MOLECULAR AND CELLULAR BIOLOGY, July 1996, p. 3658–3667 Vol. 16, No. 7
0270-7306/96/$04.00+0

Non-Mendelian, Heritable Blocks to DNA Rearrangement Are Induced by Loading the Somatic Nucleus of *Tetrahymena thermophila* with Germ Line-Limited DNA

DOUGLAS L. CHALKER* AND MENG-CHAO YAO

Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington 98104

Received 11 January 1996/Returned for modification 28 February 1996/Accepted 22 April 1996

Site-specific DNA deletion occurs at thousands of sites within the genome during macronuclear development of *Tetrahymena thermophila*. These deletion elements are usually not detected in macronuclear chromosomes. We have interfered with the normal deletion of two of these elements, the adjacent M and R elements, by loading vegetative macronuclei with these elements prior to sexual conjugation. Transformed cell lines containing the exogenous M or R element, carried on high-copy-number vectors containing genes encoding rRNA within parental (old) macronuclei, consistently failed to excise chromosomal copies of the M or R element during formation of new macronuclei. Little or no interference with the deletions of adjacent elements or of unlinked elements was observed. The micronucleus (germ line)-limited region of each element was sufficient to inhibit specific DNA deletion. This interference with DNA deletion usually is manifested as a cytoplasmic dominant trait: deletion elements present in the old macronucleus of one partner of a mating pair were sufficient to inhibit deletion occurring in the other partner. Remarkably, the failure to excise these elements became a non-Mendelian, inheritable trait in the next generation and did not require the high copy number of exogenously introduced elements. The introduction of exogenous deletion elements into parental macronuclei provides us with an epigenetic means to establish a heritable pattern of DNA rearrangement.

Programmed DNA rearrangements occur in a wide variety of organisms and play significant roles in cell differentiation. Some of the most remarkable examples of these processes are found in ciliated protozoa (reviewed in reference 24). The ciliates exhibit nuclear dualism. Polyploid macronuclei are active in gene expression, providing for the somatic functions of cells. Diploid micronuclei are transcriptionally silent in vegetative growth but provide germ line functions during sexual reproduction. This dualism is one of the simplest separations of the soma from the germ line. The conjugation process results in the loss of parental macronuclei, and new macronuclei differentiate from zygotic nuclei formed by the fusion of micronucleus-derived gametes. Macronuclear differentiation involves extensive genome reorganization (reviewed in references 24 and 33). Chromosomes are fragmented and amplified to 45 to 50 copies per macronucleus. In *Tetrahymena thermophila*, the ribosomal RNA gene, rDNA, exemplifies the rearrangement process. The *Tetrahymena* rDNA is present as a single-copy sequence in the micronuclear genome (35) and is flanked by chromosomal breakage sequences. During macronuclear development, this copy is cut out of the chromosomal DNA and is converted into a unique, head-to-head palindromic molecule with telomeric DNA added to the linear ends. This palindromic minichromosome is highly amplified and maintained at nearly 9,000 copies in each macronucleus (37; reviewed in reference 32).

In addition to chromosome fragmentation and amplification, site-specific DNA deletion events occur accurately and efficiently at some 6,000 sites per haploid genome (34), eliminating approximately 15% of the micronuclear DNA (36). Remarkably, all or nearly all copies of these deleted sequences are faithfully excised from the macronuclear genome during development. The M and R elements are the two most extensively characterized deletion elements (Fig. 1). They are adjacent to one another in the micronuclear genome, separated by about 2.7 kbp of macronucleus-destined sequence. The deleted region of the M element is either 0.6 or 0.9 kbp. These two alternative forms differ at their left endpoints by 0.3 kbp (2). The micronucleus-specific region of the R element is 1.1 kbp. The macronuclear DNA sequences at the deletion junctions show limited microheterogeneity resulting from removal of these elements (3). Some of the *cis*-acting sequences required for precise DNA deletion have been determined recently by utilizing rDNA-based transformation vectors. Vector-based micronuclear rDNA, when introduced into a developing macronucleus by microinjection (38), is faithfully processed into a palindromic minichromosome and amplified. Exogenous DNA inserted between the rDNA transcription unit and the telomere-addition site can be maintained in a macronucleus on the rDNA minichromosome. Studies of *cis*-acting sequence requirements for deletion using rDNA-based transformation vectors have shown that both the deleted region and the adjacent macronucleus-destined sequences are necessary for precise DNA deletions of the M and R elements. Regions within the macronucleus-destined DNA function as flanking regulatory sequences and determine the boundaries of deletion. For the M element, this flanking regulatory sequence is a polypurine tract, 5′ AAAAAGGGGG 3′ (A_5G_5), that lies approximately 45 bp from each deletion endpoint (16). It determines the deletion boundaries in a distance- and orientation-specific manner (15). Certain sequences flanking the eliminated region of the R element also determine the deletion boundaries from a short distance away, although the exact identities of these sequences have not yet been determined (7). Other sequences, located in the micronucleus-specific DNA, are essential to target the M and R elements for deletion. These internal

* Corresponding author. Mailing address: Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Mailstop A2-168, 1124 Columbia St., Seattle, WA 98104. Phone: (206) 667-4435. Fax: (206) 667-6526. Electronic mail address: dchalker@fred.fhcrc.org.

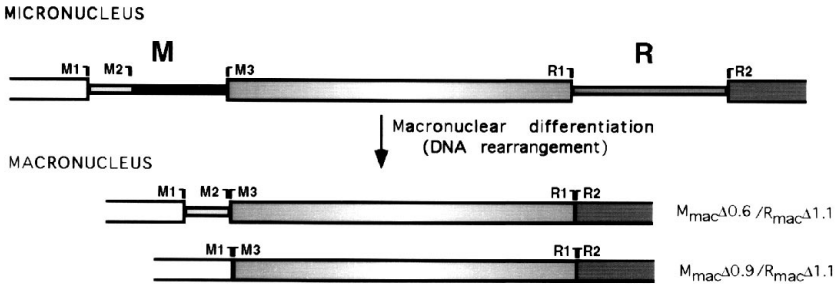

FIG. 1. M and R deletion elements. The genomic regions of the M and R elements as found in a micronucleus and a macronucleus are shown. The thin, darkly and lightly shaded boxes represent the excised portions of the M and R elements, respectively. The macronucleus-destined DNAs are shown as wide boxes. The major deletion endpoints are indicated: M1, M2, and M3 for the M element and R1 and R2 for the R element. Two alternative forms of the M element are excised during macronuclear differentiation. Joining M2 with M3 results in a 0.6-kbp deletion ($M_{mac}\Delta0.6$), while joining M1 with M3 results in a 0.9-kbp deletion ($M_{mac}\Delta0.9$). Joining R1 with R2 results in deletion of the 1.1-kbp R element ($R_{mac}\Delta1.1$).

promoting sequences function mostly independently of their orientations and distances from the deletion boundaries (31).

The purpose of eliminating these sequences from macronuclei or the roles these germ line-limited sequences may play in micronuclei are largely unknown. In *T. thermophila*, only the transcriptionally inactive micronuclei undergo mitosis, meiosis, and associated chromosome condensation. The transcriptionally active macronuclei divide by an unusual amitotic process without spindle formation or chromosome condensation. It is possible that these germ line-specific sequences participate in functions that are unique to micronuclei. To explore the functions of these micronucleus-specific DNAs, we have attempted to interfere with their possible normal activities by loading vegetative macronuclei with large numbers of deletion elements that normally reside only in micronuclei. We report below the inhibition of DNA deletion during the development of cells containing these micronucleus-specific sequences in their macronuclei. The element retained in each new macronucleus was mostly specific to the element introduced into the parental macronucleus. Remarkably, retaining these deletion elements became a non-Mendelian, inheritable trait in the next generation. Prior examples of such genetic phenomena have been observed almost exclusively in *Paramecium* spp. We report a clear molecular description of non-Mendelian inheritance in *T. thermophila* in an extensively characterized system of DNA deletion. This study provides a means both to understand the role of DNA deletion and to unravel much of the mystery surrounding the epigenetic regulation of macronuclear development in ciliates.

MATERIALS AND METHODS

Strains. *T. thermophila* HC76 [*Chx*/*Chx* (VI, cy-s)] and HC81 [*Mpr*/*Mpr* (VII, mp-s)] were used as parental cell lines in all experiments described, because these strains conjugated with a high degree of synchrony and produced a high percentage of viable progeny when crossed. HC76 and HC81 were derived from inbred B strains CU427 and CU428 (obtained from Peter Bruns), respectively, after each had undergone one round of genomic exclusion mating (1) to strain A*(III) (29). Strains CU427 and CU428 were used for anlagen transformation by electroporation as described by Gaertig and Gorovsky (14). All strains were maintained and grown as previously described (17).

