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Genetics

Molecular evidence for somatic recombination in the ribosomal DNA of *Tetrahymena thermophila*

(DNA amplification/differential replication/paromomycin resistance/restriction fragment length polymorphism/ciliate genetics)

ARNE LØVLIE*, BARBARA L. HALLER, AND EDUARDO ORIAS†

Department of Biological Sciences, University of California, Santa Barbara, CA 93106

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ABSTRACT **The ribosomal DNA (rDNA) in *Tetrahymena thermophila* is a 21-kilobase-pair palindromic DNA molecule that replicates autonomously in the macronucleus and is maintained at the level of about 10,000 copies per macronucleus. The rDNA of inbred strain C3 outreplicates the rDNA of inbred strain B in most B/C3 heterozygous macronuclei, generating macronuclei containing exclusively C3 rDNA sequences. In 1% or less of the B/C3 heterozygous macronuclei, however, rDNA sequences derived from both B and C3 strains persist in the macronucleus (co-maintainers). We report here that long-term culture of co-maintainers has yielded recombinant rDNA molecules combining sequences from both parental inbred strains. The genetic structure of such molecules also gives us virtual certainty that the differential replication of C3 rDNA with respect to B rDNA is due to the DNA sequence difference previously reported in domain 2 of the rDNA replication regions of the two strains.**

A unique characteristic of the ciliated protozoa is the separation of genetic function into two differentiated nuclei. The diploid micronucleus is not expressed and serves as the germline of the cell, while the polygenomic macronucleus serves as the somatic nucleus and determines the phenotype of the cell. The macronucleus differentiates from a mitotic sister of the micronucleus; in "higher" ciliates, such as *Tetrahymena*, this differentiation occurs only once in the life of the clone, as a postzygotic event in conjugation [reviewed by Orias (1)].

In *Tetrahymena*, the micronucleus contains five pairs of chromosomes; in contrast, the macronucleus contains about 300 acentric, autonomously replicating DNA molecular species, typically represented by 45 copies [reviewed by Karrer (2)]. The ribosomal DNA (rDNA, the rRNA gene) is the smallest known DNA molecule in the macronucleus. It is atypical in that there are about 10,000 copies per macronucleus. Structurally, it is a giant palindrome containing 21 kilobase pairs (kb) of DNA. The diploid micronucleus has a single copy of the rRNA gene integrated in the left arm of each chromosome 2. During macronuclear differentiation, the two copies are excised, made into palindromes, provided with telomeric repeats, and amplified to the level of 10,000 copies. During subsequent vegetative growth, macronuclear rDNA molecules replicate once per cell division on the average [reviewed by Yao (3)].

When progeny generated by crossing inbred strains B and C3 are tested after 50 fissions from conjugation, only the rDNA derived from strain C3 is detected in 99% of these heterozygotes (4). The origin of the rDNA is revealed by a restriction polymorphism at the 3′ nontranscribed spacer region of the molecule (Fig. 1). It is now known that both types of rDNA are comparably amplified at the time of macronuclear differentiation, and that the predominance of the C3 rDNA in most heterozygous macronuclei is due to a differential replication of C3 rDNA with respect to B rDNA during vegetative multiplication (6).

