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Alternative Processing of Sequences During Macronuclear Development in *Tetrahymena thermophila*[1]

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ABSTRACT. DNA is eliminated during development of the somatic MACronucleus from the germinal MICronucleus in the ciliated protozoan, *Tetrahymena thermophila*. Facultatively persistent sequences are a class of sequences that persist in the MAC DNA of some cell lines but are eliminated from the MAC DNA of other cell lines. One cloned MAC fragment contains a persistent sequence as well as sequences normally retained in the MAC. When this cloned fragment was used to construct MAC restriction maps of this region in cell lines whose MAC DNAs do, or do not, contain the persistent sequence, extensive variation in the map flanking this region was observed. The different DNA rearrangements of this MIC segment are epigenetically determined during or soon after MAC development. Moreover, different rearrangements may occur among the 45 copies of this MIC segment as a MAC is formed, resulting in polymorphisms that are later resolved by phenotypic assortment.

DNA elimination is a normal part of development in many eukaryotic organisms including ciliated protozoa (30, 40), crustaceans (9), insects (35), and nematodes (38). In the ciliated protozoa DNA elimination has been studied most extensively in the holotrich, *Tetrahymena thermophila* (40), and in various species of the hypotrich, *Oxytricha* (30). Recently, molecular cloning techniques have allowed us to probe the processes of DNA elimination in these ciliates. One question that these techniques can address is how tightly controlled is the process of elimination? How much variability is inherent in the system?

Tetrahymena, like most ciliated protozoa, contains a germinal micronucleus (MIC) and a somatic macronucleus (MAC) that develops from the MIC during the sexual process of conjugation (reviewed in 2). When two cells mate, their MICs undergo meiosis and one of the four meiotic products is retained in each cell. This haploid nucleus then undergoes mitosis to form migratory and stationary pronuclei; the two cells exchange migratory pronuclei, and the stationary pronucleus fuses with its partner's migratory pronucleus to form a diploid zygotic nucleus that is genetically identical in the two cells. In each conjugant, the zygotic nucleus then undergoes two rounds of mitosis. The four resulting nuclei each have a different fate: one nucleus is retained as the cell's MIC; another nucleus is destroyed and the remaining two nuclei develop independently into MACs. The two conjugating cells separate and each exconjugant undergoes cell division. At the first cell division the MIC divides but each daughter cell receives only one of the two new MACs. The four daughter cells from one mating pair are called caryonides. They have genetically identical MICs, but each caryonide contains an independently developed MAC.

The MAC has approximately 23 times as much DNA as the MIC (reviewed in 25). During MAC development, the MIC chromosomes fragment into smaller pieces averaging 600 kb long but ranging in size from 21 kb to greater than 1100 kb (7). Each piece can replicate, and except for rDNA, each of the 50 to several hundred types of molecules is present on average in 45 copies. These autonomously replicating pieces probably correspond to the assorting "units" seen in heterozygotes (28). In heterozygotes, some of the 45 copies will contain one allele and the rest will contain the other allele. Random distribution of allelic copies in successive fissions can produce a cell with a MAC that is homozygous for one of the alleles. The phenomenon is referred to as phenotypic assortment and occurs with the kinetics predicted from the random distribution of 45 copies of each type of assorting unit to each daughter nucleus (reviewed in 25).

During macronuclear development, 10–20% of the MIC genome is eliminated from the MAC. Recombinant DNA clones have been used to show that these DNA eliminations are associated with two kinds of DNA rearrangements. One such DNA alteration is the fragmentation of the MIC genome into subchromosomal fragments to which the telomeric repeat, $(C_4A_2)_n$, is added (41). The best studied site of fragmentation is at one end of the 17S and 26S rDNA locus. As this site is fragmented, some DNA sequences are eliminated before the telomeric repeat is added to the fragment ends (39, 42).

Recently, Yao et al. (43) have shown that sequences expected to be internal to the subchromosomal fragments can be eliminated by interstitial deletion. One clone of MIC DNA was found to contain three eliminated sequences, at least two of which are eliminated by breakage and rejoining of the flanking sequences. This type of deletion has been shown to occur in the vicinity of the tubulin gene (13) and also for internal MIC DNA regions containing C_4A_2 repeats (44).

Elimination of DNA sequences occurs as a result of both fragmentation and interstitial deletion in *Tetrahymena*. The process appears to be tightly controlled; however, for the few completely eliminated regions studied, some appeared to be generated in alternative ways (8, 20) and some young MAC preparations contained sequences that had not undergone deletions (43).

The hypotrichous ciliates also eliminate DNA sequences during MAC development (30). In the hypotrichs, the MAC consists of gene-sized pieces of DNA that are produced by fragmentation of the MIC chromosomes. Ninety-five percent of the MIC genome is eliminated during this fragmentation process (30). In addition, Klobutcher et al. (21) have recently identified sequences in *Oxytricha nova* that are eliminated from regions of DNA destined to become MAC fragments. These internal eliminated sequences (IES) appear to be deletions similar to those observed in *Tetrahymena* by Yao et al. (43) although the IES are much smaller.

Variability was not observed for the IES sequences although the situation is complicated because there are at least three versions of the gene in the MIC genome. Recently, Cartinhour & Herrick (14) found in the MAC of *Oxytricha fallax* a common block of DNA sequences that occurs on several different gene-sized pieces. These sequences are either different members of a

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small family of sequences or they represent alternate ways of processing a single MIC sequence. Alternative DNA rearrangements are part of several biological systems including immunoglobin switching in mammals (34), mating type interconversion in yeast (26), and antigen switchng in trypanosomes (10).

We have recently studied three eliminated families, two of which have sequences that persist in the MAC (37). These facultatively persistent sequences may persist because of weak or less accessible signals for processing at sites of elimination (36). In the present study, we have isolated a clone of MAC DNA from a cell line that contains a persistent member of a repetitive family that is eliminated from the MAC DNA of other cell lines, as well as sequences normally retained in the MAC. The latter sequences were subcloned. The MAC DNA clone and its subclone were used to discern how a single MIC segment is alternatively arranged in the MAC of six different vegetatively aged cell lines and in the MAC of the four caryonides from one mating pair. In two cases, two DNA arrangements were observed in the MAC DNA of one caryonide, and this epigenetically induced heterozygosity was resolved by the process of phenotypic assortment. These alternative arrangements may reflect variations in the deletion mechanism or they may reflect other as yet uncharacterized DNA rearrangements that occur during MAC development.

