POSITIVE SELECTION FOR MATING WITH FUNCTIONAL HETEROKARYONS IN *TETRAHYMENA PYRIFORMIS*

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ABSTRACT

Allelic assortment of *Tetrahymena pyriformis* makes possible the isolation of cells which contain both a heterozygous germ line nucleus and a somatic nucleus which expresses phenotypes of only one member of allelic pairs. Cycloheximide-sensitive segregants have been isolated from the vegetative progeny of cells heterozygous for a dominant mutation conferring resistance to cycloheximide; such segregants are called functional heterokaryons. Since progeny from crosses of these cells are cycloheximide-resistant, addition of the drug provides positive selection for successful exconjugants. The timing for expression of the new phenotype during conjugation is presented and used to identify what appear to be immature progeny of round one mating in genomic exclusion. Usefulness of functional heterokaryons in a number of genetic and developmental studies is discussed.

CONJUGATION in ciliated protozoa is both a necessary tool for genetic analysis and a potential model system in development. Sexual strains of *Tetrahymena pyriformis* contain two specialized nuclei: a germinal (micro) and a somatic (macro) nucleus. Conjugation is an ordered sequence which involves cell fusion, development of male and female pronuclei from the micronucleus, exchange and fertilization of pronuclei to form a diploid zygote nucleus, and development of new macro- and micronuclei from the zygote nucleus. Thus conjugation ultimately leads to the formation of new, recombinant nuclei, and establishes a dual system in which the same genome is maintained in very different functional and structural states (GOROVSKY 1973). Exconjugant cells with a newly formed macronucleus are considered immature, as they are unable to reenter the sexual phase. Vegetative growth of 40–80 fissions following conjugation is necessary before the cells are once again able to form pairs (NANNEY and CAUGHEY 1953; BLEYMAN 1971).

The inability to select for successful mating has posed a major problem in this system. The best mating mixtures never achieve 100% pairs, and not all pairs complete conjugation. Cells previously exposed to mutagens typically show a dramatic decrease in pair formation, and a reduced ability of isolated pairs to generate immature, viable exconjugants. In addition, nuclear reorganizations involving self-mating which have been extremely useful for genetic analyses and mutagenesis in other ciliates—for example, autogamy in *Paramecium*

aurelia (SONNEBORN 1947)—have never been found in Tetrahymena. A scheme allowing positive selection for cells with new macronuclei should facilitate the isolation of successful exconjugants in crosses, and make possible the identification of rare self-mating, whether natural or induced. Allelic assortment of dominant positively selectable markers allows the isolation of strains which make this kind of selection possible.

Allelic assortment in Tetrahymena was first described by NANNEY and DUBERT (1960) with a set of allelic variants of a surface antigen. It has been observed with such diverse genetic markers as several temperature- and saline-dependent surface antigens (PHILLIPS 1967; GRASS 1972; DOERDER 1973), enzyme variants (ALLEN 1965), drug resistance (CARLSON 1971), a heat-sensitive locus (ORIAS and FLACKS 1973), and the locus conferring resistance to cycloheximide used in this study (ROBERTS and ORIAS 1973). The phenomenon involves the appearance of cells during vegetative growth which phenotypically express only one of the two alleles present in genetic heterozygotes. Such vegetatively assorted variants have been cloned, and in all cases have produced completely stable subclones, continuing to express the phenotype of only one of the alleles. Yet these cells always behave as heterozygotes in crosses. Thus the genetic constitution of the germinal (micro) nucleus remains stable, whereas the phenotypic expression of the somatic (macro) nucleus is subject to change during vegetative growth. Because this phenomenon has been found with gene systems showing dominance (ROBERTS and ORIAS 1973; BYRNE, BRUSSARD and BRUNS, unpublished), it is possible to isolate cells heterozygous for a dominant allele that express the phenotype of the recessive allele; when heterozygotes of a dominant mutation conferring resistance to cycloheximide are used, cycloheximide-sensitive segregants can be isolated. Use of such segregants to eliminate non-conjugant progeny was suggested by ROBERTS and ORIAS (1973). This communication describes such isolates, demonstrates how they can provide positive selection for mating, and discusses their use in a number of genetic and developmental analyses.