Plasmid constructions. All recombinant DNA techniques were performed essentially as described by Sambrook et al. (25). Micronuclear DNA containing either the M or R deletion element (originally isolated on lambda clone cTt455 [34]) was ligated into the unique *Not*I restriction site in the rDNA vector pD5H8 (15). This site lies downstream of the transcribed region but upstream of the site of telomere addition of the rDNA. In order to simplify the subcloning of micronuclear DNA, the M and R elements were first inserted into the polylinker of pHSS6 (26) that is flanked by *Not*I sites. A *Hin*dIII restriction fragment from a plasmid subclone of cTt455 (2) containing 2.8 kbp of DNA from the micronuclear M locus, starting 0.24 kbp to the left and ending 1.7 kbp to the right of the eliminated sequence, was ligated to the *Hin*dIII-digested plasmid pHSS6, creating pDLCM1. The *Not*I fragment was then inserted into pD5H8, creating pDLCM4. The R element was cloned into pHSS6 as a 2.2-kbp DNA fragment starting from an *Ssp*I site 0.3 kbp to the left of the eliminated region and ending at an *Eco*RI site 0.8 kbp to the right of the eliminated region. The *Not*I fragment of the resulting plasmid, pDLCR4, was then inserted into the *Not*I site of pD5H8, creating pDLCR6. Plasmids pDLCM4 and pDLCR6 were used to generate rDNA molecules containing either the intact micronuclear forms or the rearranged macronuclear forms of the M and R deletion elements, respectively, by anlagen transformation as described below. Plasmids containing subregions of the deletion elements and the MR_{int} chimeric deletion element were constructed in similar fashions, and their construction will be described in more detail elsewhere (31). The M_{int} rDNA contains the right 0.6 kbp of the M element between nucleotides 561 and 1144 (5). The R_{int} and MR_{int} rDNAs contain the 1.1-kbp internal region of the R element between nucleotides 353 and 1413 (4).

Anlagen transformation and microinjection of macronuclei. Hybrid rDNA molecules were generated by transformation of developing cells with rDNA vectors containing the desired M and R element sequences. For anlagen transformation, CU427 and CU428 mating pairs were transformed with the rDNA plasmids described above by electroporation as described by Gaertig and Gorovsky (14). The transforming rDNAs contain the C3-type replication origin that is preferentially maintained over the endogenous B-type rDNA (22). It also contains a mutation within the 17S rRNA coding region that confers paromomycin resistance (pm-r). Whole-cell DNAs were extracted from pm-r lines and used for Southern blot analysis in order to determine whether the transformants had deleted the micronucleus-specific DNAs introduced on the vectors.

Whole-cell DNA preparations containing hybrid rDNAs were isolated from the *Tetrahymena* transformants described above and used to transform vegetatively growing HC76 and HC81 cells to pm-r as described previously (28, 38). DNAs were purified and injected at a concentration of 1 mg/ml. Injected cells were transferred and grown to saturation in drops of growth medium and replicated to medium containing 100 to 125 μg of paromomycin sulfate per ml to identify transformants, which are 5 to 20% of the injected cells. Replacement of the endogenous rDNAs with the injected rDNAs in the transformants was confirmed by Southern blot analysis.

DNA isolation and analysis. Whole-cell DNAs were isolated from *Tetrahymena* cells as previously described (4). DNAs were digested with restriction enzymes under the conditions indicated by the suppliers. Fragmented DNAs were separated by electrophoresis in 0.8 to 1.2% agarose gels. DNA fragments were transferred to nitrocellulose filters (Schleicher & Schuell) with a pressure blotter (Stratagene). DNAs immobilized on filters were hybridized to specific radiolabeled probes (11, 12) in 6× SSC (20× SSC is 3 M sodium chloride and 0.3 M sodium citrate, pH 7.0), 0.1 M Tris-Cl (pH 7.5), 0.5% sodium dodecyl sulfate (SDS), and 2× Denhardt's reagent (50× Denhardt's reagent is 1% Ficoll, 1% polyvinylpyrrolidone, and 1% bovine serum albumin) at 65°C overnight (12 to 20 h). Filters were washed two to three times in 2× SSC–0.5% SDS at 65°C for 30 min and once in 0.5× SSC–0.5% SDS at 50°C for 30 min and then exposed to X-ray film. Specific DNA probes are as follows (and diagrammed in Fig. 3): M, a *Hin*dIII restriction fragment from pDLCM3 containing 1.9 kbp of macronuclear DNA from the M element region starting 0.24 kbp to the right of the deletion junction and ending 1.7 kbp to the left of the junction; R, an *Eco*RI-*Pst*I restriction fragment from plasmid pDLCR5 containing 0.3 kbp to the right of the deletion junction and 0.8 kbp to the left of the deletion junction; cam, a 376-bp DNA fragment generated by PCR of whole-cell DNA with oligonucleotides 5′ TTTATTTTTCTTTTTTTTTTATTTTTTGAG 3′ and 5′ AGTGATGGGT TGTTCCTTAT 3′, containing sequences between 4 and 380 bp upstream of the ATG start codon of the *T. thermophila* calmodulin (CaM) gene (20). A 1.4-kbp micronucleus-specific sequence is located ~3.5 kbp upstream of the CaM-coding region.

Mating and caryonidal analysis. Parental strains HC76 and HC81 or pm-r transformants were grown, starved in 10 mM Tris-Cl (pH 7.4), and mated as previously described (16). Strains were starved separately at 30°C, and equal

