ISOLATION AND GENETIC CHARACTERIZATION OF A MUTATION AFFECTING RIBOSOMAL RESISTANCE TO CYCLOHEXIMIDE IN TETRAHYMENA

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ABSTRACT

A dominant mutation at a new locus affecting resistance to cycloheximide has been isolated by exploiting a synergistic relationship with a previously known mutation for cycloheximide resistance in Tetrahymena. The new mutation (*ChxB*) was induced in a line homozygous for *ChxA* and was recovered from that background by a new technique termed *interrupted genomic exclusion*. Segregation data from the interrupted genomic exclusion suggest that *ChxA* and *ChxB* are separate, linked loci showing 30% recombination. Minimal lethal doses of cycloheximide for the four possible combinations of the wild-type and mutant alleles of these two genes are: wild type 6 μg/ml, *ChxA* 125 μg/ml, *ChxB* 10 μg/ml, *ChxA-ChxB* 175 μg/ml.

THE biosynthesis and fate of various components of ribosomes in the ciliated protozoan *Tetrahymena thermophila* (NANNEY and McCOY 1976; formerly *T. pyriformis*, syngen 1) has recently become the subject of a number of studies. HALLBERG and BRUNS (1976) have reported that ribosomal protein synthesis and ribosome accumulation occur coordinately in exponentially growing cells, and that these rates are drastically reduced during starvation. Upon refeeding, the rate of ribosomal protein synthesis per cell increases 80-fold before any accumulation of new ribosomes is seen. In addition, different ribosomal protein complements have been isolated from cells in growth (high protein synthetic activity) and nongrowth (low protein synthetic activity) situations (HALLBERG and SUTTON 1977). Such instances of experimentally inducible changes in ribosome biosynthesis and structure suggested that Tetrahymena might be a useful model system for studying coordinated control of synthesis, and structural interrelationships of the many ribosomal components.

With the development of new techniques for the isolation and analysis of mutations in this species (ORIAS and BRUNS 1976; BRUNS, BRUSSARD and KAVKA 1976; BRUNS and SANFORD 1978), a search for genetic elements involved has been initiated. Since the phenotypes are so easily selectable, resistance to drugs known to interfere with translation seemed the most direct first step. This

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approach has been extremely successful in marking genes for ribosomal elements of prokaryotes (JASKUNAS, NOMURA and DAVIES 1974; NOMURA and JASKUNAS 1976; DABBS and WITTMANN 1976) and in yeast (MCLAUGHLIN 1974; GRANT, SCHINDLER and DAVIES 1976). Cycloheximide, a well known antagonist of protein synthesis in eukaryotes (GALE *et al.* 1972), was chosen to begin our studies.

The first two cycloheximide resistance mutations in Tetrahymena, *Chx-1* and *Chx-2*, were isolated independently (ROBERTS and ORIAS 1973; BYRNE and BRUNS 1974; BYRNE, BRUSSARD and BRUNS 1978); both are dominant, show the same phenotypic responses, and appear to be mutant alleles of the same gene (BLEYMAN and BRUNS 1977).

In order to isolate mutations in other genes (hopefully including some involved with ribosome biogenesis and function), we subjected a strain with the genotype *Chx-2/Chx-2* to mutagensis and selected cells with increased cycloheximide resistance. This report describes the induction, isolation and genetic analysis of a new dominant mutation, *ChxB*, which confers slight resistance to cycloheximide by itself, but which, in combination with the original mutation (which we must now name *ChxA*) yields resistance to extremely high cycloheximide concentrations. In addition, we have developed a new technique, *interrupted genomic exclusion*, to identify recombinants between *ChxA* and *ChxB*. The method exploits new techniques for manipulating large numbers of clones and should be of general use for genetic analyses in Tetrahymena.

MATERIALS AND METHODS

General: Readers are referred to ORIAS and BRUNS (1976) for extensive explanation of methods used.

Strains: All strains were derived from inbred strain B1868, except C* and A*, which are vegetative derivatives of families C (ALLEN 1967a) and A (WEINDRUCH and DOERDER 1975), respectively. Genetic heterokaryons (BRUNS and BRUSSARD 1974b) *ChxA2/ChxA2* (cy s) mating types II and IV, and *Mpr/Mpr* (6-mp s) mating types IV and V were used. This notation indicates that the micronucleus is homozygous for a dominant drug-resistance mutation, but that the macronucleus expresses drug sensitivity. In addition, a set of seven strains, each expressing one of the seven mating types found in this species, was used for mating-type and maturity determinations.

