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Macronuclear DNA of *Tetrahymena thermophila* exists as defined subchromosomal-sized molecules

Marsha I. Altschuler[1] and Meng-Chao Yao

Department of Biology, Washington University, St. Louis, MO 63130, USA

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ABSTRACT

Using the method of orthogonal-field-alternation gel electrophoresis, we have resolved the macronuclear DNA of Tetrahymena thermophila into a series of distinct bands. Using electrode switching intervals ranging from 10 to 70 seconds we have resolved DNA bands ranging in size from about 21 kb up to and beyond the size of yeast chromosomes VII and XV. Hybridization of Southern blots from these gels to both unique and repetitive DNA sequences shows that the macronuclear genome of T. thermophila has a precise organization. The unique sequences tested each hybridize to only one band of macronuclear DNA and the hybridization patterns seem to be identical in several inbred strains examined.

INTRODUCTION

Cells of Tetrahymena thermophila contain two distinctly different nuclei--a diploid germinal nucleus (the micronucleus) and a somatic nucleus (the macronucleus) which contains most of the micronuclear DNA sequences amplified approximately 53-fold (1,2). Considerable information has been gained about the organization of specific genes and other unique and repetitive DNA sequences in the two nuclei (3-14). Many DNA sequences in the micronucleus are known to exist in the macronucleus in altered form due to deletion and/or fragmentation events that occurred during formation of the macronuclear genome (12-15). However, the larger scale organization of the macronuclear genome is not as well understood. An important issue concerns the size of the intact macronuclear DNA. Whereas cytological observation shows that the micronuclear genome is organized into five pairs of chromosomes (16), no resolution of the macronuclear genome into discrete chromosomes has resulted from microscopic methods. Genetic observations have shown that genes linked in the micronucleus are unlinked in the macronucleus, thus suggesting that the macronuclear genome consists of fragmented micronuclear chromosomes (17). Physical evidence for chromosomal fragmentation also exists. Studies of the ribosomal RNA gene indicate that the micronuclear chromosome is fragmented at the two ends of the rDNA during macronuclear development (18, 19). Fragmentation at other sites

on the micronuclear chromosomes has also been shown (20), although it is not known how widely this process occurs in the genome. Sucrose gradient analysis of DNA from gently lysed cells indicates an average macronuclear chromosome size of 633 kb (21). Viscoelastic measurements, on the other hand, indicate that the macronuclear genome is organized mainly as large molecules up to 40,000 kb in size (the size of micronuclear chromosomes) (22). To resolve this issue, we have employed the method of pulsed field gradient gel electrophoresis (23, 24) as modified and renamed orthogonal-field-alternation gel electrophoresis by Carle and Olson (25). Using this method we are able to reveal the sizes of intact macronuclear DNA, and show that with respect to DNA fragment size the macronuclear genome shows a rather precise and stable organization.

MATERIALS AND METHODS

Strains

Stocks of strain B isolates CU399, CU401, P2f, CU427, and CU428 were obtained from P. Bruns of Cornell University. Strains A*III, C3-368V, A1873I, and F1668II were obtained from D. Nanney of the University of Illinois.

Sample preparation

Two methods of sample preparation were used.

Lysate method. Cells were grown to late-log phase ($5x10^5$ cells/ml) in 100 ml of PPYS medium (1% proteose peptone, .1% yeast extract, .003% Sequestrene). The cells were gently pelleted in a table-top clinical centrifuge, resuspended in 100 ml of 10 mM Tris, pH 7.4, and shaken gently at 30^o for five to six days. These starved cells were then pelleted by centrifugation as before and the pellet (approximately 200µl) was squirted into 200µl of NDS buffer (10 mM Tris, pH 9.5, 1% sodium dodecyl sulfate, .5 M EDTA) preheated to 65^o. The sample was kept at 65^o overnight to ensure complete lysis. Some samples were treated with .1 mg/ml proteinase K at 65^o overnight. Proteinase and non-proteinase treated samples gave identical banding patterns following gel electrophoresis and staining. Samples were then stored at 4^o until used. No change in electrophoretic behavior was observed for samples stored in this manner for up to four months.

Microbead method. The second method involved embedding cells in agarose microbeads (26). Approximately $3x10^7$ cells in late-log phase of growth were gently pelleted and mixed with an equal volume of 2% low temperature agarose (Biorad) at 37^o. Five ml of mineral oil (Squibb) preheated to 37^o was added and the mixture was mixed thoroughly at the highest setting of a vortex mixer at room temperature and then placed on ice for 15 minutes. NDS buffer was added, the microbeads pelleted, and the mineral oil supernatant removed. Lysis

and deproteinization were accomplished by incubating the microbeads in NDS buffer containing .1 mg/ml proteinase K at 60° for 2-14 hours. The beads were pelleted and washed twice with TE (10 mM Tris, 1 mM EDTA, pH 7.4) and stored in TE at 4°.

