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Genetic Interactions Among *Chlamydomonas reinhardtii* Mutations That Confer Resistance to Anti-Microtubule Herbicides

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ABSTRACT

We previously described two types of genetic interactions among recessive mutations in the *APM1* and *APM2* loci of *Chlamydomonas reinhardtii* that may reflect a physical association of the gene products or their involvement in a common structure/process: (1) allele-specific synthetic lethality, and (2) unlinked noncomplementation, or dominant enhancement. To further investigate these interactions, we isolated revertants in which the heat sensitivity caused by the *apm2-1* mutation is lost. The heat-insensitive revertants were either fully or partially suppressed for the drug-resistance caused by the *apm2-1* allele. In recombination tests the revertants behaved as if the suppressing mutation mapped within the *APM2* locus; the partial suppressors of *apm2-1* herbicide resistance failed to complement *apm2-1*, leading to the conclusion that they were likely to be intragenic pseudorevertants. The *apm2-1* partial suppressor mutations reversed $apm1^{-}apm2\text{-}1$ synthetic lethality in an allele-specific manner with respect both to $apm1^{-}$ alleles and *apm2-1* suppressor mutations. Those $apm1^{-}$ $apm2\text{-}1^{rev}$ strains that regained viability also regained heat sensitivity characteristic of the original *apm2-1* mutation, even though the *apm2-1* suppressor strains were fully heat-insensitive. The Hs^{+} phenotypes of *apm2-1* partial suppressors were also reversed by treatment with the microtubule-stabilizing agent deuterium oxide (D_2O). In addition to the above interactions, we observed interallelic complementation and phenotypic enhancement of temperature conditionality among $apm1^{-}$ alleles. Evidence of a role for the products of the two genes in microtubule-based processes was obtained from studying flagellar assembly in $apm1^{-}$ and $apm2^{-}$ mutants.

THE haploid, biflagellate green alga *Chlamydomonas reinhardtii* is extremely sensitive to killing by the herbicides amiprophos-methyl (APM) and oryzalin (ORY), which disrupt plant microtubules; it is relatively insensitive to microtubule poisons commonly used in animal cells, such as colchicine (reviewed by MOREJOHN and FOSKET 1986). We have used a genetic approach to analyze microtubule function in Chlamydomonas initially by isolating and characterizing mutants resistant to these anti-microtubule agents (JAMES *et al.* 1988, 1989; JAMES and LEFEBVRE 1989). The long-term goal of this research is to describe in detail the regulation of assembly of singlet cytoplasmic microtubules, doublet flagellar microtubules, and triplet basal body microtubules in this system.

Several unusual genetic interactions have been observed between mutations in the different herbicide-resistance loci of Chlamydomonas (JAMES *et al.* 1988, 1989; JAMES and LEFEBVRE 1989; DUTCHER and LUX 1989). These interactions include the generation of synthetic phenotypes, *e.g.*, synthetic lethality (reviewed by HUFFAKER, HOYT and BOTSTEIN 1987; and DUTCHER and LUX 1989); and unlinked noncomplementation or dominant enhancement, the failure of recessive mutations in different loci to complement one another [reviewed by FULLER *et al.* (1989) and DUTCHER and LUX (1989)]. In other systems, these phenomena have been interpreted as being due to the physical association of different gene products in multimeric structures or to the involvement of the products in the same structure or pathway. Proof that these genetic interactions can reflect protein-protein associations has been obtained in Drosophila and Saccharomyces, in which synthetic lethality and dominant enhancement have been observed between mutations in the α- and β-tubulin genes (HUFFAKER, HOYT and BOTSTEIN 1987; MATTHEWS and KAUFMAN 1987; STEARNS and BOTSTEIN 1988; HAYS *et al.* 1989). The heterodimeric association of α- and β-tubulin constitutes the basic subunit of the microtubule polymer (LUDUEÑA, SHOOTER and WILSON 1977).

We have described a number of unusual genetic interactions between mutant alleles of the *APM1* and *APM2* loci (JAMES *et al.* 1988). $apm1^{-}$ mutants are resistant to APM and ORY; the *apm2-1* mutant is resistant to APM and ORY and is strongly heat-

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sensitive. Allele-specific synthetic lethal interactions were observed between *apm2-1* and certain APM-resistant *apm1*$^-$ alleles (JAMES *et al.* 1988). These phenomena have been extended by observations of synthetic lethality in crosses between *apm1*$^-$ alleles and a second *apm2*$^-$ allele (S. W. JAMES and P. A. LEFEBVRE, unpublished data), and by a recent report of allele-specific synthetic lethality among a different group of *apm1*$^-$ and *apm2*$^-$ mutations (DUTCHER and LUX 1989). *apm1*$^-$ and *apm2*$^-$ alleles also cause dominant enhancement of their drug-resistance phenotypes in doubly heterozygous diploids (*apm1*$^-$ +/+ *apm2*$^-$) (JAMES *et al.* 1988; DUTCHER and LUX 1989). These diploids display intermediate levels of APM-resistance even though mutations at both loci are recessive to their wild-type alleles. In these diploids, however, complementation of the heat-sensitivity of *apm2-1* and of some *apm1*$^-$ alleles is observed.

Evidence that the products of the *APM1* and *APM2* loci participate in microtubule-related processes comes from several different observations. First, the *apm1*$^-$ and *apm2*$^-$ mutations cause resistance to two classes of anti-microtubule herbicides and not to a series of other inhibitors that do not affect microtubules (JAMES *et al.* 1988; JAMES and LEFEBVRE 1989). Second, *apm1*$^-$ alleles cause synergistic increases in resistance to APM and ORY when combined with an APM-resistant mutation in the α_1-tubulin gene (S. W. JAMES, C. D. SILFLOW, P. STROOM and P. A. LEFEBVRE, manuscript in preparation). Third, *apm1*$^-$ mutations have recently been reported to exhibit allele-specific interactions with mutations in *FLA10*, a locus involved in flagellar assembly (DUTCHER and LUX 1989; LUX and DUTCHER 1991). Last, *apm2*$^-$ mutants are unable to complete vegetative cell division at restrictive temperature and they show a nonconditional lethal defect in meiosis or some other aspect of zygote germination when homozygous in zygotes (JAMES *et al.* 1988; DUTCHER and LUX 1989). Since the *APM1* and *APM2* loci do not correspond to any of the four tubulin genes of *Chlamydomonas* (JAMES *et al.* 1988), they may encode microtubule-associated proteins (MAPs) or accessory proteins in a microtubule-containing complex. The *APM1* gene maps to linkage group XIX (JAMES *et al.* 1988), a linkage group that contains an unusual clustering of mutations that affect flagellar assembly or function (RAMANIS and LUCK 1986; DUTCHER 1986).

