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# Specific DNA rearrangements in synchronously developing nuclei of *Tetrahymena*

**(genome rearrangement/DNA elimination/conjugation/ciliate development)**

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**ABSTRACT** **Specific rearrangement of internal chromosomal regions occurs during development of the somatic macronucleus in *Tetrahymena thermophila* and results in elimination of germ-line (micronuclear) DNA sequences. The timing and mechanism of genome rearrangement within one particular 9.3-kilobase region, which contains three distinct eliminated sequences, were investigated. Portions of this cloned region were used as probes in Southern hybridization experiments to analyze DNA from developing macronuclei (anlagen). All three deletions were found to occur predominantly within a 2-hr time period in which the nuclear DNA contents increased from 4C to 8C (1C represents the amount of DNA present in a haploid genome). The three deletion events can occur independently because intermediate forms, having sustained one or two deletions, were detected. One of the deletions occurs in two alternative ways, resulting in two equally abundant products of different size. Because reciprocal products expected from unequal sister chromatid exchange were not detected, an intramolecular DNA splicing mechanism is suggested.**

Several examples of developmental genome rearrangement have been found in which some or all somatic nuclei rearrange and/or eliminate DNA sequences. Germ-line-specific sequences are completely eliminated from the soma of certain invertebrates (1) such as Ascarid worms (2), flies (3, 4), and copepods (5). Mammalian immunoglobulin genes undergo rearrangement in B lymphocytes, deleting internal sequences and joining previously separated regions (6). Extensive genome rearrangement including sequence elimination also occurs in various species of ciliates during macronuclear development (7–9). This process clearly has a significant role in development, but its molecular mechanism is not well understood.

The holotrich ciliate *Tetrahymena thermophila*, like most other ciliates, has one micronucleus and one macronucleus. The micronucleus serves as the germinal nucleus, and the somatic macronucleus controls the phenotype of the cell (10, 11). These two nuclei are different in many important aspects, including the structure of their genomes. Although the macronucleus contains 23 times more DNA (12), its sequence complexity is 10–20% less than that of the diploid micronucleus (13). The sequences that are specific to the micronucleus are apparently eliminated during the formation of the macronucleus (14). Some of these sequences have been cloned. Most of them appear to be moderately repetitive and widely dispersed in the micronuclear genome (15–17). Little is known about the mechanism of micronucleus-limited DNA elimination in ciliates. Elimination of the sequences flanking rDNA in *Tetrahymena* is accompanied by chromosome breakage and generation of new telomeres (15). However, a major proportion of DNA elimination in *Tetrahymena* probably involves subsequent rejoining of broken chromosomes (18). A specific example of the latter case has been analyzed in detail (18) and is illustrated in Fig. 1. The corresponding regions of the macro- and the micronuclear DNA have been cloned and compared. As the 6.8-kilobase (kb) macronuclear *Eco*RI fragment is derived from the 9.3-kb micronuclear *Eco*RI fragment, three distinct portions are eliminated, and the retained sequences are joined together in the macronuclear genome. Other examples of sequence elimination involving rejoining of retained sequences in *Tetrahymena* (19, 20) and *Oxytricha* (21) also have been identified.

