Elimination of Specific DNA Sequences from the Somatic Nucleus of the Ciliate *Tetrahymena*

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ABSTRACT *Tetrahymena* micronuclear DNA fragments have been cloned in the plasmid pBR322. One clone, pTt 2512, has been found to contain the C-C-C-C-A-A hexanucleotide repeat which is also present in the macronuclear rDNA. Further restriction enzyme digestion and hybridization studies suggest that the clone also contains sequences that are not present in the somatic macronucleus. The flanking sequences of the C_4A_2 repeats in this clone were separated into four restriction fragments, one from one side and three from the other. These fragments were used as probes for Southern hybridization to study the organizations of similar sequences in the macronucleus and micronucleus. All four fragments hybridized to many fragments of restriction enzyme digested micronuclear DNA. However, none of these hybridizations were detected in the macronucleus. Thus, these families of repetitive DNA are completely eliminated from the macronucleus. Further analysis suggested that the four different sequences may be linked at other locations of the genome. Using nullisomic strains of *Tetrahymena*, it is found that at least one of these sequences is present in more than one chromosome. Studies of various normal and star strains of *Tetrahymena* suggest that these sequences are stable in the normal micronucleus but are altered drastically in the defective micronuclei of the star strains. Eliminated DNA of similar nature has also been found in at least five other randomly selected clones of micronuclear DNA and may be present widely in the genome.

The phenomenon of DNA elimination or chromosome diminution has been observed for nearly a century. It was first found in nematodes as a part of the differentiation process leading to the formation of the somatic cells (reviewed in reference 1). Subsequently, it was also found in some crustaceans and insects (reviewed in references 2 and 3). More recently, molecular studies of this phenomenon have been made in nematodes (4, 5) and ciliates (reviewed in references 6 and 7), although the basis of this process still remains essentially unknown.

The ciliated protozoan *Tetrahymena* normally contains a macronucleus and a micronucleus in each cell. The two nuclei share the same genetic origin but are very different in structure and function (reviewed in references 7 and 8). Upon closer examination, it was found that the genetic contents of these two nuclei were not identical. For instance, the genes coding for ribosomal RNA are several hundred times more abundant in the macronucleus than in the micronucleus as a result of amplification (reviewed in reference 9). Besides gene amplification, DNA elimination also occurs. Although the macronucleus contains ~23 times more DNA than the diploid micronucleus does, only ~85% of the micronuclear DNA sequence is found in the macronucleus as determined by DNA renaturation studies (10). The remaining 15% of the micronuclear DNA sequences is presumably eliminated or underreplicated during the formation of the macronucleus.

In this study I have isolated, by cloning, some fragments of micronuclear DNA that are involved in the elimination process. Using restriction enzyme digestion and hybridization, it was found that these sequences were repetitive in the micronucleus, and the members of the repetitive families were probably dispersed throughout the genome. Furthermore, the elimination process seemed to affect all members of the repetitive families, suggesting a elimination mechanism that seemed to involve recognition of specific DNA sequences.

MATERIALS AND METHODS

Cells and Culturing Conditions

Tetrahymena thermophila inbreeding strains A, C, and F were obtained from D. Nanney, University of Illinois. *T. thermophila* wild type LWB was obtained from J. Gall, Yale University. All other strains of *Tetrahymena* used in this study were obtained from P. Bruns, Cornell University. The cells were maintained in axenic cultures as previously described (11).

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Nuclei Isolation, DNA Extraction, and Radioactive Labeling of DNA

Macronuclei and micronuclei were isolated from *Tetrahymena* in late log phase of growth using the method described by Gorovsky et al. (11). Contamination of macronuclei in the micronucleus preparation was checked with a light microscope in each isolation. Normally, no more than one macronucleus was found in every 200 micronuclei. DNA was prepared from the macronucleus or the whole cell in stationary phase by phenol extraction as described (12). Micronuclear DNA was prepared by equilibrium sedimentation in a CsCl gradient as described (10). Plasmid DNA was isolated from bacterial lysates using ethidium bromide-CsCl gradients. Phage λ DNA was made directly from agarose plate lysates after polyethylene glycol precipitation and phenol extraction. Phage DNA prepared by this method can be digested readily with restriction enzymes and labeled by nick translation, although it always contained some bacterial DNA. All DNAs were labeled in vitro with ^{32}P by nick translation (13), except the repeated hexanucleotide C-C-C-C-A-A of rDNA, which was labeled by a modified method of nick translation as described earlier (14).

