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Rearrangement of repeated DNA sequences during development of macronucleus in *Tetrahymena thermophila*

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ABSTRACT

Three clones of non-repetitive sequences and six clones containing repetitive sequences were obtained from micronuclear DNA of Tetrahymena thermophila. All the non-repetitive and three repetitive sequences had the same organization in micro- and macronuclear DNAs as revealed by blot hybridization. On the other hand, the remaining three clones with repetitive sequences had apparently different organization in the two nuclear DNAs. All these repetitive sequences showed a smear on the blot in addition to a number of discrete bands when micronuclear DNA was digested with EcoR I. In macronuclear DNAs, these sequences invariably became one or two bands and the smear disappeared. We conclude that, when a macronucleus develops from a micronucleus, the non-repetitive sequences amplify by more than 20 times with relatively few rearrangement, whereas some selected portions of repeated and/or repeat-contiguous sequences are amplified with rather extensive reorganization.

INTRODUCTION

The ciliated protozoan Tetrahymena thermophila possesses a transcriptionally inactive, diploid, germinal micronucleus and a transcriptionally active, polyploid, somatic macronucleus. During the course of sexual conjugation, the macronucleus is destroyed and resorbed, while the micronucleus undergoes meiosis. After subsequent reciprocal exchange and fusion of the two haploid genetic nuclei, a new macronucleus develops through a process of amplification of micronuclear DNA to ∿45-ploid (3,12). The multiple copies of rRNA genes in the macronucleus are generated by amplification of the apparently single gene in the micronucleus (13). There is evidence that rRNA gene amplification may be associated with chromosome breakage and DNA elimination (15).

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Renaturation kinetics of micro- and macronuclear DNA are different from each other indicating the occurrence of an unequal gene amplification in the chromoromes during the macronuclear development (5). Micronuclear DNA is composed of 80% or more of single-copy sequences whose Cotl/2 value is 200 and about 10% of repetitive sequences, while macronuclear DNA is composed of nearly one repetitive class whose Cotl/2 value is also 200. Hybridization experiments with DNA complementary to poly(A) containing RNA and micro- or macronuclear DNA indicate that the repetitive sequences in micronuclear DNA are transcribed into messenger ribonucleic acid (mRNA) from macronuclear DNA up to 30% of total cellular mRNA (5).

In order to analyze the mode and mechanism of gene amplification with possible reorganization during macronuclear development in Tetrahymena at the molecular level, we have compared the micro- and macronuclear DNAs with Southern blot hybridization using two sets of clones; one consisting solely of nonrepetitive sequences, the other containing repetitive sequences.

MATERIALS AND METHODS

Preparation of High Molecular Weight DNA from Micro- and Macronuclei and Fractionation into Repetitive and Non-repetitive DNAs

Culturing of cells and isolation of micro- and macronuclear DNA were described previously (5).

Sheared and denatured micronuclear DNA was incubated to a Cot of 10, and the repetitive DNA fraction which had become double stranded was separated on a column of hydroxylapatite and designated repetitive DNA here. The remaining single-stranded DNA was hybridized further to a Cot of 2×10^2 and the non-repetitive DNA fraction which had remained as single-stranded was separated in the same manner and regarded as non-repetitive DNA. This DNA was again hybridized extensively (to a Cot of 1×10^4) to obtain double-stranded DNA.

Preparation and Screening of the Recombinant Phage

Bacteriophage λgtWES·λB, an EK-2 vector, was propagated in Escherichia coli strain ED8656. Introduction of DNA into

ED8656 by an in vitro packaging technique was carried out according to Blattner et al. (2) on the basis of other reports (1,4,10). Cloning experiments were performed in a P2 facility according to the Guidelines set by Japanese Goverment (1978). Clones were obtained here by screening with repetitive or non-repetitive micronuclear DNA as probes.

