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# Mutants Resistant to Anti-Microtubule Herbicides Map to a Locus on the *uni* Linkage Group in *Chlamydomonas reinhardtii*

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## ABSTRACT

We have used genetic analysis to study the mode of action of two anti-microtubule herbicides, amiprophos-methyl (APM) and oryzalin (ORY). Over 200 resistant mutants were selected by growth on APM- or ORY-containing plates. The 21 independently isolated mutants examined in this study are 3- to 8-fold resistant to APM and are strongly cross-resistant to ORY and butamiphos, a close analog of APM. Two Mendelian genes, *apm1* and *apm2*, are defined by linkage and complementation analysis. There are 20 alleles of *apm1* and one temperature-sensitive lethal (33°) allele of *apm2.* Mapping by two-factor crosses places *apm1* 6.5 cM centromere proximal to *uni1* and within 4 cM of *pf7* on the *uni* linkage group, a genetically circular linkage group comprising genes which affect flagellar assembly or function; *apm2* maps near the centromere of linkage group VIII. Allele-specific synthetic lethality is observed in crosses between *apm2* and alleles of *apm1*. Also, self crosses of *apm2* are zygotic lethal, whereas crosses of nine *apm1* alleles *inter se* result in normal germination and tetrad viability. The mutants are recessive to their wild-type alleles but doubly heterozygous diploids (*apm1* +/+ *apm2*) made with *apm2* and any of 15 *apm1* alleles display partial intergenic noncomplementation, expressed as intermediate resistance. Diploids homozygous for mutant alleles of *apm1* are 4–6-fold resistant to APM and ORY; diploids homozygous for *apm2* are ts$^-$ and 2-fold resistant to the herbicides. Doubly heterozygous diploids complement the ts$^-$ phenotype of *apm2*, but they are typically 1.5–2-fold resistant to APM and ORY. From the results described we suggest that the gene products of *apm1* and *apm2* may interact directly or function in the same structure or process.

THE phosphoric amide and dinitroaniline herbicides have been classified as anti-microtubule drugs based on studies with higher plants and algae in which polymerized microtubules disappeared and microtubule-mediated processes were disrupted *in vivo* after drug treatment (PARKA and SOPER 1977; FEDTKE 1982; BAJER and MOLE-BAJER 1986). These herbicides inhibit cell division in plants and algae at very low concentrations ($10^{-8}$ to $10^{-6}$ M) but they do not affect animal or fungal cells (FEDTKE 1982).

Although the two classes of drugs are structurally distinct and differ in their chemical properties, some evidence suggests that both may act by directly binding to tubulin, the major protein of microtubules. For example, the dinitroanilines oryzalin and trifluralin bind to Chlamydomonas flagellar tubulin with an affinity similar to the binding of colchicine to animal tubulin (HESS and BAYER 1977; STRACHAN and HESS 1983). In addition, oryzalin inhibits the *in vitro* polymerization of higher plant tubulin (MOREJOHN *et al.* 1987). The phosphoric amide herbicide amiprophos-methyl (APM) also inhibits the *in vitro* polymerization of purified plant tubulin but has no effect on the *in vitro* polymerization of brain tubulin (MOREJOHN and FOSKET 1984). These findings suggest that the herbicides interact with sites on plant tubulins which are not present on animal tubulins.

In *Chlamydomonas reinhardtii*, both the dinitroaniline and phosphoric amide herbicides inhibit cell division at low micromolar concentrations; they also completely inhibit the microtubule-based process of flagellar regeneration which normally occurs after amputation of the flagella (QUADER and FILNER 1980). Accompanying the APM-induced inhibition of flagellar regeneration is a complete inhibition of tubulin synthesis (COLLIS and WEEKS 1978). The rapid increase in tubulin mRNA levels which normally occurs during flagellar regeneration is inhibited by APM (MINAMI *et al.* 1981). Chlamydomonas is an ideal system for the genetic analysis of herbicide action on microtubule function because different microtubule-based processes in this organism display extreme sensitivity to the drugs. In addition, a great deal is known about the coordinately regulated induction and expression of tubulin and other flagellar protein genes after deflagellation (reviewed by LEFEBVRE and ROSENBAUM 1986).

In this report we describe the isolation and characterization of a number of herbicide-resistant mutants. The mutants map to two unlinked loci. Several unusual interactions between mutants at the two loci have been observed. One of the two loci maps to the *uni*

The sequence data presented in this article have been submitted to the EMBL/GenBank Data Libraries under the accession number Y00607.

linkage group (ULG), a genetically circular linkage group comprising loci which affect flagellar assembly or function (RAMANIS and LUCK 1986).

