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β-Tubulin mutants of the unicellular green alga *Chlamydomonas reinhardtii*

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ABSTRACT **Two β-tubulin mutants of the unicellular green alga *Chlamydomonas reinhardtii* have been isolated on the basis of altered sensitivity to the growth-inhibitory effect of colchicine. The two mutations *col*^{R}4 and *col*^{R}15 have been found to be tightly linked, mapping to a previously unmarked site in linkage group XII. The drug-resistance phenotypes of both mutations segregated in genetic crosses with the presence of distinct, acidic variant β-tubulin isoforms found assembled into the microtubules of the flagella. Analysis of the *in vitro* translation products of total poly(A)$^+$ RNA from the mutants provided evidence that the variant proteins are altered primary β-tubulin gene products. Compared to wild type, strains carrying the mutations expressed an increased resistance to the inhibitory effects of colchicine in clonal growth, flagellar assembly, and germination of meiotic products, suggesting that the β-tubulin altered in the mutants participates in multiple microtubule functions.**

The unicellular green alga *Chlamydomonas reinhardtii* has emerged as an important experimental system for the genetic and biochemical analysis of the function and assembly of microtubules and their associated proteins. *Chlamydomonas* has several functionally distinct sets of microtubules that are expressed during various phases of the cell cycle. These include flagellar and basal body microtubules, interphase cortical microtubules, mitotic and meiotic intranuclear-spindle microtubules, and cytoplasmic microtubules that participate in nuclear and cell cleavage (1–3).

Largely due to the analysis of flagellar-defective mutants in *C. reinhardtii*, the structural organization and functions of many of the microtubule-associated proteins of the flagellum have been elucidated (for review, see ref. 4). Although >50 flagellar-related genes have been identified and mapped by mutations, none has been described for the tubulin subunits that make up the "9+2" microtubular framework. However, significant progress has been made on the molecular biology and protein biochemistry of the tubulins in the cell. The tubulin gene family in *C. reinhardtii* has been found to be small, containing only two α- and two β-tubulin genes that, by restriction mapping, are unlinked to one another (5, 6). All four of the genes have been sequenced. The α-tubulin genes code for two polypeptides that differ in their amino acid sequences by two residues (7), whereas the two polypeptides encoded by the β-tubulin genes are identical in their amino acid sequences (8).

Although, to the best of our knowledge, tubulin mutants have not been described in algae or higher plant systems, they have been isolated in other organisms (for review, see ref. 9). Many of these mutants were recovered as a class of mutations that caused altered response to antimitotic drugs. Cell division and flagellar assembly in *Chlamydomonas* have been shown (10–12) to be sensitive to several of the known tubulin-binding drugs, including colchicine, vinblastine, and podophyllotoxin. In fact, *Chlamydomonas* mutants altered in their sensitivities to one or more of these drugs have been isolated, but in no case was a molecular diagnosis made for the mutations (12–15).

In this report two mutants of *C. reinhardtii* isolated on the basis of increased resistance to colchicine are described. Evidence is presented that drug resistance in these two mutants is associated with mutations in β-tubulin.

EXPERIMENTAL PROCEDURES

Strains, Cell Culture, and Genetic Analysis. Wild-type strain 137c *mt*$^+$ and *mt*$^-$ and several marker strains used in mapping studies were obtained from the *Chlamydomonas* Stock Center at Duke University (Durham, NC). Cells were cultured at 20°C under constant light conditions in Tris/acetate/phosphate (TAP) medium (16) either as lawns on 1.5% agar plates or in liquid culture. Equilibrium labeling with [^{35}S]sulfate was achieved by growing cells as described (17) as lawns on Petri dishes containing 25 ml of low-sulfate TAP medium (medium in which the $MgSO_4$ concentration was reduced to 10% of that normally used and $MgCl_2$ was added to restore the original Mg^{2+} concentration), 2% (wt/vol) agar prewashed in distilled water, and $H_2{}^{35}SO_4$ (25 mCi/liter).