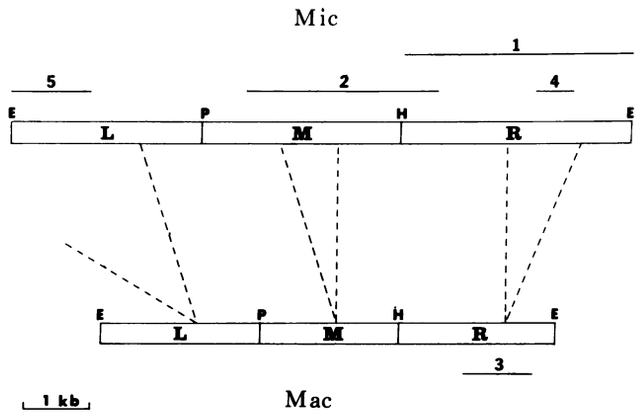


FIG. 1. Schematic representation of DNA rearrangement in one defined region of the *Tetrahymena* genome. An *Eco*RI fragment of the micronuclear DNA and the corresponding region of the macronuclear DNA have been isolated previously in clones cTt455 and cTt1107, respectively, and compared in detail (18). This diagram summarizes the relevant features of these two sequences. Both sequences, which are bounded by *Eco*RI (E) sites, are conveniently divided into three regions (*L*, *M*, and *R*) by the restriction enzymes *Hpa* II (P) and *Hha* I (H). Each region contains a segment of micronuclear-specific sequence that is deleted in the macronucleus. The dashed lines link the estimated junctions of the deletions. The left junction of the deletion in region *L* has not as yet been cloned and characterized. Five subfragments of these two cloned DNAs were used as hybridization probes in this study. They are indicated by the five lines marked number one through five.

In spite of the differences noted between micronuclear and macronuclear genomes, they both are derived *de novo* from the same progenitor genome during conjugation. When *Tetrahymena* cells of different mating type pair, their micronuclei undergo meiosis to form haploid progametic nuclei, and one progametic nucleus in each cell undergoes mitosis to form two gametic nuclei. Mating cells reciprocally exchange one of their gametic nuclei, which fuses with the partner's remaining gametic nucleus; this exchange results in both cells containing an identical diploid zygotic nucleus. The old macronucleus is degraded as new micro- and macronuclei

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Abbreviation: kb, kilobase.

develop from the mitotic products of the zygotic nucleus. The conjugation process of *Tetrahymena* can occur synchronously in large populations (22). Immature macronuclei at specific stages of development have been purified from these cells (23). These capabilities offer us a unique opportunity to analyze the molecular processes of DNA rearrangement and sequence elimination. In this study we have performed hybridization experiments to characterize the rearrangements within one particular region of the genome. The results clearly indicate that the deletion and elimination processes illustrated in Fig. 1 occur in most, if not all, genomes within a short period of time during development, are accompanied by no more than one round of DNA replication, and are most likely mediated by specific intramolecular recombination of the chromosome and rapid degradation of the deleted DNA.

## MATERIALS AND METHODS

**Cells and Culture Conditions.** *Tetrahymena thermophila* strains B1868 III, CU399, and CU401 were obtained from P. Bruns of Cornell University, and A1873 I and F1668 II were obtained from D. Nanney of the University of Illinois. The four caryonidal clones described in *Results* were isolated in this laboratory from a mating pair of A1873 I and B1868 III. The cells were maintained and grown in axenic medium as described earlier (24).

**Purification and Analysis of Nuclei.** Nuclei from vegetatively growing cells were isolated by the method as described (24). Nuclei from conjugating cells were isolated and fractionated by a method involving differential sedimentation in shallow sucrose gradients under unit gravity (23). The purified fractions were analyzed using a flow microfluorometer to determine the numbers and the DNA contents of the nuclei present.

**DNA Isolation, Enzyme Digestion, and Gel Electrophoresis.** DNA was isolated from nuclei or whole cells by a method involving phenol extraction as described (25). Restriction endonucleases were purchased from New England Biolabs or Bethesda Research Laboratories and used under conditions recommended by the supplier. At least a 2-fold excess of enzyme was used to insure complete digestion. Gel electrophoresis was carried out in a horizontal slab gel apparatus as described (15). DNA was transferred to nitrocellulose filters by the method of Southern (26) with slight modifications.