## MATERIALS AND METHODS

**Strains:** Wild-type strains used were the 137c derivatives NO $mt^+$ and NO $mt^-$ provided by U. GOODENOUGH. The following strains were provided by E. HARRIS, the *Chlamydomonas* Genetics Center, Duke University, Durham, North Carolina: CC-48 (*arg2* $mt^+$) and CC-51 (*arg7* $mt^-$), which were back-crossed to wild type three times before they were used for diploid construction; CC-1373 (*C. smithii* $mt^+$), used for RFLP analysis; CC-568 (*pf7* $mt^-$), CC-1026 (*pf3* $mt^+$), CC-1084 (*nr1, ac9* $mt^+$), and CC-29 (*ac17, can1, nic13, pf2, y1, pyr1, msr1, act2, sr1,* $mt^-$) were used for genetic mapping; *ac17* was used for centromere mapping. Strain *uni1*, used for genetic mapping, was provided by D. LUCK, The Rockefeller University, New York. Strain *vfl1*, used for genetic mapping, was provided by J. JARVIK, Carnegie-Mellon University, Pittsburgh, Pennsylvania. Strain *nit4*, used for diploid construction, was provided by E. FERNANDEZ, University of Cordoba, Cordoba, Spain.

**Culture conditions:** Minimal medium I (SAGER and GRANICK 1953) was used for all vegetative cultures and stock maintenance. When appropriate this medium was supplemented with 0.2 g/liter arginine, 22 mM sodium acetate, 80 μg/ml neamine, and varying amounts of the herbicides and other inhibitors used in this study. Solid media were prepared by the addition of appropriate quantities of agar (Gibco Laboratories, Madison, Wisconsin) which were washed and rinsed for 3–5 days before use. Cells were routinely grown at 24° in continuous light, except that cultures containing herbicides were grown on a 14-hr light/10-hr dark cycle. Cultures were illuminated with white light (4800 lux) from two F40T12CW fluorescent tubes (North American Philips Lighting Corp., Bloomfield, New Jersey). Mutants were tested for sensitivity to heat (33°) and cold (15°) in Percival incubators (Model 1-35 LVLL; Percival Mfg. Co., Boone, Iowa) with continuous illumination (5000 lux at 33°, 4000 lux at 15°) from two F20T12CW fluorescent tubes (General Electric Co., Wilmington, Massachusetts).

**Herbicides and other inhibitors:** APM (*O*-methyl-*O*-(4-methyl-6-nitrophenyl)-*N*-isopropyl-phosphorothioamidate) was obtained from Chemagro, Mobay Chemical Corp., Kansas City, Missouri. Butamiphos (*O*-ethyl-*O*-(3-methyl-6-nitrophenyl)-*N*-*sec*-butyl-phosphorothioamidate) was obtained from Sumitomo Chemical Co., Ltd., Osaka, Japan. Technical grade (95%) oryzalin (ORY; 3,5-dinitro-$N^4$,$N^4$-dipropylsulfanilamide) was provided by Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, Indiana. The herbicides were made up as 10 mM stock solutions in 100% dimethyl sulfoxide (DMSO) and stored in aliquots at −20°. Appropriate amounts were added to media after autoclaving. Because of the low solubility of ORY (~8 μM), 1% DMSO was added to agar media containing concentrations of ORY 8 μM and higher. Neamine was provided by Upjohn Corp., Kalamazoo, Michigan. All other reagents and inhibitors were the highest grade available.

**Mutagenesis and mutant isolation:** Mutagenesis with methylmethane sulfonate (MMS) (Aldrich Chemical Co.) was performed according to the method of LOPPES (1969). Light-synchronized cells ($mt^+$) grown to log phase ($2 \times 10^6$ cells/ml) with continuous aeration were collected 2–4 hr into the light cycle and resuspended in 0.02 M $KPO_4$ buffer, pH 7.0, at a density of $1-2 \times 10^7$ cells/ml. MMS was added to a final concentration of 0.026 M for 80–85 min, or 0.039 M for 60–65 min; during mutagenesis cells were stirred constantly in light at 24°. Cells were pelleted out of MMS, resuspended in 10 ml of minimal medium, and 2.5 ml aliquots were added to 100 ml of minimal medium. The plating efficiency of mutagenized cultures was measured and normalized to plating efficiencies from control (nonmutagenized) aliquots taken at the beginning of each mutagenesis experiment. In 11 experiments, treatment with 0.026 M MMS yielded 1.8–12.9% survivors, and with 0.039 M MMS, 0.5–10.9% survivors.