MATERIALS AND METHODS

Strains:

All cells were derived from the inbred strains of *Tetrahymena pyriformis*, syngen 1. Unless specified, wild-type cells were strain B1868 mating types IV and VII.

The cycloheximide-resistant strain Chx-F3 was kindly provided by C. ROBERTS and E. ORIAS of the University of California, Santa Barbara. The original mutation was isolated from the progeny of a cross between two strains which had been derived from family D and subjected to mutagenesis by treatment with N-methyl, N′-nitro, N-nitrosoguanidine. Chx-F3 is a homozygous strain, resulting from two rounds of mating with strain C*, to induce genomic exclusion (ALLEN 1967a,b).

Strain C* is a vegetative derivative of inbred family C (ALLEN 1967b).

Media:

1) All growth except where otherwise noted was supported by 1% proteose peptone (Difco).

2) Bacterized peptone: *Klebsiella aerogenes* was held in stationary phase liquid cultures in 1% proteose peptone at 15°. Just prior to use, the bacterial culture was diluted 1:100 in room temperature distilled H_2O.

Establishment of axenic cultures:

A slightly altered version of the method of ORIAS and FLACKS (1973) was employed to rid the culture of contaminating bacteria. Using a micro pipet, 5 to 10 cells were isolated into drops containing 2 mg/ml of both Penicillin G and streptomycin sulfate, in 1% proteose peptone. After 15 minutes of incubation at room temperature, all robust cells were transferred to a fresh drop of the antibiotics and again incubated at room temperature for 15 minutes. Another transfer was made into a fresh drop of the antibiotics, again selecting only vigorously swimming cells. Following two to three days' incubation at room temperature, all uncontaminated drops were isolated into tubes of sterile 1% proteose peptone.

Timed axenic matings:

The method previously described was used (BRUNS and BRUSSARD 1974a). Cells of different mating types were grown to mid log phase. Equal numbers were mixed and gently washed twice with sterile 10 mM Tris-HCl, pH 7.4. The mixture was centrifuged a final time and resuspended in fresh sterile 10 mM Tris buffer at a concentration of $6–10 \times 10^4$ cells/ml. Mating mixtures of 12 to 25 ml were put into 250 ml Erlenmeyer flasks. The flasks were immediately put on a New Brunswick Model G76 Gyrotary Water Bath Shaker, at 30°, and swirled at 200 r.p.m. for at least 2 hours, but could be held on the shaker for over 24 hours. Pair formation begins within an hour after cessation of swirling.

Pair isolation:

Pairs were isolated from mating mixtures into 0.1 ml 1% peptone drops in 100×15 mm plastic petri dishes. The drops were arranged to match the pattern of a 32-place aluminum replicator (Melrose Machine Shop, Woodlyn, Pennsylvania); the 8 peripheral prongs (2 prongs in each corner) were never used. Three days' incubation at 30° provided well grown drops; extremely dense cultures (ca. 10^6/ml) were achieved if 0.003% Sequestrene (GEIGY, ARDSLEY, New York) was added to the medium (B. C. BYRNE, unpublished).

Maturity testing:

The following method was perfected by B. C. BYRNE, and will appear elsewhere. Petri plates were established with 0.10 ml drops of 10 mM Tris buffer in the replicator pattern. Known mature cells of a non-parental and the two parental mating types were separately washed in 10 mM Tris, concentrated to 5×10^5 cells/ml, and 20 ml of each suspension put into sterile petri plates. Both the washed concentrates of knowns and the sequestrene-enriched, grown drop cultures of unknowns were replicated into the 10 mM Tris drops. The unknowns were not washed because the dilution of nutrients during replication into the Tris drops was sufficient for starvation and mating. The mixtures were then incubated at 30° and periodically observed for 3 days. Absence of any pair formation in all three mating type combinations was judged as adequate proof of immaturity.

