinetics of the micronuclear DNA of the hypotrichs Oxytricha sp. and Stylonychia lemnæ (formerly S. mytilis) demonstrated that 90–95% of the sequence complexity of the micronucleus is eliminated during macronuclear development (LAUTH et al. 1976; AMMERMANN et al. 1974). Because 70% of the Oxytricha sp. genome reassociates as a single-copy component the sequence elimination must include large amounts of unique sequence DNA (LAUTH et al. 1976).

The nuclear duality of ciliated protozoa presents interesting possibilities for genome organization. The lack of transcriptional activity of the micronucleus and the massive extent of genome rearrangements that generate the macronuclear genes suggests that the micronucleus could be the ultimate haven for “selfish DNA” (DOOLITTLE and SAPIENZA 1980; ORGEL and CRICK 1980), where interruption of genes or addition of new sequences would have minimal consequence. In studying micronuclear sequence organization we not only learn how macronuclear genes are recognized for excision but how a whole genome might evolve if not subject to the usual functional constraints.

Previous studies of Oxytricha nova micronuclear genome organization have concentrated on determining how the macronuclear destined sequences are arranged in the micronuclear genome. Thus, either total macronuclear DNA (BOSWELL et al. 1983) or individual macronuclear genes (KLOBUTCHER et al. 1986) were used to select clones from a micronuclear
genomic phage library. These studies demonstrated that the macronuclear destined sequences are clustered together with very short stretches (<550 bp) of eliminated unique sequence DNA interspersed between them. In addition, short stretches of eliminated DNA were shown to exist internally in the macronuclear destined sequences (termed Internal Eliminated Sequences, or IESs) (KLOBUTCHER, JAHN and PRESCOTT 1984; RIBAS-APARICIO et al. 1987). By selecting for clones that hybridized to macronuclear DNA, BOSWELL et al. (1983) also identified a repetitive sequence family that is interspersed with macronuclear destined sequences and comprises up to 10% of the micronuclear genome. As discussed by KLOBUTCHER et al. (1986), if macronuclear destined sequences are clustered, then large regions of the genome must be devoid of them. In this report, we have taken the approach of randomly selecting micronuclear phage clones in order to characterize the overall organization of the O. nova macronuclear genome. Thus, 80% of the clones we have analyzed do not contain macronuclear destined sequences. These clones demonstrate that eliminated repetitive sequences are not highly interspersed with eliminated unique sequences. The only evidence we have obtained for their interspersion comes from further characterization of the repetitive element previously identified by BOSWELL et al. (1983). By isolating clones containing each end of this large (approximately 24 kb) repetitive element, we have demonstrated its interspersion with eliminated unique sequences and clusters of macronuclear destined sequences.

MATERIALS AND METHODS

Enzymes and reagents: Restriction enzymes were purchased from BRL Inc. or Boehringer Mannheim and were used under conditions recommended by the supplier. [32P]dCTP was purchased from New England Nuclear (NEN). DNA polymerase I and its Klenow fragment were purchased from Boehringer Mannheim. Random hexamer primers, (dN)6, (see Labeling reactions below) was purchased from PL Biochemicals.

Plasmids and phage: The phage clones Ch 8-02, Ch 8-07, and Ch 8-010 are described in BOSWELL et al. (1983). Subclones of the 4.5A and 4.5B EcoRI fragments were kindly provided by Art Greslin (University of Colorado). The 11L and 11R probes consist of 0.9 and 2.4 kb EcoRI-BamHI fragments derived from 11 kb EcoRI fragment in Ch 8-02 that were subcloned into pUC12. The micronuclear DNA library used throughout this study consists of Sau3A partial digests of micronuclear DNA cloned into λ 47.1 and was described in KLOBUTCHER, JAHN and PRESCOTT (1984). Additional clones were constructed by inserting EcoRI partial digests of micronuclear DNA into λ phage EMBL4 (FRISCHAUFF et al. 1983). These clones were screened to obtain the phage in group B. Construction of the pMAC and LMAC clones of macronuclear sequences has been described elsewhere (KLOBUTCHER, JAHN and PRESCOTT 1984).

DNA isolation: Phage DNA was purified by standard procedures described in MANIATIS, FRITSCH and SAMBROOK (1982). Macronuclear and micronuclear DNA purification has been described elsewhere (KLOBUTCHER, JAHN and PRESCOTT 1984; JAHN 1988).

Gel transfers: The standard Southern blot procedure for denaturation, neutralization and transfer of DNA from agarose gels in 10X SSC was followed when transfers were made to nitrocellulose. The nitrocellulose blots were baked at 80°C in a vacuum oven for 2–3 hr. For transfer to Gene Screen Plus (NEN), gels were soaked in 0.4 N NaOH for 10 min and the gel was then blotted using 0.4 N NaOH as the transfer buffer. Following blotting, the membrane was rinsed in 0.2 M Tris-HCl, pH 7.5 and dried. Gels of micronuclear DNA were soaked in 0.25 N HCl for 20 min prior to transfer in NaOH as above.

