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NULLISOMIC TETRAHYMENA. II. A SET OF NULLISOMICS DEFINE THE GERMINAL CHROMOSOMES

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ABSTRACT

Crosses of a diploid *Tetrahymena thermophila* to a strain with a haploid germinal nucleus result in chromosome loss during meiosis in the haploid. The resulting monosomics can be made nullisomic by a special cross that induces homozygosis of a meiotic product of the germinal nucleus, but retention of the parental somatic nucleus. The creation and testing of single nullisomics for three of the five chromosome pairs and a triple nullisomic missing another pair is presented. Taken together, these strains make possible a series of crosses in which all but one of the chromosomes is missing in one parent. This set of nullisomics can, therefore, be used to map any mutation in Tetrahymena to a specific chromosome.

THE micronucleus in *Tetrahymena thermophila* is a specialized germinal nucleus; the cell's phenotype is encoded by a separate macronucleus (see GOROVSKY 1980 for a comparison of these nuclei). It is now possible to create heterokaryons with different genotypes in the two nuclei. Such cells always express a phenotype reflecting the genotype of the macronucleus (see ALLEN 1967 for an introduction to this approach and BRUNS and BRUSSARD 1974b and MAYO and ORIAS 1981 for applications). It has recently been shown (BRUNS and BRUSSARD 1981; BRUNS, BRUSSARD and MERRIAM 1982) that this nuclear dimorphism can be exploited to create micronuclear nullisomics, missing both copies of one of the five chromosomes.

Two general methods have been employed to generate nullisomics. Fundamental to both is the isolation of a strain with a haploid micronucleus and a functional, apparently normal, macronucleus. As PREPARATA and NANNEY (1979) have shown, these cells can be mated with a diploid. The haploid micronuclei undergo meiosis, but the resulting products of meiosis are highly aneuploid. Thus, meiosis in the haploid cell provides a mechanism for chromosome elimination.

A second process common to both procedures is genomic exclusion described by ALLEN (1967). Figure 1 outlines the events that occur when cells are mated to certain lines called "star" strains (A*, C*). The star strain micronucleus is lost at meiosis, whereas the nonstar micronucleus carries out the early events of

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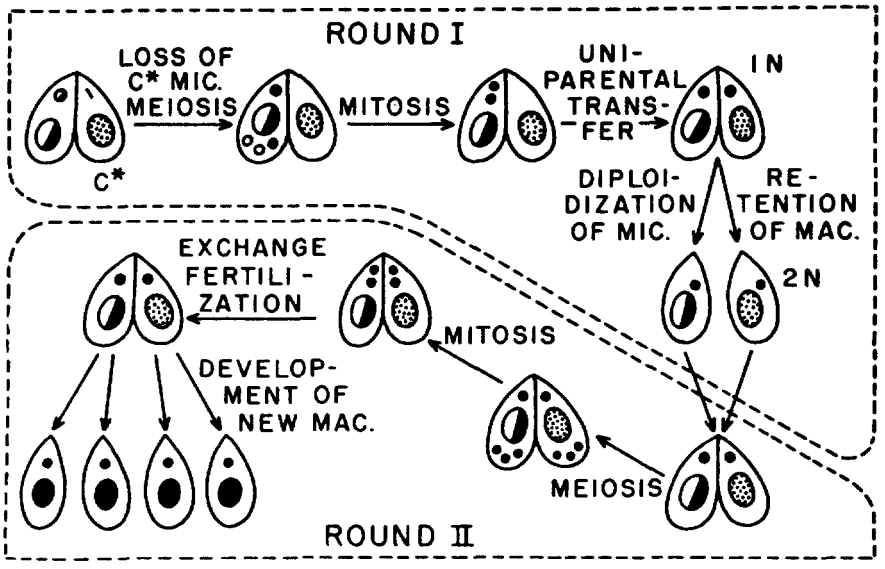

FIGURE 1.—The nuclear events associated with genomic exclusion. Round I yields genetic heterokaryons; micronuclei are derived from the nonstar parent, but each conjugant retains its parental macronucleus. Round II yields homokaryons, with all nuclei derived from the round I micronuclei (from BRUNS and BRUSSARD 1974b).

conjugation: meiosis, elimination of all but one product, mitosis of that nucleus and transfer to the star partner. Each conjugant, therefore, has an identical haploid nucleus derived from the nonstar's micronucleus. This nucleus undergoes an endoreduplication (DOERDER and DEBAULT 1975), producing an identical, fully homozygous zygote nucleus in both conjugants. As Figure 1 indicates, the next step in normal conjugation fails to occur; the old macronucleus is retained, and the conjugants separate. These two cells can be cloned (the round I exconjugant clones) and remated. As Figure 1 indicates, all of the events of normal conjugation occur at round II. Round II exconjugants have new macronuclei expressing the phenotype of the micronuclear genotype.

