A Conditional Mutant Having Paralyzed Cilia and a Block in Cytokinesis Is Rescued by Cytoplasmic Exchange in *Tetrahymena thermophila*

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ABSTRACT

Nineteen mutants that are conditional for both the ability to regain motility following deciliation and the ability to grow were isolated. The mutations causing slow growth were placed into five complementation groups. None of the mutations appears to affect energy production as all mutants remained motile at the restrictive temperature. In three complementation groups protein synthesis and the levels of mRNA encoding α-tubulin or actin were largely unaffected at the restrictive temperature, consistent with the hypothesis that mutations in these three groups directly affect the assembly of functional cilia and growth. Complementation group 1 was chosen for further characterization. Both phenotypes were shown to be linked, suggesting they are caused by a single mutation. Group 1 mutants regenerated cilia at the restrictive temperature, but the cilia were nonmotile. This mutation also caused a block in cytokinesis at the restrictive temperature but did not affect nuclear divisions or DNA synthesis. The block in cell division was transiently rescued by wild-type cytoplasm exchanged when mutants were paired with wild-type cells during conjugation (round 1 of genomic exclusion). Thus, at least one mutation has been isolated that affects assembly of some microtubule-based structures in Tetrahymena (cilia during regeneration) but not others (nuclei divide at 38°), and the product of this gene is likely to play a role in both ciliary function and in cytokinesis.

As in other eukaryotic cells microtubules in the ciliated protozoan *Tetrahymena thermophila* are involved in a variety of structures and functions: (1) cell movement (cilia and associated basal bodies and ciliary rows); (2) feeding (oral ciliature directing food into the mouth); (3) nuclear divisions (micronuclear mitosis and meiosis and macronuclear division); (4) maintenance of cell shape (cortex); and (5) nuclear movements during conjugation (R. D. ALLEN 1967; DAVIDSON and LAFOUNTAIN 1975; LAFOUNTAIN and DAVIDSON 1979; NILSSON and WILLIAMS 1966; ORIAS, HAMILTON and ORIAS 1983; ORIAS 1986; WILLIAMS and WILLIAMS 1976). The questions of how eukaryotic cells regulate the assembly of such different microtubule systems, spatially and temporally, and what gives each microtubule system its unique properties remain largely unanswered.

In an attempt to address these questions we initiated a genetic analysis of ciliary asssembly and function in Tetrahymena. Tetrahymena is amenable to genetic analyses (BRUNS 1986) and has only 1 α-tubulin and 2 β-tubulin genes (CALLAHAN, SHALKE and GOROVSKY 1984; BARAHONA *et al.* 1985; G. SHALKE and M. A. GOROVSKY, unpublished observations), so a genetic analysis is not complicated by a large number of tubulin genes. The various microtubule structures in Tetrahymena contain five α and two β protein isoforms (SUPRENANT *et al.* 1985). Thus, the Tetrahymena tubulins probably undergo numerous posttranslational modifications as do the tubulins in higher eukaryotes (PIPERNO and FULLER 1985). Tetrahymena can be deciliated, and cells regain motility within sixty minutes and regenerate a full complement of cilia within 3 hr (ROSENBAUM and CARLSON 1969; CALZONE and GOROVSKY 1982). Also, since microtubules are involved in so many different structures in Tetrahymena, it seems likely that many mutations affecting microtubule assembly should affect cellular growth and/or division. Thus, a screen for conditional mutants that fail to recover motility and feeding ability after deciliation and that fail to grow should yield a population enriched in mutations that affect microtubule assembly and/or function. However, cilia regeneration is also accompanied by large increases in protein and RNA synthesis (GUTTMAN and GOROVSKY 1979; CALZONE, ANGERER and GOROVSKY 1983; SEYFERT 1987). Mutations affecting these processes might also cause failures in cilia regeneration and growth and must be distinguished from mutations that have a direct effect on ciliary assembly or function.

In this paper we describe the isolation and preliminary characterization of thirty conditional mutants that have defects in both cilia regeneration and growth at the restrictive temperature. Nineteen of the

mutants were fertile, and the mutations were placed into five complementation groups. One complementation group (group 1) was chosen for further analysis. Genetic analyses suggested both phenotypes are caused by a single, recessive mutation. Group 1 mutants remained motile when shifted to 38°, but when deciliated and incubated at 38°, they regenerated nonfunctional cilia. Vegetative cells failed to complete cytokinesis at 38°, indicating that the gene affected by the group 1 mutation encodes a product required for cytokinesis and for the assembly of functional cilia. Interestingly, wild-type cytoplasm transferred during conjugation transiently rescues cell division arrest at 38°.

MATERIALS AND METHODS

Strains: Cu 428 is a functional heterokaryon (BRUNS and BRUSSARD 1974) homozygous in the micronucleus for an allele conferring resistance to 15 μg/ml 6-methylpurine (6 mp), but carrying the allele conferring sensitivity in the macronucleus so the strain is phenotypically 6-methylpurine sensitive. Cu 427 is another functional heterokaryon, and it is phenotypically sensitive to 25 μg/ml cycloheximide (cy) but is homozygous in the micronucleus for an allele conferring resistance to cycloheximide. A*III and B*VI strains contain a defective micronucleus and are used in genomic exclusion matings (S. L. ALLEN 1967a, b).

