Mapping Three Classical Isozyme Loci in Tetrahymena: Meiotic Linkage of *EstA* to the *ChxA* Linkage Group

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ABSTRACT

We demonstrate a reliable method for mapping conventional loci and obtaining meiotic linkage data for the ciliated protozoan *Tetrahymena thermophila*. By coupling nullisomic deletion mapping with meiotic linkage mapping, loci known to be located on a particular chromosome or chromosome arm can be tested for recombination. This approach has been used to map three isozyme loci, *EstA* (Esterase A), *EstB* (Esterase B), and *AcpA* (Acid Phosphatase A), with respect to the *ChxA* locus (cycloheximide resistance) and 11 RAPDs (randomly amplified polymorphic DNAs). To assign isozyme loci to chromosomes, clones of inbred strains C3 or C2 were crossed to inbred strain B nullisomics. *EstA*, *EstB* and *AcpA* were mapped to chromosomes *1*R, *3*L and *3*R, respectively. To test *EstA* and *AcpA* for linkage to known RAPD loci on their respective chromosomes, a panel of Round II (genomic exclusion) segregants from a B/C3 heterozygote was used. Using the MAPMAKER program, *EstA* was assigned to the *ChxA* linkage group on chromosome *1*R, and a detailed map was constructed that includes 10 RAPDs. *AcpA* (on *3*R), while unlinked to all the RAPDs assigned to chromosome *3* by nullisomic mapping, does show linkage to a RAPD not yet assignable to chromosomes by nullisomic mapping.

ISOZYMES were among the first loci investigated genetically in *Tetrahymena thermophila* (ALLEN 1960, 1961, 1965, 1968; ALLEN *et al.* 1963a,b; BORDEN *et al.* 1973). Seven loci were identified, each with two alleles: Esterase A (*EstA*), Esterase B (*EstB*), Acid Phosphatase A (*AcpA*), Tyrosine Aminotransferase A (*TatA*), NADP-Isocitrate dehydrogenase A (*IdhA*), NADP-Malate dehydrogenase (*MdhA*), and Tetrazolium Oxidase A (*TzoA*). Genetically determined variation was observed among the inbred strains (Table 1).

There are five pairs of chromosomes in the micronucleus of *T. thermophila*. With at least 40 loci identified by the early 1970's, linkage between some of these loci might have been expected. No obvious meiotic linkages were observed among six of the seven isozyme loci nor with any of the other 30–35 loci, except for one case: *EstA* and *mat* (mating type locus) (ALLEN 1964; ALLEN and GIBSON 1973; DOERDER 1973). However, even this linkage was disputed (MCCOY 1977) since crosses involving different combinations of inbred strains did not behave uniformly with regard to recombination frequency between *mat* and *EstA*. It was suggested that crosses between certain inbred strains showing linkage involved structural heterozygosity while crosses of other strains not showing linkage for the same markers lacked this structural heterozygosity. However, no gross chromosomal rearrangements could be detected during meiosis, with respect to ploidy or chromosomal morphology (MCCOY 1977). MCCOY (1977) also detected inbred strain differences with regard to a possible linkage of *EstA* and *ChxA* (cycloheximide resistance). He observed linkage when inbred strains B and C2 were crossed but not with crosses of inbred strains B and D.

In crosses involving the 40 loci known then, a total of six potential linkage groups was found (DOERDER 1973; MCCOY 1977). At least three of these linkage groups have not stood the test of time: when mapping with nullisomic strains, those supposedly linked markers mapped to different chromosomes (BRUNS and CASSIDY-HANLEY 1993).

The nullisomic strains are a unique set of strains that have proved very useful in genetic studies. They are characterized by having micronuclei (germ-line nuclei) that have lost both copies of one or more chromosomes or chromosome arms (BRUNS *et al.* 1982, 1983). They survive vegetatively because they are heterokaryons having normal somatic macronuclei. The macronucleus is the only nucleus active in gene expression and vegetative reproduction, and therefore a population of cells can be propagated indefinitely while missing one or more chromosomes in their micronuclei. During sexual reproduction the micronuclei of conjugating cells undergo meiosis. When a nullisomic strain is crossed to a normal strain, progeny with monosomic micronuclei are produced. Using this type of cross and a battery of nullisomic strains missing different chromosomes, genes can be assigned to their respective chromosomal location. To date over 100 different loci, >50 cloned

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TABLE 1

Allelic constitution of inbred strains of *T. thermophila*

Inbred strain	Isozyme locus						
	EstA	*EstB*	*AcpA*	*TatA*	*IdhA*	*MdhA*	*TzoA*
A	*B*	*B*	*A*	*S*	*S*	*S*	*F*
B	*B*	*B*	*B*	*S*	*S*	*S*	*F*
C3	*C*	*B*	*A*	*S*	*S*	*S*	*F*
C2	*C*	*C*	*B*	*F*	*F*	*F*	*F*
D	*B*	*B*	*A*	*S*	*S*	*S*	*S*

EstA, Esterase A; *EstB*, Esterase B; *AcpA*, Acid Phosphatase A; *TatA*, Tyrosine Aminotransferase A; *IdhA*, NADP-Isocitrate Dehydrogenase A; *MdhA*, NADP-Malate Dehydrogenase A; *TzoA*, Tetrazolium Oxidase A. Alleles *A*, *B* and *C* refer to the particular inbred strain in which the allele was first observed. Alleles *F* and *S* refer to the relative migration after electrophoresis: *S*, slow; *F*, fast.

sequences and 47 RAPDs (randomly amplified polymorphic DNAs) have been mapped to specific chromosomes or chromosome arms using this technique (BRUNS and CASSIDY-HANLEY 1993).