Growth Conditions: Growth medium was 1% proteose peptone (Difco) supplemented with 0.003% Sequestrene (Geigy). Stocks were maintained in 10 ml tube cultures at room temperature and transferred by loop at least every six weeks. Cells for experiments were grown on microtiter plates (Cooke Laboratory Products, Alexandria, Va.), in tubes or in flasks, all at 30°. Flask cultures were shaken at 90 rpm on a New Brunswick Gyrotary Shaker. Cell numbers were determined on a Coulter Counter (Coulter Electronics).

Cloning: Single cells or pairs were isolated by drawing them into a pulled-out pasteur pipette (60–100 μm diameter tip) and depositing them one at a time into 60 μl drops arranged in a 6×8 array on a plastic petri plate. This pattern exactly matched the pattern of wells on a microtiter plate, so that, when grown, the drop cultures on two petri plates could be replicated to one microtiter plate.

Matings: Matings were performed in 10 mM Tris-HCl pH 7.4 as previously described (BRUNS and BRUSSARD 1974a), with cells at 1.1×10^5 cells/ml. The two mating types were prestarved separately for at least two hours and mixed, or starved together on a fast shaker (200 rpm), with mating started by turning off the shaker. Mixtures were refed by making the solution up

to 1% peptone, 0.003% sequestrene six to eight hr after the beginning of conjugation, and pairs were isolated after 30 min. Matings of large numbers of clones for analytical purposes were performed as follows. Replicates of the clones to be mated were made in V-bottom microtiter plates containing 100 μl peptone per well and allowed to grow three to four days. The entire microtiter plate was centrifuged for two min at 2100 × g in a rotor equipped to carry the plates (Cooke). Supernatant fluids were removed simultaneously from all wells on a plate by a custom-made 96 channel aspirator. The cells were quickly resuspended in 100 μl per well 10 mM Tris with a multichannel dispenser (Cooke), and washed twice more in the same manner with the final resuspension in 10 mM Tris at 50 μl per well. Next, 50 μl of the appropriate prestarved tester strain was added to each well with a micropipette (Cooke), and the plates were placed at 30°. Pairs that formed were scored under a dissecting microscope four to 12 hr later. After 15 hr, 25 μl of 5% peptone, 0.015% sequestrene were added to each well. Fresh peptone-containing plates were inoculated by replica plating six hr later if additional phenotype tests were required.

Assortment: Phenotypic assortment of heterozygotes to obtain cells expressing the recessive sensitivity phenotype (BRUNS and BRUSSARD 1974b) was followed by selected subcloning. In this procedure, a pair was isolated and allowed to grow for about 18 fissions, at which time 96 subclones were isolated. These were allowed to grow and replicates were tested to identify the subclones containing sensitive cells, which were further subcloned. Every 18 fissions, 96 subclones were taken from only those subclones that had the most cells expressing the recessive phenotype in the previous test. A line was considered stable when all subclones respond homogeneously. Once isolated, stabilized subclones never assorted cells expressing the other allele.

Mating type and maturity tests: Maturity tests of unknowns were done in V-bottom microtiter plates by assaying the ability to form pairs with a mating-type I tester strain. Since the strain used in this study never expresses mating-type I, failure to form pairs with mating-type I indicated immaturity. To determine mating type, unknowns were replicated to microtiter plates such that all eight wells in a given column had the same unknown. As many as 11 unknowns (plus an empty column control) could be tested on a single plate. The cells were grown and washed as described above. Prestarved mating-type testers were added. All eight rows of the microtiter plate were utilized: rows one through seven were filled with testers for mating-types I-VII, respectively, and the last row had no tester added as a control for the rare unknowns able to undergo intraclonal mating. All mating-type and maturity test plates were scored between four and 12 hours and discarded.

Drug testing: Three concentrations of cycloheximide (cy, Sigma) were required to distinguish resistance phenotypes. The concentrations used were 140 μg per ml, 25 μg per ml and 7 μg per ml. Stock solutions were made at 7.0 mg per ml in distilled water, sterile filtered and stored at 4°. These stocks were never kept more than a week as biological activity appears to decrease under these conditions (ORIAS, personal communication). The 6-methylpurine (6-mp, Sigma) concentration used to select 6-mp r cells was 15 μg per ml. Stock solutions were 1.5 mg per ml sterile filtered, and kept in the cold. Testing of microtiter plate cultures involved adding 25 μl of 5× drug in peptone to each 100 μl well. Resistance was clearest if replicates were six to 18 hr old when the drug was added, and were observed four to seven days later.

