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Reproducible and Variable Genomic Rearrangements Occur in the Developing Somatic Nucleus of the Ciliate *Tetrahymena thermophila*

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We analyzed the extent, reproducibility, and developmental control of genomic rearrangements in the somatic macronucleus of the ciliate *Tetrahymena thermophila*. To exclude differences caused by genetic polymorphisms, we constructed whole-genome homozygotes, and we compared the homozygous progeny derived from single macronuclear differentiation events. This strategy enabled us to identify a novel form of variable rearrangement and to confirm previous findings that rearranged sequences occur at a high frequency in the *Tetrahymena* genome. Rearrangements studied here were deletions of both unique and interchromosomally dispersed repetitive DNA sequences involving DNA rejoining of internal, nontelomeric regions of macronuclear DNAs. We showed that although rearrangements of some sequence classes are reproducible among independently developed macronuclei, other specific sequence classes are variably rearranged in macronuclear development. The variable somatic genomes so produced may be the source of phenotypically variant cell lines.

A long-standing question in eucaryote development is the extent and nature of DNA rearrangement during development. Irreversible differential gene alterations have been found in a number of widely separated systems. Developmentally programmed examples of these include the chromosomal diminution found in nematodes (reviewed in reference 36), some insects and crustaceans (reviewed in references 5 and 35), and ciliates (reviewed in references 15, 24, and 25) and DNA rearrangements which occur in antibody-producing lymohocytes (reviewed in reference 34).

The ciliated protozoan *Tetrahymena thermophila* affords an excellent model system to determine the extent, nature, and developmental timing of rearrangements during development of the somatic nucleus. *T. thermophila* is a unicellular organism containing two nuclei, a transcriptionally inactive germline nucleus (micronucleus) and a somatic, amitotically dividing macronucleus which is the site of gene expression. In the sexual process, conjugation, haploid micronuclei generated by meiosis are exchanged between the paired cells and subsequently fused. A division product of the newly diploid micronucleus in each cell differentiates into a new macronucleus, replacing the old macronucleus, which is destroyed (for reviews, see references 12, 14, and 15). Since these cells subsequenly divide vegetatively by binary fission in the absence of conjugation (which requires specific culture conditions as well as interaction between two cells of different mating types), large clonal populations can be obtained at given stages of development, and pure preparations can be made of macronuclei and their parent micronuclei.

Evidence that the sequence complexity of the macronucleus has been reduced relative to the micronucleus has come from two lines of investigation. First, C_0t studies (39) showed that 10 to 20% of the sequences found in the micronucleus are absent in the macronucleus. Most of the eliminated sequences are moderately repetitive (about 100 copies per genome). Second, specific middle repetitive sequences present in the micronucleus have been shown to be eliminated in the macronucleus as detected by in situ hybridization (41) and biochemical analysis (8, 37, 38). In addition to DNA sequence elimination, the micronuclear genome has been shown by sucrose density gradient analysis to be converted in the macronucleus to defined subchromosomal linear fragments (32), with an average size of about 600 kilobases (kb). The telomeres of these molecules share a common repeated hexanucleotide sequence $(C_4A_2)n$ (40).

By comparing the arrangement of randomly chosen regions of the genome in the micronuclei and macronuclei, it is possible to ask whether DNA rearrangements are a general aspect of development in *T. thermophila* or whether the rearrangements are strictly confined to regions fated to become macronuclear telomeres. In this paper we show that developmentally controlled DNA rearrangements occur at remarkably high frequency in the *T. thermophila* genome. None of the rearranged, retained macronuclear sequences we examined are located near the macronuclear telomeres. Furthermore, by caryonidal analysis of the clonal descendents of single newly formed macronuclei, we showed that although the rearrangements of some DNA sequences are reproducible, other DNA sequences undergo variable rearrangements in macronuclear development.

MATERIALS AND METHODS

***T. thermophila* cell strains, culture conjugation, and production of whole-genome homozygotes.** Growth medium and starvation medium for culturing and mating, respectively, were as described in Blackburn et al. (6).

Mating of *T. thermophila* strains was done as described by Orias and Bruns (29). Genomic exclusion was done as described by Allen (1) and is described in the legend to Fig. 1. Parent *T. thermophila* strains used for the first round of genomic exclusion were CU324 [Chx^+/chx^+, Mpr/Mpr (chx-S, 6mp-S, mating-type IV)] and A* (defective micronucleus, wild-type macronucleus that induces genomic exclusion, mating-type III). The products of the second round of genomic exclusion were designated 17A, 17B, 17C, and 17D. 17A and 17B were derived from one exconjugant of a single

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pair, and 17C and 17D were derived from the other (see Fig. 1). *T. thermophila* strains used for nullisomic analysis are described below.

Isolation of micro- and macronuclei. The protocol for isolation of micro- and macronuclei was adapted from (17). Cells were grown from an overnight inoculum in 2 liters of PPYS (17) at 30°C with shaking to a density of 2.0×10^5 to 2.5×10^5 cells per ml. Cells were first spun at 2,800 rpm for 4 min in a Sorvall GSA rotor, leaving 50 to 100 ml of supernatant per centrifuge bottle and then respun two to three times at 2,800 rpm for 4 min in an HB-4 rotor to obtain loosely packed pellets. Loosely packed cells were washed with 100 ml of nuclei wash solution (10% sucrose, 10 mM Tris, 1.5 mM magnesium chloride [adjusted to pH 7.5 with HCl]), spun at 1,250 rpm for 4 min in an HB-4 rotor, and suspended in 180 ml of solution A (4% acacia gum [from 20% stock prespun at 16,000 × *g* for 10 min], 3% sucrose, 10 mM Tris, 1.5 mM magnesium chloride, 5 mM spermidine-trihydrochloride, 1 mM spermine-tetrahydrochloride [adjusted to pH 7.5 with HCl]) with *n*-octanol added to 1% (vol/vol) just before use. Cells were blended three times for 30 s each in a prechilled Waring blender at the lowest setting to separate the micro- and macronuclei. Cells were placed on ice between blendings. EDTA was added to 8 mM to inhibit nucleases, and the cells were blended an additional 20 s. Macronuclei were spun down by centrifugation at 3,000 rpm for 6 min in the HB-4 rotor. The micronuclei-enriched supernatant was reblended for 20 s and respun. The latter procedure was repeated two times, the supernatant was blended an additional two times for 30 s each, and the micronuclei were concentrated by spinning at 10,000 rpm for 10 min in the HB-4 rotor. The nuclei were washed in 40 ml of medium A (0.1 M sucrose, 4% acacia gum, 0.1% [wt/vol] spermidine-trihydrochloride, 5 mM EDTA [adjusted to pH 6.75 with NaOH]) and respun at 3,500 rpm in the HB-4 rotor for 10 min. Micronuclei were suspended in 25 ml of medium A, filtered from contaminating macronuclei through a 47-mm Nuclepore filter (pore size, 8 μm), and subsequently filtered through a 5-μm-pore-size Nuclepore filter. The filtration steps were done under low vacuum. The filtrate was concentrated by spinning at 3,500 rpm for 10 min and suspended in 1 ml of nuclear isolation buffer (0.06 M potassium chloride, 0.015 M sodium chloride, 0.5 mM spermidine-trihydrochloride, 0.15 mM spermine-tetrahydrochloride, 0.015 M Tris [adjusted to pH 7.4 with HCl]). At this stage, micronuclei were examined cytologically to determine the level of macronuclear contamination. All macronuclear preparations had a macronuclear DNA contamination equal to or less than 3% (routinely less than 1%) and a routine yield of 15 to 20%. Micronuclei were brought to 20 ml in nuclear isolation buffer, spun at 5,000 rpm for 10 min, and suspended in no less than 1 ml of buffer A (0.06 M potassium chloride, 0.015 M sodium chloride, 0.5 mM spermidine-trihydrochloride, 0.15 mM spermidine-tetrahydrochloride, 0.015 M Tris hydrochloride [pH 7.4]). Macronuclear pellets (from the blending centrifugations) were centrifuged for 10 min at 6,000 rpm during the filtration steps and suspended in 2 to 5 ml of buffer A. DNA was extracted from both nuclei as described for whole-cell DNA isolations, omitting RNase treatment. Breakdown was particularly affected by two factors, initial cell density and the amount of time taken to harvest cells. Cells were kept on ice between all spins and spun in prechilled rotors, bottles, and tubes. The solution volume for blending could not be reduced or shear forces destroyed the intact nuclei and greatly increased breakdown and reduced yields. Yield was most affected by cell loss during harvesting. If micronuclei were suspended in less than 1 ml of buffer A, a significant proportion (>50%) of the high-molecular-weight micronuclear DNA was irreversibly trapped in the interface after phenol extraction. Purity was most affected by the initial cell density and the amount of care taken during filtrations. Preparations of micronuclei routinely contained <1 macronucleus per 2,000 to 3,000 micronuclei as judged by light microscopic examination.

To remove contaminating carbohydrates, micronuclear and macronuclear DNAs were further purified on a CsCl gradient (gradient consisting of 5.8 ml DNA solution, to which had been added 6.35 g of CsCl and 0.7 ml of ethidium bromide [2 mg/ml], spun at 47,000 rpm for 44 h at 15°C in a Ti 50 rotor, extracted with CsCl-saturated isopropanol, and concentrated by ethanol precipitation.

