Abstract. β-tubulin of budding yeast *Saccharomyces cerevisiae* is a polypeptide of 457 amino acids encoded by the unique gene TUB2. We investigated the function of the carboxy-terminal part of yeast β-tubulin corresponding to the carboxy-terminal variable domain of mammalian and avian β-tubulins. The GAA codon for Glu-431 of TUB2 was altered to TAA termination codon by using in vitro site-directed mutagenesis so that the 27-amino acid residues of the carboxyl terminus was truncated when expressed. The mutated TUB2 gene (tub2(T430)) was introduced into a haploid strain in which the original TUB2 gene had been disrupted. The tub2(T430) haploid strain grows normally <30°C but not at 37°C. The truncation of the carboxyl terminus caused hypersensitivity to antimitotic drugs and low spore viability at the permissive temperature for vegetative growth. Immunofluorescence labeling with antitubulin antibody and DNA staining with 4',6-diamidino-2-phenylindole showed that in these cells at 37°C, formation of spindle microtubules and nuclear division was inhibited and cytoplasmic microtubule distribution was aberrant. These results suggest that functions of the carboxy-terminal domain of yeast β-tubulin are necessary for cells growing under suboptimal growth conditions although it is not essential for growth under the optimal growth conditions. Cells bearing tub2(411), a tub2 gene in which the GAA codon for Glu-412 was altered to TAA were no more viable at any temperature. In addition, a haploid strain carrying two functional β-tubulin genes is not viable.

**Materials and Methods**

**Strains**

*Escherichia coli* strain HB101 was used for construction of yeast integrating vectors. JM107 was used as a host strain of bacteriophage M13mp18 and its derivatives. Table I shows yeast strains used in this study.
DNA Manipulation and Genetic Analysis

Isolation and manipulation of plasmid DNA and Southern blot analysis are as described in Maniatis et al. (1982). Yeast media and procedures for mating, sporulation, and tetrad analysis were described previously (Sherman et al., 1981). Yeast genomic DNA was prepared according to the method of Hereford et al. (1979). Transformation of yeast cells was performed by the lithium acetate method (Io et al., 1983).

Oligonucleotide-directed Mutagenesis

The plasmid pRB168 that contained 4.3-kb Bam HI fragment of yeast genome including the entire yeast α-tubulin gene, TUB2 (Neff, et al., 1983), was a gift from Dr. D. Botstein (Massachusetts Institute of Technology). There is a Bam HI site within the coding sequence of α-tubulin corresponding to the amino acid residue, 344. pRB168 was digested with Bam HI to yield the 2.2-kb Bam HI fragment containing the 3’ half of α-tubulin sequence. The fragment was inserted into M13 mpl8 (Yanisch-Perron et al., 1985), yielding mpl8-457. Point mutations were introduced in single-strand DNA of mpl8-457 according to the method of Zoller and Smith (1984) using chemically synthesized oligonucleotides. Oligonucleotides 5′-TCT GAG GCT TAA TCT AT-3′ and 5′-GCT ACT GTA TAA GAT GAT GA-3′ were used for mutations of the codons 412 and 431 to termination codon, UAA. The receptors were selected by hybridization with the oligonucleotides and confirmed by sequencing by the dideoxy-chain-termination method (Sanger et al., 1977). The mutants isolated were designated mpl8-411 and mpl8-430, respectively.

Construction of Yeast Strains Carrying Mutant TUB2 Genes

Disruption of a TUB2 gene of diploid strain: the Kpn I-Bam HI fragment (607 bp) of the β-tubulin gene (codon 142-344) from plasmid pRB168 was cloned into plasmid pUC18 (Yanisch-Perron et al., 1985). The Bam HI fragment of LEU2 gene (2.2 kb) was then inserted as a selectable marker into a Bam HI site of the recombinant plasmid. The resulting plasmid pLT-KB was linearized with Bgl II which produced a single cut in the inserted β-tubulin sequence of the plasmid and used to transform a diploid strain, YK21.

Leu* transformants to which pLT-KB was correctly integrated at the TUB2 locus were selected by Southern blot analysis of their genomic DNAs and confirmed by tetrad analysis. In accord with the previous report (Neff et al., 1983), the disruption of the TUB2 gene resulted in a recessive lethal mutation. The transformed strain yielded in this study was designated YK21LT.