Mutagenized cells were grown under continuous light and aeration for 4–8 days. In 6 of 11 experiments, mutagenized cultures were enriched for mutants by including 0.8 μM APM or 3.5 μM ORY in the culture medium during growth. After growth in liquid, approximately $1 \times 10^6$ cells from unenriched cultures or $2 \times 10^5$ cells from enriched cultures were spread onto agar medium containing 2 μM APM (4 times the lethal dose for wild type) or 7.5 μM ORY (1.5 times the lethal dose for wild type). Resistant colonies were isolated after 10–12 days of growth on a light/dark cycle. Mutants were also obtained by plating approximately $2 \times 10^6$ nonmutagenized cells on 1.2 μM APM (approximately 2.5 times the lethal dose for wild type) and selecting for APM-resistant colonies which appeared spontaneously. Only those mutants selected from different aliquots of the original mutagenized cultures were considered to be products of independent mutation events.

**Mutant screening:** Resistant colonies picked into minimal medium in the wells of a microtiter dish (Costar #3596, Costar, Inc., Cambridge, Massachusetts) were screened for resistance to 2 μM APM, 7.5 μM ORY, and 4 μM cycloheximide on agar plates. The mutants were also tested for temperature sensitivity (33°) and cold sensitivity (15°) for growth on agar. Mutants which displayed resistance to cycloheximide were presumed to have alterations not specific to the herbicides and were not analyzed further. Single cell clones of the remaining mutants were isolated in preparation for genetic analysis. The 21 mutants examined in this study were further tested for resistance or sensitivity to butamiphos, colchicine, emetine, erythromycin, and the photosynthetic inhibitors atrazine and DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea].

**Genetic analysis:** Tetrad analysis was performed at 24° using standard techniques (LEVINE and EBERSOLD 1960). After backcrossing APM- and ORY-resistant mutants to wild type, $F_1$, $F_2$, and $F_3$ progeny were recovered and used for genetic analysis. In the case of mutant *apm2-1*, $F_5$, $F_6$, and $F_7$ progeny were used for genetic analysis. Map distance was calculated using the formula $\frac{1}{2} T + 3NPD/(PD + NPD + T)$ and distance from the centromere marker *ac17* was calculated using the equation $\frac{1}{2} T/(PD + NPD + T)$ (GOWANS 1965).

Diploids were constructed by the method of LOPPES, MATAGNE and STRIJKERT (1972), as derived from the method of EBERSOLD (1967). Double-mutant strains containing each of the herbicide-resistant mutations coupled with the auxotrophic mutation *arg7* were crossed to herbicide-resistant mutants carrying the complementing *arg2* mutations, and after 4–5 days of growth in continuous light on 2% minimal medium agar plates (−arginine), prototrophic diploid colonies were isolated. Alternatively, diploids were constructed by the method of FERNANDEZ and MATAGNE (1986), in which diploids are selected using complementing nitrate reductase defective mutants. Diploids were identified on the basis of three characteristics: arginine or nitrate prototrophy, minus mating type, and larger cell size in comparison to haploid cells (as measured using phase-contrast microscopy with an ocular micrometer). For each cross producing diploids, 8–16 diploid colonies were recovered and analyzed.

## RESULTS

**Mutant recovery and initial characterization:** After mutagenesis, 1588 putative mutants were recovered by selection with APM, and 828 by selection with ORY. Initial screening eliminated 1445 APM-selected and 265 ORY-selected mutants from the study because they were resistant to cycloheximide and were presumed to have alterations unrelated to the action of the herbicides. An additional 500 putative mutants (mostly ORY-selected) retested as wild-type, leaving approximately 200 APM- or ORY-resistant mutants for study. Of these, 14 APM-selected and 9 ORY-selected mutants were temperature sensitive (ts); 4 APM- and ORY-selected mutants were cold sensitive (cs). Several spontaneous APM-resistant mutants were also recovered. From the initial screening, all APM-selected mutants were cross-resistant to ORY, and approximately ⅓ of ORY-selected mutants were APM resistant. This report describes 21 independently isolated mutants which were cross-resistant to APM and ORY (18 MMS-induced; 3 spontaneous). As will be shown below, two unlinked Mendelian loci, designated *apm1* and *apm2*, were defined on the basis of linkage analysis and complementation tests.