In this report we describe several new genetic interactions involving mutations at the *APM1* and *APM2* loci of Chlamydomonas, and show that at least one microtubule-based process, flagellar regeneration, is affected by these mutations.

MATERIALS AND METHODS

Media and culture conditions: Minimal medium I (M medium) (SAGER and GRANICK 1953) was used for vegetative growth, matings and tetrad dissection. Media for growing diploids differed by substitution of KNO_3 (4 mM) for NH_4NO_3 as the nitrogen source. Solid media were prepared by addition of agar (Gibco Laboratories, Madison, Wisconsin) which was washed and rinsed extensively for 3–5 days before use. Media for vegetative growth were solidified with 1% (wt/vol) washed agar; media for mating and zygote dissection were solidified with 2% washed agar. Light and temperature conditions for growing Chlamydomonas were as previously described (JAMES *et al.* 1988). APM was a gift from Chemagro, Mobay Corp., Kansas City, Missouri. ORY was a gift from Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, Indiana. Taxol (TAX) was provided by MATTHEW SUFFNESS, Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland. APM and ORY were kept as 10 mM stocks in dimethylsulfoxide (DMSO) at −20°; TAX was kept as a 1.75 mM stock in DMSO at −20°. Aliquots of the drugs were thawed and added to warm (approximately 45°) medium immediately before the plates were poured. Deuterium oxide (D_2O) was obtained from Sigma Chemical Company, St. Louis, Missouri.

Strains: Genotypes and sources of strains used in this study are listed in Table 1. For backcrossing, the wild-type strains used were NO5c *mt*$^+$ *nit1*$^-$ *nit2*$^-$ and NO5d *mt*$^-$ *nit1*$^-$ *nit2*$^-$. These strains are segregants recovered after 5 backcrosses of the 137c derivative NO *mt*$^-$ *nit1*$^-$ *nit2*$^-$ to NO *mt*$^+$ *nit1*$^-$ *nit2*$^-$, with NO *mt*$^+$ as the recurrent parent. To prepare for diploid construction, herbicide-resistant mutations isolated in the NO *mt*$^+$ *nit1*$^-$ *nit2*$^-$ background (JAMES *et al.* 1988) were crossed to 21gr *mt*$^+$ nit$^+$, and nit$^+$ herbicide-resistant strains were recovered. These segregants were then crossed to SJ581-H2 *mt*$^+$ *nit4*$^-$ or SJ581-F4 *mt*$^-$ *nit4*$^-$ to obtain *nit4*$^-$ herbicide-resistant strains. SJ581-H2 and SJ581-F4 were derived from a cross of SJ532-E4 *mt*$^-$ *apm2-1* nit$^+$ (this laboratory) to EF104 *mt*$^+$ *nit4*$^-$ (provided by E. FERNÁNDEZ, University of Córdoba, Córdoba, Spain).

Genetic analysis: Techniques for matings and tetrad analysis were as described by LEVINE and EBERSOLD (1960). Zygotes were dissected manually on the same plates used for zygote germination with the aid of a Zeiss DR-C stereomicroscope at 40× magnification. Separated meiotic progeny were grown for 4–5 days at 24° in continuous light, and colonies were then transferred by toothpick into approximately 400 µl of minimal medium I in the wells of a 96-well culture dish. After 3–5 days of growth at 24° in constant light, 96-well dishes were replica-plated onto 150 × 50 mm agar dishes using an Octapettor (Corning). Heat and cold sensitivity were scored on agar plates after growth in continuous light for 4 days at 33° and 8 days at 15°, respectively. Resistance to APM or ORY was scored on agar plates after 5 days of growth at 24° on a 14-hr light/10-hr dark cycle.

Dominance/recessiveness tests and complementation analysis were performed in vegetative diploids constructed by crossing *nit4*$^-$ strains to *nit1*$^-$ *nit2*$^-$ strains and selecting nit$^+$ cells, as described by FERNÁNDEZ and MATAGNE (1986). Diploids were identified on the basis of nitrate prototrophy, minus mating type, and larger cell size in comparison to haploid cells. For each diploid construction, at least six different diploid colonies were initially recovered and analyzed. Following this, two or three diploids from each construction were clonally isolated and then retested for conditional and drug-resistant phenotypes on both NH_4NO_3- and KNO_3-containing media.

Isolation of revertants of *apm2-1*: The tight conditional-lethal phenotype of *apm2-1* was used to select spontaneous revertants capable of growing at the nonpermissive temperature (33°). Cells (2.5×10^5 and 1×10^6) from 10-ml clonal