Cycloheximide resistance of mutant lines: To determine killing doses of the drug in flasks, exponentially growing cultures were adjusted to 2 to 3 × 10^4 cells per ml, and various concentrations of drug were added. Each day, small samples were observed under a dissecting microscope, and 0.1 ml of the culture was transferred to 10 ml of fresh 1% peptone, 0.003% sequestrene. The culture was considered killed if after three days no live swimming cells were seen and no cells grew in the transfer culture. Killing doses were also determined in microtiter plates by replicating dense plate cultures to fresh plates of 1% peptone, 0.003% sequestrene, allowing six to 18 hr of growth at 30° and then adding a different drug concentration to each row. The plates were observed daily thereafter, and if all wells in a row were devoid of swimming cells after four days, the dose used on that row was considered lethal.

Mutagenesis: The method used ("short-circuit genomic exclusion") has been previously described (BRUNS, BRUSSARD and KAVKA 1976). Briefly, cells were exposed to 10 μg/ml N-methyl-

N'-nitrosoguanidine for three hr, and then mated to strain C* prior to selection, so that micronuclear (sexually heritable) mutations are brought into a new macronucleus and thus expressed phenotypically.

RESULTS

Mutagenesis: Approximately 4×10^6 mutagenized *ChxA/ChxA* (cy s, IV) cells were mated to C*III and passed through short-circuit genomic exclusion (BRUNS, BRUSSARD and KAVKA 1976). Successful short circuit progeny were selected by incubating the mating mixture in 1% peptone + 25 μg per ml cycloheximide (25-cy) for two days. Variants resistant to unusually high concentrations of cycloheximide were selected by bringing 1×10^6 of these short-circuit progeny to 140 μg per ml cycloheximide (140-cy), a concentration not tolerated by cells expressing the *ChxA* mutation alone (BYRNE and BRUNS 1974). After eight days at 30°, aliquots of this culture were observed under a dissecting microscope; we saw much cellular debris, but no live cells. This 10 ml culture was centrifuged and the pellet was resuspended in 100 ml of fresh 140-cy medium. After seven more days, single cells and dividers were observed and isolated into droplets of 1% peptone. A vigorous clone, designated SCDIO, was chosen for further study.

F_1 cross and phenotypic assortment: Clone SCDIO was mated to strain *Mpr/Mpr* (6-mp s, V), a strain homozygous in the micronucleus for a dominant mutation conferring 6-mp resistance, but with a macronucleus expressing sensitivity; pairs were isolated and cloned. The surviving clones were replicated and tested for drug resistance. Since only true progeny have macronuclei containing the *Mpr* allele, they will be uniquely resistant to 6-mp (see BRUNS and BRUSSARD 1974b for details). Cycloheximide resistance was evaluated only among the 6-mp resistant cells. Of the 94 pairs isolated, 69 (73%) lived. Of these, 62 (90%) were 6-mp resistant. Among these, 33 were 140-cy resistant, and 29 were 140-cy sensitive, but 25-cy resistant. We conclude that resistance to 140 μg per ml cy is dominant and clone SCDIO is heterozygous for the new mutation.

Phenotypic assortment, a phenomenon of the macronucleus by which cells expressing the phenotype of only one member of an allelic pair assort during vegetative growth (for a review, see SONNEBORN 1975), was employed to distinguish whether 140-cy resistance was caused by a new allele at the *ChxA* locus, or by a second-site mutation synergistic with *ChxA*. Since the original strain used for mutagenesis was homozygous for *ChxA*, the 140-cy resistant F_1 progeny would be either: (1) heterozygous for the new mutation and *ChxA*$^+$ (if the new mutation occurred at the *ChxA* locus), or (2) a double heterozygote with mutant and wild-type alleles at the *ChxA* locus and at a new locus. Since intragenic assortment has not yet been detected (BLEYMAN and BRUNS 1977; FRANKEL *et al.* 1976), we assumed that the first case would result in only two possible assorting phenotypes: 140-cy resistant and 25-cy sensitive. The second situation could result in four phenotypes (depending on the phenotype of the new mutation expressed by itself), but at least three should certainly be found: 140-cy resistant, 140-cy sensitive-25-cy resistant, and 25-cy sensitive. Four F_1 synclones (*i.e.*, the

clones of four isolated pairs) initially expressing 140-cy resistance were subcloned. In each, subclones expressing each of the three phenotypes mentioned above were isolated. Thus the intial phenotypes of the F_1 synclones, plus the phenotypes they subsequently assorted, strongly suggested that dominant mutant alleles at two separate loci were combining to give 140-cy resistance.