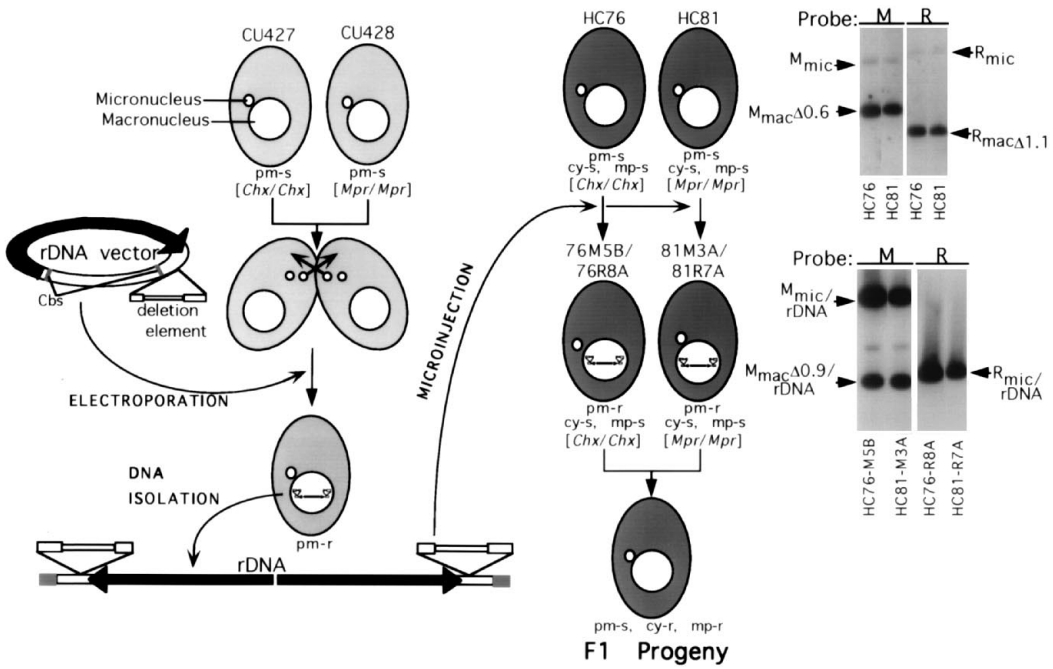

FIG. 2. Schematic representation of the method used to load the macronuclei of vegetatively growing cells with deletion elements (for details, see Materials and Methods). *Tetrahymena* cells are depicted as large, shaded ovals. The large and small open circles represent macronuclei and micronuclei, respectively. The exchange of gametic nuclei during mating is indicated by arrows within the CU427-CU428 mating pair. The name of the strain or transformed line is indicated above each cell. Relevent drug sensitivity (-s) and resistance (-r) phenotypes are given below each cell line (pm, paromomycin; cy, cycloheximide; mp, 6-methylpurine). In addition, micronuclear genotypes that allow for identification of progeny are given in brackets. Conjugating strains CU427 and CU428 were transformed with rDNA vectors containing the M or R deletion element or, in later experiments, portions of these elements. Transformants were identified by their pm-r phenotypes. Transformants that retained the micronucleus-specific DNA of each element in their mature, palindromic rDNA were identified by Southern blot analysis (not shown). These rDNAs were isolated in whole-cell DNA preparations and introduced by microinjection into the macronuclei of strains HC76 and HC81 growing vegetatively. Transformants were again identified on the basis of their pm-r phenotypes. The presence of the hybrid rDNA was confirmed by Southern blot analysis with radiolabeled probes specific to the M or R element region. A representative example of this analysis is shown to the right. Whole-cell DNA was isolated from HC76 and HC81 (top gel) or the injected lines (bottom gel). DNAs hybridized with the M-specific probe were digested with *Hin*dIII. DNAs hybridized with the R-specific probe were digested with *Bgl*II and *Eco*RI. (Both probes are specific to macronuclear DNA; see Fig. 3.) The whole-cell DNAs from HC76 and HC81 contain the expected ratio (between 1:15 to 1:20) of micronuclear fragments (M_{mic} and R_{mic}) and rearranged macronuclear fragments ($M_{mac}\Delta0.6$ and $R_{mac}\Delta1.1$) hybridizing to the corresponding probes (only the 0.6-kbp deleted form of the M element and not the 0.9-kbp deleted form is found in these strains). The major hybridizing DNAs of the HC76-M5B and HC81-M3A samples correspond to 2.8-kbp M_{mic} rDNA fragments. These injected lines also contain rDNA molecules containing the 0.9-kbp deleted form of the M element which was present in the original transformant DNA preparation used for microinjection (data not shown). The major hybridizing DNAs of the HC76-R8A and HC81-R7A samples correspond to 2.2-kbp R_{mic} rDNA fragments. Comparable amounts of whole-cell DNAs were used for each lane, but the autoradiographs shown of the HC76 and HC81 DNAs were exposed approximately 100 times longer than those for the transformed lines. Therefore, the exogenous copies of the M and R elements on the rDNAs are present in concentrations 100 times greater than the concentrations of the endogenous copies found in the macronuclear chromosomes. Once transformant lines loaded with specific deletion elements were established, they were crossed and the effects of the deletion elements on conjugation and DNA deletion were studied. True F_1 progeny were identified by their cy-r mp-r phenotypes. Cbs, chromosomal breakage sequences.

numbers of prestarved cells of different mating types were mixed to initiate conjugation. Synchrony and progression through conjugation were assessed by phase-contrast microscopy after fixation of cells in saturated $HgCl_2$–95% ethanol (2:1) (30) or by UV-epifluorescence microscopy of cells stained with 5 μg of DAPI (4′,6-diamidino-2-phenylindole) per ml. Four to five hours after mixing, mating cells were fed with equal volumes of 1× SPP (1% Proteose Peptone, 0.2% dextrose, 0.1% yeast extract, 0.003% Sequestrene) medium for 30 min to 1 h at 30°C. Individual mating pairs were isolated and transferred to individual drops of 1× SPP medium, allowed to complete conjugation, and cultivated. Successful mating resulted in progeny exhibiting resistance to cycloheximide and 6-methylpurine. Each cross was performed at least twice. Whole-cell DNA was isolated from 4 to 14 progeny lines for each cross and analyzed by Southern blot hybridization. For caryonidal analysis, exconjugants of isolated mating pairs were transferred to drops of fresh medium until the first cell division and then the four caryonides were transferred again to individual drops of medium.

Single-cell subclones of several F_1 synclonal lines were isolated into separate drops of 1× SPP medium. After cultivation, these subclones were transferred to 96-well culture plates and replica plated to fresh medium every 5 to 7 days for 2 months. After the 11th replica plating, 24 secondary subclones of each F_1 line were grown in individual drops of 1× SPP medium and then individually tested for sensitivity to cycloheximide and 6-methylpurine. Sexually mature subclones that had assorted into cell lines sensitive to both drugs were identified. F_1 heterocaryons were individually grown and allowed to mate to each other. Single mating pairs were isolated into drops of medium. After cultivation, true F_2 progeny were identified by their resistance to both cycloheximide and 6-methylpurine. DNAs were isolated from 25 F_2 lines and analyzed by Southern blot hybridization.

RESULTS

Germ line-limited DNAs in macronuclei interfere with normal DNA deletion. To explore the possible roles of DNA deletion in *T. thermophila*, we loaded cells with micronucleus-specific DNAs in order to interfere with the normal actions of these sequences. We accomplished this by introducing specific deletion elements carried on rDNA vectors into macronuclei by the method outlined in Fig. 2. These altered rDNA molecules are maintained at approximately 9,000 copies per cell, which is 4,500-fold higher than any single copy sequence in a micronucleus. Therefore, the copy number of the deletion element introduced into a macronucleus is comparable to the total number of micronucleus-specific elements present in a micronucleus (34). We inserted 2.8 kbp of DNA from the micronuclear, M element locus (M_{mic}) containing 0.9 kbp of micronucleus-specific DNA and 1.8 kbp of flanking DNA into

the rDNA transformation vector pD5H8, creating pDLCM4. This plasmid was used to transform conjugating cells. Although transformation with this type of rDNA plasmid usually results in the deletion of the micronucleus-limited sequences that are present on the vector (16), some transformants fail to carry out the deletion process and contain mature rDNA carrying intact deletion elements. These rDNA species provided us with a means to load cells with specific deletion elements that normally are absent from macronuclei.

In order to conduct subsequent experiments using cell lines with well-characterized genetic backgrounds rather than the original transformed progeny, whole-cell DNAs containing the M_{mic} rDNA minichromosomes were isolated from the transformants described above and microinjected into the macronuclei of mating strains HC76 and HC81. These strains are heterokaryons for genetic markers encoding resistance to cycloheximide and 6-methylpurine, respectively, that allow for selection of progeny after conjugation. Transformants were selected for paromomycin resistance. Southern blot analysis identified transformed lines, HC76-M5B and HC81-M3A, whose endogenous rDNAs had been mostly replaced with the injected M_{mic} rDNA (Fig. 2). These lines were used in the subsequent experiments.

Transformed lines HC76-M5B and HC81-M3A showed no obvious differences from parental strains during vegetative growth. These lines were crossed, and their progress through new macronuclear development was monitored by phase-contrast and epifluorescence microscopy and compared with the results of the matings of the uninjected HC76 and HC81 cells. The transformed lines proceeded through the stages of nuclear development normally, and the conjugating pairs separated at about the same time as pairs of uninjected strains (data not shown). No obvious abnormality in formation of the new macronuclei was detected. Individual pairs of mating cells were cloned and tested for unique progeny phenotypes (resistance to cycloheximide and 6-methylpurine) to assess the percentages of conjugants giving rise to progeny. Both the control strains (HC76 and HC81) and the transformed lines produced high percentages of viable progeny (80 to 90%). Thus, the progression of conjugation was not affected significantly by the number of copies of the micronucleus-specific M element in macronuclei.

Although nuclear differentiation appeared normal, we observed that the process of micronuclear sequence deletion was significantly affected. Whole-cell DNAs were isolated from progeny (cultivated over 25 cell divisions), and the structures of the M element loci were examined by Southern blot analysis with a probe specific to DNA flanking the element. Only a fraction (1/15 to 1/20) of a whole-cell DNA preparation is micronuclear DNA; therefore, most of the hybridizing DNA represents the progeny's macronuclear chromosomes which are present in about 50 copies per cell. The DNAs of the old macronuclei are degraded prior to cell division of the progeny. In the control mating of HC76 with HC81, the macronuclear chromosomes of the synclonal lines (the combined progeny derived from one mated pair) examined contained the two expected rearranged forms resulting from the deletion of 0.6 and 0.9 kbp of germ line-specific sequences (Fig. 3A, lanes 1 to 3). A synclonal population is known to contain either one or a mixture of both of the 0.6- and 0.9-kbp deleted forms which occur at roughly equal frequencies (2, 5). Southern blot analysis of progeny DNAs from the HC76-M5B × HC81-M3A ($M_{mic} \times M_{mic}$) cross showed that they also contained some of the two rearranged forms as expected. Interestingly, most of the lines also contained significant amounts of unrearranged DNA (Fig. 3A, lanes 4 to 6). Although the amounts of the

