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Genetics

Nuclear fusion-defective phenocopies in *Chlamydomonas reinhardtii*: Mating-type functions for meiosis can act through the cytoplasm

(karyogamy/microtubules/segregation)

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ABSTRACT **Nuclear fusion in newly formed *Chlamydomonas reinhardtii* zygotes can be inhibited by drugs that affect microtubule stability, which include colchicine, amiprophos-methyl, oryzalin, and taxol. This inhibition can be monitored genetically by the production of haploid meiotic products from conjugations between haploid and diploid parents. Such zygotes would normally produce aneuploid progeny. Inhibition of nuclear fusion by colchicine requires treatment of gametic cells both before conjugation and after formation of the zygotes. These results suggest that nuclear fusion requires dynamic microtubules. Treated zygotes formed from a haploid–diploid mating can produce six spores, but only four spores germinate to form viable haploid colonies. No contribution from the nuclear genome of the haploid parent is recovered, whereas all loci from the diploid parent are recovered. The four viable products from the diploid parent of inhibited zygotes show normal segregation of loci located on linkage groups segregating according to Mendelian laws. Levels of meiotic recombination were tested for pairs of loci on linkage groups XVIII and XIX and found to be unchanged by inhibition of nuclear fusion. Thus, similar to *Saccharomyces cerevisiae*, *C. reinhardtii* mating-type functions necessary for nuclear fusion are not nuclear limited and can act through the cytoplasm. Inhibition of nuclear fusion can be used to analyze diploid *Chlamydomonas* that cannot enter meiosis. This technique permits direct analysis of dominant mutations, dominant suppressors and enhancers, and new alleles of identified loci that have been isolated in diploid strains.**

After conjugation between *Chlamydomonas* cells of opposite mating-type, the newly fused cells can follow one of two pathways. Most cells become dormant zygotes competent to complete meiosis with the appropriate nutritional regimen (1, 2). The remainder of the cells, which represents between 2% and 10% of the successfully mated cells, become stable mitotically dividing diploid cells (3). Diploid cells, which are genotypically identical to zygotic cells, cannot be induced to enter the meiotic pathway directly to produce haploid progeny. However, diploid cells heterozygous at the mating-type locus retain the ability to mate as mating-type minus cells (4, 5). Therefore, diploid cells can re-enter the meiotic pathway by mating with haploid mating-type plus cells. Triploid zygotes are formed, and these cells complete meiosis. Asci with four spores are then formed, but only 15–20% of the spores produce colonies, and these cells are aneuploid (6). Diploid cells can also be analyzed through a tetraploid meiosis after conjugation between two diploid cells because diploid cells with a mating-type plus phenotype can be formed either by polyethylene glycol-mediated fusion (7, 8) or from a previous tetraploid meiosis (8). Four-spored asci are recovered; most spores are viable and are diploid.

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Although diploid *Chlamydomonas* cells retain the ability to mate, the completion of nuclear fusion produces a triploid cell when mated to a haploid cell. If nuclear fusion were inhibited in zygotes formed from these matings and the independent nuclei were induced to enter meiosis by signals acting through the cytoplasm, haploid meiotic progeny could then be recovered from a diploid cell. Two examples of this approach exist. (*i*) A mutant, *karl-1*, in *Saccharomyces cerevisiae* blocks nuclear fusion (9). In zygotes formed between *MAT***a**/*MAT***a** diploid cells, which cannot sporulate themselves, and *MATα* haploid cells that carry the *karl-1* mutation, nuclear fusion is inhibited. When these binucleated zygotes are placed immediately into sporulation medium, the diploid nucleus enters meiosis and four haploid progeny are produced from the diploid parent(s) (10, 11). Two haploid progeny are also recovered from the haploid parent. (*ii*) Treatment of cells with compounds that depolymerize microtubules prevents nuclear fusion. In *Saccharomyces*, treatment of mating cells with benomyl prevents nuclear fusion in newly formed zygotes (12). In *Tetrahymena*, migration of the gametic pronuclei and nuclear fusion is inhibited by a variety of antagonists of microtubule integrity (13, 14). In sea urchin embryos, colcemid or hydrostatic pressure treatment prevents nuclear migration of the male pronuclei after fertilization, and consequently nuclear fusion is blocked (15–17).