The Hind III fragment of the URA3 gene (1.170 bp) was inserted into plasmid pUC18. Into this plasmid was inserted each of the Bam HI-Sph I fragments of the three M13 phage DNAs, mpl8-411, mpl8-430, and mpl8-457. Finally, the 2.2-kbp Bam HI fragment of TUB2, which contained the 5′end half of the tubulin gene, was inserted into the Bam HI site of the above three recombinant plasmids to reconstitute the entire α-tubulin gene sequence in each of the plasmids. The resulting plasmids were designated pUT411, pUT430, and pUT457, respectively.

YK21LT cells were transformed with the three plasmid DNAs that had been linearized at a unique Sma I site within the URA3 sequence. Ura* transformants to which the plasmid DNAs were correctly integrated at the URA3 locus were selected by Southern blot analysis of their genomic DNAs.

Copolymerization of Yeast Tubulin with Porcine Brain Tubulin

Four haploid strains, SM201, YKTL411-6-d, YKTL430-5-10a, and YKTL457-4-9a, and a diploid strain, YKTL430-5, were examined. Cells of each strain were grown in 10 ml SD + Trp, Leu, Ura, and Ade, his medium at 30°C to 1 x 10⁷ cells/ml. After diluting to 5 × 10⁶ cells/ml, 1.5 x 10⁷ cells of each strain were labeled with 170 IxCi of [35S]methionine (Amerham Corp.) at 30°C for 3 h with shaking. Harvested cells were washed with 2 ml of 0.1 M Pipes (pH 6.9), 1 mM MgCl₂, 1 mM GTP, 1 mM dithiothreitol (DTT), 10 μg/ml Leupeptin, and 10 μg/ml aprotinin (buffer A), and resuspended in 150 μl buffer A, after which the cells were treated with 0.3 mg/ml zymolyase at 30°C for 30 min. The cells were then broken by vortexing with glass beads, and extracted with 100 μl buffer A. Clear supernatants were obtained from the extracts by centrifugation at 50,000 rpm for 20 min in an ultracentrifuge (model TL100; Beckman Instruments, Inc., Palo Alto, CA), and were mixed with an equal volume of 10 mg/ml purified porcine brain tubulin, a gift from Drs. H. Sakai and E. Nishida (University of Tokyo). After EGTA was added to the solutions of 1 mM, three cycles of temperature-dependent assembly (at 37°C) and disassembly (at 0°C) were carried out. Proteins copolymerized with porcine brain tubulin were resolved in two-dimensional PAGE (O'Farrell, 1975).

Immunofluorescence Microscopy

Immunofluorescence staining of yeast cells were performed according to the method described by Kilmartin and Adams (1984). A rat monoclonal antibody directed against yeast α-tubulin, MAS 077 (YL1/2)(Sera-lab), was used as the first antibody for the indirect immunofluorescence staining. FITC-labeled rabbit anti-rat IgG was used as the second antibody. Fluorescently labeled cells were observed with an Olympus fluorescence microscope BHS-RFK.
Figure 1. Carboxy-terminal amino acid sequences for yeast β-tubulin and mammalian major constitutive β-tubulin. Homologous sequences between yeast β-tubulin (Neff et al., 1983) and mammalian constitutive β-tubulin (Wang et al., 1986; Cleveland, 1987) are boxed. Isotype-specific carboxy-terminal sequences for mammalian and avian β-tubulin start at the 430th-amino acid residue (Cleveland, 1987). Glu-412 and Glu-431 indicated by arrows are the sites where termination codon was introduced.

Flow Cytofluorometry

The methods for labeling of yeast cells with propidium iodide were as described (Hutter and Eipel, 1979). Labeled cells were analyzed with a EPICS-CS (Coulter Electronics, Inc., Hialeah, FL).

Results

Construction of Mutants Expressing Truncated Yeast β-Tubulin

β-tubulin of budding yeast S. cerevisiae is a polypeptide of 457 amino acids and encoded by the TUB2 gene mapped on chromosome VI (Neff et al., 1983; Gallwitz et al., 1983). The carboxy-terminal part of yeast β-tubulin corresponding to the carboxy-terminal variable domain of mammalian and avian β-tubulins starts at Val-430 through Glu-457 (Fig. 1). The GAA codon for Glu-431 was altered to TAA termination codon by using techniques of in vitro site-directed mutagenesis (Zoller and Smith, 1984).