Labeling reactions: Phage, plasmid and micronuclear DNA were labeled by nick-translation using standard methods. Individual fragments were isolated and labeled by electrophoresing the restriction enzyme digested DNA in low melting agarose, cutting out the appropriate bands and labeling (without elution) using DNA polymerase I Klenow fragment and random hexamer primers as described by FEINBERG and VOGELSTEIN (1983).

Hybridizations: All transfers (nitrocellulose and Gene Screen Plus) were hybridized as follows. The transfer was soaked in 4X SSC (1X SSC = 0.15 M NaCl, 0.015 M Na3Citrate), 0.25% powdered milk (JOHNSON et al. 1984) for 1–3 hr at 65°C. The blot was then hybridized in 6X SSC, 0.25% powdered milk, 0.5% SDS. Aliquots of 100 μg/ml sonicated herring sperm DNA and polyA were added to the probe, which was then boiled for 10 min and added to the above mixture. The blots were hybridized with probe for 16–24 hr at 65°C after which they were washed in three or more changes of 2X SSC, 0.5% SDS followed by three or more changes of 1X SSC, 0.5% SDS at 65°C. Transfers made to Gene Screen Plus were kept moist while exposing them to X-ray film and the hybridized probe was subsequently removed by treating the membrane with NaOH as described by the manufacturer. Blots of phage DNA to Gene Screen Plus were reused up to ten times. Blots of micronuclear DNA digests were used up to five times, after which the hybridization was too weak and diffuse to be informative.

RESULTS

Randomly selected clones of micronuclear DNA: To determine the arrangement of eliminated repetitive and unique sequences relative to macronuclear destined sequences, we isolated 30 clones at random from a micronuclear DNA library constructed from Sau3A partial digests of micronuclear DNA. These clones were characterized as follows: (1) each clone was labeled by nick-translation and hybridized to Southern blots of macronuclear DNA to detect possible macronuclear destined sequences; (2) nick-translated micronuclear DNA was hybridized to Southern blots of restriction enzyme digests of the clones to determine which fragments in the clones contained repetitive sequences; and (3) the labeled clones were hybridized to Southern blots of restriction enzyme digests of micronuclear DNA to determine which family of repetitive sequences, if any, was present. The clones were classified into five groups as shown in
Table 1. Three classes of clones (I, II and III, Table 1) are especially prevalent in the micronuclear library and will be discussed individually below.

For simplicity, we refer to the clones as “unique” and “macronuclear destined” according to their behavior in the hybridization experiments described above. The following qualifications should be noted. Clones that do not hybridize to nick-translated micronuclear DNA (classes I and II, Table 1) are referred to as unique. Since this method detects sequences repeated greater than 10–50 times per genome some low copy number repeated sequences would be included in this class. Sequences that hybridized to macronuclear DNA are referred to as macronuclear destined (groups I and V, Table 1). In O. nova and Oxytricha fallax, DNA sequence analysis of macronuclear clones that contain putative precursors of macronuclear sequences detects different versions of the macronuclear sequence in different clones (KLOBUTCHER, JAHN and PRESCOTT 1984; HERRICK 1986). Each of the clones hybridizes to 3–6 size classes of macronuclear sequences. The sizes of the macronuclear sequences detected by each clone add up to enough DNA to occupy most of each insert and thus each clone appears to contain closely spaced macronuclear destined sequences. Another possible cause of hybridization to multiple size classes of macronuclear DNA molecules is “alternate” or “alternative” processing, which produces several differently sized overlapping sequences from the same region of the micronuclear genome (CARTINHOUR and HERRICK 1984; HERRICK et al. 1987a, b). Alternate processing is common in O. fallax (6 of 15 randomly selected macronuclear sequences) (CARTINHOUR and HERRICK 1984). However, we have hybridized more than twenty randomly selected macronuclear clones from O. nova to macronuclear DNA and observed only two cases of multiple size classes. Data on the macronuclear clone insert size and the sizes of macronuclear DNA molecules detected by hybridization for 15 of the macronuclear clones that we know are clones of intact macronuclear molecules or of an EcoRI fragment of an intact molecule are shown in Table 2. The other randomly selected macronuclear clones examined contained inserts unrelated in size to the macronuclear molecule detected by hybridization. The LMAC11b and LMAC5 inserts detect two size classes of macronuclear sequences. Unlike the observations in O. fallax, the different molecules detected by these O. nova clones are very close in size. Several other

### Table 1

Characterization of micronuclear DNA clones

<table>
<thead>
<tr>
<th>Types of sequences in clone</th>
<th>No. of clones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I: Unique, macronuclear-destined</td>
<td>5</td>
</tr>
<tr>
<td>Class II: Unique, eliminated</td>
<td>8</td>
</tr>
<tr>
<td>Class III: Repetitive, eliminated common family</td>
<td>15</td>
</tr>
<tr>
<td>Class IV: Repetitive, eliminated other families</td>
<td>1</td>
</tr>
<tr>
<td>Class V: Unique, macronuclear-destined with repetitive common family</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>30</strong></td>
</tr>
</tbody>
</table>