MATERIALS AND METHODS

Cell lines. Table I lists the cell lines used in this study. The various relationships among inbred strains have been discussed previously (2, 4). Cell lines BX2 and BX6 are sister clones from one mating pair of round two of genomic exclusion of B-19755 crossed to A* III and were obtained from L. Jenkins (University of Iowa). The other B strain cell lines were derived by inbreeding clones of different mating type from the same mating pair. Cell lines B7N and B4 were obtained from D. L. Nanney (University of Illinois) and had been frozen from about 1968 to 1974. Cell line B7B is the same cell line as B7N except that it was never frozen and was obtained from P. J. Bruns (Cornell University). Cell lines B7 (N or B) and B4 are caryonides that differ in mating type and were randomly isolated from the same mating pair. The construction of strain D/1, congenic with strain D, has been described previously (3). All strains, except for BX2 and BX6, were originally constructed in this laboratory.

A set of caryonides (31A–31D) was isolated from a cross of D × D/1. Caryonides 31A and 31B are the sister caryonides from one exconjugant, and caryonides 31C and 31D are the sister caryonides of the other exconjugant. After 35 fissions, each caryonide was subcloned: single cells were randomly isolated from each of the four caryonidal populations. Three cell lines were used from one caryonide (31A) while only a single cell line was used from each of the other three caryonides. This first set of subclones was allowed to divide for 40 more fissions or a total of 75 fissions. Then, a second set of subclones was made by cloning the first set of subclones; single cells were isolated from one of the descendant populations of three of the caryonides. Three cell lines were used from the 31A descendant while only one cell line was used from each of the descendants of 31B and 31D. This second set of subclones was further cloned after 40 more fissions or a total of 115 fissions. Single cells were isolated from one of the descendants of 31A, 31B, and 31D—three cell lines being used for the 31A lineage and one cell line for each of the 31B and 31D lineages. All three sets of subclones are listed in Table I and labeled to illustrate how they descended from the original set of caryonides.

The vegetatively aged, or "old," cell lines were also obtained from a cross of D × D/1. From this cross, made at the end of 1975 (A75-163), individual mating pairs were placed into separate wells of depression slides, and caryonides were randomly isolated. Serial cloning was carried out for each cell line; every 13 fissions, a single cell was isolated and allowed to divide. After ten serial isolations, the cell line was placed in tube culture. The resulting cell lines were subcultured bimothly at 23°C (1976–1978) and then monthly (1979–1985) at 16°C and are now about 1000 fissions old.

TABLE I. Tetrahymena thermophila *cell lines used in this study.*

Type of strain	Designation of lineage (names in parentheses are used in text)	
Inbred strains	A-17686 (A)	B-18684 (B4)
	B-2079X2 (BX2)	B-18684b (Bb)
	B-2079X6 (BX6)	C2-4683 (C2)
	B-18687N (B7N)	C3-3685 (C3)
	B-18687B (B7B)	D-25772a* (D)
		D/1(I_1)-5771* (D/1)
Carvonides from a cross D × D/1	82-2-31A (31A)	
	82-2-31B (31B)	
	82-2-31C (31C)	
	82-2-31D (31D)	
Subclones of caryonides (first subcloning at 35 fissions)	31A-1	31B-2
	31A-2	31C-2
	31A-3	31D-2
Subclones of caryonides (second subcloning at 75 fissions)	31A-1-a	31B-2-a
	31A-1-b	
	31A-1-c	31D-2-a
Subclones of caryonides (third subcloning at 115 fissions)	31A-1-a-4	31B-2-a-7
	31A-1-a-5	
	31A-1-a-6	31D-2-a-8
Old cell lines from a cross D × D/1 (approximately 1000 fissions old)	75-163-3C (3C)	75-163-19A (19A)
	75-163-14C (14C)	75-163-20B (20B)
	75-163-18A (18A)	75-163-21C (21C)
Young population	DI21481 (DI)	

* Cultures of these two strains were obtained from frozen samples (ATCC 30845 and ATCC 30847, respectively) stored at the American Type Culture Collection.

The "young population" was derived from a cross of D × D/1 and is labeled DI (4, 5, 37). The young population refers to cells obtained from a population of exconjugant cells that had been purified from non-mating cells by magnetic columns (12).

Growth of cultures, nuclear isolations, and DNA preparations. Cultures are maintained at 16°C in 1% proteose peptone and are now subcultured monthly. Growth of cultures for nuclear preparations has been described (6). Macronuclei were purified by the use of repeated Percoll gradients until the nuclear cross contamination was less than 1 MIC in 200 MACs. Micronuclei were purified by the use of repeated Percoll gradients until the nuclear cross contamination was less than 1 MAC in 2000 MICs (6). After nuclear isolation, DNA was purified by CsCl gradient centrifugation as already outlined (6). Nuclear DNAs were routinely screened for plasmid contamination by probing genomic blots with radioactive pBR322 (see below). In addition, all glassware and solutions used for preparing nuclear DNAs were kept separate from those used for plasmid preparation.

Genomic "library" construction and colony hybridization. A partial genomic library was constructed using MAC DNA from cell line 14C completely digested with Mbo I. The fragments were separated by electrophoresis through 1% low-melting-point agarose and sized using appropriate markers. The region of the gel that contained DNA fragments of 2.5–3.0 kb was cut out of the gel. The low-melting-point agarose was removed from the

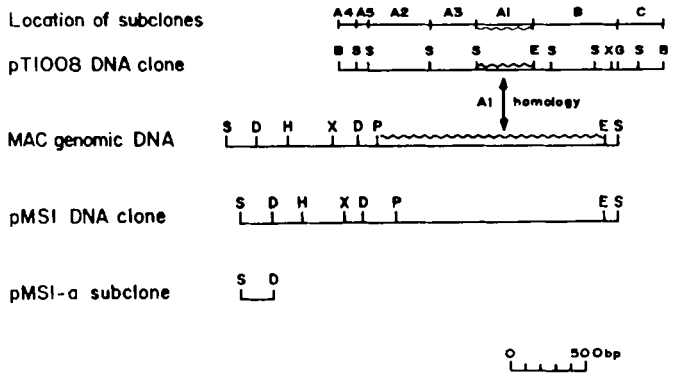

Fig. 1. Restriction map of the MIC DNA clone, pT1008, and the MAC DNA clone, pMS1. The location of the subclones of pT1008 is shown on the top line. Clone pMS1 contains a 2.6-kb MAC fragment obtained from old cell line 14C. Above the map of pMS1 is shown the restriction map of the 2.6-kb MAC fragment from 14C constructed using genomic blots. The restriction maps of pMS1 and pT1008 were constructed by direct analysis of the plasmids. The restriction sites are designated as follows: B = Bam HI, D = Hind III, E = Eco RI, G = Bgl II, H = Hpa I, P = Pvu II, S = Sau 3A and Mbo I, and X = Xba I. Within the region of the MAC sequence which is shown as a *wavy line*, there is homology to the A1 subclone of pT1008.

