

Genetic Interactions at the *FLA10* Locus: Suppressors and Synthetic Phenotypes That Affect the Cell Cycle and Flagellar Function in *Chlamydomonas reinhardtii*

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ABSTRACT

Through the isolation of suppressors of temperature-sensitive flagellar assembly mutations at the *FLA10* locus of *Chlamydomonas reinhardtii*, we have identified six other genes involved in flagellar assembly. Mutations at these suppressor loci, termed *SUF1-SUF6*, display allele specificity with respect to which *fla10*⁻ mutant alleles they suppress. An additional mutation, *apm1-122*, which confers resistance to the plant herbicides amiprofos-methyl and oryzalin, was also found to interact with mutations at the *FLA10* locus. The *apm1-122* mutation in combination with three *fla10*⁻ mutant alleles results in synthetic cold-sensitive cell division defects, and in combination with an additional pseudo-wild-type *fla10*⁻ allele yields a synthetic temperature-sensitive flagellar motility phenotype. Based upon the genetic interactions of these loci, we propose that the *FLA10* gene product interacts with multiple components of the flagellar apparatus and plays a role both in flagellar assembly and in the cell cycle.

THE flagellar apparatus of *Chlamydomonas* consists of a pair of basal bodies and their associated flagella (TUCKER 1984; LeDizet and PIPERNO 1986; HOLMES and DUTCHER 1989). In addition to the microtubules of the flagella, the majority of the cytoplasmic microtubules and the rootlet microtubules originate in the vicinity of the basal bodies at the anterior of the cell (RINGO 1967). Prior to mitosis, the flagella disassemble and the components are resorbed, basal body replication/elongation is completed, and the two pairs of basal bodies separate and migrate to opposite sides of the nucleus where they take up residence in the region of each mitotic spindle pole (JOHNSON and PORTER 1968; COSS 1974; TRIEMER and BROWN 1974; HOLMES and DUTCHER 1989). The cytoplasmic microtubules also disassemble but the rootlet microtubules remain intact. After cell division is completed, new flagella, cytoplasmic microtubules, and two new rootlet microtubules are assembled. Most evidence suggests that the basal bodies are required only for the assembly of the flagella and not for the assembly of the other microtubule systems (PICKETT-HEAPS 1971). In other words, basal bodies and centrioles are not required for mitosis (MCINTOSH 1983; BRINKLEY 1985).

Basal bodies are tubular structures composed of nine blades of triplet microtubules that are approximately 400 nm in length. Basal bodies are morphologically similar to centrioles, which are found in many but not all eukaryotic cells (KUBAI 1975; PETERSON and BERNS 1980). Basal bodies are complex organelles

by morphological and biochemical criteria. Isolated basal bodies from *Chlamydomonas reinhardtii* contain more than 200 polypeptide components (DUTCHER 1986) and isolated centrosomes from mammalian cells contain a similar number of components (KLOTZ *et al.* 1990). At least 15 different polypeptides have been identified by antisera that recognize components of this organelle (CALARCO-GILLAM *et al.* 1983; BARON and SALISBURY 1988; SNYDER and DAVIS 1988; KELLOGG, FIELD and ALBERTS 1989; KURIYAMA 1989; RAO *et al.* 1989). One approach to the study of a complex system is the application of a genetic analysis. To this end, we have initiated a genetic and phenotypic analysis of mutations that appear to affect the basal body of *Chlamydomonas* (DUTCHER, GIBBONS and INWOOD 1988; DUTCHER 1989). In this paper we report the study of mutations in the *FLA10* gene of *Chlamydomonas*. This gene was originally identified by temperature-sensitive mutations that are unable to assemble flagella at 32° (HUANG, RIFKIN and LUCK 1977; ADAMS, HUANG and LUCK 1982). We have shown that mutations at this locus have an effect on cell division and flagellar motility as well as on flagellar assembly. In addition to their requirement for flagellar assembly, basal bodies may also play a role in cell division.

MATERIALS AND METHODS

Mutant strains: The mutant strains dd-a-224, 519, and 544 were isolated in screens for temperature-sensitive flagellar assembly-defective strains of *Chlamydomonas reinhardtii* (HUANG, RIFKIN and LUCK 1977; ADAMS, HUANG and

LUCK 1982). Each of the mutant *fla10⁻* strains was backcrossed to the isogenic wild-type strain, 137c, a minimum of three times to assure that the flagellar-defective phenotype segregated 2⁺:2⁻ and was due to a single gene mutation. The *act2* (CC-1590), *arg2* (CC-48 and CC-49), and *arg7* (CC-50 and CC-51) strains were obtained from the Chlamydomonas Genetics Center (HARRIS, BOYNTON and GILLHAM 1987). The original *arg⁻* strains yielded less than 10% viable spores in crosses. Consequently, *arg⁻* spores were backcrossed to wild type (137c) a minimum of six times to generate strains that yielded meiotic spore viability of 90–99%. All haploid strains used in the construction of diploid strains were derived from these *arg⁻* strains and yielded similar spore viability. Stable diploid strains were constructed by mating strains that carried the complementary arginine markers, *arg2* and *arg7*, and selecting for prototrophic progeny (EBERSOLD 1967). These haploid parents also carried various recessive drug-resistance mutations.

The *apm1⁻* mutations were isolated in this laboratory following ultraviolet irradiation on rich medium containing 15 μ M oryzalin. They were designated as alleles at the *APM1* locus based on map distance from the *UN11* locus, failure of the *apm1-122* allele to recombine with the *apm1-7* allele from P. LEFEBVRE (JAMES *et al.* 1988) in 48 random spores, failure of the 14 *apm1⁻* alleles used in this study to recombine with each other in a total of 320 tetrads, and a failure to observe complementation in diploid strains in any of the 33 pairwise combinations of the 14 *apm1⁻* alleles isolated in this laboratory. The *pdr3-1* allele, isolated in the same selection, shows pleiotropic drug resistance phenotypes as do the original *pdr⁻* mutations (JAMES and LEFEBVRE 1989), and is unlinked to them. Resistance to oryzalin is semidominant in heterozygous *pdr3-1/PDR3⁺* diploid strains. The *apm3-1* allele, also isolated in the same selection, has not been mapped but is semidominant in heterozygous *apm3-1/APM3⁺* diploid strains. The *pdr1-1* allele was obtained as strain CC-399 from the Chlamydomonas Genetics Center.

Media and culture conditions: Rich growth medium is a modified version of medium I of SAGER and GRANICK (1953) in which the concentration of K₂HPO₄ has been raised from 0.57 mM to 1.0 mM and the trace metal solution of HUTNER *et al.* (1950) is substituted. All water used in media preparation is derived from a Milli-Q water filtration system equipped with an ultrafilter cartridge that has an effective pore size of 0.1 μ m (Millipore Corporation, Bedford, Massachusetts). Strains that are auxotrophic for arginine are grown on rich medium with one-tenth the normal concentration of ammonium nitrate, supplemented with 200 μ g/ml of arginine-HCl. Decreased concentrations of ammonium nitrate are reported to stimulate the uptake of exogenous amino acids in some algae (NORTH and STEPHENS 1971, 1972). In Volvox, nitrogen starvation elevates arginine uptake, but arginine is insufficient to support growth when supplied as the sole nitrogen source (KIRK and KIRK 1977). Oryzalin-containing plates are rich medium supplemented with 15 μ M oryzalin (courtesy of GLENN EVANS, Eli Lilly Greenfield Laboratories). M-N/5 is a low nitrogen, minimal liquid medium that lacks acetate and has one-fifth the normal concentration of ammonium nitrate of rich medium. Low sulfate medium is modified rich medium with one-tenth the normal concentration of magnesium sulfate, supplemented with magnesium chloride at a concentration of 0.28 mg/ml. Low sulfate plates contain 1.5% Difco Bacto-agar washed a minimum of five times in Milli-Q water to remove endogenous sulfates and other contaminants and air dried.