Drug testing:

1) Plate tests: Petri plates were set up with 0.07 ml drops of 1% peptone in the replicator pattern. Grown drop cultures of unknowns were replicated into the peptone drops. 2 μl of an 0.9 mg/ml sterile cycloheximide solution were added to each drop by use of a 100 μl Hamilton syringe fitted with a repeating dispenser, to make a final concentration of 25 μg/ml drug. Visual inspection of the drops with a dissecting microscope after a 3–5 day incubation at 30° was adequate to determine resistance.

2) Tube tests: Wasserman (13×100 mm) tubes with 2.5–3.0 ml 1% peptone containing 25 μg/ml cycloheximide were inoculated with 0.1–0.2 ml unknowns. Growth of resistant cells could easily be seen with the unaided eye after 5–10 days incubation at 30°.

RESULTS

ISOLATION OF A FUNCTIONAL HETEROKARYON

Strain Chx-F3 was mated in sterile conditions to inbred strain B1868. Pairs

were isolated and clones demonstrating immaturity were retained; immaturity is a feature of cells containing new macronuclei following successful conjugation. All of the progeny demonstrated the same degree of resistance to cycloheximide characteristic of the Chx-F3 parent (slightly retarded growth in 25 μg/ml of the drug). Wild-type cells (B1868) in culture conditions otherwise favoring log phase growth are killed within 24 hours at 30° by 25 μg/ml. Backcrosses of the progeny to the wild-type parental strain repeatedly produced exconjugants showing a 1:1 ratio of sensitive:resistant, confirming ROBERTS and ORIAS' (1973) demonstration that the cycloheximide resistance is due to a dominant allele.

The resultant heterozygotes were subcloned into bacterized peptone by single cell transfers. By transferring a single cell from each subclone to fresh bacterized medium, and testing the phenotype of the remaining cells in bacterized peptone containing 25 μg/ml cycloheximide, it was possible to establish and test new subclones every two to three days. By approximately 130 fissions several of the leftover cultures showed sensitivity to the drug, and these clones were retained and made axenic by penicillin-streptomycin washes. All of the rest of the work described in this paper was done using sterile conditions, 1% peptone as growth medium and sterile 10 mM Tris for the matings.

One of the sensitive subclones was backcrossed to parental strain B; when pairs were isolated, cloned and tested for immaturity, 75 immature clones developed. Testing for the phenotype of cycloheximide resistance revealed a ratio of 37 resistant: 38 sensitive. Thus the sensitive segregant was expressing the phenotype of the recessive allele, although its micronucleus was still heterozygous; the macronucleus had undergone allelic assortment. Because (1) allelic assortment is associated with just one of the two nuclei, and (2) the underlying mechanism is still unknown (WOODARD, KANESHIRO and GOROVSKY 1972), we propose that sensitive strains such as described here be called functional heterokaryons.

NOMENCLATURE FOR FUNCTIONAL HETEROKARYONS

In order to denote both the sexually heritable genotype (micronucleus) and the phenotypic expression (macronucleus), we employed the following system. (1) The genotype of the micronucleus is expressed by lower case italicized letters for each allele; a capital first letter denotes a mutant allele that is dominant to wild type. (Dominance is determined by examining the phenotype of heterozygotes within a few fissions after conjugation, before macronuclear assortment can occur.) Wild-type alleles are designated by a superscript +. (2) The phenotypic expression of the macronucleus is designated in parentheses by words or abbreviations in lower case, non-italicized letters. Mating type is indicated by roman numerals to conform to the already established practice. Thus among the cells mentioned above, the original F1 before vegetative assortment is written as *Chx-1/Chx-1*$^+$ (cy. rest., II) since it is cycloheximide-resistant and mating type II. The selected sensitive segregant is indicated as *Chx-1/Chx-1*$^+$ (cy. sens., II). If more than one marker is involved, the phenotypic designations are separated by commas, so that a mating type II functional heterokaryon for both the cyclo-

heximide marker and a dominant mutation conferring resistance to 6-methyl purine (B. C. BYRNE and P. J. BRUNS, in preparation) is denoted as *Chx-1/Chx-1*$^+$ *Mpr/Mpr*$^+$ (cy. sens., 6-mp. sens., II).