Standard techniques of crossing and tetrad analysis were used to determine segregation patterns and recombination frequencies (18). Plate-grown cells were induced to become gametes by resuspension and incubation in nitrogen-free, high salt medium (19).

Mutagenesis and Mutant Selection. Logarithmic-phase wild-type 137c *mt*$^+$ cells were pelleted and resuspended at a density of 3×10^7 cells per ml in liquid growth medium. A 10-ml aliquot of the cells was exposed to UV irradiation for 90 sec as described (17). UV-irradiated and non-irradiated cells were plated at a density of 2×10^7 cells per plate onto 1.5% agar plates containing 5 mM colchicine (Sigma) in growth medium (a concentration of colchicine that effectively inhibits clonal growth of wild-type cells). The plates were held in the dark overnight before transfer to low light (≈1000 lux). The plates were kept at 20°C under low-light conditions for 12 days. *col*^{R}15 was recovered in a mutagenesis experiment in which the UV-killing efficiency was 90% and the frequency of drug-resistant clones among survivors was 2.2×10^{-4}. In the same experiment, the frequency of spontaneous drug-resistant clones was 1.2×10^{-6}.

Protein Analyses. Flagellar axonemes were isolated as described (20). Isolated axonemal pellets were solubilized in isoelectric focusing (IEF) sample buffer (21) and loaded on gels (within 4 hr) or frozen at −80°C for later analysis. IEF slab gel electrophoresis was performed as described (21) with

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Abbreviation: IEF, isoelectric focusing.

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the following modifications: to obtain linear pH gradients in the range of 4.0–7.0, the following mixtures of LKB (Bromma, Sweden) ampholines were used: one part pH 3.5–10 ampholines, two parts pH 4–6 ampholines, two parts pH 5–7 ampholines; or one part pH 3.5–10 ampholines, four parts pH 5–7 ampholines; the amount of riboflavin added for gel polymerization was 20% of the amount indicated; and the gels were electrophoresed at 400 V for 18 hr and then at 800 V for 1 hr. IEF gels were stained in a solution of 40% (vol/vol) methanol, 4% (vol/vol) formaldehyde, and 0.015% Coomassie brilliant blue R-250 (Kodak) and destained in a solution of 15% (vol/vol) isopropanol and 7% (vol/vol) acetic acid before they were dried for autoradiography.

For immunoblot analysis, effective transfer of proteins from the IEF gels to nitrocellulose was obtained by preincubating the gels in 1% $NaDodSO_4$/20 mM Tris/150 mM glycine for 30 min with one change of the solution during the incubation period. After a brief rinse in transfer buffer [20 mM Tris, 150 mM glycine, and 20% (vol/vol) methanol], the proteins were electrophoretically transferred to 0.2-μm pore-size nitrocellulose at 3.5 V/cm for 2 hr.

For two-dimensional resolution of axonemal tubulins, 4-mm vertical strips from IEF gels were equilibrated for 15 min in 0.5% $NaDodSO_4$ (Sigma)/54.1 mM Tris sulfate, pH 6.1, prior to loading onto a second-dimension $NaDodSO_4$ gel. The second-dimension gel was formed with a stacking gel composed of 3.2% (wt/vol) acrylamide, 0.1% $NaDodSO_4$, and 54.1 mM Tris sulfate (pH 6.1) and a resolving gel consisting of a 4–11% (wt/vol) linear gradient of acrylamide made up in 25 mM Tris glycine, pH 8.3/0.1% $NaDodSO_4$. The electrode buffer was 25 mM Tris glycine, pH 8.3/0.1% $NaDodSO_4$.