Unless otherwise noted, all cells were grown under constant light at an intensity of 20 μ E/m²/sec.

Genetic analysis: Standard methods were employed for mating and for tetrad analysis (LEVINE and EBERSOLD 1960; for detailed description, see HARRIS 1989). Centromere distances were determined by the method of WHITEHOUSE (1950) and linkage analysis was performed using the method of PERKINS (1952).

Mutagenesis: *fla10⁻* mutant cells were exposed to ultraviolet (UV) irradiation or were treated with the alkylating agents; nitrosoguanidine (MNNG), ethyl methanesulfonate (EMS), or methyl methanesulfonate (MMS).

UV irradiation: The basic procedure for mutagenesis was that of LUCK *et al.* (1977). Lawns of approximately 10⁸ cells on 100-mm diameter Petri plates containing either rich or low sulfate medium, or suspensions of 10⁸ cells in 10 ml of M-N/5 medium in 100-mm diameter Petri plates, were irradiated under a General Electric 30 W germicidal UV lamp (G3T08) at a distance of 15 cm for 45 or 60 sec.

Chemical mutagenesis: Suspensions of 10⁸ cells in M-N/5 medium were incubated in appropriate concentrations of MNNG (2–50 μ g/ml), EMS (10–30 μ l/ml), or MMS (0.2 or 1.0 μ l/ml) for 15–120 min (LUX 1990). Cells were then pelleted, washed three times in M-N/5 medium, and diluted into 20 ml of rich liquid medium and incubated in the light at 32° for the isolation of suppressed strains (see below). EMS-treated cells were washed three times in 1% sodium thiosulfate in rich medium or in M-N/5 medium to inactivate the EMS.

Isolation of suppressors: Following mutagenesis, suppressors of the *fla10⁻* strains were isolated through the use of one of two schemes. The first regimen involved the enrichment for mutagenized *fla10⁻* cells that had acquired the ability to swim at the nonpermissive temperature of 32° (swimming screen). The second regimen selected for *fla10⁻* cells that had acquired the ability to mate at 32° (mating selection). This selection only required that cells be competent to assemble flagella, whether or not the flagella were functional and motile. Cells must only assemble flagella and express the appropriate surface molecules for the recognition of cells of the opposite mating type (BERGMAN *et al.* 1975; GOODENOUGH and WEISS 1975). Zygotes can be selectively recovered because their thick protective cell wall is impermeable to chloroform vapors, while the cell wall of vegetative haploid and diploid cells is not.

Swimming screen: Approximately 10⁵ to 10⁶ mutagenized cells were placed in 20 ml rich liquid medium in 25 mm \times 175 mm tubes. After 12–24 hr at 32°, the upper 2 ml of medium were transferred to 20 ml of fresh rich medium in the same size tubes at 32° until visible numbers of swimming cells became apparent, which was usually 3–4 days. At that point, the upper 2 ml were again transferred to 20 ml of fresh rich medium in order to further enrich for suppressed *fla10⁻* strains that were able to swim. Cells were then plated on solid medium to isolate single colonies. Eighteen to 24 single colonies from each plate were inoculated individually into rich liquid medium and the motility phenotype was scored at 21° and 32° to confirm the presence of a suppressor mutation. In general, only one revertant isolate from each original selection tube was retained in order to assure the independence of each suppressor allele. However, in cases where two single colonies from a particular tube yielded cells with significantly different phenotypes, both isolates were saved for further study and labeled "isolate #" and "isolate #A."

Mating selection: Mutagenized populations were made competent for mating at the restrictive temperature of 32°. Mutagenized cells were mated after nitrogen starvation either to *fla10-1* or to wild-type tester cells at 32° for approximately 12 hr to maximize the mating of suppressed

fla10⁻ cells in the population. Mixtures of mating cells were then inoculated onto rich medium plates, incubated for another 12 hr in the light at 32°, and allowed to undergo the remainder of zygotic maturation under standard conditions (HARRIS 1989). Following zygotic maturation, plates were treated with chloroform and then placed in the light at 21° to allow for germination of zygotes and growth of the resultant meiotic progeny cells. After approximately 1 week, these zygote clones were transferred into rich liquid medium and grown for 1 or 2 days at 21°. Cells were then diluted and plated on rich medium for the isolation of single colonies. These single colonies were picked into rich liquid medium and their flagellar assembly phenotypes were scored at 21° and 32°.

Identification of intragenic vs. extragenic suppressors: Each suppressed *fla10*⁻ isolate was crossed to the isogenic wild-type strain 137c. Aflagellate cells are nonmotile; when grown in liquid culture they fall under the influence of gravity and form a pellet. By contrast, wild-type cells either accumulate at the air-liquid interface or distribute themselves throughout the culture medium. Based upon this phenotypic difference at 32°, mutant *fla10*⁻ strains are easily distinguished from wild type or from suppressed strains. If only PD tetrads were recovered, then the suppressor was considered to be intragenic or linked [less than 7 centiMorgans (cM)] to the original *fla10*⁻ mutation. Those strains that yielded tetratype (TT) and nonparental ditype (NPD) tetrads were considered to contain an extragenic suppressor mutation. At least seven tetrads were examined in these crosses and an average of 33 tetrads were analyzed.

To confirm that suppression resulted from a single mutational event, strains that contained an extragenic suppressor mutation were backcrossed to the unmutagenized parental *fla10*⁻ allele. At least nine tetrads were examined in the backcrosses and an average of 19 tetrads were analyzed. Recovery of only PD tetrads indicated that suppression resulted from a single extragenic mutation. Recovery of TT or NPD tetrads indicated that suppression resulted from more than one extragenic mutation.

RESULTS

Description of the *FLA10* alleles: The dd-a-224 mutation, originally named *fla10*, was the first mutation to identify the *FLA10* locus. Two other mutations, isolates 519 and 544, are also alleles of the *FLA10* locus. The 519 mutation was initially designated *fla*⁴⁷ and mapped to linkage group IV (ADAMS, HUANG and LUCK 1982). However based on two criteria, the 519 and 544 mutations are allelic with dd-a-224. The mutations are less than 0.05 cM apart based on all pairwise combinations. In *Chlamydomonas*, 1 cM corresponds to approximately 50 kilobases (kb) of DNA on average (RANUM *et al.* 1988; P. FERRIS and U. W. GOODENOUGH, personal communication). Based on this estimate, dd-a-224, 519, and 544 map to within 2.5 kb of one another. When tested in heteroallelic diploid cells, these mutations fail to complement one another in all pairwise combinations. Because of the allelism described above, dd-a-224 has been designated as *fla10-1* and 519 and 544 have been designated as *fla10-14* and *fla10-15*, respectively.