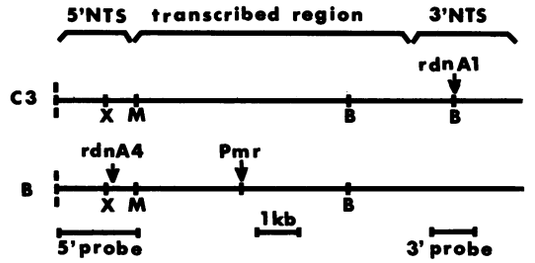

FIG. 1. Genetic and molecular maps of the rDNA in the heterozygotes generated by crossing clones of inbred strains B and C3. Only one arm of the palindrome is shown; vertical broken lines represent the palindrome center. NTS, nontranscribed spacers. Restriction sites: X, *Xba* I; M, *Msp* I; B, *Bam*HI. 5′ and 3′ probes: rDNA inserts in plasmids pTtr1 and p6J, respectively (5). *rdnA1*, Base-pair substitution resulting in an additional *Bam*HI restriction in C3 rDNA (4); *rdnA4*, 42-base-pair (bp) deletion in strain B compared to inbred strain C3 (6); *Pmr*, base-pair substitution conferring resistance to paromomycin (PM) (7). Probing *Xba* I/*Msp* I double digests with pTtr1 reveals distinct 0.8- and 0.76-kb bands for C3 and B rDNA, respectively. Probing *Bam*HI digests with p6J reveals distinct 2.5- and 3.9-kb fragments for C3 and B, respectively.

It was originally reported that, in about 1% of B/C3 heterozygous progeny, rRNA derived from both B and C3 parents could be detected (4). We call such clones "co-maintainers." In these clones, neither type of DNA showed any tendency to outreplicate the other. The lack of differential replication in co-maintainers suggested to us that they carry genetically altered rDNA molecules and raised the possibility of genetic recombination between B and C3 rDNA molecules.

The objective of this study was to characterize co-maintainers and test the idea that they might be recombinants. To recover co-maintainers more efficiently, we have used *Pmr* as a selective marker for the B type of rDNA. *Pmr* is a dominant mutation that maps to the coding region of the 17S rRNA (7, 8). We report molecular evidence of recombination in every co-maintainer investigated and show that co-maintainers can also be induced by UV radiation. Furthermore, the data obtained permits us to conclude that the cis-acting determinant for differential replication is located in the central third of the palindrome and almost certainly corresponds to the DNA sequence difference previously detected at the 5′ NTS of the rDNA molecule (6).

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Abbreviations: rDNA, ribosomal RNA gene; NTS, nontranscribed spacer; PM, paromomycin; PM^S and PM^R, PM-sensitive and -resistant.

*Permanent address: Zoologiske Institut, University of Oslo, Blindern, Oslo 3, Norway.
†To whom reprint requests should be addressed.

MATERIALS AND METHODS

Strains. B/C3 heterozygotes that stably maintain B rDNA sequences (here called B-maintainers) are rare. To select for such clones, we crossed to one another cells of two strains, SB1915 and SB1934 (Table 1). *Chx* and *Mpr* markers were included to select against cells that had failed to mate or retained their old macronucleus and against true progeny cells that resulted from self-fertilization [cytogamy (1)].

Routine Methods. Routine genetic methods were as previously described (13, 14). PP210 medium consists of 2% (wt/vol) Proteose peptone (Difco), supplemented with 10 μM $FeCl_3$ and 250 μg/ml each of penicillin and streptomycin sulfate (Sigma). Unless otherwise specified, conventional molecular methods were used, essentially as described by Maniatis *et al.* (15) and Larson *et al.* (6). Whole-cell *Tetrahymena* DNA was prepared and analyzed as described in ref. 6.

Selection of B-Maintainers. The following generalized protocol was used for the generation and selection of stable B-maintainers. Strains SB1915 and SB1934 were separately grown overnight in PP210 medium. They were then washed in 10 mM Tris·HCl, pH 7.5, and left to starve overnight at approximately 2.5×10^5 cells per ml. Mating was induced by mixing starved cells of the two strains at a total concentration of 2×10^5 cells per ml. Under these conditions mating started within 30–60 min. Times stated throughout this paper in connection with treatments of conjugating cells or exconjugants refer to hr after mixing the starved cells.

Conjugating cultures were re-fed at various times, depending on the treatment, by the addition of an equal volume of PP210 medium. The re-fed cells were immediately distributed to microtiter plates (50 μl per well) at two different concentrations: 2.5 and 50 cells per well, to determine viability and to select B-maintainers, respectively.