DNA cloning procedures. Cloning of the *T. thermophila*-Charon 28 recombinant clones and subcloning of p7H3, p7H4, and p7H5 from λTtBg7 has been described previously by Blackburn et al. (6). Clones pTtsC, pTtsN, pTts19, pTts23, and pTts25 were cloned as described by Blackburn et al. (6). pBR322 was used as the vector for the subclones p7H3, p7H4, and p7H5 of λTtbg7. pCEH15 (19) was used as the vector for clones pTtsC, pTtsN, pTts23, and pTts25.

DNA isolations. Isolation of *T. thermophila* whole-cell DNA and rDNA was done as described by Blackburn et al. (6). Charon 28 phage and Charon 28 recombinant DNA isolation and plasmid DNA isolations were also done as described previously by Blackburn et al. (6).

Digestions with BAL 31 nuclease and restriction enzymes. BAL 31 nuclease (18) was obtained from New England Biolabs. The digestion buffer used was that recommended by the manufacturer. Reactions were stopped by the immediate addition of the time-point sample to an equal volume of phenol-chloroform (1:1) in a dry ice-ethanol bath. Samples were then ether extracted and ethanol precipitated. Restriction enzymes were purchased from New England Nuclear, Bethesda Research Laboratories, or Boehringer Mannheim and were used according to the instructions of the manufacturer.

DNA blotting and hybridizations. Hybridization analyses with nitrocellulose filters were carried out by standard procedures. Hybridization analyses to diazotized APT paper were as described by B. Seed, as presented by Maniatis et al. (26). Details of the procedures are described by Blackburn et al. (6). Probes were labeled with ^{32}P to a specific activity of 10^8 cpm/μg.

RESULTS

Genomic analysis of macronuclear and micronuclear-derived cloned DNA segments. The events in conjugation of *T. thermophila* are outlined in Fig. 1, which shows that all four caryonidal cells (caryonidal set) produced from a single pair of mated cells have genetically identical micronuclei, and the macronucleus in each caryonidal cell developed independently from a mitotic sister of these identical micronuclei. An important aspect of these experiments was to eliminate background differences between clones of cells due to meiotic or mitotic recombination between polymorphic alleles or from phenotypic assortment of heterozygous alleles in macronuclei during vegetative growth of cells. To this end whole-genome homozygotes were first constructed, using the method of genomic exclusion (see legend to Fig. 1; for reviews see references 1 and 10). To examine the clonal descendants of single macronuclei, the four caryonidal cells from each of several pairs of cells from the second mating of the genomic exclusion cross were separately isolated. The

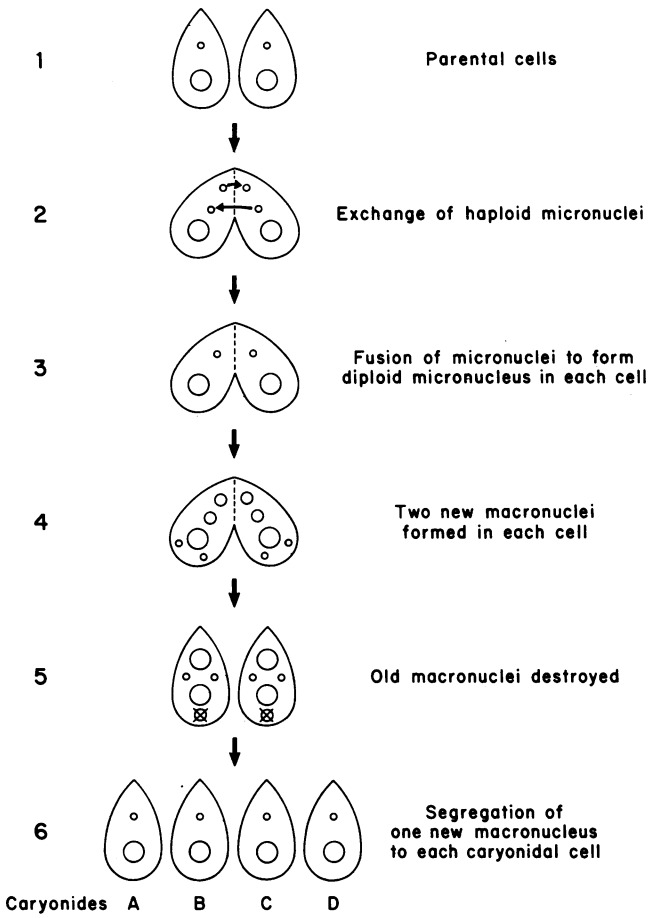

FIG. 1. Conjugation in *T. thermophila*. (Step 1) The first visible stage of conjugation occurs when the two cells of differing mating types fuse anteriorly to form a pair. The micronucleus of each cell undergoes meiosis. Three meiotic products migrate to the posterior end of each conjugant and are destroyed. The remaining product divides mitotically, producing two identical gametic pronuclei. (Step 2) Migratory gametic pronuclei are exchanged between conjugants. (Step 3) The pronuclei in each cell fuse to form a zygotic (fertilization) nucleus. (Step 4) Each zygotic nucleus divides twice mitotically. The anterior products differentiate into new macronuclei and the posterior products into new micronuclei. Conjugants each contain two new macronuclei and micronuclei. (Step 5) The old macronucleus moves to a posterior position and is eventually destroyed. The conjugants separate. One new micronucleus is destroyed in each exconjugant. The other micronucleus divides mitotically. (Step 6) Products of the first vegetative cell division (caryonides labeled A, B, C, and D each receive one new macronucleus and a copy of the new micronucleus. In the first round of genomic exclusion (ref. 1, referred to in the text), the defective micronucleus of the A* mate degenerates. Steps 1 and 2 proceed as in the normal mating, followed by a one-way transfer of the migratory pronucleus. The two pronuclei diploidize, and the old macronucleus is retained. Both exconjugants thus carry identical diploid micronuclei (homozygous for the haploid genome inherited from the single meiotic product of the normal mate) but each exconjugant expresses its original (parental) phenotype. In the second round of genomic exclusion, the exconjugants of the first round are recrossed in a normal mating (steps 1 through 6). New macronuclei are formed from the identical micronuclei. The progeny of this cross are now whole-genome homozygotes with respect to their micro- and macronuclei.

macronuclei of all these caryonides, therefore, were all developed from genetically identical, totally homozygous micronuclei. A clone of each caryonidal cell was maintained as a vegetatively dividing cell culture, and these cell clones were the source of the micro- and macronuclear DNAs described here.

To estimate the frequency and distribution of genomic rearrangement events in *T. thermophila*, a random sampling of cloned DNA segments was chosen and used to probe Southern blots of micronuclear and macronuclear genomic DNAs. If rearrangement events were associated only with chromosomal fragmentation and the formation of macronuclear telomeres, nontelomeric regions (i.e., internal regions of the macronuclear subchromosomal fragments) would not have been rearranged during macronuclear development. To address this question, macronuclear DNA segments were cloned by a strategy which selects against the cloning of telomeres. The *Tetrahymena* macronuclear DNA was partially restricted with *Bgl*II and cloned into the *Bam*HI-digested arms of Charon 28; hence, only internal regions of *Tetrahymena* macronuclear DNA should be represented in the library, since cloning of telomeric fragments requires a strategy of either S1 or BAL 31 treatment of the DNA, followed by blunt-end ligation into the cloning vector (13, 33).

In addition, micronuclear DNA partially digested with *Sau*3A was cloned into the *Bgl*II sites of vector pCEH15 (for a description of the vector, see reference 19). Restriction analysis and genomic blotting analysis indicated that none of these clones shared sequences with the macronuclear-derived clones. Each of the clones described was used to probe Southern blots of genomic digests of micronuclear and macronuclear DNAs.

It should be noted that in all cases the micronuclei and macronuclei analyzed were isolated from whole-genome homozygotes. Any changes observed in DNA organization in the results described below, therefore, cannot be the result of meiotic or mitotic recombination or of assortment during vegetative growth of heterozygous polymorphic alleles. The whole-genome homozygotes used here were also caryonidal in origin, that is, all macronuclei in each population were derived from a single micronucleus-to-macronucleus transition event. Restriction enzymes known to be unaffected by DNA methylation in *T. thermophila* were used in these analyses so the results would not be complicated by differential methylation of macronuclear and micronuclear DNAs (7, 16). Representative results are shown in Fig. 2, 3, 4, and 5. Analysis of all clones is summarized in Tables 1 and 2.

The DNA clones fell into four classes based on their fates in macronuclear development. The first class included the micronuclear-derived clones containing only repetitive DNA (clones pTts19 and pTts25). The sequences in these clones were eliminated during macronuclear development, as were all repeats in the micronuclear genome with homology to these clones (Fig. 2a). Similarly eliminated repeated sequences have been described by Brunk et al. (8), Yao (37), and Karrer (21).