Combining nullisomic deletion mapping with recombination data allowed the determination of meiotic linkage between the *mat* locus and the ribosomal RNA gene (BLEYMAN *et al.* 1992). Nullisomic mapping revealed that both *mat* and the ribosomal RNA gene are located on the left arm of chromosome *2*. Appropriate crosses revealed meiotic linkage. The distance between the two loci is estimated to be 34.3 cM (LYNCH *et al.* 1995).

This approach was used here to map three of the isozyme loci, *EstA, EstB* and *AcpA*. They were first assigned to a particular chromosome, or chromosome arm, by deletion mapping using crosses to 10 different nullisomic strains. Linkage to known RAPD loci was then tested by appropriate crosses and, when found, maps were constructed. *EstA* was assigned to the *ChxA* linkage group previously located to *1*R and built by mapping RAPD loci (BRICKNER *et al.* 1996). Here, a linkage group is defined as a group of loci such that every member shows statistically significant linkage to at least one other member of the group. As more loci are mapped, the *ChxA* linkage group should become coextensive with the entire chromosome *1*. No linkage was found between *AcpA*, located to chromosome *3*R, and six known RAPD loci on chromosome *3*, but it does show linkage to a RAPD not yet assigned to chromosomes by nullisomic mapping. *EstB*, known from this work to be located on chromosome *3*L, has yet to be tested for linkage to these RAPD loci because inbred strains B and C3 are not polymorphic for *EstB*.

MATERIALS AND METHODS

Strains: The isozyme genotypes for each of the inbred strains of *T. thermophila* are listed in Table 1.

The clones used (Table 2) were derived from various inbred strains of *T. thermophila* (ALLEN and GIBSON 1973; ORIAS and BRUNS 1975). Genotypes, phenotypes and references are given for each clone. For the nullisomic CU strains the designation N (nulli) x, where x represents the appropriate missing chromosome(s), is used to indicate the micronuclear composition of each nullisomic line. Recently thawed stocks were used in all crosses. Clone C2-2671(C) was a gift from Dr. THOMAS NERAD at the American Type Culture Collection (ATCC) and has the ATCC number 30389. The other clones listed in Table 2 have been kept frozen in the ORIAS lab (as described by FLACKS 1979) since shortly after their isolation and initial characterization in the BRUNS' lab.

Culture media: PP210 (nutritionally rich, proteose-peptone-based growth medium), 2% BP (bacterized proteose peptone used for sequentially growing and starving cells) and Dryl's buffered salts solution (used for starving cells) have previously been described (ORIAS and BAUM 1984). To select for progeny, cycloheximide (cycl) (Sigma) was added to PP210 medium at a final concentration of 15 μg/ml.

Genetic procedures: Routine procedures for the maintenance of strains and growth of cells have been previously described (ORIAS and BRUNS 1975; ORIAS and HAMILTON 1979). For long-term maintenance, stocks obtained in this work were frozen in liquid nitrogen by JUDITH DODGE ORIAS. Unless otherwise specified all the work was done at 30°.

Crosses: Cultures were sequentially grown, starved, mixed, refed, and cycloheximide-selected in Petri dishes (ORIAS and BRUNS 1975). The progeny were then grown 5–6 days in Erlenmeyer flasks (100 ml in 250-ml flasks) before extracts were prepared for isozyme phenotyping. Two-day-old cultures were used for preparing DNA when required for PCR and RAPD testing.

Isozyme phenotyping: Whole-cell extracts were made by concentrating the cells by centrifugation and rinsing them with Dryl's salts solution. The cells were then frozen-thawed six times in a dry-ice ethanol bath and stored at −20°.

Gels were made in 12% hydrolyzed potato starch (STARCHART, Smithville, TX: lots W560-2 and W562-2). The starch buffer was 30 mM boric acid-Tris, pH 7.7 for the esterases and pH 7.15 for the acid phosphatases. We had to lower the pH to 7.15 to reveal the B allele of *AcpA*, which is very sensitive to pH compared to the A (C3) allele. The end-tray buffer was 0.3 M boric acid-Tris, pH 7.34 for both the esterases and acid phosphatases. Except for the pH of the buffers, details of the technique used for starch-gel electrophoresis followed published procedures (ALLEN 1960, 1964; ALLEN *et al.* 1963a,b). Gels were run for 4 3/4 hours at 245V for the esterases and 225V for the acid phosphatases.

The gels were then sliced into pieces and marked; the pieces were placed in a tray (for the esterases) or test tubes (for the acid phosphatases), and an enzyme-specific substrate and dye were added, as previously described (ALLEN 1960, 1964; ALLEN *et al.* 1963a,b). The substrate reacts with the enzyme in the gel and the dye binds to their product *in situ*, producing visible bands. The substrates used were as follows:

