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Diversity among β-Tubulins: A Carboxy-Terminal Domain of Yeast β-Tubulin Is Not Essential In Vivo

WENDY S. KATZ AND FRANK SOLOMON*

Department of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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Sequences of genes for β-tubulins from many different organisms demonstrate that they encode highly conserved proteins but that these proteins diverge considerably at their carboxyl termini. The patterns of interspecies conservation of this diversity suggest that it may have functional significance. We have taken advantage of the properties of *Saccharomyces cerevisiae* to test this hypothesis in vivo. The sole β-tubulin gene of this species is one of the most divergent of all β-tubulins and encodes 12 amino acids which extend past the end of most other β-tubulin molecules. We have constructed strains in which the only β-tubulin gene is an allele lacking these 12 codons. We show here that this carboxy-terminal extension is not essential. The absence of these 12 amino acids had no effect on a number of microtubule-dependent functions, such as mitotic and meiotic division and mating. It did confer dominant supersensitivity to a microtubule-depolymerizing drug.

Microtubules in animal cells form a diversity of structures and are involved in a variety of specific functions, usually associated with organization of the cytoplasm and motility. However, with few exceptions, the ultrastructure of individual microtubules is highly conserved. The sources of the molecular information that specify the various conserved and unique elements of microtubule structure, organization, and function are not known.

Several models have been proposed. At present, the model most accessible to experimental test is based on the suggestion that the major protein components of microtubules, the tubulins, may contain this information in their primary sequence. For example, sequence analyses by several groups suggest that although the β-tubulin genes are highly conserved, divergence at their 3′ ends could play a part in detailed organization of microtubules (4, 22).

In this paper, we describe a direct in vivo test of this notion. *TUB2*, the single β-tubulin gene of *Saccharomyces cerevisiae*, is highly divergent at its carboxyl terminus and encodes a tail of 12 amino acids extending beyond the position where most animal β-tubulins end (15). We have constructed yeast strains in which the only β-tubulin gene is an allele lacking these extra codons. The mutant tubulin does confer dominant supersensitivity to the microtubule-depolymerizing drug benomyl. Otherwise, however, several processes known to depend on microtubules, such as mitotic and meiotic division and mating, appear to be the same in these strains as in wild-type cells. These experiments, and previous studies on the two α-tubulin genes of *S. cerevisiae* (18–20), detect no functional implications in vivo of divergent tubulin sequences.

MATERIALS AND METHODS

Plasmid constructions. DNA manipulations were performed by standard techniques (13). Enzymes were purchased from New England Biolabs, Inc. (Beverly, Mass.). Table 1 lists the yeast genes present on each plasmid used in this study. pRB429 is a YIp5 derivative containing a portion of the *TUB2* gene from codon 5 to an *Sph*I site approximately 1.4 kilobases from the carboxyl terminus (T. C. Huffaker, J. H. Thomas, and D. Botstein, J. Cell Biol., in press). pJT69 is a YIp5 derivative bearing an *Eco*RI-*Sph*I genomic fragment containing *TUB2*. pWK4 was constructed by inserting an *Xho*I-*Sal*I fragment containing *LEU2* (from YEp13) into the *Sal*I site of pJT69. The *Xho*I end of the *LEU2* fragment is approximately 640 base pairs (bp) upstream of the *LEU2* start codon. The ligation of this *Xho*I end to the *Sal*I end of the *TUB2* fragment disrupts the tubulin gene in codon 436 and introduces four new codons, followed by a stop codon and the remainder of the *LEU2* insert (1). The tubulin allele created by this fusion is designated *tub2-591*. pWK23 was constructed by subcloning a *Bam*HI-*Sph*I fragment, containing the sequence to be deleted, from pRB429 into the vector pTZ18R (Pharmacia, Piscataway, N.J.). pWK25 was the product of the deletion reaction described below. pWK28, pWK29, and pWK30 were constructed by subcloning the deletion-bearing *Bam*HI-*Sph*I fragment from pWK25 into pRB429 from which the corresponding wild-type fragment had been excised.

Strains and media. *Escherichia coli* HB101 was used for routine bacterial transformations and plasmid preparations. *E. coli* JM103 was used for production of single-stranded DNA for oligonucleotide-directed deletion and sequencing. Bacterial media were prepared as described in Maniatis et al. (13). *S. cerevisiae* strains were constructed from S288C strains obtained from D. Botstein and G. Fink (Massachusetts Institute of Technology). The genotypes of the strains described in this paper are listed in Table 1.