Each mutant *fla10*⁻ strain assembles functionally wild-type flagella at the permissive temperature of

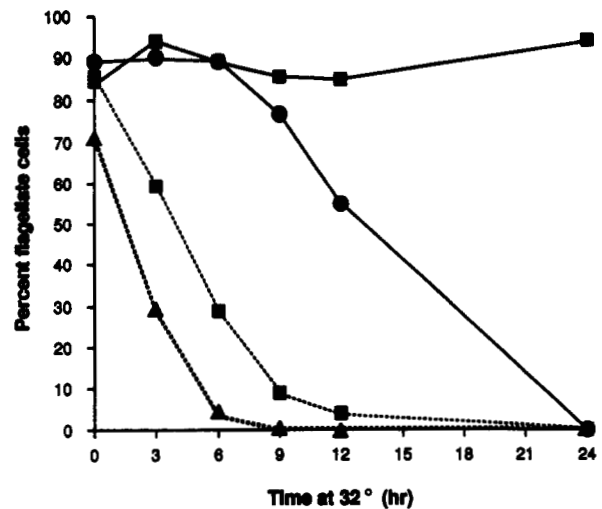


FIGURE 1.—Kinetics of flagellar loss in three *fla10*⁻ mutant alleles at the nonpermissive temperature. The *fla10-1*, *fla10-14* and *fla10-15* mutant strains were compared to the wild-type strain, 137c, at 32°. Cells were incubated in rich liquid medium at 21° overnight, shifted to 32° and the percentage of flagellate cells in each sample was determined at 3 hr intervals. For each sample, a minimum of 100 cells from three independent samples were counted and scored for the presence or the absence of flagella at each time point. *fla10-15* (triangles, dashed line); *fla10-1* (stippled squares, dashed line); *fla10-14* (solid circles, solid line); 137c (solid squares, solid line).

21° and fails to assemble flagella at the restrictive temperature of 32°. Although the strains are similar in their gross phenotypes, they differ from one another in subtle aspects of flagellar assembly and stability. Each strain displays distinct kinetics of flagellar loss following a shift from the permissive to the restrictive temperature (Figure 1). Unlike the *fla10-1* and *fla10-14* mutant alleles at the permissive temperature, populations of *fla10-15* cells contain about 30% aflagellate cells. Thus, the *fla10-15* mutation results in a weak nonconditional phenotype as well as in a strong temperature-sensitive flagellar assembly defect. The differences in the flagellar-assembly phenotypes of these three alleles suggest that each mutation results from a distinct lesion in the *FLA10* gene.

Identification of intragenic and extragenic suppressors: Suppressors of the three mutant *fla10*⁻ alleles were isolated based upon reversion of the flagellar assembly defect at 32° after treatment with a variety of mutagens (LUX 1990). Suppressors were identified in either a swimming screen or a mating selection. Of the 316 suppressed strains isolated, 100 contained extragenic suppressors.

The spectrum of intragenic vs. extragenic suppressors isolated from each *fla10*⁻ mutant strain varies dramatically among the three alleles examined (Table 1). The difference in the frequency of isolation of intragenic vs. extragenic suppressors probably represents a difference in the nature of the genetic lesion underlying each mutant allele. The *fla10-1* allele appears to be easily back-mutated to the wild-type se-

TABLE 1
Summary of suppressors by *FLA10* allele

| Allele | Screen or selection | No. of suppressors | | | |
|-----------------|---------------------|--------------------|------------|------------|--------------|
| | | Total | Intragenic | Extragenic | % Extragenic |
| <i>fla10-1</i> | Swimming | 68 | 68 | 0 | 0 |
| <i>fla10-1</i> | Mating | 116 | 113 | 3 | 3 |
| <i>fla10-14</i> | Swimming | 69 | 22 | 47 | 68 |
| <i>fla10-15</i> | Swimming | 63 | 13 | 50 | 79 |

quence or pseudoreverted by a second-site mutation within the gene, while the *fla10-14* and *fla10-15* alleles are less prone to intragenic reversion, given that the frequency of revertants recovered was similar for the three alleles.

Analysis of the extragenic suppressors: Of the 100 extragenic suppressor mutations, 67 were studied further. To confirm that a single mutation was responsible for suppression, each original suppressor strain that contained an extragenic suppressor mutation was backcrossed to the original *fla10⁻* strain from which it was isolated. In all cases, a single mutation appears to be responsible.

We attempted to identify the number of suppressor loci by complementation analysis, but as shown below some of the alleles were dominant, which made complementation unfeasible. Consequently, recombination mapping was employed in order to determine the number of suppressor loci represented in the collection of strains. Because two suppressor mutations that fail to recombine may identify a single gene or two linked genes that display similar mutant phenotypes, the number of loci identified through recombination mapping represents a minimum estimate of the number of suppressor loci identified. We determined by pairwise crosses the number of loci among the suppressors of each mutant *fla10⁻* allele. Suppressors of *fla10-1* map to one recombinational group; suppressors of *fla10-14* to two loci and suppressors of *fla10-15* to four loci (Table 2).

Representative isolates from each of the seven groups were crossed to one another to determine if any of the suppressors of one *fla10⁻* mutant allele corresponded to the loci identified as suppressors of the other mutant alleles. However, difficulties can arise from the allele specificity of some of the suppressors. If both suppressor mutations are allele-specific then the diagnostic class that will distinguish linked from unlinked allele-specific suppressors are tetrads with three nonsuppressed and one suppressed spore (3⁺:1⁻), or one nonsuppressed and three suppressed spores (1⁺:3⁻). All crosses between a *fla10-14* suppressor and a *fla10-15* suppressor gave rise to the diagnostic 3:1 and/or 1:3 tetrad classes (Table 3). In total, a minimum of six extragenic suppressor loci were identified through these pseudoreversion anal-

yses. The extragenic suppressors of the *fla10-1* allele map to a locus called *SUF1*, for *SU*ppressor of *FL*agellar assembly defect. Approximately one-half of the mutations isolated as suppressors of the *fla10-14* allele map to *SUF1* and the remainder map to an unlinked locus, *SUF2*. The loci identified as suppressors of the *fla10-15* mutation have been named *SUF3-SUF6*.

Allele specificity of suppressor alleles: All the extragenic suppressors display some degree of allele specificity with respect to which *fla10⁻* mutations they suppress (Table 4). The alleles at the *SUF1* locus fall into two classes. The first class is composed of isolates that suppress both the *fla10-1* and the *fla10-14* mutations. The three suppressors isolated in a *fla10-1* mutant background fall into this class, as do three of the *suf1⁻* alleles which were isolated in a *fla10-14* mutant background. The remaining six *suf1⁻* alleles, which suppress only the *fla10-14* mutation, make up the second class (Table 4). None of the *suf1⁻* suppressor alleles has any discernible effect on the *fla10-15* mutation. All 13 *suf2⁻* alleles are similar to the second class of *suf1⁻* alleles. Each *suf2⁻* mutation was isolated in a *fla10-14* mutant background and each suppresses only the *fla10-14* mutation.

The remaining 42 suppressors have a phenotype only with the *fla10-15* allele against which they were isolated. We tested a representative allele of the *SUF3*, *SUF4*, *SUF5* and *SUF6* loci for suppression of the *pf14a* allele. This *pf14⁻* allele contains a UGA codon at codon 21 of the coding sequence (WILLIAMS *et al.* 1989). None of these suppressor *a* mutations suppresses the motility defect of the *pf14* allele at 21° or 32°. The *suf3⁻-suf6⁻* suppressor mutations have not been characterized further at this time.

Dominance of suppressor alleles: The behavior of *suf1⁻* and *suf2⁻* mutations was examined in diploid strains homozygous for the *fla10-14* mutation and heterozygous for each suppressor allele. A subset of mutant *suf1⁻* and *suf2⁻* alleles are dominant while others are recessive to the wild-type allele (Table 4). Among the *suf1⁻* alleles, only in the class I mutations, which suppress both the *fla10-1* and *fla10-14* alleles, do we observe dominant mutations.