After 1 day, at approximate age 6 fissions, 50 μl of 2× PM medium (PP210 medium with PM at 400 μg/ml) was added to the B-maintainer selection plates (final PM concentration, 200 μg/ml). At 3-day intervals, the plates were serially replicated to PP210 medium containing cycloheximide and 6-methylpurine (each at 15 μg/ml), to complete the elimination of parental cells and self-fertilized progeny. The B-maintainer selection plates were then replicated once more to PM medium (approximate age, 36 fissions) to select the relatively stable PM-resistant (PM^R) clones (B-maintainers).

The viability plates were treated identically with two exceptions: 2× cycloheximide medium was added at age 6 fissions instead of PM (final cycloheximide concentration, 15 μg/ml) and the second PM addition was also omitted. The fraction of cross-fertilized progeny and the fraction of PM^R clones were estimated from the fraction of wells without survivors (after 6-methylpurine selection for the viability plates, and after the second PM selection for the B-maintainer selection plates, respectively), using the Poisson distribution. At least 60% of the pairs gave rise to cross-fertilized progeny.

Stock cultures of clones selected by this protocol were passaged monthly, in stock test tubes. Clonal ages are expressed as the estimated number of binary fissions after conjugation.

Testing Co-Maintainer Stability. Cells from each of six co-maintainer clones were grown for more than 100 fissions in the presence of PM as follows. The stock culture was diluted 1:100 with PP210 medium, and distributed to 96-well plates, 50 μl per well. Next day, an equal volume of 2× PM medium was added to the wells. The survivors were then serially replicated in PM medium for at least 110 fissions. The PM^R cells (designated with the suffix R) from a randomly selected well were then transferred to stock tubes, from which (*i*) whole-cell DNA preparations were made and (*ii*) 48 single cells were isolated and their PM phenotypes were tested by replication to PM medium.

For comparison, co-maintainer cultures were grown for over 100 fissions without PM as follows. Twelve subclones were isolated from the stock culture and were serially replicated in 96-well plates for at least 110 fissions. At the end of that period, the cultures were tested for PM resistance by replication to PM-containing medium. With the exception of clone D26 (which gave only PM^R subcultures) the clones showed a majority of PM^S subcultures (average, 85%; range, 63–100%). From a randomly selected PM^S subclone (designated with the suffix S), (*i*) a whole-cell DNA preparation was made and (*ii*) 48–96 single-cell isolates were tested for their PM phenotypes.

Table 1. Strains used for selecting stable B-maintainers

Strain	Genotype	Phenotype
SB1915	*rdnA4 Pmr rdnA1⁺/rdnA4 Pmr rdnA1⁺; Chx/Chx*	CHX^S, PM^S
SB1934	*rdnA4⁺ Pmr⁺ rdnA1/rdnA4⁺ Pmr⁺ rdnA1; Mpr/Mpr*	$6MP^S$, PM^S

rdnA1, Allele of rDNA in inbred strain C3 having a *Bam*HI restriction site in the 3′ NTS, which is absent from the rDNA of strain B (4); *rdnA4*, 42-bp deletion (compared to inbred strain C3) in the 5′ NTS of strain B (6, 8); *Pmr*, dominant mutation within the coding region for the 17S rRNA, conferring resistance to PM (7, 9). A map of the rDNA mutation is shown in Fig. 1. *Chx* and *Mpr*, dominant mutations conferring resistance to cycloheximide (CHX) and 6-methylpurine (6MP), respectively (10, 11). Sensitive phenotypes are indicated by ^{S}. These two strains were constructed by exploiting "pronuclear fusion failure" (12) to ensure the complete micro- and macronuclear homozygosity. Both strains contain genomic DNA sequences derived from both strain B and strain C3, but they are unlikely to be isogenic.