The second class included macronuclear-derived and micronuclear-derived clones containing homology to an additional locus in the micronuclear genome (clones λTtBg4 and pTtsN). The cloned region itself was not rearranged during development; however, the additional locus to which these clones had homology was eliminated in the macronuclear genome. Clone λTtBg4 hybridized to 15.5-, 10.5-, and 2.3-kb micronuclear *Bgl*II fragments and to 10.5- and 2.3-kb

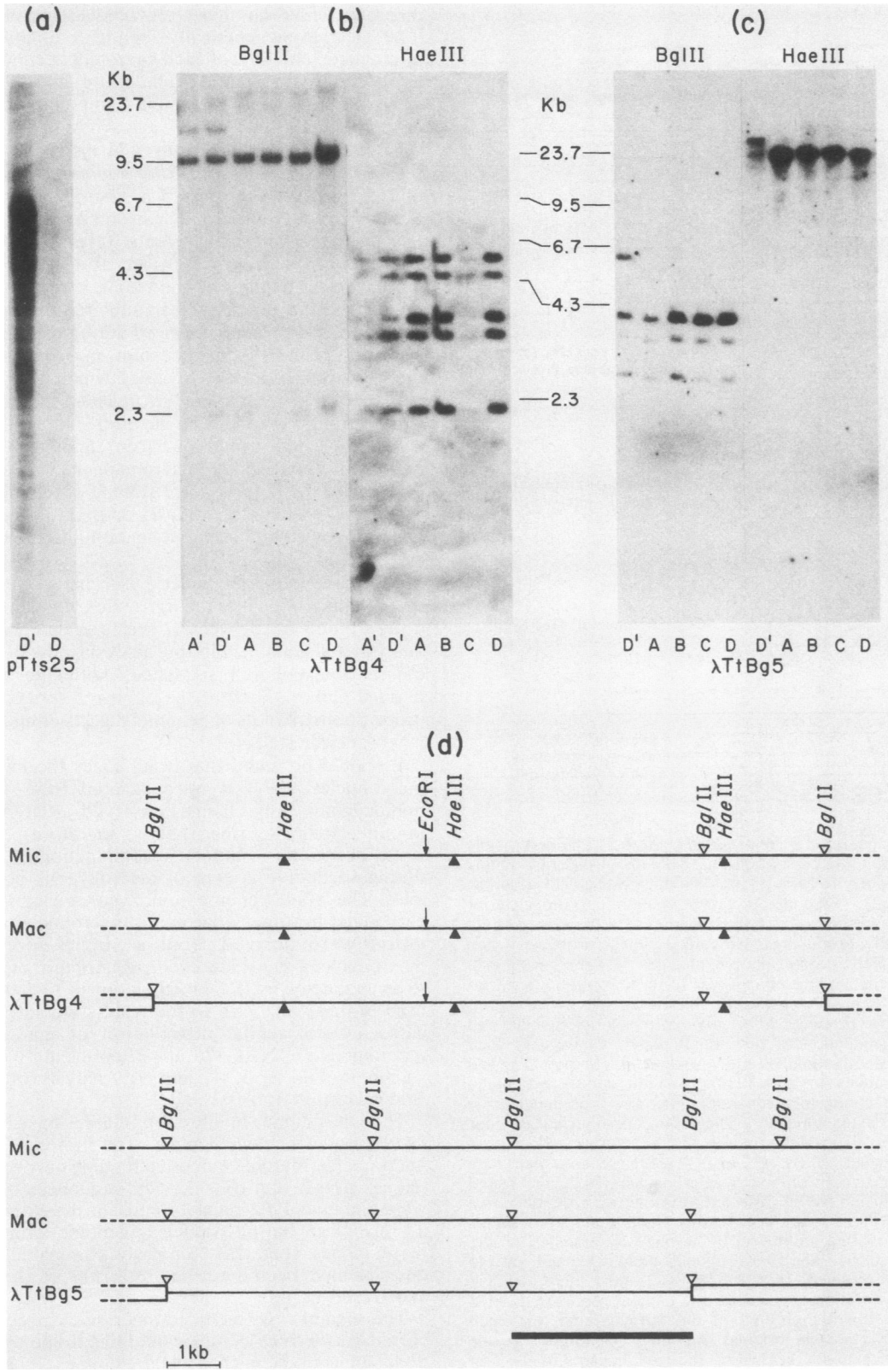

FIG. 2. Genomic micronuclear and macronuclear restriction fragments with homology to three types of processed sequences. (a through c) Nuclear DNAs from a set (set 17) of caryonidal clones were analyzed. Micronuclear DNA (10 μg) from the clonal descendants of caryonide 17A (A' lanes), and 17D (D' lanes) and macronuclear DNA from the clonal descendants of caryonide 17A (A lanes), 17B (B lanes), 17C (C lanes), and 17D (D lanes) were digested with either *Bgl*II or *Hae*III, electrophoresed for 15 h at 50 V on a horizontal 0.8% agarose gel, blotted, and hybridized with 2×10^7 cpm of ^{32}P-nick-translated DNA from either (a) clone pTts25, (b) clone λTtBg4, or (c) clone λTtBg5. (d) Restriction maps of λTtBg 4 and λTtBg5 and the micronuclear and macronuclear genomic regions represented in these clones. The rearranged *Bgl*II fragment of λTtBg5 is underlined.

macronuclear BglII fragments (Fig. 2b). Control experiments were performed to show that the 15.5-kb micronuclear *BglII* fragment, which was of much lower intensity than the 10.5-kb micronuclear fragment, was not a product of partial *BglII* digestion. The result of hybridization of λTtBg4 with Southern blots of partially *BglII*-digested micronuclear DNA was the expected array of micronuclear *BglII* fragments, including the 15.5-kb additional fragment, and the expected partial *BglII* digestion products of sizes clearly different from that of the 15.5-kb micronuclear *BglII* fragment (data not shown). The retained sequences were further analyzed by cutting micro- and macronuclear DNAs with *HaeIII*, *EcoRI* and *BamHI* and probing with λTtBg4. Figure 2b shows the micro- and macronuclear *HaeIII* fragments corresponding only to the cloned region itself. The *HaeIII* fragments hybridizing to the second micronuclear locus were not detected in this experiment. In each case the hybridizing micronuclear fragments corresponding to the cloned locus itself, and the retained macronuclear genomic fragments, were identical in size (Fig. 2b and Table 1).

The third class includes macronuclear-derived clones containing only unique DNA (clones λTtBg2, λTtBg5, and λTtBg9). One member of this class, λTtBg5, is examined in Fig. 2. Figure 2c shows that three micronuclear *BglII* restriction fragments, 5.7, 3.8, and 2.7 kb, hybridized to λTtBg5. In macronuclear DNA, the 5.7-kb fragment was absent and was replaced by a 3.4-kb *BglII* fragment. *HaeIII* digestion confirmed that the λTtBg5 sequence is rearranged in the macronucleus compared with the micronucleus. The λTtBg5 segment was contained entirely within a single 23-kb genomic macronuclear *HaeIII* fragment. This fragment contaminates the *HaeIII*-digested micronuclear DNA preparation shown in lane D′ of Fig. 2c; the micronuclear genomic *HaeIII* fragment containing the λTtBg5 fragment was >24 kb. This assignment of λTtBg5 to only a single (>24-kb) micronuclear *HaeIII* fragment is confirmed by the pattern of micronuclear genomic *BglII* fragments which matched that of λTtBg5 (Fig. 2c and d). As shown in Fig. 2c and Tables 1 and 2, the region represented in clones λTtBg2, λTtBg5, and λTtBg9 includes, or is flanked within 10 to 15 kb by, DNA rearranged during macronuclear development.

The fourth class of clones identified contain both unique and repetitive DNA (clones λTtBg7, pTtsC, and pTts23). These clones were all of micronuclear origin. For λTtBg7, the repetitive DNA represented in these clones was eliminated in the mature macronucleus (Fig. 3 and 4). The unique region of DNA represented in each of these clones was variably rearranged (see below) during the micronuclear-to-macronuclear transition. At least in the case of λTtBg7, this rearrangement occurred concomitantly with the elimination of flanking unique as well as repetitive DNA sequences. This was shown by subcloning the internal *HindIII* fragments of the insert in λTtBg7. These subclones were designated p7H3, p7H4, and p7H5 (Fig. 4b). Genomic blots with these subclones and purified subfragments of p7H3 as probes established that at least the majority of the repetitive micronuclear sequences in λTtBg7 are confined to the region represented in subclone p7H3 (Fig. 4a and b). The unique macronuclear, retained region of λTtBg7, which is <2 kb in length (data not shown), falls entirely within fragment 2 of subclone p7H3 (Fig. 4b) and is flanked on each side by two different repetitive sequences in p7H3 which are eliminated from the macronuclear genome (Fig. 4a, lanes 1, 2, and 3). The remaining two subclones, p7H4 and p7H5, are both composed entirely of unique DNA, which is also completely eliminated in the mature macronucleus, as shown for p7H4 in Fig. 4a. This elimination of a segment whose total length is over 7 kb is representative of a less common form of DNA elimination in *T. thermophila*; more commonly, smaller (<1- to 3-kb) internal blocks of DNA are eliminated during macronuclear development (38).