Restriction Endonuclease Digestion, Gel Electrophoresis, and Blot Hybridization

All restriction endonucleases used in this study were purchased either from New England Biolabs (Beverly, MA) or from Bethesda Research Laboratories (Rockville, MD). Digestion was carried out following the conditions specified by the suppliers. Twofold excess of enzyme was routinely used to ensure completion of digestion. Agarose slab gel electrophoresis was carried out in a horizontal apparatus with the gel submerged in buffer. The buffer condition of Helling et al. (15) was followed. Hind III digested phage λ DNA was routinely included in each gel as size markers. The positions of these markers are indicated by the black bars in the figures. After electrophoresis the gel was stained in ethidium bromide and visualized with a UV transilluminator. The gel was blotted immediately using the method of Southern (16). The blot was hybridized with ^{32}P-labeled, denatured DNA in 40% formamide, 4 × SSC (SSC contained 0.15 M sodium chloride and 0.015 M sodium citrate at pH 7.0), 0.1 M Tris HCl, pH 7.4, 0.5% SDS and Denhardt solution (17) at 37°C for 10–16 h. After hybridization the filter was washed extensively in 2 × SSC at room temperature and then at 65°C for at least 30 min before autoradiography.

DNA Cloning

The micronuclear DNA library previously constructed either in the plasmid pBR322 (18) or in the phage Charon 4A (19) is used in this study. The plasmid library contained micronuclear DNA digested with EcoRI and BamHI. Between 1,000 and 2,000 colonies were screened by colony hybridization (20) with labeled C-C-C-C-A-A repeats as a probe to obtain pTt 2512. The Charon 4A library contained EcoRI partially digested micronuclear DNA. 12 clones were selected randomly and analyzed by EcoRI digestion. 10 of these clones were found to contain foreign DNA and were analyzed further. To determine the linkage relationship between different repetitive families, the Charon 4A library was amplified in agar plates before being used for plaque hybridization. Approximately 9×10^4 independent clones were used for amplification. The hybridization was done following the method of Benton and Davis (21). Two replica filters were used for each probe. Only plaques hybridized in both filters were taken as positives. Four replica filters were lifted from each plate to determine the linkage between two repetitive families. Recombinant DNA cloning was carried out in P1 physical containment and EK1 host system following the guidelines of the National Institutes of Health.

RESULTS

Isolation and Restriction Mapping of pTt 2512

The extrachromosomal rDNA of *Tetrahymena* macronucleus has been known to contain the repeated hexanucleotide C-C-C-C-A-A near or at its termini (14). Similar sequences have also been found in other locations of both the macronuclear and the micronuclear genome (18). To isolate these sequences, total micronuclear DNA was digested with both the restriction enzymes EcoRI and BamHI and cloned in the plasmid vector pBR322. In this random collection of clones, the clone pTt 2512 was selected by the method of colony hybridization using the C-C-C-C-A-A repeat as a probe. Fig. 1 shows a simple restriction enzyme digestion map of pTt 2512. The micronuclear DNA insert is 7.3 kilobasepairs (kb) long and is bounded by an EcoRI and a BamHI site. The region of the clone homologous to the hexanucleotide repeats was determined by blot hybridization (Figs. 1 and 2) and was found to be within the 0.9 kb fragment (fragment d) produced by Hha I digestion.

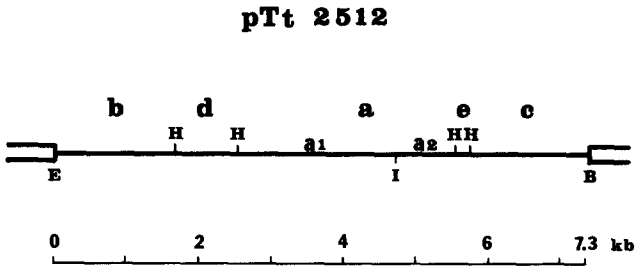

FIGURE 1 Restriction enzyme digestion map of pTt 2512. pTt 2512 contains 7.3 kb (kilobasepairs) of micronuclear DNA between the EcoRI site (*E*) and the Bam HI site (*B*) of the plasmid pBR322 (open boxes). The insert is cut by Hha I (*H*) into five fragments: *a*, *b*, *c*, *d*, and *e*. Hind III (*I*) cut this clone once to divide fragment *a* into a_1 and a_2.