RESULTS

Selection of B-Maintainers. Even though both B and C3 types of rDNA are initially amplified in B/C3 heterozygotes (6), the typical B/C3 heterozygote does not respond to selection for the B rDNA sequences when they contain the *Pmr* allele. Some *B-Pmr/C3-Pmr⁺* heterozygotes are at first phenotypically resistant to PM, but this resistance is transient, and has been lost by 35 fissions in at least 99% of the heterozygotes. This loss of resistance is due to differential replication of the C3 rDNA in the macronucleus, rather than differential multiplication of PM^S cells in the culture; such transiently resistant cells eventually die upon continuous subculturing in PM medium (data not shown). Death occurs even when heterozygotes are isolated directly into PM as early as the first postconjugation fission and serially subcultured in PM medium thereafter.

The small fraction (1% or less) of B/C3 clones that maintain PM resistance for at least 35 fissions are qualitatively more stable. We call these clones "B-maintainers." B-maintainers are efficiently selected by using the protocol described in *Materials and Methods*. On the assumption that at least some co-maintainers could be generated by recombination, we also used the B-maintainer selection protocol on UV-irradiated conjugating pairs obtained by mixing starved SB1915 and SB1934 cells (Fig. 2), since UV radiation is a universal recombinogenic agent. The data show a significant induction of B-maintainers by UV, with a peak (about 10-fold induction) when irradiated at 8 hr. This time corresponds to an early stage in the differentiation of the macronucleus anlage (reviewed in ref. 1). UV radiation is also a mutagenic agent; however, the treatment used was mild and, as will be seen below, macronuclear mutation (e.g., from *C-Pmr⁺* to *C-Pmr*) does not account for the generation of these B-maintainers.

Using a similar PM-selection protocol, we have found other agents that induce B-maintainers: $MnCl_2$, KCl, and the DNA polymerase α inhibitor aphidicolin (data not shown).

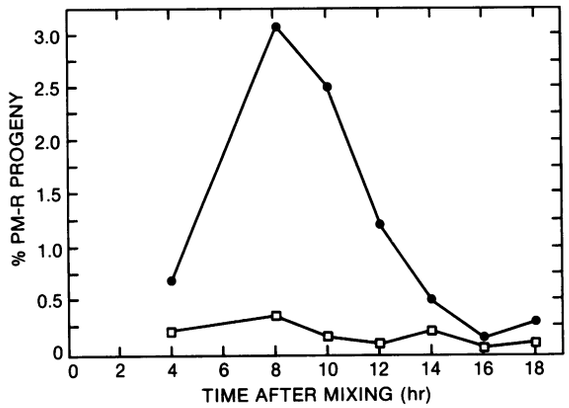

FIG. 2. Induction of B-maintainers by UV irradiation. ●, UV-irradiated; □, mock-treated controls. A 10-sec UV irradiation was given at the times indicated on the abscissa. Cells were prepared for crosses as indicated in *Materials and Methods*. Samples from the same pair of starved cultures were mixed at appropriate times so that they could be irradiated at essentially the same time. A Sylvania G15T8 germicidal lamp, located 362 mm above a shaking platform, with an interposed shutter assembly to control treatment time accurately, was used. After the treatment, 0.5-ml samples were removed to 15-cm test tubes and 0.5 ml of PP210 medium was added to each tube. The samples were then diluted, distributed to microtiter plates, and subsequently treated as indicated in *Materials and Methods*. All the manipulations surrounding the UV treatment were conducted in yellow light to avoid photoreactivation; after distribution, the 96-well plates were wrapped in aluminum foil for the subsequent 24 hr. Controls were treated in identical manner, except that the 0.5-ml samples were taken just before irradiating the companion sample. The irradiation caused 7% killing (i.e., 93% survival) averaged over the 4- to 18-hr samples.

Aphidicolin causes a peak of induction (50-fold) at 10 hr. In contrast, $MgCl_2$ decreases the frequency of B-maintainers (data not shown), whereas starvation and temperature were previously shown not to have marked effects (4).