Flagellar assembly phenotypes: Because the suppressors were isolated based upon reversion of a temperature-sensitive flagellar assembly defect, each suppressor *fla10⁻* double mutant strain was examined for its flagellar assembly behavior at various temperatures. Among the original 316 suppressed strains, two general types of flagellar assembly phenotypes were recovered. The first type consists of revertant isolates that display flagellar assembly behavior indistinguishable from that of the wild-type strain, 137c, and all are intragenic events. The second type, which consists of both intragenic and extragenic events, displays flagellar assembly phenotypes intermediate between

TABLE 2
Genetic characterization of suppressed *fla10*⁻ strains

| <i>SUF</i> locus (no. of alleles) | Parental strain used in suppressor isolation | | | Centromere distance (in cM) ^a |
|--------------------------------------|--|---|---|---|
| | <i>fla10-1</i> | <i>fla10-14</i> | <i>fla10-15</i> | |
| <i>suf1</i> (12) | 1, 2, 3 | 103, 106, 108, 116, 122, 134, 137, 139, 142 | | 8.3 ± 8.6 |
| <i>suf2</i> (13) | | 104, 107, 109, 112, 118, 119, 123, 126, 127, 130, 140, 144, 146 | | 21.8 ± 8.7 |
| <i>suf3</i> (23) | | | 400, 401, 402, 405, 409, 413, 430, 432, 433, 434, 435, 437, 438, 439, 441, 442, 443, 449, 450, 451, 453, 455, 456 | 7.6 ± 10.6 |
| <i>suf4</i> (17) | | | 401A, 408, 414, 415, 417, 418, 420, 421, 423, 424, 425, 427, 429, 431, 454, 458, 463 | 31.5 ± 14.3 |
| <i>suf5</i> (1) | | | 419 | 9.3 |
| <i>suf6</i> (1) | | | 440 | 9.3 |

^a *Suf* to centromere distance (SUF-CEN) was calculated from data obtained in backcrosses of *fla10*⁻ *suf*⁻ strains to the wild-type strain (WHITEHOUSE 1950). *FLA10* displays 27% second division segregation from its centromere at 21°. Values given are the averages from all alleles of each locus with the standard error.

^b Segregation of each *suf1*⁻ allele was examined in at least 15 tetrads to another *suf1*⁻ allele; the average number of tetrads examined was 40. Segregation of each *suf2*⁻ allele was examined in at least 14 tetrads to another *suf2*⁻ allele; the average number of tetrads examined was 23. Segregation of each *suf3*⁻ allele was examined in at least 12 tetrads to another *suf3*⁻ allele; the average number of tetrads examined was 21. Segregation of each *suf4*⁻ allele was examined in at least 12 tetrads to another *suf4*⁻ allele; the average number of tetrads examined was 16. The identification of *SUF5* and *SUF6* as separate loci is documented in Table 3.

TABLE 3

Pairwise crosses of suppressors

| Cross | Tetrad progeny suppressed spores: nonsuppressed spores | | | | |
|---|--|-----|-----|-----|----------------|
| | 4:0 | 3:1 | 2:2 | 1:3 | 0:4 |
| <i>fla10-14 suf1</i> × <i>fla10-14 suf2</i> | 206 | 235 | 291 | 0 | 0 ^a |
| <i>fla10-14 suf1</i> × <i>fla10-15 suf3</i> | 0 | 0 | 4 | 6 | 3 |
| <i>fla10-14 suf1</i> × <i>fla10-15 suf4</i> | 2 | 17 | 16 | 8 | 2 |
| <i>fla10-14 suf1</i> × <i>fla10-15 suf5</i> | 2 | 9 | 10 | 2 | 2 |
| <i>fla10-14 suf1</i> × <i>fla10-15 suf6</i> | 1 | 4 | 3 | 0 | 3 |
| <i>fla10-14 suf2</i> × <i>fla10-15 suf3</i> | 0 | 3 | 6 | 3 | 3 |
| <i>fla10-14 suf2</i> × <i>fla10-15 suf4</i> | 0 | 3 | 8 | 2 | 1 |
| <i>fla10-14 suf2</i> × <i>fla10-15 suf5</i> | 3 | 8 | 7 | 1 | 0 |
| <i>fla10-14 suf2</i> × <i>fla10-15 suf6</i> | 4 | 9 | 2 | 0 | 1 |
| <i>fla10-15 suf3</i> × <i>fla10-15 suf4</i> | 9 | 37 | 16 | 0 | 0 ^b |
| <i>fla10-15 suf3</i> × <i>fla10-15 suf5</i> | 2 | 0 | 4 | 0 | 0 |
| <i>fla10-15 suf3</i> × <i>fla10-15 suf6</i> | 9 | 4 | 1 | 0 | 0 |
| <i>fla10-15 suf4</i> × <i>fla10-15 suf5</i> | 2 | 5 | 3 | 0 | 0 |
| <i>fla10-15 suf4</i> × <i>fla10-15 suf6</i> | 8 | 21 | 8 | 0 | 0 |
| <i>fla10-15 suf5</i> × <i>fla10-15 suf6</i> | 4 | 7 | 4 | 0 | 0 |

^a Data are derived from 33 different pairwise crosses.

^b Data are derived from 5 different pairwise crosses.

TABLE 4

Allele specificity and dominance of *SUF1* and *SUF2* alleles

| Suppressor allele | Suppression of ^a | | | Dominance in diploid strains ^b |
|--|-----------------------------|-----------------|-----------------|---|
| | <i>fla10-1</i> | <i>fla10-14</i> | <i>fla10-15</i> | |
| Class I | | | | |
| <i>suf1-1, 2, 3, 103</i> | + | + | - | Dominant |
| <i>suf1-108, 122</i> | + | + | - | Recessive |
| Class II | | | | |
| <i>suf1-106, 116, 134, 137, 139, 142</i> | - | + | - | Recessive |
| <i>suf2-107, 112, 126, 130, 144, 146</i> | - | + | - | Recessive |
| <i>suf2-104, 109, 118, 119</i> | - | + | - | Dominant |
| <i>suf2-123, 127, 140</i> | - | + | - | NT ^c |

^a + indicates suppression of the mutant *fla10*⁻ allele, - indicates no suppression.

^b Suppression phenotypes were determined in diploid cells homozygous for the *fla10-14* mutation and heterozygous for each suppressor mutation.

^c Not tested.

those of the original *fla10*⁻ mutant strain and the wild-type strain. Flagellar assembly is partially restored at the restrictive temperature of 32°. The *fla10*⁻ *suf*⁻ double mutant combinations have been termed SP-strains (Slow Pelleter) based upon the kinetics of fla-

gellar loss at 32°. Whereas mutant *fla10*⁻ cells become aflagellate within 24 hr at 32° in liquid culture, SP-suppressor strains are 60–90% flagellate and motile under the same conditions. At a gross phenotypic level, the SP-suppressor strains are distinguishable from both *fla10*⁻ and wild-type cells after 24 hr at 32°. The aflagellate *fla10*⁻ cells form a pellet, wild-type cells swim and SP-suppressor strains form a dis-

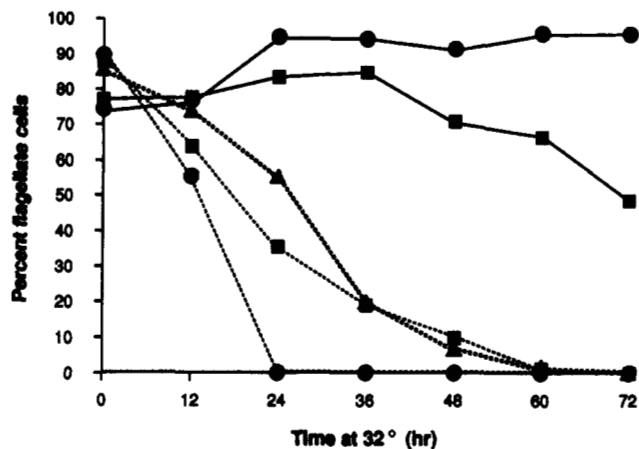


FIGURE 2.—Additivity of suppression by *suf1*⁻ and *suf2*⁻ alleles. The kinetics of flagellar loss at the nonpermissive temperature of 32° were determined for the *fla10-14 suf1-142 suf2-112* (solid squares, solid line) triple mutant strain and compared to those of the *fla10-14 suf1-142* (stippled squares, dashed line) and *fla10-14 suf2-112* (triangles, dashed line) double mutant strains, as well as the original *fla10-14* (stippled circles, dashed line) mutant and the wild-type strain, 137c (solid circles, solid line). Cells were incubated in rich liquid medium at 21° overnight. The cells were then shifted to 32° and the percentage of flagellate cells in each sample was determined at 12 hr intervals. For each sample, a minimum of 100 cells from three independent samples were counted and scored for the presence or the absence of flagella at each time point.

tinct pellet in addition to swimming cells.