Elimination of Micronuclear DNA

Although pTt 2512 was first isolated due to its homology with the C-C-C-C-A-A repeats, further analysis showed that this clone also contained sequences that were eliminated from the macronucleus. pTt 2512 was digested with Hha I and the fragments produced were isolated and used as probes to hybridize to the macronucleus and micronuclear DNA after EcoRI and BamHI digestion using the method of Southern (14). Fig. 3 shows the results of these experiments. The four Hha I fragments were separated into three fractions: a, b+c, and d. Each fraction hybridized to an array of bands in the micronuclear DNA. This result indicated that all three fractions contained sequences that were repeated many times in the genome.

A very different result was obtained when the macronuclear DNA was hybridized with the same restriction fragments (Fig. 3). No hybridization was detected when fragment a or b+c was used as a probe. Apparently, these sequences were either absent from or present in much lower quantities in the macronucleus. Fragment d, on the other hand, did hybridize with the macronuclear DNA in multiple bands, although the banding pattern was very different from that in the micronucleus. This hybridization was probably due to the presence of the C-C-C-C-A-A repeat in fragment d. The repeated hexanucleotide has been found to hybridize to different sets of bands in the two nuclei. Furthermore, the hybridization of the repeats closely resembled the hybridization of fragment d (Fig. 3).

To further investigate the phenomenon of elimination, pTt 2512 was digested with Hind III and Hha I, and the fragments a1, a2, b, and c were prepared (Fig. 1). They were then used to hybridize the macronuclear and micronuclear DNA after EcoRI digestion. All four fragments were found to hybridize to the micronuclear DNA but not to the macronuclear DNA. The hybridization patterns generated by these four fragments seemed to be different from each other. Apparently, there were at least four families of repetitive DNA present in these fragments, and all members of the repetitive families were grossly underrepresented in, or completely missing from the macronucleus.

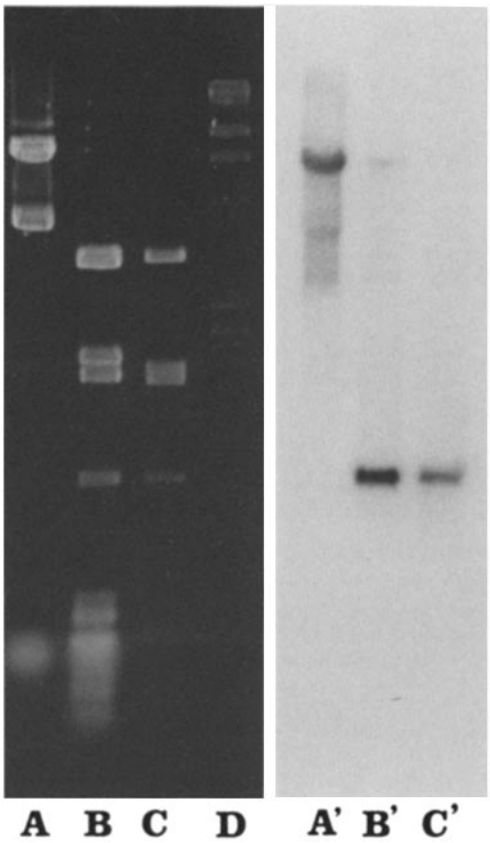

FIGURE 2 Southern hybridization of pTt 2512 with C_4A_2 repeats. pTt 2512 was digested with restriction enzymes and the fragments were separated by electrophoresis in a 1.0% agarose gel. In lane *A*, pTt 2512 was cut with EcoRI and Bam HI to separate the insert from pBR322. Lane *B* contained pTt 2512 digested with Hha I. Lane *C* contained the 7.3 kb insert of pTt 2512 digested with Hha I. Lane *D* showed Hind III digested λ DNA as size markers. The gel was blotted and hybridized with the C_4A_2 repeats of the extrachromosomal rDNA. Lanes *A'*, *B'*, and *C'* showed the hybridization result of lanes *A*, *B*, and *C*. C_4A_2 repeats hybridize with only one (fragment d) of the fragments produced by Hha I digestion.

To estimate how much the two nuclear DNAs differed in the contents of these sequences, 5 μg of macronuclear DNA was compared with 0.1 μg of micronuclear DNA or 5 μg of whole cell DNA for their abilities to hybridize. Fig. 4 shows the results of this experiment. It was clear that all or almost all the bands detected in the micronucleus were reduced 50-fold or more in the macronucleus. The slight hybridization detected in the macronucleus might actually be derived from the micronuclear contaminations, which in some cases could account for as much as 1% of the macronuclear DNA preparation.