On minimal agar medium on a 14-hr light/10-hr dark cycle the threshold lethal dose of herbicide (*i.e.*, the minimum dose required to kill 100% of cells) for wild-type cells was 5.0 μM for ORY, 0.5 μM for APM, and 0.2 μM for butamiphos, a close structural analog of APM. The threshold lethal dose for *apm1* mutants ($F_1$ or $F_2$ backcross progeny) was between 2 and 4 μm for APM (4–8-fold resistant), 0.8 and 1.5 μm for butamiphos (4–8-fold resistant), and 15–30 μm for ORY (3–6-fold resistant). $F_5$ backcross progeny clones of mutant *apm2-1* were ~3-fold resistant to the herbicides. For each mutant the fold-resistance to APM and butamiphos was equal.

The mutants showed wild-type sensitivity to six other compounds: the cytoplasmic protein synthesis inhibitors cycloheximide and emetine, the chloroplast protein synthesis inhibitor erythromycin, the anti-microtubule drug colchicine, and the photosynthesis inhibitors atrazine and DCMU. For each drug, the mutants were tested over a range of concentrations which included levels below, at, and above the threshold lethal dose for wild type. The fact that the mutants were sensitive to structurally and functionally unrelated drugs suggests that herbicide resistance does not result from general defects in permeability but does not rule out a herbicide-specific transport defect or herbicide-specific inactivation of the drugs in the mutant cells.

Mutants resistant to APM and ORY are normal in gross morphology and motility and they display near-normal growth rates. Temperature-sensitive phenotypes occur in three of the mutants in this study; of these, only *apm2-1* is a tight conditional-lethal. This mutant fails to divide at the nonpermissive temperature; within 24–30 hr at the nonpermissive temperature, the cells swell to approximately ten times normal diameter and they are bleached after 3 days. Two other mutants (*apm1-12* and *apm1-14*) are weakly temperature-sensitive; the cells typically undergo 4–6 cell divisions at the nonpermissive temperature before they begin to die and bleach. Dying cells remain normal-sized or swell only slightly.

TABLE 1

**Tetrad analysis of backcrosses between $APM^R$ $ORY^R$ mutants and wild-type**

*PD* = 2:2 $APM^R$ $ORY^R$:$APM^S$ $ORY^S$

| Selection | Mutant | PD:NPD:T |
|---|---|---|
| APM | *apm1-1* (sp)[a] | 100:0:0 |
| APM | *apm1-2* (sp) | 13:0:0 |
| APM | *apm1-3* (sp) | 30:0:0 |
| APM | *apm1-4* | 45:0:0 |
| APM | *apm1-5* | 24:0:0 |
| APM | *apm1-6* | 12:0:0 |
| APM | *apm1-7* | 127:0:0 |
| APM | *apm1-8* | 52:0:0 |
| APM | *apm1-9* | 51:0:0 |
| APM | *apm1-10* | 17:0:0 |
| APM | *apm1-11* | 29:0:0 |
| APM | *apm1-12* (ts)[b] | 130:0:0 |
| APM | *apm1-13* | 44:0:0 |
| ORY | *apm1-14* (ts)[b] | 52:0:0 |
| ORY | *apm1-15* | 56:0:0 |
| ORY | *apm1-16* | 34:0:0 |
| ORY | *apm1-17* | 35:0:0 |
| ORY | *apm1-18* | 40:0:0 |
| ORY | *apm1-19* | 22:0:0 |
| ORY | *apm1-20* | 55:0:0 |
| APM | *apm2-1* (ts)[b] | 203:0:0 |

[a] sp = spontaneous.
[b] In all cases temperature-sensitivity co-segregated with APM- and ORY-resistance.

**Crosses of resistant mutants to wild type and to other mutants:** The mutants were crossed to wild type and the tetrad progeny were scored for APM-resistance, ORY-resistance and, where appropriate, temperature sensitivity (Table 1). Each of the mutant phenotypes segregated 2:2 in all crosses. Resistance to APM and ORY always co-segregated, giving only parental ditype tetrads; the ts phenotypes always co-segregated with herbicide-resistance, leading to the conclusion that the multiple phenotypes of each mutant probably result from a single mutation.

Pairwise crosses among 18 different mutants define two unlinked loci, *apm1* and *apm2* (Table 2). With one exception, crosses among nine different *apm1* mutants gave only parental ditype tetrads. In the cross *apm1-1* × *apm1-10*, one tetratype tetrad was recovered out of 85 total tetrads. Since *apm1-1* and *apm1-10* are allelic (see Table 3), the single tetratype tetrad recovered may have resulted from intragenic recombination or gene conversion.