### Table 2

Size classes of macronuclear sequences detected by macronuclear clones

<table>
<thead>
<tr>
<th>Clone</th>
<th>Size (bp) of insert</th>
<th>Size (bp) of native macronuclear DNA hybridization</th>
<th>Size (bp) of EcoRI digested macronuclear DNA hybridization</th>
</tr>
</thead>
<tbody>
<tr>
<td>pMAC1*</td>
<td>1100</td>
<td>1100</td>
<td></td>
</tr>
<tr>
<td>pMAC2</td>
<td>700</td>
<td>700</td>
<td></td>
</tr>
<tr>
<td>pMAC6</td>
<td>3200</td>
<td>3200</td>
<td></td>
</tr>
<tr>
<td>pMAC7</td>
<td>560</td>
<td>560</td>
<td></td>
</tr>
<tr>
<td>pMAC10</td>
<td>7200</td>
<td>7200</td>
<td></td>
</tr>
<tr>
<td>pMAC11</td>
<td>3000</td>
<td>3000</td>
<td></td>
</tr>
<tr>
<td>pMAC12</td>
<td>500</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>LMAC3</td>
<td>1250</td>
<td>1250</td>
<td>1250</td>
</tr>
<tr>
<td>LMAC8</td>
<td>580</td>
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<tr>
<td>LMAC9</td>
<td>800</td>
<td>800</td>
<td>800</td>
</tr>
<tr>
<td>LMAC12</td>
<td>1700</td>
<td>2000</td>
<td>1700, 980, 750, 300</td>
</tr>
<tr>
<td>LMAC6</td>
<td>2500</td>
<td>6000</td>
<td>2500, 3200</td>
</tr>
<tr>
<td>LMAC11*</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>LMAC11B</td>
<td>1800</td>
<td>1800, 1900</td>
<td>1800, 1900</td>
</tr>
<tr>
<td>LMAC5</td>
<td>1300, 1500</td>
<td>2800, 2500</td>
<td>1300, 1500, 2500</td>
</tr>
<tr>
<td>LMAC4*</td>
<td>7400</td>
<td>7400</td>
<td>3500, 3200, 700</td>
</tr>
</tbody>
</table>

\* pMAC and LMAC are plasmid and λ phage clones, respectively. The macronuclear inserts were cloned using EcoRI linkers.
\* PMAC11 contains two inserts corresponding to different macronuclear molecules.
\* LMAC4 contains a copy of the rDNA gene.
clones detect multiple species upon EcoRI digestion of macronuclear DNA. These presumably represent different versions of the same sequence. The data in Table 2 indicates that alternate processing does not occur frequently in *O. nova* and could not explain the high frequency of micronuclear clones that hybridize to multiple size classes of macronuclear DNA.

The reassocation kinetics of the *Oxytricha* sp. micronuclear genome indicate that approximately 70% of the eliminated sequences are unique (Laught et al. 1976). This contrasts sharply with sequence elimination in Ascaris (Moritz and Roth 1976) and Tetrahymena (Yao and Gorovsky 1974) where most of the elimination involves repetitive sequences. A Cot analysis has not been done on the *O. nova* micronuclear genome. However, by characterizing the randomly selected micronuclear clones it is possible to determine whether large amounts of eliminated unique sequence DNA exist in *O. nova*. In the random sample of clones, eight (class II, Table 1) did not hybridize to macronuclear DNA and were also negative for hybridization with nick-translated micronuclear DNA. We characterized these eight further to see if they truly represented eliminated unique sequences. Five of the eight clones hybridize to EcoRI fragments in digests of micronuclear DNA that correspond in size to EcoRI fragments contained in the clone. Since these hybridizations required high specific activity probes and film exposures equivalent to using unique micronuclear sequences as probes, we believe these clones consist entirely of unique sequence DNA. The other three clones detected a high molecular weight smear of fragments in addition to a set of fragments similar in size to those inserted in the clone. The smear was more intense than the discrete bands. These three clones must include some short stretch of DNA that is repetitive and hybridizes to the high molecular weight smear. The majority of the inserted DNA in these clones behaves as unique sequences. Although we consider contamination with other DNA unlikely, as a control we also hybridized all eight of the unique sequence clones to DNA from Chlorogonium, the food organism for *O. nova*, and did not detect any hybridization.

To further determine the frequency of macronuclear destined sequences among clones containing unique or low copy number sequences and to see if we could find any evidence for their interspersion, we isolated another set of 12 phage. This group was limited to unique sequence clones by selecting for phage that failed to hybridize to micronuclear DNA labeled by nick translation. Approximately 50% of the phage in the *O. nova* micronuclear DNA library meet this criterion. The 12 clones were classified as described for the 30 random clones (Table 1). Only one of these unique sequence clones hybridizes to macronuclear DNA. This clone detects four size classes of macronuclear sequences and thus behaves as if it contains a cluster of macronuclear destined sequences. Overall, with a total of 25 clones (all of the class I and II, Table 1) that contain unique sequences, the clones containing eliminated unique sequences are more prevalent (19 of 25) than those containing macronuclear destined sequence clusters (6 of 25). This demonstrates that most of the low copy number or unique sequences that are eliminated are not interspersed with the macronuclear destined sequences and occur on their own in long stretches that are not interrupted by repetitive sequences.