FSY121 through FSY124 were constructed by transforming FSY117 with pWK28, cleaved within the *TUB2* sequence to direct integration to the *TUB2* locus (16). Transformants were screened for absence of staining by a polyclonal antibody raised against a synthetic peptide corresponding to the last 12 amino acids of TUB2. Nonstaining candidates were plated on 5-fluoro-orotic acid plates (2) and screened for absence of staining by the diagnostic antibody, together with loss of the *URA3* marker. FSY125 was constructed in parallel transformation and selections by using pRB429 instead of pWK28.

FSY126 through FSY128 are haploid segregants from a diploid constructed by mating FSY117 × FSY121.

FSY131 and two cognate strains were constructed by

* Corresponding author.

TABLE 1. Yeast strains and plasmids used

Strain or plasmid[a]	Relevant genotype or genes
Strains	
FSY117, FSY125	*MATa TUB2 his4-619 leu2-3,112 ura3-52*
FSY118	*MATα TUB2 leu2-3,112 lys2-801 ura3-52*
FSY121, FSY122, FSY123, FSY124	*MATa tub2-590 his4-619 leu2-3,112 ura3-52*
FSY126	*MATa tub2-590 his4-619 leu2-3,112 ura3-52*
FSY127	*MATα tub2-590 leu2-3,112 lys2-801 ura3-52*
FSY128	*MATa tub2-590 his4-619 leu2-3,112 ura3-52*
FSY131	*MATa/MATα TUB2/tub2-591 leu2-3,112/leu2-3,112 lys2-801/LYS2 his4-619/HIS4 ura3-52/ura3-52*
FSY136	*MATa tub2-590 ACT1:URA3:act1 his4-619 leu2-3,112*
FSY120	FSY117 × FSY118
FSY141	FSY136 × FSY118
FSY129	FSY126 × FSY127
FSY130	FSY128 × FSY127
Plasmids	
pJT69[b]	*TUB2, URA3*
pRB429[b]	Codons 5–457 of *TUB2, URA3*
pRB151[b]	Codons 188–360 of *ACT1, URA3*
pWK4	*tub2-591, LEU2, URA3*
pWK23	Codons 345–457 of *TUB2*
pWK25	Codons 345–457 of *tub2-590*
pWK28	Codons 5–457 of *tub2-590, URA3*

[a] All strains were constructed for this study. They are derived from S288C strains provided by D. Botstein and G. Fink (Massachusetts Institute of Technology). Except as noted, all plasmids listed were constructed for this study.

[b] Source: D. Botstein.

transforming FSY120 with an *Xho*I-*Sph*I fragment from pWK4, containing the *tub2-591* allele. The *TUB2* ends of the fragment direct integration to the *TUB2* locus (17). The chromosomal configuration resulting from such an integration is shown in Fig. 2. These strains were constructed to provide a disruption of β-tubulin upstream of the truncation produced by *tub2-590* and linked to an auxotrophic marker. At present we have no assay for the product of this gene, nor has the sequence of the construction been verified.

FSY136 and two cognate strains were constructed by transforming FSY121 with pRB151, cleaved to direct integration to the *ACT1* locus. They were constructed to provide an auxotrophic marker closely linked to the *tub2-590* locus. By the criteria of growth rate, sporulation, spore viability, and benomyl sensitivity, these marked strains are indistinguishable from the unmarked parental strains.

Yeast media were prepared as described in Sherman et al. (21). Histidine, leucine, lysine, and uracil were routinely added to YPD after autoclaving. Benomyl was obtained from Du Pont. A working stock of 10 mg/ml in dimethyl sulfoxide was stored in aliquots at 4°C. YPD with benomyl was prepared shortly before use by adding the working stock dropwise, with constant agitation, to YPD heated to 65°C. The drug-free control medium for the benomyl growth curves was YPD heated to 65°C with dimethyl sulfoxide added to the same concentration as in the medium containing drug. 5-Fluoro-orotic acid plates, for selection of strains which had undergone plasmid excision (2), were provided by P. Schatz.

Oligonucleotide-directed deletion. The synthetic oligonucleotide used to direct the deletion is composed of the 14 nucleotides preceding the codon for amino acid 446 of *TUB2*, immediately followed by the termination codon and the 11 subsequent nucleotides. This 28-mer was annealed to the antisense single-stranded DNA generated from pWK23, elongated with Klenow fragment, ligated, and used to transform strain JM103. Transformants were further grown on nitrocellulose filters and processed for DNA hybridization; those carrying the deletion were identified by their ability to hybridize the end-labeled oligonucleotide at high stringency. One candidate was sequenced by the dideoxy method to confirm that the extent of the deletion was correct and that the surrounding sequence was unchanged. This plasmid was designated pWK25. The tubulin allele created by the deletion was designated *tub2-590*.