Yeast DNA samples were obtained from G. Carle and were made from strain AB972 as described previously (25).

Gel electrophoresis

The methods used for orthogonal-field-alternation gel electrophoresis were as described by Carle and Olson (25). Gels were 1.5% agarose in .4x or .5x TBE buffer (1x TBE is 90 mM Tris base, 90 mM boric acid, 2.5 mM EDTA, pH 8.2). One-tenth volume of 10x dye solution (50% (w/v) ficoll, .2% bromphenol blue, .2% xylene cyanole, 400 mM EDTA) was added to the samples which were then loaded onto the gel without further dilution. Sample volumes were 10µl (about 5 µg of DNA) for 6 mm wide wells and 50-80µl (about 25-40 µg of DNA) for 33 mm wide wells. The term switching interval refers to the length of time each pair of electrodes is active (25). Following electrophoresis the gels were stained for 30 minutes in .5 µg/ml ethidium bromide and then destained and photographed.

Southern blots

Before blotting, gels were treated for 20 min in .4 M HCl followed by 20 min in denaturation buffer (1.0 M NaCl, .5 M NaOH) and 20 min in neutralization buffer (.5 M Tris-HCl, pH 7-7.5, 1.5 M NaCl). They were blotted to .45µ Nitrocellulose (Schleicher and Schuell) for 24-36 hours in 6x SSC (.9 M NaCl, .09 M sodium citrate, pH 7) following the method of Southern (27). Filters were baked at 65° for at least six hours before hybridization.

DNA labelling, hybridization, and autoradiography

All DNAs were labelled *in vitro* by nick translation (28). The radioisotopes used were ^{32}P-dATP and ^{32}P-dCTP (Amersham, 3000 Ci/mmole). The specific activity of nick-translated probes was at least $1x10^8$ cpm/µg. Filters were prehybridized at 65° for 18-24 hours in a prehybridization solution consisting of 6x SSC, 50 mM phosphate buffer, 5x Denhardt's solution (29), .1% sodium dodecyl sulfate, .01% sodium pyrophosphate, and .25 mg/ml sheared herring sperm DNA. Hybridization was carried out for 36 hours at 65° in hybridization solution consisting of 6x SSC, 20 mM phosphate buffer, .4x Denhardt's solution (29), .1% sodium dodecyl sulfate, .005% sodium pyrophosphate, and .125 mg/ml sheared herring sperm DNA. Nick-translated probes were denatured by boiling for 10 min and then added to the hybridization solution at a concentration of 10^6-10^7cpm/ml. 1x SSC was used in the prehybridization and hybridization solutions instead of 6x SSC when clone pTt2512/Hha.9 was used as the probe. Following hybridization,

filters were washed for one hour at room temperature in 2x SSC, .5% sodium dodecyl sulfate followed by two or three one-hour washes at 65° in 3x SSC, .01% sodium pyrophosphate, .05% sodium sarkosyl. Some hybridizations were washed more stringently in .6x SSC for one hour at 65°. Autoradiography was accomplished using Kodak XAR-5 X-ray film with a Dupont intensifying screen at -70° for 1-3 days.

RESULTS

The general appearance of macronuclear DNA

Figures 1 and 2 show examples of ethidium bromide stained gels and parts of gels containing Tetrahymena thermophila samples fractionated by the method of orthogonal-field-alternation gel electrophoresis (25). The overall pattern on the gels is as Carle and Olson described with the bands extending in a rather straight line across the gel although with a slight downward slope at the outer edges. The only major distortion is a bowing in of the outer margins of the gel but this does not interfere with the banding pattern observed when a single sample is electrophoresed on each half of the gel as in Figure 1. However if many different samples are to be electrophoresed on a single gel (using 6 mm wide gel wells) then only the center six or eight wells will yield

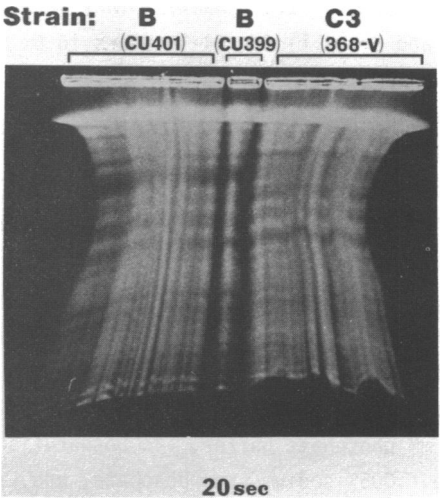

Figure 1. Overall appearance of an ethidium bromide stained OFAGE gel. Samples were electrophoresed for 16 hours at 300 v using a 20 second switching interval. The CU399 sample was loaded into a 6 mm wide well; the others were in 33 mm wide wells. The samples were made from the strains indicated above the gel wells using the lysate method of sample preparation.

clear results. Uneven distributions of DNA across a gel lane are often observed (see Fig. 1). This is probably due to the gentle handling of the lysate samples which precludes any thorough mixing. This was less of a problem when samples were prepared as agarose microbeads and could be thoroughly mixed without shearing the DNA. All of these slight distortions should be kept in mind when examining the autoradiograms in Figures 3-7.