Linkage Relationships of the Eliminated Sequences

The four repetitive families found in pTt 2512 apparently are organized in a rather complicated fashion in the micronuclear genome. They are clearly not arranged in simple tandem arrays and may be interspersed with other sequences in the genome. It is also possible that these sequences are grouped into many clusters in the genome, with each cluster containing different repeating units in different arrangements, such as the organization of some repetitive sequences in *Drosophila* (22). Whether these repetitive sequences are indeed linked were determined by the following experiments. The micronuclear DNA was digested partially with the restriction enzyme Eco RI and cloned in the phage vector Charon 4A. This genomic library was then hybridized with the repetitive sequences by the plaque hybridization method (21) to identify the clones containing the sequence of interest. In each case two different probes were used to hybridize to each set of roughly 10,000 clones. The hybridization results were compared, and the clones hybridized with each and both probes were determined. Table I summarizes these results. The majority of plaques hybridized with fragment b or fragment c also were hybridized with fragment a. Since each clone contained on the average 15 kb of the micronuclear DNA, the results indicated that most of the fragment b and fragment c sequences were located within 15 kb of the fragment a sequences. Thus, the three sequences tend to cluster in the genome. Fragment d sequences, on the other hand, did not show strong linkage with fragment a sequences. About 20% of the clones hybridized with fragment a were also hybridized with fragment d. These results were confirmed by directly isolating and characterizing several clones containing the fragment a sequences (J. Choi and M. C. Yao, unpublished observations).

The Eliminated Sequences Are Present in More Than One Chromosome

It is clear that the sequences homologous to fragment a, b, and c are closely linked. To determine whether these repetitive sequences were present as one large cluster, or distributed in more than one location in the genome, I examined their presence in a nullisomic strain of *Tetrahymena*. The triple nullisomic strain CU359 is a heterokaryon which contains a

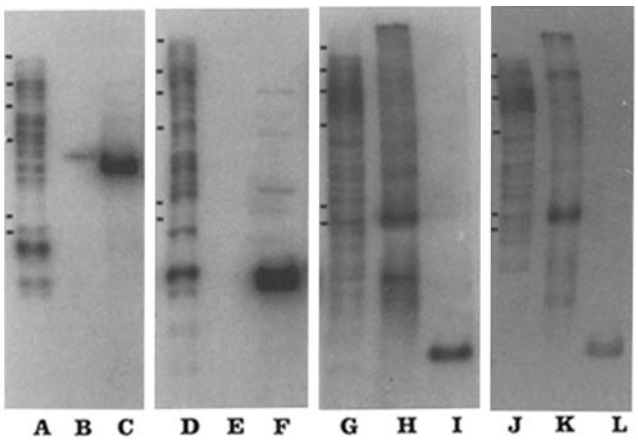

FIGURE 3 Southern hybridization of macronuclear and micronuclear DNA with pTt 2512. 2.5 μg each of macronuclear and micronuclear DNA were digested with both EcoRI and Bam HI, and 0.01 μg of pTt 2512 was digested with Hha I. The three digested DNA were run in a agarose gel, blotted, and hybridized with ^{32}P-labeled Hha I fragments of pTt 2512 or the C_4A_2 repeats of rDNA. In each set the left lane (*A*, *D*, *G*, *J*) contained micronuclear DNA, the center lane (*B*, *E*, *H*, *K*) contained macronuclear DNA, and the right lane (*C*, *F*, *I*, *L*) contained pTt 2512 DNA. The first set (lanes *A–C*) was hybridized with fragment a, the second set (lanes *D–F*) with fragment b and c, the third set (lanes *G–I*) with fragment d, and the fourth set (lanes *J–L*) with the C_4A_2 repeats of rDNA. The black bars in this and the following figures indicate the positions of the six larger fragments of the Hind III digested phage λ DNA as size markers.