POSITIVE SELECTION OF SUCCESSFUL CONJUGATION

Figure 1 briefly reviews the events associated with conjugation in Tetrahymena. Since the new macronucleus is derived directly from the newly fertilized zygote nucleus, phenotypes with normal mendelian expectations are expressed in the progeny; the phenotypes are modified by allelic assortment only after a period of growth. Thus, when the cycloheximide-sensitive functional heterokaryon is mated to wild type (also sensitive), half of the progeny will be resistant to cycloheximide.

A related paper (BRUNS and BRUSSARD 1974a) demonstrates that pair formation will begin about one hour after mixing two mating types which have been prestarved for at least two hours in 10 mM Tris. In addition, similar timing was achieved by starving the two mating types in the same flask on a shaker (see MATERIALS AND METHODS); pairs began forming about one hour after the shaker was turned off. Using the time of shaker-off as zero, normal conjugation can be characterized by timing the appearance of resistant cells in the mating mixture. Mixtures of prestarved *Chx-1/Chx-1*$^+$ (cy. sens., II) and *Chx-1/Chx-1*$^+$ (cy. sens., IV), were made at 10^5 cells/ml, and hourly 0.1 ml samples were used to inoculate tubes containing 2.5 ml of 25 μg/ml cycloheximide in 1% proteose peptone. The tubes were incubated at 30° for 4 days, and then checked for live cells. Growing cultures first appeared in those tubes inoculated with cells taken 30 hours after the shaker was turned off.

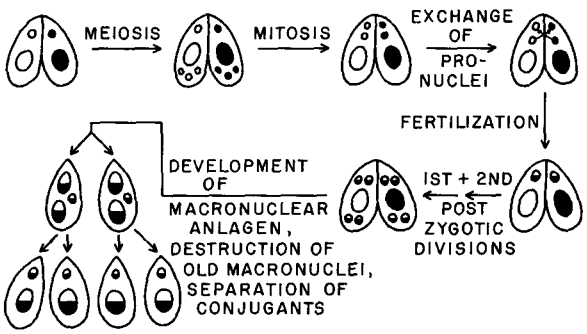

FIGURE 1.—Conjugation in *Tetrahymena pyriformis*. Each conjugant undergoes meiotic reduction of the micronucleus, retains one of the haploid products, and mitotically doubles it. Mutual exchange of haploid male pronuclei and subsequent fertilization of female pronuclei produce the diploid zygote nuclei. Two mitotic divisions (post-zygotic divisions) of the zygote nuclei are followed by (1) DNA synthesis in the macronuclear anlagen, (2) retention of one new micronucleus, and (3) loss of the second new micronucleus plus the parental macronucleus. The first cellular division segregates the new macronuclei, reconstituting the normal makeup of one macronucleus and one micronucleus.

The effect of added nutrients was tested, since matings are normally carried out in 10 mM Tris. Samples of mating mixtures were brought to 1% peptone with the addition of 10% peptone at various times; aliquots were inoculated into cycloheximide-peptone at half-hour intervals. Although appearance of the new phenotype was hastened, peptone had an effect only after time 14 hours. Resistant cells appeared at 15.5 hours when peptone was added any time between 3 and 14 hours. Figure 2 expresses these results as lag (hours from addition of peptone to first expression of resistance) *versus* time of feeding; the time when peptone has an effect is seen as a turn in the curve at 14 hours. The data from Figure 2 thus define conditions for positive selection of normal mating with this gene system. For example, if a *Chx-1/Chx-1*$^+$ (cy. sens., II), *Chx-1/Chx-1*$^+$ (cy. sens., IV) mixture is fed at time 6 hours, resistant progeny will be available 10 hours later. Samples of the cycloheximide-selected cells from many of the time points in these experiments were tested for ability to form pairs, and were found to be immature in all cases.