**Dominance/recessiveness tests and complementa-**

TABLE 2

**Tetrad analysis of pairwise crosses between APM$^R$ ORY$^R$ mutants**
$PD$ = 4:0 APM$^R$ ORY$^R$:APM$^S$ ORY$^S$
$NPD$ = 2:2 APM$^R$ ORY$^R$:APM$^S$ ORY$^S$
$T$ = 3:1 APM$^R$ ORY$^R$:APM$^S$ ORY$^S$

| | *apm1-1* | *apm2-1 (ts)* |
|---|---|---|
| *apm1-1* (sp)[a] | 11:0:0 | 5:6:48[b] |
| *apm1-2* (sp) | 54:0:0 | |
| *apm1-3* (sp) | 64:0:0 | 7:15:68[b] |
| *apm1-5* | | 0:0:16[b] |
| *apm1-6* | 62:0:0 | |
| *apm1-7* | 73:0:0 | 8:8:64[b] |
| *apm1-10* | 84:0:1 | |
| *apm1-11* | 25:0:0 | |
| *apm1-12* (ts) | | 2:3:36[b] |
| *apm1-13* | | 4:3:37[b] |
| *apm1-14* (ts) | | 2:2:25[b] |
| *apm1-15* | | 8:5:35[b] |
| *apm1-16* | | 2:1:32[b] |
| *apm1-17* | 78:0:0 | 1:0:5[b] |
| *apm1-19* | 85:0:0 | 9:5:36[b] |
| *apm1-9* | | 7:5:31[c] |
| *apm1-18* | | 5:6:33[c] |
| *apm2-1* (ts) | | Zygotic lethal |

[a] sp = spontaneous.
[b] Indicates complete synthetic lethality of progeny with inferred genotype *apm1 apm2*.
[c] Indicates high viability of double-mutant progeny; *i.e.*, all complete tetrads recovered.

TABLE 3

**Pairwise complementation tests between APM$^R$ ORY$^R$ mutants**

| | *apm1-3* | *apm1-5* | *apm1-7* | *apm2-1* |
|---|---|---|---|---|
| WT | 0.3[a] | 0.3 | 0.3 | 0.4 (TS$^+$) |
| *apm2-1* | | | | 0.9 (ts$^-$) |
| *apm1-1* | 1.8 | 1.5 | 1.2 | 0.8 (TS$^+$) |
| *apm1-2* | | 1.2 | 0.9 | |
| *apm1-3* | | 1.6 | 1.2 | 0.6 (TS$^+$) |
| *apm1-4* | | 1.5 | 1.2 | |
| *apm1-5* | 1.2 | 1.8 | 1.8 | 0.7 (TS$^+$) |
| *apm1-6* | 1.2 | 1.2 | 1.2 | 0.7 (TS$^+$) |
| *apm1-7* | 1.5 | 1.5 | 1.5 | 0.8 (TS$^+$) |
| *apm1-8* | 1.5 | | | 0.9 (TS$^+$) |
| *apm1-9* | 1.5 | 1.8 | 1.5 | 0.8 (TS$^+$) |
| *apm1-10* | 1.2 | 1.8 | 1.5 | 0.6 (TS$^+$) |
| *apm1-11* | | 1.7 | 1.5 | |
| *apm1-12* | 1.5 | 1.8 | 1.2 | 0.6 (TS$^+$) |
| *apm1-13* | 1.8 | 2.0 | 1.7 | 0.8 (TS$^+$) |
| *apm1-14* | 1.3 | | | 0.6 (TS$^+$) |
| *apm1-15* | 1.5 | | | 0.8 (TS$^+$) |
| *apm1-16* | | 1.8 | 1.2 | |
| *apm1-17* | | 0.9 | 0.9 | |
| *apm1-18* | 1.5 | 1.5 | 1.2 | 0.8 (TS$^+$) |
| *apm1-19* | 1.5 | 1.5 | 1.2 | 0.6 (TS$^+$) |
| *apm1-20* | 0.9 | 1.7 | 1.2 | 0.6 (TS$^+$) |

[a] Indicates threshold lethal dose of APM (μM) for given combination of alleles in diploid strain; resistance was quantified by testing diploids at 0.2- and 0.3-μM increments, over the range 0.2–2.0 μM APM.