Molecular Analysis of B-Maintainers. To determine the nature of the rDNA molecules present in B-maintainers, we exploited two restriction fragment length polymorphisms (Fig. 1) that distinguish B and C3 rDNAs: at the 3′ NTS, C3 has an additional *Bam*HI restriction site (16), while at the 5′ NTS strain B has a 42-bp deletion compared to C3 (6, 8). Whole-cell DNA from 24 B-maintainers of spontaneous origin derived from several experiments and 11 induced by UV (treatment 8 hr after conjugation; Fig. 2) was prepared, at an approximate age of 66 fissions. The DNA was digested with the appropriate restriction enzymes, separated by agarose gel electrophoresis, Southern blotted to a Nytran membrane (Schleicher & Schuell), and probed with the appropriate ^{32}P-labeled DNA probes (see *Materials and Methods* and Fig. 1).

The data (Fig. 3) indicate the existence of two classes of B-maintainers. Those of the first class—e.g., clone I6 in Fig. 3—show no evidence of possessing any C3 sequences at either end and, of course, they express B-derived PM resistance. The second class of B-maintainers is the co-maintainer class—e.g., clone J9 in Fig. 3—showing rDNA sequences derived from both C3 and B parental DNA. Four out of 24 spontaneous B-maintainers and 2 out of 11 UV-induced B-maintainers were co-maintainers. The remaining clones were pure B-maintainers. It is clear that overall the pure B-maintainers make up the majority (83%) of B-maintainers and that there are no striking differences in the relative proportions of the two classes of B-maintainers among the UV-treated and control samples.

The replication region in the 5′ NTS of the *T. thermophila* macronuclear rDNA molecule contains two highly conserved domains, called domains 1 and 2 (5, 6). All 35 B-maintainers were tested for the presence of an unusual number of domains

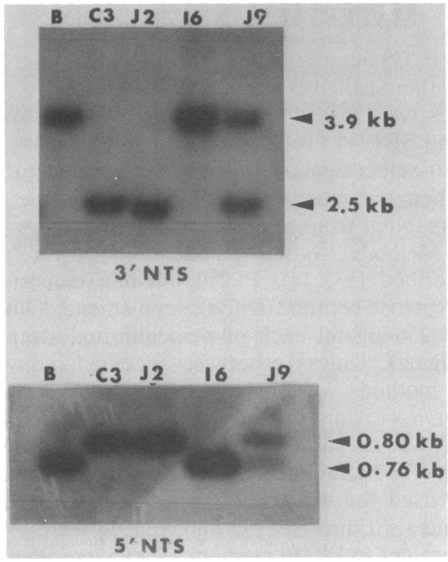

FIG. 3. Molecular analysis of representative B-maintainers. Southern blot analysis of the rDNA of co-maintainers selected so as to illustrate different types of B-maintainers. Lanes: B and C3, parental inbred strains; J2, typical B/C3 heterozygote; I6 and J9, B/C3 heterozygous progeny selected by the B-maintainer protocol. (*Upper*) rDNA digested with *Bam*HI and probed with labeled p6J. (*Lower*) rDNA doubly digested with *Xba* I and *Msp* I; probed with pTtr1.

(after DNA digestion with *Alu* I, which cuts neither domain but cuts on either side of the domain region), with negative results (data not shown).

Do Co-Maintainers Show Long-Term Stability? To determine the long-term stability of the co-maintainers selected by our protocol, we serially subcultured them for an additional 110 fissions in either the continuous presence or the continuous absence of PM, as indicated in *Materials and Methods*, and we analyzed whole-cell DNA from (*i*) a randomly selected PM^R subculture from the PM growth series and (*ii*) a randomly selected PM^S subclone from the control growth series.

The results of the molecular analysis of one set of subclones, derived from cultures of clone J9, and grown for 110 fissions with and without PM, are shown in Fig. 4. These results, as well as those obtained for the other five co-maintainers investigated, are summarized in Table 2. Clear trends were observed when all the six clones were compared. *All* the subclones remained or became homogeneous C3-type for the 5′ NTS, regardless of whether they were derived from cultures grown with or without PM. In contrast, the sequence composition at the 3′ NTS depends strongly on the previous treatment of the cultures. Selection for PM resistance promoted the maintenance of a B-type 3′ NTS, albeit in a mixture with C3 3′ NTS sequences in all but one case (clone A3). In subclones of cultures subcultured for 110 fissions without PM, the trend was in the direction of decrease or disappearance of B-derived 3′ NTS sequences.