HOMOZYGOUS FUNCTIONAL HETEROKARYONS

When a functional heterokaryon such as *Chx-1/Chx-1*$^+$ (cy. sens., II) is crossed with wild type, only half the progeny will be resistant heterozygotes; the rest will be homozygous recessive, and therefore sensitive. The efficiency of such matings can be doubled by crossing to strains which have micronuclei homozygous for the selectable dominant allele, but which still have macronuclei expressing the recessive phenotype.

Figure 3 illustrates the steps associated with genomic exclusion, a phenomenon initiated by conjugation with strain C* (ALLEN 1967a, b). Round-one conjugation involves the unidirectional transfer of a non-C* haploid pronucleus following loss of the C* micronucleus at meiosis. Diploidization of the identical haploid pronuclei leads to homozygosis of the non-C* micronuclear genome. Since macro-

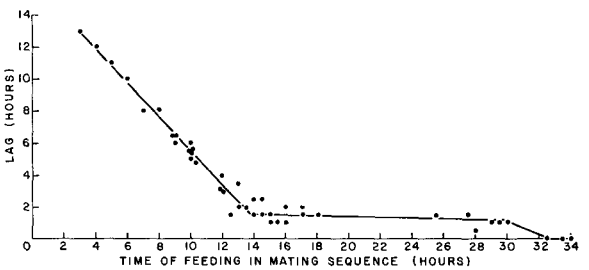

FIGURE 2.—Time of the appearance of resistant cells after the addition of nutrients during conjugation. *Chx-1/Chx-1*$^+$ (cy. sens., II) and *Chx-1/Chx-1*$^+$ (cy. sens., IV) were starved together in 10 mM Tris on a culture shaker for 10–14 hours; the shaker was turned off at time 0. Peptone was added at the indicated times, and 0.1 ml samples of the fed cultures were used to inoculate cycloheximide tubes at 0.5 or 1 hour intervals. Resistance was judged by growth in the cycloheximide tubes after five days. Lag is the time in hours between the addition of food and the inoculation of the first cycloheximide tube to show growth. Points in the curve are from a large series of experiments designed with overlapping times.

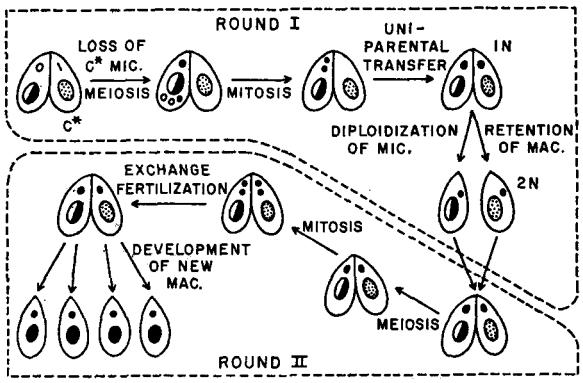

FIGURE 3.—Genomic exclusion. Round I mating involves loss of the C* micronucleus, but normal meiosis and mitosis in the non-C* conjugant. The non-C* male pronucleus is transferred, resulting in haploid germinal nuclei in both cells which become diploid by either endoreduplication or mitosis and fertilization (ALLEN 1967b). Lack of development of the macronuclear anlagen, retention of the macronuclei, and separation of the conjugants yields mature single cells with parental phenotypes, which can remate for round II. Round II follows the normal course of events (as in Figure 1) in both conjugants. ⊙ = C* macronucleus; ◒ = Heterozygous macronucleus from normal parent.

TABLE 1

Genetic analysis of homozygous functional heterokaryon

A. Origin of F1

Chx-1/Chx-1 (cy. sens. II) × *Chx-1⁺/Chx-1⁺* (cy. sens. VII)

Pairs isolated	Viable clones	Immature clones	Resistant : Sensitive (Among immature clones)
87	77	68	68 : 0

B. Backcross of 13 F1 to wild-type parent

Chx-1/Chx-1⁺ (cy. rest.) × *Chx-1⁺/Chx-1⁺* (cy. sens.)