**tion analysis:** Heterozygous diploids (*apm1*/+ or *apm2*/+) were constructed with all mutants using *arg7* and *arg2* auxotrophic markers. Several of the same diploids were prepared using strains carrying *nit1*, *nit2* and *nit4* auxotrophic markers. Several homozygous diploid strains carrying the wild-type alleles at the *apm1* and *apm2* loci (+/+) were also constructed and used as controls. The threshold lethal dose of APM for heterozygous *apm1*/+ diploids and the control (+/+) diploids was 0.3 μm, and the ts phenotypes of *apm1* mutants were fully complemented by the wild-type allele, demonstrating that all the mutants are completely recessive. Heterozygous *apm2-1*/+ diploids died at 0.4–0.5 μm APM, and their ts phenotypes were fully complemented by the wild-type allele, demonstrating that *apm2-1* is almost fully recessive.

Pairwise complementation tests using diploids constructed with *arg7* and *arg2* auxotrophic markers define 20 alleles of *apm1* and one conditional-lethal (ts) allele of *apm2* (Table 3). Diploids containing any pair of mutant *apm1* alleles were 3–6 times as resistant to APM and ORY as *apm1*/+ diploids, but the weak ts phenotypes of *apm1-12* and *apm1-14* were usually complemented in *apm1*/*apm1*·(ts) diploids. Diploids homozygous for *apm2-1* were ts-lethal and approximately 2-fold resistant relative to *apm2-1*/+ heterozygotes. Alleles of *apm1* fully complemented the ts phenotype of *apm2-1* when *apm1* +/+ *apm2-1* double-heterozygotes were constructed. However, these same double-heterozygotes containing the *apm2-1* allele and any of 15 mutant *apm1* alleles exhibited partial intergenic noncomplementation, expressed as intermediate resistance to APM and ORY. At 0.4 and 0.5 μM APM, concentrations which kill *apm1*/+ and *apm2*/+ diploids, the double-heterozygote colonies grew vigorously and appeared unaffected by the herbicide. These diploids were 1.5–2 fold resistant relative to heterozygous *apm2*/+ diploids; none showed resistance greater than that of homozygous *apm2-1* diploids. Using *nit1*, *nit2* and *nit4* auxotrophic markers for diploid construction, the same results were obtained for doubly heterozygous diploids containing *apm2-1* and any of 14 *apm1* alleles. Because the mutants are completely recessive, or nearly so, and because the double heterozygotes express intermediate levels of resistance, some form of interaction between the two loci may be indicated.

**Zygotic lethality is observed in self crosses of *apm2-1*:** Zygotes from self crosses of *apm2-1* failed to germinate (<0.02%), whereas crosses of nine *apm1* alleles *inter se* resulted in normal germination and tetrad viability (Table 2). Ten complete tetrads recovered from a cross of $F_6$ *apm2-1* × WT were analyzed for co-segregation of zygotic lethality by crossing all the $F_7$ tetrad progeny to $F_7$ *apm2-1* $mt^+$ and $F_7$ *apm2-1* $mt^-$ tester strains. For all ten tetrads, the zygotic lethal phenotype co-segregated with *apm2-1* progeny (ts$^-$, APM$^R$ ORY$^R$), demonstrating close linkage of the zygotic lethal phenotype to *apm2-1*. Self-crosses of *apm2-1* mated well to produce abundant

zygotes which were smaller (approximately one-half to two-thirds the diameter) than zygotes from crosses of *apm2-1* to wild type or to alleles of *apm1*. Some zygotes appeared to swell slightly during incubation under conditions which normally induce swelling and germination, and rare zygotes (<0.02%) were observed to germinate. Adding 0.25 $\mu$M APM, 0.4 $\mu$M APM, or 1.5 $\mu$M ORY to the agar media did not prevent the observed zygotic lethality.

Even though zygotic lethality occurs in self crosses of *apm2-1*, stable vegetative diploids are easily obtained from these self-crosses. Therefore, the wild-type gene product of *apm2-1* must be required for some zygote-specific process or function, such as maturation, induction of germination, or meiosis. However, this gene must also provide a function(s) essential for vegetative growth because, at the nonpermissive temperature, haploids and diploids homozygous for *apm2-1* express a strong lethal phenotype.

**Allele-specific synthetic lethality in crosses between *apm1* and *apm2-1*:** In addition to the unusual observation of partial intergenic noncomplementation between mutant loci, we observed complete synthetic lethality in crosses between *apm2-1* and 11 alleles of *apm1* (Table 2). That is, double-mutant progeny (*apm1 apm2*) were inviable, whereas other tetrad members (*apm1* +,+ *apm2*, or ++) survived. The only complete tetrads recovered were parental ditypes (2 *apm1*/+:2 +/*apm2*). The majority of tetrads recovered were tetratypes and had three survivors (*apm1* +, + *apm2*, and ++). All nonparental ditypes were recovered as dyads in which both surviving tetrad products were phenotypically wild type (++).