The ability to resolve diploid cells into haploid cells in *Chlamydomonas* would be useful in many kinds of genetic analyses. I therefore sought a practical method for obtaining meiotic products from *Chlamydomonas* diploids. This paper presents such a method, in which nuclear fusion in *Chlamydomonas* is inhibited by colchicine and other agents that affect microtubules. All these drugs inhibit cell division and assembly of flagellar axonemes in *Chlamydomonas*, but they do not affect the stability of assembled flagellar microtubules (ref. 18, and unpublished observations). Early stages of conjugation in *Chlamydomonas* can be inhibited by colchicine at 10 mg/ml (19).

MATERIALS AND METHODS

Genetic Analysis. Genetic analysis was performed as described (2, 20, 21). Diploid strains were constructed using complementing *arg2* and *arg7* mutations by the method of Ebersold (3). The *arg2* and *arg7* strains were obtained from F. Lux (University of Colorado).

Drug-Treated Matings. Gametic cells were grown as described (21, 22). Cells were scraped from agar plates into low-nitrogen medium (1) for gametogenesis. Sterile solutions of colchicine (or other compounds) were added 1 hr after resuspension; this interval allows flagella to assemble. In other experiments, timing of the addition and time before plating were varied. After plating zygotes, the plates were exposed to light for 24 hr at 21°C and then stored in the dark for 4 days before exposure to chloroform vapors. Zygotes were dissected onto Sager and Granick medium I (1) with 2% agar containing arginine at 2 μg/ml and one-tenth the normal

concentration of ammonium nitrate. The percentage of conjugation is reduced with colchicine to ≈10–60% of control values.

RESULTS

Characterization of Colchicine-Treated Zygotes. The meiotic progeny from untreated diploid × haploid zygotes have been characterized as reported elsewhere (6). These data show that most spores that form macroscopic colonies from a triploid meiosis are aneuploid; few triploid zygotes produce four viable spores. Therefore, production of at least four viable meiotic products was chosen as the first assay for inhibition of nuclear fusion.

Diploid and haploid gametic cells treated with colchicine (see below) produced an increased number of tetrads that formed four macroscopic colonies. These colonies contained $>10^4$ cells after 5 days of growth at 21°C. The faster cell-division time distinguished these colonies from most progeny of an untreated triploid meiosis. The tetrads were characterized by analyzing (*i*) segregation of genetic markers from the diploid parent, (*ii*) appearance of genetic markers from the haploid parent, and (*iii*) viability of spores in a meiotic test cross. The diploid parent used in these experiments was heterozygous for five unlinked loci and for the intra-allelic complementing alleles at the *arg2/arg7* locus (23). The haploid parent was wild type for all scored loci and carried the mt^+ allele at the mating-type locus (Table 1).

In 668 out of the 681 asci with four viable spores produced after colchicine treatment, all spores required arginine. In each of these asci, two arginine-requiring spores carried the *arg2* allele and the other two spores carried the *arg7* allele. The remaining five loci segregated $2^R:2^S$ (where R and S represent the two alleles of the diploid parent) in 670 of 681 tetrads tested. Therefore, these colonies contain all the genetic markers contributed by the diploid parent, and the distribution of the genetic markers suggests that the diploid cells underwent a standard meiosis in 664 of the 681 tested asci that had four viable spores. At this level of resolution, no genetic markers were recovered from the haploid parent. The frequency of the minority class of asci with four viable spores that do not show $2^R:2^S$ segregation for the six loci suggests that they represent the rare triploid zygotes that completed nuclear fusion and produced four viable products. This frequency (17 of 2100 tetrads) was similar to the frequency seen in other experiments (data not shown). All 17 aberrant tetrads contained more than one marker that failed to segregate $2^R:2^S$.