Genetic techniques and transformation. Yeast genetic methods were as described in Sherman et al. (21). Transformations were done by the lithium acetate method. Sporulations were carried out in 1% potassium acetate, with no supplements.

Antibody preparation. Synthetic peptides were purchased from Peninsula Laboratories (Belmont, Calif.). The peptides were coupled to keyhole limpet hemocyanin via an amino-terminal cysteine and used to immunize rabbits as described by Schatz et al. (18).

Immunofluorescence. Cells were fixed in 3.7% formaldehyde at room temperature. Fixed cells were permeabilized by a 1-h incubation at 37°C with 0.05% glusulase (Du Pont Pharmaceuticals, Wilmington, Del.) and 5 μg of Zymolyase (Miles Laboratories, Naperville, Ill.) per ml in 0.1 M sodium phosphate, pH 6.5, containing 1.2 M sorbitol and 0.01% β-mercaptoethanol (L. Pillus, Ph.D. thesis, Massachusetts Institute of Technology, Cambridge, 1986). Permeabilized cells were washed with 0.1 M sodium phosphate, pH 6.5, and stained for immunofluorescence essentially as described by Kilmartin and Adams (9). All antibodies were diluted 1:2,000 in phosphate-buffered saline with 0.1% bovine serum albumin. The second antibody was fluorescein isothiocyanate-conjugated goat anti-rabbit serum (Cappel).

Protein preparation and Western blot (immunoblot) analysis. Yeast cells were grown to a density of approximately 10^7 cells per ml, harvested by centrifugation, and washed with phosphate-buffered saline. The cells were suspended in phosphate-buffered saline containing the protease inhibitors phenylmethylsulfonyl fluoride, tosyl phenylalanine chloromethyl ketone, pepstatin A, leupeptin, and aprotinin, lysed by vortexing with glass beads, mixed with glycerol (to 10% final concentration), sodium dodecyl sulfate (to 2%), dithiothreitol (to 0.1 M), and bromophenol blue (to 0.001%), and boiled for 5 min. Portions were stored at −80°C and reboiled immediately before loading the gel. Gel electrophoresis and Western blotting were performed as described by Fridovich-Keil et al. (5).

Growth rate analysis. Cultures were inoculated with logarithmically growing cells. Aliquots were quantitated during growth by measuring the optical density at 600 nm. Alternatively, formaldehyde-fixed cells were washed, lightly sonicated, and counted on either a Coulter counter or a hemacytometer. At the end of each growth curve, samples were examined by immunofluorescence to confirm their β-tubulin phenotype.

RESULTS

Construction of two truncated yeast β-tubulins. To construct *tub2-590*, we removed the last 12 codons of *TUB2* by oligonucleotide-directed deletion, as described in Materials and Methods. The product of the deletion reaction was confirmed by sequencing. The truncated sequence was then subcloned into a yeast vector to create the construct used for

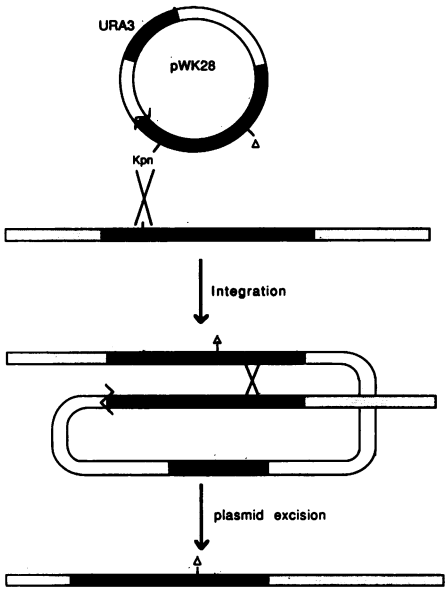


FIG. 1. Chromosomal integration of *tub2-590* and excision of vector DNA. Heavy shading indicates the *tub2* insert on the plasmid and the homologous chromosomal sequence, while light shading indicates the flanking chromosomal sequence. Unshaded regions indicate sequence derived from pBR322, and the *URA3* sequence is shown in black. Δ, Site of the deletion. A jagged line indicates the 5′ deletion of the *tub2* insert on the plasmid.