Culture, deciliation and mating conditions: All strains were grown in modified Neff's medium containing one third of the proteose peptone (Oxoid), yeast extract (Difco), and glucose (Baker) that is in regular Neff's (EVERHART 1973). Starvation medium was 10 mM Tris-HCl, pH 7.4. For matings cells were starved and mixed essentially as described by MARTINDALE, ALLIS and BRUNS (1982). Cells were starved, deciliated and allowed to regenerate as described by CALZONE and GOROVSKY (1982). Single cell manipulation, drop culture, microtiter plate culture, and replica plating were as described in ORIAS and BRUNS (1976).

Genetic nomenclature: The nomenclature proposed by BRUNS and BRUSSARD (1974) has been followed. For example, Cu 428 is Mpr/Mpr (mp-s, VII) which designates micronuclear genotype (6-methylpurine resistant/6-methylpurine resistant) and macronuclear phenotype (6-methylpurine sensitive, mating type VII). Cu 427 is Chx/Chx (cy-s, VI).

Mutagenesis: Mutagenesis was performed essentially as described in ORIAS and BRUNS (1976). A log phase culture of Cu 428 was exposed to 10 μg/ml nitrosoguanidine (Sigma) for 3 hr at room temperature, washed three times with growth medium (modified Neff's), and allowed to recover overnight with shaking at 28°. Mutagenized Cu 428 cells and normal Cu 427 cells were starved, mixed and incubated at 28°. Five hours after cells were mixed, an equal volume of concentrated Neff's (containing 6X the amounts of proteose peptone, yeast extract and glucose, and 2X the normal salts found in modified Neff's) was added to induce cytogamy (ORIAS, HAMILTON and FLACKS 1979), and the culture was incubated for 60 min. The culture was then diluted to essentially normal osmotic strength with 62.5 volumes of modified Neff's and incubated overnight at 28°, after which 6-methylpurine was added to a final concentration of 30 μg/ml. This concentration of 6-methylpurine is somewhat toxic to cells heterozygous for 6-methylpurine resistance so the fraction of cells in the culture that are homogyzgous for resistance is increased (P. BRUNS, unpublished observations). Clones derived from isolated pairs (synclones) were tested for resistance to 6-methylpurine and cycloheximide to determine the percentage of cells that had undergone cytogamy. Approximately 14% of the pairs underwent cytogamy.

Isolation of mutants: Mutagenized cells were starved at 28°. At various times over a period of 3 days, aliquots of approximately 10^6 cells were removed, deciliated and placed in a 38° incubator for 5–6 hr to allow cilia to regenerate. Shortly before the regenerating cells were screened, an equal volume of a prewarmed 1:250 dilution of India ink (Higgins) in starvation buffer was added to each flask, and the flasks were incubated another 30 min at 38°. Single cells that neither swam nor had black food vacuoles (oral ciliature is required for feeding) were identified using bright field optics on an Olympus dissecting microscope and were hand isolated using a braking pipette with a microtip. Each putative mutant was sucked up and blown out of the pipette 3–4 times. If the cell still did not swim it was deposited in a drop of medium on a Petri plate. Putative regeneration mutants were incubated in a moist chamber for several days at 28°. Approximately 800 cells survived, regenerated cilia and grew into clones. These clones were transferred to microtiter plates containing media and incubated at 28°.

The putative mutants were replica plated into microtiter plates and incubated overnight at 28° or 38°. Growth was scored by inspection using a dissecting microscope. Microtiter plate wells with obviously fewer cells at 38° than 28° were scored as temperature-sensitive for growth.

Complementation groups: Nineteen of the thirty mutant lines were fertile. Each was mated with strains A*III or B*VI, and exconjugants were isolated. Exconjugants from matings involving * (star) strains have undergone round 1 of genomic exclusion (S. L. ALLEN 1967a, b) and have new, homozygous micronuclei derived from the micronucleus of the non * member of the pair but retain their original macronuclei and phenotypes (including their original mating types). When round 1 exconjugants are mated to each other or to other non-* cells, they proceed normally through nuclear development and a new macronucleus develops from the zygotic nucleus. Round 1 exconjugants from each mutant strain were mated to round 1 exconjugants from every other mutant strain. Progeny were treated with 30 μg/ml 6-methylpurine to kill nonconjugants whose cytoplasm and macronucleus were derived from the * strain. Surviving cells were replicated to new microtiter plates and incubated at 38° overnight, after which wells were scored for wild-type growth. Nonconjugants derived from the mutant (non-*) parent and exconjugants from mutants in the same complementation group fail to grow.