We present here the construction and karyotypes of a set of strains, each missing one or several of the chromosome pairs. Since crosses of diploids to nullisomics yield viable monosomic progeny (the Tetrahymena macronucleus can withstand this degree of genetic imbalance), these nullisomic lines provide a set of standards that make it possible to assign genes to any chromosome in the genome.

MATERIALS AND METHODS

Strains: Table 1 lists the strains used to construct the nullisomics in this study. All of these strains were derived from several mating types of inbred strain B1868. Table 2 lists all of the loci used in the crosses to define the chromosomes in the nullisomics. *T. thermophila* was formerly known as *Tetrahymena pyriformis*, syngen 1 (NANNEY and MCCOY 1976).

Media: The growth medium (PPYS) was 1% proteose peptone (Difco), 0.1% yeast extract (Difco), and 0.003% Sequestrine (Geigy). Growth medium in Petri or microtiter plates had 250 μg/ml each of

TABLE 1

Strains

Designation	Genotype (phenotype)
CU291	*ChxA cdaA* (cy. r, temp s, IV)
CU336	*Mpr/Mpr* (6-mp. s, VII)
B18683	Wild type (III)
B18684	Wild type (IV)
B18687	Wild type (VII)
A*, C*	Strains with defective micronuclei, but wild type macronuclei that induce genomic exclusion (see text).

As previously suggested (BRUNS and BRUSSARD 1974b), the letters preceding the parentheses designate the micronuclear genotype of the cell; the letters inside the parentheses represent the phenotype expressed by the macronucleus, and the Roman numerals stand for the mating type.

TABLE 2

Genetic loci used to define chromosomes in nullisomic lines

Set	Locus	Mutant phenotype
1	*ChxA2*	Cycloheximide resistant
2	Mpr	6-Methylpurine resistant
3	*tsA1*	Temperature sensitive
	ts-21	Temperature sensitive
	pig-4	Tyrosine dependent pigment producer
4	*cdaA1*	Temperature sensitive cell division arrest
	cdaF1	Temperature sensitive cell division arrest
	psmB1	Temperature sensitive pseudomacrostome; altered oral morphology
5	*fatD1*	Temperature sensitive fat cell body
	disA1	Temperature sensitive disorganized cortex
	cdaD1	Temperature sensitive cell division arrest
	cdaD2	Temperature sensitive cell division arrest
	cdaH1	Temperature sensitive cell division arrest
	cdaH2	Temperature sensitive cell division arrest

Original references for these loci are cited in BRUNS (1982).

penicillin and streptomycin sulfate added. The antibiotics were prepared as a 1000× stock solution which was sterile filtered and stored frozen in small aliquots. The starvation medium was 10 mM Tris-HCl pH 7.4 (Sigma) as originally suggested in BRUNS and BRUSSARD (1974a) and extensively studied in WELLNITZ and BRUNS (1979). Growth and selective media are discussed in depth in ORIAS and BRUNS (1976).

Matings: As previously described (BRUNS and BRUSSARD 1974a), cells were prepared for mating by washing once in 10 mM Tris and resuspending in Tris at a final concentration of 1.2×10^5 cells/ml. Cells were allowed to starve 8–18 hr. Mass matings were made by mixing equal numbers of the prestarved parents in Erlenmeyer flasks at a ratio of mating mixture to flask volume of at least 1:10. The mixtures were usually incubated without shaking at 30°, although room temperature was used for crosses when temperature-sensitive strains were used. Timed ratings from which round I pairs were isolated were usually begun by allowing the parents to starve together in a fast-shaken flask; a time-operated switch turned the shaker off, after which pairing began (BRUNS and PALESTINE 1975; ARES and BRUNS 1978). Round I pairs were isolated 6–8 hr after the shaker was turned off. To cross nullisomic strains by each other, cultures of each were starved in 10 mM Tris at 1×10^5 cells/ml

overnight. For mating, 0.5 ml of each prestarved parental strain was added to an 18- × 150-mm culture tube.

Progeny from crosses to the marked strains listed in Table 2 always expressed a unique drug resistance, derived from a dominant mutation contributed by the nullisomic micronucleus. They were identified or selected by addition of the appropriate drug. Single pairs were isolated and cloned as previously described (SCHOLNICK and BRUNS 1982). Mass matings were performed in tubes, by mixing 1 ml of each prestarved parental strain.