None of the rearranged macronuclear sequences identified in this study was in a macronuclear telomeric region. To test for the proximity of the cloned macronuclear segments to macronuclear telomeres, macronuclear DNA was digested with BAL31 nuclease, and then digested with the restriction enzyme *BglII*, Southern blotted, and probed with the genomic clones λTtBg4, λTtBg5, λTtBg9, p7H3 (a subclone of λTtBg7; see Fig. 4b), pTtsC, and pTtsN. Because macronuclear telomeres are sensitive to shortening with BAL31 nuclease (40), if the sequence represented on the clone were close to a macronuclear telomere, the *BglII*

TABLE 1. Genomic mapping of cloned DNA sequences

Clone	Nuclear genome[a]	Restriction fragments (kb)				Chromosomal location
		BglII	*HaeIII*	*EcoRI*	*BamHI*	
λTtBg2	mic	8	18			1
		3.9	5.6			
	mac	8	16.8			
		2.4	5.6			
			22			
λTtBg4	mic	15.5	5.2	15.5	24	1
		10.5	4.5	6.4		
		2.3	3.5			
			3.2			
			2.0			
	mac	10.5	5.2	15.5	24	
		2.3	4.5	6.4		
			3.5			
			3.2			
			2.0			
λTtBg5	mic	5.7	>24			
		3.8				
		2.7				
	mac	3.8	23			
		3.4				
		2.7				
λTtBg7	mic	R[b]		R		1
	mac	3.5–4.1 (V)[c]	>24			
λTtBg9	mic	8.9	>16			1
		4.5	5.2			
			1.8			
			1.3			
	mac	8.9	16			
		2.6	5.2			
			1.8			
			1.3			
pTtsC	mic		R			ND[d]
	mac		4.5–5.0 (V)			
pTtsN	mic		15			ND
			6.4			
	mac		6.4			
pTts19	mic	R				1
	mac	E[e]				
pTts23	mic	R				ND
	mac	3.7–4.1 (V)				
pTts25	mic	R				ND
	mac	E				

[a] mac, Macronuclear; mic, micronuclear.
[b] R, Repetitive.
[c] V, Variably rearranged in macronucleus.
[d] ND, Not done.
[e] E, Eliminated.

TABLE 2. Extent of rearrangement of *T. thermophila* genome

Clone	Source	Insert length (kb)	Minimum length of genomic DNA sampled (kb)[a]	Type of cloned sequence		Developmental rearrangement/ elimination	Minimum length of DNA eliminated (kb)[b]
				Unique	Repetitive		
λTtBg2		11.0	18	+	−	Yes	1.5
λTtBg4	mac	12.9	24	+	−	No[c]	−[c]
λTtBg5		10	>24	+	−	Yes	2.3
λTtBg9		10	23	+	−	Yes	1.9
pTtsN		1.0	6.4	+	−	No[c]	−[c]
pTts19		3.0	3.0	−	+	Yes	3.0
pTts25	mic	1.0	1.0	−	+	Yes	1.0
pTsC		2.0	6.9	+	+	Yes	ND[d]
pTts23		5.6	8.2	+	+	Yes	4.5
λTtBg7		10.7	>24	+	+	Yes	8.3

[a] Minimum amounts of genomic DNA sampled are estimated from the largest genomic restriction fragments detected.
[b] Estimates are minimal estimates assuming simplest case, i.e., observed rearrangement due to elimination. Additional genomic sequences not considered for clones containing repetitive DNAs.
[c] Only the genomic locus from which the clone was derived is considered here; see text.
[d] ND, Not determined.

fragment on which the region of homology was located would become progressively shorter with time. There was no evidence that BAL31 shortened the fragments hybridizing to any of the clones (data not shown). In a control experiment, the terminal 3.1-kb *Bgl*II fragment of the macronuclear rDNA was progressively shortened and finally completely eliminated during the course of the BAL31 treatment. The cloned DNA sequences examined are therefore ≥3.1 kb from a telomeric end.

Both reproducible and variable rearrangement occur during macronuclear genomic reorganization. The regions of DNA represented in the clones of the first three classes of cloned sequences described above are reproducibly rearranged. This is demonstrated in Fig. 2, in which the clonal descendants of the four caryonidal products of a single mating were analyzed with these DNA clones as hybridization probes. In every case the rearrangements associated with a given probe occurred identically in every caryonidal population, indicating that these rearrangements are highly specific. Identical results were found for three additional caryonidal sets analyzed. The reproducibility of these rearrangements further argues that random processing or rearrangements of macronuclear DNA sequences do not occur in the course of somatic growth.

In contrast, the rearrangements associated with the fourth class of sequences (λTtBg7, pTtsC, and pTts23) do not occur reproducibly in the four caryonidally derived cell populations originating from a single mating pair. Figures 3 and 4 show the variable rearrangement of the λTtBg7 sequence. These results were confirmed and extended by comparing rearrangements of the sequences in λTtBg7, pTtsC, and pTts23 in sets of caryonidal clones from three additional different crosses (data not shown), making a total of 16 caryonidal clones examined. For each of these cloned sequences, there was a limited set of possible sizes of the macronuclear restriction fragment carrying the retained segment. Thus, for λTtBg7, although a total of 16 different caryonidal clones were analyzed, only six discrete fragment sizes were observed. Variability in genomic processing was also detected during analysis of some nullisomic strains of *T. thermophila* (see below and Fig. 6).

Chromosomal distribution of rearranged sequences. Sequences represented in the clones described here were mapped to the five pairs of chromosomes which make up the diploid genotype of the micronucleus. *T. thermophila* strains which have been cytologically and genetically determined to lack both micronuclear copies of chromosomes 2, 3, and 5 (CU359), chromosomes 3, 4, and 5 (CU358), chromosome 3 (CU361), chromosome 4 (CU357), and chromosome 5 (CU354) have been isolated (10). Using this set of nullisomic strains, one can, by elimination, designate a band in a Southern blot of micronuclear DNA to a particular chromosome. Micronuclear and macronuclear DNAs were isolated from each of the strains CU359 (nulli 2, 3, 5), CU358 (nulli 3, 4, 5), CU361 (nulli 3), CU357 (nulli 4), and CU354 (nulli 5). The micro- and macronuclear DNAs were digested with *Bgl*II transferred to nitrocellulose, and hybridized with each of the cloned DNA probes. Figure 5 shows an example of the results obtained with λTtBg7. Four of the six *Bgl*II fragments in nulli 2, 3, 5 micronuclear DNA matched those of λTtBg7 (bands indicated by arrows in Fig. 5, lane 1; see map in Fig. 4b). From this result, λTtBg7 can be deduced to be located on either chromosome 1 or 4. However, the corresponding hybridizing fragments, including the 2.9-kb *Bgl*II fragment known to contain the unique, macronuclear retained region of λTtBg7 (Fig. 4), were also detected in the micronuclear DNA of the nulli 3, 4, 5 strain (Fig. 5, lane 3). Therefore λTtBg7 maps to chromosome 1. Repetitive sequences in λTtBg7 also mapped to at least chromosomes 2 and 5 as well as chromosome 1 (Fig. 5). Note that the nullisomic strains analyzed in this experiment are redundant for certain chromosomes. For this reason, although hybridization to CU357 (nullisomic for chromosome 4) was quite weak, chromosomal assignment could still be made due to detectable hybridization in CU358 (which is also nullisomic for chromosome 4 as well as for chromosomes 3 and 5).

Clones λTtBg4, λTtBg5, λTtBg7, λTtBg9, pTtsN, and pTts19 all mapped to chromosome 1; i.e., the unique portions of the sequences contained in these clones were present in the micronuclei of all nullisomic strains examined. Only chromosome 1 should be present in all of the nullisomic micronuclei. It is not clear why chromosome 1 appeared to be overrepresented in the random sampling of cloned DNAs used for these experiments. The repeats of pTts25 were dispersed to at least chromosomes 1, 2, 4, and 5, and pTts19 contained sequences present, at a minimum, on chromosomes 1, 2, and 4 (data not shown).

