

ISOLATION AND CHARACTERIZATION OF MUTATIONS IN THE β -TUBULIN GENE OF *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

Of 173 mutants of *Saccharomyces cerevisiae* resistant to the antimetabolic drug benomyl (Ben^R), six also conferred cold-sensitivity for growth and three others conferred temperature-sensitivity for growth in the absence of benomyl. All of the *ben*^R mutations tested, including the nine conditional-lethal mutations, were shown to be in the same gene. This gene, *TUB2*, has previously been molecularly cloned and identified as the yeast structural gene encoding β -tubulin. Four of the conditional-lethal alleles of *TUB2* were mapped to particular restriction fragments within the gene. One of these mutations was cloned and sequenced, revealing a single amino acid change, from arginine to histidine at amino acid position 241, which is responsible for both the Ben^R and the cold-sensitive lethal phenotypes. The terminal arrest morphology of conditional-lethal alleles of *TUB2* at their restrictive temperature showed a characteristic cell-division-cycle defect, suggesting a requirement for tubulin function primarily in mitosis during the vegetative growth cycle. The *TUB2* gene was genetically mapped to the distal left arm of chromosome VI, very near the actin gene, *ACT1*; no *CDC* (cell-division-cycle) loci have been mapped previously to this location. *TUB2* is thus the first cell-division-cycle gene known to encode a cytoskeletal protein that has been identified in *S. cerevisiae*.

MICROTUBULES are complex structures found in all eukaryotes and thought to function in a wide variety of cellular processes. These include the formation of the spindle on which chromosomes segregate during mitosis and meiosis. Microtubules are composed primarily of two polypeptide subunits, α - and β -tubulin, which form the heterodimer tubulin. The ability to polymerize microtubules *in vitro* has permitted study of their properties in pure form and an understanding of their structure and polymerization *in vitro* (SNYDER and MCINTOSH 1976; KIRSCHNER 1980). However, the relationship between these *in vitro* properties and the *in vivo* functions of microtubules has remained unclear, although interesting hypotheses have been suggested (for example, see MARGOLIS, WILSON and KIEFER 1978).

In budding yeast, *Saccharomyces cerevisiae*, there appears to be a single essential gene encoding β -tubulin. In a previous report (NEFF *et al.* 1983), we described the identification of this gene by molecular cloning using homology

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with a β -tubulin gene from chicken. DNA sequence analysis revealed more than 70% homology in amino acid sequence. Recent advances in the biochemistry of yeast microtubules indicate that they behave *in vitro* very similarly to those of mammals (KILMARTIN 1981). Thus, yeast presents an attractive opportunity for relating microtubule biochemistry *in vitro* to function of microtubule assemblies *in vivo* through the isolation and phenotypic characterization of mutations in the β -tubulin structural gene. One of the first questions that can be answered by such mutant isolations is whether conditional-lethal tubulin mutants, as might be expected, show a cell-cycle-specific arrest and thereby fall into the class of mutations used to genetically define the yeast cell cycle (see PRINGLE and HARTWELL 1981 for review).

The antimitotic drug benomyl is known to bind to tubulin and inhibit the growth of many lower eukaryotes, such as fungi (DAVIDSE and FLACH 1977). By selection of resistance to benomyl, mutations in tubulin have been isolated in *Aspergillus nidulans* (SHEIR-NEISS, LAI and MORRIS 1978; MORRIS, LAI and OAKLEY 1979). Here, we describe the isolation of benomyl-resistant and conditional-lethal mutations in the yeast gene encoding β -tubulin (*TUB2*), a fine structure physical map of the positions of four conditional-lethal alleles of *TUB2*, as well as the DNA sequence of one of them. In addition, we describe morphological features of arrest of such mutants at their restrictive temperature that show that *tub2* mutants are cell-division-cycle (*cdc*) mutants as defined by HARTWELL (1974). By mapping the *TUB2* gene very near to *ACT1* on chromosome VI, we show that *TUB2* is a previously undescribed cell-cycle gene, the first in *S. cerevisiae* for which there is a clear cytoskeletal basis for the cell-cycle-specific defect.