Pair isolation, subcloning and microtiter plate manipulations: Matings in microtiter plates were used for maturity tests and matings of many clones. Clones to be tested were replica plated into microtiter plates containing 0.1 ml/well of PPYS and grown overnight at 30°. Culture medium was removed with a custom designed 96-place aspirator (Lansing Industries, Ithaca, New York) without centrifuging the plates, the fresh 10 mM Tris was added (0.1 ml/well) using a 12-channel manifold (Dynatech). This procedure was repeated twice, except the final addition of Tris was 0.05 ml/well. The cells were starved overnight in the plates. The tester strain was grown normally in PPYS in a flask, washed in Tris and starved in a flask at 1×10^5 cells/ml overnight. For mating, 0.05 ml of the starved tester was added to each of the microtiter wells. For timed pair isolation, 0.05 ml 3× PPYS was added after 6–8 hr at 30°, and 0.5 hr later a small aliquot (0.1 μl) was removed from the top of each well and added to a shallow 10-μl drop of PPYS in a Petri plate. Pairs were isolated from this drop into similar PPYS drops in Petri plates as previously described (SCHOLNICK and BRUNS 1982); subsequent clones were replica plated to microtiter plates.

Drugs: Drug doses used for selecting cycloheximide and 6-methyl purine-resistant strains were 25 μg/ml and 15 μg/ml, respectively. These drugs were maintained as 500× stock solutions in 95% ethanol (cycloheximide) or distilled water after sterile filtering (6-methyl purine).

Cytology: Chromosomes were visualized by fixation in Schaudinn's fluid plus acetic acid and staining in Giemsa, by the method previously described (BRUNS and BRUSSARD 1981) except for the following. The original procedure calls for cells to be washed in 70% ethanol after fixation and states that the cells can then be kept in the ethanol for several days. We have found that the chromosomes spread better if the time the cells are kept in the ethanol is kept to a minimum.

RESULTS

Chromosome 5 nullisomics

Figure 2 outlines all of the crosses used to create chromosome 5 nullisomics. The method used to generate nullisomics was to first cross the haploid strain CU291 with the diploid CU336 to generate monosomics. As Table 1 indicates, CU291 has a haploid nucleus containing the markers *ChxA* and *cdaA*. *ChxA* is a dominant mutation that confers resistance to cycloheximide (cy) (BLEYMAN and BRUNS 1977); *cdaA* (FRANKEL *et al.* 1976) is a recessive temperature-sensitive morphological mutation. CU336 has a wild-type macronucleus and a micronucleus homozygous for the dominant mutation *Mpr*, which, when expressed, confers resistance to 6-methyl purine (6-mp) (BYRNE, BRUSSARD and BRUNS 1978). Thus, F_1 progeny were selected by growth in cy plus 6-mp. A doubly resistant clone (X007) which was phenotypically wild type for *cdaA* was grown to maturity and crossed with strain C*, and round I exconjugants were isolated.

The exconjugants that received the cy, 6-mp-resistant parental macronucleus were crossed to fresh C* at 30°, and samples were fixed 5 hr after mixing the prestarved parents. The preparations were stained as previously described (BRUNS and BRUSSARD 1981), and the micronuclear chromosome content was examined. Since C* loses its micronucleus at meiosis, it is possible to distinguish between the two conjugants.

One of the round I exconjugants (line X019-7) had four of the five chromo-

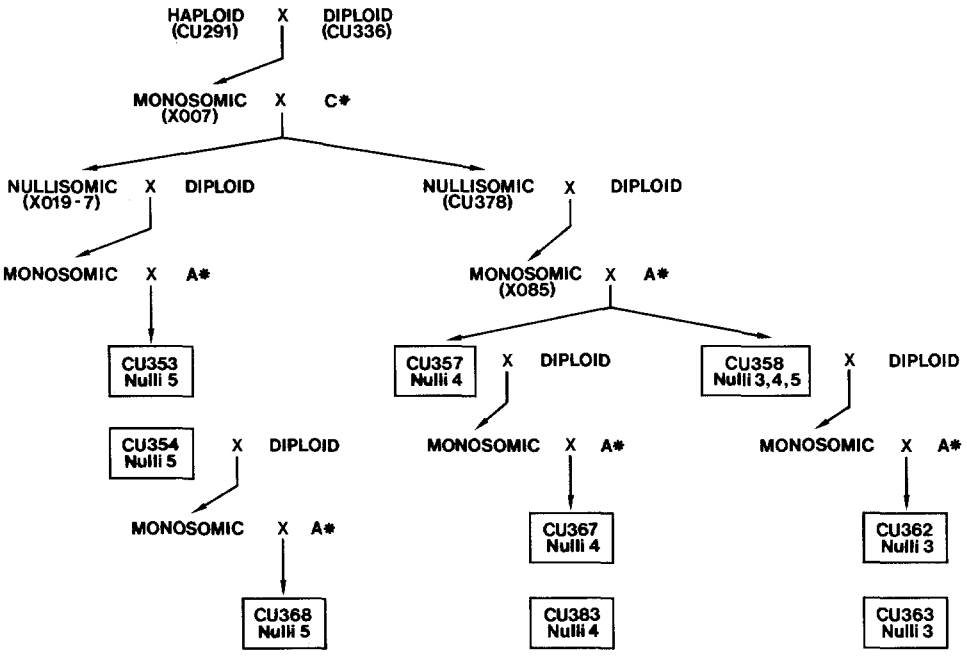

FIGURE 2.—Crosses performed to create nullisomics by first crossing a haploid with a diploid. See text for details.