sample with Elutip-d's (Schleicher and Schuell, Inc.) as described (32), with modifications. The DNA was ligated into the Bam HI site of pBR322. The 5′ phosphates of the linearized plasmid were removed with calf intestinal alkaline phosphatase (Boehringer, Mannheim) to prevent recircularization of the plasmid. The ligation mixture was transformed into *E. coli* (HB101) and ampicillin-resistant transformants were selected. These transformants were screened by a modified colony-hybridization procedure (17). The probe used to screen the library was a nick-translated insert from D × D/1 MIC DNA clone pT1008, isolated from low-melting-point agarose gels as described above. Potential colonies were selected and rescreened.

Plasmid preparation and nick translation. The plasmids that were used in this study include pT1008 and two of its subclones, pT1008-A1 and pT1008-B (see Fig. 1 for a map of pT1008 and the location of the subclones). The two subclones are referred to in the text as "A1" and "B". Plasmid pT1008 contains MIC DNA derived from the progeny of a cross of congenic strains D and D/1. It was selected for its smaller size from a group of clones that gave an intense reaction during colony hybridization to a probe of total MIC DNA but a minimal reaction when probed with total MAC DNA (37). To prepare subclones, subfragments were cut out of pT1008 by employing the enzymes indicated in Fig. 1. Plasmid DNA was isolated by a modification of the SDS/high-salt-cleared lysate procedure (18), followed by CsCl/ethidium bromide gradient centrifugation in a vertical rotor. The DNA was labeled in vitro with α ^{32}P dATP by a modified nick translation procedure routinely giving about 10^8 cpm/μg (23, 31). Unincorporated radioactive nucleotides were removed by chromatography through Sephadex G-50.

Restriction enzyme digestion and gel electrophoresis. Digestions using ten-fold excess of enzyme were carried out by the method of Maniatis et al. (22) using restriction enzymes purchased from Bethesda Research Laboratories, New England Biolabs, or Amersham. Submarine gel electrophoresis was carried out with 12-inch-long gels and a Tris-borate buffer system containing ethidium bromide (19, 24, 29). As size markers, either ϕX 174 DNA digested with Hind II or λ DNA digested with Ava I and Bgl II was used. The λ DNA digested with Ava I and Bgl II generates visible fragments of the following sizes (in kb): 14.9, 8.8, 5.5, 4.3, 3.7, 2.44, 2.23, 1.93, 1.64, 1.44, 1.07, 0.54, and 0.48. Dashes in all figures are estimates of size and are derived from standard curves using the above size markers.

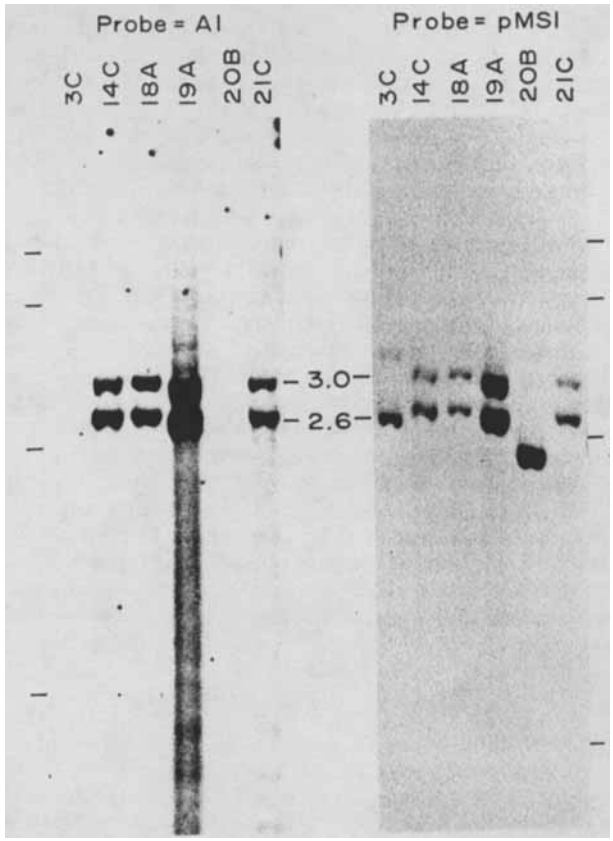

Fig. 2. Genomic blots of MAC DNAs digested with Mbo I. Each lane contains 5 μg of MAC DNA from one of the six old cell lines, 3C, 14C, 18A, 19A, 20B, and 21C. One set of blots was probed with DNA subclone A1. The other set of blots was probed with pMS1. The 3.0- and 2.6-kb fragments are the methylated and unmethylated versions of the same MIC segment. The dashes with each blot represent 7.5, 5.0, 2.5, and 1.0 kb.

Genomic blotting and hybridization. The DNA was transferred from the gels to nitrocellulose filters by the method of Southern (33), with modifications. Prehybridization, hybridization, and washing were all done at 62°C. These conditions were chosen due to the 25% GC content of *Tetrahymena* DNA (1). The filters were presoaked in 3× SSC for ½ h (SSC = 0.15 M NaCl, 0.015 M sodium citrate) and then prehybridized for 5 h in 10× Denhardt's solution (15) with 3× SSC, 0.1% SDS, and 25 μg/ml of denatured calf thymus DNA. Then the prehybridization mixture was replaced with fresh, denatured prehybridization and probe mixture (1–2 × 10^6 cpm/ml), and hybridization was continued for at least 36 h. Filters the size of a full-sized gel received 4 × 10^7 cpm. The filters were washed six times in 2× SSC, 1% SDS for 20 min, then once in 0.2× SSC, 1% SDS for 30 min, and finally in 2× SSC for 10 min. The filters were dried for 2 h and exposed to Kodak XAR-5 film at −80°C with an intensifying screen. The length of exposure depended on the amount of radioactivity on the filter.