Interactions between *SUF1* and *SUF2* alleles: Behavior of the *suf*⁻ alleles was examined in pairwise combinations with the *fla10-14* mutation, which can be suppressed by all *suf1*⁻ and *suf2*⁻ alleles. Triple mutant strains, *fla10-14 suf1*⁻ *suf2*⁻, were constructed and flagellar assembly and macroscopic motility were examined at the restrictive temperature of 32°. The parental *fla10-14* strain as well as representative *fla10-14 suf1*⁻ and *fla10-14 suf2*⁻ strains were included as controls for the comparison of relative amounts of suppression conferred by each combination of suppressor alleles. Examination of 39 of the 156 possible pairwise combinations of *suf1*⁻ and *suf2*⁻ alleles revealed certain trends. Most pairwise combinations of *suf1*⁻ and *suf2*⁻ alleles (34 of 39) appear to have an additive effect on suppression of the *fla10-14* mutation. The flagellar defect of the *fla10-14* mutation in these triple mutant cells is more efficiently suppressed than in any *fla10-14 suf*⁻ double mutant cells. These *suf1*⁻ *suf2*⁻ double mutant combinations are "Super Suppressors" (designated SS) of the *fla10-14* mutation. The exceptions are combinations that contained the *suf1-137* allele. Five of the ten combinations with *suf1-137* looked like the parents and the other five were additive. Comparisons of the kinetics of flagellar loss in nonsuppressed, singly suppressed, and doubly suppressed *fla10-14* strains are presented in Figure 2.

Genetic interactions with *APM* loci: Many mutations in *Chlamydomonas* have been identified that confer resistance to the plant herbicides amiprofos-

TABLE 5

Interactions of *fla10*⁻ alleles with the *apm1-122* mutation

| Allele | Synthetic phenotype | Condition | Segregation of interacting loci ^a |
|-----------------|---------------------|-----------------------|--|
| <i>fla10-1</i> | Arrested | Cold-sensitive | 29:0:73 |
| <i>fla10-14</i> | Slow growth | Cold-sensitive | 56:1:100 |
| <i>fla10-15</i> | Slow growth | Cold-sensitive | 25:0:35 |
| <i>fla10-16</i> | Aberrant motility | Temperature-sensitive | 11:0:17 |

^a Numbers given are for parental ditype:nonparental ditype:tetratype tetrads, respectively. Tetrad data presented in each case are derived from a cross of each *fla10*⁻ mutant allele to a *apm1-122 FLA10* mutant strain.

methyl and oryzalin (JAMES *et al.* 1988). HESS and BAYER (1977) demonstrated that each of these herbicides binds to the β -tubulin molecule of *Chlamydomonas in vitro*, however their intracellular targets are unknown. Although the tubulin genes would be likely targets for mutations that confer resistance to these compounds, genetic mapping and restriction fragment length polymorphism (RFLP) analysis have demonstrated that neither the *APM1*, the *APM2* (JAMES *et al.* 1988; RANUM *et al.* 1988), the *APM3* (S. K. DUTCHER and K. MUEH, unpublished observations), the *PDR1*, *PDR2* (JAMES and LEFEBVRE 1989) nor the *PDR3* (S. K. DUTCHER and K. MUEH, unpublished observations) locus encodes either an α - or a β -tubulin polypeptide.

One allele at the *APM1* locus, *apm1-122*, results in a cold-sensitive synthetic phenotype in combination with *fla10*⁻ mutant alleles (Table 5). *fla10-1 apm1-122* double mutant cells display a dramatic nonlethal arrest phenotype at 16° or 21°. Following meiosis of zygotes from a cross of *fla10-1 APM1* \times *FLA10 apm1-122* cells, the spores bearing both mutations undergo only two mitotic divisions at 16° or 21° and arrest as colonies of four cells. Shifting these arrested cells to 32° rescues the arrest phenotype, even after as long as two weeks of restrictive temperature. These rescued double mutant cells divide at rates comparable to those of the wild-type strain 137c as judged by colony size. Two lines of evidence support the conclusion that the arrest phenotype is due to the *fla10-1 apm1-122* double mutant combination. First, in a cross of *fla10-1* by *apm1-122*, the cold-sensitive arrest phenotype was present in 29 tetrads out of a total of 102. In each recombinant tetrad, the three nonarrested spores included one temperature-sensitive aflagellate, oryzalin-sensitive spore (*fla10-1 APM1*⁺); one biflagellate, oryzalin-resistant spore (*FLA10*⁺ *apm1-122*); and one biflagellate, oryzalin-sensitive spore (*FLA10*⁺ *APM1*⁺). Mapping of the two mutations responsible for the arrest phenotype indicates that the two loci are separated by approximately 30 cM. This distance corre-

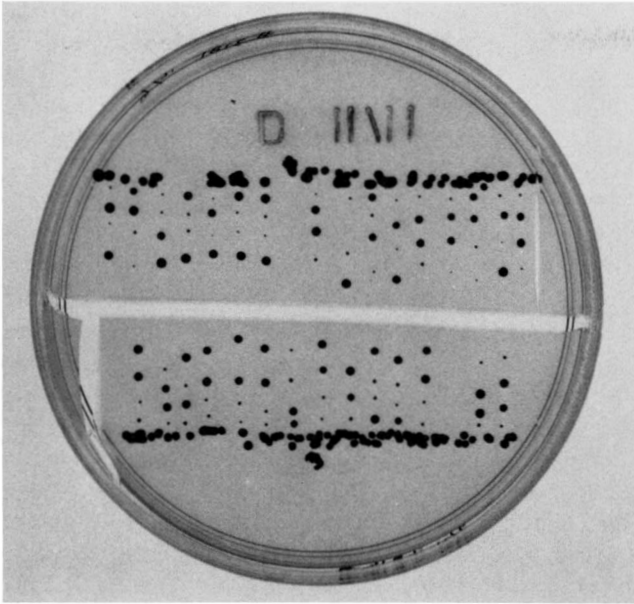


FIGURE 3.—Cell division phenotype of *fla10-15 apm1-122* double mutant strains at 21°. Tetrads from a cross of *fla10-15* by *fla10-15 apm1-122* dissected and grown at 21°. *fla10-15 apm1-122* double mutant spores give rise to small colonies, while *fla10-15* spores give rise to large colonies. In each tetrad, large and small colonies segregate 2:2. The dark streaks above and below the dissected tetrads are made up of zygotes that were spread on the plate but were not dissected.

sponds to the map distance between the *FLA10* and *APM1* loci (JAMES *et al.* 1988). Three of the spores that show a conditional arrest phenotype were grown at 25° and mated to wild-type cells. In 20 tetrads from each of these matings, the *fla10-1* mutant phenotype and the *apm1-122* mutant phenotype were recovered (data not shown). Second, analysis of 30 tetrads from each of eight spontaneous revertants of the arrest phenotype at low temperature reveals that the reversion of the arrest phenotype maps to the *APM1* locus. Loss of the oryzalin resistance phenotype in some revertants (data not shown) strongly supports the conclusion that the *apm1-122* mutation and not some other gene is responsible for the mutant phenotype in combination with the *fla10-1* mutation.