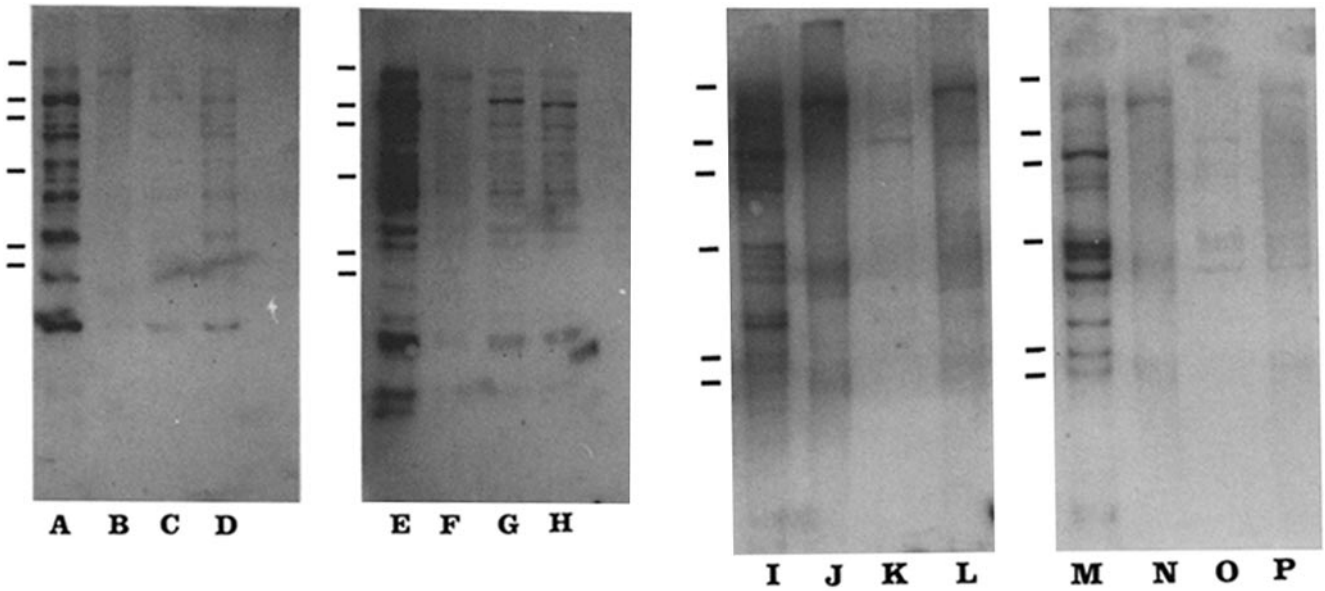

FIGURE 4 Southern hybridization of pTt 2512 subfragments with different amounts of macronuclear and micronuclear DNA. Various *Tetrahymena* DNAs were digested with EcoRI, run in a 1.0% agarose gel, blotted, and hybridized with four labeled fragments of pTt 2512 produced by Hind III and Hha I digestion. Each set of hybridization contained (from left to right) 1 μg of micronuclear DNA (lanes *A*, *E*, *I*, and *M*), 5 μg of macronuclear DNA (lanes *B*, *F*, *J*, and *N*) 0.1 μg of micronuclear DNA (lanes *C*, *G*, *K*, and *O*) and 5 μg of whole cell DNA (lanes *D*, *H*, *L*, and *P*). The first set was hybridized with fragment b (lanes *A–D*), the second set with fragment a1 (lanes *E–H*), the third set of fragment c (lanes *I–L*), and the fourth set with fragment a2 (lanes *M–P*). The hybridization patterns in the four sets were different from each other. Hybridization with 0.1 μg of micronuclear DNA is equal to or higher than with 5 μg of macronuclear DNA. The single band showed up in lane *J* and lane *N* was near the limit mobility region of the gel. Hybridizations to these bands were not always detected.

TABLE I

Linkage Relationships between the Repetitive Family of Fragment a and Other Families of pTt 2512 by Plaque Hybridization

	Experiment		
	1*	2‡	3‡
Fragment used as probe	a/b	a/c	a/d
Plaques hybridized with each probe	195/105	58/66	67/95
Plaques hybridized with both probes	65	42	14

* ~10,000 plaques were hybridized.
‡ ~5,000 plaques were hybridized.

normal macronucleus and a micronucleus believed to be missing three pairs of chromosomes (P. Bruns, personal communication). Micronuclear DNA was prepared from this cell and hybridized with Hha I fragment a of pTt 2512. The results are shown in Fig. 5. It is apparent that some of the bands found in the normal strain are missing from the nullisomic strain. The nullisomic strain seems to contain only a subset of this repetitive family. Thus, this sequence must be present in more than one chromosome in the normal genome.

Eliminated DNA in Normal and Defective Strains of Tetrahymena

Although the eliminated DNA is selectively removed from the macronucleus during development, it is rather stable in the micronucleus during vegetative and sexual passages. This property is best seen when different strains of *T. thermophila* were compared. Strains A, B, C, and F were four inbreeding lines and strain LWB was a wild type not related to the inbreeding lines. As shown in Fig. 6, the organizations of the sequences homologous to Hha I fragment a of pTt 2512 were very similar among the five strains examined. It is apparent that members of this repetitive family are rather stable and are not like the unstable genetic elements found in *Drosophila* or yeast (23, 24).