RESULTS

A persistent sequence present in some cell lines. We have previously identified the repetitive family, C-B-A1, which is present

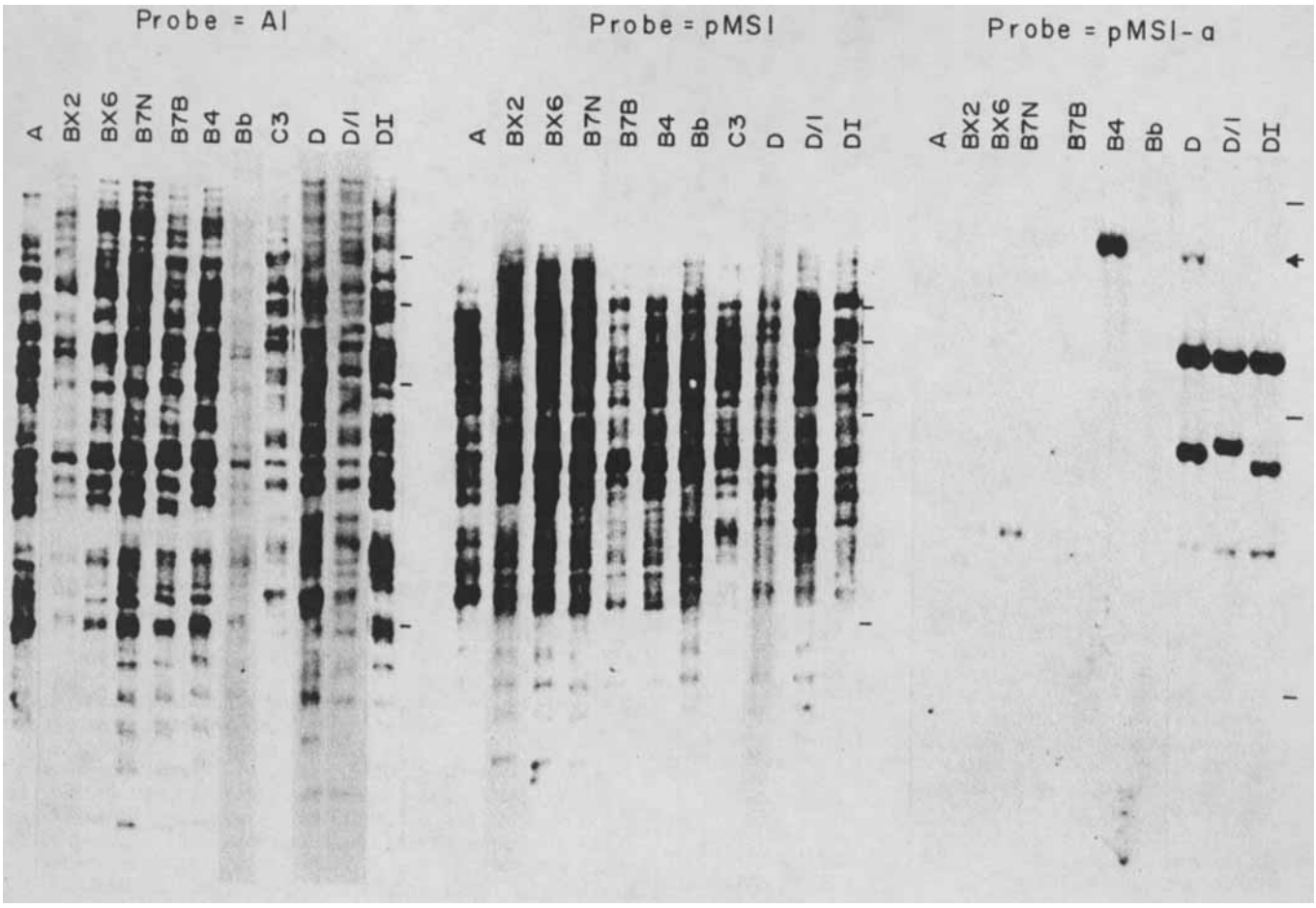

Fig. 3. Genomic blots of MIC DNAs digested with Hind III. Each lane contains 5 μg of Hind III-digested MIC DNA from the cell lines of the inbred strains as labeled. One set of blots was probed with A1; one set was probed with pMS1, and one set was probed with 1-a. The dashes on the right of the left and middle blots represent 7.5, 5.0, 2.5, and 1.0 kb; the dashes on the far right of the 1-a blot represent 10.0, 5.0, and 1.0 kb. The high molecular weight band in B4 and D (arrowhead) and in B7N (very faint) hybridizes to pBR322 and is thus apparently due to low levels of contamination by a plasmid related to pBR322. *No other DNAs used in this study were contaminated.* The autoradiographs of the genomic blots probed with A1 and pMS1 were exposed for 16 h while the autoradiograph of the genomic blot probed with 1-a was exposed for seven days.

in approximately 300 copies in the MIC genome and is almost completely eliminated from the MAC genome (36, 37). This family was identified by its homology to DNA subclones C, B, and A1 from a clone of MIC DNA, pT1008 (see the top two lines of Fig. 1 for a map of pT1008 and the location of the subclones). We have found that sequences that hybridize to DNA subclone C are completely eliminated while three fragments that hybridize to subclone B are present very faintly in all MACs. Other sequences that hybridize to subclone B, as well as sequences hybridizing to subclone A1, are well represented in the MAC of some of the cell lines tested but are completely absent from others (see Table I for the cell lines used in this study). For instance, DNA subclone A1 hybridized to two fragments (3.0 and 2.6 kb) in genomic blots of Mbo I-digested MAC DNA from four vegetatively aged cell lines of a hybrid strain that was produced by crossing congenic strains D and D/1, but did not hybridize to any MAC fragments in two other "old" cell lines of the same strain (Fig. 2). The DNA subclone B also hybridized to the 3.0-kb fragment (data not shown). The 3.0-kb fragment is the methylated version and the 2.6-kb fragment the unmethylated version of the same MIC DNA segment in these MAC DNAs (unpubl. observ.). Using Mbo I, Sau 3A, and Dpn I, we could show that methylation occurs on both strands of DNA at this site but that only half of the DNA molecules are methylated. Since the 2.6- and 3.0-kb fragments are only present in the MAC of four of the six old cell lines, it was of interest to clone one of the MAC fragments to investigate the region of homology to the C-B-A1 family as well as any flanking sequences that may exist on the fragment.

Isolation of a clone containing the MAC sequence. An incomplete genomic library containing Mbo I fragments ranging in size between 2.5 and 3.0 kb of MAC DNA from cell line 14C was screened with the insert of pT1008. A clone, pMS1, was isolated from this library on the basis of its homology to pT1008.