Protein synthesis: Cultures in log growth at 28° were divided into two parts. One half was incubated at 28° with 20 μCi/ml [^{3}H]lysine (Amersham 40 Ci/mmole) and 100 μl samples were spotted onto Whatman GFA filters at 0, 15, 30 and 60 min. The remaining half was incubated for 4 hr at 38° and then pulsed with [^{3}H]lysine as described above. Cells were counted in each culture at 0, 30 and 60 min after addition of isotope. GFA filters containing cells were placed into cold 10% trichloroacetic acid (TCA) and left for several hours. Filters were then washed 2X with cold 10% TCA (10 min/wash), 2X with 70% ethanol (10 min/wash), 2X with 95% ethanol (10 min/wash), and air dried overnight. Filters were incubated in 0.9 ml NCS-tissue solubilizer (Amersham) plus 0.1 ml H_2O overnight at 45°. Ten milliliters of scintillation fluid containing 36 μl glacial acetic acid was added to each tube, and radioactivity was determined in a Beckman scintillation counter.

RNA dot blots: Wild-type and mutant cells in log growth

were incubated at 28° or 38° for 4 hr. Cells were then fixed and prepared for whole cell dot blots as described by YU and GOROVSKY (1986). Duplicate spots of 2000, 4000 and 8000 cells were made on poly-L-lysine coated glass fiber filters, and the filters were hybridized to ^{32}P-labeled antisense RNAs made from *Tetrahymena thermophila* α-tubulin and actin genes cloned into pGem2 (Promega Biotech). The actin gene was a gift from RONALD PEARLMAN (York University). The tubulin gene was cloned by SU-MAY YU in our laboratory (S. M. YU and M. A. GOROVSKY, unpublished observations). The dots were cut out of the filter, treated, and radioactivity was determined as described above.

Cell fixation: Cells were fixed either by the addition of formaldehyde (Baker) to 1% or by a modified Schaudin's (2 parts saturated $HgCl_2$, 1 part 99% ethanol, 0.01 vol glacial acetic acid). After fixation with modified Schaudin's, cells were washed 2X in 70% ethanol (10 min/wash), then resuspended in 99% ethanol:glacial acetic acid (3:1).

Microscopy: Single cell manipulations were done using dark field on an Olympus SZH dissecting microscope. Phase, Nomarski and fluorescence microscopy were done using an Olympus BH-2 compound microscope equipped with an Olympus 35 mm camera. Nuclei were visualized by staining formaldehyde fixed cells with 0.001 mg/ml 4′-6-diamidino-2-phenylindodihydrochloride (Dapi; Polysciences).

RESULTS

Isolation of mutants: A culture of *Tetrahymena thermophila* (Cu 428) was mutagenized, and cytogamy was induced to obtain cells that were homozygous in the micronucleus for any induced mutations and that were expressing those mutations in the macronucleus (ORIAS, HAMILTON and FLACKS 1979). These cells were starved and deciliated, and approximately eight hundred putative mutants that did not regain motility at 38° were isolated. We are interested in mutations that affect other structures and processes in addition to cilia or ciliogenesis, so the putative mutants were screened for lack of growth at 38°. After two rounds of screening we identified 56 mutants that failed to grow at 38°. These clones were rescreened for inability to resume motility at the restrictive temperature following deciliation. Thirty of the 56 mutants remained nonmotile. All of the mutants behaved normally or nearly so at 28°, and when ciliated, all mutants remained motile after incubation for several hours at 38°. The mutants were designated SG for slow growing.

Complementation groups: Nineteen of the 30 mutants were fertile, and the mutations were placed into 5 complementation groups (data not shown). Group 1 has 2 members: groups 2, 3 and 4 have 1 member each, and group 5 has 14 members. Since all of the mutants were isolated after a single mutagenesis with a period of growth allowed before screening, we do not know how many independent mutations are represented in complementation groups 1 and 5.

RNA and protein metabolism in mutant cells: Cilia regeneration and growth could be affected by mutations that affect RNA or protein synthesis as Tetrahymena fails to regain full motility after deciliation when incubated with cycloheximide or actinomycin D (GUTTMAN and GOROVSKY 1979). To distinguish such mutations from those that cause defects specifically in ciliary assembly and growth, the rates of protein synthesis and the amounts of α-tubulin mRNA were measured in mutants at the permissive (28°) and the restrictive (38°) temperatures.

As shown in Table 1, the rate of protein synthesis was higher at 38° than at 28° in wild-type cells, and in mutant cells from groups 1, 3 and 5. These results suggest that the growth defect exhibited by these mutants is not due to some general defect in the translation machinery, in mRNA synthesis or in energy production. In contrast, [^{3}H]lysine incorporation was dramatically lower in group 2 and group 4 mutants incubated at 38° than in the same mutants at 28°. The low rates of translation in these cells probably accounts for their failure to grow and to regain motility at the restrictive temperature. As group 2 and group 4 mutants swim actively at 38°, the mutations probably do not affect energy production.