TABLE 2

Strains used

Clone	Inbred strain	Micronuclear genotype[a]	Mating type	Macronuclear phenotype	Reference
A*III	A	Defective, not transmissible	III	Wild type	1
SB1969	B	*mat-2/mat-2 ChxA2/ChxA2*	II	cycl-S	1
C2-2671	C2	*mat-3/mat-3*	I	Wild type	2
C3-3685	C3	*mat-3/mat-3*	V	Wild type	1, 2
C3-4911	C3	*mat-3/mat-3*	I	Wild type	3
C3-4916	C3	*mat-3/mat-3*	VI	Wild type	3
CU354	B	*mat-2/mat-2,* N-*5, ChxA2/ChxA2*	IV	cycl-S	4
CU357	B	*mat-2/mat-2,* N-*4 ChxA2/ChxA2, Mpr/Mpr*	IV	cycl-S, 6mp-R	4
CU361sb[b]	B	*mat-2/mat-2,* N-*3,4 ChxA2/ChxA2, Mpr/Mpr*	IV	cycl-S, 6mp-R	5
CU371	B	*mat-2/mat-2,* N-*1L,2R ChxA2/ChxA2, Mpr-null*	IV	cycl-S, 6mp-R	6
CU372	B	*mat-2/mat-2,* N-*1L,3 ChxA2/ChxA2*	IV	cycl-S	6
CU374	B	*mat-null,* N-*2L,4L ChxA2/ChxA2, Mpr/Mpr*	IV	cycl-S, 6mp-R	5
CU377	B	*mat-null,* N-*2L,3,4L ChxA2/ChxA2*	IV	cycl-S	6
CU378	B	*mat-2/mat-2,* N-*3R,4,5 ChxA2/ChxA2*	V	cycl-S	6
CU380sb[b]	B	*mat-2/mat-2,* N-*3,4,5 ChxA2/ChxA2*	IV	cycl-S	7
CU389	B	*mat-2/mat-2,* N-*1L,2R,5 ChxA2/ChxA2*	VII	cycl-S	8

[a] See Table 1 for isozyme genotypes.

[b] sb, Santa Barbara maintained stock CU361 was originally described as N-*3* (BRUNS *et al.* 1983); however, the strain maintained in our laboratory under the same name (now renamed CU361sb) behaves as N-*3,4* (GUTIERREZ and ORIAS 1992; BRICKNER *et al.* 1996). CU380 is listed as N-*3*R,*4,5* in CASSIDY-HANLEY *et al.* (1994). The N-*3,4,5* assignment for the strain frozen in our laboratory under the same name (now designated CU380sb) was as originally communicated to us by the BRUNS laboratory. References: 1, BLEYMAN *et al.* 1992; 2, ORIAS and BRUNS 1975; 3, BRICKNER *et al.* 1996; 4, BRUNS *et al.* 1983; 5, GUTIERREZ and ORIAS 1992; 6, BRUNS *et al.* 1982; 7, this work; 8, CASSIDY-HANLEY *et al.* 1994.

α-naphthyl propionate (*EstA*), α-naphthyl butyrate (*EstB*), and sodium α-napthyl phosphate (*AcpA*) (all from Sigma). The dye-couplers used were Fast Blue RR Salt (esterases) and Fast Garnet GBC (acid phosphatase), both from Sigma. Gels were photographed using a Polaroid Land camera under transillumination. Typical gel patterns for each *EstA, EstB* and *AcpA* genotype are illustrated in the photographs shown in Figure 1.

RAPD genotyping: For use in PCR whole-cell DNA was prepared as described by LARSON *et al.* (1986). The RAPD method of WILLIAMS *et al.* (1990) was used to identify, map and target DNA polymorphisms except that two primers were used in each reaction instead of one. Inbred strains B and C3 were used and B+,C− polymorphisms were scored in the progeny of various types of crosses of these strains. The details of these procedures are described in LYNCH *et al.* (1995) and BRICKNER *et al.* (1996). RAPDs found here to be linked to isozyme genes are described in Table 3.

Linkage maps: To look for linkage between the isozyme loci and other previously mapped loci, a panel of randomly segregating F_2 homozygotes was used (panels 1 and 2 in LYNCH *et al.* 1995). These homozygotes were obtained by allowing progeny of a B/C3 cross to undergo genomic exclusion using the A* strain. Genomic exclusion is a process that utilizes a cross with a so-called "star" strain to produce progeny that are homozygous at every locus (ALLEN 1967). Each member of the panel is a whole-genome homozygote, derived from an independent haploid meiotic product of a B/C3 heterozygous F_1; alleles at each heterozygous locus segregate in the panel in a 1:1 ratio. Details of the construction and nomenclature of these panels are given in LYNCH *et al.* (1995). The F_2 data were analyzed using the computer program MAPMAKER (LANDER *et al.* 1987) to detect linkage and to order linked loci into the maximum likelihood linkage map. The order within a linkage group is that which gives the lowest log-likelihood for the F_2 data, as determined by MAPMAKER. For statistical significance the likelihood given by this order