somes. It was missing both copies of the smallest chromosome; we have proposed (BRUNS and BRUSSARD 1981) that this genotype be designated Nulli 5. This strain was then crossed to a wild-type diploid, pairs were isolated and immature double drug-resistant clones retained. True products of cross-fertilization should be hemizygous for all markers on chromosome 5 but heterozygous for markers on other chromosomes. Cells with heterozygous macronuclei undergo phenotypic assortment when grown. This phenomenon (see SONNEBORN 1975 for a discussion) involves, in a heterozygous clone, the appearance of cells stably expressing the phenotype of either allele. In this instance, subclones of the monosomic were established which were both cy and 6-mp sensitive; the monosomics must have been heterozygous for both *ChxA* and *Mpr*.

The assorted monosomics were then crossed with strain A*, and round I exconjugants were isolated. With respect to chromosome 5, we expect nullisomics and diploid micronuclei to result. Moreover, we expect equal frequencies of mutant and wild-type homozygotes for the two unlinked markers (*ChxA* and *Mpr*) which are not on chromosome 5, since they were heterozygous in the monosomics.

We assayed the micronuclear content of the round I exconjugants by crosses to wild-type diploids. Since wild type is sensitive to both drugs, and the round I exconjugants retain their sensitive assorted macronuclei, resistant progeny of the round I by wild-type cross were detected by adding the drug after mating.

We established clones that had one or the other of the resistance mutations

in the micronucleus. The round I exconjugants of these strains were then remated (round II), and the appropriate drug was added. Ability to produce drug-resistant progeny when crossed with a diploid, but failure to produce drug-resistant progeny when taken through round II, is characteristic of a nullisomic strain. Strains that yield these results were then crossed to fresh A* and examined cytologically. Two of these, each missing a pair of chromosomes, were retained. Since both of these lines are round I exconjugants from the same monosomic, they have identical macronuclei (sensitive to both drugs, mating type IV). One of the lines was homozygous for *Mpr* (CU353); one was homozygous for *ChxA* (CU354). Both of these strains have been crossed to a number of strains homozygous for a variety of mutations (see Table 2). Both yield the same results; progeny uniformly express the mutant phenotypes of only some of the genes (set 5). We, therefore, assign the genes that are uncovered by these nullisomics to chromosome 5.

To produce a chromosome 5 nullisomic with a different mating type, CU354 was crossed to a wild-type diploid strain (see Figure 2). The resulting progeny were cy resistant, but, again, sensitive subclones could be isolated following phenotypic assortment. These progeny have new macronuclei and, therefore, are independent for mating type. A mating type II clone was chosen and a sensitive subclone isolated. These cells were then crossed to A* and round I exconjugants again isolated, and a clone (CU368) containing the *ChxA* mutation but missing both copies of chromosome 5 was identified by the strategy outlined before. As predicted, this strain uncovers the same set of recessive markers uncovered by the other chromosome 5 nullisomics.

Chromosome 4 nullisomics

The same basic protocol was used to obtain chromosome 4 nullisomics. All of the crosses used to establish these strains are outlined in Figure 2. The monosomic progeny clone (X007) of the original cross of haploid to diploid (CU291 × CU336) outlined in the previous section was actually multiply monosomic. In addition to the nullisomic 5 round I exconjugants described in the previous section, a complex nullisomic was isolated; this strain has been named CU378. As Figure 3A indicates, when this strain was mated and fixed for cytology, two full-size and one small chromosome pair were seen. Subsequent genetic analysis has revealed that this strain is apparently missing both copies of chromosomes 4 and 5 and is missing both copies of the right arm of chromosome 3 (E. V. MERRIAM and P. J. BRUNS, unpublished results). Of importance here is that this strain was crossed to a wild-type diploid to create a multiple monosomic (X085), and this strain was again crossed to A*, and round I exconjugants were collected. A strain was retained that could not yield viable round II progeny, could yield viable progeny when crossed with either a diploid or CU354 (the Nulli 5) and had four pairs of chromosomes. This strain (CU357) is homozygous for both *ChxA* and *Mpr* and has a macronucleus that expresses cy sensitivity and 6-mp resistance. We have arbitrarily labeled the missing chromosome pair in this strain number 4. To construct nullisomic 4 strains with different mating types and a fully wild-type macronucleus for the drug-resistant phenotypes, we