Isolation of RNA and *in Vitro* Translation. Total RNA was prepared as described (22) with the following modifications: RNA precipitates were washed twice with 2 M LiCl to remove polysaccharides (23) before they were further purified by centrifugation through a CsCl cushion (24). Poly(A)$^+$ RNA was isolated by two cycles of oligo(dT)-cellulose chromatography (25). Poly(A)$^+$ RNA was translated *in vitro* by using a rabbit reticulocyte lysate system (Amersham; nuclease-treated and mRNA-dependent) with added [^{35}S]methionine (Amersham).

RESULTS

Isolation and Genetic Segregation of the *col*^{R}4 and *col*^{R}15 Mutations. Mutants *col*^{R}4 and *col*^{R}15 were originally isolated as strains that expressed the capacity for clonal growth on agar plates containing 5 mM colchicine. Both mutants were recovered in a background of wild-type strain 137c *mt*$^+$, *col*^{R}4 as a spontaneous mutation and *col*^{R}15 following UV irradiation. The original mutant isolates were back-crossed to wild-type strain 137c *mt*$^-$, and in tetrad analysis the drug-resistance phenotype of each of the mutants segregated 2:2 as expected for single-gene mutations. F_1 daughter cells carrying the *col*^{R}4 and *col*^{R}15 mutations were crossed to each other, and in an analysis of 83 tetrads no recombination events were observed, indicating that *col*^{R}4 and *col*^{R}15 define either a single gene or two tightly linked genes. As seen in Fig. 1, the *col*^{R}4 and *col*^{R}15 mutations were mapped to a previously unmarked site on the right arm of linkage group XII.

***col*^{R}4 and *col*^{R}15 Express a Wild-Type and a Variant β-Tubulin Assembled into the Flagellar Microtubules.** *col*^{R}4 and *col*^{R}15 were initially identified as altered in their β-tubulin content in a two-dimensional gel electrophoretic analysis of the tubulin subunits found in flagellar axonemes isolated from the mutants. Fig. 2 contains autoradiograms of the two-dimensional resolution of ^{35}S-labeled α- and β-tubulin subunits found in wild-type and mutant axonemes. Wild-type axonemes have been reported (22, 26, 27) to contain a single β-tubulin subunit and one major and one minor α-tubulin isoform. Under the conditions of IEF used in this study, a single β-tubulin species was also detected in wild-type axonemes. However, in contrast to previous studies, an additional minor component was resolved in the α-tubulin region. Axonemes from the mutants *col*^{R}4 and *col*^{R}15 contained an α-tubulin pattern identical to wild type, but, in contrast to wild type, two β-tubulin isoforms were present. In experiments in which nonradioactive wild-type axonemes were coelectrophoresed with mutant ^{35}S-labeled axonemes the more basic β-tubulin isoform in each of the mutants comigrated with the single β-tubulin found in wild type (data not shown). These data indicate that both mutants express a wild-type β-tubulin and a variant, more acidic β-tubulin assembled into the microtubules of the flagellar axonemes. The *col*^{R}4 variant β-tubulin migrated in the IEF dimension to a more acidic pI value than the *col*^{R}15 variant and slightly faster than the wild-type β-tubulin isoform in the $NaDodSO_4$ dimension.

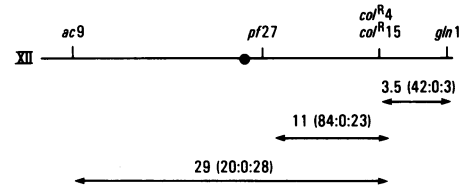

FIG. 1. Genetic mapping of *col*^{R}4 and *col*^{R}15 to linkage group XII. *col*^{R}4 and *col*^{R}15 mutants were initially crossed to strains singly or multiply marked for mutations in 22 mapped genes. On the basis of tetrad analysis, statistically significant linkage was found for only one of the genes tested, *pf*27 on linkage group XII (46:0:16, PD:NPD:TT). Second-division segregation frequencies from crosses of *col*^{R}4 and *col*^{R}15 to the two closely centromere-linked markers, *ac* 17 and *pf* 2, gave a consistent estimated gene–centromere distance of 13 cM (37:44:29, PD/NPD/TT). Tetrad analysis of two-point and three-point crosses in which *col*^{R}4, *col*^{R}15, *pf*27, *gln* 1, and *ac* 9 were segregating placed the *col*^{R} mutations on the right arm of linkage group XII between *pf*27 and *gln* 1. In the figure the intervals between arrowheads designate the map distances (cM), and the numbers in parentheses are the numbers of observed PD/NPD/TT tetrads. PD, Parental ditype; NPD, nonparental ditype; TT, tetratype.