Table 1. Viability of meiotic products from colchicine-treated cells

Tetrad number	Four-spored tetrads, %	Tetrads dissected, no.	Genotype				
			mt	*eryl*	*sprl*	*act2*	*flal0*
10–1	96	68	P	+	+	–	–
10–2	94	64	P	–	+	+	–
10–3	97	66	M	+	–	+	+
10–4	98	65	M	–	–	–	+
11–1	90	65	P	–	+	+	–
11–2	92	68	P	–	–	+	+
11–3	0	0*	M	+	+	–	+
11–4	0	0*	M	+	–	–	–
12–1	95	66	M	+	+	–	+
12–2	97	64	P	–	+	+	+
12–3	98	55	P	+	–	+	–
12–4	95	53	M	–	–	–	–
25–1	95	63	P	+	–	–	+
25–2	97	65	M	+	+	+	–
25–3	93	68	P	–	+	+	–
25–4	98	61	M	–	–	–	+
27–1	93	60	M	–	–	+	–
27–2	97	59	P	+	+	–	+
27–3	93	63	P	+	+	–	+
27–4	96	61	M	–	–	+	–

Meiotic products from five tetrads were obtained after treatment of parental strain 10000 (*arg2/arg7 act2/ACT2 eryl/ERYl sprl/SPRl flal0/FLAl0 mtM/mtP*) and strain 137 mt^+ with 10 mM colchicine for 4 hr before mating and for 2 hr after mixing of the gametes. Each product was mated to either strain 137 mt^+ or strain 137 mt^-, and products were analyzed. M and P represent mt^- and mt^+, respectively.

*These spores mated with high efficiency but failed to sporulate. No meiotic spores were detected in >5000 zygotes.

Cells from five of the tetrads (discussed above) that showed $2^R:2^S$ segregation were mated to a wild-type strain (137 mt^+ or 137 mt^-); viability of the meiotic products in this test cross was measured, and segregation of the five drug-resistance loci was monitored. In the progeny from four of the five tetrads, >93% of the spores produced macroscopic colonies after 5 days at 21°C (Table 1). For one tetrad (no. 11), two spores produced at least 90% viable progeny, whereas zygotes from matings with the other two spores failed to sporulate. This failure to complete meiosis is not understood, but the phenotype is not that of the aneuploid cells produced by a triploid meiosis. In the remaining 18 crosses that did sporulate, genetic markers segregated as expected for monosomic chromosomes. Strains that were resistant to a drug segregated $2^R:2^S$ spores; strains that were sensitive to a drug segregated $0^R:4^S$ spores (data not shown). The 18 spores that sporulated appear to be euploid by the criteria of meiotic viability and segregation of genetic loci.

The strategy of using the production of four viable meiotic products as an assay for inhibition of nuclear fusion seems valid. By the genetic criteria presented, these progeny apparently result from two meiotic divisions of the diploid nucleus and contain no contribution from the haploid nucleus.

Inhibition of Nuclear Fusion. Algae are unusually resistant to the effects of colchicine (19, 24), perhaps due to a permeability barrier. The drug concentration required to inhibit cell division is high compared with that required for mammalian cells. Diploid and haploid *Chlamydomonas* cells were treated separately with 10 mM colchicine during gametic differentiation in liquid medium for differing periods, mated in the presence of the drug, and plated onto 4% agar medium 1 hr after mixing gametic cells. Plating of cells dilutes the colchicine present. Colchicine was added 4, 3, 2, 1, and ½ hr before mixing gametic cells and at time of mixing (0 hr). The percentage of tetrads that produced four macroscopic colonies after 5 days of growth was monitored. Without colchicine, only one ascus out of 131 tetrads dissected produced four viable spores. These four spores were not euploid by the criterion of segregation of loci from the diploid parent. Increasing time of colchicine exposure increased the percentage of zygotes that produced four viable colonies (Fig. 1). After 4-hr exposure, 33% of dissected zygotes produced four viable colonies. These spores showed $2^R:2^S$ segregation of the five loci from the diploid parent in 319 of 322 tetrads with four viable spores. Increasing time in colchicine to 12 hr before mixing of gametic cells did not increase the percentage of asci that produced four viable colonies beyond the level seen with 4-hr treatment (39 of 121 dissected tetrads).