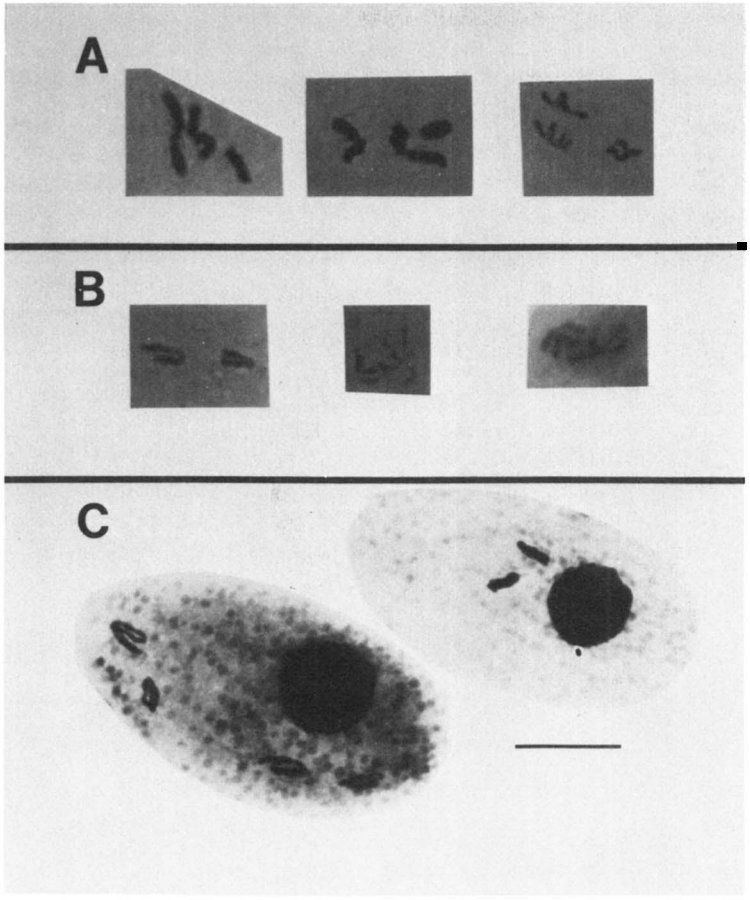

FIGURE 3.—Giemsa-stained preparations at 5.5 hr in conjugation (30°) showing micronuclear chromosomes at several stages in meiosis (see MARTINDALE, ALLIS and BRUNS 1982 for a timing of the stages in conjugation). As described previously (BRUNS and BRUSSARD 1981) the strains to be examined were crossed with strain A*. Since A* loses its micronucleus early in the process, the identity of each conjugant in every pair could be determined; chromosomes are shown only from the nonstar cell. Staining was as previously described (BRUNS, BRUSSARD and MERRIAM 1982). All photomicrographs are at the same magnification. The bar indicates 10 μm. A, Karyotype of strain CU378. Two normal-sized metacentrics and a smaller telocentric are evident. B, Karyotype of the triple nullisomic, CU358. The two metacentric chromosomes are numbers 1 and 2. C, Karyotype of the triple nullisomic CU359. The two metacentric chromosomes are numbers 1 and 4. The large, darkly stained object in each of the cells is the macronucleus.

crossed CU357 to a wild-type diploid, generating a double drug (cy and 6-mp)-resistant monosomic, which expressed mating type VI. This line was subcloned, and a sensitive assorter to both drugs was retained. This clone was then crossed to A*, and round I exconjugants were isolated and two differently marked Nulli 4 lines retained. CU367 has a micronucleus that is homozygous *ChxA*$^+$ but homozygous *Mpr*. CU383 is homozygous for both *ChxA* and *Mpr*. Both strains

have the double drug-sensitive, mating type VI macronucleus from the monosomic parent.

All three Nulli 4 lines uncover the same recessive mutations in crosses to marked strains. These markers are a different set (set 4, Table 2) than those uncovered by the Nulli 5 lines. Set 4 loci are, therefore, defined as being on chromosome 4.

Chromosome 3 nullisomics

Two Nulli 3 lines were established by procedures much like those described before. The crosses involved are included in Figure 2. The same multiple monosomic (X007) derived from the original haploid by diploid cross served as a source of nullisomics for chromosome 3. As mentioned before, CU378, which was derived after mating to strain C*, appears to have lost only part of one of its chromosome pairs (Figure 3A). This complex nullisomic was crossed to a wild-type diploid, and, as described in the previous section, a resulting multiply monosomic progeny clone (X085) was crossed to A*, and round I exconjugants were retained. In addition to the Nulli 4 (CU357) described before, this cross yielded a strain (CU358) that cytological examination revealed was missing three pairs of chromosomes (Figure 3B). When crossed with marker strains, it uncovers all markers uncovered by the Nulli 5's and the Nulli 4's, and an additional set (set 3, Table 2). We conclude that it is nullisomic for chromosomes 4 and 5 as well as another, which we have labeled chromosome 3. Set 3 loci, therefore, are located on chromosome 3.