However, drastic alterations in the organizations of these sequences can be seen in the defective strains known as star strains. Star strains were originally isolated from the normal strains because of their altered mating behaviors (25, 26). They were able to pair with normal strains but were unable to produce fertile gametic nuclei. Genetic studies suggested that the star strains might contain defective micronuclei.

Whole cell DNAs were isolated from four star strains, A star, A3 star, C star, and F star, and analyzed by the hybridization method described earlier. The result is shown in Fig. 7. The organizations of the repetitive sequence homologous to fragment Hha I-a of cTt 2512 were strikingly different between the star strains and the normal strain, and also among the different star strains. In general, fewer bands were found in the star strains. A3 star and F star contained similar but not identical sets of bands, and they might be subsets of the ones found in the normal strain. A star contained two prominent bands, one of which was not detected in the normal strain. The C star strain showed no hybridization and apparently contained little or no sequence homologous to this probe. It is clear that all the star strains examined contain defective micronuclear genomes. The defects apparently involve losses and possibly rearrangements of DNA sequences which are specific to the micronucleus.

The Occurrence of Micronuclear Specific Sequences

The four repetitive DNA families found in cTt 2512 are not the only sequences that are specifically eliminated from the macronucleus. These kinds of sequences apparently exist

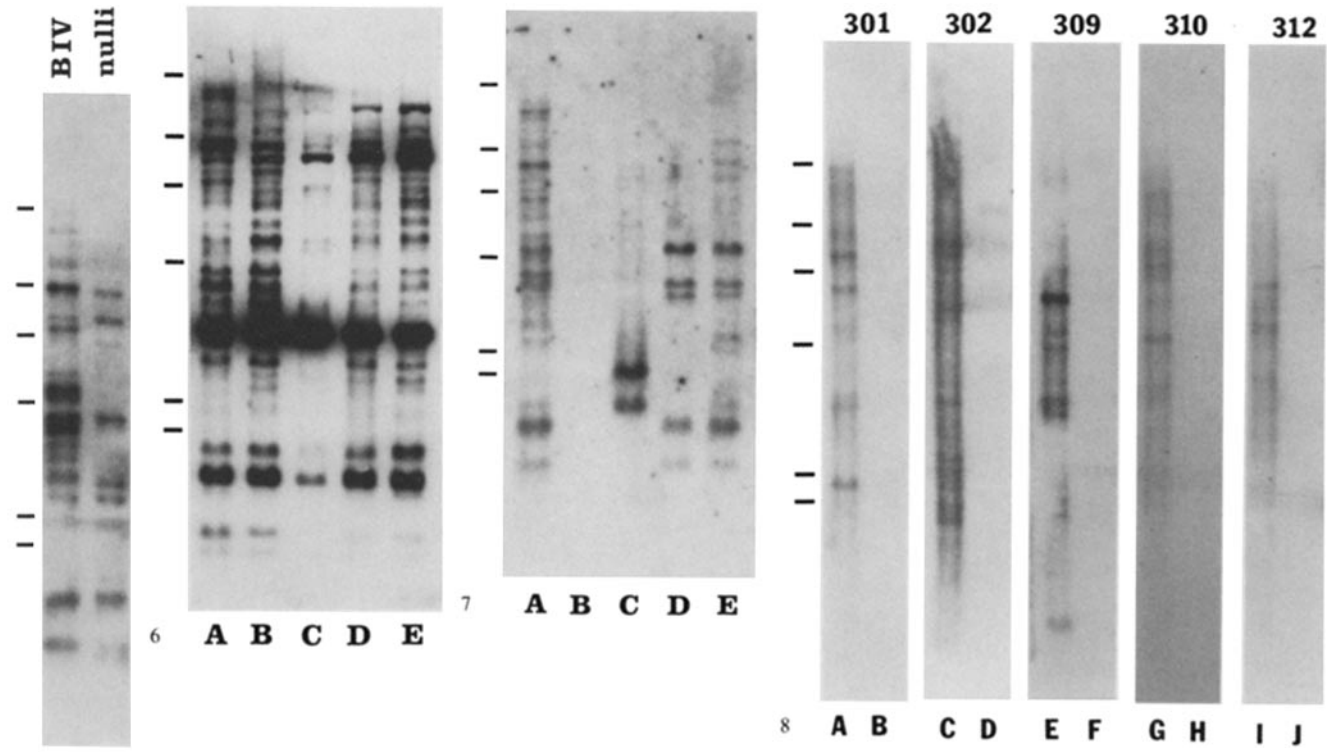

FIGURE 5 Southern hybridization of pTt 2512 fragment a with DNA from nullisomic strain of *Tetrahymena*. Micronuclear DNA were isolated from strain BIII which contained a normal micronucleus and strain CU359 which contained a nullisomic micronucleus lacking chromosomes 2, 3, and 5. The DNA were digested with EcoRI, separated in a 0.7% agarose gel, and hybridized with fragment a of pTt 2512 in a Southern blot. Lane *A* contained DNA from BIII and lane *B* contained DNA from CU359. A few bands in lane *A* were clearly absent from lane *B*.

FIGURE 6 Southern hybridization of different normal *Tetrahymena* strains with fragment a of pTt 2512. Roughly 5 μg each of whole cell DNA from various strains of *T. thermophila* was digested with EcoRI, separated in a agarose gel, and hybridized with fragment a of pTt 2512 in a Southern blot. DNA from the four inbreeding strains BIII, A1837, C1564, and F1668 were in lanes *A–D*, respectively. Lane *E* contained DNA from the wild strain LWB. Only minor differences were seen in the banding patterns among these strains. The relative hybridization intensities of these bands varied somewhat in different experiments, depending on the conditions used.