TABLE 1

Chlamydomonas strains

Name	Genotype	Source
NO5c	mt^+ *nit1 nit2*	P. LEFEBVRE
NO5d	mt^- *nit1 nit2*	P. LEFEBVRE
21gr	mt^+ nit^+	J. ROSENBAUM
SJ581-H2	mt^+ *nit4*	This laboratory
SJ581-F4	mt^- *nit4*	This laboratory
SJ56-A2	mt^+ *apm1-1 nit1 nit2*	This laboratory
SJ63-F9	mt^- *apm1-2 nit1 nit2*	This laboratory
SJ566-D3	mt^- *apm1-3 nit1 nit2*	This laboratory
SJ566-E8	mt^+ *apm1-3 nit1 nit2*	This laboratory
SJ296-A8	mt^+ *apm1-4 nit1 nit2*	This laboratory
SJ296-A10	mt^- *apm1-4 nit1 nit2*	This laboratory
SJ225-F9	mt^- *apm1-5 nit1 nit2*	This laboratory
SJ203-C2	mt^+ *apm1-6 nit1 nit2*	This laboratory
SJ203-C8	mt^- *apm1-6 nit1 nit2*	This laboratory
SJ652-A12	mt^+ *apm1-7nit1 nit2*	This laboratory
SJ652-A4	mt^- *apm1-7 nit1 nit2*	This laboratory
SJ181-A7	mt^- *apm1-8 nit1 nit2*	This laboratory
SJ667-F10	mt^+ *apm1-9 nit1 nit2*	This laboratory
SJ667-F2	mt^- *apm1-9 nit1 nit2*	This laboratory
SJ225-E5	mt^+ *apm1-10 nit1 nit2*	This laboratory
SJ225-E11	mt^- *apm1-10 nit1 nit2*	This laboratory
SJ243-G7	mt^- *apm1-11 nit1 nit2*	This laboratory
SJ296-F2	mt^- *apm1-12 nit1 nit2*	This laboratory
SJ552-E2	mt^+ *apm1-12* nit^+	This study
SJ791-B7	mt^+ *apm1-12 nit4*	This study
SJ791-F6	mt^- *apm1-12 nit4*	This study
SJ154-C9	mt^+ *apm1-13 nit1 nit2*	This laboratory
SJ154-H10	mt^- *apm1-13 nit1 nit2*	This laboratory
SJ272-A4	mt^+ *apm1-14 nit1 nit2*	This laboratory
SJ272-A6	mt^- *apm1-14 nit1 nit2*	This laboratory
SJ137-B2	mt^- *apm1-15 nit1 nit2*	This laboratory
SJ560-F2	mt^+ *apm1-16 nit1 nit2*	This laboratory
SJ560-F1	mt^- *apm1-16 nit1 nit2*	This laboratory
SJ86-B2	mt^- *apm1-17 nit1 nit2*	This laboratory
SJ656-A9	mt^+ *apm1-18 nit1 nit2*	This laboratory
SJ656-D5	mt^- *apm1-18 nit1 nit2*	This laboratory
SJ95-A6	mt^- *apm1-19 nit1 nit2*	This laboratory
SJ668-C6	mt^+ *apm1-21 nit1 nit2*	This study
SJ684-G10	mt^- *apm1-21* nit^+	This study
SJ702-D10	mt^+ *apm1-21 nit4*	This study
SJ702-C2	mt^+ *apm1-21 nit4*	This study
SJ702-C11	mt^- *apm1-21 nit4*	This study
SJ531-A12	mt^+ *apm2-1 nit1 nit2*	This laboratory
SJ531-A7	mt^- *apm2-1 nit1 nit2*	This laboratory
SJ532-E4	mt^- *apm2-1* nit^+	This laboratory
SJ581-G3	mt^+ *apm2-1 nit4*	This laboratory
SJ581-C8	mt^- *apm2-1 nit4*	This laboratory
SJ569-D2	mt^- *apm2-1 nit1 nit2*	This study
SJ720-A8	mt^- *apm2-3* nit^+	This study
Spontaneous revertants of *apm2-1* isolated in this study:		
SJ2001-A6	mt^+ $apm2\text{-}1^{rev}$ *nit1 nit2*	
SJ2001-A8	mt^+ $apm2\text{-}1^{rev}$ *nit1 nit2*	
SJ2001-A10	mt^+ $apm2\text{-}1^{rev}$ *nit1 nit2*	
SJ2001-B3	mt^+ $apm2\text{-}1^{rev}$ *nit1 nit2*	
SJ2001-B11	mt^+ $apm2\text{-}1^{rev}$ *nit1 nit2*	
SJ2001-C2	mt^+ $apm2\text{-}1^{rev}$ *nit1 nit2*	
SJ2001-C4	mt^+ $apm2\text{-}1^{rev}$ *nit1 nit2*	
SJ2001-C8	mt^+ $apm2\text{-}1^{rev}$ *nit1 nit2*	
SJ2001-E2	mt^+ $apm2\text{-}1^{rev}$ *nit1 nit2*	
SJ2001-E3	mt^- $apm2\text{-}1^{rev}$ *nit1 nit2*	
SJ2001-E5	mt^- $apm2\text{-}1^{rev}$ *nit1 nit2*	
SJ2001-F12	mt^- $apm2\text{-}1^{rev}$ *nit1 nit2*	
SJ2001-G3	mt^- $apm2\text{-}1^{rev}$ *nit1 nit2*	
SJ2001-G9	mt^+ $apm2\text{-}1^{rev}$ *nit1 nit2*	

liquid cultures grown in continuous light at 24° were plated onto M agar, or onto M agar + 0.7 μM APM or 1.2 μM APM, and plates were incubated in continuous light at 33° for 8–10 days before recovering revertant colonies. Single-cell clones of independently isolated revertants were obtained for phenotypic and genetic analysis.

Flagellar regeneration: These experiments were performed using methods described by LEFEBVRE *et al.* (1978). To test the effects of temperature on regeneration, the *apm2*$^-$ mutants were preincubated for 90 min at the nonpermissive temperature (34°) before deflagellation, and this temperature was maintained throughout the experiment. In experiments to test the effects of APM on regeneration, the drug was added to cultures within one minute after deflagellation.

RESULTS

Drug resistance and conditional phenotypes associated with mutant *apm1*$^-$ alleles: Chlamydomonas exhibits extreme sensitivity to the phosphoric amide herbicide APM and the dinitroaniline herbicide ORY. On agar media, wild-type cells die at 0.5 μM APM and 5.0 μM ORY. Relative to wild type, strains carrying any of 20 previously characterized *apm1*$^-$ alleles (JAMES *et al.* 1988) or one new allele (*apm1-21*) were 4–8-fold resistant to APM and 3–6-fold resistant to ORY. The new *apm1-21* mutation is allelic to other *apm1*$^-$ mutations because it shows tight linkage to two *apm1*$^-$ alleles (*apm1-1* and *apm1-7*; $n \geq 35$), and because it fails to complement other *apm1*$^-$ alleles (described below). Weak conditional phenotypes (heat and/or cold sensitivity) were associated with 11 *apm1*$^-$ alleles. Strains carrying one of three alleles, *apm1-3*, *-12* and *-14*, were weakly heat-sensitive; the cells typically underwent 5–10 cell divisions at the nonpermissive temperature (33°) and swelled before bleaching and dying. Strains bearing one of three *apm1*$^-$ alleles, *apm1-5*, *-8*, and *-21*, expressed weak cold-sensitive phenotypes characterized by poor growth and/or abnormally swollen cells at 15°; and five *apm1*$^-$ alleles, *apm1-6*, *-7*, *-10*, *-13* and *-14*, caused weak sensitivity to both high and low temperatures, although these strains grew normally at 24°.