Recovery of DNA from Agarose Gel

Extraction of DNA from agarose gel was effected by the method of Tabak & Flavell (11). Recombinant DNA and cloned DNA were digested with EcoR 1 and Hind II, respectively, and electrophoresed on a 1% agarose gel. Hydroxylapatite was packed in a trough which had been made adjacent to the DNA band, and the DNA was electrophoresed into the hydroxylapatite, which was taken out from the trough. The hydroxylapatite was poured on the top of Sephadex G-50 and the DNA eluted with 0.4 M phosphate buffer, pH 6.8.

Radioactive Probes

In this study, several probes were used for the hybridization experiments. Repetitive micronuclear DNA, nonrepetitive micronuclear DNA, cloned DNA fragments and endonuclease Hind II fragments of clone 5 DNA were labeled by the nick translation technique with α^{32}P-dCTP as precursor (10).

Gel Electrophoresis and Blot Hybridization

Restriction endonuclease digestion, agarose gel electrophoresis, Southern transfer and hybridization were carried out as described elsewhere (9,15).

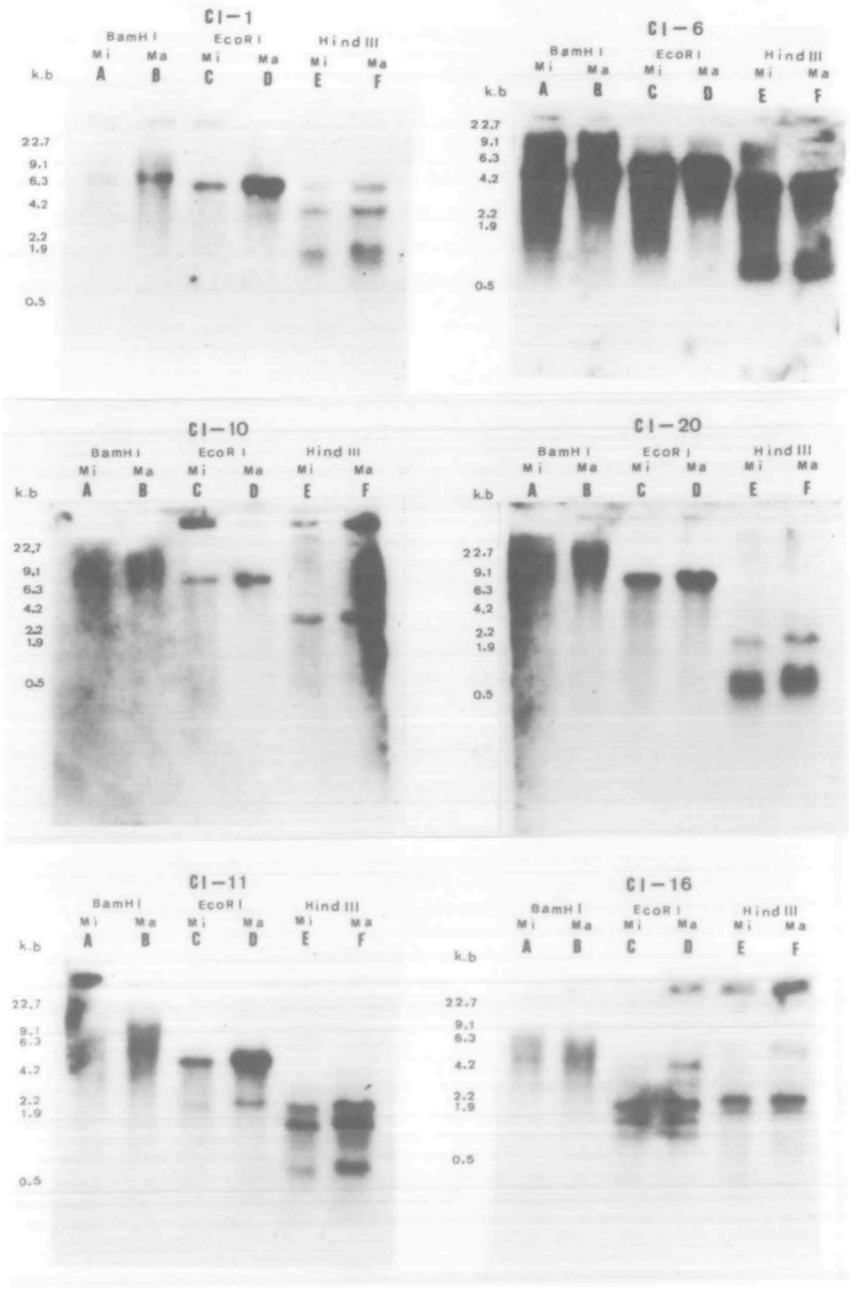
Enzymes

EcoR I, BamH I and Hind III were purchased from Takara-Shuzo Co. Other enzymes were purchased from Bethesda Research Laboratories, Inc.

RESULTS

1. Sequences which show the same pattern between micro-and macronucleus

The sequences cloned could be divided into two categories; one those which showed the same pattern between micro- and macronuclear DNA and the other those which showed some differences. In Figure 1, the blotting patterns were presented of the clones



which did not show detectable differences between micro-and macronuclear DNA. As cloned DNAs were EcoR I fragmens of micronuclear DNA, if the sequence existed only once in the genome, one hybridization signal should be expected at the position of the cloned fragment when EcoR I digested micronulear DNA is blotted. On the contrary, when a repetitive sequence was used as a probe, additional