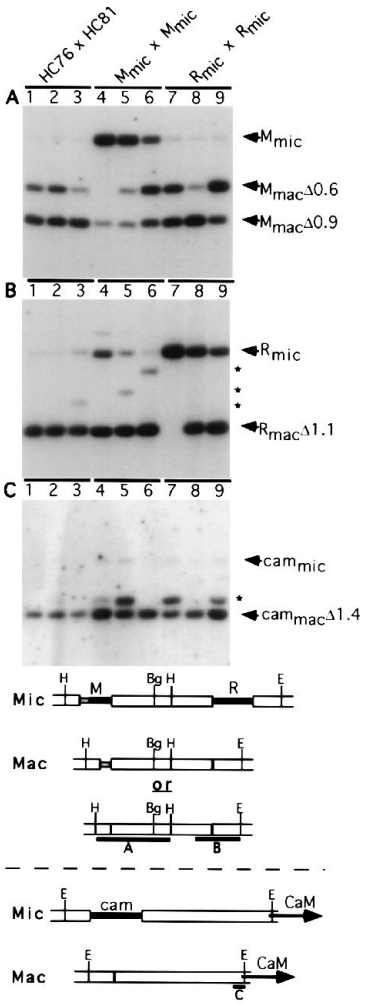

FIG. 3. Southern blot analysis of DNA from progeny derived from mating parents whose macronuclei were loaded with micronuclear M or R elements. Whole-cell DNA was isolated from three, representative synclonal populations from each mating: lanes 1 to 3, HC76 × HC81; lanes 4 to 6, HC76-M5B × HC81-M3A ($M_{mic} \times M_{mic}$); lanes 7 to 9, HC76-R8A × HC81-R7A ($R_{mic} \times R_{mic}$). DNAs were digested with restriction enzymes, fractionated by electrophoresis in agarose gels, and then transferred to nitrocellulose filters. DNAs on filters were hybridized to specific, radiolabeled, macronuclear DNA fragments and exposed to X-ray film. (A) DNAs were digested with *Hin*dIII and hybridized to an M-specific probe; (B) DNAs were digested with both *Eco*RI and *Bgl*II and hybridized with an R-specific probe; (C) DNAs were digested with *Eco*RI and hybridized to a cam-specific probe. The hybridizing DNA fragments representing the micronuclear forms (M_{mic}, R_{mic}, and cam_{mic}) and major macronuclear forms ($M_{mac}\Delta0.6$ and $M_{mac}\Delta0.9$, the 0.6- and 0.9-kbp deletions of the M element, respectively; $R_{mac}\Delta1.1$, the 1.1-kbp deleted form of the R element; and $cam_{mac}\Delta1.4$, the 1.4-kbp deleted form of cam) are indicated to the right of each panel. The M_{mic} *Hin*dIII fragment is 2.8 kbp; the R_{mic} *Eco*RI-*Bgl*II fragment is 3.3 kbp; and the cam_{mic} *Eco*RI fragment is 6.6 kbp. The positions of some DNA fragments of unique size representing aberrant deletion events are indicated by the asterisks. A schematic diagram showing the genomic region containing both the M and R elements and the region containing the cam element is given at the bottom of the figure. The wide, open boxes depict macronucleus-destined DNAs. The narrow, shaded boxes represent the M, R, and cam deletion elements as indicated. The micronuclear form of each region is displayed on the top line. The macronuclear forms are given below. The orientation of the CaM gene is designated by the arrow pointing in the direction of transcription. Restriction endonuclease sites used for the Southern blot analyses are indicated: H, *Hin*dIII; Bg, *Bgl*II; and E, *Eco*RI. The DNA fragments that were radiolabeled and used as M-, R-, and cam-specific probes are indicated by the bars labeled A, B, and C, respectively, to correspond to the three panels of Southern blots.

unrearranged DNA varied among synclonal lines, on average, about half of the total number of chromosomal copies of the M element remained unrearranged. Some lines completely failed to delete the M elements, containing only the micronuclear form of the M element (data not shown). The failure to rearrange the M element is distinctly different from what has been observed in normal matings. The unrearranged form of the M element has never been detected in the macronuclei of typical laboratory strains or their progeny (2, 5).

The presence of the chromosomes containing the M element in newly differentiated macronuclei appears to result from an epigenetic block of the deletion process rather than through conventional genetic inheritance. Parental macronuclei are not known to transmit genetic material to the progeny. The rDNA carrying the M element in a macronucleus is not transmitted to the progeny, since they are sensitive to paromomycin (data not shown). Furthermore, wild-type M element DNAs that could find entry into differentiating macronuclei would be expected to be eliminated, as observed when deletion elements are microinjected into anlagen (16).

In order to determine whether this phenomenon is limited to the M element, we crossed strains containing micronuclear R element DNAs (R_{mic}) in their macronuclei. These strains were generated in the same manner as the M_{mic} rDNA strains (Fig. 2). A R_{mic} rDNA hybrid, containing 1.1 kbp of micronucleus-specific DNA and 1.1 kbp of flanking DNA, was introduced into the macronuclei of strains HC76 and HC81 by microinjection, generating transformed lines HC76-R8A and HC81-R7A, respectively. These transformants were crossed, and deletions of the R element were examined in the progeny. Southern blot analysis showed that the synclonal progeny from the HC76 × HC81 control mating contained only the rearranged form of the R element in their macronuclei (Fig. 3B, lanes 1 to 3) as expected (4). In contrast, the progeny from the HC76-R8A × HC81-R7A (R_{mic} × R_{mic}) mating contained significant amounts of the unrearranged, micronuclear form of the R element (Fig. 3B, lanes 7 to 9). More than half of the approximately 50 macronuclear copies retained the 1.1-kbp, micronucleus-specific region. Thus, for both elements tested, loading parental cells with the micronuclear forms of DNA deletion elements interfered with normal DNA deletion during macronuclear development.

Deletion elements in macronuclei primarily interfere with their own deletion. In order to address whether the observed block of DNA deletion was specific to the element present in the parental macronuclei, we used Southern blot analysis to examine the effect of the R element on M element deletion and, likewise, the effect of the M element on R element deletion. Progeny from the HC76-R8A × HC81-R7A mating showed no observable failure to delete the M elements during development (Fig. 3A, lanes 7 to 9). Progeny from the HC76-M5B × HC81-M3A mating showed an observable but minor failure to delete the R elements. The level of unrearranged R elements remaining in the HC76-M5B × HC81-M3A progeny (Fig. 3B, lanes 4 to 6) is much lower than that remaining in the HC76-R8A × HC81-R7A progeny (Fig. 3B, lanes 7 to 9). Nevertheless, the ability of the M element in a parent to block R element deletion in the progeny clearly demonstrates that the observed interference with deletion is an epigenetic phenomenon.

To assess further this specificity, we examined a nonadjacent deletion element that is located ~3.5 kbp upstream of the calmodulin (CaM) gene (20). The presence of the M or the R element in a parental macronucleus did not result in overall failure to delete the 1.4-kbp micronucleus-specific DNA of the CaM-associated (cam) element (Fig. 3C, lanes 1 to 9) (although the level of aberrant deletion may be increased [discussed below]). The M and R elements also showed no obvious interference with the elimination of the repetitive germ line-limited sequences present in clone p2512 (data not shown), which is distributed in approximately 200 regions of the *Tetrahymena* micronuclear genome (34). Overall, these results show that DNA sequences present in parental macronuclei have a specific epigenetic influence on the rearrangements of the same sequences in developing macronuclei.

Although deletion of the M and R elements is quite precise, as observed on Southern blots in the studies described above, rare, aberrant deletion events that utilize novel endpoints were observed. These aberrant deletion events (observed as DNA fragments of unique sizes on Southern blots) appeared more frequently in the progeny of cells containing deletion elements in their macronuclei (e.g., Fig. 3B, lanes 5 and 6) than in the progeny of normal matings (e.g., Fig. 3B, lane 3). We also detected possible aberrant deletion of the cam element, resulting in a unique cam-hybridizing band on Southern blots. This aberrant deletion was observed in both the progeny of the control matings and matings of the cells containing M and R elements (data not shown). It appeared to be in greater abundance in the progeny of the latter, albeit in a limited sample examined. These results indicate that the precision as well as the efficiency of deletion is affected by the presence of micronucleus-specific DNA in parental macronuclei.

Micronucleus-limited sequences are sufficient to inhibit normal DNA deletion. The hybrid rDNAs in the above-described matings contained both the micronucleus-limited sequences and the flanking macronucleus-destined sequences. Both regions contain *cis*-acting sequences important for developmentally regulated DNA deletion. The macronucleus-destined sequences contain flanking regulatory sequences that determine the boundaries of deletion (7, 14, 15), and the deleted regions contain internal promoting sequences required to stimulate deletion of a particular DNA sequence (31). In order to determine whether the entire locus or just one of its components is necessary to inhibit DNA deletion during macronuclear development, we constructed hybrid rDNAs containing various portions of the M and R elements (Fig. 4, top). These rDNAs contained either (i) the macronucleus-destined, M element sequences corresponding to either the 0.6-kbp ($M_{mac}\Delta0.6$) or 0.9-kbp ($M_{mac}\Delta0.9$) deleted form of the M element; (ii) the right 0.6-kbp micronucleus-specific region of the M element (M_{int}); (iii) the 1.1-kbp micronucleus-specific region of the R element (R_{int}); or (iv) a chimeric deletion element that contains the 1.1 kbp of micronucleus-specific R element DNA replacing the right 0.6-kbp deleted region of the M element (this chimeric element can be accurately deleted when introduced into developing anlagen [31]). Each rDNA was injected into the macronuclei of HC76 and HC81 cells, and their presence was confirmed by Southern blot analysis (data not shown).