Several criteria were used to confirm that the insert corresponded to the 2.6-kb fragment. First, a restriction map of the insert was made and compared to a restriction map of the 2.6-kb fragment in the MAC (Fig. 1) that had been constructed by digesting MAC DNA with Mbo I and another enzyme, blotting it, and probing with pT1008. Not only are the correct restriction sites present in pMS1, but no restriction sites are present on the clone that were not predicted from genomic blot analysis of the

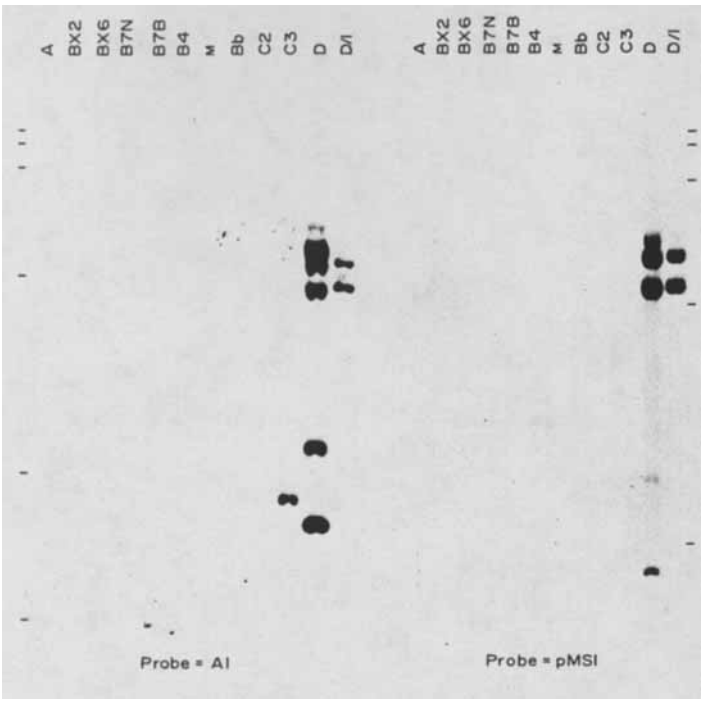

Fig. 4. Genomic blots of MAC DNAs digested with Mbo I. Each lane contains 5 μg of Mbo I-digested MAC DNA from the cell lines of the inbred strains as labeled. One set of blots was probed with A1; one set was probed with pMS1. The dashes with each blot represent 10.0, 7.5, 5.0, 2.5, and 1.0 kb. Lane M contains marker DNA.

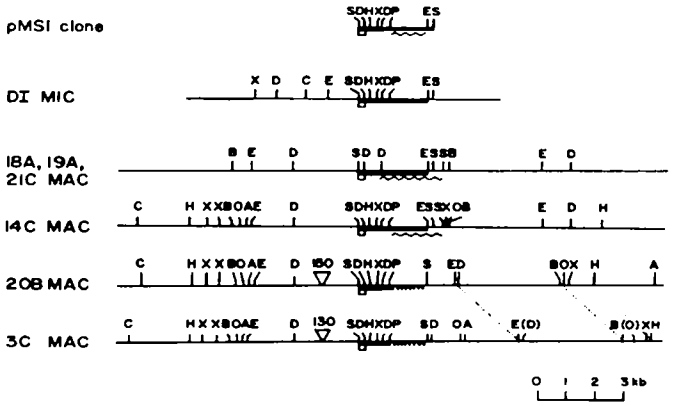

Fig. 5. Restriction map of a genomic region in the MIC, six different MACs, and the clone pMS1. MIC and MAC restriction maps were constructed using genomic blots probed with pMS1, 1-a, and A1; pMS1 was mapped by direct analysis of the plasmid. The restriction sites which were identified are designated as follows: A = Ava I, B = Bgl II, C = Cla I, D = Hind III, E = Eco RI, H = Hpa I, O = Xho I, P = Pvu II, S = Sau 3A and Mbo I, and X = Xba I. The *heavy line* in the restriction maps represents sequences with homology to pMS1. The *dotted line* represents regions in which the homology to pMS1 is not complete. The *wavy lines* represent regions that contain homology to subclones A1 or B of pT1008. The *boxes* below the maps represent the region of homology to 1-a. The restriction maps of the MAC from 18A, 19A, and 21C were not constructed with as many restriction enzymes as the map of the MAC of 14C; however, all sites that were identified in these three MACs were also found in 14C. The regions to the left of the cloned region vary slightly in the MACs of 3C and 20B. These variations are shown as insertions (not drawn to scale) between the Hind III and Sau 3A sites in 20B and 3C. Except for these insertions, the left flanks of all MACs are the same, but they differ from the left flank of the MIC. Therefore, not only does rearrangement occur, but it is exceedingly precise. The right flanks of the MACs of the six cell lines vary extensively. Since the right flank of the MIC cannot be mapped with the present probes, we do not know if similarity to one of the MAC maps occurs. Thus, rearrangement occurs, but it may not always take place.

2.6-kb fragment. Second, the clone hybridized to the A fragment of pT1008, which contains A1, but not to the B or C fragments of pT1008. Third, pMS1 hybridized to the Mbo I 2.6- and 3.0-kb fragments in the MAC of the four cell lines known to contain these fragments (Fig. 2).

pMS1 contains a member of the C-B-A1 family. To prove that pMS1 contains a member of the C-B-A1 family, pMS1 was used as a probe against MIC and MAC DNA. Genomic blots of Hind III-digested MIC DNA from several inbred strains were probed with pMS1 or A1 (Fig. 3). Both A1 and pMS1 hybridized to many fragments in the MIC of all the inbred strains that were tested. Moreover, the banding patterns of A1 and pMS1 were very similar.

Genomic blots of Mbo I-digested MAC DNA from several inbred strains were probed with A1 and pMS1, and the banding patterns were compared (Fig. 4). Neither probe hybridized to any fragments in the MAC of cell lines from inbred strains A, B, or C2. Both A1 and pMS1 hybridized to C3, D, and D/1 although the banding patterns of the two probes were similar, but not identical. The signal given by the smaller fragments was weaker for pMS1 than for A1, suggesting that pMS1 does not contain 100% of the sequences found in A1.

When pMS1 was used to probe genomic blots of MAC DNA digested with Mbo I from the six old cell lines (Fig. 2), the clone hybridized to the 2.6- and 3.0-kb fragments present in the MAC of four of the old cell lines, as expected. In addition, it hybridized to new fragments in the MAC of the other two cell lines. The two new fragments in cell line 3C and the major new fragment in cell line 20B must be homologous to sequences in pMS1 other than the A1 sequence since the A1 sequence did not hybridize to MAC DNA from these two cell lines. The presence of these new fragments suggests that pMS1 contains flanking sequences that are normally present in the MAC.