The strategy for constructing a haploid strain bearing the mutated β-tubulin gene is shown in Fig. 2. Using a diploid strain, one of the paired TUB2 genes was disrupted by integration of a plasmid pLT-KB. A plasmid composed of the mutagenized TUB2, designated tub2(T430), and URA3 was transfected to the diploid strain at ura3 on chromosome V, yielding a diploid transformant (YKLT430-5) of TUB2/tub2-(disrupted) on chromosome VI and tub2(T430)/- on chromosome V (see Fig. 2). A haploid strain bearing tub2(disrupted) and tub2(T430) could be identified as LEU2 URA3 haploids recovered by sporulation of strain YKLT430-5 (Fig. 2).

As a control, the GAA codon for Glu-412 was altered to TAA so that the carboxyl terminus of β-tubulin was truncated within the region corresponding to the constant domain of mammalian and avian β-tubulins (Figs. 1 and 2). This truncated tub2(T411) was introduced into chromosome V of the above TUB2/tub2(disrupted) diploid strain, yielding YKLT411-6. We had expected that a haploid strain in which TUB2 is replaced by tub2(T411) could not be viable. As another control, the complete TUB2 gene was introduced to chromosome V in the same way (YKLT457-4).

YKLT457-4, YKLT430-5, and YKLT411-6 cells were separately induced to sporulate and subjected to tetrad analysis (Table II). From YKLT457-4, both LEU2 URA3/tub2(disrupted)/TUB2(T457) and leu2 ura3 were recovered by sporulation whereas LEU2 ura3 or leu2 URA3/TUB2(TUB2(457)) were not. Unexpected was the lethality associated with leu2

Figure 2. Strategy for expression of truncated yeast β-tubulin in haploid strains. A diploid strain YK21 was transformed with a plasmid pLT-KB linearized within the TUB2 coding region to disrupt a copy of the wild-type TUB2. The resultant YK21LT was transformed with a plasmid pUT430 containing the mutagenized tub2 gene that was linearized in URA3 marker. The resulting diploid strain YK21LT430(YKLT430) was induced to sporulate to obtain haploid strains bearing only the truncated β-tubulin gene. Painted boxes in the plasmids represent TUB2 protein coding sequence and thick lines represent its franking sequences. White boxes represent marker genes. Thin lines represent the vector pUC18 sequence. Restriction enzyme recognition sites are indicated for Bgl II (Bg), Bam HI (B), Kpn I (K), Sph I (Sp), Stu I (St), and Hind III (H).
Table II. Tetrad Analysis of Diploid Transformants Carrying Mutated TUB2 Gene

| Diploid transformant | Spore phenotype | Spore Viability |
|---------------------|----------------|----------------|
|                     | Leu/Ura*       | Viable | Nonviable |
| YKLT457-4           | +/− tub2       | 0 11   |           |
|                     | −/− TUB2       | 9 0    |           |
|                     | +/− tub2/TUB2(T457) | 9 0 | |
|                     | −/− TUB2/TUB2(T457) | 0 11 | |
| YKLT430-5           | +/− tub2       | 0 11   |           |
|                     | −/− TUB2       | 9 0    |           |
|                     | +/− tub2/tub2(T430) | 9 0 | |
|                     | −/− TUB2/tub2(T430) | 0 11 | |
| YKLT411-6           | +/− tub2       | 0 10   |           |
|                     | −/− TUB2       | 8 0    |           |
|                     | +/− tub2/tub2(T411) | 0 8 | |
|                     | −/− TUB2/tub2(T411) | 10 0 | |

* Leu + indicates that TUB2 gene is disrupted with LEU2 fragment. Ura + indicates that the mutagenized TUB2 or tub2 gene exist in ura3 locus. See text.

**URA3**, which indicates that a haploid bearing two functional TUB2 genes could not survive. A haploid strain showing phenotype of LEU + URA +, YKLT457-4-9a (tub2(disrupted)-TUB2(T457)) was isolated and used for further study. From YKLT430-5, both LEU2 URA3 and leu2 ura3 were recovered, suggesting that the product of tub2(T430) is functional. A haploid strain, YKLT430-5-10a (tub2(disrupted)TUB2(T430)), was thus isolated. In addition, no recovery of leu2 URA3 haploid indicates that a haploid bearing one TUB2 and one tub2(T430) did not survive. tub2(T430) was thus functionally equivalent to TUB2 in this regard too. Tetrad analysis of YKLT411-6 clearly indicated that the product of tub2(T411) is not functional.