Each HC76 transformant line was then crossed with the HC81 transformant containing identical hybrid rDNAs. Conjugating pairs were isolated, and the resulting progeny were cultivated. DNA was prepared and examined for rearrangements of the chromosomal M and R elements by Southern blot analysis (Fig. 4). Again, intact M_{mic} sequences, carried on the rDNAs, primarily blocked deletion of the M element and R_{mic} sequences inhibited rearrangement of the R element (Fig. 4A and B, lanes 3 to 6). When lines containing the M_{mic} rDNA were crossed with lines containing the R_{mic} rDNA, the synclonal progeny often failed to delete both elements (Fig. 4A and B, lanes 7 to 8).

The macronuclear forms of the M element, $M_{mac}\Delta0.6$ or

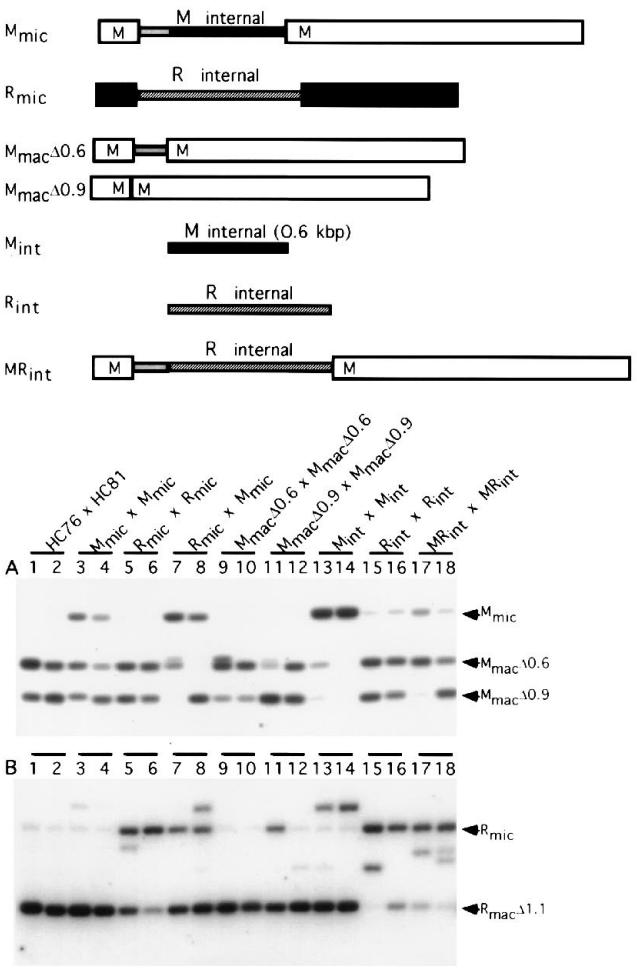

FIG. 4. The internal deleted region is sufficient to interfere with DNA deletion. DNAs isolated from representative synclonal progeny derived from mating parents whose macronuclei were loaded with various micronuclear and/or macronuclear sequences of the M and R elements were examined by Southern blot analysis. The M and R element sequences present in the hybrid rDNAs of the parents are diagrammed schematically at the top of the figure. The wide boxes represent macronucleus-destined DNAs, and the narrow boxes indicate the internal, deleted sequences. The wide, open box represents sequences immediately flanking the M element, and the wide, shaded box represents sequences flanking the R element. The narrow, solid box indicates the internal (germ line-limited) region of the M element eliminated in the 0.6-kbp deletion event, while the narrow, shaded box indicates the additional 0.3 kbp eliminated in the 0.9-kbp deletion of the M element. The narrow, hatched box represents the internal, deleted sequence of the R element. DNAs were isolated from two independent subclonal populations for each mating. The first two lanes of each gel contain DNAs from a pair of synclones descended from mating HC76 × HC81. Each successive pair of lanes contains DNAs from two synclonal progeny derived from mating strains whose macronuclei harbor hybrid rDNAs containing micronuclear and/or macronuclear DNAs of the M and R elements. The DNAs present in the hybrid rDNAs of the parents are listed above the lanes. The DNAs in gel A were digested with *Hin*dIII and hybridized to the M-specific probe. The DNAs in gel B were digested with *Bgl*II and *Eco*RI and hybridized with the R-specific probe. The hybridizing DNA fragments corresponding to the micronuclear forms and major rearranged macronuclear forms are indicated as described in the legend to Fig. 3.

$M_{mac}\Delta0.9$, were tested for their ability to inhibit M element deletion. Both of these DNAs contain the flanking regulatory sequence A_5G_5. These forms of the M element are normally present in macronuclei, but they number only about 50 copies per cell rather than the 9,000 copies found when they are carried on the hybrid rDNAs. Neither macronuclear form, when present at a high copy number, showed any effect on deletion of the M element (Fig. 4A, lanes 9 to 12). In addition, the ratio of the 0.6-kbp deletion to the 0.9-kbp deletion in new macronuclei was not significantly altered despite the relative abundance of a single rearranged form present in parent cells. In contrast, crossing cell lines that contained the 0.6-kbp internal, deleted region produced progeny in which M element deletion was significantly impaired (Fig. 4A, lanes 13 and 14). The progeny also contained uniquely sized DNA fragments hybridizing to the R-region probe, indicative of aberrant deletion of the R element (Fig. 4B, lanes 13 and 14). The internal 1.1-kbp micronucleus-specific region of the R element was sufficient to interfere with the deletion of the R element in the progeny examined (Fig. 4B, lanes 15 and 16), but had little effect on the deletion of the M element, confirming the specificity of this effect (Fig. 4A, lanes 15 and 16). To investigate this point further, we tested the effect of a chimeric deletion element containing the internal region of the R element inserted between the macronuclear sequences flanking the M element. This chimeric element significantly inhibited R element deletion during development (Fig. 4B, lanes 17 and 18), again showing the sufficiency of the germ line-specific region to block deletion. In at least one synclone examined, some failure of M element deletion was also observed (Fig. 4A, lane 17). Overall, the results obtained from crossing cell lines containing the various portions of the M and R elements show that the internal sequences normally deleted during development, and not the sequences immediately surrounding them, epigenetically influence macronuclear development in a specific way when these sequences remain in a parental macronucleus.

Interference with DNA deletion exhibits cytoplasmic dominance. The synclonal population produced from each mating pair consists of four caryonidal lines, i.e., descendants of four cells (caryonides) each with independently formed macronuclei. This occurs because two macronuclear anlagen develop in each partner, and these two anlagen are distributed to the two daughters of the first cell division after pair separation. In the analyses described above, the macronuclei of both mating partners contained the introduced deletion elements; therefore, all four new macronuclei of synclones are formed in similar cytoplasmic environments with regard to the excess of micronucleus-specific DNA elements. Cytoplasmic mixing occurs during conjugation of *T. thermophila* (23). If the block of DNA deletion we have observed is mediated through cytoplasmic components, we might expect that deletion elements present in the macronucleus of a single parent would affect DNA deletion occurring in the other partner. Alternately, the normal mating partner might be able to rescue the deletion block caused by germ line-limited DNA within the mating partner's macronucleus.