bands together with smears could also be seen on EcoR I digested micronuclear DNA. Clone 1, 10 and 20 showed only one band each at the sites where the cloned sequences migrated (Fig. 1). Clones 6, 11 and 16 showed two to several bands in EcoR I digests. All these patterns, however, did not differ between micro-and macronuclear DNA. We regard that these sequences exist a few times in the micronuclear genome but amplify in the same way as non-repetitive sequences without rearrangement. However, the possibility remains that some of these bands are created by DNA modification occurred at some EcoR I cleavage sites (8). Clone 16 DNA showed almost the same patterns between micro- and macronuclear DNA digested with BamH I, EcoR I and Hind III. Although we regard the slight differences found in EcoR I and Hind III digests to be due to experimental variations, the possibility of reorganization of this sequence cannot be ruled out. The bands detected with different clones were summarized in Table 1. Thus all of the nonrepetitive sequences obtained here including sequences which showed a few bands without smears were amplified from micronucleus to macronucleus without detectable reorganization.

2. Sequences which show difference between micro-and macronucleus

Clones 4, 13 and 5 DNAs (Fig. 2) obtained by screening

Figure 1. Hybridization patterns of labeled clone 1, 6, 10, 20, 11 and 16 DNAs with micro- and macronuclear DNAs of T. thermophila digested with BamH I, EcoR I, or Hind III.

Nuclear DNAs were digested with each endonuclease, electrophoresed on a gel, and hybridized as described in the text. Lane A, micronuclear DNA digested with BamH I; Lane B, macronuclear DNA digested with BamH I; Lane C, micronuclear DNA digested with EcoR I; Lane D, macronuclear DNA digested with EcoR I; Lane E, micronuclear DNA digested with Hind III; Lane F, macronuclear DNA digested with Hind III. Hind III digested λDNA fragments were used as size markers.