To determine whether any of the mutations affect tubulin gene expression, the levels of message encoding α-tubulin and actin (as control) were measured in mutants in each complementation group at 28° and 38° (YU and GOROVSKY 1986). The results are shown in Table 1. Wild-type cells (Cu 428) incubated at 38° for 4 hr showed a slight decrease in the level of message coding for actin and a slight increase in the level of message coding for α-tubulin compared to cells incubated at 28°. Cells in group 2 and group 4 incubated at 38° for 4 hr showed a striking decrease in the levels of both α-tubulin and actin messages. Again, these results suggest that the mutations in these groups are not specific for ciliary assembly or growth. The relative message levels in group 3 and 5 mutants at 28° and 38° were not dramatically different from the relative message levels observed in wild-type cells incubated at the two temperatures. We think the small differences we did observe occurred because the cells were arrested in a particular growth state (see below) and not because the mutations directly affect α-tubulin or actin message levels. In group 1 mutants actin and tubulin message levels increased more in cells shifted to 38° than did message levels in wild-type cells. This is consistent with the observation that the rate of protein synthesis also increased more in group 1 cells shifted to 38° than it did in wild-type cells. We do not know the reason(s) for these observations, but because the RNA levels and the protein synthesis rate were always higher in group 1 mutants incubated at 38°, it seems unlikely that the differences in message levels are the cause of the abnormal phenotypes. Thus, the group 1 mutation as well as the group 3

TABLE 1

Relative protein synthesis rates and actin and α-tubulin message levels in mutants incubated at permissive or restrictive temperatures

Group	Representative	Relative rate of protein synthesis at 38°[a]	Relative amount of actin message at 38°[a]	Relative amount of α-tubulin message at 38°[a]
Wild-type	Cu 428	1.713	0.559	1.240
1	SG 17	4.077	3.684	2.394
2	SG 21	0.300	0.059	0.186
3	SG 60	1.201	1.360	3.840
4	SG 62	0.035	0.189	0.209
5	SG 79	1.666	1.838	0.716

[a] Calculated as the rate or message amount at 38° divided by the rate or amount at 28°.

TABLE 2

Relative cell numbers of mutant cultures incubated at 28° or 38°

		Relative cell number[a] at time:				
			2.5 hr		5.0 hr	
Group	Representative	0 hr[b]	28°	38°	28°	38°
Wild-type	Cu 428	1.00	1.98	1.90	3.21	3.99
1	SG 17	1.00	1.88	1.56	3.20	1.65
2	SG 21	1.00	1.66	0.98	2.42	0.93
3	SG 60	1.00	1.70	1.62	2.65	2.79
4	SG 62	1.00	2.09	1.17	3.71	1.11
5	SG 79	1.00	2.22	1.44	3.95	1.55

[a] Relative cell number is the number of cells/ml at a timepoint divided by the number of cells/ml in the same culture at time = 0.
[b] At time = 0 hr one culture was divided and equal volumes incubated at 28° or 38°.

and 5 mutations appear to affect specifically the assembly of functional cilia and growth.

Growth: All of the mutants were originally identified as having slow growth by inspection of microtiter plate cultures. A more rigorous examination of their growth at non-permissive temperature was undertaken in the hope of obtaining additional insights into the nature of the mutations. Cultures of mutant cells from each complementation group were grown at the permissive temperature (28°) until they were in early log growth. Half of each culture was shifted to 38°, and growth was monitored.

The results are shown in Table 2. Wild-type cells (Cu 428) grew as well or slightly better at 38° than at 28°. Groups 1 and 5 divided for a short time after the shift to 38°. Group 2 and group 4 did not divide at all after the temperature shift. Group 3 divided normally at the restrictive temperature for at least 5 hr but apparently stopped dividing and began to die sometime before 24 hr since few cells remained in the culture after that time (data not shown). These observations suggested that the group 1 and group 5 mutations cause cell cycle or cell division arrest, and those mutants were selected for further study. The group 5 mutants are discussed elsewhere (PENNOCK, THATCHER and GOROVSKY 1988), and the group 1 mutants are described in this paper.

Genetics of the group 1 mutation: SG 14 cells were mated with Cu 427 cells, and multiple F_1 synclones were established. Synclones are clones derived from a single mating pair and thus are genetically identical. However, each synclone can contain multiple mating types and can be used to produce F_2 synclones [see BRUNS (1986) and ORIAS (1986) for reviews of Tetrahymena conjugation and genetics]. Four F_1 synclones were tested for the ability to grow at 38° and for the ability to regain motility after deciliation and incubation at 38°. All four behaved like wild-type cells, indicating the mutation(s) is recessive. In addition, 166 F_2 synclones were established from a mating involving a single SG 14 F_1 synclone, and 146 F_2 synclones were established from a mating involving a single SG 17 F_1 synclone. All were tested for growth at 38°. Twenty percent of the F_2 synclones established from each mating failed to grow, indicating the slow growth phenotype segregates as if caused by a single, recessive mutation in both SG 14 and 17 strains (χ^2 analysis yields a P value of 0.15 in each case). To determine whether slow growth and inability to regenerate functional cilia are linked, 38 F_2 synclones (derived from a SG 14 F_1 synclone) homozygous for slow growth were starved; deciliated and allowed to regenerate at 28° or 38° as described previously (PENNOCK, THATCHER and GOROVSKY 1988). All 38 synclones failed to regain motility at 38°, indicating the two phenotypes are linked.