Interallelic complementation and phenotypic enhancement among alleles of the *APM1* locus: The weak conditional defects caused by *apm1*$^-$ alleles were analyzed in diploids carrying different combinations of conditional and nonconditional alleles. All *apm1*$^-$ alleles were fully recessive both for APM and ORY resistance and for temperature-conditional growth. *apm1*$^-$/*apm1*$^-$ diploids containing the cold-sensitive *apm1-21* allele and one of 18 different *apm1*$^-$ alleles displayed interallelic complementation or phenotypic enhancement of conditional traits (Table 2). Seven different Cs^+ *apm1*$^-$ alleles complemented the Cs^- defect associated with *apm1-21*. However, eleven other *apm1*$^-$ alleles, four of which were fully Cs^+, failed to complement the weak Cs^- defect caused by *apm1-21*, as expected if the conditional *apm1-21* de-

TABLE 2

Interallelic complementation of temperature-conditional phenotypes among *apm1*$^-$ mutations

Control diploids:	15°	24°	33°	0.3 μM APM (24°)
+/+	+	+	+	S
apm1-7 (cs/hs)/+	+	+	+	S
apm1-12 (hs)/+	+	+	+	S
apm1-13 (cs/hs)/+	+	+	+	S
apm1-14 (hs)/+	+	+	+	S
apm1-21 (cs)/+	+	+	+	S

apm1$^-$/*apm1*$^-$ diploids:[a]	*apm1-21* (cs-)			*apm1-12* (hs-)		
	15°	24°	33°	15°	24°	33°
apm1-1	−	+	+	+	+	+
apm1-2	+	+	+	±	+	+
apm1-3 (hs)	±	+	+	+	+	±
apm1-4	+	+	+	+	+	±
apm1-5 (cs)	−	+	±	+	+	+
apm1-6 (cs/hs)	−	+	±	ND	ND	ND
apm1-7 (cs/hs)	−	+	−	+	+	−
apm1-8 (cs)	−	+	±	+	+	±
apm1-9	+	+	+	+	+	+
apm1-10 (cs/hs)	−	+	±	±	+	−
apm1-11 (hs)	ND	ND	ND	±	+	−
apm1-12 (hs)	+	+	−	+	+	−
apm1-13 (cs/hs)	−	+	−	+	+	−
apm1-14 (cs/hs)	−	+	−	+	+	−
apm1-15	−	+	+	+	+	+
apm1-16	−	+	+	+	+	±
apm1-17	+	+	+	+	+	±
apm1-18	+	+	+	+	+	+
apm1-19	+	+	+	+	+	±
apm1-21 (cs)	−	+	+	±	+	−

Key: − = no growth; cells enlarge, fail to divide, and bleach after 3–5 days at the restrictive temperature; ± = weak growth, cells abnormally swollen, but divide slowly; + = wild-type growth; ND, not determined.

[a] All *apm1*$^-$/*apm1*$^-$ diploid strains were resistant to APM (0.7 μM) at 24°.

fect resides in the *APM1* gene. Surprisingly, the Cs$^-$ phenotype of *apm1-21* was strongly enhanced in the majority of Cs$^-$ diploid strains, *i.e.*, diploid cells swelled rapidly, and failed to divide or divided only once at the restrictive temperature (15°). Furthermore, *apm1-21* (cs) failed to complement the heat-sensitive defects caused by *apm1-6* (cs/hs), *apm1-7* (cs/hs), *apm1-12* (hs), *apm1-13* (cs/hs), and *apm1-14* (cs/hs). These diploids were much more heat-sensitive than the corresponding Hs$^-$ haploids. The diploids swelled greatly and failed to divide or divided only once at the nonpermissive temperature (33°). Also, two diploids, *apm1-21/apm1-5* and *apm1-21/apm1-8*, exhibited moderate heat-sensitivity characterized by poor growth and swollen, yellowish cells even though these *apm1*$^-$ alleles did not cause heat sensitivity in haploids. Additional diploid strains constructed with the Hs$^-$ *apm1-12* allele and one of 18 different *apm1*$^-$ alleles displayed a range of phenotypes similar to those described above (Table 2). Collectively, these data reveal a complex pattern of interallelic complementation and phenotypic enhancement that suggest some type of interaction among *apm1*$^-$ gene products.

Phenotypic analysis of spontaneous revertants of *apm2-1*: The tight hs-lethal phenotype of *apm2-1* allowed selection of spontaneous Hs$^+$ revertants capable of growth at the restrictive temperature (33–34°). Thirty-one revertant strains representing at least 9 independent isolates were recovered from minimal medium without drug supplements; 15 revertants representing at least five independent isolates were recovered from medium supplemented with 0.7 μM or 1.2 μM APM.

Two classes of spontaneous Hs$^+$ revertants of *apm2-1* were recovered: (1) In class I revertants, the APM^r, ORY^r, and Hs$^-$ phenotypes were reverted; these strains were indistinguishable from wild-type. Twenty-one class I revertants comprising at least 8 independent isolates were recovered; (2) class II revertants showed full reversion of the Hs$^-$ phenotype, but showed only partial reversion for the APM^r and ORY^r phenotypes. These isolates grew as well as wild type at 33–35°, but displayed levels of APM and ORY resistance one-third to one-half that of *apm2-1* strains. Twenty-five class II revertants comprising at least six independent isolates were recovered.