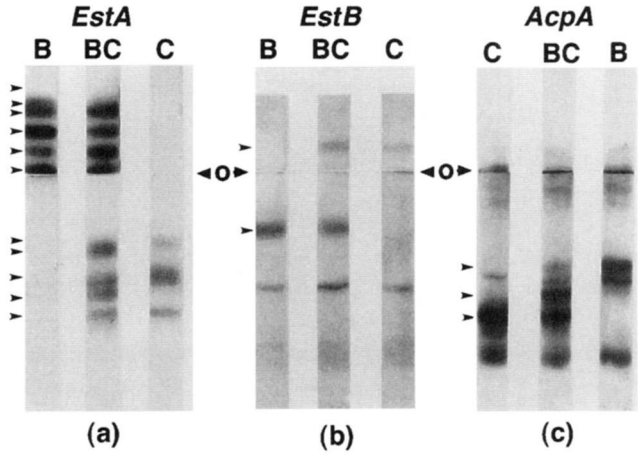

FIGURE 1.—Photograph of gels of isozymes specified by *EstA* (A), *EstB* (B), and *AcpA* (C). B, BC and C: inbred strain B (homozygotes), B/C F_1 hybrids (heterozygotes) and inbred strain C2 or C3 (homozygotes). Small arrowheads, polymorphic bands; O and big arrowheads, electrophoretic origin. The cathode is at the top. The esterases and acid phosphatases are enzyme families with only some members showing genetic variation (ALLEN 1960, 1961, 1965; ALLEN *et al.* 1963a,b). Under various electrophoretic conditions 20–25 family members are visualized; the members of the family vary under different physiological conditions and pH, and show specificity for different substrates, inhibitors, or activators. There are four to five isozymes for the *EstA* alleles, which segregate as a unit. The *AcpA* heterozygote has a prominent hybrid molecule in addition to forms seen in each homozygote. In the photograph the prominent anodal band does not belong to the *AcpA* group.

TABLE 3

List of RAPDs used

RAPD	Primers		Size	Location
1JO14	A2	D1	0.45	*1*R
1JO15	A13	D8	0.7	*1*R
1JO18	A2	D17	0.4	*1*R
1JP12a	A2	B11	0.4	*1*R
**1XS61R*	C6	D13	1.5	*3*R

RAPDs are named with initials of those who identified and mapped them (see LYNCH *et al.* 1995). JO, JUDITH ORIAS; JP, JUSTIN PHILLIPS; XS, XUEYU SHEN. Other RAPDs used are described in BRICKNER *et al.* (1996). Primers are from RAPD primer kits obtained from Operon Technologies Inc. Size of the polymorphic band is in kb, approximately. Location: micronuclear chromosome arm location. **1XS61R* is a "reverse" RAPD, *i.e.*, it gives a band with C3 but not with B DNA; it was mapped to *3*R only by meiotic linkage to *AcpA* (this work). All other RAPDs used are B+,C3−.

must be at least 3 log units greater than that given by the next best order.

RESULTS

Assignment of isozyme loci to chromosome: Inbred strain C3 differs from inbred strain B at the *EstA* and *AcpA* loci, while inbred strain C2 differs from strain B at the *EstA* and *EstB* loci (see Table 1). To assign isozyme loci to their respective chromosome, clones of inbred strains C3 (C3-3685, C3-4911, and C3-4916) and C2 (C2-2671) were crossed to a panel of 10 inbred strain B nullisomic strains that differ in which chromosomes have been lost from the micronucleus (see Table 2). The resulting progeny were heterozygous for all chromosomes or chromosome arms except for those missing in the nullisomic parent, for which the progeny were hemizygous for the C3- (or C2-) derived chromosome. A panel of monosomics was thus created containing at least one panel member hemizygous for every chromosome except *1*R. By determining the phenotype of each member, the location of the gene being investigated could be deduced. If the progeny show the presence of only the C3 strain isozyme(s), then the gene is located on a B-derived chromosome missing in the monosomic strain.

Isozyme phenotypes of monosomic progeny were determined by starch-gel electrophoresis of frozen-thawed whole-cell extracts. Photographs of the results of the crosses of C2 with the B strain nullisomics are shown in Figure 2 for *EstA* and *EstB*. A heterozygous pattern is observed for *EstA* in all of the C2/B nulli crosses. Thus, *EstA* must be located on *1*R since this chromosome is not missing in any member of this panel and other chromosome arms are ruled out by the data. A similar result was observed in the crosses of C3 with the B strain nullisomics. All the results of monosomic tests are summarized in Table 4. For *EstB* note that the pure C2 allele pattern is observed in the progeny of the nullisomic strains missing the entire chromosome *3* but not in those missing *3*R (Figure 2). We conclude that *EstB* is located on chromosome *3*L. *AcpA* appears to be located on the right arm of chromosome *3* (Table 4). This inference was drawn from the results of the crosses of C3 to eight of the B strain nullisomics, where a hemizygous pattern is observed only in the progeny of nullisomics lacking chromosome *3* or *3*R (CU378).