**DNA Labeling and Hybridization.** The micronuclear (cTt455) and macronuclear (cTt1107) genomic clones have been described (18). DNA for probes 1, 3, and 5 (Fig. 1) were obtained by cutting out segments of low-melting-temperature agarose (Bio-Rad) gels containing the DNA fragments and purifying as described (18). DNA for probes 2 and 4 were subcloned in plasmids pEMBL8+ (27) in host JM103 (28) or pUC13 in host JM83 (29), respectively, and the entire recombinant plasmids were used as probes. Probes were labeled by nick-translation (30). Hybridization was for 16–24 hr at 37°C in 0.6 M NaCl/0.06 M NaOAc, pH 7.0/0.1 M Tris·HCl, pH 7.4/40% (vol/vol) formamide/0.5% NaDodSO$_4$/Denhardt solution (31). Unless given otherwise in the figure legend, filters were washed after hybridization under moderately stringent conditions (0.3 M NaCl/0.03 M NaOAc for 1 hr at 65°C) before autoradiography on Kodak NoScreen film or XAR-5 film with a DuPont intensifying screen.

**Scanning Densitometry.** Relative hybridization intensities of bands within a lane were determined by measuring light absorbance of the autoradiogram with a Quick Scan Model 1052 densitometer (Helena Laboratories, Beaumont, TX).

## RESULTS

**Purification and Characterization of Specific Nuclei from Conjugating Cells.** Beginning about 9 hr after conjugation is initiated (by mixing starved cells of different mating type), three classes of nuclei are present and can be separated by sedimentation at unit gravity (23). These are old (parental) macronuclei, developing micronuclei, and developing macronuclei (macronuclear anlagen). Identification and purification of each class of nuclei are based on their sizes and DNA contents. Immature macronuclei are intermediate in size and less dense than the other two nuclei (23) due to considerable swelling prior to endoreplication of the genome (32).

DNA contents of anlagen as well as the extent of contamination by micronuclei and old macronuclei in anlagen preparations were determined by flow microfluorometry (23). Relative DNA contents of the anlagen and micronuclei indicated that anlagen purified from 9-, 10-, and 12-hr cells were virtually all at the 4C stage, whereas by 14 hr 70% were at the 8C stage and only 30% were still at the 4C stage (1C represents the amount of DNA present in a haploid genome). Although micronuclei constituted the majority of contaminating nuclei, they contributed very little to the purified DNA due to their low DNA contents. Contamination by old macronuclei was greatest at the earliest stages (9–10 hr) and decreased with time as the old macronucleus was degraded. Estimated from their numbers and DNA contents, the old macronuclei contributed about 30% of DNA in the 9- and 10-hr anlagen preparations, and about 20% in the 12-hr preparation. Contamination of anlagen preparations from 14-hr mating cells by either old macronuclei or new micronuclei was negligible.

**Deletion of Sequences from Region *R*.** Among the differences detected between micro- and macronuclear genomes is the absence or underrepresentation of some micronuclear sequences in the macronucleus, as exemplified in the micronuclear sequences contained in clone cTt455 (see the introduction and Fig. 1). For convenience, the micronuclear and macronuclear forms of this sequence have been arbitrarily divided into three regions, each containing one of the three deletions. Region *R* is bounded by the right *Eco*RI and the *Hha* I sites, region *M* by the *Hha* I and *Hpa* II sites, and region *L* by the *Hpa* II and the left *Eco*RI sites. Various restriction fragments and plasmid subclones of the micronuclear (cTt455) and macronuclear (cTt1107) genomic clones were used as hybridization probes in the experiments reported here (Fig. 1).