The various tubulin isoforms in wild type and the *col*^{R} mutants could also be resolved and identified in one-dimensional IEF slab gels. Fig. 3 *Left* contains an autoradiogram of the nitrocellulose transfer of ^{35}S-labeled axonemal polypeptides from wild type and *col*^{R}4 and *col*^{R}15 mutants resolved by IEF. The identity of the β-tubulin bands was verified in immunobinding experiments with a rabbit anti-β-tubulin antibody (28). Fig. 3 *Right* shows the anti-β-tubulin immunoperoxidase staining pattern of the nitrocellulose transfer seen on the *Left*.

From a visual examination of the autoradiograms shown in Figs. 2 and 3 and the immunoperoxidase staining pattern shown in Fig. 3, the relative intensities of the β_{wt}- and β_v-tubulin isoforms found in the mutant axonemes were very similar. This was confirmed by cutting out and quantitating radioactivity in the gel regions containing the β_{wt}- and β_v-tubulins from IEF slab gels. From several independent experiments the distribution of total β-tubulin radioactivity between the β_{wt} and β_v isoforms was 0.48:0.52 for *col*^{R}4 axonemes and 0.44:0.56 for *col*^{R}15 axonemes.

Cosegregation of the Colchicine-Resistance Phenotypes of *col*^{R}4 and *col*^{R}15 and the Presence of the Variant β-Tubulins Assembled into the Axonemes. The IEF patterns of the tubulin subunits found in isolated axonemes from each daughter clone of 24 complete tetrads from crosses in which *col*^{R}4 and *col*^{R}15 were segregating (12 tetrads for *col*^{R}4 and 12 tetrads for *col*^{R}15) were examined. In every instance, two out of the four daughters from each tetrad expressed the variant β-

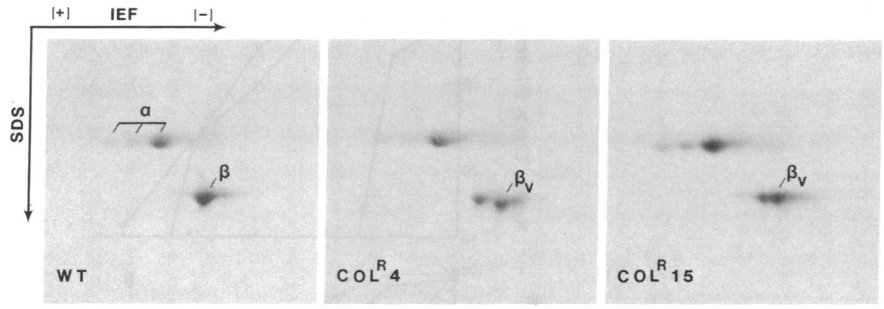

FIG. 2. Autoradiograms of the two-dimensional resolution of ^{35}S-labeled α- and β-tubulin subunits found in isolated flagellar axonemes from wild-type strain 137c and strains carrying the *col*^{R}4 and *col*^{R}15 mutations. SDS, NaDodSO$_4$; WT, wild type.

tubulin isoform assembled into the axonemes, and invariantly, the same two out of the four daughters showed colchicine resistance for growth. Fig. 4 illustrates the results for four complete tetrads from a cross in which *col*^{R}15 was segregating.