The unique micronuclear rRNA gene, which is processed

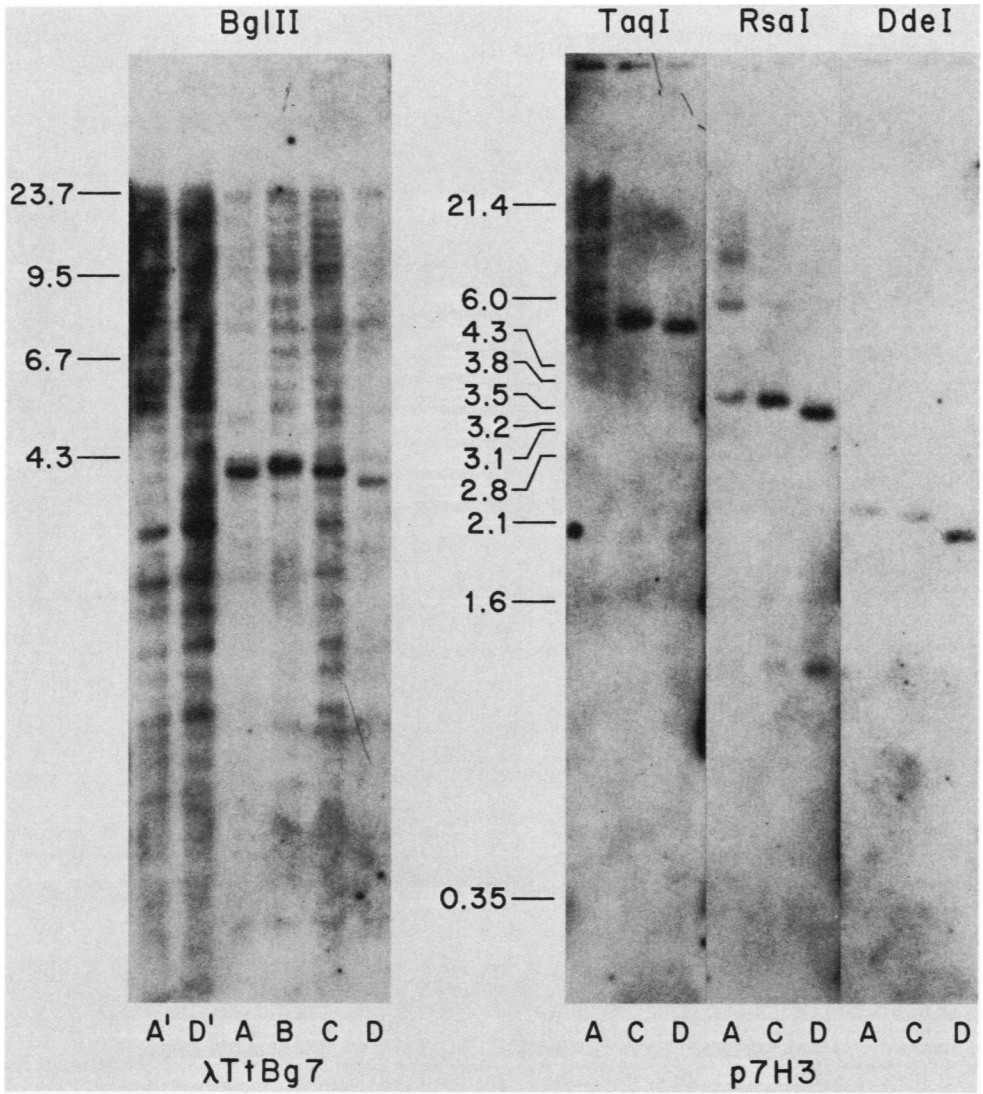

FIG. 3. Variability in retained macronuclear restriction fragments with homology to λTtBg7. (a) Micronuclear DNA (10 μg) from the clonal descendants of caryonide 17A (A′ lanes) or 17D (D′ lane) and 10 μg of macronuclear DNA from caryonide 17A (A lanes), 17B (B lane), 17C (C lanes), and 17D (D lanes) were digested with *Bgl*II, blotted to APT paper and hybridized to 2×10^7 cpm of ^{32}P-nick-translated λTtBg7. Detection of middle repetitive sequences in the macronuclear DNA lanes is due largely or completely to the micronuclear DNA contaminating the DNA preparations; see part b). (b) Highly purified macronuclear DNA (10 μg) from the clonal descendants of caryonide 17A (A lanes), 17C (C lanes), and 17D (D lanes) were digested with *Taq*I, *Rsa*I, or *Dde*I, electrophoresed for 13.5 h in a 1.2% agarose gel, blotted, and hybridized to 2×10^7 cpm of ^{32}P-nick-translated p7H3, a subclone of λTtBg7 (see text and legend to Fig. 4b). The *Taq*I and *Rsa*I digests of caryonide 17A (A lanes) were partial, accounting for the high-molecular-weight-hybridizing bands seen in these lanes. Sizes in kilobases of molecular weight marker fragments are indicated on the sides of the autoradiograms.

during macronuclear development, has been shown by three methods to map to chromosome 2 (9, 10a; E. Orias, M. Koller, J. Shampay, and E. H. Blackburn, unpublished results). Processing of unique DNA sequences, therefore, is not confined to a single chromosome. These results also show that a given eliminated middle repetitive sequence can occur on several different chromosomes, in agreement with previous findings (21, 37).

Genomic reorganization is developmental-stage specific. Variation between caryonides observed with the fourth class of clones (λTtBg7, pTtsC, and pTts23) has two possible (and potentially additive) sources. Caryonides could be variably processed at the time at which the new macronucleus is formed after sexual conjugation or rearrangements could take place during subsequent vegetative propagation. To determine the contribution of these distinct developmental stages to variable processing, the time of rearrangement and somatic stability of the sequences with homology to clone λTtBg7 was analyzed. The approach used for this analysis is summarized in Fig. 6a. Two single caryonidal cells 80A and 80C (from a single cross of whole-genome homozygotes 17A and 17D) were isolated as described above. Whole-cell DNA was isolated from the vegetative descendants of each of the two caryonides at 25 generations after mating (the earliest stage at which sufficient DNA could be obtained for biochemical analysis). An aliquot of the 25-generations time point from each caryonidal clone population was reserved and allowed to continue vegetative divisions for a total of 100

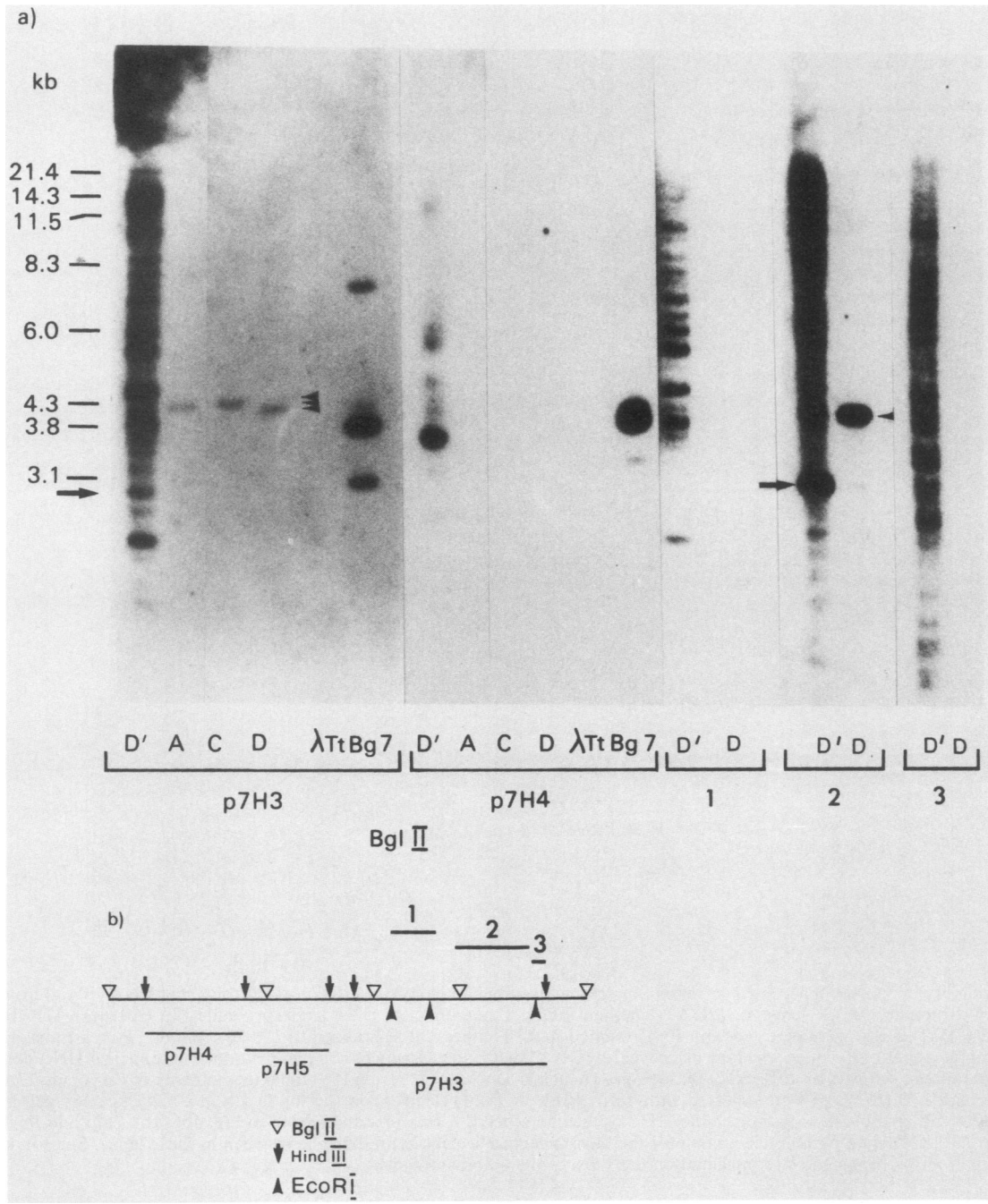

FIG. 4. Only a portion of the sequence of λTtBg7 is retained in the macronuclear genome. (a) Micronuclear DNA from the clonal descendants of caryonide 17D (D' lanes) and macronuclear DNA from the clonal descendants of caryonides 17A (A lanes), 17C (C lanes), and 17D (D lanes) and a dilution of λTtBg7 were digested with *Bgl*II, electrophoresed on a horizontal 0.8% agarose gel, blotted, and hybridized to 2×10^7 cpm of either the ^{32}P-nick-translated clone p7H3, clone p7H4, the *Eco*RI fragment marked 1 in Fig. 4b, the *Bgl*II-*Eco*RI fragment marked 2 in Fig. 4b, or the *Eco*RI-*Hin*dIII fragment marked 3 in Fig. 4b. Sizes in kilobases of marker fragments are indicated on the side of the figure. The arrows next to the blots probed with clone p7H3 or fragment 2 of p7H3 indicate the 2.9-kb micronuclear *Bgl*II fragment which contains the entire retained macronuclear region (indicated by arrowheads in blots probed with p7H3 and fragment 2; data not shown). (b) Restriction map of λTtBg7. Regions subcloned in p7H3, p7H4, and p7H5 are indicated by bars under the map; restriction fragments 1, 2, and 3 are indicated by bars over the map.

generations. Micronuclear and macronuclear DNA were isolated from the 100-generations time point. The arrangement of the region of DNA represented in clone λTtBg7 at each of these stages was analyzed by using genomic blotting techniques.