Genetic analysis of spontaneous *apm2-1* revertants: Representative Hs$^+$ revertants from class I and class II were crossed to wild type, and the tetrad progeny were analyzed for the segregation of drug resistance and temperature-conditional lethality. Only parental ditype (PD) tetrads were recovered from crosses of eight independently isolated revertants to wild type ($n \geq 14$, $n \leq 49$ for each cross), indicating that reversion of the heat-sensitive phenotype was caused by events in the *APM2* locus (true reversion or intragenic pseudoreversion) or in a gene(s) closely linked to *APM2* (linked extragenic suppressor). The weak APM^r ORY^r phenotypes of each class II revertant segregated 2:2 as single Mendelian mutations, and these intermediate drug-resistance traits were recessive to wild type in heterozygous (*apm2-1*^{rev}/+) diploids (Table 3).

Diploids constructed by crossing *apm2-1* to each of nine different revertants were analyzed for temperature-conditional and drug-resistance phenotypes (Table 3). Diploids containing any of three class I *apm2-1*^{rev} alleles (Hs$^+$ APM^s ORY^s) and *apm2-1* fully complemented the Hs$^-$ defect of *apm2-1*, and exhibited nearly wild-type sensitivity to APM. Together with the data showing linkage of these *apm2-1*^{rev} alleles to the *APM2* locus, the complementation data suggest that the class I revertant alleles are caused by true reversion events or by intragenic second-site mutations.

Diploids made by crossing each of six different class

TABLE 3

Diploid analysis of *apm2-1* revertants

Diploid	Hs phenotype	Threshold lethal dose of APM (μM)[a]	Interpretation
Controls:			
+/+ (WT)	+	0.4	
apm2-1/+	+	0.5–0.6	Recessive
apm2-1/apm2-1	−	1.0–1.2	
Class I (Hs^+ APM^S ORY^S) revertants:			
R^{A8}/*apm2-1*	+	0.6–0.7	Complements *apm2-1*
R^{A10}/*apm2-1*	+	0.6–0.7	Complements *apm2-1*
R^{G9}/*apm2-1*	+	0.6–0.7	Complements *apm2-1*
Class II (Hs^+, weak APM^r ORY^r) revertants:			
R^{A6}/+	+	0.4–0.5	Recessive
R^{C4}/+	+	0.4–0.5	Recessive
R^{C2}/+	+	0.4–0.5	Recessive
R^{E3}/+	+	0.4–0.5	Recessive
R^{E5}/+	+	0.4–0.5	Recessive
R^{G3}/+	+	0.4–0.5	Recessive
R^{A6}/*apm2-1*	±	0.7–0.9	Partial complementation
R^{C4}/*apm2-1*	±	0.7–0.9	Partial complementation
R^{C2}/*apm2-1*	−	1.0–1.2	Noncomplementation
R^{E3}/*apm2-1*	−	1.0–1.2	Noncomplementation
R^{E5}/*apm2-1*	−	1.0–1.2	Noncomplementation
R^{G3}/*apm2-1*	−	1.0–1.2	Noncomplementation

Key: + = Hs^+; wild-type growth at 33°; ± = weak growth at 33° (cells abnormally swollen, divide slowly); − = hs-lethal at 33° (cells enlarge and fail to divide, phenotype identical to *apm2-1*).

[a] Indicates the minimum dose of APM required to kill 100% of cells. Resistance was quantified by plating cells on plates containing APM at the following concentrations: 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.2 and 1.5 μM APM.

II *apm2-1* revertants (Hs^+, weak APM^r ORY^r) to *apm2-1* (*apm2-1^{rev}/apm1$^-$*) failed to complement the *apm2-1* mutant phenotypes, displaying levels of APM resistance and heat sensitivity similar to that of *apm2-1/apm2-1* homozygotes. Since the mutations in class II revertants are closely linked to the *APM2* locus, these complementation data are consistent with either of two interpretations: (1) they may be intragenic second-site mutations, in which case the *apm2-1* mutation may be considered dominant to the suppressed *apm2-1* allele; or (2) they may be recessive extragenic suppressor mutations in a gene(s) that is closely linked to *APM2*.

Unusual allele-specific interactions between *apm1⁻* mutations and *apm2-1^{rev}* alleles: Because *apm1⁻* mutations exhibited allele-specific synthetic lethality with *apm2-1* (James *et al.* 1988), it was of interest to determine whether *apm2-1^{rev}* alleles would reverse synthetic lethality in *apm1⁻ apm2-1^{rev}* double mutants. The class I (Hs^+ APM^s ORY^s) *apm2-1^{rev}* allele R^{A10} reversed synthetic lethality in two cases tested (Table 4). In contrast, double mutants of the class II (Hs^+, weak APM^r ORY^r) *apm2-1^{rev}* allele R^{C2} with five different *apm1⁻* alleles were not viable at restrictive temperature. In crosses to two other alleles, however, synthetic lethality was reversed, *i.e.*, the double mutants survived and, in addition, a new phenotype was observed. These two strains (*apm1-3 apm2-1^{RC2}* and *apm1-17 apm2-1^{RC2}*) were as heat-sensitive as the *apm2-1* allele, even though strains bearing *apm2-1^{RC2}* alone were fully heat-insensitive. Two other class II suppressor mutations, R^{A6} and R^{E3}, reversed synthetic lethality and uncovered heat sensitivity when combined with *apm1⁻* alleles (Table 4). In each case where synthetic lethality was reversed by the partial suppressor allele, a new hs-lethal phenotype was observed only in the inferred *apm1⁻ apm2-1^{rev}* progeny. Revertant R^{C2} was also crossed to two *apm1⁻* alleles, *apm1-9* and *apm1-18*, that did not exhibit synthetic lethality with the original *apm2-1* mutation but instead produced viable double mutants (James *et al.* 1988). Nonetheless, inferred double mutants carrying the R^{C2} allele and *apm1-9* or *apm1-18* displayed strong hs-lethality. In sum, certain combinations of *apm2-1^{rev}* alleles and *apm1⁻* alleles reversed *apm1⁻ apm2-1* synthetic lethality. In addition these strains no longer possessed the heat-resistance associated with the *apm2-1^{rev}* allele, but showed a restoration of the heat-sensitivity caused by the original *apm2-1* mutation. These phenomena were allele-specific with respect to both the *apm1⁻* allele and to the *apm2-1* revertant allele.