Interrupted genomic exclusion, analysis of Round I micronuclei: Following phenotypic assortment, a subline sensitive to both cycloheximide and 6-mp and expressing mating type II was isolated from an F_1 synclone, and was mated to cells of strain A*III, which is also sensitive to both drugs. The two parents were starved together without pair formation on a fast shaker (BRUNS and BRUSSARD 1974a). Peptone was added to the mating mixture six hours after the fast shaker was halted, and 256 pairs were isolated and incubated at 30°. Six hours later (12 hours after the shaker was halted) 225 of the drops contained exactly two cells, and each of these was isolated into adjacent drops. Of these, both exconjugants were successfully cloned from 89 pairs (40%). Master plates were made by replicating grown drops to a microtiter plate.

The nuclear events of crosses to strains such as A* have been previously described (ALLEN 1967a,b), and are summarized in Figure 1. The A* micronucleus degenerates at meiosis, a haploid product of meiosis in the F_1 conjugant is duplicated mitotically, and one of the resulting nuclei is transferred to the A* conjugant. Since the nuclei become diploid by endoreduplication (ALLEN 1967a,b), the two exconjugants have identical homozygous micronuclei. Because new macronuclei do not develop from the new micronuclei at this round of mating, all the round I exconjugants have parental phenotypes. All the exconjugant clones from the cross of the F_1 by A* in this study were mature and had retained their parental phenotypes: both exconjugants from each isolated pair were sensitive to both drugs, and only one exconjugant clone was able to pair with a mating type II tester. Finally, because the micronuclear genome of each synclone (pair of exconjugant clones) represents a unique meiotic product of the F_1, a 1:1 ratio of synclones containing one or the other of the two alleles from any locus heterozygous in the F_1 is expected. Thus, the genetic basis of the 140-cy

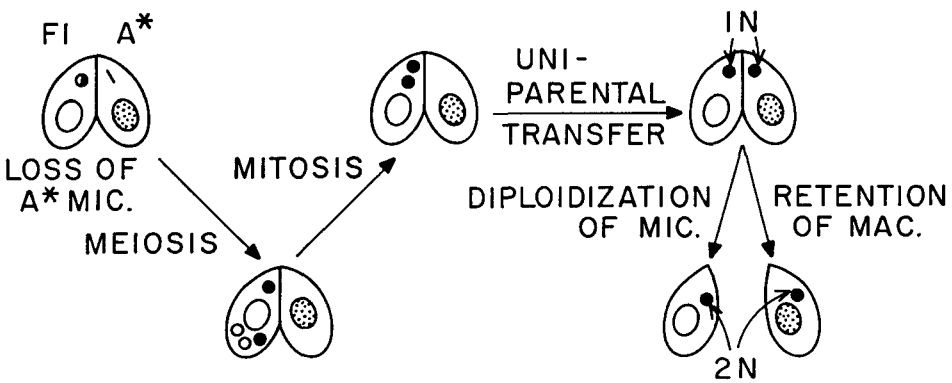

FIGURE 1.—Nuclear events during round I mating in genomic exclusion. See text for details.

resistance, and evidence for linkage of any new mutations to known markers in the F_1 was investigated by analyzing the genotypes of round I micronuclei.

An analysis of round I micronuclei was performed in the following way. As indicated in Figure 2, exconjugant clones were replicated into V-bottom microtiter plates, grown, and mated to two different strains. Replicate set one was crossed to a heterocaryon for the *ChxA* allele: *ChxA/ChxA* (7-cy s, II)). After allowing sufficient time for the completion of conjugation, peptone was added, the cells were allowed to grow, and two replicates were made. The first was taken to 15 μg per ml 6-mp, the second to 140 μg per ml cy. Replicate set two was mated with wild type expressing mating type IV, and tested for growth in 25 μg per ml cy.