Group 1 mutants regenerate paralyzed cilia: To determine whether group 1 mutants fail to regenerate cilia or regenerate nonfunctional cilia at 38°, cells from a culture of F_2 synclones derived from SG 14 were deciliated, incubated at 28° or 38° and examined. Surprisingly, although >95% of the cells incubated at 38° were nonmotile, they had what appeared to be a nearly full complement of full-length cilia that were not beating (Figure 1). These observations indicate the group 1 mutation affects a molecule required for ciliary function but not for assembly. Since vegetative cells remained motile for at least twenty-four

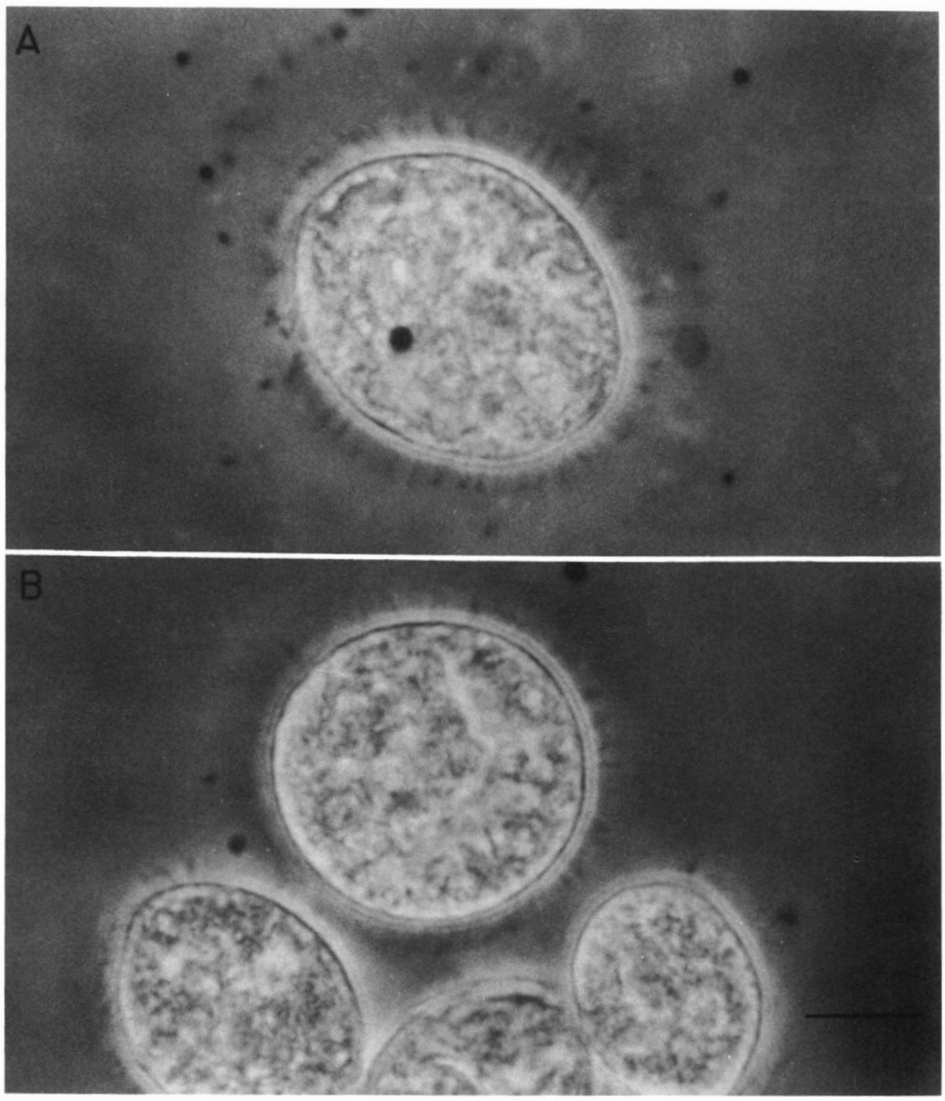

FIGURE 1.—Regeneration of cilia in group 1 mutants at 28° and 38°. Cells from a culture of F_2 synclones derived from SG 14 were deciliated and incubated for six hours at 28° (A) or 38° (B). Cells were fixed and observed with phase contrast optics. More than 95% of the cells incubated at 28° were motile, while fewer than 5% of the cells incubated at 38° were motile (bar = 10 μm).

hours after being shifted to 38°, cilia assembled at the permissive temperature must be unaffected at the restrictive temperature.