To test whether higher or lower concentrations of colchicine or treatment of only one parent would alter the percentage of affected cells, each parent was exposed to concentrations from 0 to 14 mM for 4 hr before mating, and the mixed gametes were plated 1 hr later (Table 2). Treatment of only one parent was sufficient to achieve levels of inhibition comparable to treatment of both parents. Treat-

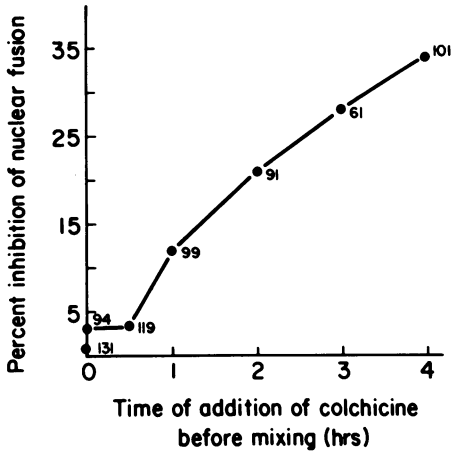

FIG. 1. Effect of colchicine addition at various times during gametogenesis on the percentage of zygotes that show inhibition of nuclear fusion. Colchicine was added 4 (n = 101), 3 (n = 61), 2 (n = 91), 1 (n = 99), and ½ (n = 119) hr before mixing of the gametic cells and at the time of mixing (0 hr, n = 94). n = Number of tetrads analyzed. Numbers beside each data point in the figure indicate the number of zygotes dissected. The untreated control is indicated by the point at 131.

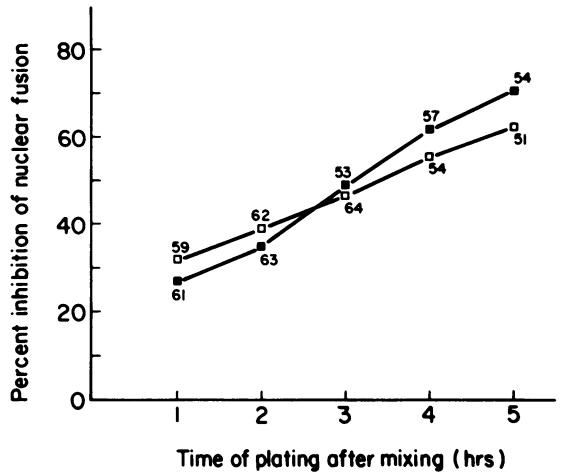

FIG. 2. Effect of colchicine for various periods after cytoplasmic fusion on percentage of zygotes in which nuclear fusion was inhibited. For each point an average of 58 tetrads was analyzed. ■, Cells treated with 4 mM colchicine; □, cells treated with 10 mM colchicine.

ment of either the diploid or the haploid parent with 4–10 mM did not produce significantly different percentages of asci with four viable spores. However, colchicine treatment of 12 mM and 14 mM reduced the percentage of zygotes that produced four viable colonies (Table 2), and the percentage of zygotes that sporulated was reduced (data not shown). Because the assay monitors production of viable colonies, reduction by higher concentrations of colchicine may reflect the deleterious effects of colchicine rather than any increase in completion of nuclear fusion.

To test whether the effect of colchicine was reversible, cells treated 3 hr or 4 hr before mating were washed with fresh medium before mixing the two gametic cultures. The percentage of zygotes that produced four viable spores dropped to 3.2% (n = 63) and 3.4% (n = 59) for the cultures treated for 3 and 4 hr, respectively, before mating. Although this percentage is higher than for untreated controls, it was dramatically reduced relative to cells exposed continuously to colchicine and is comparable to the percentage seen when colchicine was added at the time of mating. Treated zygotes appeared to recover after colchicine was removed. In addition, this result suggests that colchicine is required both before and after mixing of the gametic cultures to inhibit nuclear fusion.