Sixteen of the 30 clones contain repetitive sequences and 15 of these 16 (class III, Table 1) show a pattern of hybridization to micronuclear DNA similar to that seen by Boswell et al. (1983) and shown in Figure 1. These clones give related patterns of hybridization to EcoRI digested micronuclear DNA. All but one of these clones detect large restriction fragments of 11, 10, 9 and 7.4 kb in size in addition to several smaller fragments. Boswell et al. (1983) showed that a repetitive 11 kb EcoRI fragment isolated from a micronuclear phage clone hybridized to fragments 11, 10, 9 and 7.4 kb in size in EcoRI digested micronuclear DNA indicating that this repetitive element is found in these four different arrangements. In ethidium bromide stained gels of EcoRI digested micronuclear DNA, several intense bands are visible above a background smear, indicating the presence of a high copy number of fragments of certain sizes. As demonstrated previously by Boswell et al. (1983) these prominent bands are identical in size to those detected by hybridization with the repetitive sequence clones. As seen in Figure 1 the pattern of smaller fragments differs from clone to clone. Similar combinations of these small hybridizing fragments are seen with several of these clones.
These additional hybridizing bands suggested that either the element hybridizing to the 11, 10, 9 and 7.4 kb bands is frequently associated with other repetitive elements that hybridize to the other fragments or that the element is very large and a given phage clone contains only a portion of the element, resulting in variable hybridization patterns from clone to clone. In the latter case the 11, 10, 9 and 7.4 kb hybridizing sequences must occur more frequently in the clones than the other portions of the element. The results described below establish that these repetitive sequences comprise one very large element that is interspersed with both macronuclear destined sequences and eliminated unique sequences.

**Isolation of clones containing the repetitive sequence family:** To determine the structure of this repetitive element, we isolated and mapped a series of phage clones that encompass the entire element. A consensus map of the element was defined from the restriction maps of the phage clones and from genomic mapping data obtained by hybridization of the phage clones to restriction enzyme digested micronuclear DNA. Since this repetitive element is large and highly abundant, restriction site polymorphisms and deletions/insertions abound and produce variability in the restriction maps. Even with restriction fragments observed in micronuclear DNA (where we are detecting the most abundant versions) several maps could be derived. Our strategy for isolating the clones and all of their restriction maps are illustrated (Figures 2–4 and 6) in relation to the consensus arrangement of the largest possible form of the element. Each of the hybridization probes used to define the internal structure of the element and the fragments these probes hybridize to are identified in the figures by a different shading design (see Figure 2A and subsequent figures). Throughout this report we will refer to hybridization probes used to define a given region of the element by the size of the “consensus” EcoRI fragment from that region, as shown at the top of each figure.

Individual fragments were isolated from clones as shown in Figure 2A for use as hybridization probes specific for different regions of the repetitive element. Since there is variation in restriction maps from clone to clone for this element, we did not necessarily use a fragment as probe that corresponds in size to the “consensus” fragment size. Hybridization of each of these probes to EcoRI digested micronuclear DNA is shown in Figure 2B. Each fragment hybridizes to one or more size classes of EcoRI fragments. The 4.7 and 11R probes show the greatest heterogeneity with respect to EcoRI sites. The size of the most abundant or largest (when more than one band of high abundance is seen) EcoRI fragment detected by each probe is used in the consensus map. For instance, when the 4.4 kb EcoRI fragment from clone C-A (Figure 2A) is hybridized to EcoRI digested micronuclear DNA it detects fragments of 4.7, 3.5, 1.9, 1.2 and 0.7 kb in size (Figure 2B). Hybridization to the 4.7 kb is of equal or greater intensity than the other sizes. Hybridizations to genomic digests and mapping of the phage clones demonstrates that all of the smaller fragments homologous to this probe are derived from the 4.7-kb EcoRI fragment by deletions or by addition of new EcoRI sites (restriction site polymorphisms). Thus, 4.7 is used as the fragment size in this portion of the consensus restriction map. The variability of other regions of this element relative to the consensus map will be discussed below.

The phage isolated to encompass this repeat comprise seven groups that were selected using “chromosome walking” methods as shown in Figure 3. In those cases where we wanted to walk along the element, the phage were isolated by selecting for phage that hybridized to one end of the previously characterized region of the repeat while selecting against hybridi-
that extends farther than the 4.7-like sequences containing fragments hybridizing to the 1.7 probe immediately leftward (groups D and F, Figure 4) and any clone extending beyond the 4.5-like sequences has fragments hybridizing to the 2.7 probe immediately rightward (clones A13 and A16 in Figure 4). Except for the presence of two versions, where either 2.7- or 4.5-like sequences can be adjacent to 11R sequences, the internal order of these hybridizing regions is maintained in all of the phage we have isolated and mapped (80 total).