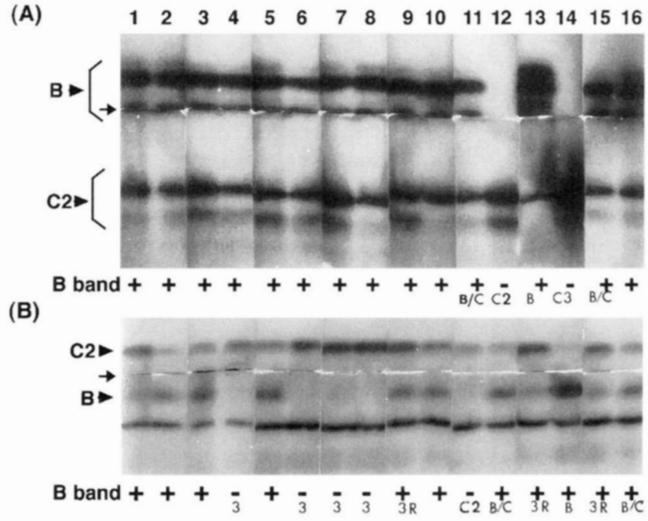

FIGURE 2.—Nullisomic deletion mapping of *EstA* and *EstB* genes. Arrowheads (and braces for *EstA*), location of polymorphic bands; arrow, electrophoretic origin; + and − below each gel, scoring for presence of B strain isozyme(s). (A) *EstA* maps to *1*R. Lanes 1–10 and 16: F_1 of C2 crossed respectively to N*5;* N*4;* N*3,4;* N*1*L,*2*R; N*1*L,*3;* N*2*L,*4*L; N*2*L,*3,4*L; N*3,4,5;* N*1*L,*2*R,*5;* N*3*R,*4,5;* and N*3,4,5*. Lanes 11 and 15: B × C2. Lanes 12–14: C2, B, and C3, respectively. Note that all monosomic lanes show the B-derived bands; this eliminates all but the *1*R chromosome. (B) *EstB* maps to *3*L. Lanes 1–10, 13, 15: F_1 of C2 crossed respectively to N*4;* N*5;* N*1*L,*2*R; N*3,4;* N*2*L,*4*L; N*1*L,*3;* N*3,4,5;* N*2*L,*3,4*L; N*3*R,*4,5;* N*1*L,*2*R,*5;* N*3*R,*4,5;* and N*3*R,*4,5*. Lanes 11, 12, 14, and 16: C2, B × C2, B, B × C2. Note that the B-derived band is present in all monosomic lanes except those that include *3*.

Linkage of *EstA* to the *ChxA* linkage group: A number

TABLE 4

Summary of nullisomic mapping of three isozyme loci

	EstA		*EstB*	*AcpA*
Nulli's	X C3	X C2	X C2	X C3
CU354 (N *5*)	+	+	+	+
CU357 (N *4*)	+	+	+	+
CU361sb (N *3, 4*)	+	+	−	−
CU371 (N *1L, 2R*)	+	+	+	+
CU372 (N *1L, 3*)	ND	+	−	ND
CU374 (N *2L, 4L*)	+	+	+	+
CU377 (N *2L, 3, 4L*)	ND	+	−	ND
CU378 (N *3R, 4, 5*)	+	+	+	−
CU380sb (N *3, 4, 5*)	+	+	−	−
CU389 (N *1L, 2R, 5*)	+	+	+	+
Deduced location	*1*R		*3*L	*3*R

ND, not determined. +, presence or −, absence of B allele.

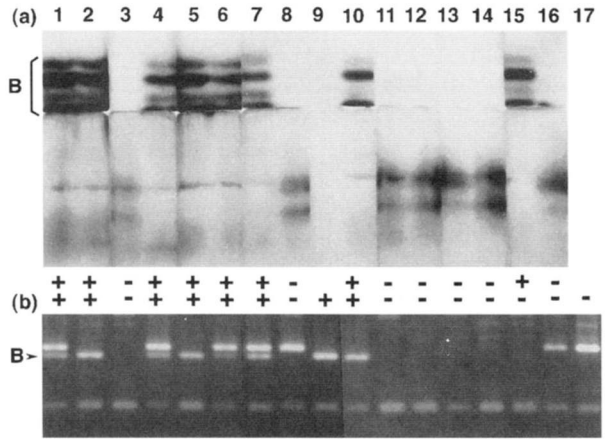

FIGURE 3.—Linkage between *EstA* and RAPD *1JB12*. Photographs showing the segregation of *EstA* (starch gel) (A) and *1JB12* (agarose gel) (B) among B/C3 segregants. The B-derived bands are marked with a brace for *EstA* and with an arrowhead for *1JB12*. + and −, scoring for presence of B-derived bands for *EstA* (upper line) and *1JB12* (lower line). Lanes 1–8 and 10–16: panel 2 members SB (2315C, 2370C, 2402C, 2403C, 2411C, 2412C, 2417C, 2435C, 2479P, 2395P, 2460P, 2461A, 2466A, 2473P, 2302P), respectively. Lanes 9 and 17: B and C3 for *1JB12* and blank for *EstA*. Segregant SB2473P (lane 15) is the only recombinant seen in this figure. Note, that as expected, none of the segregants are isozyme heterozygotes.

of RAPDs assigned to chromosome *1*R have been mapped relative to *ChxA* (BRICKNER *et al.* 1996; J. ORIAS, X. SHEN and E. ORIAS, unpublished observations), a locus previously assigned to *1*R. This mapping utilized panels of Round II (genomic exclusion) segregants from B/C3 heterozygotes (panels 1 and 2 in LYNCH *et al.* 1995). Each member of a panel is a whole-genome homozygote, derived from an independent haploid meiotic product of a B/C3 heterozygous F_1. Alleles at each heterozygous locus segregate in a 1:1 ratio in the panel (LYNCH *et al.* 1995). RAPD genotypes of panel members were determined by PCR amplification using primers specific to the relevant polymorphisms previously mapped to chromosome *1* and phenotyped using agarose-gel electrophoresis. Isozyme patterns of panel members were determined by starch-gel electrophoresis of frozen-thawed whole-cell extracts. The F_2 segregants were homozygous for either the "B" or "C3" *EstA* allele, as illustrated in the photographs of starch gels of a sample of 15 F_2 segregants (Figure 3A). The segregation of RAPD *1JB12* is illustrated in the photographs of agarose gels for the same panel of F_2 segregants (Figure 3B).