MATERIALS AND METHODS

Media and genetic analysis: Media, methods of mating, sporulation and tetrad analysis were as described by SHERMAN, FINK and LAWRENCE (1974).

Materials: Benomyl, 98.6%, was a generous gift from O. ZOEBISCH, E. I. Dupont de Nemours and Company, Inc., and was stored as an 8 mg/ml stock in dimethyl sulfoxide at -20° . The stock was added to warm medium, with vigorous swirling to prevent precipitation of the benomyl, just prior to pouring plates. Restriction enzymes, T4 DNA ligase, DNA polymerase I, Klenow fragment and other DNA modifying enzymes were purchased from New England Biolabs. Zymolyase 60,000 was purchased from Miles Laboratories, Inc., and 4'-6-diamidino-2-phenylindole (DAPI) was purchased from Accurate Chemical Company and was stored as a 1 mg/ml stock in sterile water at 4° in the dark. Purified α -factor was the generous gift of JEREMY THORNER.

E. coli and yeast transformation: *E. coli* transformation and preparation of plasmid DNA by equilibrium banding in CsCl, ethidium bromide was as described by DAVIS, BOTSTEIN and ROTH (1980). *E. coli* strains DB6329 and DB6507 were used throughout. (DB6329 is also known as HB101 and is *recA*; DB6507 carries a Tn5 insertion in the *pyrF* gene of DB6329.) The uracil auxotrophy conferred by this *pyrF* mutation can be complemented by the yeast *URA3* gene found on many yeast cloning vectors. Recombinant *E. coli* strains were propagated on LB medium (DAVIS, BOTSTEIN and ROTH 1980) containing 100 μ g/ml ampicillin (Bristol-Myers, Inc.).

Yeast transformation was by the spheroplasting method (HINNEN, HICKS and FINK 1978), except in the case of mapping *tub2* alleles using gapped plasmids, when the method of LiAc transformation (first described by ITO *et al.* 1983) was used. For LiAc transformation we used the protocol described by KUO and CAMPBELL (1983), except that the cells were washed once with water before plating. In some experiments, sterile glycerol was added to a concentration of 15% (v/v) to the

TABLE 1

Strain list

Number	Genotype	Source
DBY473	<i>MATα his4-619</i>	G. R. FINK
DBY640	<i>MATα ade2-1</i>	F. SHERMAN
DBY880	<i>MATα cdc6 his2 his6 his7 adel ade2 ade6 lys2 lys9 leu1 leu2 ura3 trp1 met2 met14 aro7 arg1 arg4 ilv3 asp5</i>	G. KAWASAKI
DBY945	<i>MATα lys2-803</i>	Our laboratory
DBY1034	<i>MATα his4-539 lys2-801 ura3-52</i>	Our laboratory
DBY1368	<i>MATα his4 tub2-150</i>	This study
DBY1369	<i>MATα his4 ura3 tub2-216</i>	This study
DBY1372	<i>MATα lys2 tub2-207</i>	This study
DBY1373	<i>MATα his4 ura3-52 tub2-104</i>	This study
DBY1374	<i>MATα his4 ura3-52</i>	This study
DBY1395	<i>MATα his4-539 ura3-52</i>	This study
DBY1384	<i>MATα his4 ura3-52 tub2-104</i>	This study

All strains are in the S288C genetic background (see text), with the exception of DBY880. The strains constructed for this study are either the products of standard crosses and tetrad analysis and/or mutant isolations described in the text.

competent cells just before addition of DNA and was frozen at -70° . Frozen competent cells were thawed slowly on ice for use, and the transformation protocol continued normally, without removing the glycerol. We found that cells could be frozen in this way for at least several weeks without significantly affecting their transformation efficiency or the outcome of the mapping experiments.