Cytokinesis but not nuclear division or DNA replication is affected by the group 1 mutation: To determine whether all arrested group 1 cells in a culture exhibit the same terminal phenotype, SG 17 cells were incubated at 38°, fixed and observed with the compound microscope. Greater than 80% of the mutants incubated without shaking had a dumbbell shaped appearance (see Figure 2 for an example), indicating that SG 17 cells arrest during cytokinesis. During routine culturing of Tetrahymena, the cultures are shaken. Under these conditions arrested cells tended to separate, and only approximately 20% of the cells retained the dumbbell configuration. Separation did not appear to release cells from division arrest as separated cells failed to initiate cytokinesis a second time (Table 2). Under all conditions the cells remained motile, and cilia appeared to beat normally. Arrested cells contained a new oral apparatus just posterior to the cleavage furrow, indicating that new cilia had been assembled at the nonpermissive temperature (data not shown). It seems likely that the cilia in the new oral apparatus were nonfunctional as were cilia regenerated at 38°, although we were unable to test this directly. These observations suggested the group 1 mutation causes cell cycle or cell division arrest.

To determine whether the mutation affects nuclear division, arrested, fixed cells were stained with Dapi. Figure 2 shows that the macronucleus had completed amitotic division at 38° even though cells did not complete cytokinesis. The micronucleus also completed division, and in a large number of cells, appeared to initiate a second division. In cultures of mutants (SG 17) incubated at 28°, 7.5% of the cells had either dividing micronuclei or multiple micronuclei. In cultures incubated at 38° for 4 hr, 30% of the dividing or recently divided cells (or cell halves) had

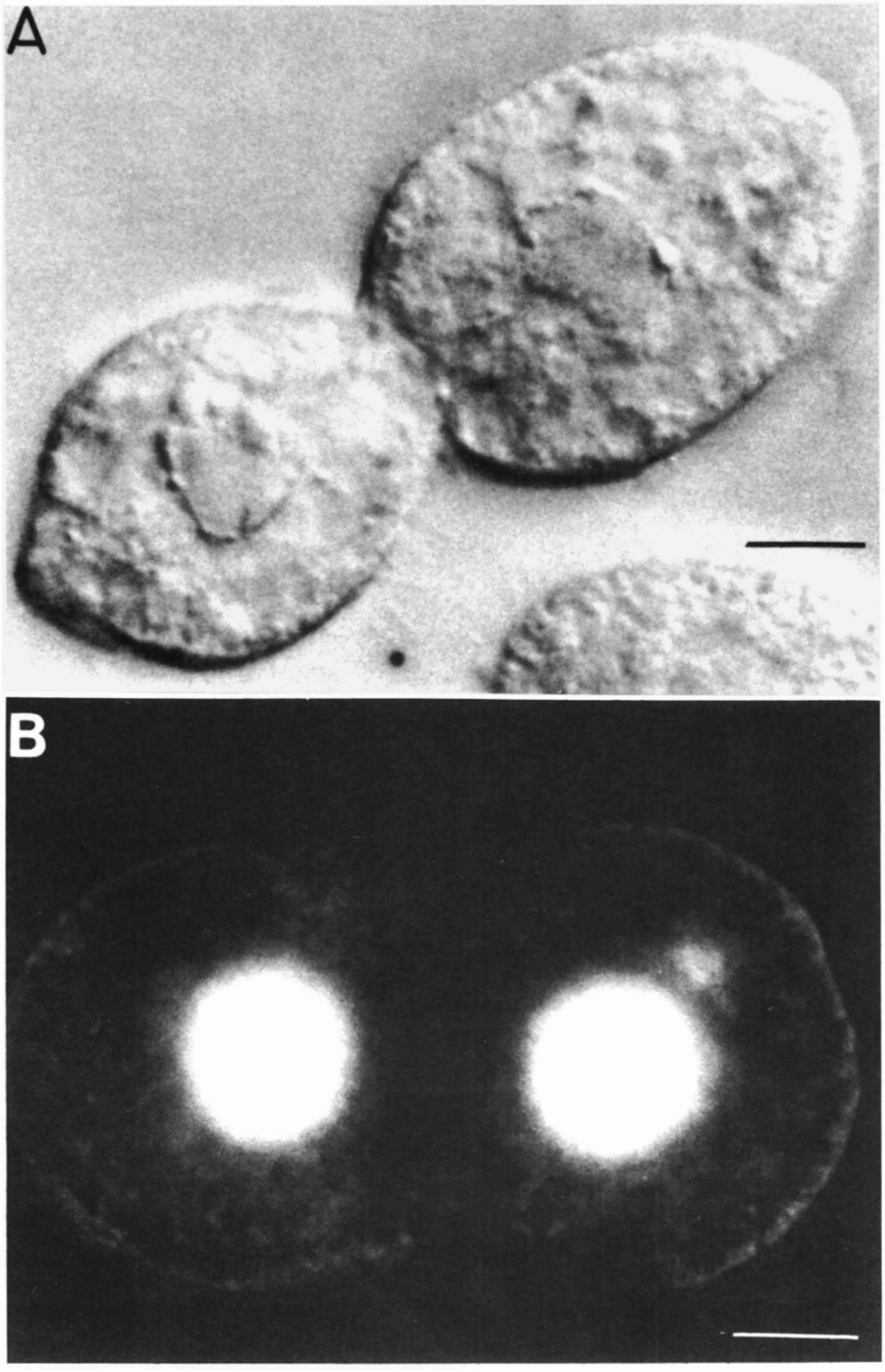

FIGURE 2.—Cellular and nuclear morphology of SG 17 incubated at 38°. A culture of SG 17 cells in logarithmic growth was shifted to 38°; after four hours aliquots were removed and fixed with formaldehyde and observed with Nomarski optics (A) or stained with Dapi and examined with the fluorescence microscope (B) (bar = 10 μm).

dividing or divided micronuclei. Thus, nuclear divisions continued in mutants arrested at 38°, indicating that the mutation caused cell division arrest but not true cell cycle arrest.