**Evidence for Expression of Truncated β-Tubulin**

The above results indicate that β-tubulin devoid of the variable domain, designated βT430, is functional for cell growth. If this is indeed the case, then the above haploid strain with the genotype LEU2 URA3 and leu2 ura3 must express only the truncated β-tubulin. To investigate this point, cells were labeled with [35S]methionine at 30°C for 3 h. A clear supernatant fraction of the cell lysate (1.3 x 10⁶ cpm/μl) was mixed with an equal volume of 10 mg/ml of purified porcine brain tubulin in polymerizing buffer. The mixture was incubated at 37°C for 1 h to allow polymerization, after which polymerized tubulin was pelleted down. An aliquot of the pellet was resolved by two-dimensional PAGE (IEF/SDS-PAGE; O'Farrell, 1975). Two α-tubulin spots and one β-tubulin spot (pI = 5.03–5.07; Mr = 51,000) were detected in a wild-type (data not shown) and haploid strain expressing βT457 (Fig. 3 a). We have detected three tubulin-like spots in cell extracts of a haploid expressing tub2(T430), two of which were identified to be α-tubulin according to their position (Kilmartin, 1981). The third spot indicated by an arrowhead in Fig. 3 b (pI = 5.33–5.37; Mr = 48,000), together with the β-tubulin spot in Fig. 3 a, were cut out from the dried gel and subjected to peptide mapping by partial proteolysis with Staphylococcus aureus V8 protease (Fig. 4). The peptide mapping result clearly indicated that the third spot is a β-tubulin-related protein, βT430. The positional shift revealed by βT430 on IEF/SDS-PAGE could be accounted for by the truncation. This result clearly indicated that read-through of the 431st TAA codon did not occur in this strain. Both normal β-tubulin and βT430 were expressed in a heterologous diploid strain of TUB2/tub2(T430) (Fig. 3 c). The spot of normal β-tubulin from the diploid cells appeared to be less dense than the spot of βT430 (Fig. 3). The reason for this is unclear at present. These results indicate that βT430 is the only β-tubulin expressed in the haploid strain containing tub2(T430) and is not different from normal β-tubulin in the copolymerizability with porcine brain tubulin.
Figure 4. Peptide mapping of (a) the β-tubulin and (b) the truncated β-tubulin, β-T430. The spot of normal β-tubulin (Fig. 3 a) and that of the truncated β-tubulin (Fig. 3 b) were cut out from the gels and subjected to peptide mapping with *S. aureus* V8 protease (25 ng in lane a and 5 ng in lane b) according to the method of Cleveland et al. (1977). An arrow indicates the location of undigested β-tubulin.

lin (Fig. 3, a vs. b, and c). In extracts from heterologous diploid cells of *TUB2*/tub2 (*T411*), only normal β-tubulin was detected by copolymerization with porcine brain tubulin (data not shown).

**Characterization of the Haploid Strain Expressing Truncated β-Tubulin**

In all the above tests for viability and growth, cells were incubated at 30°C. Haploid (YKLT430-5-10a) cells expressing only βT430 grew normally at 25 or 30°C. However, we have unexpectedly found that YKLT430-5-10a cells did not grow continuously at 37°C. As can be seen in Fig. 5 A, these cells stopped growth upon shift-up of the temperature from 30 to 37°C. Both viable cell numbers as determined by colony formation (Fig. 5 A) and DNA synthesis (Fig. 5 C) continued to increase up to one generation time (2 h) after the shift-up but gradually ceased thereafter. On the other hand, protein synthesis continued at least 4 h after the shift-up (Fig. 5 E), although the colony forming ability began to decrease before the decrease in protein synthesis. Colony forming units decreased to one-tenth, 6 h after the shift-up (Fig. 5 A).