The *col*^{R}4 and *col*^{R}15 Variant β-Tubulins are Altered Primary β-Tubulin Gene Products. To distinguish between the possibilities that the variant β-tubulins in *col*^{R}4 and *col*^{R}15 represented altered primary β-tubulin gene products or were new isoforms generated by posttranslational modification of a wild-type β-tubulin, poly(A)$^+$ RNA was isolated from wild-type and mutant cells and translated *in vitro* in a reticulocyte cell-free system. Since it was shown (22) that the abundance of tubulin mRNAs in *Chlamydomonas* is the highest in cells that are in the process of regenerating their flagella, the poly(A)$^+$ RNA used in the *in vitro* translations was isolated from gametic cells 40 min after deflagellation. Fig. 5 contains autoradiograms of one-dimensional IEF slab gels in which wild-type and mutant ^{35}S-equilibrium-labeled axonemes and [^{35}S]methionine-labeled *in vitro* translation products of isolated poly(A)$^+$ RNA were resolved.

As reported (22), a single β-tubulin isoform was synthesized *in vitro* from poly(A)$^+$ RNA isolated from deflagellated wild-type cells (Fig. 5*B*, lane 1). The *in vitro*-synthesized β-tubulin migrated to the same position in the IEF gel as the β-tubulin found assembled into wild-type axonemes (Fig. 5*A*, lane 1). *In vitro* translation of poly(A)$^+$ RNA from both *col*^{R}4 (Fig. 5*B*, lane 2) and *col*^{R}15 (Fig. 5*B*, lane 3) revealed two β-tubulin mRNAs—one that encoded a wild-type β-tubulin and the other that encoded a more acidic β-tubulin that comigrated with the variant β-tubulin found assembled into the mutant axonemes (Fig. 5*A*, lanes 2 and 3). From a quantitative analysis of densitometric scans of the gel (Fig. 5*B*), the amount of total β-tubulin synthesized *in vitro* from poly(A)$^+$ RNA isolated from the mutants was similar to that observed for wild type [(*col*^{R}4 $\beta_{wt} + \beta_v$)/wild-type β_{wt} = 1.08; (*col*^{R}15 $\beta_{wt} + \beta_v$)/wild-type β_{wt} = 1.36]. The distribution of total β-tubulin radioactivity between the *in vitro*-synthesized β_{wt}- and β_v- tubulins was 0.51:0.49 for *col*^{R}4 RNA and 0.44:0.56 for *col*^{R}15 RNA.

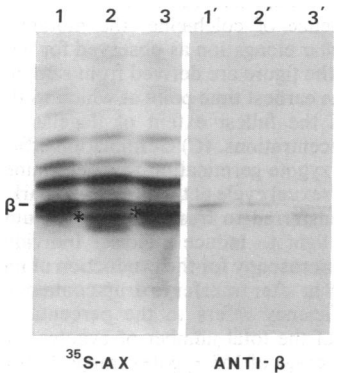

FIG. 3. Nitrocellulose transfer of axonemal tubulins isolated from wild-type strain 137c (lanes 1 and 1′), and strains carrying the *col*^{R}4 (lanes 2 and 2′) and *col*^{R}15 (lanes 3 and 3′) mutations and resolved by one-dimensional IEF slab gel electrophoresis. (*Left*) Autoradiogram. The mutant variant β-tubulins are indicated (*). (*Right*) Immunoperoxidase staining of the same nitrocellulose blot after incubation with a rabbit anti-β-tubulin antibody.

Colchicine-Resistance Phenotypes of *col*^{R}4 and *col*^{R}15. Fig. 6 contains dose–response curves for the effects of colchicine on the relative cloning efficiency (*A*), flagellar regeneration efficiency (*B*), and germination efficiency (*C*) in wild-type strains and strains carrying the *col*^{R}4 and *col*^{R}15 mutations. As reported (11, 12), millimolar concentrations of colchicine are required to inhibit mitotic cell divisions and flagellar regeneration in haploid wild-type *Chlamydomonas* cells. In this study we have observed that, in addition to vegetative growth and flagellar assembly, the germination of meiotic products from diploid zygotes produced in matings of wild-type strain 137c *mt*$^+$ × 137c *mt*$^-$ was also sensitive to colchicine in a dose-dependent manner.