To get single nullisomics for chromosome 3, the triple nullisomic was crossed to a wild-type diploid, yielding cy- and 6-mp-resistant progeny. Again, drug-sensitive assorters were isolated; *ChxA* and *Mpr* cannot be on chromosomes 3, 4 or 5, since this strain is monosomic for these chromosomes. This strain was then crossed to A*, and round I exconjugants were isolated. These exconjugant clones included a number of multiply nullisomic strains. In addition, two isolates contained four pairs of chromosomes, failed to uncover any chromosome 4 or 5 markers but did uncover the new set of markers uncovered by the triple nullisomic CU358 (the Nulli 3, 4, 5). Therefore, we conclude that these two lines are nullisomic for chromosome 3. One of these strains (CU362) is homozygous for *ChxA* and has the monosomic macronucleus. The other strain (CU363) is homozygous for both *ChxA* and *Mpr* and inherited the macronucleus of the A* parent.

The following section describes an entirely separate method to create nullisomics. A third nullisomic for chromosome 3 is included.

Chromosome 2 nullisomic

A different approach to constructing nullisomics is outlined in Figure 4. In this series, the haploid was crossed directly with C*, and round I pairs were isolated. Of 88 isolated pairs, only one viable synclone which had undergone meiosis was recovered. Most of the isolates (79 of 88) were inviable. This seems reasonable, since haploid by star crosses often yield amicronucleate progeny that then fail to grow. At meiosis, the haploid frequently loses all of its

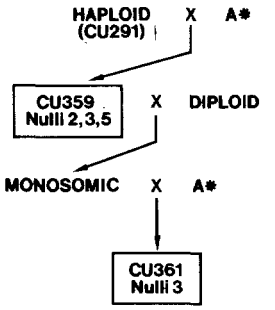

FIGURE 4.—Crosses performed to create nullisomics by crossing the haploid with a star strain. See text for details.

chromosomes, and the star conjugant always loses its micronucleus; under these conditions, neither conjugant would provide any micronuclear genome to the progeny. Although the nullisomics described before demonstrate that an entire micronuclear genome is not necessary for viable cells, no one has yet reported a viable amicronucleate *T. thermophila* with a normal macronucleus. Of the remaining nine viable synclones, eight had not undergone round I. The one line that had undergone round I was crossed to A* and examined cytologically at meiosis. As Figure 3C indicates, it has two chromosome pairs. When crossed with wild type, it yields cy-resistant progeny; it must have retained the *ChxA*-containing chromosome. When crossed with a strain homozygous for *Mpr*, the progeny are 6-mp resistant, but no sensitive subclones could be established by phenotypic assortment; the nullisomic strain must have lost the chromosome bearing Mpr^{+}. Since neither *ChxA* nor *Mpr* are missing in the nullisomics for chromosomes 3, 4 or 5, this triple nullisomic has lost one chromosome pair of the remaining two and has retained the other. We have arbitrarily labeled the *Mpr*-bearing chromosome missing in this strain chromosome 2. Upon crossing with strains containing mutations on chromosome 3, 4 or 5 (see Table 2), markers on chromosomes 3 and 5 were uncovered, but markers on 4 were not; the strain (CU359) must be nullisomic for chromosomes 2, 3 and 5.

The multiple nullisomic (CU359) was crossed to a diploid (CU336), and resulting monosomic progeny were cloned. Since CU336 has a micronucleus that is homozygous for the chromosome 2 marker *Mpr*, these progeny are heterozygous for the chromosome 1 and 4 markers, *ChxA* and *cdaA*, respectively, but hemizygous for *Mpr*. As expected, subclones stably expressing the wild-type phenotypes for *ChxA* and *cdaA* were found after phenotypic assortment, but no 6-mp-sensitive clones were ever seen. A subclone expressing the phenotype cy sensitive, temperature resistant, 6-mp resistant, and mating type IV was retained. It was crossed to A*, and round I clones were established. One of these clones (CU361) was retained because it was unable to yield round II progeny, did yield cy, 6-mp-resistant progeny when crossed to a diploid, failed to uncover any chromosome 4 or 5 markers, but uncovered markers that CU358 (Nulli 3, 4, 5) had uniquely uncovered (see Table 2, set 3). Cytological examination reveals four pairs of chromosomes. We conclude that it has a micronu-

cleus that is homozygous for *ChxA* and *Mpr* and is nullisomic for chromosome 3.