Table 1 presents all the results of this analysis, and indicates, as explained below, the genotype revealed in the micronuclei of the corresponding round I exconjugant clones. Since both the assortment data presented above, and the results of this analysis indicate the presence of a dominant mutation at a locus separate from *ChxA*, we have named the new mutation *ChxB*. Resistance to 6-mp in cross one indicated that the round I exconjugant clone had retained, and become homozygous for, the *Mpr* allele during round I of genomic exclusion. Resistance to the 140 μg per ml cy in cross one indicated the presence of the *ChxB* mutation in the round I micronuclei, but demonstrated nothing about the presence or absence of the *ChxA* allele, since the other parent in this cross was homozygous for *ChxA*. The test with 25 μg per ml cy in cross two indicated the *ChxA* constitution of the corresponding round I exconjugants. Resistant cells demonstrated the presence of the *ChxA* mutation in the round I micronuclei; a well containing only sensitive cells indicated round I micronuclei homozygous for the wild type allele. A phenotype of the *ChxB* mutation alone was identified

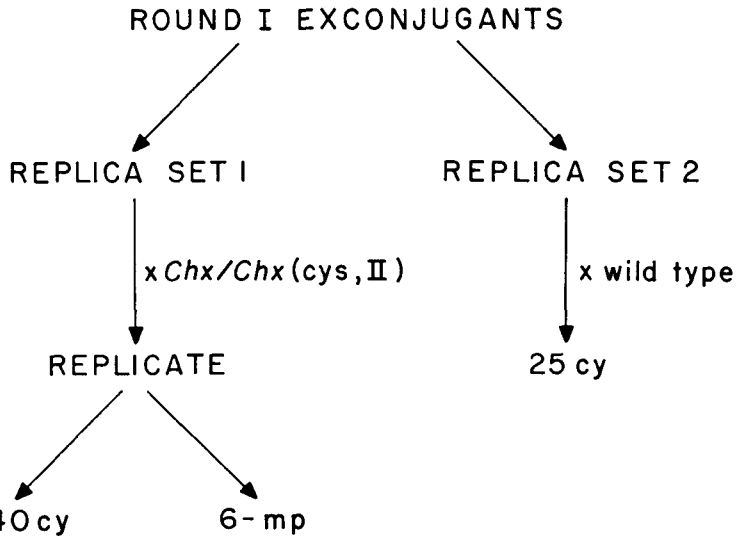

FIGURE 2.—Manipulations of round I exconjugant clones to identify micronuclear genotypes.

TABLE 1

Interrupted genomic exclusion

Round I exconjugants testcrossed by: Cross 1 *Chx/Chx* (cy s, II) 6-mp test	140-cy test	Cross 2 Wild type 25-cy test	Allelic constitution of round I micronucleus			Number
+	+	+	*Mpr*	*ChxA*	*ChxB*	11
+	+	—	*Mpr*	*ChxA*+	*ChxB*	5
+	—	+	*Mpr*	*ChxA*	*ChxB*+	6
+	—	—	*Mpr*	*ChxA*+	*ChxB*+	10
—	+	+	*Mpr*+	*ChxA*	*ChxB*	16
—	+	—	*Mpr*+	*ChxA*+	*ChxB*	8
—	—	+	*Mpr*+	*ChxA*	*ChxB*+	9
—	—	—	*Mpr*+	*ChxA*+	*ChxB*+	24
					Total	89

by noting many instances of exconjugants resistant to 140 μg per ml cy in cross one, but sensitive to 25 μg per ml cy in cross two; thus this new mutation by itself does not confer high levels of resistance. Similarly, *ChxB*+/*ChxB*+ with the *ChxA* mutation yielded a unique, identifiable set of phenotypes: sensitive to the 140 μg per ml cy in cross one, but resistant to the 25 μg per ml in cross two. Finally cross two provides a test to eliminate all pairs of exconjugant clones that had not actually performed the micronuclear events of round I. All instances of successful round I events yield pairs of exconjugant clones that give identical results in this test; all instances of a pair retaining parental micronuclei would yield one 25-cy resistant and one 25-cy sensitive exconjugant clone in this test. Since none of the paired exconjugant clones differed in the 25-cy test in cross two, we conclude that all had completed round I micronuclear events. Table 1 lists these various phenotypes and genotypes, and presents the number of exconjugant pairs in each class.