The *fla10-14* and *fla10-15* mutations also display a cold-sensitive cell division cycle phenotype in combination with the *apm1-122* mutation, although the mutant phenotype is less severe. *fla10-14 apm1-122* and *fla10-15 apm1-122* double mutant cells display a decreased colony size as compared to the wild-type strain at 16° or 21° (Figure 3). At 16° the wild-type strain has a doubling time of approximately 20 hr while in the *fla10-14 apm1-122* and *fla10-15 apm1-122* double mutant strains the doubling time is increased to approximately 40 hr based on plating of logarithmically growing liquid cultures onto solid medium over the course of 5 days.

These slow growth phenotypes are allele-specific and locus-specific with respect to *apm*⁻ mutations. Of

14 mutant *apm1*⁻ alleles tested, *apm1-122* is the only one that confers a synthetic phenotype in combination with mutations at the *FLA10* locus. In addition, none of the single alleles tested at the *APM2*, *APM3*, *PDR1* or *PDR3* loci has an effect in combination with the *fla10-1* mutation.

In addition to the slow growth phenotypes, *apm1-122* results in a synthetic temperature-sensitive flagellar motility defect in combination with the *fla10-16* allele. The *fla10-16* allele is an intragenic revertant of *fla10-1* (DUTCHER and LUX 1989). The *fla10-16 apm1-122* double mutant strain is phenotypically wild type at temperatures ranging from 16° to 25°. At 32°, the double mutant cells assemble full length flagella that display a defective waveform. These flagella twitch in an uncoordinated fashion, which renders the cells nonmotile at the restrictive temperature of 32°. No growth phenotype is observed at any temperature.

Two lines of genetic evidence support the conclusion that the novel flagellar motility phenotype results from an interaction between the *apm1-122* mutation and the *fla10-16* allele, and not between *apm1-122* and some otherwise silent mutation in the *fla10-16* strain. First, in a cross of *fla10-16* by *apm1-122*, one of four spores displayed both the motility phenotype and oryzalin resistance in 17 of 28 tetrads (Table 5). In these recombinant tetrads only one spore with wild-type motility was resistant to oryzalin. In the 11 non-recombinant tetrads, all four spores displayed wild-type motility and oryzalin-resistance segregated 2^s:2^r. This observed segregation suggests that the two mutations responsible for the motility defect are linked and map to within approximately 30 cM of one another. Second, when motility-defective, oryzalin-resistant spores were crossed to the *fla10-14* mutant strain, the oryzalin-resistant spores from 69 tetrads displayed either the temperature-sensitive motility phenotype of the original parent, or a cold-sensitive slow growth phenotype characteristic of *fla10-14 apm1-122* double mutant cells. These data indicate that the mutation responsible for the motility defect in combination with the *apm1-122* mutation maps to within 0.7 cM of the *fla10-14* mutation. Based upon these observations, we conclude that it is the *fla10-16* mutation, rather than some other genetic lesion, that generates the synthetic temperature-sensitive motility phenotype in combination with the *apm1-122* mutation.

The *fla10-16* mutation was classified as an intragenic revertant of the *fla10-1* mutation because in backcrosses to the wild-type strain, no spores with the *fla10-1* mutant phenotype were recovered ($n = 76$). Further support for this notion derives from the observed coreversion of both the temperature-sensitive flagellar phenotype of the *fla10-1* mutation, and the cold-sensitive arrest phenotype in combination with

the *apm1-122* mutation. Interaction of the *fla10-16* mutation with the *apm1-122* mutation indicates that it must be a pseudorevertant rather than a true revertant of the *fla10-1* mutation. While interactions between mutations at the *FLA10* and *APM1* loci can affect flagellar motility as well as cell division, specific interactions of the *fla10-16* mutation with *apm1-122* demonstrate that the effect on cell division is separable from that on flagellar function.

The *fla10-16* mutation is the only intragenic revertant of *fla10⁻* examined for these interactions, and it is the only *fla10⁻* mutant allele that displays a flagellar motility phenotype in combination with the *apm1-122* mutation. At lower temperatures, the *apm1-122* mutation has no effect on flagellar motility in combination with either the *fla10-14* or the *fla10-15* allele. Both double mutant strains display wild-type flagellar assembly and motility at permissive temperatures for the *fla10⁻* mutations. *fla10-1 apm1-122* double mutant cells that have been grown at 32°, the permissive temperature for growth, and shifted to 16° assemble functional flagella. At 32°, each of the *apm1-122 fla10⁻* double mutant strains is aflagellate and displays the temperature-sensitive flagellar phenotype characteristic of the mutant *fla10⁻* allele.

Dominance of the conditional arrest phenotype:

We monitored colony size as a measure of the cell cycle time in a variety of heterozygous diploid strains to determine if the synthetic arrest phenotype was dominant or recessive. We examined strains heterozygous for *fla10-1* and *apm1-122*, homozygous for *fla10-1* but heterozygous for *apm1-122*, and homozygous for *apm1-122* but heterozygous for *fla10-1*. As controls we compared these diploid strains to wild-type cells and to diploid strains homozygous for *fla10-1* and *apm1-122*. Strains that are heterozygous for both the *fla10-1* and the *apm1-122* allele show no slow growth phenotype. The strains homozygous for *fla10-1* and heterozygous for *apm1-122* show a slight slow growth phenotype; they produce slightly smaller colonies than do wild-type cells. The reciprocal strain, which is heterozygous for *fla10-1* and homozygous for *apm1-122*, has an obvious slow growth phenotype. The synthetic arrest phenotype is not dominant but the *fla10-1* allele appears to be semidominant in the presence of two copies of the *apm1-122* allele.

Phenotypes in combination with suppressor alleles: Because the *suf1⁻*, *suf2⁻*, and *apm1-122* mutations interact with mutations at the *FLA10* locus in different ways, we asked whether the *suf⁻* and *apm1-122* mutations interact with one another in triple mutant combinations with the *fla10-14* mutation. Triple mutant strains constructed with *fla10-14 apm1-122* and various *suf⁻* alleles display the slow growth phenotype characteristic of the *fla10-14 apm1-122* double mutant strain at 16°. Thus, the suppressors of

the *fla10-14* flagellar assembly phenotype have no effect on the cold-sensitive growth phenotype conferred by the *fla10-14 apm1-122* double mutant combination (Table 6). In addition, none of the *suf⁻* alleles tested affects the herbicide resistance phenotype of the *apm1-122* allele.

However, there is an interaction between the *apm1-122* mutation and mutations at the *SUF1* and *SUF2* loci. The *apm1-122* mutation alters the efficiency of suppression by most *suf1⁻* and *suf2⁻* alleles tested (Table 6). In 10 of 21 triple mutant strains tested, the suppressor activity is completely abolished by the presence of the *apm1-122* mutation. These triple mutant cells are aflagellate at 32° and are phenotypically indistinguishable from *fla10-14* mutant cells. In eight triple mutant strains, the suppressor activity is not abolished, but it is reduced dramatically. These triple mutant cells display some flagellar assembly at 32°, although the percentage of flagellate cells ($n = 200$) is reduced approximately sixfold as compared to the *fla10-14 suf⁻* double mutant strains. Of these, the *suf1-108* allele is unique; the few flagella that are assembled display aberrant motility. In three cases, complete suppression is observed. From these observations it is clear that mutations in these four genes interact with one another.