Judging from an increase in viable cell number as determined by colony forming ability, after the shift-up, 60-70% cells divided once at 37°C within 2 h. In addition, staining with 4',6'-diamidino-2-phenylindole (DAPI) of these cells revealed that cells containing dividing nuclei transiently increased to 40% 2 h after the shift-up (Fig. 6 and Table III). These results indicated that the high temperature allowed nuclear division during this period but greatly reduced its rate so that cells undergoing nuclear division were enriched. The majority of them completed nuclear division at 37°C within 4 h after the shift-up. However, bud formation was initiated before cell division, yielding a quadripartite form consisting of two large-budded cells, each of which contains only one nucleus. 40-60% of large-budded cells existed as quadripartite cells in this case. These quadripartite cells might have lost colony forming ability as shown in Fig. 5 A. Flow-cytomfluorometric analysis indicated that the amount of DNA contained in nucleus of such a large-budded cell was more than that contained in a cell of diploid strain (Fig. 7). Cells subjected to flow-cytomfluorometric analysis had been briefly sonicated and confirmed by microscopy to be mostly (>99%) single. These results indicated, therefore, that initiation of the second nuclear division after the shift-up was inhibited whereas new round of DNA replication unexpectedly continued. This is compatible with the result of [14C]uracil incorporation into DNA (Fig. 5 C). Without agitation, quadripartite cells frequently divided into two large-budded cells, suggesting that cell division was not strictly inhibited at

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1. **Abbreviation used in this paper**: DAPI, 4',6'-diamidino-2-phenylindole.
37°C. Probably for the same reason, large-budded cells divided, yielding "aploid" cells that lack a nucleus (Fig. 8, e and f). As described above, protein synthesis was not immediately inhibited at 37°C, giving rise to an enlargement of cell body. Long axes of these large budded cells were 1.3–1.8 times as long as those of growing large-budded cells at 30°C (Fig. 8).

**Microtubules of Cells Expressing Truncated β-Tubulin**

Since YKLT430-5-10a cells express β-tubulin lacking the carboxy-terminal 27 amino acids, it would be possible that abnormal properties associated with this strain at 37°C could be accounted for by a possible abnormality of microtubules consisting of βT430 as a unique β-tubulin component. We have examined this possibility by indirect immunofluorescence using tubulin-specific antibody. Cells of a wild-type strain grown separately at 30 and 37°C and YKLT430-5-10a cells grown at 30°C revealed normal distribution of microtubules (Fig. 8).

When YKLT430-5-10a cells, grown at 37°C, were processed for immunofluorescence staining, quadripartite cells were dissociated into two large-budded cells during digestion with zymolyase and β-glucuronidase. Staining with DAPI revealed that only one nucleus is present in one large-budded cell. Immunofluorescence results indicated that one large-budded YKLT430-5-10a cell contained one bright spot decorated by tubulin antibody from which cytoplasmic microtubules emanated and run into the large bud (Fig. 8 d).

**Table III. Effect of Temperature Shift-up on Nuclear Morphology of Yeast Cells Expressing Truncated β-Tubulin**

|                  | Cells containing dividing nuclei (incubation time after shift-up in h) |
|------------------|------------------------------------------------------------------------|
|                  | 0  | 1  | 2  | 3  | 4  |
| Haploid          |    |    |    |    |    |
| YKLT457-4-9a     | 5.5| 4.2| 5.1| 5.2| 5.8|
| YKLT430-5-10a    | 4.9| 9.5| 40.1|16.9|11.2|

Exponentially growing YKLT430-5-10a and YKLT457-4-9a cells were shifted to 37°C and incubated for the indicated periods. The cells were washed twice with 0.1 M phosphate buffer (pH 6.5) and fixed with 3.7% formaldehyde at room temperature for 1 h. The cells were stained with 0.2 μg/ml DAPI. The results were expressed as the percentage of cells containing dividing nuclei in total cells (>200).
majority of YKLT430-5-10a cells grown at 37°C were abnormal in their intracellular distribution. Some of the microtubules curved along the cell periphery and others were dispersed in the cytoplasm.