For each of the three cellular functions assayed, strains carrying the *col*^{R}4 and *col*^{R}15 mutations showed an increased resistance to colchicine over that observed in wild type, with the levels of increased resistance being very similar for the two mutations. In Fig. 6*C* it should be noted that heterozygous *col*^{R}4*COL*^{R}4 and *col*^{R}15*COL*^{R}15 zygotes expressed an intermediate level of drug resistance for germination as compared to homozygous *col*^{R}4*col*^{R}4 and *col*^{R}15*col*^{R}15 zygotes and heterozygous *col*^{R}4*col*^{R}15 zygotes. This observation that the drug-resistance phenotypes of the mutants are expressed in the presence of the wild-type allele in diploid zygotes indicates that *col*^{R}4 and *col*^{R}15 are codominant mutations.

In addition to studying the colchicine-resistance phenotypes of *col*^{R}4 and *col*^{R}15, we have also screened the mutants for secondary phenotypes that might be expressed in the absence of colchicine. Thus far, we have not detected any alterations in the temperature-dependent growth character-

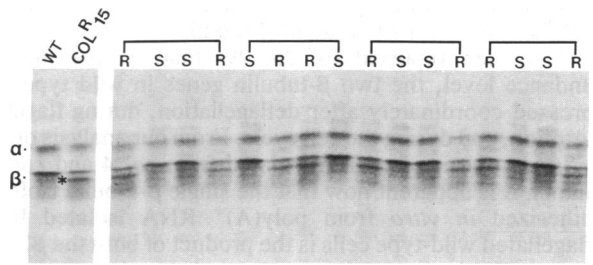

FIG. 4. Autoradiogram of an IEF slab gel that resolves the tubulin subunits found in ^{35}S-labeled axonemes isolated from each daughter clone of four complete tetrads derived from a mating of wild-type (WT) 137c × *col*^{R}15. The meiotic products of a single tetrad are bracketed, and the colchicine phenotypes for each of the products are indicated as resistance (R) or sensitivity (S). For reference, the tubulin patterns of the parental strains are seen at the left with the *col*^{R}15 variant β-tubulin indicated (*).

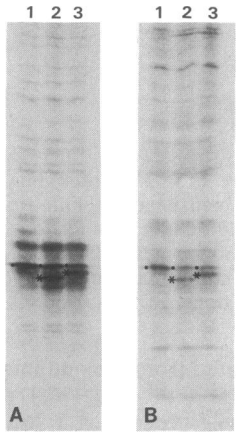

FIG. 5. Autoradiograms of IEF slab gels. In each lane, the position of β_{wt}-tubulin isoform (·) and the mutant β_v-tubulin isoforms (*) are indicated. (*A*) Resolution of *in vivo* ^{35}S-labeled axonemal polypeptides isolated from wild type (lane 1), *col*^{R}4 (lane 2), and *col*^{R}15 (lane 3). (*B*) Resolution of [^{35}S]methionine-labeled polypeptides synthesized *in vitro* from poly(A)$^+$ RNA isolated from wild type (lane 1), *col*^{R}4 (lane 2), and *col*^{R}15 (lane 3).

istics or flagellar motility properties of either *col*^{R}4 or *col*^{R}15 mutants.