Several groups have observed that nuclear fusion is delayed after cytoplasmic fusion for 1½–4 hr (19, 25, 26); therefore, a period may exist between cytoplasmic fusion and nuclear fusion in which colchicine must be present to inhibit nuclear fusion. To test this possibility, cells were treated for 4 hr before mixing the gametic cultures, and the cultures were plated 1, 2, 3, 4, or 5 hr after mixing. At two different concentrations of colchicine, the percentage of zygotes increased linearly, which showed inhibition of nuclear fusion as a function of time between mixing and plating of the zygotes (Fig. 2). Cultures that received colchicine at the time of mixing and were plated 5 hr later produced only 3.2% asci with four viable spores (n = 94). In summary, it appears that nuclear fusion can be inhibited by colchicine treatment and that the efficiency of this inhibition is correlated to time of colchicine exposure both before and after mating. But there is not a single window period during which colchicine is essential.

No genetic markers were recovered from the haploid parent in the above-described crosses. Although no viable colonies contain markers from the haploid parent, six-spored asci were seen in colchicine-treated haploid × diploid crosses in *Chlamydomonas*. Two of these spores were never viable; these two spores were about one-third to one-fifth the diameter of the other four spores, and they failed to divide (n = 460). Whenever these small spores were seen, the other four spores were products of the diploid nucleus. However, these small spores were not seen from every zygote that produced four viable haploid colonies (only 230 of 680 zygotes produced small spores). This failure to see six-spored asci in every nuclear-fusion-inhibited zygote may have reflected their small size, failure to package the nuclear contents into spores, or loss of mechanical integrity.

Table 2. Effect of colchicine dosage on nuclear fusion

Per diploid parent, mM	Per haploid parent, mM					
	0	4	6	8	12	14
0	**0.8** (123)	**9** (47)	**16** (50)	**20** (64)	**19** (47)	**16** (47)
4	**20** (30)	**13** (65)	**11** (64)	**22** (64)	**9** (64)	**10** (52)
6	**22** (45)	**16** (55)	**25** (64)	**23** (64)	**13** (66)	**13** (54)
8	**27** (47)	**16** (25)	**20** (61)	**12** (43)	**25** (48)	**12** (32)
10	**29** (41)	**20** (64)	**23** (64)	**30** (43)	**18** (48)	**19** (32)
12	**30** (33)	**13** (31)	**19** (42)	**9** (32)	**13** (47)	**10** (46)
14	**15** (31)	**14** (63)	**0** (11)	**21** (33)	**4** (45)	**9** (44)

Cells were treated as described in Table 1 except that cells were plated 1.0 hr after mixing gametes. Cells were in colchicine for 5.0 hr. Boldface numbers indicate percentage of zygotes dissected that had four viable spores as assayed by growth after 5 days at 21 °C. Numbers in parentheses indicate number of dissected tetrads.

Table 3. Effects of other compounds on nuclear fusion

Compound	Concentration, μM	Inhibition of nuclear fusion, %	Tetrads dissected, no.
Amiprophos-methyl	5	21	57
Oryzalin	10	19	62
Taxol	100	11	192

Compounds were added to cultures of strain 16012 (*arg2/arg7 act2/ACT2 eryl/ERYl sprl/SPRl mtM/mtP*) and strain 137 mt^+ 1 hr after gametogenesis began, and cells were plated 4 hr after mixing the two gametic cultures.

Effects of Other Antagonists. Several agents that affect microtubule integrity were assayed for inhibition of nuclear fusion. Amiprophos-methyl and oryzalin cause depolymerization of microtubules in higher plants (27–29). Gametic cells were treated with 5 μM amiprophos-methyl and 10 μM oryzalin for 4 hr before mating and for 5 hr after mixing gametic cells. Both compounds at the concentrations tested inhibited cell division (data not shown). Inhibition of nuclear fusion was seen, but the percentage of affected zygotes was lower than in the colchicine-treated cells (Table 3).

Taxol stabilizes microtubules from many organisms *in vitro* (30); however, taxol also inhibits cell division (31). Taxol inhibited *Chlamydomonas* cell division (data not shown) and nuclear fusion at a concentration of 100 μM (Table 3).