Fig. 2*a* shows the autoradiograph of a Southern blotting experiment in which purified micronuclear, macronuclear, and anlagen DNAs from cells at various times after initiation of conjugation were double-digested with *Hha* I and *Eco*RI, fractionated by electrophoresis, blotted to nitrocellulose, and hybridized to probe 1 (probe 1 is the micronuclear form of region *R*, Fig. 1). We have shown (18) that 1.1 kb of DNA is deleted from the center of region *R* in the macronucleus. The 3.4-kb micronuclear form of region *R* predominated in the anlagen at 10 hr (Fig. 2*a*, lanes A and B). By 12 hr of conjugation, a significant amount of the 2.3-kb macronuclear form of region *R*, ≈40% of the hybridization as measured by scanning densitometry, appeared in the anlagen (Fig. 2a, lane C). The levels of contamination by old macronuclear DNA were roughly 30% and 20% in the 10- and 12-hr anlagen preparations, respectively. Thus, the increased proportion of macronuclear-type fragment in 12-hr vs. 10-hr anlagen must have been due to deletion of micronucleus-limited sequences from region *R* in ≈20% of the anlagen genomes. Rearrangement at a lower level was difficult to assess by this method but probably had occurred at earlier times (see the following sections). Anlagen DNA from 14-hr cells (Fig. 2*a*, lane D) showed virtually complete processing of the chromosomes to the macronuclear form. Hence, most of the rearrangement in this region occurred between 12 and 14 hr of mating. The DNA content of ≈70% of the anlagen increased 2-fold, from 4C to 8C, during the same period. The lack of detectable intermediate forms or variants at any time sug-

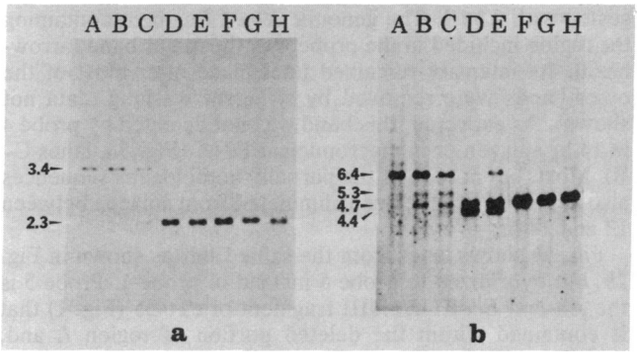


FIG. 2. Analysis of DNA deletion from region *R* (*a*) and region *R* + *M* (*b*). Approximately 2 μg of various DNAs were digested with both *Eco*RI and *Hha* I (*a*) or *Eco*RI and *Hpa* II (*b*) to completion, fractionated in a 1% (*a*) or 0.7% (*b*) agarose gel, and blotted to a nitrocellulose filter. Each filter was incubated with $4 \times 10^6$ cpm of $^{32}$P-labeled probe 1 (Fig. 1) and washed under moderately stringent conditions as described. Numbers along the side indicate fragment sizes in kb. DNA in lanes A–E and lane H were from mating cultures at various times after mixing the parental strains, CU399 and CU401. DNA in lanes F and G were from nonmating cells of the same strains. Lanes: A, 10-hr micronuclear DNA; B–D, 10-, 12-, and 14-hr anlagen DNA, respectively; E, 24-hr whole-cell DNA; F, CU399 macronuclear DNA; G, CU401 macronuclear DNA; H, 10-hr macronuclear DNA.

gests that the sequence elimination and rearrangement process in region *R* occurs in a direct and invariant fashion.

**Deletion of Sequences from Region *M*.** Fig. 2*b* shows the results of an experiment similar to that shown in Fig. 2*a* but with *Hpa* II/*Eco*RI double digestion of the genomic DNAs instead of *Hha* I/*Eco*RI double digestion. Thus, the detected restriction fragment in this case included region *M* (the *Hpa* II–*Hha* I fragment; see Fig. 1) in addition to region *R*. Region *M* had been shown previously to sustain a 0.9-kb deletion of micronucleus-limited sequences (18). The micronuclear form of region *R* + *M*, a 6.4-kb *Eco*RI–*Hpa* II fragment, predominated until 14 hr after mating strains were mixed, at which time it was replaced by the macronuclear forms. Therefore, the deletion in region *M* was shown to occur during the same 2-hr time period in which most of the deletions in region *R* occurred.