Second, we have determined the number of phage that hybridize to each probe in the micronuclear DNA library using replica plaque lifts to determine the coincidence of hybridization with the various probes. Linkage of these hybridizing regions as one large element implies that neighboring regions of the repeat would occur at high frequency in the same phage clone. In addition, for internal regions of the element, if a fragment lying in one direction is not in the same phage, then we would expect to find hybridization to the neighboring fragment from the other direction. We therefore determined the frequency that 4.7-like sequences are surrounded by 11L and 1.7 like sequences and the frequency that 4.5-like sequences are surrounded by 11R and 2.7 DNA sequence homology. We also included 11SAL hybridization in our determinations because maps of the clones (groups D and F, Figure 4) and of genomic digests indicate that the 11L region is deleted in some versions of the element leaving 4.7 and 11SAL-like sequences adjacent to each other. Likewise, the presence of two different versions of the element in the group A clones suggested that we should see a high coincidence of 11R and 2.7-like sequences without coincident hybridization to the 4.5 probe.

The chart in Figure 5 demonstrates that the majority of the clones in the micronuclear library that contain this repetitive element show the pattern of hybridization expected from our consensus map of the different hybridizing regions. Most of the phage hybridizing to the 1.7 probe also hybridize to the 4.7 probe (1.7+4.7+ in Figure 5). The majority of the phage that hybridize to the 4.7 probe also hybridize to the 11L probe (4.7+11L+). Those 4.7 positive phage that fail to hybridize to the 11L probe are positive for either the 11SAL or 1.7 probes (4.7+11L−11SAL+ or 4.7+11L−1.7+). At the right end of the element nearly all of the phage positive for hybridization with the 4.5 probe also hybridize with the 11R probe (4.5+11R+). Half of those that are 11R negative hybridize to the 2.7 probe (4.5+11R−2.7+). Hybridization with the 2.7 probe shows that phage containing the 4.5 kb region between 11R and 2.7 hybridizing regions (2.7+11R+4.5+ and 2.7+11R−4.5+) are equally prevalent to those lacking the 4.5 positive region (2.7+11R+4.5−). Approximately

<table>
<thead>
<tr>
<th>CONSENSUS MAP</th>
<th>PHAGE GROUP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>C</td>
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<td></td>
<td>D</td>
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<td></td>
<td>E</td>
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<tr>
<td></td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>G</td>
</tr>
</tbody>
</table>

**Figure 3.**—Strategy for the isolation of phage clones encompassing the entire repetitive element. Phage clones were isolated by probing the *O. nova* micronuclear DNA library with the hybridization probes shown. Phage were isolated to “walk” along the repetitive element (groups A, B, C, D, and E) by screening for positive hybridization to one probe (+) with negative hybridization to a second probe (−). In cases where additional phage were isolated to verify our consensus genomic map, phage were selected that hybridized to more than one probe (groups A, F and G). The consensus map of the repetitive element is shown at the top of the figure.
half of the phage hybridizing to the 2.7 probe did not hybridize to the other portions of the element and presumably extend into nonrepetitive DNA. This points out that it was much easier to "walk" rightward from the end of the element into adjacent nonrepetitive DNA than it was to move leftward. Many fewer clones in the library (2% vs 40%) contain the 1.7 end without the 4.7 and 11 hybridizing regions than contain the 2.7 hybridizing region without the 4.5 or 11.

Finally, we have hybridized each of the probes to Southern blots of single and double restriction enzyme digests of micronuclear DNA. Although the fragments detected are large and multiple fragments are detected with each probe, the 1.7, 4.7 and 11L probes all hybridize to similar patterns of BamHI, SalI and XhoI fragments (data not shown) indicating that these large fragments span from within the 11 kb EcoRI fragment leftward to conserved sites within the repetitive end. Similarly the 11R, 4.5 and 2.7 probes show related patterns of hybridization to BamHI, BglII, SalI and XhoI digested micronuclear DNA. Unfortunately, the complexity of the patterns of bands seen with each probe did not allow the construction of a consensus map for these other restriction enzymes.

**Multiple versions of the repetitive element:** As mentioned previously, both the restriction maps of the clones and the genomic hybridizations demonstrate heterogeneity in this repetitive element. Some of the alternate forms are equally prevalent in the genome. Variability in the middle of the element results in the presence of 11, 10, 9 and 7.4 kb EcoRI fragments in micronuclear DNA. This variation is seen in the restriction maps of the clones as well. For example, comparison of the XhoI-EcoRI maps of clones G6 and G8 to that of G2 and G7 (Figure 4) shows a loss of sequences lying in the 11SAL hybridizing regions of G2 and G7. In both G7 and G3, new EcoRI sites are present toward the right end of the 11 kb hybridizing regions. The small EcoRI fragments hybridize to 11R and correspond to fragments hybridizing in 11R in digests of micronuclear DNA (see the 11R lane in Figure 2B). At the left end of the element, in all cases where smaller fragments hybridizing to the