Interactions with other *APM1* alleles: Although *apm1-122* is the only *apm1⁻* mutant allele tested that interacts with mutations at the *FLA10* locus, this does not preclude the interaction of other *apm1⁻* mutant alleles with mutations at the *suf1⁻* and *suf2⁻* loci. Therefore, we asked if any *apm1⁻* mutant alleles result in an alteration of suppressor function similar to the abolition of suppression observed in *fla10-14 apm1-122 suf⁻* triple mutant strains. The effects of six other *apm1⁻* mutant alleles were examined in triple mutant cells. Of 150 possible combinations of *apm1⁻*, *suf1⁻*, and *suf2⁻* mutant alleles, 90 were examined. In most combinations *apm1⁻* mutant alleles appear to have no effect upon the suppressor activity of *suf1⁻* and *suf2⁻* mutant alleles. However, suppressor activity was abolished in the case of four *apm1⁻ suf⁻* combinations.

DISCUSSION

The *FLA10* locus of *Chlamydomonas* plays a role in proper flagellar assembly and cell division. This gene is of interest, in part, because it maps to linkage group XIX, an enigmatic chromosome of approximately six to nine megabases in size (HALL, RAMANIS and LUCK 1989) that displays several novel genetic properties (DUTCHER 1986; RAMANIS and LUCK 1986). We have performed classical pseudoreversion analyses of mutant alleles at the *FLA10* locus. One underlying rationale in a pseudoreversion analysis is that if two proteins interact, a deleterious mutation in one protein can be suppressed by a compensating

TABLE 6
fla10⁻ suf⁻ apm1-122 interactions

| Genotype | | | Growth phenotype at 21° ^a | Flagellar assembly at 32° ^b |
|-----------------|-----------------|---|---|---|
| <i>FLA10</i> | <i>APM1</i> | <i>SUF</i> | | |
| + | + | + | + | F |
| + | <i>apm1-122</i> | + | + | F |
| <i>fla10-1</i> | + | + | + | A |
| <i>fla10-14</i> | + | + | + | A |
| <i>fla10-15</i> | + | + | + | A |
| <i>fla10-16</i> | + | + | + | F |
| <i>fla10-1</i> | <i>apm1-122</i> | + | - | A |
| <i>fla10-14</i> | <i>apm1-122</i> | + | -/+ | A |
| <i>fla10-15</i> | <i>apm1-122</i> | + | -/+ | A |
| <i>fla10-16</i> | <i>apm1-122</i> | + | + | T |
| <i>fla10-14</i> | <i>apm1-122</i> | <i>suf1-1, 2, 106, 116, 122, 137, 142</i> | -/+ | A |
| | | <i>suf2-107, 126, 130</i> | | |
| <i>fla10-14</i> | <i>apm1-122</i> | <i>suf1-103, 134, 139</i> | -/+ | f |
| | | <i>suf2-109, 112, 144, 146</i> | | |
| <i>fla10-14</i> | <i>apm1-122</i> | <i>suf1-108</i> | -/+ | f, T |
| <i>fla10-14</i> | <i>apm1-122</i> | <i>suf2-104, 118, 140</i> | -/+ | F |

^a +, wild-type growth; -, arrest; -/+, increased cell cycle time. Growth at 32° is wild-type for all mutant strains tested.

^b F, flagellate; A, aflagellate; T, twitching motility; f, reduced number of cells with assembled flagella. Flagellar assembly at 21° is wild-type for all mutant strains tested.

mutation in the interacting protein. Through such analyses we have isolated a total of 316 suppressors of mutations at the *FLA10* locus, derived from three different *fla10⁻* mutant alleles. In sum, 216 intragenic revertants as well as 100 extragenic suppressor mutations were isolated. Sixty-seven of these extragenic suppressors identify six genes, which have been named *SUF* loci, for *SUP*pressor of *FLA*gellar assembly defect. These mutations display allele specificity with respect to which mutant *fla10⁻* alleles they suppress. None of the extragenic suppressor mutations has a phenotype in the presence of the wild-type *FLA10* allele. Although some suppressor mutations might have been expected to display a mutant flagellar assembly phenotype of their own, suppressors that display no phenotype are common in both yeast (BOTSTEIN and MAURER 1982; MOIR *et al.* 1982; NOVICK, OSMOND and BOTSTEIN 1989) and in *Chlamydomonas* (DUTCHER, GIBBONS and INWOOD 1988).

Another mutation, *apm1-122*, was also found to interact with mutations at the *FLA10* locus, resulting in several interesting pleiotropic phenotypes in combination with *fla10⁻* mutant alleles. The interactions between *fla10⁻* alleles and the *apm1-122* mutation suggest that the *FLA10* gene may influence not only flagellar assembly, but progress through the cell cycle. The *FLA10* gene may also be involved in the proper segregation of chromosomes at mitosis as monitored by the rate of chromosome loss (F. G. LUX and S. K. DUTCHER, manuscript in preparation).

Nature of the *FLA10-APM1* interaction: Mutations at the *FLA10* locus in combination with a specific

mutant *apm1⁻* allele result in the generation of synthetic phenotypes. These synthetic phenotypes include cold-sensitive slow growth phenotypes and a temperature-sensitive flagellar motility defect. Based upon the data presently available, we propose that the *apm1-122* and *fla10⁻* mutant gene products physically interact to produce the observed synthetic phenotypes through the formation of a deleterious complex at the restrictive temperatures.

Two lines of evidence support the suggestion that the synthetic phenotypes result from the interaction of the two mutant gene products. First, they are allele-specific with respect to both the *apm1⁻* and the *fla10⁻* mutations involved. Thus, the synthetic phenotypes are not due merely to the presence of mutations in the *APM1* and *FLA10* genes, but depend upon the specific combination of mutant alleles at each locus. As in the case of suppressor mutations, the allele specificity of the mutant phenotypes is suggestive of a specific physical interaction between mutant gene products.

Second, the observed arrest phenotypes are conditional cold-sensitive phenotypes. The *apm1-122* allele is not conditional while the three *fla10⁻* alleles are temperature-sensitive mutations. At high temperature it is likely that the mutant *fla10⁻* gene products are nonfunctional for flagellar assembly and that this alteration in the *fla10⁻* gene product is responsible for alleviation of the growth phenotypes associated with *fla10⁻ apm1-122* double mutant cells.

The *apm1-122* mutation also results in a temperature-sensitive flagellar motility phenotype in combi-

nation with the *fla10-16* mutation. This motility phenotype indicates that an interaction occurs between these two mutant alleles at 32°, and that a mutant *apm1-122* gene product must be present at temperatures from 16° to 32°. Such synthetic phenotypes may serve as a useful diagnostic tool in determining whether other intragenic revertants of *fla10*⁻ alleles are true revertants or are second-site pseudorevertants.

It is interesting that *fla10-1 apm1-122* double mutant cells are arrested for growth at the nonpermissive temperature, while the *bald2* strain, which apparently fails to assemble basal bodies or assembles only rudimentary basal bodies, has a wild-type rate of cell division (GOODENOUGH and ST. CLAIR 1975). Therefore, an apparent absence of basal bodies does not block the cell cycle. If we postulate that the *FLA10* gene product affects the function of the basal body, based on the flagellar assembly defect, then we must assume that the arrest phenotype of the double mutant cells is not caused by a loss of basal body function. Two alternatives are suggested. The synthetic arrest phenotype may result from a novel interaction of the mutant *fla10-1* and *apm1-122* gene products to form a toxic complex. This complex would interfere with progress through the cell cycle in some way at lower temperatures but would be inactivated at the higher temperature. Alternatively, the synthetic phenotype could be generated by the inactivation or alteration of the *apm1-122* gene product by the mutant *fla10*⁻ gene products. This model is based on the observation that recessive conditional lethal *apm1*⁻ alleles have been isolated (JAMES *et al.* 1988), and the possibility that the *APM1* gene is essential. The first model for the generation of synthetic phenotypes by the formation of toxic products predicts that the arrest phenotype would be dominant or semidominant and that the arrest phenotype may be different from that of *apm1*⁻ alleles. In essence, this type of toxic product is analogous to a gain of function mutation, or to the acquisition of a novel activity. The second model predicts that the arrest phenotype would be recessive in heterozygous diploid strains and the arrest phenotype would be similar to the arrest phenotype observed for the conditional lethal *apm1*⁻ alleles. The results of dominance tests and of the arrest phenotypes suggest that the second model is incorrect because the conditionality is semidominant and the arrest phenotypes appear to be different. The conditional lethal mutant *apm1* cells continue to increase in size and become bleached (JAMES *et al.* 1988), whereas the double *fla10*⁻ *apm1-122* mutant cells remain small and are flagellated (our unpublished observations).