Two equally abundant fragments were seen in 14-hr anlagen and 24-hr total cellular DNA, which contained mainly anlagen DNA at the 8C stage. The two fragments were 4.7 and 4.4 kb in size (Fig. 2*b*, lanes D and E). Since two alternative sizes of macronuclear region *M*, differing by 0.3 kb, were seen with several different restriction enzyme digestions (data not shown), the variation was not simply due to restriction site polymorphism. Therefore, the doublet seen in 14- and 24-hr samples indicated that deletion of DNA from region *M* can result in elimination of two alternative lengths of DNA sequence.

Only the 4.7-kb form of region *R* + *M* was seen in the parental macronuclei (Fig. 2*b*, lanes F–H). When DNAs of eight other inbred lines or caryonidal clones were compared, it was found that in some strains only one or the other of the two alternative macronuclear forms was detected, and in other strains both forms exist, though not always in equal amounts (Fig. 3). Both forms were present in three cultures (Fig. 3, lanes E–G) grown for ≈26 vegetative generations from isolated daughter cells (caryonides) of a conjugating pair. Therefore, both forms must have coexisted in the same macronucleus in at least three of the four caryonides. It is likely that both types of deletion occur in most anlagen. Assortment of those two types probably occurs during subsequent propagation of the exconjugant to give rise to cells with only one type of this DNA.

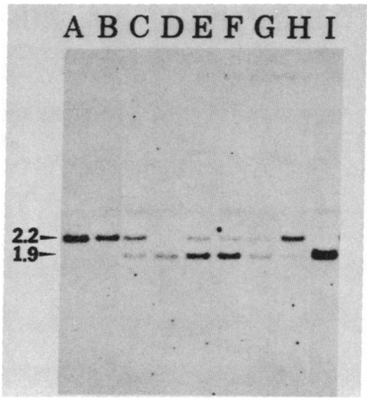


FIG. 3. Strain variation in deletion from region *M*. Macronuclear or whole-cell DNA from various strains was digested to completion with *Hin*dIII, fractionated by electrophoresis in a 1% agarose gel, and blotted to nitrocellulose, and the filter was incubated with $1.5 \times 10^6$ cpm of a $^{32}$P-labeled plasmid subclone containing the 2.8-kb micronuclear *Hin*dIII fragment designated probe 2 (Fig. 1). Probe 2 encompasses the micronucleus-limited sequences in region *M* as well as flanking sequences retained in the macronucleus. The filter was washed under stringent conditions (0.03 M NaCl/0.003 M NaOAc at 65°C for 1 hr). The faint, largest (2.8 kb) fragment detected in whole-cell DNA is the micronuclear form. Two fragments, 2.2 and 1.9 kb in size, were found in macronuclear genomes. Lanes: A, CU399 macronuclear DNA; B, CU401 macronuclear DNA; C, 24-hr CU399 × CU401 mating, whole-cell DNA; D–G, caryonidal set from A1873 I × B1868 III mating, whole-cell DNA; H, F1668 II whole-cell DNA; I, B1868 III whole-cell DNA.

**The Deletions in Regions *R* and *M* Can Occur Independently.** As noted above, deletion in region *R* had already occurred in approximately 20% of the anlagen genomes purified from 12-hr cells. Little if any deletion in region *M* occurred by this time, however, since the 4.7-kb band detected in 10- and 12-hr samples (Fig. 2*b*, lanes B and C) can be accounted for largely by contamination by parental macronuclei. Since both regions *M* and *R* were included on the same *Hpa* II–*Eco*RI restriction fragment, asynchrony in the occurrence of deletion in region *R vis-a-vis* region *M* would result in a fragment intermediate in size between the micronuclear and macronuclear forms. Judging from its intensity and size, the 5.3-kb fragment seen in 10- and 12-hr samples (Fig. 2*b*, lanes B and C) could have been the expected intermediate that contained a deletion in region *R* but not in region *M*. Hybridization of the same DNA samples with probe 4 supported this conclusion. Probe 4 consists entirely of micronucleus-limited sequences from region *R* (Fig. 1), and its failure to hybridize to the 5.3-kb fragment (data not shown) indicated that micronucleus-limited sequences from region *R* had been deleted from this fragment.