DISCUSSION

In this report the isolation and characterization of two colchicine-resistant mutants in *C. reinhardtii* have been described in which drug resistance has been shown to cosegregate with specific alterations at the protein level in β-tubulin. Three lines of evidence strongly indicate that *col*^{R}4 and *col*^{R}15 are missense mutations in the coding sequence of either the β1- or β2-tubulin genes in *Chlamydomonas*, which in wild type encode identical proteins (8). (*i*) The two mutations are associated with distinct, acidic variant β-tubulin isoforms found assembled into cellular microtubules in approximately equal molar ratios with a wild-type β-tubulin isoform. (*ii*) *In vitro* translation of poly(A)$^+$ RNA isolated from the mutants yields both wild-type and variant β-tubulins at the same ratio as observed in isolated microtubules. (*iii*) *col*^{R} phenotypes of both mutations are codominantly expressed in heterozygous mutant diploid zygotes. Preliminary results from hybrid-select translation of mutant poly(A)$^+$ RNA with cDNAs for the β1- and β2-tubulins in *Chlamydomonas* suggest that *col*^{R}4 and *col*^{R}15 variant β-tubulins are the products of the β2-tubulin gene. Definitive evidence of the identity of the specific β-tubulin gene altered in the mutants and the precise nature of the genetic lesions should be derived through the isolation and characterization of cDNA clones for the mutant β-tubulins.

It has been reported (6, 23, 29) that, at the mRNA abundance level, the two β-tubulin genes in wild type are expressed coordinately after deflagellation, during flagellar resorption, and during the cell cycle. From our analysis of the *in vitro* synthesis of mRNA isolated from *col*^{R}4 and *col*^{R}15 mutants, it is apparent now that the single β-tubulin isoform synthesized *in vitro* from poly(A)$^+$ RNA isolated from deflagellated wild-type cells is the product of both the β1 and β2 genes. Furthermore, the protein products of the two genes are coordinately assembled into the microtubules of the flagella.

Thus far, it has only been demonstrated at the biochemical level that the *col*^{R}4 and *col*^{R}15 variant tubulins are assembled into the microtubules of the flagella. However, the observation that the mutations confer an increased resistance to the inhibitory effects of colchicine on vegetative cell divisions and the germination of meiotic products suggests that the β-tubulin gene product altered in the mutants is likely to participate in additional microtubule functions specifically associated with mitotic and meiotic cell divisions. With gene-specific nucleic acid probes (8) and the gene-specific