Recombination in Colchicine-Treated Zygotes. Recombination distances on linkage group XVIII between *anil* and *sprl* and on linkage group XIX between *fla10* and *pf10* were monitored in several different crosses. Diploids heterozygous for all four mutations and diploids heterozygous for linkage group XVIII or XIX loci were treated with colchicine for 4 hr before mating to wild-type haploid cells (strain 137 mt^+). Distances were compared with the same interval in zygotes made from haploid parents of the diploid strains. Recombination was not changed in the diploid parent by treatment with colchicine compared with the level seen in the parental zygotic crosses (Table 4).

Evidence for Cytoplasmic Fusion. Were markers from cytoplasmic organelles recovered from zygotes in which nuclear fusion was inhibited? The chloroplast genome can easily be marked with a number of drug-resistance mutations (32, 33). For this experiment, the haploid strain was marked with a chloroplast mutation that conferred resistance to streptomycin at concentrations of 100 μg/ml (34). Euploid colonies that arose from nuclear-fusion-inhibited zygotes, as monitored by the regular segregation of nuclear markers, and aneuploid colonies that arose from zygotes that underwent a triploid meiosis were scored for the cytoplasmic streptomycin-resistance phenotype. Among aneuploid spores, 78 of 105 (74%) were resistant to streptomycin; this value is similar to previously reported data (35). Among euploid tetrads, the segregation patterns of $4^R{:}0^S$ (12 tetrads), $3^R{:}1^S$ (15 tetrads), and $2^R{:}2^S$ (8 tetrads) were found; 78% of these spores were resistant to streptomycin. No $1^R{:}3^S$ or $0^R{:}4^S$ tetrads were found. Thus, chloroplast markers of the haploid parent are recovered in the meiotic progeny that contain nuclear information from only the diploid nucleus. This result proves that cytoplasmic fusion occurred between the haploid and the diploid parents. In addition, segregation of the chloroplast appeared unaffected by nuclear fusion; similar recovery of the two parental contributions was seen in both classes of zygotes.

DISCUSSION

Zygotes produced by conjugation between haploid and diploid *Chlamydomonas* cells that have been treated with antagonists of microtubule integrity show an elevated frequency of viable meiotic progeny. This elevated frequency of viable spores apparently results from an inhibition of nuclear fusion between the diploid and haploid nuclei after cytoplasmic fusion. Inhibition can occur after treatment of one or both parents, and the greatest inhibition is seen when the cultures are treated both before and after mixing. Several lines of genetic evidence support this conclusion. (*i*) The recovered progeny have the genetic markers of the diploid parent in the cross, and these spores, by all criteria tested, appear to be haploid and euploid. (*ii*) Diploid cells are not induced to enter meiosis by treatment with colchicine alone; cells must mate to produce viable progeny (data not shown). Further evidence for the mating requirement is the recovery of cytoplasmic markers from the haploid parent in meiotic progeny of all such inhibited zygotes scored. (*iii*) In drug-inhibited crosses between two diploid parents, eight viable progeny are recovered. These progeny appear haploid, and assortment of markers between the two parents is not seen (S.K.D. and J. A. Holmes, unpublished work).

Table 4. Recombination distances in colchicine-treated zygotes

mt^- parent Genotype	Ploidy	Recombination distance, cM XVIII	XIX
anil–sprl, pf10–fla10 / *ANIl–SPRl, PF10–FLA10*	D	8.6 (54)	18.5 (54)
anil–sprl / *ANIl–SPRl*	D	9.2 (38)	
pf10–fla10 / *PF10–FLA10*	D		19.0 (63)
anil–sprl, pf10–fla10	H	9.8 (102)	18.6 (102)
anil–sprl	H	8.2 (98)	
pf10–fla10	H		19.2 (99)

Each strain was mated to 137 mt^+. Diploid (D) strains were treated with 10 mM colchicine for 4 hr during gametogenesis and for 1 hr after mixing of the gametic cells; haploid (H) strains were not treated with colchicine. The region between *anil–sprl* is on linkage group XVIII, and the region between *pf10–fla10* is on linkage group XIX. cM, centimorgans. Numbers in parentheses indicate *n*.