**Deletion of Sequences from Region *L*.** The experiment shown in Fig. 4*a* extended the analysis to include the entire *Eco*RI fragment (region *R* + *M* + *L*) since the samples analyzed in this experiment were digested only with *Eco*RI. The same probe (probe 1) was again used. No additional macronuclear bands were detected in 14- and 24-hr samples, in which the rearrangements for all three regions appeared to be complete. Thus, as in region *R* and in contrast to region *M*, no evidence exists for variation in the length of the DNA sequences deleted from region *L*. At 9, 10, and 12 hr, an 8.2-kb fragment was seen, which could have resulted from deletion in region *R* prior to deletion in regions *M* and *L*. A smaller (7.9 kb), less-abundant fragment (Fig. 4*b*, arrowhead in lane C) was also detected in a similar experiment (Fig. 4, legend). Since this intermediate has only been seen with *Eco*RI digestion alone, it could be due to some degree of asynchrony in the elimination of sequences from region *L* relative to the other two deletions.

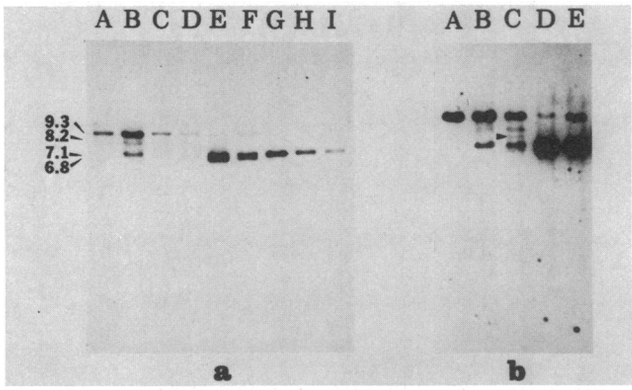


FIG. 4. Analysis of deletion in all regions ($R + M + L$). Various DNAs were digested with *Eco*RI alone to completion, fractionated by electrophoresis in a 1.0% (*a*) or 0.5% (*b*) agarose gel, and blotted to nitrocellulose filters. The filter in *a* was incubated with $6 \times 10^6$ cpm of $^{32}$P-labeled probe 1, and the filter in *b* was incubated with $4 \times 10^7$ cpm of $^{32}$P-labeled probe 3 (Fig. 1). All samples were from mating cultures of CU399 × CU401 except lanes G and H of *a*, which were from nomating cells of the same strains. Lanes in *a*: A, 12-hr micronuclear DNA; B–E, 9-, 10-, 12-, and 14-hr anlagen DNA, respectively; F, 24-hr whole-cell DNA; G, CU399 macronuclear DNA; H, CU401 macronuclear DNA; I, 10-hr macronuclear DNA. Lanes in *b*: A, 10-hr micronuclear DNA; B–D, 10-, 12-, and 14-hr anlagen DNA, respectively; E, 24-hr whole-cell DNA.

**Other Sequences Related to the Micronucleus-Limited Sequences in cTt455 Are Also Eliminated During the Same Time Period.** If Southern blots of micronuclear genomic DNA that had been hybridized with probe 1 were not washed with sufficient stringency after hybridization, a background smear was seen (data not shown). When probe 4 (which consists entirely of micronucleus-limited sequences) was used, distinct bands were recognizable above the general background smear, indicating that there were many other sequences related to some extent to probe 4 sequences. Fig. 5*a* shows a low-stringency Southern hybridization of probe 4 to micronuclear, anlagen (12 and 14 hr), and macronuclear DNA digested with *Xba* I. The genomic *Xba* I fragment containing the region included in the probe was the major band (arrowhead). Its intensity remained unchanged after most of the other bands were removed by stringent washing (data not shown). As expected, this band was not detected by probe 4 in 14-hr anlagen or in macronuclear DNA (Fig. 5*a*, lanes C–E). Most, but not all, of the partially homologous sequences also appeared to have been eliminated from anlagen between 12 and 14 hr.