Altered Properties Revealed at 25°C by Cells Expressing the Truncated β-Tubulin

As described above, YKLT430-5-10a cells grow at 25°C at almost the same rate as wild-type or YKLT457-4-9a cells. However, YKLT430-5-10a cells were found to be much more sensitive at 25°C than wild-type or YKLT457-4-9a cells to antimitotic drugs, thiabendazole or methyl 2-benzimidazole-carbamate, when the sensitivities were determined on the plates containing these drugs (Table IV). YKLT430-5-10a cells were not cold sensitive.

tub2(T430) strains were able to mate at either 25 or 30°C. Resulting tub2(T430)/tub2(T430) homozygous diploid cells sporulated when incubated at 25 or 30°C in a sporulation medium. However, the frequency of incomplete tetrads, two or three spores per ascus, was found to be relatively high as compared to TUB2/TUB2 homozygous diploid cells (data not shown). Similarly, TUB2/tub2(T430) heterozygous diploid cells showed also this unusual spore formation (data not shown).

Discussion

In this study, we have shown that yeast cells that uniquely express a truncated β-tubulin lacking 27-amino acid residues at the carboxyl terminus (βT430) normally grow below 30 but not at 37°C. We constructed the above yeast strain by disrupting the TUB2 gene on chromosome VI and inserting a
mutagenized TUB2 in the URA3 locus on chromosome V. Cells having the complete TUB2 on chromosome V instead of the mutated TUB2 grow normally at 37°C, excluding the possibility that the abnormal properties associated with cells expressing βT430 is due to a possible positional effect of the tub2(T430) located on chromosome V. We thus conclude that the truncation of β-tubulin is the only cause that gives rise to the temperature-sensitive growth of cells expressing βT430.

Judging from the result of copolymerization of βT430 with an excess amount of purified porcine brain tubulin, βT430 is essentially polymerizable in vitro at 37°C. Fluorescence microscopic observations revealed the presence of abnormally aligned cytoplasmic microtubules in YKLT430-5-10a cells incubated at 37°C. Furthermore, electron microscopic observations suggested that individual microtubules of cells expressing βT430 were at least morphologically normal (unpublished observations). These results are consistent with previous results that β-tubulin whose carboxyl terminus was enzymatically removed is polymerizable (Serrano et al., 1984b; Bhattacharyya et al., 1985).

It should be noted, however, that microtubules of YKLT430-5-10a are somewhat functionally abnormal even at 25°C at YKLT430-5-10a cells were shown to be hypersensitive to antimitotic drugs. The defect associated with βT430 at 37°C was recessive to normal β-tubulin and βT430. Since the polymerizability of βT430 examined in vitro did not appear significantly lower than that of its normal counterpart, both normal β-tubulin and the truncated β-tubulin could be copolymerized into microtubules in the above diploid cells. The above fact clearly suggests that a decrease in the number of β-tubulin carboxyl termini to one-half per unit length of microtubule does not significantly affect cell growth. However, the frequency of spore formation of the above heterozygous (TUB2/tub2-1430) diploid cells was lower even at 25°C than that of homozygous (TUB2/TUB2) diploid cells. A clear interpretation of these results must await further investigations.

Phenotypes of the truncated β-tubulin gene tub2(T430) are similar in some properties to but different in other properties from two mutations related to microtubules that have been reported previously. The tub2-104 mutant, originally isolated as an antimitotic drug-resistant mutant, occurred in the TUB2 gene and displayed cold sensitivity for growth (Thomas et al., 1984). tub2-104 cells were synchronously arrested at the restrictive temperature within one cell-cycle time, yielding large-budded cells containing undivided and unelongated nuclear DNA (Thomas et al., 1984). Differently from the tub2(T430) mutation, microtubules largely disappeared at the restrictive temperature in tub2-104 cells. The ndcl-1 mutant originally isolated as a cold-sensitive mutant displayed a defect in nuclear division but allowed the cell cycle to progress for several generations at the restrictive temperature, yielding a number of "aploid" cells (Thomas et al., 1984). Being similar to the tub2(T430) mutation, ndc1-1 cells grown at the restrictive temperature have well-developed cytoplasmic microtubules (Thomas et al., 1984). A phenotypically similar mutant of β-tubulin to the tub2(T430) was also found with fission yeast Schizosaccharomyces pombe, which caused a defect in nuclear division at the restrictive temperature (Hiraoka et al., 1984). In addition, the kar1 mutants defined by mutations in a different locus from the three tubulin genes, is somewhat similar to the truncation of the carboxyl terminus of β-tubulin as both showed defect in nuclear division (Rose and Fink, 1987). Furthermore, the disruption of the TUB3 gene, one of the two α-tubulin genes of the yeast, caused hypersensitivity to benomyl and poor spore viability (Schatz et al., 1986b) both of which were shown for the tub2(T430) strain at the permissive temperature.