Requirements for the Inhibition of Nuclear Fusion. Inhibition can be achieved with a variety of drugs, which share the common property that they affect the integrity of dynamic microtubules; the assembly of flagellar microtubules is not disrupted by these drugs (18, 19). However, it is possible that other cellular processes besides microtubules are being affected by these drugs and that nuclear fusion is affected only indirectly by these treatments.

If microtubules were directly involved, the observation that taxol inhibited nuclear fusion suggests that the ability of microtubules to disassemble is also required for completion of nuclear fusion. The process of nuclear migration and subsequently nuclear fusion may depend on microtubule disassembly to let the two nuclei move together.

Segregation of the Chloroplast Genome. Chloroplast mutations are recovered at similar frequencies from the nuclear fusion-inhibited zygotes and the triploid zygotes. Most progeny have the cytoplasmic phenotype of the haploid mt^+ parent as expected (35). This similarity between the two classes of zygotes suggests that there is no nuclear-limited gene product responsible for the unequal inheritance of the chloroplast genomes. Mitochondrial inheritance was not studied, but it is reasonable to suspect that inhibition of nuclear fusion would not alter the inheritance from the pattern seen in triploid zygotes.

Segregation of Linkage Group XIX. The behavior of linkage group XIX was analyzed in zygotes that failed to fuse their

nuclei. Linkage group XIX is unusual in that its genetic map is circular, there is a clustering of genes that participate in microtubule-based functions, and meiotic recombination is temperature-sensitive (36, 37). This linkage group segregates as expected for a nuclear linkage group in standard crosses. By use of alleles of *pf10* and *fla10*, segregation of linkage group XIX was monitored in cells that failed to complete nuclear fusion to ask whether this group behaved differently from other nuclear linkage groups. When diploid strains heterozygous for *fla10* (Table 1), heterozygous for *pf10* and *fla10* (Table 4), and homozygous for *fla10* (data not shown) were crossed to haploid strains carrying the corresponding wild-type alleles, no additional copies of the wild-type alleles were seen in the progeny. A cross between a diploid homozygous for *fla10* and a haploid strain carrying the *vfl1* mutation (38) did not produce any tetrads with the wild-type allele of *fla10* in 46 nuclear fusion-inhibited tetrads (unpublished data). These observations suggest, but do not prove, nuclear localization for linkage group XIX.

Haploid Nuclei in Inhibited Zygotes. The behavior of haploid nuclei in inhibited zygotes differs between *Chlamydomonas* and *Saccharomyces*. In *Chlamydomonas*, the haploid parent fails to produce viable spores. One explanation is that the haploid nucleus attempts to complete meiosis I by itself. If the chromosomes remain as univalents, they may move at random to each pole to form two incomplete meiosis I products; the cells may not then attempt meiosis II. On the other hand, in yeast, progeny from the haploid nucleus are recovered, and disomic chromosomes undergo meiotic levels of recombination. The haploid nucleus of yeast has been postulated to undergo recombination and then a meiosis II division, thus producing two viable meiotic products (10). The ability to skip the meiosis I division in yeast may be unusual.

Application of Nuclear-Fusion Inhibition. A practical benefit of the technique described here is the genetic manipulation of diploid cells that it allows. In diploid strains it is possible to isolate new alleles of existing loci (39) and to isolate dominant mutations (6) as well as dominant suppressors and enhancers of defined loci. Resolution of diploid strains by a triploid meiosis takes three to four generations to approach euploidy (6), and mutations that affect viability may be difficult to detect. However, using nuclear-fusion-defective phenocopies, diploid strains can be analyzed easily in a single cross. Mutations that have a lethal phenotype can be detected in such phenocopies, whereas they would remain undetected in triploid meioses. Furthermore, this technique can be used to analyze strains that have incurred chromosome-loss events or mitotic recombination and to verify the chromosome complement (F. Lux and S.K.D., unpublished work).