FIGURE 7 Southern hybridization of DNA from star strains of *Tetrahymena* with fragment a of pTt 2512. Roughly 5 μg each of whole cell DNA was prepared from four star strains and one normal strain of *T. thermophila*, digested with EcoRI, separated in a 0.7% agarose gel, and hybridized with fragment a of pTt 2512 in a Southern blot. Lane *A* contained DNA from the normal strain BIV. Lanes *B*, *C*, *D*, and *E* contained DNA from strains C star, A star, A3 star, and F star, respectively. No hybridization was detected in C star. The other three star strains contained fewer bands than the normal strain does.

FIGURE 8 Southern hybridization of macronuclear and micronuclear DNA with other eliminated sequences. Five phage λ clones with micronuclear DNA inserts were labeled and hybridized with EcoRI digested macronuclear and micronuclear DNA in a Southern blot. Lanes *A*, *C*, *E*, *G*, and *I* contained micronuclear DNA and lanes *B*, *D*, *F*, *H*, and *J* contained macronuclear DNA. Lanes *A* and *B* were hybridized with cTc 301, *C* and *D* with cTt 302, *E* and *F* with cTt 309, *G* and *H* with cTt 310, and *I* and *J* with cTt 312. No hybridization was detected in the macronucleus in all five cases, except in cTt 302 where a minor band was found.

widely in the micronucleus. Two other cloned micronuclear DNA fragments isolated earlier have also been shown to contain sequences that are repetitive in the micronucleus but are absent from the macronucleus (19, 27). To further investigate this problem, 10 additional clones of micronuclear DNA were analyzed. These clones were selected randomly from a micronuclear genomic library constructed using the phage vector Charon 4A. The micronuclear DNA in this library was digested partially with the restriction enzyme EcoRI before cloning. Each clone contains roughly 15 kb of micronuclear DNA. These clones were radioactively labeled and used as probes to detect the homologous sequences in the macronuclear and micronuclear DNA by the hybridization method of Southern. Seven of the ten clones hybridized to numerous bands of the micronuclear DNA. The banding patterns generated by five of these clones were quite different, as shown in Fig. 8. The other two clones, cTt 304 and cTt 305, gave results similar to that of cTt 301 and may contain the same repetitive sequence. All seven clones failed to hybridize to the macronuclear DNA under the same condition, with the exception of cTt 302 which hybridized to a single band. Thus, most of the repetitive sequences homologous to these cloned DNA are eliminated from the macronucleus. The single band detected by cTt 302 may be the only exception to this rule, although it is also possible that this hybridization is due to the presence of some unrelated sequence in cTt 302. The other three of the ten randomly selected clones hybridized to very few bands in both nuclear DNAs and probably contained only single copy sequences.