F1 number	Pairs isolated	Viable clones	Immature clones	Resistant : Sensitive (Among immature clones)
1	22	22	19	12 : 7
2	20	19	17	8 : 9
3	20	19	19	12 : 7
4	20	19	19	11 : 8
5	20	20	19	12 : 7
6	20	19	18	10 : 8
7	20	20	17	9 : 8
8	20	18	18	8 : 10
9	20	19	19	10 : 9
10	20	19	19	9 : 10
11	20	19	18	9 : 9
12	20	18	17	10 : 7
13	10	10	10	5 : 5
Total	252	241	229	125 : 104*

* $\chi^2 = 1.93$ $P = 0.16$

nuclear retention normally follows round-one conjugation, these "exconjugants" express parental phenotypes. Round-two pairs are formed by the round-one "exconjugants" and the normal sequence of conjugation proceeds this time. The resulting exconjugants are immature and can express new recombinant phenotypes.

Chx-1/Chx-1$^+$ (cy. sens. II) was mated with C* (cy. sens., III) and round-one pairs were isolated into peptone 10 hours after the beginning of pair formation; the resulting clones had parental phenotypes (cycloheximide-sensitive mating types II and III). To identify and eliminate clones with micronuclei homozygous for *Chx-1*$^+$, replicas of all the clones were grown for several days, washed twice in 10 mM Tris, and allowed to self-mate (complete round two) for 20 hours. An equal volume of 2% peptone was then added to each, and six hours later 0.1 ml of the culture was added to 2.5 ml of 25 μg/ml cycloheximide in 1% peptone. Single cells were isolated from round-one clones whose replicas grew in the cycloheximide test, since only cells homozygous for *Chx-1*$^+$ would be unable to form resistant cells after extensive mating. One such isolate was shown to be the desired homozygous heterokaryon *Chx-1/Chx-1* (cy. sens., II), by a genetic analysis which consisted of a cross to wild type (strain B), and subsequent backcrosses, again to strain B (Table 1).

SELECTION FOR SUCCESSFUL EXCONJUGANTS FOLLOWING ROUND-ONE GENOMIC EXCLUSION

ALLEN (1971) presented data from crosses of C* to a strain heterozygous for two unlinked markers. Although 1:1 ratios of homozygotes:heterozygotes for both markers were expected following two rounds of mating in genomic exclusion, she found an excess of homozygotes for both (139:101 and 143:97). On the other hand, when the homozygote:heterozygote ratio for either marker was evaluated only among the heterozygotes of the other marker, good 1:1 ratios were found (46:51 and 50:51). She concluded that the unexpected ratios were caused by an inflation of homozygotes, and proposed two possible mechanisms: occasional lack of separation of conjugants between rounds one and two, and preferential remating of round-one exconjugants.

A possible cause of the eccentric ratios was examined by timing the onset of new phenotypic expression in matings of functional heterokaryons. *Chx-1/Chx-1*$^+$ (cy. sens., II) and C* (cy. sens., III) were washed out of peptone into 10 mM Tris and starved on the fast shaker as described in MATERIALS AND METHODS. The shaker was halted at time zero; at time 10 hours the mixture was taken to 1% peptone by the addition of 10% peptone. Hourly 0.2 ml samples were inoculated into 5 ml of 25 μg/ml cyclohexamide in 1% proteose peptone from time 10 to 20 hours. Prior to the 16 hour sample, no cells grew in the cycloheximide. Each sample from 16 hours on grew in the cycloheximide; all were immature. When peptone was added any time at or after 3 hours, resistant, immature cells consistently grew in the 16 hour sample. True exconjugants were recovered when peptone was added very shortly after the formation of round-one pairs, although feeding prevents any further pair formation (BRUNS and BRUS-

SARD 1974a). We conclude that immature resistant cells result at the same time, whether the cycloheximide-sensitive functional heterokaryon is mated with wild-type strain B or defective strain C*.