To determine whether DNA replication is affected by the group 1 mutation, mutants were labeled with [^{3}H]thymidine after preincubation at the restrictive temperature, and DNA synthesis was assayed by autoradiography. More than 95% of the nuclei were heavily labeled (see Figure 3 for an example), indicating that DNA replication continues in mutants well after cytokinesis has been arrested.

Wild-type cytoplasm rescues the cell division block in group 1 mutants: Mutant cells were mated with cells from strain A*III and allowed to undergo round 1 of genomic exclusion. During round 1 of genomic exclusion cells pair and retain their old macronuclear genomes. Cytoplasm is also exchanged since wild-type cytoplasm has been shown to restore 'normal' phenotype to mutants (DOERDER and BER-

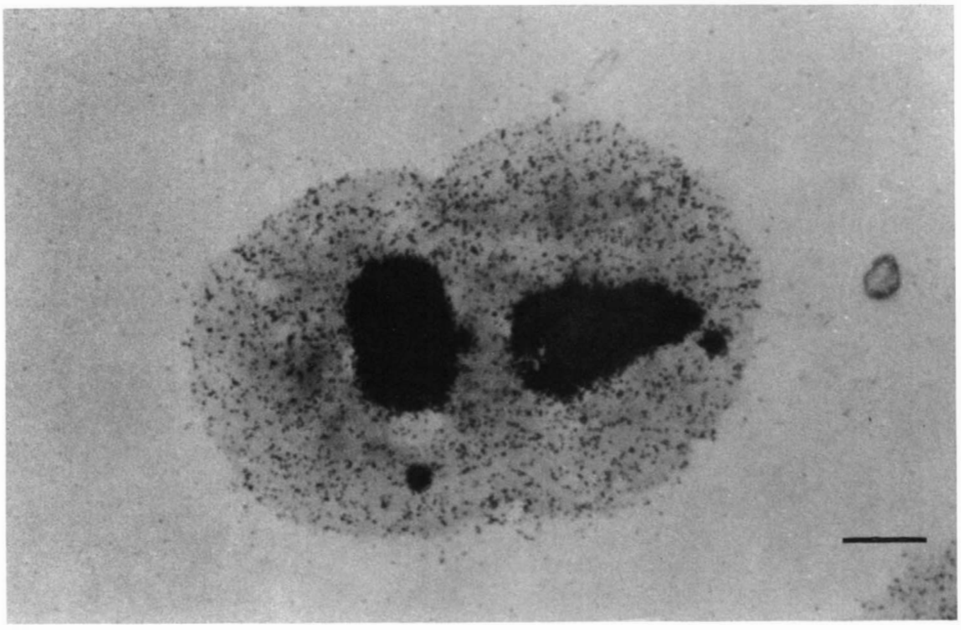

FIGURE 3.—[^{3}H]Thymidine incorporation into SG 17 cells after incubation at 38°. A culture of SG 17 cells in logarithmic growth was shifted to 38° or kept at 28° and incubated for 3.6 hr (the equivalent of one generation at 28°). [^{3}H]Thymidine was added to a final concentration of 20 μCi/ml, and the culture was incubated for an additional 3.6 hr. Cells were then harvested, fixed with modified Schaudin's and prepared for autoradiography. The cell shown was exposed 4 days at 4°. The large, labeled regions are macronuclei, and the two smaller, labeled regions are micronuclei (bar = 20 μm).

KOWITZ 1987; SATIR, REICHMAN and ORIAS 1986). Thus, any change in the phenotype of the mutant cell after round 1 of genomic exclusion must be due to wild-type cytoplasm transferred from the A*III member of the pair.

After mixing starved mutant and A* strains, pairs were isolated into growth medium and incubated at 20° until the cells separated. Exconjugants were isolated into drops and incubated at 38° overnight after which the number of cells in each drop was determined. Approximately one-half of the exconjugants divided numerous times, and the cells were not counted. These cells presumably contained * cytoplasm since * cells incubated at 38° grow normally (data not shown). In contrast, the remaining half of the exconjugants (presumably containing mutant cytoplasm) divided an average of one time before arresting (n = 170). Individual (unmated) mutant cells transferred from starvation buffer to growth medium and incubated at 38° did not divide at all (n = 38), nor did exconjugants from a mutant × mutant mating that were incubated in growth medium at 38° (n = 33). These results indicate that wild-type cytoplasm passed from the * cell to the mutant cell during pairing and that the transferred cytoplasm contained enough rescuing activity to support one cell division.