DISCUSSION

Using cloned micronuclear DNA as tools, the nature of DNA elimination in *Tetrahymena* has been examined. Hybridization with pTt 2512 suggests that these sequences are eliminated from, and not simply underreplicated in, the macronucleus. This is based mainly on the observation that there is at least a 50-fold difference between the abundances of these sequences in the two nuclei. Since an average macronucleus contains 23 times more DNA than a diploid micronucleus does, there is

probably less than one haploid set of these sequences in one macronucleus. In fact the difference is probably far greater than 50-fold if one takes into consideration the contamination of micronuclear DNA in the macronuclear preparation. It is not unlikely that all the hybridization detected in the macronucleus is due to micronuclear contamination. One support for this argument is found in the studies of the star strains. Hybridization of whole cell DNA from these strains gives no indication that the normal macronuclei of these cells contain any of these sequences, which, if present, could easily be distinguished from the abnormal banding patterns contributed by the micronucleus.

It is intriguing that when elimination occurs it occurs to all or almost all the members of a given repetitive DNA family. So far, this phenomenon has been observed in all the eliminated sequences studied, including pTt 2512, cTt 301, cTt 302, cTt 309, cTt 310, cTt 312, and pTt 2837, which has been reported earlier (27). It is likely that this property is shared by most of the eliminated sequences in *Tetrahymena*. Unless these sequences are clustered in one or very few regions of the genome, this observation suggests that the elimination process may involve recognition of specific DNA sequences.

The eliminated sequences do not exist as one single cluster in the genome and may be widely distributed. Although the different repetitive families represented by pTt 2512 are closely linked, they probably exist as many clusters in different regions of the genome, with each cluster containing different repeating units in different arrangements. The fact that they are found in more than one chromosome offers a strong support for this argument. Although the other cloned eliminated sequences have not yet been analyzed in detail, they are probably not in one large cluster and may also be widely distributed. This argument is supported by the fact that although the eliminated sequences comprise only ~15% of the gemone (10), they are found in at least five out of ten randomly selected clones of micronuclear DNA. If the eliminated sequences are organized in clusters much longer than 15 kb, one should not find them in such a high frequency. In fact in one case where the boundaries are known, the eliminated region is only ~2.8 kb in size (19).

It is not known how similar the members of a given repetitive family are. However, all hybridizations were done under moderately stringent conditions, and the hybrids formed were reasonably stable. For fragment a of pTt 2512, the hybridization pattern remained unchanged after washing the hybridized filter in 0.1 × SSC at 65°C.

The clone pTt 2512 apparently contains sequences homologous to the tandemly repetitive hexanucleotide C-C-C-C-A-A found in the termini of rDNA (14). This hexanucleotide repeat seems to exist in many clusters in both the macro- and the micronucleus, although their restriction banding patterns are rather different. Recently, it has been found that the C_4A_2 repeats are located near free ends of the macronuclear DNA but are present in the internal regions of the micronuclear chromosome (28). How this change occurs is still not known. It is interesting that in pTt 2512 the flanking sequences in both sides of the hexanucleotide repeat are eliminated. Apparently, this particular cluster of the hexanucleotide repeat can not exist in the same genetic context in the macronucleus. The simplest assumption would be that it is also eliminated. However, it is also possible that this particular cluster of repeat is moved to a different location in the macronucleus. Although most C_4A_2 repeats are not closely linked with the eliminated sequences represented by pTt 2512, recent studies on additional clones containing C_4A_2 repeats indicated that in the micronucleus these sequences tended to be flanked by sequences which were eliminated from the macronucleus (R. Yokoyama and M. C. Yao, unpublished observations). Thus, there seems to be an interesting relationship between DNA elimination and the rearrangement of C_4A_2 repeats in *Tetrahymena*.

New macronuclei are formed during conjugation. During this process the DNA in the macronuclear anlage increases gradually. From cytological study it is not possible to tell when elimination takes place (29). Recently, using *in situ* hybridization techniques, it was found that elimination of pTt 2512 and pTt 2837 sequences might begin during conjugation before the new macronucleus divided (R. Yokoyama and M. C. Yao, submitted). These observations ruled out the possibility that the micronuclear-specific sequences simply failed to replicate in the macronucleus and were diluted out subsequently through vegetative growth.

The function of these germ-line specific DNAs is not known. They are not likely needed for vegetative growth, since all the star strains examined showed gross abnormality in the organization of at least one of these sequences, and in the C star strain this particular sequence was completely missing. The star strains are able to grow normally as vegetative cells but are unable to generate fertile gametic nuclei during mating. It is possible that the germline-limited DNA is involved in the meiotic process. However, since the star strains may also be defective in other aspects, it is not possible to tell whether these two observations are actually related. The function of the germline specific DNA is likely to be complex. Hopefully, with some of these sequences now isolated, one may begin to ask simple questions such as whether and when these sequences may be transcribed.

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