DISCUSSION

Positive selection for successful conjugation can be used in a number of diverse developmental and genetic studies. Appearance of resistant cells in mating mixtures of functional heterokaryons can be employed to time the expression of new phenotypes during conjugation. Appropriately timed drug addition should also allow selection of cells effectively synchronized in later stages of conjugation. For example, prestarved *Chx-1/Chx-1*$^+$ (cy. sens., II) and *Chx-1/Chx-1*$^+$ (cy. sens., IV) can be mixed at time 0, fed at 10 hours, and have cycloheximide and radioactively labeled amino acids added at time 15.5 hours. Since this is the first time cycloheximide-resistant cells can be recovered from such a cross (Figure 2), incorporation of label into protein should only occur in a developmentally synchronized population of cells which paired at the first opportunity, and carried out conjugation at maximal speed. Preliminary studies (unpublished) indicate that functional heterokaryons of a different dominant drug-resistant mutation generate resistant progeny 10 hours after mixing prestarved cells, a time when macronuclear anlagen are still developing. Studies are underway to relate expression of the new phenotypes to exact stages in conjunction.

Functional heterokaryons should also make mutant isolation by the method of CARLSON (CARLSON 1971; DOERDER 1973) more efficient. This method involves treatment of wild-type cells of two mating types with a mutagen, followed by mating, growth to allow allelic assortment, and selection. The method assumes that a recessive mutation, although heterozygous, will be expressed in some of the vegetative progeny as a result of allelic assortment. One problem with this method is that nonconjugant parental cells will grow as well as, and possibly better than, mutant cells, swamping out interesting mutants during the necessary outgrowth. Use of functional heterokaryons for the required mating will limit growth to true exconjugants. Use of homozygous functional heterokaryons should further increase the efficiency of this approach.

Functional heterokaryons also make possible a search for natural or induced self-mating, which would allow the direct expression of recessive mutations without allelic assortment. Preliminary studies subjecting newly formed pairs to increased ionicity, added nutrients, or fast shaking have been performed with functional heterokaryons; these conditions have previously been shown to prevent pair formation (BRUNS and BRUSSARD 1974a). Although treatment two hours after the beginning of pair formation appeared to break apart all pairs, resistant immature cells were recovered. Current studies are underway to investigate the consequences of interrupting mating after the start of meiosis, but before exchange of pronuclei. In contrast with mating mixtures, no treatment or growth condition has thus far generated resistant cells for single mating type suspensions of sensitive heterokaryons, reaffirming the irreversibility of allelic repression.

Finally, at least some of the immature resistant cells isolated from mating mixtures with C* must be successful homozygous progeny of round-one mating. The timing studies of gene expression in progeny macronuclei suggest that macronuclear development does not always abort after round-one mating; immature cells develop with a macronucleus derived from the newly formed homozygous zygote nucleus. ORIAS and FLACKS (1973) report what appears to be a similar occurrence after mating a homozygote for the recessive *ts-2* with C*, and isolating round-one pairs seven hours later. Both exconjugant clones of one of the viable pairs so isolated expressed the recessive phenotype and were not fully mature. All these results imply that there may be a critical point in conjugation (after fertilization, but before macronuclear anlagen development) beyond which development may or may not proceed. This critical point would control entry into the process whereby the new genome in the zygote nucleus is committed to a new somatic nucleus. If the zygote nucleus arose by unusual means (for example the diploidization in crosses with C*), macronuclear development of the new genome is perhaps only rarely attempted. Conversely, in crosses between wild-type stocks, at least some "non-conjugants" (mature, parental products of isolated pairs) may be cells with recombinant micronuclei, but parental macronuclei. When describing the need for careful maturity testing, NANNEY (1963) noted that, "The cells from some pairs separate without forming new macronuclei, even though in some of these pairs the micronuclei have undergone some division." In any case, positive selection for cells which have undergone genomic exclusion without macronuclear retention makes possible the isolation of cells with new, homozygous macronuclei following mutagenesis, and in genetic analyses. A related paper (BRUNS and BRUSSARD 1974b) describes a scheme for the isolation of recessive induced mutations using this selective approach.

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