T. thermophila samples were electrophoresed alongside yeast chromosome preparations and lambda DNA HindIII digests using switching times from 10 to 70 seconds. Figure 2 shows representative results of ethidium bromide stained gels. It is apparent that the majority of the whole cell DNA migrates in these gels as distinct bands. The size of the DNA spans the size range of the yeast chromosomes. The smallest size DNA observed runs approximately the same as the 23.6 kb lambda HindIII fragment (data not shown). Tetrahymena DNA which migrates slower than the eleventh yeast DNA band is observed (this band corresponds to yeast chromosomes VII and XV (25)). From genetic linkage map data, yeast chromosomes VII and XV are estimated to be 1100 kb in size (30). The average size of macronuclear DNA reported from sucrose gradient analysis (633 kb)

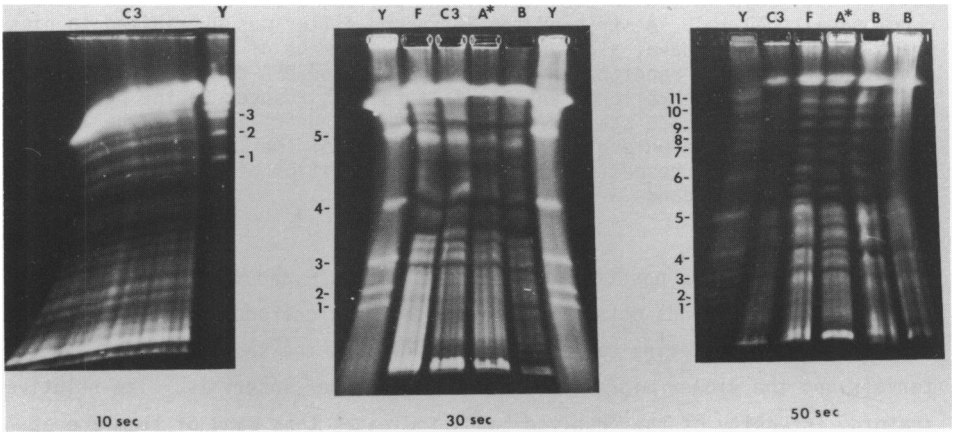

Figure 2. Effect of different switching intervals on the resolution of macronuclear DNA in inbred strains. The samples were electrophoresed at 300 v for 18, 19, and 20 hours using 10, 30, and 50 second switching intervals respectively (indicated below each gel). The lanes marked "Y" contain DNA from yeast strain AB972. The yeast DNA bands are numbered as in Carle and Olson (ref. 25). The Tetrahymena samples were prepared by the microbead method and were made from the strains indicated above the gel wells (the B strains used were CU427 for the 30 second gel, and in the 50 second gel from left to right, P2f and CU428). Only parts of the gels are shown: the left half of the 10 second gel and the middle six wells of the 30 and 50 second gels.

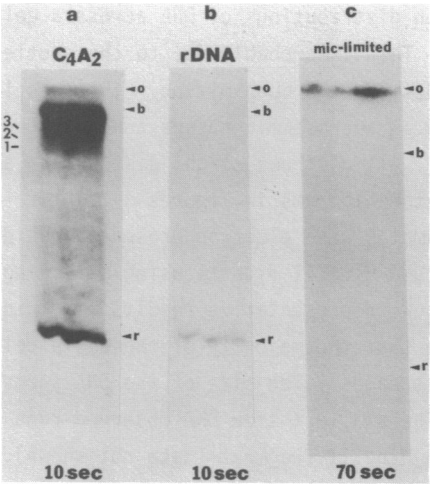

Figure 3. Hybridization to telomeric repeats, rDNA, and a micronucleus-limited sequence. Southern blots were made from gels containing lysates from strain CU399 electrophoresed at 300 v for 18 hours using a 10 second switching interval (a and b) or for 17 hours using a 70 second switching interval (c). A strip of the filter from the 10 second gel was hybridized to a nick-translated CCCCAA-containing clone (pTt2512/Hha.9) (a), and then, following removal of this probe, rehybridized to nick-translated pTt122, a subclone of the extra-chromosomal rDNA (b). A strip of the 70 second filter was hybridized to nick-translated pTt2512/Hha-a, a gel-purified HhaI fragment of clone pTt2512 which contains a copy of a repetitive micronucleus-limited DNA sequence (c). Arrows indicate three landmarks to orient the filters with respect to the patterns seen in ethidium bromide stained gels: "o", "b", and "r" indicate the locations of the gel wells, the unresolved DNA near the top of the lane, and the smallest DNA band (the rDNA), respectively. In 3a, the position of the three fastest migrating yeast DNA bands are shown to the left.