yeast transformation (Fig. 1). The vector has no replication origin, so it must undergo chromosomal integration to be maintained in the yeast cell. It carries the *URA3* gene as a selectable marker and codons 4 to 445 of *TUB2*. As diagrammed in Fig. 1, chromosomal integration at the *TUB2* locus produced a truncated β-tubulin gene expressed from the endogenous promoter. This β-tubulin allele was designated *tub2-590*. All coding and flanking sequences were identical to those of the wild-type gene except for the 12-codon deletion. Codons 1 to 4 and all of the 5′ noncoding sequence were deleted from the vector-borne copy of *TUB2*, so that only one β-tubulin gene was expressed, since expression of two copies of *TUB2* can be toxic to haploid cells (unpublished observations).

To construct *tub2-591*, we disrupted the *TUB2* gene at a *Sal*I site in codon 436, inserting the *Xho*I-*Sal*I *LEU2* fragment from YEp13. The cohesive ends of *Sal*I and *Xho*I are complementary; the sequence resulting from their ligation encodes four amino acids, followed by a nonsense codon, approximately 640 bp of *LEU2* 5′-flanking sequence, and the remainder of the *LEU2* gene (1). The ligation destroys both endonuclease recognition sites at the *Sal-Xho* junction. The disrupted sequence was then excised as an *Xho*I-*Sph*I fragment to produce the fragment used for yeast transformation. Homologous recombination at the *TUB2* locus (17) replaced the wild-type gene with the *tub2-591* allele, closely linked to *LEU2*, as illustrated in Fig. 2.

***tub2-590* transformants contain truncated β-tubulin.** We transformed FSY117, a haploid strain, directing integration to the *TUB2* locus by linearizing the vector at a *Kpn*I site in the amino-terminal region of *TUB2* (16). Transformants were selected by their ability to grow on medium lacking uracil. Both the *tub2-590*-bearing plasmid and the control plasmid bearing the wild-type carboxyl terminus gave normal efficiencies of transformation. We examined transformants by

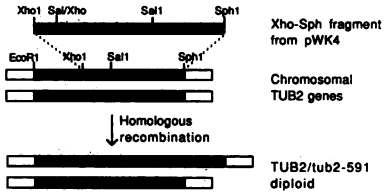


FIG. 2. Chromosomal integration of *tub2-591*. Heavy shading indicates the *tub2* sequence on the transforming fragment and the chromosomal *TUB2* sequence, while light shading indicates the flanking chromosomal sequence. The *LEU2* insert is shown in black.

immunofluorescence, screening for absence of staining by a polyclonal antiserum that specifically recognizes the carboxyl terminus of wild-type yeast β-tubulin (3). This antiserum was raised against a synthetic peptide corresponding to amino acids 446 to 457, the 12 amino acids that are removed by the *tub2-590* deletion. Five of 11 transformants screened showed few or no cells stained by the antiserum. A possible cause of the mixed populations of stained and unstained cells is restoration of expression of the wild-type β-tubulin in some cells, by events such as plasmid loopout or gene conversion. To obtain a pure, stable population of cells that expressed the truncated gene and contained no other *TUB2* sequence, we plated the five candidate strains on 5-fluoroorotic acid. By killing cells that express the vector-borne *URA3* gene, this drug allowed us to select cells that have lost the vector (2). Depending on the site of recombination, excision of the vector can leave either the wild-type or the truncated gene. When recombination occurred downstream from the deletion, the vector-borne copy of *TUB2* was lost, leaving no possibility of reversion to the wild-type carboxyl terminus (Fig. 1). Twelve of 20 strains obtained by this selection and screened by immunofluorescence showed no staining by the anti-wild-type antiserum. We further characterized four of these strains, FSY121, -122, -123 and -124, descended from four independent transformants, and strains derived from them. When backcrossed with wild-type cells, strains FSY121, -123, and -124 gave diploids that sporulated normally. FSY122, when mated with the wild type, gave diploids that failed to sporulate. This strain was not studied further. FSY125 is a control strain transformed with pRB429, which has the wild-type carboxyl terminus, and carried through the same selections as the experimental strains.

We constructed *TUB2/tub2-591* strains by transforming the wild-type diploid with the *Xho*I-*Sph*I replacement fragment from pWK4. Homologous recombination at the *TUB2* locus gave the diploid shown in Fig. 2 (17). Transformants, selected by their ability to grow in the absence of added leucine, arose at normal frequency. They were characterized by determining the auxotrophies of haploid segregants obtained from sporulation and tetrad dissection. We have no assay for the *tub2-591* gene product.