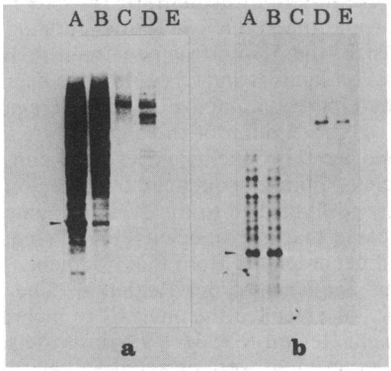


FIG. 5. Elimination of related sequences. Approximately 2 μg of various DNAs were digested with *Xba* I (*a*) or *Eco*RI and *Hpa* II (*b*) to completion, fractionated by electrophoresis in a 1.0% (*a*) or 0.7% (*b*) gel, and blotted to nitrocellulose, and the filters were incubated with $^{32}$P-labeled probe 4 at $2 \times 10^6$ cpm (*a*) or probe 5 at $2 \times 10^7$ cpm (*b*). Both of these probes are micronucleus-limited sequences of cTt455 (Fig. 1). The filters were washed in 0.3 M NaCl/0.03 M NaOAc at room temperature (*a*) or in 0.06 M NaCl/0.006 M NaOAc/40% (vol/vol) formamide/0.01 M Tris·HCl, pH 7.4 at 37°C (*b*). More stringent washing conditions (data not shown) allowed hybridization only to sequences included in the probes (arrowheads), suggesting that the other hybridizing sequences were related but not identical to those in cTt455. Lanes: A, 12-hr micronuclear DNA; B, 12-hr anlagen DNA; C, 14-hr anlagen DNA; D, CU399 macronuclear DNA; E, CU401 macronuclear DNA.

Fig. 5*b* shows lanes from the same filter as shown in Fig. 2*b*, but hybridized to probe 5 instead of probe 1. Probe 5 is the left-end *Eco*RI–*Hin*dIII fragment of cTt455 (Fig. 1) that is contained within the deleted portion of region *L* and, therefore, consists entirely of micronucleus-limited sequences. The band representing the genomic micronuclear region *L* (Fig. 5*b*, arrowhead) was virtually absent in 14-hr anlagen and in macronuclear DNA, confirming that elimination of micronucleus-limited DNA in this region occurred between 12 and 14 hr of conjugation. Probe 5 detected partially homologous sequences, represented by the minor bands seen in Fig. 5*b*, which could be removed by washes of higher stringency (data not shown). As with the probe 4-related sequences, most sequences related to probe 5 also were eliminated predominantly between 12 and 14 hr. The bands seen in Fig. 5*a*, lanes C and D, and Fig. 5*b*, lanes D and E, show that not all sequences related to probe 4 or probe 5 are micronucleus-limited. The sequences related to probes 4 and 5 have not as yet been cloned and characterized.

## DISCUSSION

Taken together, the hybridization experiments reported here show that, in the great majority of anlagen, all of the micronucleus-limited DNA in the region contained in clone cTt455 and in many related sequences is deleted and eliminated during a brief period of macronuclear development during which the total nuclear DNA content increases from 4C to 8C. Other critical events in macronuclear development begin during this same period, including deposition of macronucleus-specific histones in anlagen (33) and expression of the anlagen genome (11, 35). Although DNA rearrangement has been observed in a wide variety of organisms, it is rarely found at such a high frequency and in such good synchrony. Thus, *Tetrahymena* offers us an excellent system in which to examine directly the biochemical events associated with DNA rearrangement.