To investigate the above possibility, HC76-M5B and HC81-M3A were each crossed with their uninjected partners HC81 and HC76, respectively. Likewise, HC76-R8A and HC81-R7A were also crossed with their respective uninjected partners. Several pairs from each mating were isolated. Exconjugants were separated, and caryonides were isolated after the first cell division and cultivated. DNA was prepared, and the deletions of the M and R elements were examined by Southern blot analysis (Fig. 5). All four caryonides from the control HC76 × HC81 mating pair had completely excised their M elements from the macronuclear genome as expected (Fig. 5A, lanes 1 to 4). For two caryonidal sets from the HC76-M5B × HC81 cross, one set showed failure to remove M sequences in all four caryonides (Fig. 5A, lanes 5 to 8) while the other showed failure to delete the M elements in three of four caryonides (Fig. 5A, lanes 9 to 12). Similarly, for two caryonidal sets from

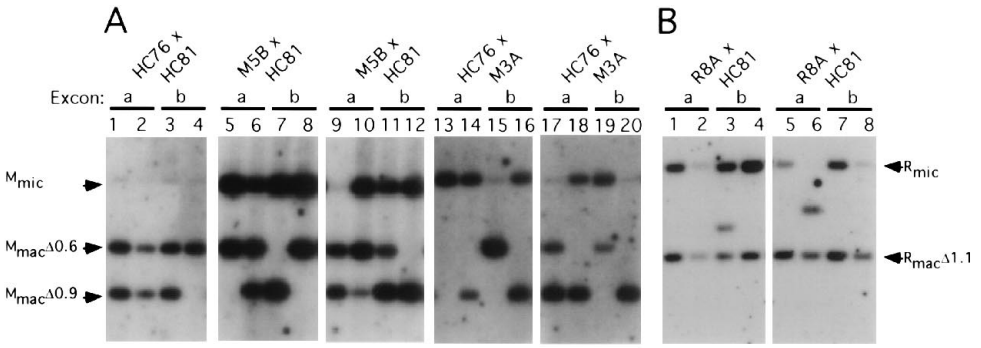

FIG. 5. Caryonidal analysis of mating strains in which a single partner is loaded with micronuclear M or R element DNA. HC76 and HC81 were mated together (lanes 1 to 4) or crossed with a mating partner containing the M_{mic} or R_{mic} hybrid rDNA (lanes 5 to 20). Exconjugants of individual pairs were isolated and followed until the first cell division. The two cells from this caryonidal division were then separated and maintained as separate cell lines. DNAs were isolated from the four caryonidal lines descended from each mated pair. After digestion with *Hin*dIII (A) or *Bgl*II and *Eco*RI together (B), DNAs were fractionated on agarose gels, transferred to nitrocellulose, and hybridized with the M-specific probe (A) or R-specific probe (B). Hybridized filters were analyzed by autoradiography. Each set of four lanes contains DNAs isolated from four independent caryonides. The first two lanes of each set contain DNAs from the caryonidal lines derived from one exconjugant (a) and the second two lanes contain DNAs from the other exconjugant (b). The names of the two strains mated are given above each set of four lanes. The bands corresponding to micronuclear and macronuclear forms of the M and R element loci are indicated as in Fig. 3.

a cross of HC76 with HC81-M3A, we observed failure of M element deletion in three of four (Fig. 5A, lanes 13 to 16) and two of four (Fig. 5A, lanes 17 to 20) caryonides. In the latter case, the two affected caryonides were derived from different exconjugants. We performed similar caryonidal analysis with strains containing the R_{mic} rDNA. Two sets were examined from the mating of HC76-R8B and HC81. The number of deletions of the R elements was significantly reduced in three of four caryonides of one set (Fig. 5B, lanes 1 to 4) and in two of four caryonides (derived from different exconjugants) of the other set (Fig. 5B, lanes 5 to 8). All four caryonides from the one set analyzed from the HC76 × HC81-R7A cross showed failure to delete the R element (data not shown). Thus, in each caryonidal set from these matings, at least one caryonidal line originating from each exconjugant showed failure to delete the M or R element. Therefore, the presence of micronucleus-limited sequences in a parental macronucleus elicits a cytoplasmic dominant influence on the macronuclear development of its mating partner, resulting in the inhibition of normal DNA deletion.

Failure to delete micronucleus-specific DNA is heritable. The macronuclei of normal cells are devoid of micronucleus-specific sequences. Nevertheless, by introducing the M and R elements on hybrid rDNAs, we have been able to produce cell lines in the F_1 generation that have retained these micronucleus-specific DNAs in their macronuclear genomes. To determine whether the block of DNA deletion behaved as a heritable trait in these cells, we mated the F_1 progeny lines that contain a significant proportion of unrearranged copies of the M element in their macronuclear DNAs (Fig. 6). In contrast to the parental strains that contained approximately 9,000 extra copies of the M element, these F_1 cells contained about 50 or fewer copies in their macronuclei. Five F_1 cell lines were mated in pairwise combinations with one another to produce F_2 progeny. Individual mating pairs were isolated and cultured, and their DNAs were examined for the deletion of M elements by Southern blot analysis. The analysis performed on 13 of 25 F_2 synclonal lines is shown in Fig. 5. Much as we observed with the F_1 generation, many of these F_2 progeny failed to delete the M elements during macronuclear formation. The F_2 cell lines showed various amounts of unrearranged M elements in their macronuclei. We did not observe any obvious correlation between the amount of M element deletion in the F_2 macronuclei and the amount of rearranged sequences in the parental (F_1) macronuclei. For example, lines $F_{1\text{-}2}$ and $F_{1\text{-}3}$ have few or no deleted forms of the M element in their macronuclei; however, their two synclonal progeny examined were only slightly impaired in M element deletion. On the other hand, lines $F_{1\text{-}4}$ and $F_{1\text{-}5}$ contain some amount of deleted copies of the M element (approximately one-fourth to one-third of their M element copies are rearranged), but their four synclonal progeny examined exhibited extensive failure to delete the M element. These results indicate that the block of DNA deletion that was created by epigenetic perturbation is perpetuated in

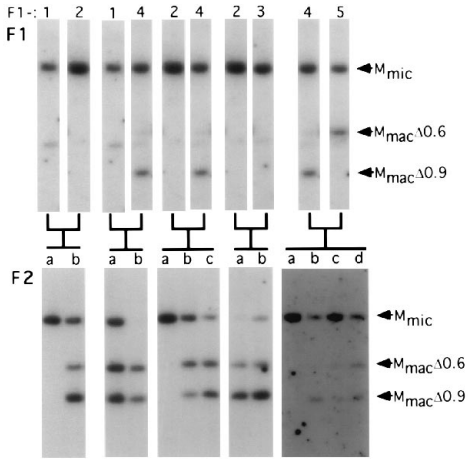

FIG. 6. Southern blot analyses of F_2 progeny show failure to efficiently delete M elements. Five F_1 progeny that failed to delete the M element were grown to sexual maturity, starved, and mated. Whole-cell DNAs were isolated from the resulting F_2 synclonal progeny. DNAs were digested with *Hin*dIII, fractionated by electrophoresis, blotted to nitrocellulose, and hybridized with the M-specific probe. Autoradiographs of hybridized filters are shown. The top gel shows the hybridization pattern of the F_1 cells used for mating to give rise to the F_2 lines. The F_1 macronuclei contain either the micronuclear form of the M element only or a combination of micronuclear and deleted forms lacking the internal 0.6 or 0.9 kbp. For each of the five pairwise F_1 matings, two to four synclonal progeny (labeled a to d) were analyzed and the results are shown below the analysis of their respective parents. The bands corresponding to micronuclear and macronuclear forms of the M element are indicated to the right as in Fig. 3.

these cells though a sexual generation and has become a non-Mendelian, inheritable trait.

DISCUSSION

Non-Mendelian inheritance in macronuclear differentiation. In the above report, we have shown that the presence of micronucleus-specific deletion elements in parental macronuclei blocks normal DNA deletion during new macronuclear development in *T. thermophila*. When either the M or R element was maintained at a high copy number on macronuclear rDNAs, it significantly inhibited the deletion of chromosomal copies of the M or R element, respectively. Since parental macronuclei do not transmit genetic material during conjugation, the macronuclear genome of the progeny is clearly affected in a nongenic way by the abnormal presence of these deletion elements. This induced failure of DNA deletion exhibits clear cytoplasmic dominance. The presence of micronucleus-specific sequences in a single parent of a conjugating pair is sufficient to cause failure to remove these sequences in all four caryonidal progeny. Furthermore, the failure to remove a particular deletion element becomes a heritable property of the progeny lines and does not require the excessively high copy numbers of the deletion element carried on the rDNAs. This phenomenon is a clear example of non-Mendelian inheritance in *T. thermophila* described at the molecular level. DNA deletion of the M and R elements is one of the best-understood examples of DNA rearrangements in ciliates and therefore provides an excellent system to explore the nucleocytoplasmic interactions that permit a parental macronucleus to affect inheritance.

The genetic enigma of non-Mendelian, nuclear inheritance was recognized in studies of mating-type determination in *Paramecium* spp. (reviewed in reference 27). The early observation was that the primary determinant is the existing mating type, either O or E, of each partner. In sexual reproduction, the caryonidal progeny descending from the O conjugant differentiate to express mating type O and the progeny descending from the E conjugant differentiate to express E, despite each conjugant having identical zygotic genetic information. However, if significant cytoplasmic exchange occurs, macronuclei that develop in an O cytoplasm can differentiate to express E. Although these long-standing observations clearly demonstrate that mating-type differentiation involves a cytoplasmic component determined by parental macronuclei, the molecular basis for this phenomenon is not yet understood.