The mutant *Mpr* allele was recovered in the progeny less frequently than its wild-type allele (32 *Mpr*:57 *Mpr*+), for unknown reasons. Since the phenotype of the round I micronuclear genotype was not expressed until the testcross was performed, the bias should not represent a vegetative selection for the wild-type allele, but rather some event at meiosis. No linkage can be detected when the frequency of recombinants to parentals for *Mpr* and either *ChxA* or *ChxB* is evaluated. For both *Mpr-ChxA* and *Mpr-ChxB*, 49 of the 89 synclones were recombinant (χ^2 for this deviation from 1:1 = 0.91, $p = 0.36$). On the other hand, *ChxA* and *ChxB* seem to be linked; only 28 of the 89 synclones were recombinant (χ^2 for this deviation from 1:1 = 12.24, $p < 0.0005$).

Two of the clones identified by the testcrosses as having the micronuclear genotype *Mpr*+/*Mpr*+ *ChxA*+ *ChxB*/*ChxA*+ *ChxB* were crossed to wild type and the *ChxA ChxB*+/*ChxA ChxB*+ (7-cy s, IV) heterokaryon. Progeny of the cross to wild type were initially 25-cy sensitive—7-cy resistant, and assorted 7-cy sensitive cells when grown. Progeny of the crosses to the heterokaryon were initially 140-cy resistant, and assorted 7-cy sensitive progeny with growth.

Round II genomic exclusion: To effect expression of the homozygous genome contained in the micronuclei of round I exconjugants, the two exconjugant clones of each pair that had been determined to have *ChxB* in their germ line were remated; in contrast to round I mating, this time macronuclear development occurs. Replicates of seven pairs from each of the round I synclones were tested for immaturity and then drug resistance. Many of these matings did not yield healthy progeny. Often immature progeny were obtained only by exploiting the drug-resistance markers contained in these lines. Thus progeny could be selected from mass matings by addition of the appropriate drug (cy in various doses for all, 6-mp for some), since the parents for all these crosses were phenotypically sensitive to both drugs. On the other hand, there were pairs of round I exconjugant clones containing all combinations of *ChxB* plus the other two allelic pairs (*ChxA* or *ChxA*+ and *Mpr* or *Mpr*+) that yielded immature progeny expressing the expected phenotypes from isolated pairs, without selection. Thus it was possible to isolate strains with the micronuclear genotypes *ChxA ChxB/ChxA ChxB*, and *ChxA*+ *ChxB/ChxA*+ *ChxB* in cells with macronuclei expressing the phenotypes 140-cy resistant, and 25-cy sensitive—7-cy resistant, respectively. That these clones were true progeny was further verified by the subsequent mating types expressed at maturity; all allelic combinations had at least one clone expressing a nonparental mating type. Since all of the progeny from these round I matings grew well, but still expressed the expected drug resistances, the poor performance of the other round II progeny probably was caused by general damage to the genome by the heavy mutagen treatment, rather than by a lethality specifically associated with the new mutation, *ChxB*.

The resistance phenotypes: Round II progeny, homozygous for *ChxB*, with and without the *ChxA* mutation, were used to measure the drug resistance phenotype of the four homozygous combinations of these two loci. Table 2 presents this comparison. Cells homozygous for *ChxB* alone are only slightly more resistant to cy than wild type, but not at all as resistant as the *ChxA* homozygotes. This phenotype (resistant to 7-cy, but sensitive to 25-cy) is also expressed in heterozygous progeny of backcrosses of the *ChxA*+ *ChxB/ChxA*+ *ChxB* homozygotes to wild type. Resistance of the double mutant is higher than that of either of the single mutants.

TABLE 2

Cycloheximide resistance of four homozygous strains (in μg/ml)

Strain		Minimum lethal dose*		Dose used to distinguish phenotypes
		In flasks	In microtiter plates	
ChxA+	*ChxB*+	6	6	
ChxA+	*ChxB*	10–12	9	7
ChxA	*ChxB*+	125	130	25
ChxA	*ChxB*	175–200	180	140

* See MATERIALS AND METHODS for definitions.