Immunological identification of the *tub2-590* gene product. An antipeptide antiserum raised against amino acids 434 to 445, the predicted carboxy-terminal 12 amino acids of the truncated β-tubulin, provided a positive assay for the *tub2-590* strains. As expected, the anti-wild-type antiserum recognized only the wild-type gene product, both by immunofluorescence and on Western blots (Fig. 3 and 4). Surprisingly, the antiserum raised against the penultimate peptide recognized primarily the truncated protein and not the wild-type protein. Figure 3 shows immunofluorescent staining of haploid (FSY117 and -124) and diploid (FSY120

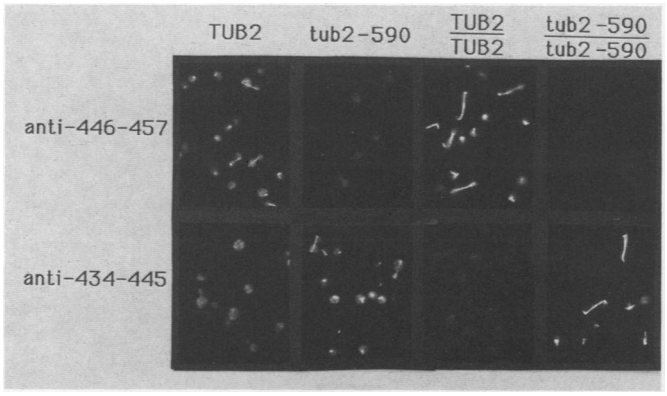


FIG. 3. Immunofluorescent staining of fixed cells. The strains shown in this figure are FSY117 (column 1), FSY124 (column 2), FSY120 (column 3), and FSY129 (column 4). Each row of four panels shows cells stained with the antibody indicated at left (against codons 446 to 457 or codons 434 to 445).

and -129) cells expressing either the wild-type or the truncated β-tubulin, fixed and stained with antibodies specific for each of the β-tubulins. Each strain was stained brightly by one antiserum or the other, but not by both. In some experiments, the antiserum raised against the penultimate peptide showed very faint staining on wild-type cells.

Figure 4 shows Western blots on protein from four diploid strains: FSY120 (*TUB2/TUB2*), FSY131 (*TUB2/tub2-591*), FSY141 (*TUB2/tub2-590*), and FSY129 (*tub2-590/tub2-590*). Each set of three lanes was loaded with 1, 2, or 4 μl of total cell extract from a given strain. On the blot probed with the anti-wild-type antiserum (Fig. 4A), a β-tubulin band was detected in protein from diploid strains that had one or two copies of the wild-type gene but not in protein from a strain homozygous for the truncated gene. In a parallel blot probed with the antiserum raised against the penultimate peptide, a tubulin band was detected only in the two strains containing the *tub2-590* allele (Fig. 4B). Another blot was probed with an antibody that recognizes histidinyl-tRNA synthetase (provided by I. Chiu), as a control for relative protein loads

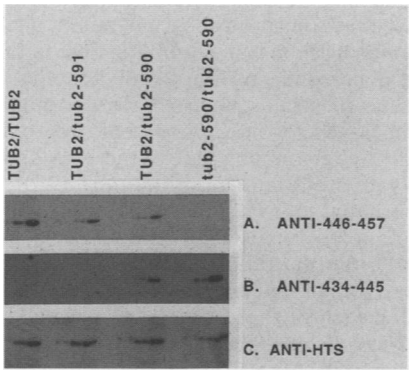


FIG. 4. Western blot analysis of whole-cell extracts from diploid cells. Each set of three lanes was loaded with 1, 2, and 4 μl of cell extract from the indicated strain. The strains analyzed were FSY120 (*TUB2/TUB2*), FSY284 (*TUB2/tub2-591*), FSY141 (*TUB2/tub2-590*), and FSY129 (*tub2-590/tub2-590*). The three panels show the tubulin region from three blots performed in parallel. (A) Probed with an antibody raised against amino acids 446 to 457 of TUB2; (B) probed with an antibody raised against amino acids 434 to 445 of TUB2; (C) probed with an antibody raised against histidinyl-tRNA synthetase (HTS).