An initial connection between non-Mendelian inheritance and DNA rearrangements occurring during macronuclear development was provided by the *Paramecium* mutant d48. The *A* surface protein gene is not expressed in d48 strains, because the *A* gene is missing from macronuclei (10). The *A* gene is present in micronuclei, but a defect in DNA processing during development results in the loss of the end of the macronuclear chromosome containing the gene (13). This defect is heritable, but it is determined cytoplasmically within each mating partner. Nucleoplasm from a wild-type cell (19) or even cloned portions of the *A* gene itself (21, 39) are sufficient to rescue the mutant phenotype in subsequent generations. Nonetheless, the molecular mechanism for producing and rescuing the d48 mutation remains unknown.

A recent observation has further established a possible link between non-Mendelian inheritance and DNA rearrangements in *Paramecium* spp. The excision of a 222-bp internal eliminated sequence interrupting the *G* gene of *Paramecium tetraurelia* is subject to epigenetic regulation (9). The rearrangement state, with or without the internal eliminated sequence, of the *G* gene within progeny is determined by the state of the gene in parental macronuclei. That study has shown that developmentally programmed DNA deletion occurring in *Paramecium* spp. can be influenced by parental macronuclei, as we have shown above, quite independently, for deletion elements in *T. thermophila.*

Examples of non-Mendelian inheritance (excluding organellar inheritance) in organisms other than *Paramecium* spp. are very limited. A possible precedent for nucleocytoplasmic interactions affecting inheritance has been genetically described in studies of mutants defective for *SerH* gene expression in *T. thermophila* (8). Expression of some of these mutants could be restored by the exchange of wild-type cytoplasm during conjugation. Proper *SerH* expression in the progeny was dependent upon its prior expression in parental macronuclei. Although defects in the developmental processing of the *SerH* gene have been proposed to explain the behavior of these mutants, the molecular basis for this inheritance is still unknown. Our study clearly demonstrates that DNA deletion in *T. thermophila* can be affected by the rearrangement state of the same sequence in parental macronuclei. It shows that the ability of parental macronuclei to influence DNA rearrangement is not limited to the *Paramecium* genus and could be a common property of ciliates, as *Tetrahymena* spp. and *Paramecium* spp. are fairly distant evolutionarily (6, 18). Our description of non-Mendelian inheritance in the well-studied system of *Tetrahymena* deletion elements is an important step toward dispelling the mystery of these epigenetic phenomena.

Mechanisms and control of DNA deletion. The inhibition of DNA deletion of the M and R elements provides us with a better understanding of the mechanism of deletion as well. First, we have found that the cell can distinguish among different deletion elements. The M and R elements greatly inhibit their own deletion, but neither element severely blocks rearrangement of the other element or a third (cam) element. This element specificity is not absolute, however, since the M element does interfere with R element deletion, albeit to a much lesser extent than with its own deletion. Because we have examined only a few of the several thousand deletion elements, it remains possible that other, unexamined elements are affected also.

From this study, it is clear that the sequences entirely within the micronucleus-specific DNA are sufficient to distinguish one element from another. The internal, deleted region of the R element blocks R element deletion even in the context of the M element macronucleus-destined sequence (the MR_{int} chimeric element). Although the DNAs immediately flanking deletion elements are sufficient to determine the boundaries of deletion (these sequences are different between the M and R elements) (16), M element-flanking DNA when present at a high copy number had little or no effect on deletion. This perhaps is not surprising, since these sequences are normally found in macronuclei. *trans*-acting factors that function at these flanking regulatory sequences may not be able to enter old macronuclei, or the recognition of these sequences may require the action of *cis*-acting sequences found within the deletion elements. In support of the second possibility, a low level of interference with M element deletion was observed in matings of cells containing the MR_{int} chimeric element whereas no interference was detected when the M element-flanking DNA alone was present in the parental rDNA.

Although the M, R, and cam elements appear to be mostly distinct from one another, it seems unlikely that each of the estimated 6,000 deletion elements is unique in regard to its deletion requirements. It is possible that there are different classes of deletion elements, each of whose members has sim-

ilar or identical requirements for deletion. Alternatively, each element may contain several sequence components that interact with different *trans*-acting factors. The combined action of these components would be necessary to promote efficient deletion of each element. Any particular component may be shared among some elements, but most elements would have a unique combination of these components. Therefore, each element would specifically interfere with its own deletion and also interfere with the deletion of other elements to different extents if they have some common components. Such partially overlapping deletion requirements may explain the ability of the M element to interfere somewhat with R element deletion, especially if the shared component is more important for R element deletion than for the M element. Partial interference may also explain the increase in aberrant deletion events observed. While interfering with most or all of the *cis*-acting sequence components results in complete failure of deletion, inhibiting the action of fewer components may result in aberrant deletion. Consistent with this view, multiple regions of the M element internal region appear to be required for full deletion activity (31).

One of the most striking properties of this phenomenon is the dominant nature of the interference. Cytoplasm is known to be exchanged in mating *T. thermophila* organisms. By radiolabeling macromolecules in one partner of a mating pair, McDonald (23) showed that thorough mixing of cytoplasms can be obtained between the time cells initially pair and the developmental stage during which DNA deletion occurs. If the effect we have observed is transmitted through cytoplasms, it must be initiated sufficiently early in the developmental process in order to permit cytoplasmic exchange between partners. The interference with DNA deletion we have observed here must be quite strong to affect the opposite mating partner. Despite this strength, the effect appears somewhat variable since not all copies of a deletion element are affected nor are both nuclei that differentiated in the same cell equally blocked. One possible explanation could be that developing nuclei are sensitive to the block during a limited period.

The epigenetic block of DNA deletion that we have observed is manifested as a heritable trait. The F_1 lines that retained deletion elements in their macronuclei produced progeny (the F_2 lines) that also failed to delete these elements. In this respect, this phenomenon is similar to examples of non-Mendelian inheritance observed in *Paramecium* spp. At first appearance, it seemed somewhat surprising that the F_2 progeny also showed failure to delete the M elements. The F_1 lines contained $\leq$50 copies of the M_{mic} sequences, and yet their progeny appeared to be as affected in DNA deletion as the progeny (the F_1 lines) that came from parents containing thousands of M_{mic} copies. However, it is possible that other deletion elements which we have not examined are retained in the macronuclei of these F_1 lines, resulting in a much higher, effective copy number of deletion elements. It is clear that the presence of deletion elements in parental macronuclei serves as an epigenetic means of establishing a heritable rearrangement state. In addition, the ability to retain specific DNA deletion elements in macronuclei gives us the potential to study these usually germ line-limited sequences and thereby to elucidate their normal micronuclear functions and their potential actions during development.

The mechanisms evoked to explain the inhibition of deletion must account for the element specificity, the cytoplasmic dominance, and the heritability that we have observed. The simplest interpretation is that micronucleus-specific sequences in the parental macronucleus sequester *trans*-acting factors necessary for DNA deletion. Such factors must be in limited supply, must be able to pass through the conjugation junction formed between mated cells, and must be able to enter parental macronuclei. To account for the observed element specificity, the factors used for M element deletion must be mostly different from those required for R element deletion. The cross-interference of R element deletion caused by the M element could result from a common factor that recognizes a component found in both elements, as discussed earlier. The limiting factors sequestered must be expressed early to allow diffusion from the wild-type conjugant in order to account for the dominant nature of the block. It is formally possible that the deletion elements themselves escape from old macronuclei and diffuse to the wild-type conjugating partners to compete for the limiting factors.

Putative sequestration of *trans*-acting factors would require only passive roles of the deletion elements. However, we cannot exclude the possibility that these sequences play more active roles by sending signals to the developing macronuclei directing which sequences are to be retained in the progeny. These signals could be encoded by the deletion elements themselves, thus achieving the element specificities observed. The presence of an unrearranged form would send an incorrect signal, thus affecting normal deletion. This model can account for inheritance in subsequent generations, since once a particular DNA sequence is found in macronuclei, the signal could be sent repeatedly.

The potential involvement of a signal in the deletion process raises the possibility that DNA rearrangement is directed by a nucleic acid template molecule. This template, sent from old macronuclei, would provide replicas of the parental rearrangement patterns to be copied into developing macronuclei. Our data argue against this possibility. If a template guides deletion, we would expect that progeny macronuclei would reflect the DNA composition of the parent. Contrary to this expectation, mating cells that contained either the 0.6- or 0.9-kbp deleted form of the M element on high-copy-number rDNA vectors did not give rise to progeny having a large proportion of the deleted form present in the parent. Furthermore, mating F_1 lines (e.g., $F_{1\text{-}2} \times F_{1\text{-}3}$) that contained only the unrearranged form of the M element gave rise to progeny that contained deleted forms of the M element without the preexistence of a template for that form in these cells. These results provide convincing evidence against the participation of a template guiding normal deletion.