The synthetic lethal interaction between *apm1* and *apm2* mutants is allele-specific, because two additional *apm1* alleles (*apm1-9* and *apm1-18*) produced viable double mutants with *apm2-1*. These double mutants were recovered with high frequency and appeared normal except that double mutants grew at a substantially slower rate compared to other tetrad progeny. The occurrence of allele-specific synthetic lethality between the *apm1* and *apm2* loci may indicate an interaction between the gene products. To the extent that crosses of *apm1* alleles or *apm2-1* to wild-type (Table 1), to two other ORY$^R$, APM$^S$ ts-lethal loci, and to a third APM$^R$ORY$^R$ locus show normal viability (data not shown) the lethal interaction between *apm1* and *apm2* appears to be specific for these two loci.

**The *apm1* locus maps to the *uni* linkage group (ULG):** The *apm1* locus is closely linked to two loci in the centromere-distal region of the genetically circular ULG (Table 4). Mapping by two-factor crosses places *apm1* ~4 cM from *pf7* (paralyzed flagella), and ~6.5 cM from *uni1* (uniflagellate cells). The *pf7* locus was previously mapped to a position 4 cM centromere-proximal to *uni1* (Ramanis and Luck 1986); therefore, we have assigned *apm1* to a centromere-proximal position relative to *uni1*. Three-factor crosses involving *apm1*, *uni1* and *pf7* were not performed due to the difficulty of obtaining sufficient numbers of tetrads for analysis.

TABLE 4

Mapping of *apm1* on the *uni* linkage group

*sun1* — ~25 cM — *pf7* — 4 cM — *uni1* — 15 cM — *fla9* (*apm1* bracketed near *pf7*)

| | Segregation | |
|---|---|---|
| | *uni1* | *pf7* |
| *apm1-1* | 67:0:10 (6.5 cM) | |
| *apm1-7* | 58:0:8 (6.1 cM) | 107:0:9 (3.9 cM) |
| *apm1-12* | 23:0:4 (7.4 cM) | |
| Total | 148:0:22 (6.5 cM) | 107:0:9 (3.9 cM) |

TABLE 5

Mapping of *apm2* on linkage group VIII

*nr1* — ~10 cM — *pf3* — 23 cM — *vfl1* — 4 cM — O (*apm2* bracketed between *vfl1* and centromere)

| | Segregation | | |
|---|---|---|---|
| | *nr1* | *pf3* | *vfl1* |
| *apm2-1* | 30:0:40 (28.6 cM) | 66:0:53 (22.3 cM) | 86:0:4 (2.2 cM) |

**The *apm2* locus maps near the centromere of linkage group VIII:** The *apm2* locus is linked to three loci on linkage group VIII (Table 5). Mapping by two-factor crosses places *apm2* ~29 cM from *nr1* (neamine-resistance), ~22 cM from *pf3* (paralyzed flagella), and ~2.2 cM from *vfl1* (variable flagellar number). The centromere distances for *nr1* of 30 cM (E. Harris, unpublished results), for *pf3* of 23 cM (Hastings *et al.* 1965), and for *vfl1* of 4 cM (Adams, Wright and Jarvik 1985) predict that *apm2* is closely linked to the centromere. Centromere linkage was verified by crossing *apm2-1* to the centromere marker *ac17*. The *PD*:*NPD*:*T* ratio for the cross *ac17* × *apm2-1* was 45:43:4, which corresponds to a centromere distance of approximately 2.2 cM. From these results it is likely that *apm2* resides midway between *vfl1* and the centromere on linkage group VIII.

**The *apm1* and *apm2* loci are not structural genes for $\alpha$ or $\beta$ tubulin:** Because some studies suggest that APM and ORY may interact directly with plant tubulins (Morejohn and Fosket 1984; Morejohn *et al.* 1987), we determined whether mutations in the *apm1* or *apm2* loci are located in any of the four tubulin genes of *C. reinhardtii* (Silflow and Youngblom 1986). We compared the segregation of the four tubulin genes using restriction fragment length polymorphisms (RFLPs) (L. P. W. Ranum, M. D. Thompson, P. A. Lefebvre and C. D. Silflow, unpublished data) with the segregation of the *apm1* and *apm2* loci.