(21) corresponds to a position between yeast bands 5 and 6.

The number of bands observed varies with the electrode switching interval, with the faster migrating bands better resolved at the shortest switching intervals and the slower migrating bands at the longer intervals. The relative staining intensity of the resolved bands indicates that many of them are at least doublets. Using the ten second gel in Figure 2 to count up the number of bands migrating faster than the first yeast band, the 30 second gel for bands between the first and fifth yeast bands, and the 50 second gel for slower migrating bands, a total of 44 bands of DNA have been resolved. If each band were to contain a single type of DNA molecule, then summing the product of each band multiplied by its estimated molecular weight accounts for 27,000 kb of DNA at most. A macronuclear haploid genome equivalent of .18 pg (that is,

85% of the micronuclear genome content of .21 pg) translates into 173,400 kb of DNA. Thus, much of the macronuclear DNA in our gels remains unresolved as either very high molecular weight material near the top of the gel or as co-migrating bands throughout the gel.

Figures 1 and 2 also demonstrate that the sizes of the DNA molecules observed among various inbred T. thermophila strains are quite similar. With the large number of bands present in these gels it is difficult to say that the banding patterns are identical, yet at least the major bands and the areas where there are no bands are positioned the same for all the strains examined. We have compared five strain B isolates (CU399, CU401, CU427, CU428, and P2f) at several different switching intervals and found no significant differences in their DNA patterns. The other inbred strains have been compared to strain B and to each other only for the switching intervals shown except for strains A*III and A1873I which were also compared to strain B using 70 and 40 second switching intervals respectively.

Hybridization to telomeric sequences, rDNA, and a micronucleus-limited sequence

The DNA banding patterns are highly reproducible in samples prepared from the same strain at different times and is also apparently identical in samples made from different inbred strains (Fig. 1 and 2). Thus, it is unlikely that the observed DNA bands are artifacts of the experimental procedures due to breakage or degradation of larger molecular weight DNA during sample preparation. Support for this argument is provided by hybridization of Southern blots to probes containing various cloned macronuclear DNA sequences. The first probe used contains CCCCAA repeats. Tandem repeats of this hexanucleotide are known to be located near the ends of macronuclear chromosomes as judged by their BAL31 nuclease sensitivity (20). Figure 3a shows the hybridization of a C_4A_2-containing probe to an array of the fastest migrating DNA bands. The hybridization extends from the fastest migrating band ("r") (which hybridizes quite strongly), throughout the area of band resolution (molecular sizes of 21 kb to about 260 kb) where all bands hybridized to a similar extent, to the top of the gel lane where most of the DNA ("b") remains unresolved and thus hybridization to the probe is strong. Thus, it appears that the small DNA molecules that are resolved in this 10 second gel each contain at least one, presumably telomeric, C_4A_2 repeat and therefore most likely represent intact macronuclear DNA molecules and not random degradation products.

From previous studies which showed that the palindromic rDNA molecule was the smallest molecule in the macronucleus, it seemed likely that the fastest migrating band seem in the ethidium bromide stained gels would contain this

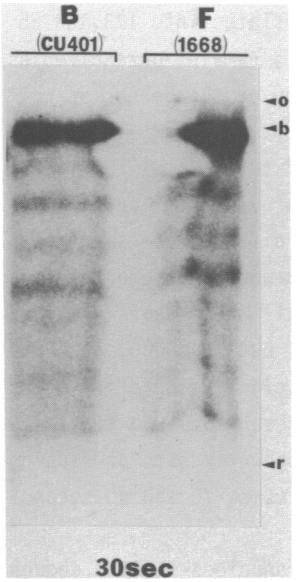

Figure 4. Hybridization to a 5S gene clone. A gel containing two 33 mm wide wells in which lysates prepared from strains B (on the left) and F (on the right) were loaded was electrophoresed for 16 hours at 300 v using a 30 second switching interval. The gel was blotted and a strip cut from the center of the filter was hybridized to nick-translated clone pDP5 obtained from D. Pederson (Univ. of Rochester). This pBR322 clone contains a 280 bp BamH1 micronuclear DNA fragment consisting of a 5S gene and its A-T rich spacer. The markers "o", "b", and "r" are described in the legend to Figure 3.

21 kb molecule (31). Hybridization of a cloned segment of the Tetrahymena rDNA to Southern blots of these gels yields a single band of hybridization in lysates prepared from either growing or starved cells of strain B and the band that hybridizes is the fastest migrating one (Figure 3b).