For decades, the intriguing phenomenon of non-Mendelian inheritance has remained a challenging genetic enigma peculiar to *Paramecium* spp. Recent molecular descriptions of this process have begun to deepen our understanding, but a mechanism that would explain this mode of inheritance is still wanting. We have shown that parental macronuclei can impose specific constraints on developmentally programmed DNA rearrangements in *T. thermophila*. Therefore, non-Mendelian inheritance is a phenomenon not limited to *Paramecium* spp. and could be a common genetic property among ciliates. Using the molecular tools available in *T. thermophila* and a well-studied process of DNA rearrangement, we can move forward to a clearer understanding of the molecular basis of these remarkable effects on genetic inheritance.

ACKNOWLEDGMENTS

We thank C. H. Yao for providing *Tetrahymena* DNA preparations used in this study and Joseph Frankel and Robert Coyne for critical readings of the manuscript.

This work was supported by National Research Service Award GM16315 to D.L.C. and U.S. Public Health Service grant GM26210 to M.-C.Y. from the National Institutes of Health.

REFERENCES

1. **Allen, S. L.** 1967. Cytogenetics of genomic exclusion in *Tetrahymena*. Genetics **55:**797–822.
2. **Austerberry, C. F., C. D. Allis, and M. C. Yao.** 1984. Specific DNA rearrangements in synchronously developing nuclei of *Tetrahymena*. Proc. Natl. Acad. Sci. USA **81:**7383–7387.
3. **Austerberry, C. F., R. O. Snyder, and M. C. Yao.** 1989. Sequence microheterogeneity is generated at junctions of programmed DNA deletions in *Tetrahymena thermophila*. Nucleic Acids Res. **17:**7263–7272.
4. **Austerberry, C. F., and M.-C. Yao.** 1987. Nucleotide sequence structure and consistency of a developmentally regulated DNA deletion in *Tetrahymena thermophila*. Mol. Cell. Biol. **7:**435–443.
5. **Austerberry, C. F., and M.-C. Yao.** 1988. Sequence structures of two developmentally regulated, alternative DNA deletion junctions in *Tetrahymena thermophila*. Mol. Cell. Biol. **8:**3947–3950.
6. **Baroin-Tourancheau, A., P. Delgado, R. Perasso, and A. Adoutte.** 1992. A broad molecular phylogeny of ciliates: identification of major evolutionary trends and radiations within the phylum. Proc. Natl. Acad. Sci. USA **89:** 9764–9768.
7. **Chalker, D. L., A. Wilson, A. La Terza, C. Kroenke, and M. C. Yao.** Unpublished data.
8. **Doerder, F. P., and M. S. Berkowitz.** 1987. Nucleo-cytoplasmic interaction during macronuclear differentiation in ciliate protists: genetic basis for cytoplasmic control of *SerH* expression during macronuclear development in *Tetrahymena thermophila*. Genetics **117:**13–23.
9. **Duharcourt, S., A. Butler, and E. Meyer.** 1995. Epigenetic self-regulation of developmental excision of an internal eliminated sequence in *Paramecium tetraurelia*. Genes Dev. **9:**2065–2077.
10. **Epstein, L. M., and J. D. Forney.** 1984. Mendelian and non-Mendelian mutations affecting surface antigen expression in *Paramecium tetraurelia*. Mol. Cell. Biol. **4:**1583–1590.
11. **Feinberg, A. P., and B. Vogelstein.** 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132:**6–13.
12. **Feinberg, A. P., and B. Vogelstein.** 1984. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Addendum. Anal. Biochem. **137:**266–267.
13. **Forney, J. D., and E. H. Blackburn.** 1988. Developmentally controlled telomere addition in wild-type and mutant paramecia. Mol. Cell. Biol. **8:**251–258.
14. **Gaertig, J., and M. A. Gorovsky.** 1992. Efficient mass transformation of *Tetrahymena thermophila* by electroporation of conjugants. Proc. Natl. Acad. Sci. USA **89:**9196–9200.
15. **Godiska, R., C. James, and M. C. Yao.** 1993. A distant 10-bp sequence specifies the boundaries of a programmed DNA deletion in *Tetrahymena*. Genes Dev. **7:**2357–2365.
16. **Godiska, R., and M. C. Yao.** 1990. A programmed site-specific DNA rearrangement in *Tetrahymena thermophila* requires flanking polypurine tracts. Cell **61:**1237–1246.
17. **Gorovsky, M. A., M. C. Yao, J. B. Keevert, and G. L. Pleger.** 1975. Isolation of micro- and macronuclei of *Tetrahymena pyriformis*. Methods Cell Biol. **9:**311–327.
18. **Greenwood, S. J., M. Schlegel, M. L. Sogin, and D. H. Lynn.** 1991. Phylogenetic relationships of Blepharisma americanum and Colpoda inflata within the phylum Ciliophora inferred from complete small subunit rRNA gene sequences. J. Protozool. **38:**1–6.
19. **Harumoto, T.** 1986. Induced change in a non-Mendelian determinant by transplantation of macronucleoplasm in *Paramecium tetraurelia*. Mol. Cell. Biol. **6:**3498–3501.
20. **Katoh, M., M. Hirono, T. Takemasa, M. Kimura, and Y. Watanabe.** 1993. A micronucleus-specific sequence exists in the 5′-upstream region of calmodulin gene in *Tetrahymena thermophila*. Nucleic Acids Res. **21:**2409–2414.
21. **Kim, C. S., J. R. Preer, and B. Polisky.** 1994. Identification of DNA segments capable of rescuing a non-Mendelian mutant in *Paramecium*. Genetics **136:** 1325–1328.
22. **Larson, D. D., E. H. Blackburn, P. C. Yeager, and E. Orias.** 1986. Control of rDNA replication in *Tetrahymena* involves a cis-acting upstream repeat of a promoter element. Cell **47:**229–240.
23. **McDonald, B. B.** 1966. The exchange of RNA and protein during conjugation in *Tetrahymena*. J. Protozool. **13:**277–285.
24. **Prescott, D. M.** 1994. The DNA of ciliated protozoa. Microbiol. Rev. **58:** 233–267.
25. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
26. **Seifert, H. S., E. Y. Chen, M. So, and F. Hefron.** 1986. Shuttle mutagenesis: a method of transposon mutagenesis for Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA **83:**735–739.
27. **Sonneborn, T. M.** 1977. Genetics of cellular differentiation: stable nuclear differentiation in eukaryotic unicells. Annu. Rev. Genet. **11:**349–367.
28. **Tondravi, M. M., and M. C. Yao.** 1986. Transformation of *Tetrahymena thermophila* by microinjection of ribosomal RNA genes. Proc. Natl. Acad. Sci. USA **83:**4369–4373.
29. **Ward, J., and M.-C. Yao.** Unpublished data.
30. **Wenkert, D., and C. D. Allis.** 1984. Timing of the appearance of macronuclear-specific histone variant hv1 and gene expression in developing new macronuclei of *Tetrahymena thermophila*. J. Cell Biol. **98:**2107–2117.
31. **Yao, C.-H., and M.-C. Yao.** Unpublished data.
32. **Yao, M.-C.** 1982. Amplification of ribosomal RNA gene in *Tetrahymena*, p. 127–153. *In* H. Busch and L. Rothblum (ed.), The cell nucleus, vol. 12. Academic Press, Inc., New York.
33. **Yao, M.-C.** 1989. Site-specific chromosome breakage and DNA deletion in ciliates, p. 715–734. *In* D. E. Berg and M. M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.
34. **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.
35. **Yao, M. C., and J. G. Gall.** 1977. A single integrated gene for ribosomal RNA in a eucaryote, *Tetrahymena pyriformis*. Cell **12:**121–132.
36. **Yao, M. C., and M. A. Gorovsky.** 1974. Comparison of the sequences of macro- and micronuclear DNA of *Tetrahymena pyriformis*. Chromosoma **48:**1–18.
37. **Yao, M. C., A. R. Kimmel, and M. A. Gorovsky.** 1974. A small number of cistrons for ribosomal RNA in the germinal nucleus of a eukaryote, *Tetrahymena pyriformis*. Proc. Natl. Acad. Sci. USA **71:**3082–3086.
38. **Yao, M.-C., and C.-H. Yao.** 1989. Accurate processing and amplification of cloned germ line copies of ribosomal DNA injected into developing nuclei of *Tetrahymena thermophila*. Mol. Cell. Biol. **9:**1092–1099.
39. **You, Y., J. Scott, and J. Forney.** 1994. The role of macronuclear DNA sequences in the permanent rescue of a non-Mendelian mutation in *Paramecium tetraurelia*. Genetics **136:**1319–1324.