DISCUSSION

We isolated 30 conditional *Tetrahymena thermophila* mutants that fail to regain motility after deciliation and that fail to grow. Nineteen of the mutants were fertile, and the mutations causing the slow growth phenotype were placed into five complementation groups. At the restrictive temperature two complementation groups (2 and 4) showed decreased levels of protein synthesis and reduced levels of RNAs coding for both actin and α-tubulin, suggesting the group 2 and group 4 mutations do not specifically affect cilia regeneration and growth. In contrast, protein synthesis in group 1, 3 and 5 cells appeared to be largely unaffected at the restrictive temperature, and the relative levels of message coding for α-tubulin and actin at 38° and 28° were not very different from the relative levels observed in wild-type cells incubated at the two temperatures. The small differences from the wild-type response that were obsrved seem unlikely to be the cause of the mutant phenotype. Thus, by a process of elimination, the mutations in complementation groups 1, 3 and 5 are likely to be in genes encoding molecules directly involved in ciliary assembly or function and in cell growth or division. This is clearly the case for group 1. When incubated at 38° group 1 mutants failed to grow and regenerated nonfunctional cilia. Interestingly, cilia assembled at 28° by group 1 cells remained motile after cells were shifted to 38°. Thus, once beating cilia are formed, the group 1 gene product is either unnecessary or is stabilized against thermal inactivation.

Both the failure to regenerate cilia and the failure to grow appear to be caused by a single, recessive mutation in group 1 mutants because the two phenotypes segregated together in all 38 F_2 progeny tested in an organism where tight linkage of genes known to be on the same chromosome is difficult to demonstrate (BRUNS 1986). In addition, both the time required to regenerate cilia and the time required to traverse the cell cycle is longer in group 1 cells incubated at 28° than it is in wild-type cells incubated at the same temperature (data not shown), indicating both phenotypes are caused by molecules that are partially

inactivated at 28° and completely inactivated at 38°. These observations argue strongly that both phenotypes are caused by one mutation; actual proof awaits isolation of the mutant gene product and complementation of both phenotypes.

We expected that our screen would yield mutations that affect growth by inhibiting processes known to require microtubules (*e.g.*, nuclear division, nuclear migration, stomatogenesis, etc.), and indeed, the group 5 mutation appears to do that (PENNOCK, THATCHER and GOROVSKY 1988). Surprisingly, however, group 1 mutants fail to complete cytokinesis at the restrictive temperature. If, as seems likely, a single mutation is responsible for both phenotypes, characterization of the group 1 gene product should lead to novel insights into a cytoplasmic component required for ciliary motility and cytokinesis. The only current candidate for such a product is actin because it has been localized to the division furrow of many cell types (SCHROEDER 1973), and a protein very similar to muscle β-actin has been found in Chlamydomonas axonemes (PIPERNO and LUCK 1979).

The group 1 gene product does not seem to be required for macro- or micronuclear division, DNA replication, protein synthesis, RNA synthesis or energy production. It is worth noting that macronuclei in cells arrested in cytokinesis divide completely (Figure 2), indicating that the amitotic macronuclear division is an active process that can be completed independently of cytokinesis. This confirms observations made previously by FRANKEL, NELSEN and JENKENS (1977).

The cell division arrest phenotype of group 1 resembles that of the CdaC2 mutant described by FRANKEL, NELSEN and JENKINS (1977). However, the CdaC2 mutant (kindly supplied by JOSEPH FRANKEL) complements the group 1 mutation, and CdaC2 also regenerates functional cilia at 38° (data not shown).

Group 1 mutants, like mutants having defective exocytosis (SATIR, REICHMAN and ORIAS 1986) and improper serotype antigen expression (DOERDER and BERKOWITZ 1987), can be rescued by wild-type cytoplasm received during conjugation with a wild-type cell, raising the possibility that the defective group 1 gene product can be identified by microinjection of wild-type cytoplasmic fractions or solubilized ciliary components into mutant cells with rescue scored as at least one cell division at 38°. Similar approaches have worked well in Paramecium (HAGA *et al.* 1984a, b), and microinjection into Tetrahymena cells is now routine (TONDRAVI and YAO 1986).

In summary, we have described a genetic screen designed to isolate mutations in genes required for growth and for the assembly and/or function of cilia in Tetrahymena. Mutations in two of the five complementation groups (groups 1 and 5) identified in this first application of the screen appear to be good candidates for mutations of the type being sought. Analysis of the group 1 mutants clearly demonstrates that the mutation affects the assembly of functional cilia but not all of the other microtubule-based systems (*e.g.*, those required for nuclear division). Because group 1 mutations are also blocked in cytokinesis, the existence of a component common to ciliary motility and cell division also seems likely. Since group 1 mutants can be rescued by cytoplasmic exchange, it should be possible to purify and characterize the group 1 gene product.

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