Sixty-two F_2 segregants were included in the linkage analysis. Of this group 44 were tested for *EstA:* 23 had the B allele and 21 the C3 allele. Thus, the *EstA* alleles segregated in the expected 1:1 ratio. The F_2 data were then analyzed using MAPMAKER (LANDER *et al.* 1987) and subjected to two and three point analysis. *EstA* showed LOD scores that exceeded 5.0 for linkage in two point crosses with *1JB14, 1JB30, 1JB12,* and *JB22,* and 3.0 with *ChxA* and *1KN2,* where 3.0, and above, is considered statistically significant (see Table 5). Only one of the five point orders of the 10 markers was statistically significant (*i.e.,* LOD for order >3.0): *1JB12—ChxA—1KN2—1JO14—1JO18.* The map given in Figure 4 shows the maximum likelihood order for this linkage group (*EstA, ChxA,* and 10 RAPDs), as determined using MAPMAKER. But it should be clear that a different order of the other markers with respect to the "robust five" is statistically possible until additional segregants are tested. For the best order, maximum likelihood map distances in cM were computed from the recombination percentages using the MAPMAKER program; the values are also shown on the map (Figure 4).

Tests of linkage of *AcpA* to RAPD loci on chromosome *3*: Six RAPDs were initially localized to chromosome *3* (*1JB16, 1JB21, 1JB26, 1JB35, 1JB15,* and *1Jb36;* BRICKNER *et al.* 1996). All but the first two have recently been assigned to the left arm of chromosome *3* using progeny generated from crosses of B/C3 F_1s to our battery of B strain nullisomics (D. ZEILINGER, T. J. LYNCH and E. ORIAS, unpublished results). A total of 66 B/C3 F_2 segregants was included in the linkage study, 39 of which were screened for their *AcpA* phenotypes (see Figure 5 for photographs of the gels of 14 representative F_2 segregants). When subjected to linkage analysis using MAPMAKER, no linkage of *AcpA* to any of the above RAPDs was observed. However, *AcpA* shows statistically significant linkage to *1XS61R,* a RAPD more recently identified (X. SHEN and E. ORIAS, unpublished results), which cannot be mapped with our monosomics because it is a "reverse" RAPD, *i.e.,* it shows a band with C3 DNA but not B DNA.

Partial deletion of chromosome *1*R: While mapping RAPD *1KN3* with panel 3 segregants (LYNCH *et al.* 1995) we observed that RAPD *1KN2,* displayed using the same primer combination but located in *1*R, was also segregating in this panel. This was unexpected, because panel 3 was generated by a procedure, described in detail in LYNCH *et al.* (1995), that is equivalent to crossing the B × C3 F_1 to strain CU374, which is N2L, *4*L. Thus for loci on *2*L or *4*L half of the panel members should be B type hemizygotes and half should be C3 type hemizygotes. By contrast, for loci on the other chromosome arms, the B band derived from the nullisomic parent should be present in every panel member. We tested additional *1*R RAPDs; those that showed 1:1 segregation in panel 3 are restricted to a connected set of six loci (Figure 4). For the rest of the tested *1*R loci, the panel 3 members all showed the B-derived band as expected (data not shown). The presence of the B band was observed in all panel members tested for loci on non-*2*L, *4*L chromosome arms, as expected. We infer the occurrence of a partial *1*R deletion in the micronucleus of the sample of strain CU374 used to construct panel 3. This serendipitous deletion mapping strengthens the order in the maximum likelihood map of Figure

TABLE 5

Two-point recombination frequencies and LOD scores for loci in the *ChxA* linkage group

	1JB12	*EstA*	*1JB30*	*1JB14*	*ChxA*	*1KN2*	*1JO14*	*1JB22*	*1JB8*	*1JP12a*	*1JO18*
EstA	0.05										
	9.15										
1JB30	0.17	0.11									
	4.20	*5.38*									
1JB14	0.15	0.10	0.02								
	6.19	*6.91*	*10.30*								
ChxA	0.18	0.11	0.05	0.02							
	5.23	*6.48*	*8.87*	*14.39*							
1KN2	0.29	0.20	0.18	0.15	0.11						
	2.09	*3.55*	*3.77*	*5.96*	*14.80*						
1JO14	0.36	0.30	0.26	0.23	0.17	0.07					
	0.94	1.50	2.10	*3.64*	*9.45*	*23.63*					
1JB22	0.29	0.18	0.16	0.19	0.16	0.13	0.11				
	1.22	2.51	*3.38*	*2.72*	*3.38*	*5.87*	*6.48*				
1JB8	0.31	0.21	0.19	0.22	0.19	0.11	0.11	0.02			
	1.00	2.11	2.93	2.33	2.93	*6.98*	*6.73*	*12.05*			
1JP12a	0.32	0.22	0.23	0.26	0.23	0.11	0.14	0.09	0.06		
	0.87	1.92	2.14	1.64	2.14	*6.73*	*5.63*	*7.95*	*9.30*		
1JO18	0.47	0.39	0.33	0.37	0.33	0.16	0.21	0.20	0.20	0.18	
	0.03	0.30	0.74	0.47	0.74	*4.87*	*3.36*	*3.77*	*3.97*	*4.40*	
1JO15	0.40	0.37	0.33	0.30	0.27	0.21	0.38	0.34	0.30	0.28	0.14
	0.26	0.40	0.74	1.07	1.48	2.11	0.37	0.62	1.07	1.31	*3.44*

The top and bottom numbers at each intersection are the maximum likelihood recombination frequency and the LOD score (log of the odds against independent segregation), respectively. LOD scores > 3, the threshold of statistical significance, are italicized.