Yeast strains: All yeast strains used in obtaining, backcrossing and analyzing the mutants described here were derived from a set of essentially isogenic S288C strains provided by G. R. FINK. The *ura3-52* allele was backcrossed into this S288C background ten times by B. OSMOND and M. CARLSON (personal communication). Thus, all mutant strains and their parents are theoretically isogenic at all other loci. The strain DBY880, used to map *TUB2*, is not in S288C background, but was used only for mapping purposes. All yeast strains, their genotypes and sources are listed in Table 1.

Isolation of mutants: Cells of strain DBY640 or DBY945 were spread for single colonies on YEPD plates, and each single colony was resuspended in sterile water. About 10^6 cells from each resuspension were spread on a YEPD plate containing 40 μ g/ml benomyl. A single colony was isolated from each plate, purified by streaking on YEPD and tested for growth under a variety of conditions. This procedure ensured the isolation of independent spontaneous mutations.

Gel-electrophoresis and DNA manipulations: Restriction digests were performed according to the supplier's recommendations. Agarose gel electrophoresis of DNA fragments, gel-transfer hybridization experiments and other DNA manipulations were performed with minor modifications by the methods described by DAVIS, BOTSTEIN and ROTH (1980). DNA sequence analysis was performed as described by MAXAM and GILBERT (1980). Restriction fragments were end-labeled for sequence analysis at their 3' ends by filling 5' overhangs with the appropriate α - P^{32} -labeled nucleotide with the Klenow fragment of DNA polymerase I. Sequencing of the *tub2-104* allele was performed by end-labeling at the *Bam*HI or the *Xho*I sites in pJT71 and sequencing the entire region between them from each end.

Yeast DNA preparation: Small-scale yeast DNA preparations were made by the following method: yeast cells were grown to saturation in 5 ml of YEPD, centrifuged, resuspended in 1 ml of water, transferred to a 1.5-ml microfuge tube, centrifuged 5 sec in a microfuge, washed once and resuspended in 1 ml of 1 M Sorbitol, 50 mM sodium phosphate, 5 mM dithiothreitol, pH 7.5. Zymolase 60,000 was added to a final concentration of 100 μ g/ml, and the tube was incubated

for 60 min at 30° with gentle rocking. The resulting spheroplasts were centrifuged for 10 sec in a microfuge, and the pellet was resuspended gently in 0.5 ml of 50 mM EDTA, 0.3% SDS, pH 8.5. The tube was heated to 65° for 20 min, then 100 μ l of 5 M potassium acetate was added, chilled on ice for 20 min and centrifuged for 10 min in a microfuge at 4°. The supernatant was poured to a new tube, filled with room temperature 95% ethanol, mixed by inversion and immediately centrifuged at room temperature in a microfuge for 15 sec. The supernatant was poured off, the pellet was drained for 5 min and was then resuspended carefully in 300 μ l of 20 mM Tris, 2 mM EDTA, pH 8.0. Pancreatic RNase (DNase free) was added to a concentration of 20 μ g/ml and was incubated at 37° for 1 hr. Then, 200 μ l of 20% SDS and 167 μ l of isopropanol (room temperature) were added, mixed by inversion and incubated at room temperature for 5 min. The tube was centrifuged for 15 sec at room temperature in a microfuge, the supernatant was removed and the pellet was drained well and resuspended in 160 μ l of 20 mM Tris, 2 mM EDTA, pH 8.0. To this was added 40 μ l of 1 M NaCl, and the sample was extracted twice with buffer-saturated distilled phenol. To the remaining aqueous phase was added 100 μ l of water, 30 μ l of 3 M sodium acetate and 600 μ l of 95% ethanol at room temperature. This was incubated for 2 min at room temperature and was centrifuged for 15 sec in a microfuge. The supernatant was removed and the pellet was drained well and resuspended in 50 μ l of 10 mM Tris, 1 mM EDTA, pH 8.0.