The rDNA compositions of all the PM-selected subclones imply the occurrence of genetic recombination: exclusively C3-derived sequence at the 5′ NTS, PM resistance (B-derived) at the 17S rRNA coding region, and a 3′ NTS also derived from B in some of the molecules. A recombination event in the interval between the 5′ NTS polymorphism and *Pmr* accounts for the structure of these molecules. Mutation is excluded as an explanation, since one would have to invoke either (*i*) the joint occurrence of independent mutations in C3 rDNA to create *Pmr* and the *Bam*HI site or (*ii*) the insertion of dozens of base pairs in B rDNA to restore full replication function.

Selection for *Pmr* also selects for the maintenance of a B-derived 3′ NTS, while in the absence of selection the rDNA

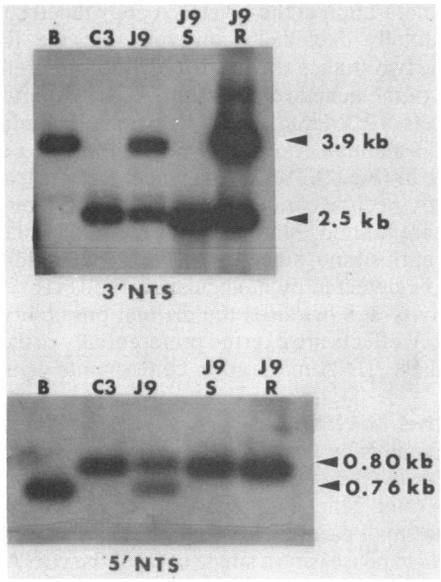

FIG. 4. Molecular analysis of subclones of co-maintainer clone J9, grown with and without PM. Lanes: B and C3, parental controls. J9, tested at 66 fissions. J9S and J9R, PM^S and PM^R subclones isolated after growing about 110 fissions in PP210 medium without and with PM, respectively. Restriction digests and probes were as in Fig. 3.

population tends to become not only PM^S but also biased in the direction of C3-derived 3' NTS sequences. These trends imply that recombination events are not frequent and thus the genetic linkage between the *Pmr* site (in the 17S rRNA coding region) and the 3' NTS tends to be maintained.

DISCUSSION

Macronuclear and rDNA Recombination. This work demonstrates the occurrence of genetic recombination and linkage in the macronuclear rDNA molecule of *T. thermophila*. Genetic assortment of macronuclear markers has been commonly observed in this organism, but not linkage (reviewed in ref. 17), presumably because the markers under study were located on separate, independently assorting, autonomously replicating molecular species. The existence of somatic recombination in the *Tetrahymena* macronucleus was predicted in 1973 (18) to account for the segregation of novel isozymes of the acid phosphatase P-11. In this study we have followed three markers, two restriction polymorphisms and *Pmr*, all located on the same macronuclear DNA molecular species. The genetic recombination observed here must be accomplished by the cutting and intermolecular rejoining of rDNA molecules.

Table 2. Summary of the molecular analysis of the rDNA molecules of subclones of co-maintainers grown for about 110 fissions in the presence and absence of PM

Clone	100 fissions, no PM (S)	Initial	100 fissions, in PM (R)
	5' NTS analysis		
J9*	C3 only	C3 = B	C3 only
I1	C3 only	C3 > B	C3 only
A3	C3 only	C3 >> B	C3 only
D7	C3 only	C3 >> B	C3 only
D6	C3 only	C3	C3 only
J7*	C3 only	C3	C3 only
	3' NTS analysis		
J9*	C3 only	C3 < B	C3 = B
I1	C3 only	C3 = B	C3 < B
A3	C3 = B	C3 = B	Only B
D7	C3 only	C3 = B	C3 < B
D6	C3 > B	C3 = B	C3 = B
J7*	C3 > B	C3 = B	C3 < B