Performance of the nullisomics

The single nullisomics for chromosome 3, 4 and 5 and the multiple nullisomic missing chromosome 2 (Nulli, 2, 3, 5) make a set of standards that can be used in crosses to assign any marker to a chromosome. Mutations not uncovered by crosses to any of these strains are assumed to be on chromosome 1. We have not yet isolated a strain nullisomic for chromosome 1 (see DISCUSSION). To demonstrate that the rest of the genome is intact in the single nullisomics, they were mated to each other, and the ability to yield progeny was analyzed. Table 3 lists the micronuclear genotypes and macronuclear expressed phenotypes of all of these strains and presents the results of these crosses. Since none of these strains can yield progeny in round II genomic exclusion, we assume progeny from any of these crosses must be the result of cross-fertilization. Because each strain has either *ChxA* or *Mpr* or both in its micronucleus and a macronucleus expressing sensitivity for at least one of these, drug resistance was used as the assay for progeny.

Most of the crosses involved strains expressing different mating types. As detailed in MATERIALS AND METHODS, about 5×10^4 appropriately starved cells of each strain were mixed, and incubated at 30° for 1 day. Growth medium was then added, and after 24 hr at room temperature the culture was diluted in growth medium containing the appropriate drug. The next day an aliquot from each tube was transferred to fresh drug-containing medium, and both drug-containing cultures were scored for drug resistance after 3 and 7 days at 30°.

Some of the strains to be crossed expressed the same mating type and would, therefore, not pair. This problem was overcome by using strain A* to perform a process we call marker exchange. In these crosses, the two strains to be tested (such as CU361 and CU354, see Table 2) were prestarved and mixed with prestarved A* in a 1:1:2 ratio, respectively. This mixture was allowed to mate for 24 hr at 30°. By this time, round I has been completed and the exconjugants are randomly remating. Since mating type is strictly controlled by the macronucleus, all of the round I exconjugants express parental mating types. In this example, progeny of a cross between CU361 and CU354 are recovered if an A* that had mated at round I with CU361 mates at round II with CU354. Similarly, an A* undergoing round I mating with CU354 but round II with CU361 yields progeny that have received a haploid genome from both CU354 and CU361. The A* has effectively served as a temporary carrier of genes between two strains expressing the same mating type. All of the progeny in any single mating mixture should be the same since the two nonstar parents are homozygous. Although some of the round II pairs will consist of round I clones of different mating types but identical genotypes, this is lethal for nullisomics; progeny of these crosses are never recovered. Viable progeny can only result from the pairing of round I cells derived from different nullisomic strains. Again, as in the standard crosses, the appearance of drug resistance was used to identify progeny. Table 3 shows that, except for two strain CU357 crosses, all crosses between nullisomics for a different chromosome produced progeny. As ex-

TABLE 3

Performance of the nullisomics

Missing chromosome pair	CU strain No.	CU strain No.[a]									Micronucleus	Macronucleus[b]		
		361	362	363	357	367	383	368	354	353		cy	6-mp	Mt
3	361		−	−	−[c]	+	+	+	+[c]	−[c]	Chx Mpr	S	R	IV
	362			−	+	+	+	+	+	+	Chx +	S	S	VII
	363				+	+	+	+	+	+	Chx Mpr	S	WR	III
4	357					−	−	+	+[c]	+[c]	Chx Mpr	S	R	IV
	367						−[c]	+	+	+	+ Mpr	S	S	VI
	383							+	+	+	Chx Mpr	S	S	VI
5	368								−	−	Chx +	S	S	II
	354									−[c]	Chx +	S	S	IV
	353										+ Mpr	S	S	IV

[a] +, progeny recovered; −, no progeny recovered.
[b] Abbreviations: S = sensitive, R = resistant, WR = weak resistant, Mt = mating type.
[c] Achieved by marker exchange (see text).

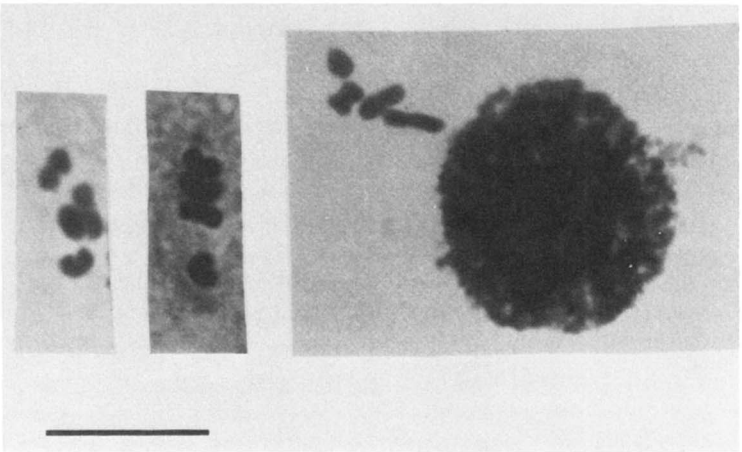

FIGURE 5.—Resulting chromosomes after addition of cy to a mating mixture. Preparation as in Figure 3. The chromosomes on the left, center and right are from clones, respectively: CU362 (Nulli 3), CU357 (Nulli 4) and CU353 (Nulli 5). The bar indicates 10 μm. The macronucleus from the CU353 preparation is included for size comparison.

pected, all crosses between strains nullisomic for the same chromosome failed to yield progeny. We conclude from this and cytogenetic preparations (for examples see Figure 5) that these lines are each missing only one chromosome pair but are otherwise normal (for chromosomes 3, 4 and 5). The two unexpected negative matings (CU357 by CU361 and CU353) suggest that these three strains share some recessive lethals which cannot be simply lost chromosomes since all of them give viable progeny in crosses to the other nullisomics.