This procedure yielded about 5 μ g of high molecular weight DNA, which was essentially RNA free, could be cut with restriction enzymes and was suitable for gel-transfer hybridization experiments. Yeast plasmid DNA is also purified by this technique and could be recovered in *E. coli* by transformation with 0.5–5% of the volume of such a preparation of yeast DNA, selecting ampicillin resistance. For recovery of integrated plasmids, the yeast DNA was cleaved with the appropriate restriction enzyme, phenol extracted and ethanol precipitated and ligated at a concentration of 0.5 μ g/ml overnight at 14° to favor intramolecular ligation products. The ligation mixture was either phenol extracted and ethanol precipitated or was directly used to transform *E. coli*, selecting ampicillin resistance. Many such integrated plasmid recoveries always gave the expected products when their structure was analyzed from plasmid DNA prepared from *E. coli*.

Generation of DNAs for fine-structure physical mapping: pJT71 was used for most of the mapping experiments. This plasmid contains an intact wild-type *TUB2* gene subcloned on an *EcoRI-SphI* fragment into the same sites on YIp5 (see Figure 2). All DNAs for transformation into yeast were generated by digestion with at least a ten-fold excess of the appropriate enzymes. The cut DNA was phenol extracted and ethanol precipitated twice before transformation of yeast and was run on an agarose gel to assess completeness of the restriction digests.

A large amount of each DNA (10 μ g) was generated at one time and was stored frozen at –20° in 10 mM Tris, 1 mM EDTA, pH 8.0. In this way, when a particular gapped DNA was used in a mapping experiment (usually 0.25 μ g/transformation) and a negative result was obtained with one *tub2* allele, this confirmed that the DNA had been cut to completion. Unique *SalI*, *KpnI*, *ClaI*, *XhoI*, *BamHI*, and *HindIII* sites lie within the *TUB2* gene in pJT71. These were used to generate seven of the 10 "deletion DNAs" illustrated in Figure 2. In addition, four *BglII* sites lie scattered throughout the *TUB2* insert on this plasmid. Cleavage to completion with *BglII* removed the entire functional *TUB2* gene from the plasmid backbone, leaving a few hundred bases of homology at each end of the *TUB2* insert to recombine with the yeast chromosome upon transformation. This gapped plasmid was used as a negative control to demonstrate that deletion of the entire gene results in failure to recover wild-type recombinants (deletion DNA no. 1 in Figure 2 and Table 3). pJT71 cut only once with *SalI* was used as a positive control, because none of the *TUB2* sequences were deleted (deletion DNA no. 2). Yeast transformations for these experiments were done with the mixture of fragments generated by restriction digest. Thus, the wild-type *TUB2* information is always present in each transformation; the only parameter that was altered was whether that sequence was covalently attached to the plasmid *URA3* gene, which was used to select transformants. Although theoretically the wild-type fragment may sometimes cotransform with *URA3* (even when not covalently linked), this event appears to be extremely rare (fewer than 1/1,000 of total transformants, based on the sum of all of our data).

Two of the deletions shown in Figure 2 (8 and 9) terminate at *BglII* sites that are not unique in the plasmid. These were generated by the following method: pJT71 was cleaved partially with *BglII* and was phenol extracted and ethanol precipitated. The 3' ends were filled with the Klenow

fragment of *E. coli* DNA polymerase I (ROSE and BOTSTEIN 1983), followed by phenol extraction and ethanol precipitation. Phosphorylated *Bam*HI linkers (10-mers, Collaborative Research) were ligated to the DNA (linker:pJT71 DNA molar ratio, about 50:1), excess linkers were removed by agarose gel electrophoresis, the ends were trimmed back to a single linker by cleavage with *Bam*HI and the molecules were ligated intramolecularly as described by ROSE and BOTSTEIN (1983). This procedure resulted in new plasmids that were fusions of the *TUB2 Bam*HI site to one of the four *Bgl*II sites, with a *Bam*HI linker at the site of fusion. Deletion DNAs 8 and 9 were generated from two such subclones by cleavage with *Bam*HI. The molecules generated in this fashion differ in two ways from the simple restriction cleavages described above (deletions 1–7): first, they do not contain the sequences in the gapped region (they are true deletions); second, they have at one end a very short region of nonhomology to the yeast chromosomal sequence at *TUB2* that is contributed by the linker insertion. Interestingly, this nonhomology appeared to have no effect on the frequency of yeast transformation or the mapping data derived from these subclones. Thus, it is likely that this mapping technique could be rendered more generally useful by the generation of such linker insertion/deletion constructs in a more random way, producing deletion intervals that do not depend on the fortuitous location of restriction sites.