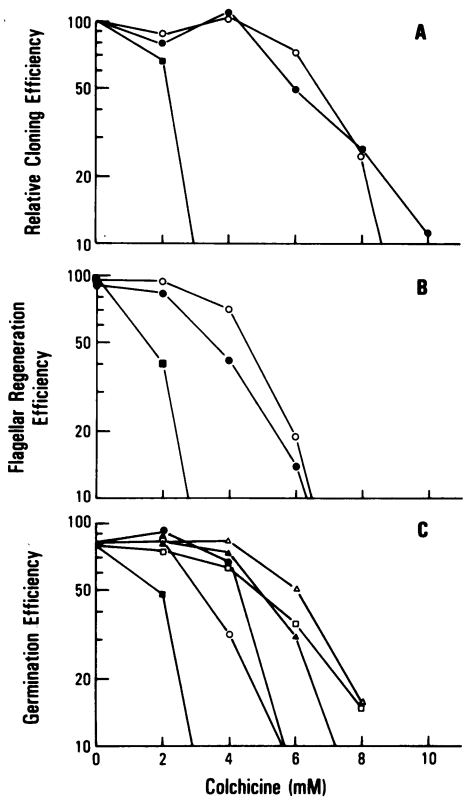

FIG. 6. Comparative colchicine dose–response curves for wild-type 137c and strains carrying the *col*^{R}4 or *col*^{R}15 mutations. (*A* and *B*) Haploid wild type (■), *col*^{R}4 (○), and *col*^{R}15 (●) cells are shown. (*C*) Diploid zygotes produced by matings of wild type × wild type (■), *col*^{R}4 × wild type (○), *col*^{R}15 × wild type (●), *col*^{R}4 × *col*^{R}4 (△), *col*^{R}15 × *col*^{R}15 (□), and *col*^{R}4 × *col*^{R}15 (▲). (*A*) Relative cloning efficiency. For each data point 10^3 cells were inoculated onto growth medium agar plates (200 cells per 90-mm plate) containing each drug concentration. The relative cloning efficiency for the various strains was determined by dividing the number of clones that grew in the presence of colchicine by the number of clones that grew in the absence of the drug. (*B*) Flagellar regeneration efficiency. Logarithmic-phase vegetative cultures were experimentally deflagellated (20); the cells were pelleted and resuspended in liquid growth medium containing various colchicine concentrations. Aliquots of the cells were fixed at 1-hr intervals for up to 6 hr after deflagellation. The fixed samples were examined by light microscopy to assess the extent of flagellar regrowth. The flagellar lengths on 100 cells were scored as 0, 1/4, 1/2, 3/4, or full length. The number of cells in each category was multiplied by 0, 1/4, 1/2, 3/4, or 1, respectively, and summed to yield a flagellar regeneration efficiency score. In the absence or presence of colchicine, the mutants showed similar kinetics for flagellar elongation as observed for wild type. The data points shown in the figure are derived from samples fixed 2 hr after deflagellation, the earliest time point at which wild type and the two mutants showed the fullest extent of flagellar outgrowth at the various drug concentrations. (*C*) Germination efficiency. The effect of colchicine on zygote germination was determined by introducing the drug into the sexual cycle at the time when dark-matured zygotes are normally transferred to fresh growth medium agar plates and exposed to the light to induce meiosis. Individual zygotes were scored by light microscopy for the production of meiotic products at 18 hr and up to 48 hr after transfer to drug-containing plates and light. Germination efficiency refers to the percentage of zygotes that germinated out of the total number of zygotes analyzed. For each data point, an average of 153 zygotes (range 113–192 zygotes) were analyzed.

protein products expressed in col^R4 and col^R15 mutants, it should now be possible to study in detail the coordinate and/or differential expression of the two β-tubulin genes in *Chlamydomonas* from transcription to assembly of the protein products during the cell cycle and into the various microtubular systems already identified within the cell.

The observation that colchicine resistance in both col^R4 and col^R15 is associated with altered β-tubulins parallels results obtained in other systems in which tubulin mutants isolated on the basis of altered sensitivities to either colchicine or benzimidazole compounds have almost exclusively been associated with mutations in the β-tubulin rather than α-tubulin subunits (30–33). Although there is no direct biochemical evidence of how the col^R4 and col^R15 mutant β-tubulins confer an increased resistance to the effects of colchicine, it has been observed (M. J. Schibler and B.H., unpublished results) that both col^R4 and col^R15 mutants express a genetically cosegregating cross-resistance to several chemically distinct microtubule-disrupting agents. These include vinblastine, the antimitotic herbicides pronamide and amiprophos-methyl, and the dinitroanilines oryzalin, trifluralin, profluralin, and ethafluralin. These results indicate that the col^R4 and col^R15 mutations are likely to affect the stability and/or assembly of the microtubules rather than necessarily altering the drug-binding characteristics of the tubulin.

An important observation reported here is that the colchicine-resistance phenotypes for the col^R4 and col^R15 mutations are codominantly expressed in heterozygous mutant diploid zygotes. These results suggest that the col^R4 and col^R15 variant β-tubulin genes may represent selectable markers that could be useful in the development of a homologous DNA-mediated transformation system in *Chlamydomonas*. The col^R4 β-tubulin genes have been cloned from a mutant genomic DNA library for this purpose (B.H. and J. Hicks, unpublished results).

Finally, it should be noted that although only low-affinity binding of colchicine to plant-derived tubulins has been reported (34) and 100- to 1000-fold higher concentrations of colchicine are required to inhibit growth in plant cells compared to animal cells (35), the recovery of β-tubulin mutants in *Chlamydomonas* on the basis of altered sensitivity to the drug provides strong evidence that the *in vitro* effects of colchicine in plant cells involve a specific interaction with plant tubulins.

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