Due to the large size of micronuclear chromosomes and the fact that micronuclear DNA is present in the cell at only 1/11th the amount of the macronuclear DNA, we did not expect the micronuclear DNA to contribute to the DNA bands resolved in our gels. To determine the location of the micronuclear DNA, we used as a hybridization probe a sequence from clone pTt2512 which is repetitive in the micronucleus and totally absent from the macronucleus (7). As shown in Figure 3c, hybridization is seen only at the origin of gels run at even a very long switching interval. No hybridization is seen to the slowest migrating bands of DNA ("b") that entered the gel. Thus, the micronuclear chromosomes do not contribute to the DNA pattern resolved on gels with switching intervals of up to 70 seconds.

Hybridization to 5S genes

The organization of 5S gene clusters in the micronucleus and macronucleus of T. thermophila has been studied in detail. In both nuclei the genes are arranged as tandem repeats consisting of a 120 bp gene sequence and a 160 bp spacer region (8,9). Approximately 35 clusters of 5S genes have been identified with only a few differences seen between the micronucleus and macronu-

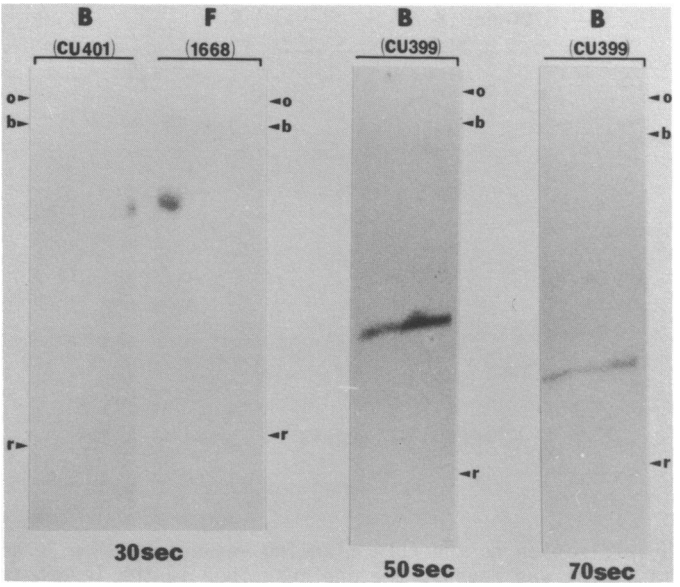

Figure 5. Hybridization of a histone H4 gene clone. Southern blots were made from gels that had been electrophoresed at switching intervals of 30, 50, and 70 seconds for 16, 18, and 17 hours respectively. Strips from the filters were hybridized with nick-translated clone p508.8, a histone H4 gene clone (ref. 11). The 30 second blots are noncontiguous filter strips made from the same 30 second gel described in the legend to Figure 4: the B strain strip is from the left third of the gel, the F strain strip is from the right third.

cleus. The clusters are found on 4 of the 5 micronuclear chromosomes (10). We hybridized a 5S gene clone to Southern blots of gels with 20, 30, 40, and 50 second switching intervals. In the result shown in Figure 4 for strain B, at least eight bands of varying intensity bybridize to the probe. At a 50 second switching interval (data not shown) the top band splits into two bands of about equal intensity, thus yielding a total of nine bands of hybridization encompassing nearly the entire size range of the macronuclear chromosomes. The lysate from strain F clearly shows a hybridization pattern similar to strain B as do strains A and C3 (data not shown).

Hybridization to unique sequence probes

The most informative data on the organization of the macronuclear genome comes from hybridizations using unique DNA sequence clones as probes. If there is only one way in which the micronuclear genome is processed to make the macronuclear genome, then a unique gene should hybridize to only one DNA band. To explore this possibility, we have used four different unique DNA sequences as

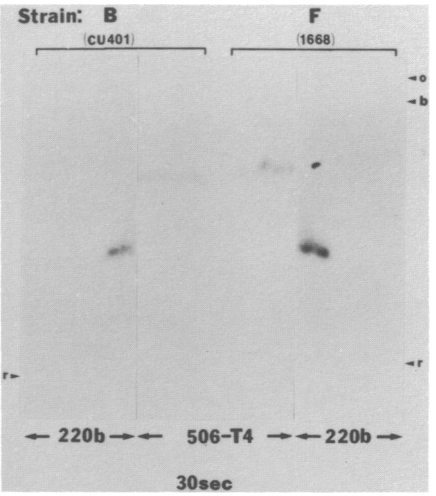

Figure 6. Hybridization to the rDNA flanking sequences. The 30 second gel from which this blot was made is the one described in the legend to Figure 4. The center section of the filter was hybridized to nick-translated clone pTt506-T4 which contains a .8 kb TaqI fragment of micronuclear DNA located to the left of the integrated rDNA gene. The left and right margins of the filter were hybridized to nick-translated clone pTt220b which contains a 3.5 kb insert of micronuclear DNA (ref. 18) located to the right of the integrated rDNA gene.

hybridization probes. Each represents a different DNA processing event occurring during formation of the macronucleus: the histone H4 gene is not rearranged (11), the two rDNA flanking sequences undergo fragmentation (15,19), and cTt1107 undergoes internal deletion events (12,13).