Sequences that flank this C-B-A1 member. The homology of pMS1 to A1 was predicted to lie between the Pvu II and Eco RI sites from the mapping experiments carried out on the 2.6-kb MAC fragment using pT1008 as probe (Fig. 1). To isolate possible flanking sequences, the 300-bp fragment between the Mbo I (Sau 3A) and Hind III site on the left was subcloned. This clone, pMS1-a (referred to as 1-a) does not hybridize to pT1008. Clone 1-a was used as a probe against genomic blots of various MIC and MAC DNAs. When genomic blots of Hind III-digested MIC DNAs from various inbred strains were probed with DNA subclone 1-a (Fig. 3), 1-a hybridized to the genomes of most all of the cell lines tested. In these lines, the hybridization pattern consisted of only a few fragments even after exposure of the autoradiograms for a week, instead of many fragments as was the case for the C-B-A1 sequence family after exposure for a few hours.

The DNA subclone 1-a was also used as a probe against genomic blots of Mbo I-digested MAC DNA from the old cell lines. The subclone gave identical results to those of the clone pMS1 already shown in Fig. 2.

Restriction maps of the MAC sequence in six old cell lines. Genomic blots of MAC DNAs from the old cell lines were digested with various combinations of restriction enzymes and probed with pMS1 or 1-a. These genomic blots were used to construct detailed restriction maps of the MAC sequence from the old cell line 14C, which contains the 2.6-kb Mbo I fragment

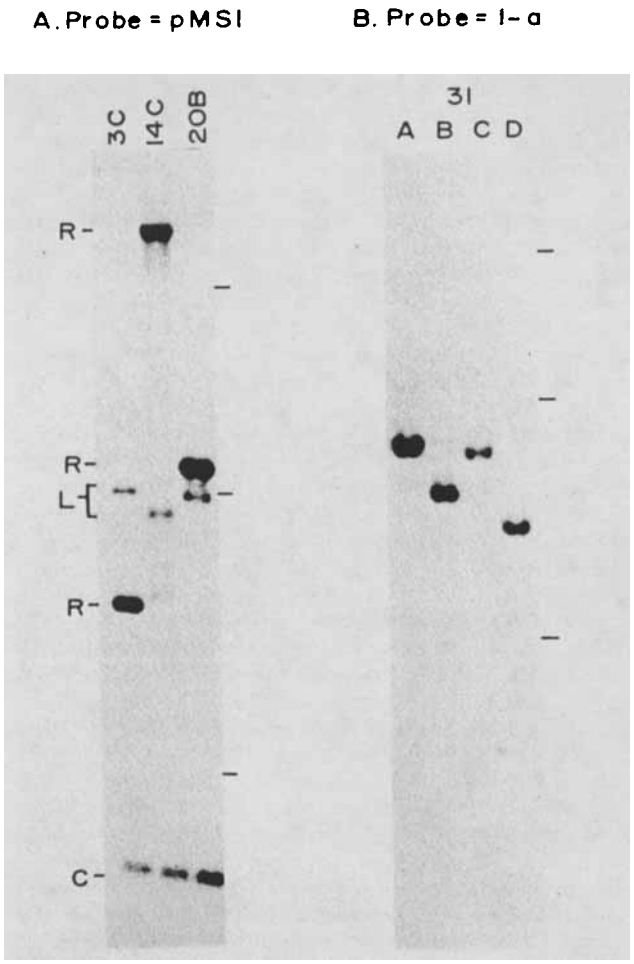

Fig. 6. Genomic blots of Hind III-digested MAC DNA. A. MAC DNAs probed with pMS1. Each lane contains 5 μg of MAC DNA from 3C, 14C, or 20B. The 0.6-kb band from the cloned region is labeled C. The bands that extend to the left of the cloned region are labeled L. The bands that extend to the right of the cloned region are labeled R. The dashes on the right represent 5.0, 2.5, and 1.0 kb. B. Genomic blots of the caryonide set 31A-D probed with 1-a. Each lane contains 5 μg of MAC DNA. The dashes on the right represent 5.0, 2.5, and 1.0 kb.

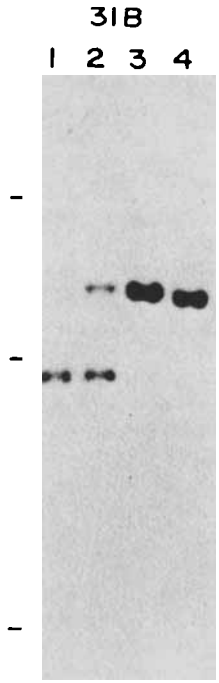

Fig. 7. Genomic blots of the 31B caryonidal subclones probed with DNA subclone 1-a. Each lane contained 5 μg of MAC DNA digested with Hind III. 1 = 31B, 2 = 31B-2, 3 = 31B-2-a, 4 = 31B-2-a-7. The dashes to the left of each blot represent 3.0, 2.0 and 1.0 kb.

and its methylated version, the 3.0-kb Mbo I fragment, as well as the MAC sequences from 3C and 20B, which do not contain the 2.6- or 3.0-kb fragments (Fig. 5). Three other old cell lines (18A, 19A, and 21C) also contain the 2.6- and 3.0-kb fragments in their MAC DNAs. These MAC DNAs were not mapped as completely as that from 14C; however, the restriction sites that were mapped are identical in position to those of 14C, suggesting that these four old cell lines, all of which have the 2.6- and 3.0-kb fragments, have identical restriction maps for the MAC sequence represented by pMS1.

Both pMS1 and 1-a were also used to construct the restriction maps of the MAC sequences in cell lines 3C and 20B. Clone pMS1 can be used in this analysis because it contains sequences that are not part of 1-a, yet its homology to A1 does not interfere with the mapping since A1 does not hybridize to these MAC DNAs. The sequences of pMS1 help to extend the restriction map to the right as drawn in Fig. 5.

The detailed restriction maps of cell lines 14C, 3B, and 20B show that the three MAC sequences are identical within the 1 kb of MAC sequence that is part of the cloned sequence, pMS1, between the Sau 3A and Pvu II sites. The restriction sites to the left of the cloned region vary slightly in their distances from the cloned region in different MACs. The restriction sites to the right of the cloned region vary a great deal. All three of these features are apparent when genomic blots of Hind III-digested MAC DNAs are probed with pMS1. The 0.6-kb band (labeled C in Fig. 6A) is from the cloned region and is the same size in all three MAC DNAs. The 2.2–2.35-kb bands, which vary between MAC DNAs (labeled L in Fig. 6A), represent the region to the left of the cloned region while the most intense bands (labeled R in Fig. 6A) vary a great deal and represent the region to the right of the cloned region.