TABLE 2. Doubling times of *tub2-590* haploids[a]

Strain	Allele	Doubling time (h)		
		25°C	37°C	11°C
FSY117	*TUB2*$^+$	2.4	3.0	16.6
FSY118	*TUB2*$^+$	2.7	3.4	18.2
FSY124	*tub2-590*	2.7	3.4	18.5
FSY128	*tub2-590*	3.1	3.9	21.0

[a] Doubling times were derived from plots of increase in cell number with respect to time, measured over three generations of logarithmic growth.

(Fig. 4C). These data confirm the immunofluorescence results, demonstrating the presence of the truncated tubulin and the absence of wild-type tubulin in the *tub2-590/tub2-590* strain. They also show that the levels of the wild-type and *tub2-590* proteins in the heterozygous diploids were about half of the levels observed in the homozygous diploids. That is, coexpression did not significantly affect the level of either gene product.

Growth characterization of the *tub2-590* haploid. Table 2 shows doubling times for wild-type and *tub2-590* haploid strains at the standard incubation temperature, 25°C, as well as at 11 and 37°C. The growth rates of the wild-type and *tub2-590* strains and one of the mutants were very similar, although the doubling time for another mutant was slightly greater. Repetitions of this experiment with other strains showed even smaller differences in growth rate. At the end of each growth curve experiment, cells from each culture were fixed and stained to confirm their β-tubulin phenotype. At the level of resolution afforded by immunofluorescence, microtubule structures in *tub2-590* cells grown at any of the above temperatures were indistinguishable from those of wild-type cells. Figure 3 shows representative fields of haploid and diploid cells grown at 25°C and stained with serum against wild-type or truncated β-tubulin. An analysis of mother-bud size ratio distributions showed that *tub2-590* and wild-type strains in logarithmic growth at 25 or 11°C had the same fractions of their populations in a given stage of the cell cycle, indicating that *tub2-590* cells are not compensating for a mitotic handicap by compressing other phases of the cell cycle (Fig. 5).

Mating and sporulation. We crossed FSY121 through FSY124 with FSY118, an isogenic wild-type strain. All four of these *tub2-590* haploids mated with qualitatively normal efficiency, yielding healthy diploids. FSY126, -127, and -128, *tub2-590* progeny of the first cross, also mated normally both

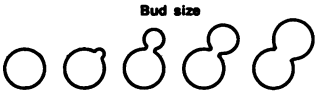
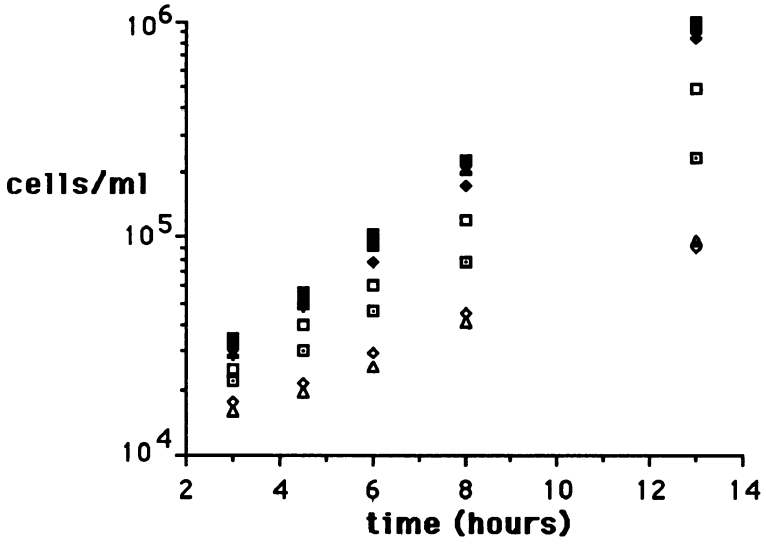
Strain	Temperature	Bud size					Doubling time (hours)
							
TUB2	26°	46%	21%	17%	12%	4%	1.8
tub2-590	26°	50%	16%	18%	12%	4%	1.8
TUB2	11°	68%	9%	10%	8%	5%	18
tub2-590	11°	68%	9%	11%	8%	4%	18

FIG. 5. Cell cycle distribution of haploid cells grown at 26 or 11°C. The strains analyzed were haploid progeny of a heterozygous diploid produced by mating FSY118 × FSY121. Their Tub2 phenotypes were determined by immunofluorescence. Bud size was determined from photographs of cells in logarithmic growth, visualized by phase-contrast microscopy. Each value is the average of values obtained for two strains.