S and R indicate, respectively, the PM^S and PM^R subclones derived from the co-maintainer clones listed in the first column. *, Co-maintainers obtained from UV-irradiated cultures. The relative amounts of B- and C3-derived sequences are qualitative, based on visual examination of autoradiographs: =, > (or <), and >> indicate approximately ratios in the ranges 1:1 to 2:1, 2:1 to 10:1 (or 1:2 and 1:10) and greater than 10:1, respectively. The 48 subclones of the R cultures were in every case all PM^R, with the sole exception of J9R; 47 subclones of J9R were PM^R, while one contained some PM^S cells. The 48–96 subclones of the S cultures were all PM^R in every case, with the exception of the D7S culture; 2 out of 83 D7S subclones were PM^R, while the other 81 were PM^S.

Our selection scheme permitted us to identify recombination events between *Pmr* and a restriction polymorphism in the 5' NTS, separated by about 3 kb. The frequency of recombination is in the order of 0.01 event per progeny macronucleus. This frequency may not be representative of that of markers located on other more typical macronuclear DNA molecules for the following reasons.

(*i*) We probably detected recombinants only if the primary recombination event occurred when there were few C3 rDNA molecules—i.e., early during rDNA amplification in the macronucleus anlage; otherwise the fraction of recombinant molecules would not have significantly influenced the PM resistance phenotype.

(*ii*) rDNA molecules are special in that they become sequestered (and possibly concentrated) into nucleoli, each containing at least 20 rDNA molecules, depending on particular estimates of the number of nucleoli per macronucleus (19, 20). The frequency of genetically consequential recombination should depend on whether or not (and for how long) nucleoli become and remain genetically mixed in a heterozygous macronucleus.

(*iii*) The still-unknown events responsible for rDNA maturation (e.g., excision or palindrome formation) may in themselves be recombinogenic; if so, the class of recombinational events observed here could be special.

Macronuclear rDNA recombination is not limited to the strains or conditions used in this study. Analogous phenomena have been detected by P. Yaeger, D. D. Larson, E. H. Blackburn, and E.O. in a study of laboratory-induced mutations affecting rDNA replication (unpublished observations) and by Yu *et al.* (8) after transformation by microinjection with a circular DNA plasmid.

The rDNA molecule is palindromic. Thus, unless recombination were to involve a highly concerted reaction simultaneously affecting both arms, or occurred prior to palindrome formation, we expect that the primary products of recombination should be heteropalindromes—i.e., rDNA molecules with genetically different arms. The first co-maintainers we reported were examined for heteropalindromic structure with negative results (4), but heteropalindromes have been generated by recombination with an exogenously supplied circular plasmid (8). On the other hand, clone A3R (Table 2) clearly must be a recombinant homopalindrome. Homopalindromes can in principle be generated from heteropalindromes by secondary intramolecular recombination or gene conversion events, regardless of whether or not nucleoli quickly become subclones of rDNA molecules.

Co-Maintainers and Differential Replication. Our initial aim was to investigate further the apparent inconsistency between the lack of differential replication observed among the co-maintainers described earlier (4) and the conclusion of differential replication of C3 with respect to B rDNA in typical heterozygotes (6). Our data, indicating that all six co-maintainers investigated have recombinant rDNA molecules, resolve the apparent contradiction.

The nature of the recombinants observed is instructive. The recombinant molecules are the product of a double selection: (*i*) the imposed growth selection for the *Pmr* allele, derived from the B parent, and (*ii*) the natural selection for the determinant for differential rDNA replication derived from the C3 parent. The structure of the recombinants (i.e., 5′ NTS of C3 origin and *Pmr* site and 3′ NTS of B origin; Table 2) maps the determinant for differential replication to the *Pmr*-proximal central segment of the palindrome. Assuming that the highly conserved rRNA coding region is not involved in replication control, this leaves the 5′ NTS as the probable location of this determinant. This makes it virtually certain that differential replication is due to the nucleotide sequence differences identified in the replication region of the 5′ NTS (6).