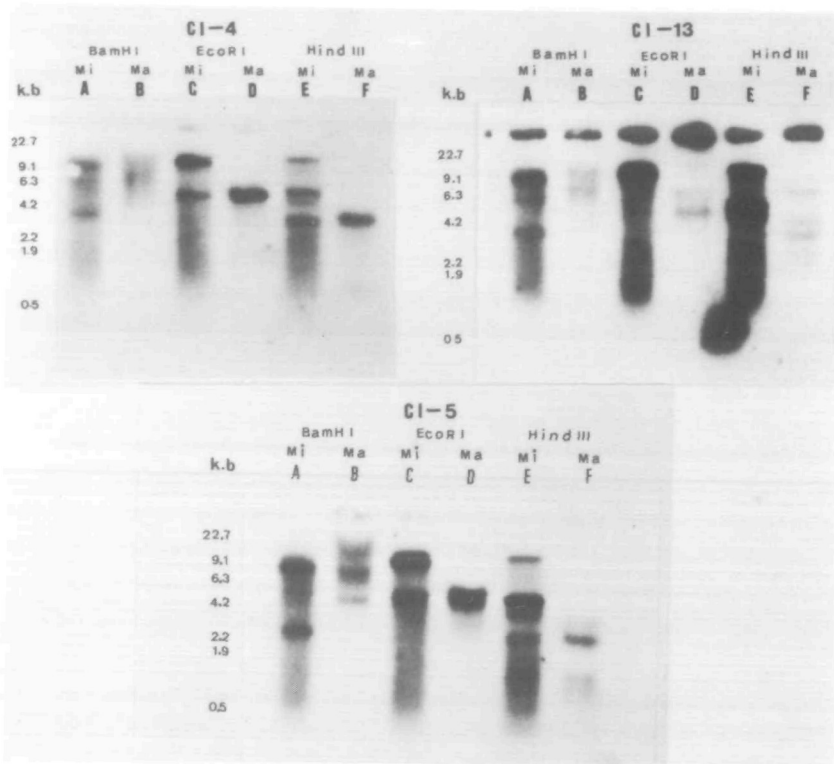


Figure 2. Hydridization patterns of labeled clone 4, 13 and 5 DNAs with micro- and macronuclear DNAs of T. thermophila digested with BamH I, EcoR I or Hind III.

Nuclear DNAs were digested with each endonuclease, electrophoresed on a gel and hybridized as indicated in the text. Lane A, micronuclear DNA digested with BamH I; Lane B, macronuclear DNA digested with BamH I; Lane C, micronuclear DNA digested with EcoRI; Lane D, macronuclear DNA digested with EcoR I; Lane E, micronuclear DNA digested with Hind III: Lane F, macronuclear DNA digested with Hind III. Hind III digested λDNA fragments were used as size markers.

with repetitive micronuclear DNA as probe, showed very different patterns between micronuclear and macronuclear DNAs when digested with either BamH I, EcoR I or Hind III. The sizes of these clones were 5.8, 4.6 and 5.4 kb long, respectively. When mironuclear DNA was digested with EcoR I, all these cloned DNA probes revealed two to several bands together with rather broad smears (Table 2 and Fig. 2). On the contrary, when

Table 1

Probe		Cl-1	Cl-6	Cl-10	Cl-20	Cl-11	Cl-16
DNA		Mic. & Mac.	Mic. & Mac.	Mic. & Mac.	Mic. & Mac.	Mic. & Mac.	Mic. & Mac.
k.b.	BamH I	6.9	11.0	16.5	21	14	6.9
			4.7			6.0	4.2
	EcoR I	6.3	6.3	8.0	7.2	5.4	5.0
			4.0			3.1	2.0 1.6 1.2
	Hind III	7.6	3.7	4.0	2.9	3.0	6.9
		3.9	0.7		1.6	2.4	2.5
		1.9				1.0	2.0

macronuclear DNA was digested with EcoR I, one (clone 4 and 5) and three (clone 13) bands were found but all the smears disappeared (Table 2 and Fig. 2). With other restriction enzymes, similar relationship was found; namely, the smears that were detected in micronuclear DNA were not found in macronuclear DNA digest and the number of the discrete bands decreased, too. These results indicate that only certain selected portions of these repetitive DNA sequences or repeat contiguous sequences were amplified with concurrent rearrangement

Table 2

Probe		Cl-4		Cl-13		Cl-5	
DNA		Mic. & Mac.		Mic. & Mac.		Mic. & Mac.	
k.b.	BamH I	18	23	13	19	9.3	18
		6.9	9.1	5.8	7.6	5.5	7.0
		3.5		3.0		2.4	4.5
	EcoR I	20		18	8.3	13	
		5.8	6.3	4.6	4.6	5.4	4.6
				2.2		3.9	
				1.2			
	Hind III	20		19		18	
		6.9	4.2	5.0	7.6	4.6	3.3
		5.4		3.9	3.9	3.6	2.5
		4.0		3.0	3.2	2.5	1.4
				2.2	2.2		

and some middle or highly repetitive sequences were virtually eliminated during macronuclear development.