Histone H4 gene. A plasmid clone p508.8 which contains a T. thermophila micronuclear histone H4 gene was obtained from G. Bannon (Univ. of Rochester) (11). This gene has been mapped to micronuclear chromosome 4. Figure 5 shows hybridization of this clone to Southern blots of gels run with various switching intervals. A single band hybridizes in all cases and, as expected, with longer switching intervals its position in the gel moves closer to the rDNA band. Hybridizations were done to DNA from strains A and C3 (not shown), B, and F. The single band of hybridization seen in all cases is to DNA of the same mobility.

rDNA flanking sequences. The conversion of the single integrated copy of the rDNA located on chromosome 2 in the micronucleus to an extrachromosomal palindromic molecule in the macronucleus has been an object of intense investigation (5,31,32). The macronuclear fate of the micronuclear sequences flanking the rDNA has been examined. Following excision of the rDNA from the chromo-

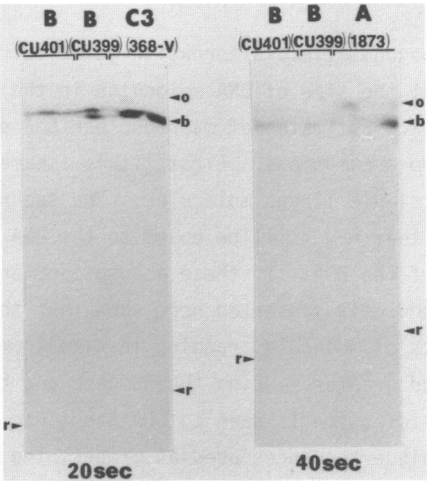

Figure 7. Hybridization to cTt1107. The center sections of filters blotted from 20 and 40 second gels were hybridized to nick-translated subclone pUC1a (a pBR322 clone containing an EcoR1 macronuclear DNA fragment corresponding to Region R of cTt1107 (ref. 13)). The 20 second filter was made from the gel shown in Figure 1. The 40 second filter was made from a gel containing two 33 mm wide wells and a center 6 mm wide well containing lysates made from the strains indicated.

some, the right flanking sequence undergoes some DNA elimination (approximately 2.8 kb of DNA is lost) and is finally stabilized as a chromosomal free end presumably by telomere addition (18). The left flanking sequence undergoes a smaller amount of DNA elimination and also becomes a free end (19). In the hybridization shown in Figure 6, clones representing the two flanking sequences have been hybridized to sections of the same filter to clearly show that the two sequences are located on different macronuclear DNA molecules. No obvious difference is seen once again in the sizes of the DNA to which these sequences hybridize in strains B, F, C3, and A.

cTt1107. Macronuclear clone cTt1107 contains a DNA sequence which differs in size from its micronuclear counterpart by about 3 kb due to three separate DNA deletions that occur in this sequence during macronuclear development (11). We have used a subclone containing the right third of the macronuclear clone in hybridizations to determine its macronuclear location. Figure 7 shows that this probe hybridizes to a large piece of DNA. With switching intervals up to 50 seconds (data not shown), the DNA to which this sequence hybridizes still does not migrate away from the bulk chromatin ("b") band. Strains B, C3, F, and A show hybridization to the same sized DNA.

DISCUSSION

The method of orthogonal-field-alternation gel electrophoresis has given us a definitive look at the size of DNA molecules in the macronucleus of Tetrahymena thermophila. Estimates of macronuclear DNA size from this gel system are based on two assumptions. First, it is assumed that the macronuclear DNA consists of simple linear molecules. The second assumption is that any residual proteins that may still be bound to the DNA in the samples do not affect the migration of the DNA. If these assumptions are correct then the ethidium bromide stained gels presented here show that the macronuclear DNA of T. thermophila consists of molecules ranging in size from the 21 kb rDNA molecules up to 1100 kb and greater. Using the 5S gene and the unique sequence clones as probes, hybridization is seen to DNA bands encompassing the entire size range. Of the unique sequences used as probes, the cTt1107 subclone hybridized to the slowest migrating DNA band (1100 kb or larger in size) and the right rDNA flank hybridized to DNA approximately 350 kb in size. Interestingly, the histone H4 gene and the left rDNA flank both hybridized to a band representing DNA of approximately 600 kb in size. It is most likely that these two sequences, which are derived from micronuclear chromosomes 4 and 2, are in two separate molecules which have similar electrophoretic mobilities rather than residing in a single DNA molecule.