Caryonidal inheritance of the MAC sequence. Because of the different arrangements surrounding this cloned sequence in different MAC DNAs, it was of interest to determine (a) if rearrangements occur early in the life cycle; (b) if they are genetically or epigenetically determined; and (c) how much variation occurs in the rearrangement process. The MAC DNAs from a set of four caryonides (31A–31D) from a cross of congenic strains D and D/1 were digested with Hind III and probed with 1-a (Fig. 6B). Each of the caryonides has a major fragment that hybridizes to 1-a, with three different sizes observed among the caryonides. Minor fragments hybridizing to 1-a are also present in 31A, 31B, and 31D, some of which appear to be "real" and are not partial digestion products since they appear in replicate gels using different digestions and are located in positions where major fragments appear in descendant cell lineages. Caryonidal subclones were also prepared for each of the four caryonides and genomic blots of Hind III-digested MAC DNA from these subclones were probed with 1-a. The major fragment of 31A was present in all nine subclones examined and the major fragment of 31C was present in the one subclone that was available

(data not shown); however, the MAC patterns of the subclones from 31B and 31D show variation. The main fragment in 31B is lost from the third and fourth caryonidal subclones and a minor species in 31B becomes more intense in successive subclones and is the only detectable band in 31B-2-a (lane 3) and 31B-2-a-7 (lane 4, Fig. 7). The caryonidal subclones of 31D show a major fragment which is the same size in 31D, 31D-2-a, and 31D-2-a-8 while in 31D-2 a different major fragment is present that is similar in size to a minor fragment present in 31D (data not shown). Similar results were obtained when the DNAs were digested with Eco RI (data not shown).

Restriction map of one MIC sequence. To determine the DNA arrangement of the MAC-cloned sequence, pMS1, in the MIC of the hybrid strain that was produced by crossing congenic strains D and D/1 (labeled DI in Table I), a restriction map was constructed of the MIC sequence that gives the most intense reaction when probed with 1-a (Fig. 3). Although cross-hybridizing bands were present, they were much less intense than those we were following and did not interfere with their mapping. The results of this mapping are shown in Fig. 5. Within the cloned region, the map of the MIC is identical to the restriction map of pMS1 and to the MAC sequence from 14C. The restriction map on the left of the cloned region in the MIC is, however, completely different from the MAC restriction maps for at least 3.5 kb. The restriction map on the right of the cloned region in the MIC is not complete because pMS1 can not be used as a probe against MIC DNA since it has homology to the C-B-A1 family, which is present in approximately 300 copies in the MIC (37).

DISCUSSION

In this study we have investigated a region of the MIC genome containing a facultatively persistent sequence that is retained in the MAC of some cell lines but eliminated from the MAC of other cell lines. A cloned MAC fragment from this region contains a member of the C-B-A1 repetitive family that persists, as well as flanking sequences. Restriction maps of the MAC region from cell lines with and without the persistent sequence were constructed using this clone and a subclone. The restriction maps show a large amount of variation in the flanks of this region, suggesting that there are alternative ways of processing this region during MAC development. Each DNA arrangement appears to be determined epigenetically as the MAC develops. A MAC can also develop which contains more than one type of DNA arrangement in the 45 copies of this MIC segment. This epigenetically induced heterogeneity is then resolved by the process of phenotypic assortment.

A facultatively persistent sequence from the C-B-A1 family that is retained in the MAC DNA of some cell lines occurs on a 2.6-kb Mbo I fragment, or a 3.0-kb Mbo I fragment when methylated. The 2.6-kb fragment is present in clone pMS1 and consists of sequences with homology to A1 as well as flanking sequences. This has been verified by several criteria including restriction mapping, hybridization to the DNA clone pT1008, which contains a member of the C-B-A1 family, and hybridization to genomic blots of MIC and MAC DNAs. The hybridization patterns of MIC DNA from various inbred strains were very similar when probed with DNA subclone A1 or pMS1. The two probes also gave a similar response when hybridized to genomic blots of the MAC DNAs from the same strains. The similarities in patterns suggest that pMS1 contains a sequence with homology to the C-B-A1 family. The absence of hybridization of pMS1 to the MAC DNAs of the cell lines of inbred strains A and B that were investigated suggests that the flanking sequences within pMS1 are absent from these strains in the MAC.

The member of the C-B-A1 family that is present in the MAC of cell line 14C has several restriction sites in common with the member of the C-B-A1 family that is part of pT1008.The restriction sites starting at the Eco RI site and extending to the right are very similar to the restriction sites within DNA subclone B of pT1008. Subclone B does not hybridize to the 2.6-kb Mbo I fragment; however, it does hybridize to the 3.0-kb Mbo I fragment which is the 2.6-kb fragment in its methylated state (unpubl. observ.). The presence of restriction sites and homologous sequences from DNA subclone B suggests that the sequence that persists in the MAC is a member of the C-B-A1 family, which includes both the A1 and B sections of the family.

Subclone 1-a was derived from pMS1 and contains a sequence that flanks the persistent sequence. There are MIC-specific copies present in the cell lines of most all inbred strains tested; however, another copy of the 1-a sequence is present only in inbred strain D and its derivatives and is retained in the MAC of these same cell lines. It is this MAC sequence that varies as to whether it is associated with the A1 persistent sequence.

Several results suggest that the 1-a region is present in a single copy per genomic equivalent in the MAC that contains 45 genomic equivalents. The genomic blots that were used to construct the restriction maps of the MAC region did not show band heterogeneity in any of the MAC DNAs investigated. This lack of heterogeneity allowed us to map the restriction sites for 10 kb in each direction and suggests that a single region was being mapped. Furthermore, the alternative DNA arrangements in the MAC DNAs of these cell lines suggest that the region is present in the MAC in a single copy. Otherwise, the DNA rearrangement in two or more regions would have to be coordinated so that the same arrangement occurs in every copy of the region. Given the variability of these DNA arrangements, this seems unlikely.

The restriction map of the MAC region that is homologous to 1-a is identical in the four cell lines that contain the A1 sequence. This map is also identical to the maps of the MAC region in the two cell lines that do not contain A1 in the vicinity of 1-a on the left end of pMS1 and to the left of the cloned fragment, except for the small insertions between the Mbo I and Hind III sites. To the right of the cloned fragment the MAC restriction maps of these two cell lines differ greatly from those of the cell lines that have the A1 persistent sequence; moreover, they differ from each other by the addition of a 2.2-kb segment in one of the two lines.