TUB2 GENOTYPE	BENOMYL (ug/ml)	DOUBLING TIME (HR)
■ +/+	0	2.0
■ +/591		2.0
◆ +/590		2.1
▲ 590/590		2.0
□ +/+	7.5	2.3
⊡ +/591		2.9
◇ +/590		4.2
△ 590/590		3.7

FIG. 6. Growth of diploid cells in the presence and absence of benomyl. The strains shown in this figure are FSY120 (+/+), FSY284 (+/591), FSY141 (+/590), and FSY129 (590/590). Solid symbols indicate growth in drug-free medium; open symbols indicate growth in benomyl. At time zero, cells in logarithmic growth were inoculated into the indicated media. Samples taken at each time point were formaldehyde-fixed for later quantitation on a Coulter counter.

with wild-type cells and with each other. With one exception, the heterozygous and homozygous diploids yielded the same number of tetrads as wild-type diploids and produced spores of normal viability; the diploid arising from the cross FSY122 × FSY118 did not sporulate and was not studied further. Haploid progeny of *TUB2/tub2-590* heterozygotes showed 2:2 segregation of diagnostic antibody staining. Together with the normal viability of the segregants, this indicates that the *tub2-590* strains have not accumulated unlinked suppressors in order to survive.

All three *TUB2/tub2-591* diploids sporulated normally. Viability segregated 2:2 in the haploid progeny, and the *LEU2* marker segregated with the inviable spores. The inviable spores germinated but died after one to three divisions. This demonstrates that the *tub2-591* gene cannot support haploid growth.

Strains containing the mutated β-tubulin alleles are supersensitive to benomyl. A number of β-tubulin alleles confer supersensitivity to the microtubule-destabilizing drug benomyl (Huffaker et al., in press). We found that strains containing *tub2-590* were supersensitive to benomyl over a range of concentrations. Figure 6 shows the growth rates, in the presence or absence of benomyl (7.5 μg/ml), of diploid strains of each β-tubulin genotype. This concentration of benomyl had only a small effect on wild-type cells but a pronounced effect on strains bearing mutant alleles of *TUB2*. All four strains had the same doubling time in the absence of drug. In the presence of 7.5 μg of benomyl per ml, the doubling time of strains heterozygous or homozygous for *tub2-590* increased to the same extent, approximately twofold. Although the doubling time of the *TUB2/tub2-590* strain was slightly greater than that of the *tub2-590/tub2-590* strain in the experiment shown, in two repetitions of this experiment the doubling times of *TUB2/tub2-590* heterozygotes were slightly less than those of *tub2-590/tub2-590* homozygotes. The average values from three determinations were the same for each of these two β-tubulin genotypes, 3.9 h. *TUB2/tub2-591* strains were only slightly more sensitive to benomyl than wild-type strains. The same relative sensitivities were observed for a different set of strains of each genotype. At higher concentrations of benomyl, the doubling times of wild-type and mutant cells increased to the same extent. The Western blot data (Fig. 4) indicate that the difference in benomyl sensitivity between *TUB2/tub2-590* and *TUB2/tub2-591* strains was probably not simply a consequence of the level of the wild-type gene product, which was apparently the same in both heterozygotes. All four strains showed a variety of abnormal microtubule structures

after exposure to benomyl. We could detect no clear difference between strains in the types of abnormalities seen or their frequencies.

Benomyl supersensitivity (Ben^{ss}) segregated 2:2 in the progeny of *TUB2/tub2-590* heterozygotes. To confirm that benomyl sensitivity was a reliable marker for the *tub2-590* allele, we stained these segregants with the diagnostic antibodies. Of seven Ben^{ss} strains tested, seven were stained only by the antiserum that recognizes the truncated tubulin; five of five Ben^{+} strains tested were stained only by the antiserum that recognizes the wild-type tubulin.

DISCUSSION

Arguments for a role for the primary sequences of tubulins in specifying microtubule diversity arise from several considerations. One is the occurrence of divergent tubulins in situations where unique microtubule structures also occur. Testes in *Drosophila*, which produce sperm, and the erythropoietic lineage in chickens, which elaborates marginal bands, both contain unique β-tubulins (8, 14). Another consideration is the striking pattern of homologies among β-tubulin sequences, highly conserved over large regions, yet quite divergent in the 3′ ends. Moreover, the divergence at the 3′ ends fits a pattern: distinct tubulins from several different species are homologous in this region (22). An in vitro binding experiment suggests that the carboxyl terminus of β-tubulin may have enhanced affinity for microtubule-associated proteins (11). However, the simple predictions of this model are apparently confounded. There is clear evidence that divergent β-tubulins can occur in a wide variety of structures, both specialized and ordinary (6–8, 10, 12, 14, 23).