By 25 generations after mating, the unique retained region of clone λTtBg7 was rearranged and most or all of the repetitive sequences with homology to clone λTtBg7 were eliminated in both caryonidal populations (Fig. 6b). No further rearrangements were detected at or beyond 100 generations after mating. The size of the unique retained *Bgl*II fragment with homology to clone λTtBg7 differed in

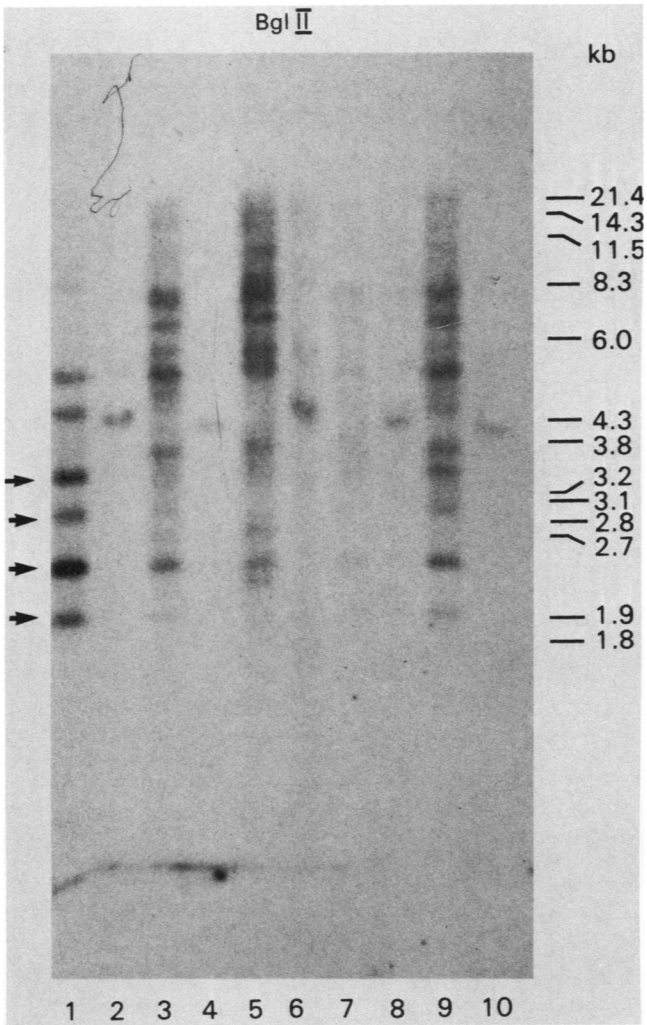

FIG. 5. Nullisomic mapping of λTtBg7. Micronuclear and macronuclear DNAs from strains which are nullisomic in the micronucleus for various germline chromosomes were digested with *Bgl*II, electrophoresed in a horizontal 0.8% agarose gel at 50 V for 15 h, blotted, and hybridized with 4×10^7 cpm of nick-translated λTtBg7. Micronuclear DNA (lane 1) and macronuclear DNA (lane 2) from CU359 (nulli 2, 3, 5); micronuclear DNA (lane 3) and macronuclear DNA (lane 4) from CU358 (nulli 3, 4, 5); micronuclear DNA (lane 5) and macronuclear DNA (lane 6) from CU361 (nulli 3); micronuclear DNA (lane 7) and macronuclear DNA (lane 8) from CU357 (nulli 4); micronuclear DNA (lane 9) and macronuclear DNA (lane 10) from CU354 (nulli 5) are shown. The arrows at the side of the figure indicate the 3.4-, 2.9-, 2.4-, and 2.0-kb micronuclear genomic fragments which are the genomic *Bgl*II fragments cloned in λTtBg7 (see Fig. 4b). A 10-μg amount of DNA was loaded per lane. Sizes in kilobases of marker fragments are indicated.

the caryonidally derived 80A and 80C populations, consistent with the previous caryonidal analysis. Interestingly, in caryonidal clone 80C at 25 generations the retained sequence of λTtBg7 was in two ~4-kb macronuclear bands, one of which was depleted by 100 generations. This result suggests that the two chromosomal homologs each rearranged this region of DNA differently in the single macronucleus of caryonide 80C. Subsequent unequal cell growth rates might explain the depletion of one of the bands at 100 generations. Digestion of the same DNA preparations with *Hae*III instead of *Bgl*II (Fig. 6b, lanes 7 to 12) confirmed that these rearrangements take place before 25 generations. In separate experiments with these DNA preparations (data not shown), the region of p7H3 which is retained in the macronucleus was found to be located in the lowest intense (~1 kb) micronuclear *Hae*III fragment seen in lanes 7 and 8, Fig. 6b. Clones p7H4 and p7H5, but not p7H3, hybridized to the 7-kb *Hae*III fragment (Fig. 6b, lanes 7 and 8), and the 3.5-kb *Hae*III fragment in lanes 7 and 8 hybridized only to p7H5. In the macronuclear genomic DNA the retained region of p7H3 is therefore entirely within the ~24-kb *Hae*III fragment (Fig. 6b, lanes 9 and 12), which was too large to allow caryonidal size differences to be detected. However, the absence of the ~1-kb micronuclear *Hae*III fragment in lanes 9 to 12 confirmed that the rearrangement event had taken place by 25 generations. The *Bgl*II and *Hae*III digestion results together showed that the caryonidally variable rearrangement event of the clone λTtBg7 sequence occurs within 25 generations of mating, and that no further rearrangement events occur for at least 100 generations after mating. In addition, the identical pattern of micronuclear restriction fragments homologous to λTtBg7 in 80A and 80C and their parents 17A and 17D indicates that these sequences are stable in the germline.

Mating type in *T. thermophila* is determined during macronuclear development (27). In a model for mating-type differentiation, Orias (27) has suggested that this differentiation involves a variable rearrangement-deletion in each developing macronucleus, such rearrangements being influenced by cell culture conditions at the time of differentiation. Orias and Baum (28) have shown that mating-type differentiation in *T. thermophila* is strongly influenced by delayed refeeding of conjugating cells during macronuclear development. Because other genomic changes have been observed to occur in the period 10 to 24 h after initiation of mating (3, 30, 41), we tested the effect of changing the physiological state of cells developing new macronuclei by altering cell culture conditions. In preliminary experiments, we have found that the relative proportions of different size classes of the variably rearranged region of λTtBg7 are also strongly influenced by delaying refeeding of conjugating cells from 14 to 24 h after initiation of cell conjugation (data not shown). Furthermore, as described above, there is a limited set of alternative processed forms of the λTtBg7 retained regions among the independently derived macronuclear DNAs. We note that this situation is very similar to the fixed number of alternative mating types possible in mating type differentiation of *T. thermophila*. Although λTtBg7 itself is not the mating type locus, as it maps to a different chromosome from the mating type locus (9), this work provides clear molecular evidence for the type of variable genomic rearrangement suggested to occur in mating-type differentiation.

DISCUSSION

Extent and nature of DNA rearrangement in *T. thermophila* macronuclear development. The results reported here confirm and extend previous reports examining the extent of DNA rearrangement in the macronucleus relative to the micronucleus, in which DNA rearrangements were shown to occur at a high frequency during development (20, 21, 37, 38). However, a question left unresolved by previous studies was whether any of the changes observed were attributable to genetic polymorphisms. The approach taken here ensures that such background differences in clonal populations were not present, first, because whole-genome homozygotes were constructed by genetic procedures for this analysis. Gener-