D_2O reversed *apm2-1* suppressor phenotypes: Because *apm1⁻* and *apm2⁻* mutations confer resistance to microtubule destabilizing drugs, it is possible that the mutations hyperstabilize microtubules and/or mi-

TABLE 4

Allele-specific suppression of *apm1*$^-$ *apm2-1* synthetic lethality by some *apm2-1*^{rev} alleles

Revertant	Class		*apm1*$^-$ allele	No. of tetrads analyzed[a]
Crosses which reversed synthetic lethality[b]:				
R^{A10}	I	×	*apm1-3*	44
R^{A10}	I	×	*apm1-7*	14
R^{C2}	II	×	*apm1-3*	44[c]
R^{C2}	II	×	*apm1-17*	51[c]
R^{A6}	II	×	*apm1-7*	14[c]
R^{E3}	II	×	*apm1-3*	57[c,d]
R^{C2}	II	×	*apm1-9*[e]	62[c]
R^{C2}	II	×	*apm1-18*[e]	40[c]
Crosses which failed to reverse synthetic lethality[f]:				
R^{C2}	II	×	*apm1-7*	54
R^{C2}	II	×	*apm1-13*	57
R^{C2}	II	×	*apm1-14*	13
R^{C2}	II	×	*apm1-15*	39
R^{C2}	II	×	*apm1-16*	53

[a] PD = 2:2 weak APM^r ORY^r: strong APM^r ORY^r; NPD = 2:2 strong APM^r ORY^r:APM^S ORY^S; T = 1:2:1 weak APM^r ORY^r:strong APM^r ORY^r:APM^S ORY^S.

[b] Each of the three class II revertant strains used in crosses to *apm1*$^-$ alleles were F_1 progeny (weak APM^r ORY^r) obtained from crosses of original revertants by wild-type.

[c] Inferred double mutant progeny (*apm1*$^-$ *apm2-1*^{rev}) displayed a heat-sensitive lethal phenotype characteristic of the original *apm2-1* allele.

[d] Of the 55 inferred double mutant progeny recovered from this cross, 6 displayed no conditional phenotype, 15 exhibited weak temperature sensitivity, and the remainder were strongly hs-lethal.

[e] These *apm1*$^-$ alleles did not exhibit synthetic lethality with *apm2-1* but instead produced viable double mutants (JAMES *et al.* 1988).

[f] In crosses which produced synthetic lethality, the PD:NPD:T ratios were inferred from the phenotypes of the survivor progeny.

TABLE 5

D_2O reverses the phenotypes of partial suppressor mutants of *apm2-1*

Strain	Control (no D_2O) 15°	24°	33°	40% D_2O 15°	24°	33°
NO5c (WT)	+	+	+	+	+	+
SJ581-F4 (WT)	+	+	+	+	+	+
apm2-1[a]	±	+	−	±	(+)	−
Class I *apm2-1* revertants (Hs^+ APM^S ORY^S):						
R^{A8}	+	+	+	+	+	+
R^{A10}	+	+	+	+	+	+
R^{G9}	+	+	+	+	+	+
Class II *apm2-1* pseudorevertants [Hs^+ APM^r (weak) ORY^r (weak)]:						
R^{A6}	+	+	+	+	+	±
R^{C4}	+	+	+	+	+	(+)
R^{C2}	+	+	+	+	+	−
R^{E3}	+	+	+	+	+	−
R^{E5}	+	+	+	+	+	−
R^{G3}	+	+	+	+	+	−

Key: + = wild-type growth; (+) = growth slightly inhibited, cells appear normal; ± = growth inhibited, cells abnormally swollen and divide infrequently; − = no growth; cells enlarge, fail to divide and bleach after 2–4 days.

[a] Three different *apm2-1* strains were tested and showed identical phenotypes: SJ531-A7, SJ531-A12 and SJ581-C8.

crotubule complexes. Mutations that hyperstabilize microtubules typically confer supersensitivity to microtubule-stabilizing agents (OAKLEY and MORRIS 1981; SCHIBLER and CABRAL 1985; OAKLEY, OAKLEY and RINEHART 1987). We therefore tested *apm1*$^-$ and *apm2*$^-$ mutants and *apm2-1* revertants for growth in the presence of the microtubule-stabilizing agents TAX or D_2O. TAX binds to and stabilizes microtubules (SCHIFF and HORWITZ 1980); D_2O stabilizes microtubules and other polymeric structures by strengthening hydrophobic interactions (ITOH and SATO 1984). Chlamydomonas cells are very sensitive to TAX, swelling and dying at approximately 10–15 μM on agar media. TAX-supersensitive (sst) mutations in β-tubulin have been described for Chlamydomonas (SCHIBLER and HUANG 1991) and a TAX^{sst} mutation in Chlamydomonas α-tubulin has also been characterized (S. W. JAMES, C. D. SILFLOW, P. STROOM and P. A. LEFEBVRE, manuscript in preparation). In addition, TAX-resistant mutations in two non-tubulin loci of Chlamydomonas have been described (JAMES *et al.* 1989). All *apm1*$^-$ and *apm2*$^-$ mutants and *apm2-1* revertants showed wild-type sensitivity to TAX, and at concentrations up to saturation (approximately 30 μM) TAX had no effect upon the conditional phenotypes of any of the mutants tested. In contrast, 40% D_2O reversed the heat-resistance of most partial *apm2-1* suppressors, causing them to express strong heat sensitivity characteristic of the *apm2-1* mutant (Table 5). In addition, 40% D_2O slightly enhanced the weak Cs^- and Hs^- traits caused by most conditional *apm1*$^-$ alleles (data not shown). The full reversal of the heat resistance associated with *apm2-1*^{rev} alleles by *apm1*$^-$ mutations and D_2O suggests that the *apm1*$^-$ gene products and D_2O may exert their effects via a similar mechanism. The absence of TAX-related phenotypes for any mutant suggests that the *apm1*$^-$ and *apm2*$^-$ mutations do not act by hyperstabilizing microtubules, or they hyperstabilize microtubules in such a way that TAX does not exacerbate the hyperstabilization.