RFLPs were identified for each of the four tubulin genes between *C. reinhardtii* and *Chlamydomonas smithii* (HOSHAW and ETTL 1966) and crosses were made between the *C. reinhardtii apm1* and *apm2* mutants and *C. smithii*. None of the four tubulin genes cosegregated with the *C. reinhardtii* APM-resistance phenotypes. For the cross *apm1-11* (*C. reinhardtii*) × *C. smithii*, six APM-resistant progeny were examined for segregation of the $\alpha$1- and $\alpha$2-tubulins using RFLPs; for the $\beta$1- and $\beta$2-tubulins, eight resistant progeny were analyzed. For each tubulin gene, the following ratios of *C. reinhardtii* vs. *C. smithii* RFLP bands were observed: $\alpha$1 and $\alpha$2—1 *C. reinhardtii*:5 *C. smithii*; $\beta$1 and $\beta$2—4 *C. reinhardtii*:4 *C. smithii*. Similarly, RFLP analysis of 4 resistant progeny from the cross *apm2-1* (*C. reinhardtii*) × *C. smithii* showed segregation of APM-resistance with tubulin genes from both parents. Therefore, the *apm1* and *apm2* loci are not structural genes for alpha or beta tubulin. Clearly it is possible to produce resistance to APM and ORY without altering the primary structure of the tubulins.

## DISCUSSION

In this report we describe a new locus, *apm1*, which is the first drug-resistance marker mapped to the *uni* linkage group (ULG). We also describe two types of unusual interactions between *apm1* and another new locus, *apm2*, which maps to linkage group VIII.

The recently described ULG exhibits three unusual characteristics by which it differs from the 18 other linkage groups in *C. reinhardtii*: (1) it has an internally consistent, circular genetic map; (2) recombination frequencies between loci on the ULG but not other linkage groups display a striking temperature-dependence; and (3) all 12 previously identified ULG loci affect flagellar assembly or function (RAMANIS and LUCK 1986). It has been suggested that the ULG loci may contribute to the structure and function of basal bodies because (1) many ULG mutants are conditionally flagella-less or possess short flagellar stubs, consistent with phenotypes expected of basal body-defective mutants; and (2) *uni1*, a uniflagellate mutant, exhibits ultrastructural defects in a basal body region called the transition zone (HUANG *et al.* 1982). Apart from being the first drug-resistance marker for the ULG, *apm1* is also the first ULG locus for which a role in flagellar assembly or function is not obvious from mutant phenotypes.

Alleles of *apm1* and *apm2-1* (ts) are recessive, yet doubly heterozygous diploids (*apm1 +/+ apm2-1*) display substantial resistance to the herbicides. Although the ts phenotype of *apm2-1* is complemented in doubly heterozygous diploids, we suggest that the intermediate levels of resistance expressed by these diploids may result from the interaction of the two mutant gene products in a common structure or process. The occurrence of intergenic noncomplementation between recessive mutations at different loci is uncommon. Examples of such interactions have included (1) mutations in $\beta$-tubulin and mutant nontubulin genes in Drosophila (RAFF and FULLER 1984); (2) mutations in four loci which are believed to encode cuticle structural proteins in *Caenorhabditis elegans* (KUSCH and EDGAR 1986); and (3) mutations in two unlinked loci which suppress the ethanolamine requirement of *cho1* lipid biosynthetic mutants in *Saccharomyces cerevisiae* (ATKINSON 1985). In these cases, failure of recessive mutants at different loci to complement has been interpreted as due to the interaction of mutant gene products to form multimeric structures.

The possible intergenic interaction between *apm1* and *apm2* is further suggested by the allele-specific synthetic lethality observed between double mutants of *apm1* (11 out of 13 alleles) and *apm2-1*. Such lethality is not observed in crosses of *apm1* or *apm2* to wild type and to mutants at three other herbicide-resistant loci (data not shown). An alternative explanation for partial intergenic noncomplementation and synthetic lethal interactions between *apm1* and *apm2* may be that, instead of acting together in a single process or structure, the gene products give rise to pleiotropic effects which overlap. Consideration of this possibility may be warranted on the basis that the product of the *apm2* locus is involved in at least two functions, one of which is zygote-specific and one of which is essential for vegetative growth.

Although the functions of the two herbicide-resistant loci are unknown, we suggest that the data may be consistent with a role for these genes in the structure or function of microtubule-based processes. Further characterization of these mutants by genetic analysis and immunofluorescence microscopy with antitubulin antibodies will be used to determine which, if any, microtubule-based structures or processes are affected by these mutants.

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