4, and adds a sixth marker, *1JO15,* to the "robust order" of the *1*R set.

DISCUSSION

Chromosome location of isozyme loci: Crosses of clones of inbred strains C3 or C2 to inbred strain B nullisomics facilitated the chromosome assignment of three of the isozyme loci. *EstA* is assigned to the right arm of chromosome *1, EstB* to the left arm of chromosome *3,* and *AcpA* to the right arm of chromosome *3.* None of the nullisomics in the set used here is missing chromosome *1*R. Thus assignment to *1*R is by default; however, it is based on the positive evidence that the polymorphic band is present in every monosomic strain missing a different chromosome arm (BRICKNER *et al.* 1996). The finding of linkage to *ChxA,* a locus classically assigned to *1*R (BRUNS and CASSIDY-HANLEY 1993) supports the validity of that assignment.

Two strains, maintained in our laboratory since shortly after they were first isolated and first characterized in the BRUNS laboratory, show genetic behavior inconsistent with their originally published designations. They have been given the "sb" prefix to indicate the changed assignment (see Table 2). The N*3,4* assignment (rather than N*3*) for CU361sb was already published (GUTIERREZ and ORIAS 1992). The new assignment for CU380sb, as N*3,4,5* rather than N*3*R,*4,5,* represents the simplest change that brings into consistency the results of CASSIDY-HANLEY *et al.* (1994), BRICKNER *et al.* (1996), the results presented here for *EstB,* and the RAPD mapping of chromosome *3* RAPDs by D. ZEILINGER, T. J. LYNCH and E. ORIAS, (unpublished results).

The basis for these differences in genotype of strains maintained in the two laboratories is not clear. Spontaneous loss of chromosomes from the micronucleus is known to occur during vegetative growth in *T. thermophila* (reviewed in ALLEN and GIBSON 1973); the lack of gene expression from the micronucleus precludes selection against chromosome loss. To avoid micronuclear chromosome loss, valuable clones are frozen under liquid nitrogen as early as possible in their clonal life. The nullisomic strains have been kept frozen in the ORIAS laboratory since shortly after their isolation and initial characterization in the BRUNS laboratory.

The assignment of *EstA* to chromosome *1*R lays to rest some of the early mapping controversy, at least for crosses of inbred strains C2, or C3, and inbred strain B (ALLEN 1964; DOERDER 1973; MCCOY 1977). *EstA* clearly cannot be linked to the *mat* locus since *mat* is located on the left arm of chromosome *2* (BLEYMAN *et al.* 1992). Moreover, the assignment of *EstA* to *1*R confirms MCCOY's contention (MCCOY 1977) that *EstA* is linked to *ChxA* (for his C2 × B crosses).

A map of chromosome *1*R: ChxA and 12 RAPDs

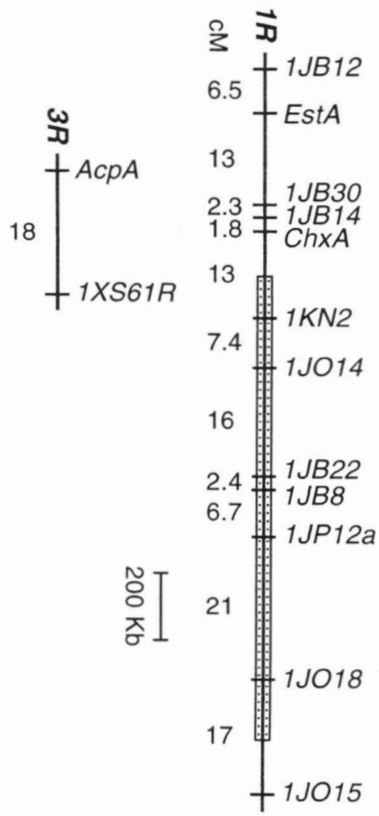

FIGURE 4.—Linkage maps of relevant segments of linkage groups in *1*R (*EstA*) and *3*R (*AcpA*). At least 45 segregants were tested for each locus, except for *J015* (only 32). The cM values represent recombination frequencies corrected for undetected multiple crossovers by the MAPMAKER program using the Haldane equation. The two-point linkage of all adjacent loci (Table 5) and the five-point order, *1JB12*—*ChxA*–*1KN2*—*1JO14*—*1JO18*, are statistically significant at the LOD 3.0 level. The shaded bar represents a partial *1*R deletion detected among members of meiotic segregant panel 3 (see text). The physical distance scale in kb is based on a preliminary estimate of 20 kb/cM for chromosome 2L (LYNCH *et al.* 1995).

(*1JB5, 1JB8, 1JB12, 1JB14, 1JB22, 1JB28, 1JB30, 1JB34, 1JO14, 1JO15, 1JO18,* and *1KN2*) map to the right arm of chromosome *1* (BRICKNER *et al.* 1996; J. ORIAS, X. SHEN and E. ORIAS, unpublished observations). *EstA,* also on *1*R, shows statistically significant linkage (LOD > 3.0) to *1JB12, 1JB14, 1JB22, 1JB30, 1KN2* and *ChxA*. The maximum likelihood estimate is 17.1 cM for the distance between *EstA* and *ChxA* (see Figure 4). For the C2 × B cross MCCOY (1977) observed 20% recombination (28 recombinants out of a total of 141 F_2 segregants when the C2/B heterozygote was crossed to C* and Round II progeny were selected). Thus our estimate of distance is not significantly different from his. The current map of the *ChxA* linkage group in chromosome *1*R (Figure 4) totals 108 cM and encompasses about 2200 kb of DNA, based on a preliminary estimate of 20 kb/cM by LYNCH *et al.* (1995).