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**Figure 4.**—Restriction maps of phage encompassing the repetitive element. Representative maps of clones isolated by screening the micronuclear DNA library are shown. The alphabetical designation of each clone refers to the phage groups shown in the screening strategy (Figure 3). Maps of clones from group A are aligned with the consensus map at the EcoRI site at the right end of the 11-kb EcoRI fragment. The other maps are aligned with the left EcoRI site. Hybridization to the 1-5 and 11R probes are shown above the hybridization to XhoI-EcoRI fragments (EcoRI = ●; XhoI = △).
4.7 probe occur (clones D88–90, F201–205 and A8, 9, 11, 12), the DNA sequence homology to 11L is missing. We have not mapped the internal regions of the 4.7-kb fragment in enough detail to know what parts are missing, but it seems possible that a large deletion could remove the rightward half of the 4.7 fragment and the leftmost portion of the 11-kb region. A similar sized deletion could account for the 2.7 hybridizing region at the left end. Ten clones that were isolated from the right end, ten clones that were isolated by walking "leftward" from the 1.7 hybridizing region (phage group E) and "rightward" from the 2.7 hybridizing region (phage group B) contain unique sequence DNA (fragments designated without any design). The black areas indicate hybridization to nick-translated micronuclear DNA which detects additional repetitive sequences, with the region hybridizing to the 1.7 or 2.7 probes shown above the micronuclear DNA hybridization. The clones that have stars next to the number contain sequences that hybridize to macronuclear DNA (EcoRI = +; XhoI = Δ).

**Preliminary characterization of the repeat element end points:** As mentioned earlier, the ends of the repeat element were defined by continuing the "walk" until inserts in the isolated phage contained unique sequence DNA adjacent to the repetitive sequences. Digests of all of the clones were probed with unique sequences (Figure 6, group E clones). In all of the clones selected with the 1.7 probe, the repetitive sequences extend beyond the 1.7 hybridizing region. When these clones are hybridized to EcoRI digested micronuclear DNA, no conserved EcoRI fragment is detected by this adjacent DNA. We do, however, detect an increased background smear of hybridization with these clones. Currently, at this end, the terminus of the element is not defined relative to any conserved restriction sites. We found that 5 of 10 and 4 of 10 of the clones hybridizing to the 2.7 and 1.7 ends, respectively, hybridized to macronuclear DNA sequences. Thus, this element is frequently associated with macronuclear destined sequences. Three of these clones show evidence of clustered macronuclear destined sequences: two hybridize to three size classes and one hybridizes to five size classes of macronuclear DNA. It should be noted that most of the clones isolated in groups B and E do not contain an EcoRI fragment of the consensus 2.7- or 1.7-kb size. It seems possible that our selection scheme for these groups of phage yields primarily nonconsensus arrangements because of a bias in cloning. Nevertheless, clones in groups B (hybridizing to 2.7 but not 11R) and E

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**Figure 5:** Determination of linkage of different portions of the repetitive element by hybridization to replica plaque lifts. Four replica transfers of 1000 to 1200 phage from the 0. nea micro- nuclear library plated on 150-mm petri plates were hybridized with either the 1.7, 4.7, 11L and 11SAL probes of the 2.7, 4.5, 11R and 11SAL probes. The replica lifts were then compared to determine the number of phage that hybridized to the combinations of probes shown. Data was averaged for replica transfers from three different plates. The combinations of probes used and the percentages of hybridizing phage are displayed relative to the consensus map. The bottom of the chart includes the percentages of phage that hybridized to each probe by itself.

**Figure 6:** Phage containing the ends of the repetitive element. These two groups of phage represent the ends of repetitive element. Most of the phage isolated by walking "leftward" from the 1.7 hybridizing region (phage group E) and "rightward" from the 2.7 hybridizing region (phage group B) contain unique sequence DNA (fragments designated without any design). The black areas indicate hybridization to nick-translated micronuclear DNA which detects additional repetitive sequences, with the region hybridizing to the 1.7 or 2.7 probes shown above the micronuclear DNA hybridization. The clones that have stars next to the number contain sequences that hybridize to macronuclear DNA (EcoRI = +; XhoI = Δ).
(hybridizing to 1.7 but not 4.7) are present as 0.5% and 1%, respectively, of the phage in the library. Thus we do not believe that these examples of junctions between the element and unique sequence DNA are exceptional. By examining more examples of clones hybridizing to the 1.7 and 2.7 probes without selecting against hybridization to the 11R or 4.7 probes, we may find more examples of clones containing the consensus size EcoRI fragments.

We have examined two other possible characteristics of the termini of this element. To look for end to end DNA sequence homology as possible direct or inverted repeats, we labeled each of the fragments mapping rightward from the 2.7 hybridizing region in the group B clones shown in Figure 6 and determined whether they hybridized to phage plaques that hybridized to the 1.7 probe ("left end") on a replica transfer of the micronuclear library. None of these "right end" fragments hybridized to plaques that hybridized with the "left end" probe. (There is also no cross-hybridization between the 1.7 and 2.7 EcoRI fragments). As a second possibility, we looked for hybridization to G_4T_4 or C_4A_4 repeats at the ends of the element. In both Tetrahymena thermophila and O. fallax, macronuclear telomeric repeat sequences have been found at the ends of repetitive elements and are proposed to have arisen during transposition of the elements (Cherry and Blackburn 1985; Herrick et al. 1985). In O. fallax, the small size of these telomeric repeats allowed hybridization only with a (G_4T_4)_2 oligonucleotide and not a (C_4A_4)_2 oligonucleotide (Herrick et al. 1985). We used the same oligonucleotides and hybridization conditions to determine whether telomeric repeats were present at the ends of this repetitive element. We probed replica plaque lifts of the O. nova micronuclear DNA library and looked for hybridization of either oligonucleotide to a plaque that hybridized to either the 1.7 or 2.7 probes. Although the frequencies of G_4T_4-positive, C_4A_4-negative and G_4T_4-positive, C_4A_4-positive plaques are very similar to that observed in O. fallax (Herrick et al. 1985), none of these positively hybridizing plaques corresponded to those with DNA sequence homology to the left or right end probes.