Gene transfection experiments permit a more direct test of the functional limitations implicit for β-tubulins of a particular primary sequence. Those experiments fail to identify any restrictions on β-tubulin activity in animal cells. For example, a chimeric β-tubulin gene, the carboxy-terminal 25% of which is encoded by the highly divergent carboxy-terminal domain of the yeast β-tubulin gene, assembles into all microtubules of an animal cell with an efficiency indistinguishable from that of endogenous tubulins (3). A similar result is obtained when full-length divergent animal β-tubulin genes are introduced into animal cells in which they are not ordinarily found: no segregation of these foreign gene products is detectable (7, 10).

However, these experiments in animal cells only demonstrate that the foreign β-tubulins can coassemble with the endogenous complement of tubulins normally expressed. They do not show that these distinct tubulins are sufficient to perform all the functions of the host cell microtubules. To test functional sufficiency rather than compatibility, these experiments have to be performed in cells where expression of the endogenous tubulins can be eliminated.

The molecular genetics of *S. cerevisiae* offer the opportunity to perform such an experiment. In addition, *S. cerevisiae* contains one of the most divergent β-tubulins yet identified. The *TUB2* gene encodes a protein that is 12 amino acids longer than most β-tubulins, although it contains regions highly homologous to other β-tubulins. Together, these features of the yeast system allow us to assay the precise functional roles of the conserved and divergent regions of *TUB2*.

The results described in this paper demonstrate that haploid cells containing only the truncated allele *tub2-590* are in most respects indistinguishable from strains containing the wild-type gene. By the criteria of growth rate, sensitivity to low and high temperature, mating and sporulation, and microtubule organization, the loss of the terminal 12 amino acids has no impact on any of a series of known microtubule functions.

This outcome is a rare example of a negative result that is more readily interpretable than a positive one. In this case, we can conclude that the carboxy-terminal tail is not essential for microtubule function. In the case of the *tub2-591* allele, which proved insufficient for normal growth, we are not able to distinguish whether that domain is directly involved in an essential interaction or simply plays a role in maintaining the proper configuration of the rest of the molecule. The heterozygous *TUB2/tub2-591* diploid used in this study (Fig. 2) contains a *tub2* gene in which a fragment containing the *LEU2* gene was inserted after codon 435. The haploid progeny of this diploid segregated 2:2 with respect to viability. However, without characterization of the product of this gene, an unambiguous interpretation of this experiment is not possible.

The one phenotype of the *tub2-590* allele which we can detect is hypersensitivity to benomyl. Over a range of concentrations of this microtubule-depolymerizing drug, *TUB2/tub2-590* heterozygotes, as well as diploids homozygous for *tub2-590*, showed the same slower growth in the presence of the drug. The dominant effect could reflect destabilization of microtubules containing the truncated β-tubulin. Alternatively, the *tub2-590* protein could have an enhanced affinity for the drug, which either causes destabilization of the copolymer or poisons assembly. The experiments to date cannot distinguish between these possibilities, and indeed it would be difficult to do so without an in vitro system suitable for analyzing the properties of yeast microtubules.

The present analysis was preceded by two other studies of the functional implications of tubulin primary sequences in *S. cerevisiae*, analyzing the structure and function of the two α-tubulin genes. Those two genes, *TUB1* and *TUB3*, are 90% homologous; that is similar to the degree of homology found among many α-tubulins. They appear to differ quantitatively, however. At sufficient levels of expression, the two gene products are functionally interchangeable (19, 20). We also noted that these two α-tubulin proteins are identical at a region in their amino termini which is the site of significant divergence among some α-tubulins. Therefore, it was possible that their functional equivalence was due to tight conservation at this crucial region. However, the precise sequence in that region is not essential for function, since we were able to produce linker insertions of up to 17 amino acids without disrupting activity (18). Here we have shown that the most striking feature of the divergent carboxy-terminal domain of the yeast β-tubulin is also functionally silent. Each of these experiments represents stringent in vivo tests. If there is a role for the blocks of extraordinary divergence among tubulins, it is not essential for any known microtubule function in yeast cells and will be manifest at a more subtle level than those we have tested. Perhaps similar gene replacement experiments in metazoa, which express a greater diversity of microtubule organelles, will reveal these roles.

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