Nevertheless, the truncation of the carboxyl terminus of β-tubulin showed unique phenotypes. When exponentially growing, YKLT430-5-10a cells were shifted to the high temperature DNA synthesis followed by nuclear division appeared to be permitted for almost one cell-cycle round, although the rate of nuclear division was greatly reduced (Fig. 6). Though DNA synthesis continued, the second cycle of nuclear division was inhibited but bud formation occurred, yielding quadripartite cells. These results seem to be consistent with the idea that the initiation of nuclear division is restricted by the tub2(T430) mutation. In exponentially growing culture of YKLT430-5-10a cells, a certain population of unbudded cells and all small-budded cells may have been committed to nuclear division. Thus, the first round of nuclear division proceeded at the restrictive temperature but the second did not. The truncation reduced, in addition, the rate of nuclear division, suggesting that the carboxyl terminus of β-tubulin might function also during nuclear division. It was recently shown that perturbation of the diversified region near the amino terminus of yeast α-tubulin did not alter growth, mating, and meiosis of yeast cells (Schatz et al., 1987). Since the diversified region of α-tubulin differs from that of β-tubulin in their positions along the whole amino acid sequences, requirements of their functions for cellular events could differ from each other.

Apart from the issues raised by the βT430 mutation, two more interesting points related to the function of β-tubulin

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**Table IV. Sensitivity of Yeast Mutant Expressing Truncated β-Tubulin to Antimitotic Drugs***

| Strain      | 0 | 10 | 20 | 30 | 50 | 60 | 80 | 100 | 5  | 10 | 20 | 40 |
|-------------|---|----|----|----|----|----|----|-----|----|----|----|----|
| SM201       | + | +  | +  | +  | +  | +  | ±  | −   | +  | ±  | −  | −  |
| YKLT457-4-9a| + | +  | +  | +  | +  | +  | ±  | −   | +  | +  | ±  | −  |
| YKLT430-5-10a| + | +  | ±  | −  | −  | −  | −  | ±   | −  | ±  | −  | −  |

* Colony formation was examined on YPD plates containing thiabendazole or methyl 2-benzimidazolecarbamate at the indicated concentrations. + and − indicate that colonies appeared and did not appear after incubation at 25°C for 3 d, respectively. YPD plate contains 1% yeast extracts, 2% bacto-peptone, 2% glucose, and 2% Bacto Agar.

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**Notes:**

- The Journal of Cell Biology, Volume 107, 1988
- Table IV.
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- Mutagenized TUB2 in the URA3 locus on chromosome V.
- Cells having the complete TUB2 on chromosome V instead of the mutated TUB2 grow normally at 37°C, excluding the possibility that the abnormal properties associated with cells expressing βT430 is due to a possible positional effect of the tub2(T430) located on chromosome V. We thus conclude that the truncation of β-tubulin is the only cause that gives rise to the temperature-sensitive growth of cells expressing βT430.
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**Phenotypes of the truncated β-tubulin gene tub2(T430) are similar in some properties to but different in other properties from two mutations related to microtubules that have been reported previously. The tub2-104 mutant, originally isolated as an antimitotic drug-resistant mutant, occurred in the TUB2 gene and displayed cold sensitivity for growth (Thomas et al., 1984). tub2-104 cells were synchronously arrested at the restrictive temperature within one cell-cycle time, yielding large-budded cells containing undivided and unelongated nuclear DNA (Thomas et al., 1984). Differently from the tub2(T430) mutation, microtubules largely disappeared at the restrictive temperature in tub2-104 cells. The ndcl-1 mutant originally isolated as a cold-sensitive mutant displayed a defect in nuclear division but allowed the cell cycle to progress for several generations at the restrictive temperature, yielding a number of "aploid" cells (Thomas et al., 1984). Being similar to the tub2(T430) mutation, ndc1-1 cells grown at the restrictive temperature have well-developed cytoplasmic microtubules (Thomas et al., 1984). A phenotypically similar mutant of β-tubulin to the tub2(T430) was also found with fission yeast Schizosaccharomyces pombe, which caused a defect in nuclear division at the restrictive temperature (Hiraoka et al., 1984). In addition, the kar1 mutants defined by mutations in a different locus from the three tubulin genes, is somewhat similar to the truncation of the carboxyl terminus of β-tubulin as both showed defect in nuclear division (Rose and Fink, 1987). Furthermore, the disruption of the TUB3 gene, one of the two α-tubulin genes of the yeast, caused hypersensitivity to benomyl and poor spore viability (Schatz et al., 1986b) both of which were shown for the tub2(T430) strain at the permissive temperature.