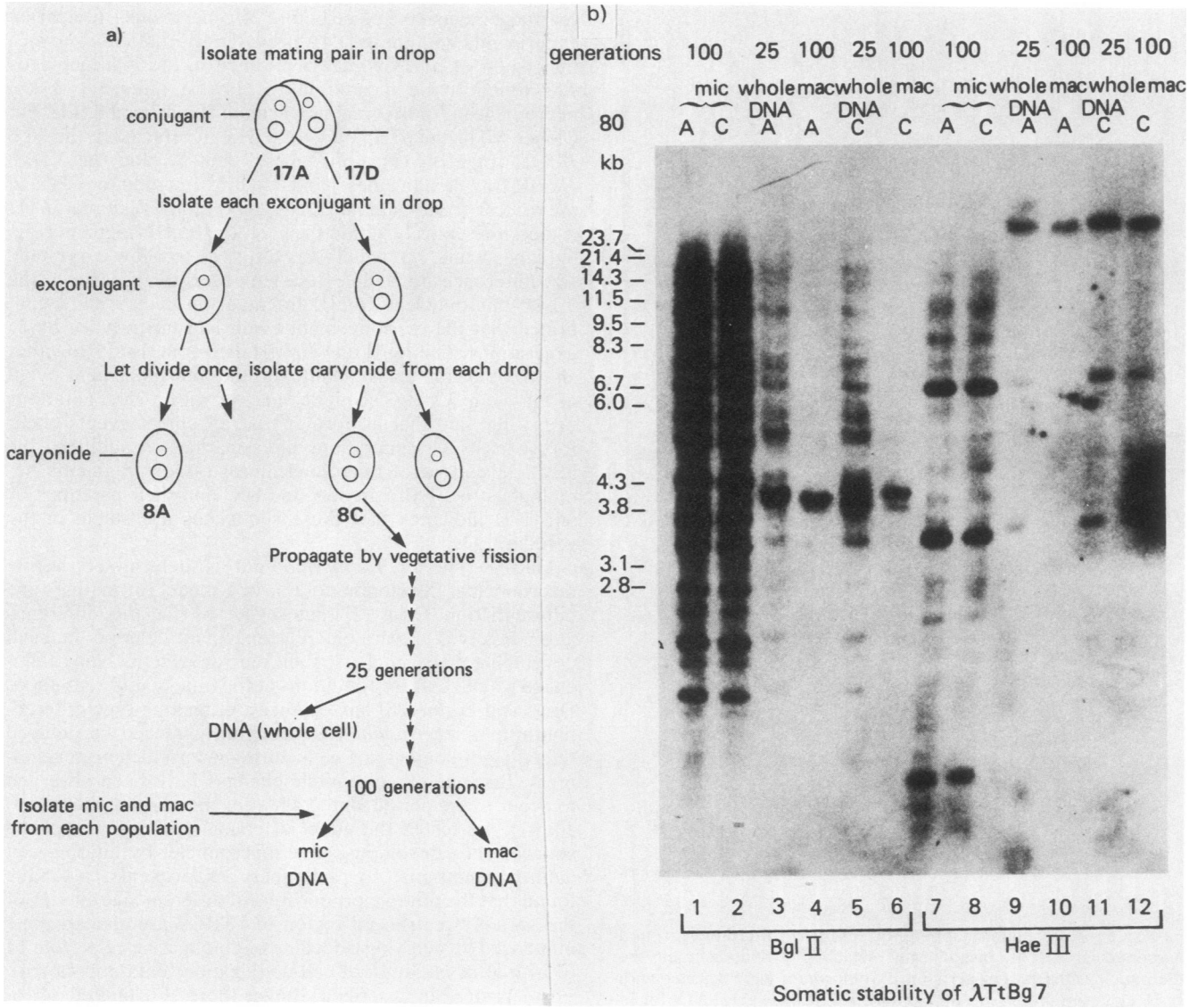

FIG. 6. Somatic stability of sequences homologous to λTtBg7. (a) Strategy for determining timing of rearrangement and the somatic stability of clone λTtBg7 sequences. The clonal descendants of caryonides 17A and 17D (which had different mating types) were conjugated. Caryonides 80A and 80C, which independently developed new macronuclei in separate cell cyoplasms, were isolated in drops of liquid culture medium and propagated. (b) Whole-cell DNA isolated from caryonides 80A and 80C 25 generations after mating, and micronuclear (mic) and macronuclear (mac) DNAs isolated from caryonides 80A and 80C 100 generations after mating were digested with either *Bgl*II or *Hae*III, electrophoresed for 15 h at 50 V on a horizontal 0.8% agarose gel, blotted, and hybridized with 2×10^7 cpm of ^{32}P-nick-translated λTtBg7 DNA. *Bgl*II-digested micronuclear DNA isolated from caryonides 80A (lane 1) and 80C (lane 2) 100 generations after mating; *Bgl*II-digested whole-cell DNA isolated from caryonides 80A (lane 3) and 80C (lane 5) 25 generations after mating; *Bgl*II-digested macronuclear DNA isolated from caryonide 80A (lane 4) and 80C (lane 6) 100 generations after mating; *Hae*III-digested micronuclear DNA isolated from caryonides 80A (lane 7) and 80C (lane 8) 100 generations after mating; *Hae*III-digested whole-cell DNA isolated from caryonides 80A (lane 9) and 80C (lane 1) 25 generations after mating; *Hae*III-digested macronuclear DNA isolated from caryonides 80A (lane 10) and 80C (lane 12) 100 generations after mating. A 10-μg amount of DNA was loaded per lane. Sizes in kilobases of molecular weight marker fragments are indicated on the side of the figure.

ating whole-genome homozygotes eliminates possible polymorphisms and aneuploidy which may have arisen during laboratory maintenance in inbred strains. Such aneuploidy has been found previously (P. Bruns, personal communication and J. Shampay and E. H. Blackburn, unpublished data). Second, sets of single caryonidal cells were individually isolated from a single cross of the whole-genome homozygotes. We have found the use of caryonidally derived clonal populations to be of critical importance for the interpretation of rearrangement events which can occur differently in independently developed macronuclei derived from genetically identical micronuclei or even within a single macronucleus.

In this study, 10 cloned DNAs were analyzed for DNA processing concomitant with the formation of a new macronucleus and in subsequent vegetative growth. The results summarized in Tables 1 and 2 show that all of the clones analyzed hybridized to genomic DNA which had

undergone a processing event during the development of the mature macronucleus. The genomic sequences of two of these clones, λTtBg4 and pTtsN, did not undergo rearrangement themselves but had a limited region of homology to another genomic locus which was rearranged. The remaining clones included, or were flanked by, rearranged DNA sequences.

In other investigations, 5 of 18 (37), 3 of 9 (20), and 13 of 20 (38) randomly selected cloned micro- and macronuclear DNA segments contained eliminated sequences. In many of these clones the eliminated sequences were repetitive in the micronuclear genome. Karrer (21) found that 16 of 68 randomly chosen micronuclear clones contained middle repetitive sequences found only in the micronucleus. Our results on the type and frequency of genomic rearrangements are thus in agreement with previous findings.

A minimum estimate can be made for the amount of eliminated DNA in each of the clones analyzed here (with the exception of clone pTtsC, as the extent of eliminated DNA was not determined for this clone) (Table 2). When the amount of DNA eliminated was compared to the total amount of DNA sampled (i.e., the estimate included data from genomic restriction fragments which extend beyond the borders of the cloned inserts), a minimum of 22.5 kb of the 138.5 kb of micronuclear DNA sampled here was estimated to be eliminated during macronuclear development. This amount represents 16.2% of the total micronuclear DNA sampled, in good agreement with the previous estimate of 10 to 20% based on C_0t studies (39).

In all cases tested, the rearrangement events described in this work were not closely associated with the generation of macronculear telomeres. Because the average size of a macronuclear molecule is about 600 kb (32), macronuclear telomeric regions are too rare to account for the high frequency of rearrangement events reported in this paper. The elimination events, since they are internal in all the cases analyzed here, must therefore be accompanied by DNA recombination, whereby noneliminated sequences become newly juxtaposed in the macronuclear genome.

We have shown that repetitive eliminated sequences are dispersed between chromosomes. These results agree with those from similar analyses of specific eliminated sequences in nullisomic strains, in which it was found that for a total of seven clones analyzed eliminated repetitive sequences were chromosomally dispersed (21, 37). Universal elimination of dispersed repetitive sequences may provide a mechanism for coordinating the DNA processing of their unrelated but flanking regions of DNA. Such processing events may provide a system whereby functionally related genes are coordinately regulated through the introduction (or elimination) of upstream or downstream regulatory regions. A corollary of this hypothesis is that a primary function of eliminated sequences is in the DNA rearrangements occurring concomitantly with their elimination rather than the possible micronuclear-specific products which such sequences might encode.

Only a few known structural genes of *T. thermophila* have been tested for somatic processing. Histone genes are not rearranged (4). DNA rearrangement has been observed in regions flanking the α-tubulin gene, although not in the gene itself (11). Similarly, the closest rearrangement events associated with the single micronuclear rRNA gene occur further than 1.5 kb from each end of the transcription unit (23, 30), although clearly the amplification of the rDNA molecules is important in rRNA gene expression. Kimmel and Gorovsky (22), Allen et al. (2) and Pederson et al. (31) have shown that 5S rRNA genes are included in regions of DNA which are rearranged during macronuclear development, although these rearrangements differ between 5S rRNA clusters and are therefore unlikely to be related to 5S rRNA gene transcription. These data suggest that macronuclear gene expression in *T. thermophila* is not regulated in a straightforward manner by intragenic rearrangement events during macronuclear development.

Developmental control of genomic reorganization. We have shown that the processing of genomic regions which include or flank only unique DNA is highly specific and reproducible between independently developed macronuclei, arguing that random recombinations of macronuclear sequences do not continue throughout the somatic life of the clonal population. Similarly, the fragmentation of the micronucleus into subchromosomal macronuclear fragments has been shown to be highly nonrandom (6).

In addition to the reproducible type of genomic rearrangement, analysis of a variable type of genomic rearrangement is reported here. In λTtBg7, a unique region flanked by different middle repetitive sequences in the micronucleus is shown here to be variably processed in different macronuclei. The rearrangement event in each nucleus occurs before 25 generations after mating and most likely occurs during the time of other known rearrangement events in the developing macronucleus (3, 30, 41). Furthermore, this investigation provides evidence that each homologue is processed independently. Two additional cloned sequences out of the ten randomly chosen cloned DNAs examined in this report were also variably rearranged, suggesting that this variability is quite common.