Determining the copy number of the repetitive element: We have quantitated the amount of the repetitive element in the genome by hybridizing different phage clones containing the element to dilutions of EcoRI digested micronuclear DNA electrophoresed and blotted side by side with dilutions of digests of each of the page (Figure 7). We used three different clones (A11, A13 and F206, restriction maps shown in Figure 4) in order to include all regions of the element. Comparison of the hybridization of phage insert fragments to the EcoRI fragments in micronuclear DNA indicates that the different regions of the element are present in similar amounts. Comparing the intensity of hybridizations of different dilutions yielded values of 9–12% as the amount of the micronuclear genome comprising the element, or a range of 2250–3000 copies per genome (assuming 6 × 10^8 bp as the genome size, based on the published value for Oxytricha sp.) (Lauth et al. 1976).

**DISCUSSION**

Characterization of randomly chosen genomic clones provides an alternative to reassociation kinetics as a method of determining repetitive sequence content and interspersion in the genome. The random clone method was recently used to characterize the Arabidopsis thaliana genome and yielded interspersion data that was not available from reassociation kinetic data (Prutt and Meyerowitz 1986). In ciliates, this is a particularly useful method for determining the interspersion of eliminated and retained (macronuclear) sequences in addition to repetitive and unique sequences. Prutt and Meyerowitz (1986) have discussed the importance of complete genomic representation in the library and of adequate sample size in using this method to study sequence organization. The micronuclear DNA library used in this study was sufficiently large to be representative and in addition has been successfully screened for at least eight single copy genes. Under-representation of only one class of sequences has been noted: clones of sequences homologous to C_4A_4 repeats are present at one-tenth the number expected. However, evidence suggests that these repeats are telomeric in O. nova (Jahn 1988) as observed previously for O. fallax (Dawson and Herrick 1984) and therefore would not be clonable by the usual methods used for producing genomic libraries. We thus believe that the interspersion trends visible in this random sample should be representative.
of the genome. On the other hand, our sample of clones is not large enough to make estimates of the amounts of repetitive or unique sequences or the number of macronuclear-destined sequence clusters: 30 clones of 15-kb average size account for 0.075% of the micronuclear genome. We therefore interpret our results as demonstrating three major aspects of micronuclear genome organization, corresponding to the three major classes of clones obtained: (1) a high prevalence of a large (approximately 24 kb) repetitive element, (2) clustering of macronuclear destined sequences, and (3) the occurrence of long stretches of eliminated unique sequence DNA.

The isolation of clones encompassing all of the repetitive element allowed us to determine its copy number and interspersion in the micronuclear genome. At the resolution of the hybridization probes we used to define the element, there is no internal redundancy. Thus, it does not appear to be derived from a block of tandemly repeating units. The element is much larger than any interspersed middle repetitive elements we have seen described (reviewed by Bouchard 1982). Because of its size, it would be very interesting to know how this element has become dispersed throughout the O. nova genome. In an attempt to determine whether it has any transposable element like properties, we tried to find inverted or direct repeats at the ends of the element. By hybridization with terminal fragments, we were unable to detect any DNA sequence homology between the ends of the element. However, the terminal repeats of transposable elements or retrotransposons can be very short sequences. These would go undetected in our hybridizations. It is also possible that many copies of the element have lost their termini. The fact that most of the terminal clones we isolated are lacking the consensus arrangement of EcoRI sites may mean they are lacking the termini. Knowing the structure of the termini will require more detailed mapping and DNA sequence analysis of the element endpoints. As a further investigation into the dispersal of this element, we also plan to determine whether all or portions of it are found in the micronuclear genome of other Oxytricha sp.

The frequency of clones containing this repetitive element indicates that it comprises a majority of the interspersed repetitive DNA in the O. nova micronuclear genome. This does not mean that this is the only repetitive sequence element in the O. nova genome: there could be families of smaller elements present in fewer copies (less than 1000 copies per genome) that would go undetected by our random sample. Two other families have been identified in clones that were selected by hybridization to C<sub>4</sub>A<sub>4</sub> repeats (C. L. Jahn, unpublished data). These are both present in lower copy number (100–1000/genome) and have not been characterized further. We have also identified three families of tandemly repeating sequences that comprise approximately 10% of the genome (C. L. Jahn, unpublished data). These families occur in large tandem arrays and therefore cannot be dispersed to very many locations.