The fact that the rearrangements occur without multiple rounds of DNA replication and prior to nuclear division has placed stringent restrictions on the possible mechanisms involved. Processes such as unequal crossover between homologous chromosomes or sister chromatids could not be responsible for this kind of alteration. Unequal crossover has been considered as a possible mechanism in the rearrangement of immunoglobulin genes in mice, primarily because deleted sequences (i.e., possible reciprocal products of unequal crossover) persist in some B-cell lines (36). These sequences are not found in all B-cell lines, but this could be due to segregation of crossover products and selective or random loss of cells containing these products during subsequent passage of the B-cell lines. In *Tetrahymena* all of the chromosomes in the population go through the same change. Therefore, it is not possible to involve any mechanism that requires chromosome assortment or loss resulting from differential cell growth to explain the absence of deleted sequences. Although there is no conclusive evidence, the deletions are most likely the result of simple intramolecular recombination.

Significant amounts of deleted sequences do not persist in extrachromosomal forms during nuclear development or they would have been detected in these experiments. This is

true for all three deleted regions contained in cTt455 and most other partially homologous sequences in the genome. Thus, if these sequences are removed from the chromosome as defined segments, they must be degraded rapidly. Whether this degradation process is sequence specific or is related to the conformation of the removed segment is not known. It is also possible that these sequences are degraded while connected to the rest of the chromosomes, with their elimination being an integral part of the deletion process.

The rearrangements observed here occur during a time when the total nuclear DNA is replicated once, from 4C to 8C. It is conceivable that concomitant replication is required for the rearrangement. However, analysis of region *R* indicates that the replication of nuclear DNA is not in good synchrony with the rearrangement. At the 12-hr point, 20% of the chromosomes have already been rearranged, but virtually all of the nuclei are still at the 4C stage; by the 14-hr point, the rearrangement is essentially complete, and yet only 70% of the nuclei have reached the 8C stage. If the timing of DNA replication in this region does not differ significantly from the bulk of the genome, these data suggest that rearrangement in *Tetrahymena* can probably occur without concomitant replication of DNA.

The presence of three rearrangement regions in close proximity allows analysis of the temporal relationship between rearrangements of neighboring sites. Minor, but significant, fractions of the chromosomes at the 10- and 12-hr points appeared to have only one or two of the three regions deleted. Thus, regions that are no more than 1–3 kb apart could be rearranged at times different enough to be detected by our method. These data strongly suggest that, in at least some cells, the period of time required to complete all three rearrangements is significant. Thus, the spread of the rearrangement processes into a 5-hr period of time (9–14 hr after cell mixing) is not entirely the result of asynchrony in conjugation. There also seems to be a temporal order in the rearrangement. This argument is based on the observation that chromosomes with rearranged region *R* predominate among intermediates apparently containing only one rearranged region. It is likely that region *R* is the first one among the three to be rearranged. The other two regions could also be rearranged in a fixed order, although firm support for this point is still lacking.

The rearrangement of DNA occurs at very high specificity. In each hybridization experiment, millions of independently developing nuclei are analyzed, and yet the rearranged chromosomes are quite homogeneous in structure. Although it is not known whether the processes are invariant at the nucleotide level, they clearly are well regulated and may reflect an important biological function and/or a precise molecular mechanism. The only exception to this rule appears in region *M*, in which two alternative products are produced. The two products differ by 0.3 kb in size and are probably the result of deletion at alternative sites. Both types of deletion occur in the same nucleus, since they are found in caryonidal clones that are descendents of single macronuclear anlagen. The difference in rearrangement is probably not the result of difference in chromosomal origin. The parental strains used here are homozygous inbreeding lines that have an identical genetic origin and are not expected to generate heterozygous offspring. These two types of rearranged products appear to assort during somatic propagation, such that in most established lines only one type is present. This phenomenon has an interesting implication. It appears that *Tetrahymena* has a mechanism to generate DNA polymorphism in a genetic clone. It has been estimated that there are >5000 rearrangement sites in a genome (18). If this type of polymorphism occurs widely, enormous variation could be generated among individuals in a clone. In a way it is much like the polymorphism of B lymphocytes in a mouse. If the genomic variation has any functional consequence, it undoubtedly would play a crucial role in the survival and the evolution of this organism. In fact, the strategy of mating type determination of this species may rely on such a mechanism (34).

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