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1. Sager, R. & Granick, S. (1954) *Ann. N.Y. Acad. Sci.* **56,** 831–838.
2. Levine, R. P. & Ebersold, W. (1960) *Annu. Rev. Microbiol.* **14,** 197–216.
3. Ebersold, W. T. (1967) *Science* **157,** 447–449.
4. Smith, G. & Regnery, D. G. (1950) *Proc. Natl. Acad. Sci. USA* **36,** 246–248.
5. Sager, R. (1955) *Genetics* **40,** 476–489.
6. Dutcher, S. K. & Gibbons, W. (1988) *J. Phycol.* **24,** 230–236.
7. Matagne, R. F., Deltour, R. & Ledoux, L. (1979) *Nature (London)* **278,** 344–346.
8. Galloway, R. E. & Goodenough, U. W. (1985) *Genetics* **111,** 447–461.
9. Conde, J. & Fink, G. R. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 3651–3655.
10. Klar, A. J. S. (1980) *Genetics* **94,** 597–605.
11. Dutcher, S. K. (1980) *Mol. Cell. Biol.* **1,** 245–253.
12. Delgado, M. A. & Conde, J. (1984) *Mol. Gen. Genet.* **193,** 188–189.
13. Hamilton, E. P. & Suhr-Jenssen, P. B. (1980) *Exp. Cell Res.* **126,** 391–396.
14. Orias, J. D., Hamilton, E. P. & Orias, E. (1983) *Science* **222,** 181–184.
15. Zimmerman, A. M. & Silberman, L. (1964) *Biol. Bull.* **127,** 355–357.
16. Zimmerman, A. M. & Zimmerman, S. (1967) *J. Cell Biol.* **34,** 483–488.
17. Longo, F. J. & Anderson, E. (1968) *J. Cell Biol.* **39,** 339–368.
18. Rosenbaum, J. L., Moulder, J. E. & Ringo, D. L. (1969) *J. Cell Biol.* **41,** 600–619.
19. Minami, S. A. & Goodenough, U. W. (1978) *J. Cell Biol.* **77,** 165–181.
20. Perkins, D. D. (1952) *Genetics* **38,** 187–197.
21. Dutcher, S. K., Gibbons, W. & Inwood, W. B. (1988) *Genetics*, in press.
22. Luck, D. J. L., Piperno, G., Ramanis, Z. & Huang, B. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 3456–3460.
23. Matagne, R. F. (1978) *Mol. Gen. Genet.* **160,** 95–99.
24. Pickett-Heaps, J. D. & Spurck, T. P. (1982) *Eur. J. Cell Biol.* **28,** 77–82.
25. Cavalier-Smith, T. (1974) *J. Cell Sci.* **16,** 529–556.
26. Kuroiwa, T., Nakamura, S., Sato, C. & Tsubo, Y. (1985) *Protoplasma* **125,** 43–52.
27. Morejohn, L. C. & Fosket, D. E. (1984) *J. Cell Biol.* **99,** 141–147.
28. Morejohn, L. C. & Fosket, D. E. (1984) *Science* **224,** 874–876.
29. Bajer, A. S. & Mole-Bajer, J. (1986) *Ann. N.Y. Acad. Sci.* **466,** 767–784.
30. Schiff, P. B., Fant, J. & Horwitz, S. B. (1979) *Nature (London)* **277,** 665–667.
31. Schiff, P. B. & Horwitz, S. B. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 1561–1565.
32. Grant, D. M., Gillham, N. W. & Boynton, J. E. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 6067–6071.
33. Mets, L. J. & Geist, L. J. (1983) *Genetics* **105,** 559–579.
34. Sager, R. (1954) *Proc. Natl. Acad. Sci. USA* **40,** 356–363.
35. Matagne, R. F. & Mathieu, D. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 4780–4783.
36. Dutcher, S. K. (1986) in *Extrachromosomal Elements in Lower Eukaryotes*, eds. Wickner, R. B., Hinnebusch, A., Lambowitz, A. M., Gunsalus, I. C. & Hollaender, A. (Plenum, New York), pp. 303–325.
37. Ramanis, Z. & Luck, D. J. L. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 423–426.
38. Adams, G. M. W., Wright, R. L. & Jarvick, J. L. (1985) *J. Cell Biol.* **100,** 955–964.
39. Dutcher, S. K. (1988) in *Cell Movement*, eds. Warner, F. R. & McIntosh, J. R. (Liss, New York), Vol. 2, in press.