From our results it seems probable that all the macronuclear DNA exists as fragments of the micronuclear chromosomes. The hybridization results with the rDNA flanking sequences and the histone H4 gene show that micronuclear chromosomes 2 and 4 are certainly fragmented. From the Southern hybridization using a subclone of pTt2512 (a micronucleus-limited DNA sequence) as probe, we know that the micronuclear chromosomes remain at the origin of these gels. Visual inspection of the ethidium bromide stained gels shows that the DNA remaining at the origin is a small fraction of the total DNA present in the sample. If one of the micronuclear chromosomes remained intact in the macronucleus, it would account for 18% of the DNA in the sample. There is not enough DNA seen at the origin of the stained gel to accomodate this plus the 9% of the DNA in the samples that is micronuclear in origin. Since there is no evidence either in the ethidium bromide stained gels or in the autoradiograms for significant degradation of the DNA, the low amount of DNA at the origin most likely reflects the actual amount of chromosome-sized DNA in the cell and not a depletion of this size class due to shearing during isolation and analysis. Thus, we conclude that all the macronuclear DNA exists as subchromosomal pieces.

An approximation of the number of DNA fragments that make up the macro-

nuclear genome can be obtained from the ethidium bromide stained gels. The macronuclear genome size is about 173,400 kb, yet a simple addition of the size of each band resolved in our gel system accounts for only about 15% of this. The rest of the DNA could be accounted for by large molecules that remain unresolved at the top of the gel in the bulk chromatin ("b") band seen in the stained gels. If this were so and the average size of these DNAs were 2000 kb, there would be about 70 DNA fragments in addition to the 44 visualized as bands in the gel. If the average size were larger, there would be fewer additional bands. Alternatively, the unaccounted-for DNA could be distributed throughout the gel, meaning that each of the 44 resolved bands would consist not of one type of DNA but of several comigrating pieces of DNA. If so, there could be a few hundred DNA fragments comprising the macronuclear genome. Thus, from these initial observations it would seem that the DNA in the T. thermophila macronucleus could consist of at least 50 and probably no more than a few hundred subchromosomal fragments.

No significant variation is seen in the overall DNA banding pattern or in the specific hybridizations seen within strain B and between other inbred strains. This similarity is interesting since small-scale differences in DNA processing are known to exist in these strains. For example, one of the developmentally deleted regions of cTt1107 can vary in size by .3 kb within and between strains (13). Based on this first look at the macronuclear molecular karyotype, there are apparently no larger scale versions of such developmental variations in the macronuclear genome which could drastically alter the sizes of the macronuclear DNA pieces. That is, chromosomes are not processed into significantly different sized pieces of DNA in different cell lines or processed in more than one way in a single macronucleus. In addition, this lack of variability suggests that there is not a rampant amount of ongoing rearrangement of the somatic DNA that occurs after the macronucleus is first formed. This somatic genome stability contrasts with the germinal genome which is known to undergo changes in DNA content and karyotype with time (10,33).

The organization of the macronuclear DNA in the hypotrichous ciliates, Oxytricha, Euplotes, and Stylonichia, and the holotrichous ciliate Glaucoma chattoni has been described. The macronuclear rDNA structure is similar in the hypotrichs and G. chattoni, being an amplified linear DNA sequence encoding a single copy of the rDNA per molecule (34,35). In T. thermophila the rDNA is the smallest molecule detected in the macronucleus and is a 21 kb palindrome carrying two rDNA copies per molecule (31). G. chattoni and T. thermophila both have CCCCAA repeats at the ends of their macronuclear

DNA whereas the hypotrichous ciliates have CCCCAAAA repeats (20,35,36). The hypotrichous ciliates eliminate over 90% of their micronuclear DNA upon forming a macronucleus; T. thermophila retains at least 85% of the micronuclear genome in its macronucleus. In the hypotrichs the macronuclear DNA ranges in size from .5 to 20 kb (37). In G. chattoni it ranges from 2.1 kb to greater than 100 kb (35). Here we have reported that T. thermophila macronuclear DNA ranges from 21 kb to greater than 1100 kb. These facts point out that organisms with the same binucleate blueprint for their cellular organization can differ greatly in the molecular details of their genome organization.

The use of nullisomic strains to assign genes and cloned DNA sequences to micronuclear chromosomes in strain B has proven a major contribution in understanding the micronuclear genome organization in Tetrahymena thermophila (38, 39). Orthogonal-field-alternation gel electrophoresis now offers the opportunity to study the chromosomal organization of the macronuclear genome. With the combined methodologies of genetic engineering and classical genetics, the germinal and somatic genomes of T. thermophila can both be explored not only at the DNA sequence level but at the chromosomal level as well.