The restriction map of the MIC sequence with the most intense reaction to 1-a is identical, not only to the restriction map of DNA subclone 1-a but also to the entire restriction map of the clone, pMS1, and to the corresponding MAC sequence from cell line 14C. This similarity suggests that this sequence is indeed the MIC progenitor of the MAC region. It also implies that the A1 persistent sequence which is present in the MAC of 14C is also adjacent to the 1-a region in the MIC and that no rearrangement has occurred within the 1a-A1 region as the MAC developed in cell lines 14C, 18A, 19A, and 21C; however, rearrangement has occurred to the left of the 1-a region during MAC development. Except for the small insertions in two of the cell lines, the maps of the left flanks of all the MACs are the same, but they differ from the map of the left flank of the MIC; at least 3.5 kb of MIC DNA appears to have been replaced with other genomic sequences. Furthermore, the site of alteration appears to be located between the Mbo I and Hind III sites just to the left of the pMS1-like region. This is the same location where the small insertions occur. Thus, not only do rearrangements occur on the left, but they are relatively precise and can be localized to a specific region of MIC DNA. The right flanks of the MACs of the six cell lines vary extensively but, since the

right flank of the MIC cannot be mapped with the present probes, we cannot tell whether there is similarity to one of the MAC maps. Thus, rearrangement occurs on the right flank, but it may not always take place.

Since the 1-a region is present in single copy in the MAC, examining caryonides allows us to ask if rearrangements occur early in the life cycle and how much variation occurs in the rearrangement process. When probed with 1-a, a different-sized Hind III fragment is seen in at least three of the MACs from a set of four genetically identical caryonides from a D × D/1 cross. Thus, rearrangements must occur during development of the MAC or within the 15–20 fissions post-conjugation needed to produce a sufficiently large population of cells for preparing MAC DNA. The differences in size of the fragments seen suggests that there is epigenetic variation in the process of rearrangement.

The extent of variation in the rearrangement process can be further assessed by sampling descendant caryonidal lineages. Some of these lineages showed polymorphisms—fragments of more than one size, which varied quantitatively. These polymorphisms probably result from different DNA arrangements occurring in the 45 copies of the 1-a MIC segment within one developing MAC and are resolved by phenotypic assortment during vegetative growth following conjugation. In the analysis of the genomic blots of the caryonidal subclones, a fragment is lost when none of the 45 copies of this segment has the fragment. A fragment is stable when all 45 copies of this segment contain the same fragment. Caryonide 31C and its subclone both appear to have one stable DNA arrangement for the sequence when it persists in the MAC. Caryonides 31A, 31B, and 31D contain several DNA arrangements; however, all but one DNA arrangement is lost during serial subcloning, and the remaining DNA arrangement appears to be stable. Analysis of the caryonides also suggests that more than two alternatives (DNA arrangements) can be phenotypically assorted since three to four different sized fragments are stabilized in different cell lines. This has previously been shown genetically by Nanney & Preparata (25) for triploids. It also occurs in diploids for the mating type locus, even in homozygotes, where as many as three types (and three DNA rearrangements) can emerge from a single MAC (28).

Alternative DNA arrangements in one region of the MAC genome demonstrate that a large amount of variation can occur during DNA elimination in MAC development. Several functions have been suggested for sequences that are eliminated during MAC formation in *Tetrahymena*. Either MIC sequences are eliminated so that genes can be activated in the MAC or these sequences represent MIC chromosomal functions involved in mitosis or meiosis that need to be removed or inactivated. The A1 family of sequences is facultatively persistent, meaning that a particular MAC sequence may, or may not, be present in those few cell lines in which persistence occurs. Thus, complete elimination does not appear to be required whatever the function of these sequences. For the 1a-A1 MIC segment considerable variation in the processing of these sequences during MAC development appears to be tolerated.

Alternative DNA arrangements in a genome are known to play important roles in immunoglobin switching in mammals (34), mating type determination in yeast (26), and surface antigen variation in trypanosomes (10). Mating type determination and surface antigen variation both occur in *Tetrahymena* although the molecular basis of these phenomena have not been studied in much detail; however, mating type determination in *Tetrahymena* has been likened to immunoglobin switching in mammals (27). Surface antigen variation has been studied at the molecular level in the related holotrich, *Paramecium*. It may involve DNA rearrangement (11) although Forney et al. (16) found that no rearrangement occurs within several kilobases of two of the genes coding for immobilization antigens in different serotypes. We do not know if the DNA rearrangements observed in this study are associated with mating type determination or surface antigen variation in *Tetrahymena*; however, it is clear that alternative processing of a single MIC segment does occur during MAC development and that this type of event should engender considerable variation in DNA content, arrangement, and function in the mature MAC.

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Cytoplasmic Inheritance of Temperature Sensitivity in a Wild Stock of *Paramecium primaurelia*[1]

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ABSTRACT. One stock, GBC, has a maximum temperature of growth about 5°C lower than other recently collected stocks of *Paramecium primaurelia*. The resistant stocks (R) are able to grow continuously at 35°C while the sensitive stock (S) cells die within 48 h. The F_1s of R × S crosses exhibited a cytoplasmic pattern of inheritance and all F_2-by-autogamy lines derived from the S cytoplasmic parent are sensitive. The F_2-by-autogamy lines cytoplasmically descended from the R parent were predominantly (93%) R in the initial assay. Upon reinvestigation one year later, only 64% of these lines were R, 9% were S, and 27% had a new phenotype, weak (W), intermediate between R and S. Backcrosses of W lines to both R and S strongly suggest that the W lines have normal cytoplasm (i.e. R) but also have nuclear gene(s) for temperature sensitivity that are derived from the original S stock. The delayed manifestation of the W phenotype is not understood.

TEMPERATURE is the environmental variable most often controlled and manipulated in biological laboratories. Induced mutations to temperature sensitivity and/or temperature-conditional phenotypic expression are abundant, usually recessive, and distributed among a very large number of genes. This laboratory experience and the obvious environmental heterogeneity in temperature might lead one to expect that temperature-tolerance variation would be common in wild stocks of a species. Genetic analysis of temperature-sensitive wild stocks in *Paramecium biaurelia* (12) and *P. tetraurelia* (6) found, in both cases, that a single recessive gene produced the temperature sensitivity. Overall, however, the variation within sibling species is low compared to the variation among species. In *Tetrahymena*, sibling species averages differed by as much as 8.0°C in tolerance to high temperature but the maximum difference between stocks within a species was 2.2°C (9). This pattern, lower

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