3. Structural analysis of clone 5

The 5.4-kb clone 5 DNA fragment was excised from the recombinant phage λ. Endonuclease Hind II cut this DNA into five fragments of 2.57, 1.69, 0.54, 0.36 and 0.20 kb long designated A, B, C, D and E, respectively. The isolated fragments were run on a gel, blotted and hybridized with labeled repetitive sequences of micronuclear DNA. Fig. 3 shows that all the Hind II fragments of clone 5 DNA were hybridized with repetitive sequences in micronuclear DNA. The data indicate that at least one of the middle or highly repetitive sequences in micronuclear DNA were present in every fragment. However, since fragments A, B and C did not cross-hybridize

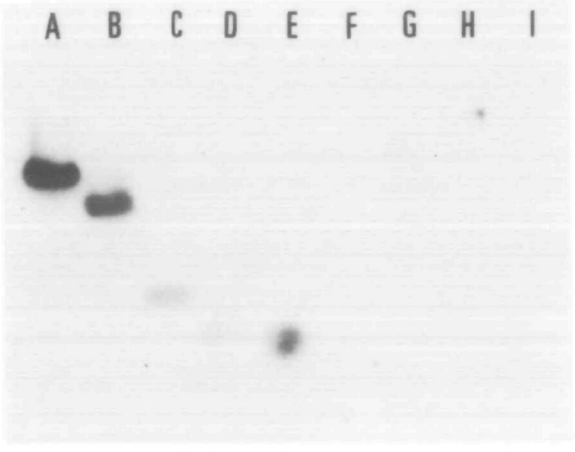


Figure 3. Hybridization of Hind II fragments of clone 5 DNA with repetitive sequences in micronuclear DNA.

Repetitive DNA fraction of micronuclear DNA was prepared and nick-translated as indicated in Materials and Methods. Hind II fragments of clone 5 DNA were electrophoresed on a gel, and hybridized with the above probe as indicated in the text. Lane A, Hind II A fragment (2.57-kb); Lane B, Hind II B fragment (1.69-kb); Lane C, Hind II C fragment (0.36-kb); Lane E, Hind II E fragment (0.20-kb); Lane F, clone 1 DNA; Lane G, clone 6 DNA; Lane H, clone 10 DNA and larger two fragments of λgtWES·λB DNA digested with EcoR 1; Lane I, clone 20 DNA and larger two fragments of λgtWES·λB DNA digested with EcoR 1. The faint bands at the position of λgtWES·λB DNA fragments may be backgrounds.