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[1]Present address: Department of Biology, Williams College, Williamstown, MA 01267, USA

REFERENCES

1. Woodard, J.E., Kaneshiro, E., and Gorovsky, M.A. (1972) Genetics 70, 251-260.
2. Yao, M.-C. and Gorovsky, M.A. (1974) Chromosoma 48, 1-18.
3. Gorovsky, M.A. (1980) Ann. Rev. Genet. 14, 203-239.
4. Yao, M.-C. and Gall, J.G. (1979) J. Protozool. 26, 10-13.
5. Yao, M.-C., Blackburn, E., and Gall, J. (1981) J. Cell Biol. 90, 515-520.
6. Yokoyama, R. and Yao, M.-C. (1984) Nucl. Acids Res. 12, 6103-6116.
7. Yao, M.-C. (1982) J. Cell Biol. 92, 783-789.
8. Kimmel, A.R. and Gorovsky, M.A. (1978) Chromosoma 67, 1-20.
9. Pederson, D.S., Yao, M.-C., Kimmel, A.R., and Gorovsky, M.A. (1984) Nucl. Acids Res. 12, 3003-3021.
10. Allen, S.L., Ervin, P.R., McLaren, N.C., and Brand, R.E. (1984) Mol. Gen. Genet. 197, 244-253.
11. Bannon, G.A., Bowen, J.K., Yao, M.-C., and Gorovsky, M.A. (1984) Nucl. Acids Res. 12, 1961-1975.

12. Yao, M.-C., Choi, J., Yokoyama, S., Austerberry, C.F., and Yao, C.-H. (1984) Cell 36, 433-440.
13. Austerberry, C.F., Allis, C.D., and Yao, M.-C. (1984) Proc. Natl. Acad. Sci. USA 81, 7383-7387.
14. Callahan, R.C., Shalke, G., and Gorovsky, M.A. (1984) Cell 36, 441-445.
15. King, B.O. and Yao, M.-C. (1982) Cell 31, 177-182.
16. Ray, C. (1956) J. Protozool. 3, 88-96.
17 Allen, S.L. (1965) Brookhaven Symp. Quant. Biol. 18, 27-51.
18. Yao, M.-C. (1981) Cell 24, 765-774.
19. Yao, M.-C., Zhu, S.-O., and Yao, C.-H. (1985) Molec. Cell. Biol. 5, 1260-1267.
20. Yao, M.-C. and Yao, C.-H. (1981) Proc. Natl. Acad. Sci. USA 78, 7436-7439.
21. Preer, J.R., Jr. and Preer, L.B. (1979) J. Protozool. 26, 14-18.
22. Williams, J.B., Fleck, E.W., Hellier, L.E., and Uhlenhopp, E. (1978) Proc. Natl. Acad. Sci. USA 75, 5062-5065.
23. Schwartz, D.C., Saffran, W., Welsh, J., Haas, R., Goldenberg, M., and Cantor, C.R. (1982) Cold Spring Harbor Symp. Quant. Biol. 47, 189-195.
24. Schwartz, D.C. and Cantor, C.R. (1984) Cell 37, 67-75.
25. Carle, G.F. and Olson, M.V. (1984) Nucl. Acids Res. 12, 5647-5665.
26. Jackson, D.A. and Cook, P.R. (1985) EMBO J. 4, 913-918.
27. Southern, E. (1975) J. Mol. Biol. 98, 503-517.
28. Rigby, P.W., Dieckmann, J.M., Rhodes, O., and Berg, P. (1977) J. Mol. Biol. 113, 237-251.
29. Denhardt, D.T. (1966) Biochem. Biophys. Res. Comm. 23, 641-646.
30. Mortimer, R.K. and Schild, D. (1980) Microbiol. Rev. 44, 519-571.
31. Karrer, K. and Gall, J.G. (1976) J. Mol. Biol. 104, 421-453.
32. Pan, W.-C. and Blackburn, E.H. (1981) Cell 23, 459-466.
33. Weindruch, R.H. and Doerder, F.P. (1976) Mech. Ageing Develop. 4, 263-269.
34. Swanton, M.T., Greslin, A.F., and Prescott, D.M. (1980) Chromosoma 77, 203-215.
35. Katzen, A.L., Cann, G.M., and Blackburn, E.H. (1981) Cell 24, 313-320.
36. Klobutcher, L.A., Swanton, M.T., Donini, P., and Prescott, D.M. (1981) Proc. Natl. Acad. Sci. USA 78, 3015-3019.
37. Swanton, M.T., Heumann, J.M., and Prescott, D.M. (1980) Chromosoma 77, 217-227.
38. Bruns, P.J. and Brussard, T.E.B. (1981) Sci. 213, 549-551.
39. Bruns, P.J., Brussard, T.B., and Merriam, E.V. (1982) Acta Protozool. 22 (Part 1), 31-44.