One of the recombinants generated in this study (clone A3R, Table 2) has proven useful as a donor for rDNA transformation. This clone, renamed AL11, possesses a C3-derived 5′ NTS (maximum replication advantage) and the *Pmr* allele. Cells whose macronuclei are pure for the *rmm1* mutation in the rDNA (6) are in principle the best recipients for rDNA transformation, since they are at the bottom of the replication advantage hierarchy. *rmm1* rDNA was derived by mutation from C3 rDNA; since AL11 has a B-derived 3′ NTS, this allows unambiguous verification that putative transformants with *rmm1* as the recipient are not, in reality, the consequence of mutation. These advantages have been exploited in demonstrating transformation of *Tetrahymena* by electroporation with native AL11 rDNA molecules (E.O., D. D. Larson, Y.-F. Hu, G.-L. Yu, J. Karttunen, A.L., B.L.H., and E. H. Blackburn, unpublished results) and by microinjection with a circular plasmid that includes one arm of AL11 rDNA (8).

Pure B-Maintainers. The occurrence of two rare phenomena in the developing macronuclear anlage can explain pure B-comaintainers, when suitable account of the replication advantage of C3 rDNA is taken: (*i*) failure of the C3 rDNA micronuclear copy to mature correctly in the macronuclear anlage; (*ii*) a great shortage of C3 rDNA molecules when amplification is completed, due to a delay in the maturation of the micronuclear C3 rDNA copy or in the initiation of its amplification. rDNA molecules are distributed unequally, as evident by the segregation of PM^S cells from Pmr/Pmr^+ heterozygotes of inbred strain B (V. Merriam and P. J. Bruns, personal communication). Thus, in our case, macronuclear division could stochastically generate, by unequal division, rare descendants whose macronuclei are pure for B rDNA.

The ratio of pure B-maintainers to co-maintainers is much higher in this than in the previous study (4). A very important difference is the use of PM to select B-maintainers in this study; two rounds of PM selection were used, the first coming at 6 fissions. Since macronuclei in which no C3 rDNA was amplified should be recovered with equal efficiency regardless of selection, we should not have expected an increase in the relative frequency of this class in this study. In contrast, pure B-maintainers generated by the combination of biased amplification and unequal distribution are efficiently recoverable in the presence of PM selection, but not in its absence. In the absence of PM selection, they are likely to be diluted out by their more numerous fission sisters, in which even a few C3 rDNA molecules are eventually able to outreplicate the B rDNA molecules. Thus the difference in the ratio of pure B- to co-maintainers observed in the two studies suggests that macronuclei in which very few copies of the C3 rDNA were amplified were much more frequent than macronuclei in which the maturation of the C3 rDNA copy failed completely. It is not totally excluded, however, that the differences between the two studies are due to undefined differences in the remainder of the genetic background of the parental clones.

The Effects of UV Irradiation. UV irradiation induced both classes of B-maintainers. The UV effect showed a peak of activity at 8 hr (Fig. 2). At this time the macronuclear anlagen have already been generated and have begun their morphological differentiation. Maturation of the rDNA may also be underway at this time, since by 9 hr enough palindromes are present to be detected by molecular methods (16). The peak of UV activity at 8 hr raises the distinct possibility that the observed UV effects are exerted preferentially or exclusively on the earliest rDNA maturation components or intermediates.

Perspectives on rDNA Genetics. It seems remarkable that the rRNA gene is by now the best *genetically* characterized gene in *Tetrahymena* and is the source of important discoveries concerning genetic events of the developing macronucleus. In no small part this is due to the the uniqueness of *T. thermophila* in possessing a single copy of the rRNA gene per haploid genome in the germline (4, 21) and to the high copy number of the rDNA molecular species in the macronucleus, which facilitates molecular characterization. Thus the rDNA is potentially a powerful system for the study of recombination mechanisms in the *Tetrahymena* macronucleus.

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