Interactions between *SUF1* and *SUF2* alleles: When examined in most pairwise combinations with one another, mutations at the *SUF1* and *SUF2* loci

appear to be additive in their suppression of the *fla10-14* mutation. The additivity of these mutations suggests that these two genes do not act by the same mechanism and/or that they are not null alleles in a single linear dependent pathway involved in flagellar assembly. The *suf1-137* mutation is the only allele at the *SUF1* or *SUF2* loci tested that does not result in the generation of a Super Suppressor phenotype in combination with mutations at the other suppressor locus. The fact that five double mutant combinations display the same suppressor phenotype as the single suppressor mutations indicates that *suf1-137* is different from the other *suf1*⁻ mutant alleles examined. However, we do not know how this allele differs from other alleles. There is no obvious pattern associated with the *suf2*⁻ alleles that are not additive in combination with the *suf1-137* allele that would allow us to predict the phenotype of other *fla10-14 suf1-137 suf2*⁻ triple mutant strains.

Interactive suppression: It is likely that suppression of *fla10*⁻ mutant alleles results from allele-specific interactions between mutant gene products. Under this model, *SUF1* and *SUF2* encode gene products that interact with the *FLA10* gene product. Suppression would occur through the interaction of mutant *suf1*⁻ and *suf2*⁻ products with mutant *fla10*⁻ products to restore partial function at the restrictive temperature. The *fla10-1* and *fla10-14* mutations may share some common structural or functional defects, which would account for the suppression of both alleles by a subset of *suf1*⁻ alleles. The remaining *suf1*⁻ alleles and all *suf2*⁻ alleles would recognize alterations unique to the *fla10-14* mutation.

Additional support for an interactive model of suppression derives from the observation that certain *apm1*⁻ alleles dramatically reduce the suppressor activity of both *SUF1* and *SUF2* mutant alleles. These effects are allele-specific, both with respect to the *APM1* allele and the *SUF1* and *SUF2* alleles involved. The reduction or the abolition of suppressor function by *apm1*⁻ mutant alleles may be explained by a conformational block to appropriate *fla10*-suppressor interaction. The interaction of the *apm1-122* product with the mutant *fla10*⁻ product may physically block the accessibility of the suppressor gene products and thus prevent proper suppressor action. The retention of some suppressor activity by certain *suf1*⁻ and *suf2*⁻ alleles could be explained by the specific nature of their interaction with the *fla10-14* mutation and with the *fla10-14 apm1-122* complex. In essence, the allele specificity is a result of the specific physical interactions between the mutant gene products of the *FLA10*, *SUF1*, *SUF2* and *APM1* genes.

Dominance of suppressor alleles: It is intriguing that both dominant and recessive mutations at the *SUF1* and *SUF2* loci suppress the *fla10-14* mutation to

the same extent, which results in the generation of a slow pelleting (SP) phenotype at the nonpermissive temperature of 32°. In other systems, extragenic suppressor mutations in a particular gene generally define either recessive or dominant alleles, but not both (MOIR *et al.* 1982; ADAMS and BOTSTEIN 1989; NOVICK, OSMOND and BOTSTEIN 1989).

In general, dominant alleles are considered to be the result of gain of function mutations, and recessive alleles are considered to be the result of loss of function mutations. Interactive suppressors might be expected to be dominant, as the interaction of two mutant gene products can be considered a gain of function, which confers a new activity upon a protein complex that results in a more wild-type phenotype. Recessive suppressors, which may result from a loss of function, could act by relieving a deleterious interaction between the original mutant gene product and the wild-type suppressor gene product. Gain of function and loss of function mutations in the same gene are generally expected to confer opposite mutant phenotypes (MULLER 1932). For example, dominant and recessive alleles of several genes in *Caenorhabditis elegans* (TRENT, WOOD and HORVITZ 1988) and in *Drosophila* (WELSHONS 1965; LEWIS 1978; STRUHL 1981; WAKIMOTO and KAUFMAN 1981) result in opposite mutant phenotypes. However, mutations at the *Dorsal* locus may constitute an exception to this general rule (NUSSLEIN-VOLHARD 1979; NUSSLEIN-VOLHARD and WIESCHAUS 1980). Although the dominant and recessive mutant phenotypes of *Dorsal* mutations are similar, they are not identical as is the case for mutations at the *SUF1* and *SUF2* loci.

One model that explains the generation of identical dominant and recessive mutant phenotypes is a strict requirement for a specific dosage of wild-type product. This model seems unlikely based on the allele specificity of the interactions between the *SUF1*, *SUF2*, *APM1* and *FLA10* alleles. The dominant behavior of a subset of alleles is more consistent with an interactive model if the dominance is considered in terms of the affinity of mutant and wild-type gene products for binding to a target. If the mutant suppressor gene products have a higher affinity than the wild-type gene products for binding to a target (the mutant *fla10-14* gene product in this case), then these mutant suppressor alleles would be dominant. If the wild-type suppressor gene products have a higher affinity for binding to the mutant *fla10-14* gene product, then the mutant suppressor alleles would be recessive. In the haploid cell only the mutant suppressor gene product would be produced and there would be no competition for interaction with the mutant *fla10-14* gene product, so dominant and recessive alleles would have the same mutant phenotype. In the diploid cell heterozygous for a suppressor mutation, the compe-

tion between the wild-type and the mutant suppressor gene product would determine whether or not suppression is observed. Another corollary of this model is that the suppression of *fla10*⁻ alleles is independent of the affinity of the *suf*⁻ product for the *fla10*⁻ product. This suggests that the wild-type *FLA10* and *SUF* gene products normally interact in the cell.

Motility-defective mutations in the *MotA* gene of *Escherichia coli* display similar dominance behavior to the *suf1*⁻ and *suf2*⁻ mutations (BLAIR and BERG 1990). The *MotA* gene is thought to encode a component of the flagellar motor complex in *E. coli* (BLAIR and BERG 1988). The dominance and recessiveness of *motA*⁻ mutant alleles have been explained in terms of a competition between mutant and wild-type gene products for a limited number of sites of interaction with the flagellar motor complex.

The original mutations at the *FLA10* locus suggested that this gene is important for flagellar assembly. In this role, it appears that the *FLA10* gene product can interact with at least six other genes that are defined by the six *SUF* loci. In some respects this large number of genetically interacting components is surprising. It suggests that the *FLA10* gene product is central to flagellar assembly. Furthermore, the interactions between *fla10-16* and *apm1-122* suggest that these mutations can perturb flagellar function as well.

In addition to its role in flagellar assembly, the *FLA10* gene product must interact with gene products that are involved in the cell cycle as revealed by its interactions with the *apm1-122* mutation. It seems unlikely that *APM1* itself is a component of the basal body but it may be a component of the more broadly defined microtubule organizing center of the cell. Identification of these genes and their products will help to understand the relationships between the completion of the cell cycle and flagellar assembly and the role played by basal bodies in these processes.

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