DISCUSSION

As the number of mutant genes in Tetrahymena increases, already characterized mutations can be used to isolate new mutations. This paper presents the isolation of a new mutation affecting cycloheximide resistance from an already resistant background. Evidence to be reported elsewhere (SUTTON, ARES and HALLBERG 1978) has revealed that both *ChxA* and *ChxB* mutants have ribosomes with altered cycloheximide resistance *in vitro*. Thus, by mutagenizing a *ChxA* background and requiring higher resistance, we have isolated a new ribosomal cycloheximide-resistance mutation, *ChxB*. We are continuing to seek second-site mutations that enhance or suppress the phenotype of mutations known to affect ribosomes, in order to identify genes involved with ribosomal synthesis and/or structure.

As the number of mutant genes in Tetrahymena increases, new problems in performing genetic analyses arise. The approach of using enhancement or suppression of a given genotype to isolate new mutant genes with associated functions is hampered by the problem that the phenotype of the new mutation by itself is unknown. Another problem arises during linkage testing between complementing mutations with identical phenotypes: double mutants cannot be distinguished simply from single mutants. Examples of such situations include temperature sensitivity, auxotrophy, and two mutations with mutually exclusive phenotypes, such as heat sensitivity for growth and a specific surface antigen expressed only at high temperature (as the T antigen, see PHILLIPS 1967). The specific technique presented here, interrupted genomic exclusion, simplifies these analyses by creating a collection of clones with recombinant micronuclei homozygous for the various combinations of the parental genotype, but with macronuclei still expressing parental phenotypes. Thus testcrosses to identify the allelic composition of the micronucleus of each of these clones can be done en masse (no need for pair isolations) and immediately (no need to grow cultures to maturity).

The technique may be summarized by dividing it into three steps: (1) isolating vegetative segregants of the F_1 expressing the appropriate phenotype for all markers, (2) crossing to a "star" strain (A* or C*) and cloning the two round I exconjugants from each of many pairs, and (3) analyzing the micronuclei of the exconjugants by the appropriate mass testcrosses. Once the allelic constitution of each micronucleus is known, the appropriate pair of exconjugants can be saved for complementation testing with any other round I exconjugant. Since all round I conjugants contain homozygous micronuclei, and since the two exconjugant clones from each pair express two different mating types, at least one of the round I exconjugants from any pair can be mated with any other clone, no matter what its mating type. Finally, the round I exconjugants of a pair may be mated with each other (round II) to generate a strain with the same homozygous genome in micro- and macronucleus.

The linkage relationship suggested by the interrupted genomic exclusion data is considered tentative, for two reasons. First, differences in recombination fre-

quency may exist between crosses involving "star" strains and normal conjugation, and second, one parent of the F_1 line used had been heavily mutagenized. This does not detract from the ability of the method to direct linkage relationships; it implies that the specific relationship between *ChxA* and *ChxB* should be rechecked by both "star" crosses and normal testcrosses after a series of backcrosses insures that residual mutagen damage is minimal.

Finally, the 1:1 ratio of 140-cy r:140-cy s obtained in the F_1 indicates that the cells recovered following mutagenesis and short-circuit genomic exclusion were heterozygous at the *ChxB* locus. This differs with previous observations of the recovery of cells homozygous for a recessive drug-resistance mutation (BRUNS, BRUSSARD and KAVKA 1976) and a large number of independent temperature-sensitive mutations (BRUNS and SANFORD 1978) following mutagenesis and short-circuit genomic exclusion. McCOY (unpublished) passed an unmutagenized strain, heterozygous for two codominant alleles at the *H* locus (H^E/H^D) (see NANNEY and DUBERT 1960 for description of this locus) through short-circuit genomic exclusion and found that out of 200 progeny, no heterozygotes were recovered. Whatever the origins of the original 140-cy r isolate, it seems clear that, although most cells recovered from short circuit genomic exclusion are the result of self-fertilization (probably endoreduplication of a haploid nucleus), occasional cases of heterozygotes can arise, at least following mutagenesis. Thus, although short-circuit genomic exclusion is very useful for isolating mutants, it may not be entirely reliable for genetic analyses. On the other hand, analyses of "normal" genomic exclusion (ALLEN and LEE 1971; ALLEN 1967a,b; McCOY 1973) have indicated that only homozygous round I micronuclei arise from that process.

In conclusion, the method of interrupted genomic exclusion may prove useful in organizing the expanding catalogue of mutations in Tetrahymena. Exploiting the division of nuclear function in Tetrahymena, and independently manipulating the two nuclei through round I of genomic exclusion and phenotypic assortment, should simplify genetic analysis of a number of useful mutations.

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