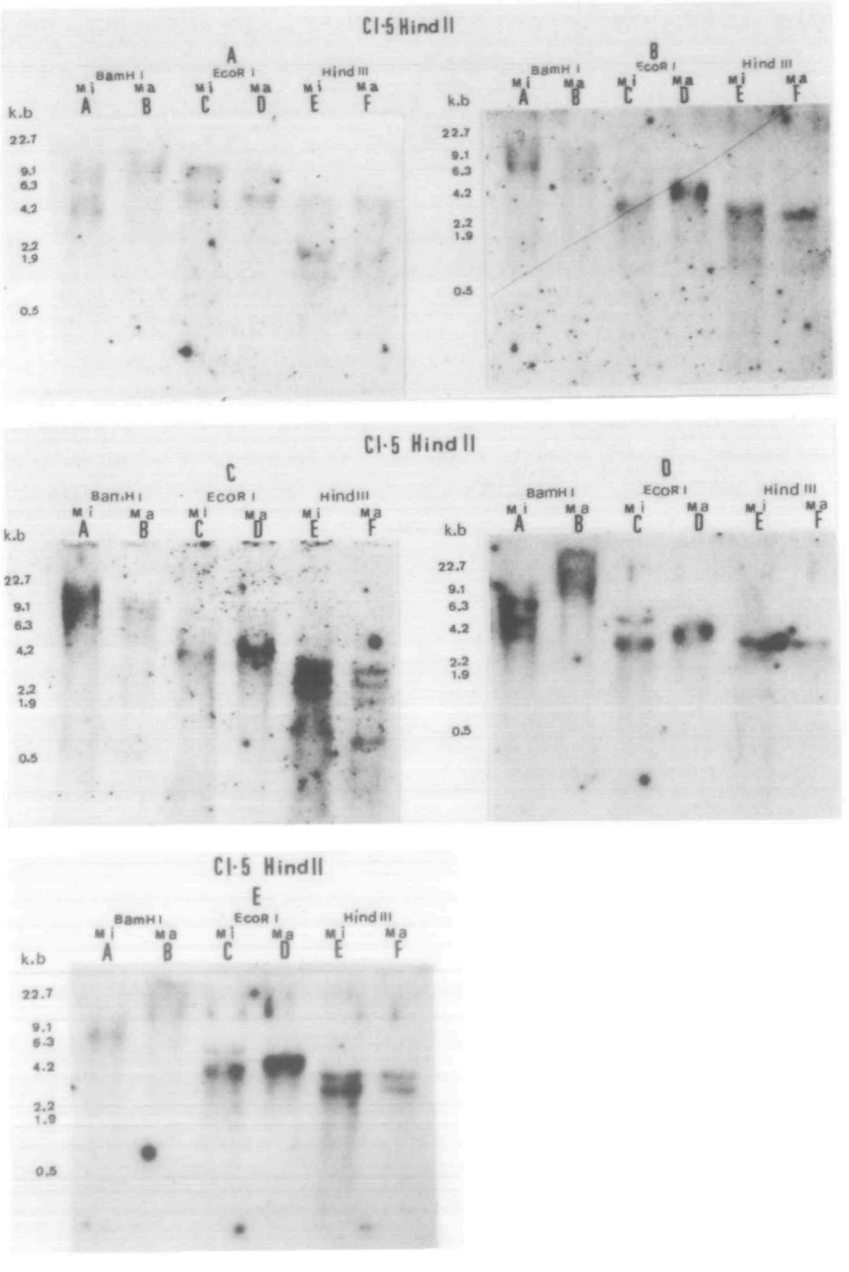
each other (data not shown), repetitive sequences dispersed in clone 5 DNA must represent different families. This experiment establishes that at least cloned DNA contains various repetitive sequences along the whole length of the clone, although the presence of some repeat-contiguous non-repetitive sequences cannot be ruled out.

4. Blot Hybridization with Hind II fragments

Next, these Hind II fragments of clone 5 were used as probes for blot hybridization. When micronuclear DNA was digested with EcoR I, two or three bands at 13, 5.4 and 3.9 kb were always found with the probes of A, B, C, D and E fragments, whereas only one band was seen at 4.6 kb when EcoR I digest of macronuclear DNA was analyzed with these probes (Fig. 4). With other restriction enzymes (BamH I and Hind III), most bands could be explained by the fact that these fragments were the parts of the clone 5 DNA, except for a few vague bands which might be ascribed to some technical failure. In any event, these results confirm that all of these fragments were rearranged in macronuclear DNA but none of the fragments were eliminated completely from macronuclear DNA. The smears which were characteristic of micronuclear DNA (Fig. 2) were less well detected in these experiments due to the shorter exposure time used to prevent higher background.