Nevertheless, the truncation of the carboxyl terminus of β-tubulin showed unique phenotypes. When exponentially growing, YKLT430-5-10a cells were shifted to the high temperature DNA synthesis followed by nuclear division appeared to be permitted for almost one cell-cycle round, although the rate of nuclear division was greatly reduced (Fig. 6). Though DNA synthesis continued, the second cycle of nuclear division was inhibited but bud formation occurred, yielding quadripartite cells. These results seem to be consistent with the idea that the initiation of nuclear division is restricted by the tub2(T430) mutation. In exponentially growing culture of YKLT430-5-10a cells, a certain population of unbudded cells and all small-budded cells may have been committed to nuclear division. Thus, the first round of nuclear division proceeded at the restrictive temperature but the second did not. The truncation reduced, in addition, the rate of nuclear division, suggesting that the carboxyl terminus of β-tubulin might function also during nuclear division. It was recently shown that perturbation of the diversified region near the amino terminus of yeast α-tubulin did not alter growth, mating, and meiosis of yeast cells (Schatz et al., 1987). Since the diversified region of α-tubulin differs from that of β-tubulin in their positions along the whole amino acid sequences, requirements of their functions for cellular events could differ from each other.

Apart from the issues raised by the βT430 mutation, two more interesting points related to the function of β-tubulin...
emerged in this study. First, our results suggest that yeast β-tubulin lacking the carboxyl 46-amino acid residues (βT41) might not be polymerizable. Alternatively, βT41 may be unstable in cells, causing the lethality of the strain carrying tub2(41I). An application of immune precipitation with anti-β-tubulin antibody would be necessary to examine the problem as to whether βT41 is expressed as a stable and soluble protein in YKL741I (TUB2/tub2 (T41I)) cells. Second, interesting is also the finding that a haploid strain carrying two functional β-tubulin genes is not viable. As a haploid strain of TUB2tub2 (T430) is also not viable, βT430 is functionally equivalent to the complete β-tubulin also in this regard. The DNA fragment we used for construction of the mutants contained a copy of the complete YPTI gene (Gallwitz et al., 1983; Neff et al., 1983; Schmitt et al., 1986; Segev and Botstein, 1987; see Materials and Methods). However, this could not have affected the principal findings of this study because the complete TUB2 gene used for construction of YKL7457-4-9a strain in the control experiments also contained the YPTI gene. We suppose that the lethality of the TUB2TUB2 or TUB2tub2 (T430) haploid strain would be related to the previous result that cells that received a multicopy vector carrying the wild-type β-tubulin gene were not viable (Schatz et al., 1986b; Hiraoka et al., 1984).

This work was originally designed to analyze the function(s) the carboxyl-terminal variable domain of mammalian and avian β-tubulins has. Bond et al. (1986) have suggested by expressing a chicken-yeast chimeric β-tubulin in cultured cells that the artificially diversified carboxyl terminus of β-tubulin does not restrict the activities of microtubules in vivo. It was also shown that a chicken-yeast chimeric β-tubulin, in which 15-amino acid residues of the carboxy-terminal domain were deleted, is able to polymerize into microtubular structures in mouse 3T3 cells (Fridovich-Keil et al., 1987). However, these results do not exclude the possibility that the carboxyl terminus of β-tubulin might have specific functions in living animal cells because endogenous mouse β-tubulin is expressed in 3T3 cells in addition to the chimeric tubulins. The results of the present study clearly demonstrated that the carboxy-terminal part of yeast β-tubulin is not strictly required for the in vivo functions of microtubules under appropriate conditions. We have also demonstrated, however, that it is necessary for cells growing under suboptimal conditions such as at relatively high temperatures or for survival of cells exposed to antimicrobial drugs. It is possible that naturally diversified carboxyl termini of β-tubulins in higher vertebrates might differently affect functions and stability of microtubules under specific conditions.

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