Cytology of the nullisomics

We have observed that the findings of KACZANOWSKI (1981) are very useful for chromosome counting in the nullisomics. He reported that inhibitory levels of cy added during conjugation result in conjugants arrested at various stages in the process. We have found condensed chromosome pairs if we allow matings to proceed 4.5 hr in 10 mM Tris HCl at 30° and then add enough cy to take the mating mixture to 2.5 μg/ml of the drug. The mixture is incubated for an additional 2.5 hr at 30° and then fixed for cytology (see MATERIALS AND METHODS). Figure 5 presents the chromosomes of single nullisomics for chromosomes *3*, *4* and *5* in matings incubated in cy. Although the chromosomes are so condensed that there is little morphological detail, they are extremely easy to count. The frequency of cells with these chromosomes is so high it appears that the chromosomes must be accumulating in this configuration.

DISCUSSION

We present here the construction of a set of nullisomics, missing one or more than one chromosome pair. We have used the nullisomics to arbitrarily define and number the micronuclear chromosomes. Since the macronucleus directs most, if not all, of the phenotype, we have been able to construct cells missing most of the micronuclear genome, as long as a genetically complete macronucleus is present.

It is worthwhile to note that we have never eliminated all of chromosome *1*. Most of the strategies reported here would have failed to yield chromosome *1* nullisomics since the haploid strain CU291 carries the dominant mutation *ChxA*, and this marker was used extensively to select for progeny; *ChxA* is on chromosome *1*. We have attempted several alternate approaches to recover aneuploids and have also now identified instances of spontaneous chromosome loss (M. I. ALTSCHULER and P. J. BRUNS, unpublished results). Again, we have not yet found a strain missing all of chromosome *1*. On the other hand, in a related paper (E. V. MERRIAM and P. J. BRUNS, unpublished results). We shall present the isolation of strains missing part (left or right arm) of a number of chromosomes, including chromosome *1*. The inability to isolate all of chromosome *1* may indicate an obligate need for some of this chromosome. It has been shown (YAO and GOROVSKY 1974) that 10–15% of the micronuclear sequences are not found in the macronucleus. It may be that some chromosome *1* DNA sequences are necessary for vegetative growth. Until more haploids with different markers are isolated, or other approaches tried more extensively, it remains an open question whether chromosome *1* can be eliminated from a *T. thermophila* with a normal macronucleus. In any case, the nullisomic strains we have isolated indicate that chromosome *2*, *3*, *4* or *5* can be eliminated with no apparent hindrance to vegetative growth.

All of the nullisomics presented here can be crossed to a diploid and viable progeny recovered with new macronuclei derived from aneuploid zygote nuclei. This striking ability of the macronucleus to absorb rampant genetic imbalance may be a consequence of the amitotic division of this nucleus. The nucleus has no segregation-based mechanism to ensure that vegetative progeny get a uniform

allotment of the parental genes. Phenotypic assortment suggests a random partitioning of the macronuclear alleles during division (see ORIAS and FLACKS 1975 for a discussion). However, subclones do not abruptly die, implying that total content of all genetic loci may be somehow regulated. It has been suggested (PREER and PREER 1979) that this regulation may be based on a replicative mechanism with some means to ensure a balanced number of the various nuclear chromosomes by extra or suppressed rounds of DNA replication. It has been noted (E. ORIAS, personal communication) that a mechanism like that found in bacterial plasmids to control copy number is possible. In crosses with nullisomics, the plastic nature of the macronucleus makes possible recovery of progeny with macronuclei derived from zygote nuclei containing extremely varied degrees of aneuploidy.

The chromosome preparations treated with cy provide a simple method to visualize the number of chromosome pairs found in strains. The development implications of the ability of cy to block conjugation at discrete steps, with chromosomes condensed in ways not normally seen, are exciting but beyond the scope of this study.

Finally, this set of nullisomics is important for gene mapping. A related paper (E. V. MERRIAM and P. J. BRUNS, unpublished results) will present the isolation of clones missing chromosome arms, and another (M. I. ALTSCHULER, D. DEVORE and P. J. BRUNS, unpublished results) will present the construction of strains with homozygous micronuclear deletions. All of these strains have made possible the construction of a map (BRUNS 1982) of almost 100 micronuclear markers in a relatively short time period.

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