Variable genomic rearrangement has important implications for the generation of phenotypic diversity in macronuclei. Our findings on variably rearranged sequences show striking parallels to previous observations on the determination of mating type in *T. thermophila*. Like the variable rearrangements described here, differentiation of mating type in this species is also determined independently in different caryonides early in macronuclear development, and once determined, is somatically stable (reviewed in reference 27). Furthermore, the ratio of mating types determined in a population can be influenced strongly by changing cell culture conditions during a period of early macronuclear development (28). Likewise, we found that changing the same cell culture conditions in the same time period is important in determining the distribution of variable rearrangements of the λTtBg7 sequence. If the variably rearranged sequences such as those analyzed here are involved in gene expression, each new macronucleus developed provides an opportunity for its vegetative progeny cells to express a new phenotype. In *T. thermophila* differential DNA rearrangement may therefore provide a route by which cells add considerably to the variety of phenotypes which can be produced by normal meiotic recombination.

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LITERATURE CITED

1. **Allen, S. L.** 1967. Cytogenetics of genomic exclusion in *Tetrahymena*. Genetics **55**:797–822.
2. **Allen, S. L., P. R. Ervin, N. C. McLaren, and R. E. Brand.** 1984.

The 5S ribosomal RNA gene clusters in *Tetrahymena thermophila*: strain differences, chromosomal localization, and loss during micronuclear ageing. Mol. Gen. Genet. **197**:244–253.

3. **Austerberry, C. F., C. D. Allis, and M. C. Yao.** 1984. Specific DNA rearrangements in synchronously developing nuclei of *Tetrahymena*. Proc. Natl. Acad. Sci. U.S.A. **81**:7388–7387.
4. **Bannon, G. A., J. K. Bowen, M.-C. Yao, and M. A. Gorovsky.** 1984. *Tetrahymena* H4 genes: structure, evolution and organization in macro- and micronuclei. Nucleic Acids Res. **12**: 1961–1975.
5. **Beermann, S.** 1977. The diminution of heterochromatic chromosome segments in *Cyclops* (Crustacea, Copepoda). Chromosoma **60**:297–344.
6. **Blackburn, E. H., M. Budarf, P. B. Challoner, J. M. Cherry, E. A. Howard, A. L. Katzen, W. C. Pan, and T. Ryan.** 1983. DNA termini in ciliate macronuclei. Cold Spring Harbor Symp. Quant. Biol. **97**:1195–1207.
7. **Bromberg, S., K. Pratt, and S. Hattmann.** 1982. Sequence specificity of DNA adenine methylase in the protozoan *Tetrahymena thermophila*. J. Bacteriol. **150**:993–996.
8. **Brunk, C. F., S. G. S. Tsao, C. H. Diamond, P. S. Ohashi, N. N. G. Tsao, and R. E. Pearlman.** 1982. Reorganization of unique and repetitive sequence during nuclear development in *Tetrahymena thermophila*. Can. J. Biochem. **60**:847–853.
9. **Bruns, P. J.** 1983. *Tetrahymena thermophila*. Genet. Maps **2**:178–181.
10. **Bruns, P. J., T. B. Brussard, and E. V. Merriam.** 1983. Nullisomic *Tetrahymena*. II. A set of nullisomics define the germinal chromosomes. Genetics **104**:257–270.

10a. **Bruns, P. J., L. Martin, A. L. Katzen, and E. H. Blackburn.** 1985. A drug-resistant mutation in the ribosomal DNA of *Tetrahymena*. Proc. Natl. Acad. U.S.A. **82**:2844–2846.

11. **Callahan, R. C., G. Shalke, and M. A. Gorovsky.** 1984. Developmental rearrangements associated with a single type of expressed α-tubulin gene in *Tetrahymena*. Cell **36**:441–445.
12. **Elliott, A. M.** 1973. Life cycle and distribution of *Tetrahymena*, p. 259–288. *In* A. M. Elliott (ed.), Biology of *Tetrahymena*, Dowden, Hutchinson and Ross Inc., Stroudsburg, Pa.
13. **Emery, H. S., and A. M. Weiner.** 1981. An irregular satellite sequence is found at the termini of the linear extrachromosomal ribosomal DNA in *Dictyostelium discoideum*. Cell **26**:411–419.
14. **Gorovsky, M. A.** 1973. Macro- and micronuclei of *Tetrahymena pyriformis*: a model system for studying the structure and function of eukaryotic nuclei. J. Protozool. **20**:19–25.
15. **Gorovsky, M. A.** 1980. Genome organization and reorganization in *Tetrahymena*. Annu. Rev. Genet. **14**:203–239.
16. **Gorovsky, M. A., S. Hattmann, and G. L. Pleger.** 1973. [^{6}N] methyl adenine in the nuclear DNA of a eukaryote, *Tetrahymena thermophila*. J. Cell Biol. **56**:697–701.
17. **Gorovsky, M. A., M.-C. Yao, J. B. Keevert, and G. L. Pleger.** 1975. Isolation of micro- and macronuclei of *Tetrahymena*. Methods Cell Biol. **9**:311–327.
18. **Gray, H. B., Jr., D. A. Ostrander, J. L. K. Hodnett, R. J. Legerski, and D. C. Robberson.** 1975. Extracellular nucleases of *Pseudomonas* Bal 31. I. Characterization of single-strand specific deoxyribonuclease and double-strand deoxyribonuclease activity. Nucleic Acids Res. **2**:1459–1492.
19. **Hagen, C. E., and G. J. Warren.** 1983. Viability of palindromic DNA is restored by deletions occurring at low but variable frequency in plasmids of *Escherichia coli*. Gene **24**:317–326.
20. **Iwamura, U., M. Sakai, and M. Muramatu.** 1982. Rearrangement of repetitive DNA sequences during development of macronucleus in *Tetrahymena thermophila*. Nucleic Acids Res. **10**:4279–4291.
21. **Karrer, K.** 1983. Germline-specific DNA sequences are present on all five micronuclear chromosomes in *Tetrahymena thermophila*. Mol. Cell. Biol. **3**:1909–1919.
22. **Kimmel, A. R., and M. A. Gorovsky.** 1978. Organization of the 5S RNA genes in macro- and micronuclei of *Tetrahymena pyriformis*. Chromosoma **67**:1–20.
23. **King, B. O., and M.-C. Yao.** 1982. Tandemly repeated hexanucleotide at *Tetrahymena* rDNA free end is generated from a single copy during development. Cell **31**:177–182.
24. **Klobutcher, L. A., C. L. Jahn, and D. M. Prescott.** 1984. Internal sequences are eliminated from genes during macronuclear development in the ciliated protozoan *Oxytricha nova*. Cell **36**:1045–1055.
25. **Lawn, R. M., J. M. Heumann, G. Herrick, and D. M. Prescott.** 1978. The gene sized DNA molecules in *Oxytricha*. Cold Spring Harbor Symp. Quant. Biol. **42**:483–492.
26. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning; a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Orias, E. O.** 1981. Probable somatic DNA rearrangement in mating type determination in *Tetrahymena thermophila*: a review and a model. Dev. Genet. **2**:185–202.
28. **Orias, E. O., and M. P. Baum.** 1984. Mating type differentiation in *Tetrahymena thermophila*. Strong influence of delayed refeeding on conjugating pairs. Dev. Genet. **4**:145–158.
29. **Orias, E., and P. J. Bruns.** 1975. Induction and isolation of mutants in *Tetrahymena*. Methods Cell Biol. **13**:247–282.
30. **Pan, W.-C., and E. H. Blackburn.** 1981. Single extrachromosomal RNA gene copies are synthesized during amplification of the rDNA in *Tetrahymena*. Cell **28**:595–604.
31. **Pederson, D. S., M.-C. Yao, A. R. Kimmel, and M. A. Gorovsky.** 1984. Sequence organization within and flanking clusters of 5S ribosomal RNA genes in *Tetrahymena*. Nucleic Acids Res. **12**:3003–3021.
32. **Preer, J., and L. Preer.** 1979. The size of macronculear DNA and its relationship to models for maintaining genic balance. J. Protozool. **26**:14–18.
33. **Shampay, J., J. W. Szostak, and E. H. Blackburn.** 1984. DNA sequences of telomeres maintained in yeast. Nature (London) **310**:154–157.
34. **Tonegawa, S.** 1983. Somatic generation of antibody diversity. Nature (London) **302**:575–581.
35. **White, M. J. D.** 1954. Animal cytology and evolution. 2nd ed. Cambridge University Press, Cambridge, England.
36. **Wilson, E. B.** 1928. The cell in development and heredity. Macmillan, New York.
37. **Yao, M.-C.** 1982. Elimination of specific DNA sequences from the somatic nucleus of the ciliate *Tetrahymena*. J. Cell. Biol. **92**:782–787.
38. **Yao, M.-C., J. Choi, S. Yokoyama, C. F. Austerberry, and C.-H. Yao.** 1984. DNA elimination in *Tetrahymena*: a developmental process involving extensive breakage and rejoining of DNA at defined sites. Cell **36**:433–440.
39. **Yao, M.-C., and M. A. Gorovsky.** 1974. Comparison of the sequences of macro- and micronuclear DNA of *Tetrahymena pyriformis*. Chromosoma **48**:1–18.
40. **Yao, M.-C., and C.-H. Yao.** 1981. Repeated hexanucleotide CCCCAA is present near the free ends of macronuclear DNA of *Tetrahymena*. Proc. Natl. Acad. Sci. U.S.A. **78**:7436–7439.
41. **Yokoyama, R. W., and M.-C. Yao.** 1982. Elimination of DNA sequences during macronuclear differentiation in *Tetrahymena thermophila*, as detected by in situ hybridization. Chromosoma **85**:11–22.