Effects of APM on flagellar regeneration: One major advantage of Chlamydomonas as an experimental system is the ability to induce assembly of flagellar microtubules by amputating the flagella. After deflagellation, the two flagella regenerate rapidly, achieving full length (10–12 μM) within 60–90 min (ROSENBAUM and CHILD 1967; for a review, see LEFEBVRE and ROSENBAUM 1986). Flagellar regeneration can be completely inhibited by addition of appropriate amounts of APM or ORY to deflagellated cells (QUADER and FILNER 1980). The possible role of the *APM1* and *APM2* gene products in the assembly of flagellar microtubules was examined in three *apm1*$^-$ mutants (*apm1-1*, *apm1-7*, and *apm1-19*) and *apm2-1*.

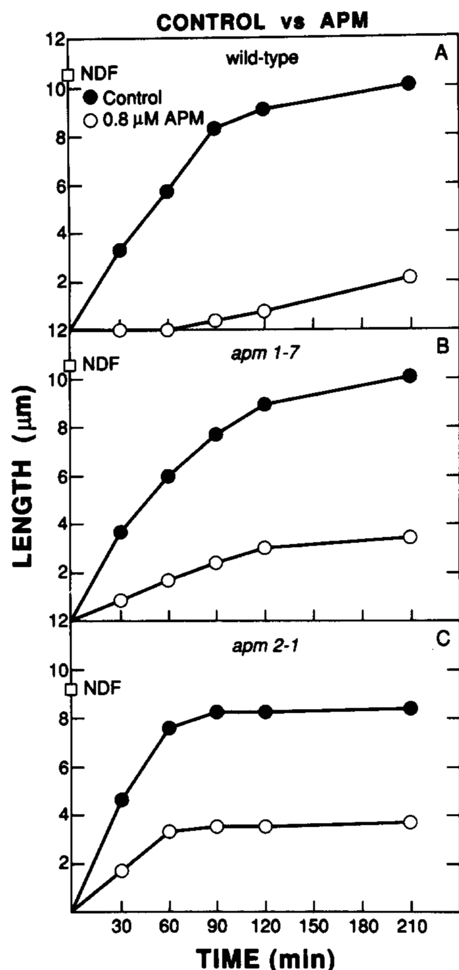

FIGURE 1.—The rates of flagellar regeneration of mutant and wild-type cells in the presence and absence of 0.8 μM APM. Flagella from 50–75 cells were measured for each sample. Pre-deflagellation lengths are given on the flagellar length axis above the zero time point. (A) wild-type NO5c-; (B) mutant *apm1-7*; (C) mutant *apm2-1*.

Because the *apm1⁻* mutants behaved similarly for a given treatment, data obtained for one representative mutant are presented for each experiment.

The *apm1⁻* mutants regenerated with wild-type kinetics in the absence of drug treatment (Figure 1, A and B). *apm2-1* mutant cells also regenerated flagella rapidly (Figure 1C), and these cells regenerated with similar kinetics at the permissive and nonpermissive temperatures (24° and 33°, respectively). *apm1⁻* and *apm2-1* mutants did, however, differ from wild type during regeneration of flagella in the presence of APM. Partial resistance to the inhibitory effects of APM on flagellar regeneration was observed. *apm1⁻* and *apm2-1* mutants regenerated ¼-⅓ length flagella (3–4 μM) during treatment with 0.8 μM APM, the minimum dose required to inhibit regeneration of wild type by >95% (Figure 1). Regeneration of APM-treated mutants began immediately and plateaued at approximately 90–120 min. By comparison, APM-treated wild-type cells began regeneration at 90–120 min, and flagella eventually grew to approximately ⅕ length (Figure 1A).

DISCUSSION

The suggestion that the *APM1* and *APM2* gene products interact physically was originally based upon two observations from genetic analysis: (1) recessive *apm1⁻* and *apm2⁻* mutations exhibit unlinked noncomplementation or dominant enhancement, and (2) allele-specific synthetic lethality occurs between mutations in the two genes (JAMES *et al.* 1988). Dominant enhancement may result from the physical association of mutant gene products to form a poison or deleterious product. The deleterious product may exert its effect by functioning abnormally in a cellular process, as appears to be the case for interacting α- and β-tubulin mutations and non-tubulin mutations in Drosophila (reviewed by FULLER *et al.* 1989; REGAN and FULLER 1990). A deleterious combination may also produce a nonfunctional complex, and thus effectively reduce the dosage of functional complex below a critical threshold. For instance, dominant enhancement between certain mutant α- and β-tubulins in Saccharomyces has been ascribed to a reduced dosage of functional α-β heterodimers (STEARNS and BOTSTEIN 1988). Alternatively, dominant enhancement may result from the accumulation of defects in a pathway or process, as exemplified by the interaction of unlinked mutations to suppress *cho1* lipid biosynthetic mutants in Saccharomyces (ATKINSON 1985).

The expression of new synthetic phenotypes as a result of combining different mutations may be explained in three ways: (1) direct association of two gene products to produce either a nonfunctional product or a product that functions abnormally, as exemplified by the synthetic lethal interaction between α- and β-tubulin mutations in Saccharomyces (HUFFAKER, HOYT and BOTSTEIN 1987); (2) the elimination of gene products whose functions are redundant, as in the case of synthetic lethality between mutations in the two α-tubulin genes of Saccharomyces (STEARNS and BOTSTEIN 1988); and (3) cumulative effects of mutations in different pathways or processes.

The mechanism governing the genetic interactions of the *APM1* and *APM2* genes of Chlamydomonas is not known. However, in this report we describe a number of new allele-specific interactions among mutations in these genes and suggest that these interactions may reflect altered stability of the structure/complex in which the mutant products participate. Furthermore, we present evidence that the gene products are involved in the microtubule-based process of flagellar assembly.