DISCUSSION

According to reassociation kinetics, the micronuclear DNA of Tetrahymena thermophila consists of significant amounts of highly repeated, moderately repeated, and single copy or non-repetitive sequences. On the other hand, the macronuclear DNA is composed mainly of one moderately repeated class of DNA whose Cot 1/2 value is close to that of the nonrepetitive sequences of the micronucleus, although the presence of a few percent of highly repeated sequences is also apparent (5). In this study, we isolated clones of nonrepetitive sequences and those with repetitive sequences in micronuclear DNA and compared the organization of these sequences in micro- and macronuclei of this organism by blot hybridization. Since all the cloned nonrepetitive sequences had the same structure in both nuclear



DNAs, we suspect that many of the nonrepetitive sequences in micronuclear DNA amplify without appreciable reorganization (Fig. 1). These sequences may contain structural genes for proteins. Because some cloned sequences which were not repetitive but showed a few bands, showed the same blotting pattern in both nuclei (Fig. 1), we suppose some sequences existing a few copies in micronuclear DNA, were amplified with the same structure in macronuclear DNA. These sequences may also be structural genes whose base sequences resembling each other. On the other hand, some repetitive and/or repeat-contiguous sequences, cloned in this experiment, drastically changed their organization in macronucleus and at the same time decreased its content in macronucleus (Fig. 2). It is apparent that all these complex changes could not be explained by DNA modification alone (8). Ciliates are perhaps unique in showing extensive genome reorganization during development. In Tetrahymena, elimination of roughly 15% of the genome has been reported (14). More extensive reorganization has been observed in another group of ciliates, the hypotrichs, in which the majority of the genomic DNA is eliminated, and the remaining part in the macronucleus is broken down into gene-sized molecules (6). Moreover the junction of ribosomal DNA were eliminated from the macronucleus (15).

It is tempting to speculate that in Tetrahymena some of the highly repeated DNA in micronucleus is a satellite-type DNA concerned with mitotic movement of chromosomes of micronuclei. Such DNA may not be necessary so much in the macronucleus

Figure 4. Hybridization patterns of Hind II A, B, C, D and E fragments of clone 5 DNA with micro- and macronuclear DNA of T. thermophila digested with BamH I, EcoR I and Hind III.

Nuclear DNAs were digested with each endonuclease, electrophoresed on a gel, and hybridized with one of the labeled probes as indicated. Lane A, micronuclear DNA digested with BamH I; Lane B, macronuclear DNA digested with BamH I; Lane C, micronuclear DNA digested with EcoR I; Lane D, Macronuclear DNA digested with EcoR I; Lane E, micronuclear DNA digested with Hind III; Lane F, Macronuclear DNA digested with Hind III. Hind III digested λDNA fragments were used as size markers.

which divides amitotically and may be discarded partially when a macronucleus develops. Some repeated classes may represent certain regulatory sequences for structural genes. These sequences may not have to be amplified to the same extent as the unique structural genes themselves, since they are already present abundantly in micronucleus. It is also possible that other selected repetitive and repeat-contiguous sequences amplify for the purpose of efficient expression. The reorganization of repeated sequences demonstrated in this study suggests the existence of complicated gene amplification mechanisms in macronuclear formation before effective transcription and expression of the genome occur.

We studied more closely the clone 5 DNA, whose sequence was drastically reorganized in macronuclei (Fig. 2) and contained repetitive sequences (Fig. 3). In order to locate the repetitive sequences in clone 5 DNA, we have cut it by Hind II into 5 fragments and tested for the presence of repetitive sequences in each fragment. However, it was found that all the 5 fragments contained some repetitive sequences (Fig. 3). These observations were confirmed by a reciprocal blot hybridization (Fig. 4). Whereas the clone 5 DNA was 5.4 kb long in micronuclear DNA, its sequence was replaced with a 4.6 kb long counterpart in macronuclear DNA. So some sequences in clone 5 DNA must have been lost in macronuclear DNA. It is noteworthy that all five Hind II fragments of clone 5 DNA (5.4 kb) could hybridize with the only 4.6 kb EcoR I band in macronuclear DNA indicating that each Hind II fragment contained some repetitive sequence homologous to that of 4.6 kb band. This could only be explained by rearrangement of repetitive sequences occurred within clone 5 DNA. This will be demonstrated by comparing the sequences of clone 5 and of 4.6 kb fragment DNA which is now under cloning.

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