We expected to define an interspersion pattern of eliminated and retained sequences that reflects the band by band breakdown of the micronuclear chromosomes during macronuclear development (Prescott and Murty 1973). If the clusters of macronuclear sequences were evenly distributed throughout the chromosomal bands and vesicles that are visible in the developing macronucleus, we would expect approximately seven macronuclear sequences per cluster [see Klobutcher et al. (1986) for calculations]. The clusters would then occupy stretches of at least 20–30 kb and the eliminated sequences would occur in 200–300 kb stretches between the clusters. To date, macronuclear sequence clusters have not been observed adjacent to long stretches of eliminated unique sequences. We would expect clones that spanned such a region to contain a large stretch of eliminated unique sequence DNA contiguous with a region that hybridizes to a macronuclear sequence at one end of the clone. These would appear different from the other clones by virtue of their hybridization to single macronuclear sequences. It seems quite likely that we have not screened enough clones to find this type of interspersion (only one clone demonstrating interspersion of the repetitive element with macronuclear sequences was obtained in the random selection).

The only evidence we have for a genome-wide interspersion pattern comes from the characterization of the repetitive element. The isolation of clones containing junctions of the element with neighboring unique sequence DNA demonstrates that this element is interspersed with both eliminated unique sequences and macronuclear destined sequences. Our current picture of its interspersion includes the two possibilities shown in Figure 8. Since we have isolated clones containing continuous blocks of up to 25 kb of either clustered macronuclear destined sequences or eliminated unique sequences we assume that either can be adjacent to the element. With this assumption, we can define the sequence organization of regions approximately 45–50 kb in length (Figure 8). Several questions need to be answered to determine how these two patterns are arranged relative to each other: (1) how large are the clusters of macronuclear destined sequences and the stretches of eliminated unique sequence DNA, (2) are long stretches of eliminated unique sequence DNA ever adjacent to macronuclear sequence clusters, and (3) does the repetitive element interrupt clusters or does it border clusters? With respect to this last question, the repetitive element...
from *O. fallax* that contains C_4A_4 repeats at its termini (TBE1) has been shown to interrupt a possible macronuclear destined sequence (HERRICK et al. 1985, 1987a). In addition, we have identified a repetitive element in *Euplotes crassus* that interrupts the macronuclear sequence clusters (C. L. JAHN, L. NILLES and M. KRIKAU, unpublished data). Thus it seems possible that the *O. nova* element is occurring within macronuclear sequence clusters. One surprising feature of the interspersion of this repetitive element is that it is approximately equally distributed between the two types of arrangements shown in Figure 8. Since the macronuclear sequences represent 10% or less of the micronuclear genome the repetitive element must be preferentially associated with macronuclear destined sequences. We have observed a similar interspersion pattern for the highly prevalent interspersed repetitive element family identified in *E. crassus* (C. L. JAHN, L. NILLES and M. KRIKAU, unpublished data).

The characterization of a random sample of micronuclear clones presented here and the previous studies of clones hybridizing to macronuclear sequences (BOSWELL et al. 1983; KLOBUTCHER et al. 1986) indicate a segregation of sequences to be eliminated from those that are retained in addition to a low level of interspersion of the eliminated repetitive and unique sequences. These eliminated sequences each occur in very long stretches with similar stretches of DNA carrying clusters of macronuclear destined sequences. Only a small amount of eliminated DNA occurs within these clusters. The organization of the *O. nova* genome differs significantly from what is observed in random samples of micronuclear clones from *T. thermophila*. Analyses similar to those reported here demonstrate that eliminated sequences 1–10 kb in size are dispersed throughout the *T. thermophila* genome, occurring on the average, once every 30–40 kb (KARRER 1983; YAO et al. 1984; HOWARD and BLACKBURN 1985). These eliminated sequences have been compared to IESs in hypotrichous ciliates because they require breakage and rejoining of the chromosome for their removal (YAO et al. 1984; AUSTEBERRY and YAO 1987). The end result of the elimination processes in Tetrahymena and Oxytricha are very different. In Tetrahymena, 10–20% of the genome is eliminated (YAO and GOROVSKY 1974), producing approximately 100 molecules averaging 600 kb in size (ALTSCHULER and YAO 1985; CONOVER and BRUNK 1986). Most of the elimination is internal to the resulting molecules. In Oxytricha, 90% or more of the genome is eliminated producing approximately 20,000 molecules averaging 2.2 kb in size (LAUTH et al. 1976; SWANTON, HEUMANN and PRESCOTT 1980). The IESs account for less than 1% of the eliminated sequences (RIBAS-APARICIO et al. 1987). Thus, most of the eliminated sequences are external to the resulting molecules. The segregation of the eliminated and retained sequences in Oxytricha suggests there are two kinds of eliminated sequences in the micronuclear genome; (1) IESs which require breakage and rejoining, and (2) sequences occurring outside the macronuclear sequence clusters, which would not require rejoining of DNA. The different kinds of sequences could be eliminated by different mechanisms.

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**LITERATURE CITED**


DOOLITTLE, W. F., and C. SAPIENZA, 1980 Selfish genes, the


Yao, M-C., and M. A. Gorovsky, 1974 Comparison of the sequences of macro- and micronuclear DNA of Tetrahymena pyriformis. Chromosoma 48: 1–18.


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