The results from D_2O experiments may shed some light on the nature of the *APM1-APM2* interaction. D_2O stabilizes microtubules and other structural com-

plexes by strengthening hydrophobic interactions (ITOH and SATO 1984). For example, in Aspergillus D_2O suppresses cold-sensitive α-tubulin mutations that apparently destabilize microtubules. In addition, D_2O restores the heat-sensitivity of the microtubule-hyperstabilizing *benA33* β-tubulin mutation in strains that also carry Cs^- α-tubulin suppressors of *benA33*. These results were interpreted to mean that D_2O acted to hyperstabilize microtubules by strengthening hydrophobic associations between α- and β-tubulins (OAKLEY, OAKLEY and RINEHART 1987). Treatment with D_2O of strains bearing *apm2-1*^{rev} alleles restored them to heat sensitivity. This reversal of the heat resistance of *apm2-1* suppressor-bearing strains may simply reflect phenotypic enhancement of *apm2*$^-$ mutants by conditions that promote microtubule assembly, such as high temperature and D_2O. Thus, the original *apm2-1* mutation may cause heat sensitivity by hyperstabilizing microtubule complexes. However, this hypothesis is contradicted by results from experiments with the microtubule-stabilizing drug TAX. If APM2 directly affects microtubule stability, then the *apm2-1* mutation may be expected to confer supersensitivity to TAX and *apm2-1* revertant phenotypes may be reversed by treatment with TAX. The absence of TAX-related phenotypes among *apm2*$^-$ mutations suggests that the *APM2* product does not directly influence microtubule stability, but rather it may be a component of another structure/complex. This structure may be associated with the microtubule cytoskeleton, as suggested by the observations that the *APM2* product is required for asexual cell division, for meiosis/zygote germination, and is involved in flagellar assembly.

The ability of certain *apm1*$^-$ mutations to phenocopy the effects of D_2O upon *apm2-1*^{rev} alleles suggests that the *APM1* gene product may function like D_2O with respect to the *APM2* product. In other words, *apm1*$^-$ mutations may cause hyperstabilization of the *apm2-1* product or a complex in which these products participate. Perhaps the synthetic lethal interaction between *apm1*$^-$ and *apm2*$^-$ mutations and the synthetic restoration of heat-sensitivity to *apm2-1*^{rev} alleles by *apm1*$^-$ alleles reflect strengthened hydrophobic associations between the defective *apm1*$^-$ and *apm2*$^-$ gene products. These results may also reflect the cumulative hyperstabilizing effects of mutations in the two genes upon the same complex. The allele specificity of these interactions and the dominant enhancement between the mutations makes it unlikely that the gene products specify redundant functions or that they act through different structures or pathways. Null mutations in the two genes could be used to distinguish whether the gene products interact directly or indirectly in the same structure/process (STEARNS and BOTSTEIN 1988; FULLER *et al.* 1989), but such mutations are so far unavailable.

Interallelic complementation and phenotypic enhancement were observed among *apm1*$^-$ mutations. The weak temperature-conditional defects caused by certain *apm1*$^-$ alleles were fully complemented by some nonconditional *apm1*$^-$ alleles, whereas other alleles caused the conditional defects to become more severe in *apm1*$^-$/*apm1*$^-$ diploids. The failure of some nonconditional *apm1*$^-$ alleles to complement conditional *apm1*$^-$ mutations suggests strongly that the weak Cs^- or Hs^- traits resulted from defects in the *APM1* locus, and the complex pattern of interallelic complementation and phenotypic enhancement suggests some type of interaction between *APM1* products. Other examples of interallelic complementation have been interpreted as being due to the association of gene products to form multimers (KAPULER and BERNSTEIN 1963; CRICK and ORGEL 1964; MAJUMDER *et al.* 1981). The unusual complementation behavior of *apm1*$^-$ alleles may be explained by interaction of the gene products to form multimeric complexes. Alternatively, they may be components of heteromultimeric structural complexes such as flagella or basal bodies that are composed of many repeating substructures. In this case the *APM1* gene products may not self-associate, but rather may be incorporated at regular intervals into repeated substructures within, *e.g.* the flagellum or basal body. Interallelic complementation has been observed among mutations in two flagella-related genes of Chlamydomonas, *LIS1* (light-induced suppressor) and *BOP1* (bypass of *PF10*). Mutations in these genes were obtained as suppressors of the paralyzed flagella mutation *pf10-1* (DUTCHER, GIBBONS and INWOOD 1988).

The results from flagellar regeneration experiments suggest that the *APM1* and *APM2* gene products play a role in the microtubule-based process of flagellar assembly. APM and ORY inhibit flagellar regeneration and cause flagellar resorption in Chlamydomonas (QUADER and FILNER 1980). *apm1*$^-$ and *apm2-1* mutants rapidly regenerated ¼ to ⅓-length flagella in the presence of APM at a concentration that completely inhibited regeneration of wild-type cells for the first 100 min after deflagellation. However, regeneration to full length was not observed. The ability of *apm1*$^-$ and *apm2-1* mutants to regenerate rapidly but only partially during treatment with APM may be explained in terms of a length equilibrium that results from the presence in mutant cells of both APM-sensitive and APM-resistant flagellar components. In addition to the *APM1* and *APM2* products, α- and β-tubulins are sites of action for APM and ORY, as judged from the observations that semidominant missense mutations in Chlamydomonas α- and β-tubulin genes confer resistance to the herbicides and that these tubulin mutants display altered flagellar

regeneration kinetics in the presence of anti-microtubule drugs (BOLDUC, LEE and HUANG 1988; SCHIBLER and HUANG 1991; S. W. JAMES, C. D. SILFLOW, P. STROOM and P. A. LEFEBVRE, manuscript in preparation). Therefore, incomplete regeneration of APM-treated mutant cells may reflect a balance between an APM-resistant component (*e.g.*, APM1) and several APM-sensitive components that are involved in flagellar assembly (*e.g.*, APM2, α-, and β-tubulin). These data support a model for the *APM1* and *APM2* products as components of flagella or basal bodies which, when mutated, modify the sensitivity of microtubules or flagella to the herbicides.

Additional evidence for a flagellar function for APM1 has come from studies by LUX and DUTCHER (1991) that revealed allele-specific interactions between mutations in the *APM1* and *FLA10* (conditionally flagellaless) loci. When the APM-resistant *apm1-122* mutation was combined with an intragenic pseudorevertant allele of *fla10-1*, the resulting double mutant expressed a synthetic heat-sensitive flagellar motility defect. This finding suggests a direct association between the two gene products (LUX and DUTCHER 1991) and indicates that APM1 may be required for flagellar function.

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