Partial deletion of chromosome *1*R: A surprising result was the discovery of a spontaneous partial deletion of chromosome *1*R inferred to have been inherited from

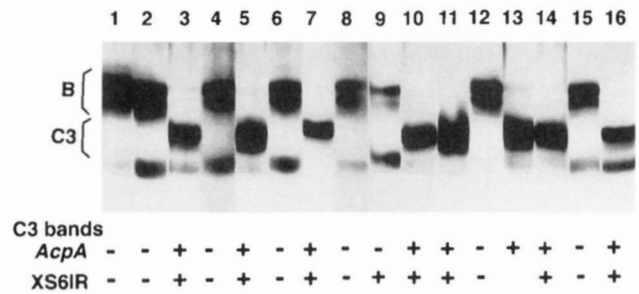

FIGURE 5.—Segregation of *AcpA* and linkage to RAPD *1XS61R*. Braces, polymorphic isozyme bands; + and −, scoring for presence of C3-derived band for *AcpA* (upper line) and *1XS61R* (lower line; agarose gel not shown). Lanes 1–14: strains SB (2352A, 2355A, 2373A, 2378A, 2406A, 2409A, 2432A, 2374C, 2377C, 2391C, 2400C, 2401C, 2426C, 2437C), respectively. Lanes 15 and 16: B and C3 parental cultures. One recombinant is seen in this group of segregants. Note that, as expected, none of the segregants are isozyme heterozygotes.

parental strain CU374 by most, if not all, of the members of meiotic segregant panel 3. The CU374 sample used to make the monosomics for mapping, thawed out independently from that used to make panel 3, shows no evidence of having this deletion. Since only a small fraction of thawed Tetrahymena cells survive (FLACKS 1979), a small clone fraction carrying the deletion may have been accidentally subcloned en route to constructing panel 3. The occurrence of spontaneous micronuclear deletions, revealed by the loss of 5S rRNA clusters, was observed earlier (ALLEN *et al.* 1984). Recently we have shown that the connected sequence of RAPDs from *1KN2* to at least *1JB8,* and possibly including *1JP12a* and *1JO18* (Figure 4), shows genetic coassortment in the macronucleus and probably is located physically on the same macronuclear autonomously replicating piece (LONGCOR *et al.* 1996). Although this connection may be purely coincidental, it raises the interesting possibility that the ends of partial *1*R deletion in the micronucleus may be related to chromosome breakage sequences (YAO *et al.* 1990) that function during macronuclear differentiation in conjugation.

A map of chromosome *3*: Mapping with the nullisomic strains places *AcpA* on the right arm of chromosome *3*. No statistically significant linkage was detected between *AcpA* and other chromosome *3* RAPDs except for the "reverse" RAPD, *1XS61R* (X. SHEN and E. ORIAS, unpublished results). *EstB,* assigned to chromosome *3*L, could not be tested for linkage to the *3*L RAPDs because inbred strains B and C3, the basis of those RAPDs, have indistinguishable *EstB* alleles. Inbred strain C2 was used in crosses to generate B/C2 heterozygotes; however, the degree of similarity of the DNA polymorphisms seen in C3 with those in C2 has not as yet been determined.

Mapping strategy: The most significant aspect of this paper is the demonstration of an effective method for mapping loci and obtaining reliable meiotic linkage data in *T. thermophila.* By coupling nullisomic mapping with

meiotic mapping, loci known to be located on a particular chromosome or chromosome arm can be tested for recombination. This approach was first used for the *mat* locus and the ribosomal RNA gene, both located on chromosome *2*L (BLEYMAN *et al.* 1992). With the advent of RAPD markers, this approach was extended to detect linkage groups in chromosome *2*L (LYNCH *et al.* 1995), *1*R, *3*L and *5* (BRICKNER *et al.* 1996). An earlier version of these chromosome assignments was communicated to CASSIDY-HANLEY and BRUNS (1993). In this work, this approach has been used to add *EstA* to and to refine the *1*R linkage group (Figure 4).

Our strategy for linkage mapping of isozyme loci took advantage of the existence of natural B-C3 differences at the *EstA* and *AcpA* loci and the availability of B-C3 meiotic segregant panels. *T. thermophila* laboratory-induced mutants, by agreement, are normally isolated in inbred strain B genetic background. Linkage testing of such mutants requires crossing the mutant to C3, isolating an *ad hoc* panel of meiotic segregants from the F_1, and testing with RAPDs assigned to the same chromosome as the mutation of interest. Knowing the chromosome assignment of the mutation and the RAPDs saves the work of testing the *ad hoc* recombinant panel with all known RAPDs.

For those isozyme loci, such as *EstB, TatA, IdhA, MdhA,* and *TzoA,* where the B and C3 alleles are identical, the first step is to map the isozyme gene to chromosomes. To do this, monosomics are generated by crossing B strain nullisomics with strains that have non-B isozyme variants. This is just what we did for *EstB* using a cross of strain C2 to a panel of strain B nullisomics. To place one of these isozyme genes (*e.g., EstB*) in a linkage group, one would next test B-C3 RAPDs in *3*L to see if they also are polymorphic between inbred strains B and C2. B-C2 RAPDs so detected would then be tested for linkage to *EstB* by using a panel of meiotic segregants from a B/C2 heterozygote.

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