Two-micron mapping: The 2 μ mapping method of FALCO and BOTSTEIN (1983) was used to map the *TUB2* locus. The plasmid pRB120 carries a fragment of yeast 2 μ DNA and thus can replicate autonomously in yeast. Integrants of this plasmid into the yeast chromosome were easily isolated since the *TUB2*-containing insert carried by pRB120 is lethal on a freely replicating 2 μ plasmid, presumably due to high copy number (data not shown). Thus, transformation of yeast selecting Ura⁺ yielded only stable integrants that had a copy number of one due to integration.

Identification of the auxotrophic mutant uncovered in the mapping experiment was complicated by the fact that there were three different heterozygous His⁻ auxotrophic markers in the diploids, marking three different chromosomes, *his2*, *his4* and *his7*. The identity of the mutation uncovered was established by "rare mating" the segregants to haploid tester strains carrying either *his2*, *his4* or *his7*. The rare-mating test was carried out for each diploid to be tested with all three testers (*his2*, *his4* and *his7*), and the results were always internally consistent.

Calculation of map distance from tetrad data was by the method of MORTIMER and SCHILD (1982). Correction for distortion at long distances was made from MORTIMER and SCHILD's plot of the equations derived by SNOW (1979).

Cell cycle arrest: Cells were grown to 2–5 $\times 10^8$ cells per milliliter under permissive conditions in liquid YEPD. The culture was shifted to the restrictive temperature by placement in a rotary water bath preequilibrated to the appropriate temperature. Microscopic observations were made following 5–10 sec of sonication to separate cells. Pilot experiments indicated that this amount of sonication is sufficient to separate cells without breaking a significant fraction of them. Observations of live or fixed cells gave the same results.

RESULTS

Isolation of benomyl resistant mutants: Yeast strains in the genetic background S288C were used throughout this work. These strains are resistant to benomyl up to a concentration of about 20 μ g/ml at 26° on YEPD medium, but are inhibited for growth at a concentration of 40 μ g/ml. Resistance shows strong dependence on growth temperature: at 37° wild type shows considerable resistance even to high levels of benomyl (40 μ g/ml), whereas at 17° wild type shows increased sensitivity, being inhibited by 5–10 μ g/ml benomyl. We isolated a set of 173 spontaneous independent mutants, some derived from DBY473 and others from DBY945 (Table 1), resistant to 40 μ g/ml benomyl (Ben^R) at 26° (see MATERIALS AND METHODS). Sixty-five of these strains were crossed to an appropriate wild-type S288C strain, and diploids were isolated to assess the recessiveness of the Ben^R phenotype. Fifteen mutants showed partial or complete dominance, and the remaining 50 showed complete or

nearly complete recessiveness for their drug-resistance phenotype. Six of the Ben^R mutants that showed no conditional-lethal phenotype (see below) were backcrossed to a wild-type strain and were shown to segregate 2:2 in tetrads for their Ben^R phenotype, indicating that a single nuclear lesion caused the Ben^R phenotype.

All 173 mutant strains were screened for secondary phenotypes acquired along with Ben^R. Eight mutants had become slow growing or unable to grow at 14° on YEPD; these will be referred to as cold-sensitive (Cs). Three other mutants failed to grow on YEPD at 37°; these will be referred to as temperature-sensitive (Ts). All of these strains could grow on YEPD at 26° without benomyl, as well as with 40 µg/ml benomyl. However, another mutant (150) grew very poorly at 26° without benomyl, and the addition of 40 µg/ml benomyl improved its growth rate at 26° (we call this phenotype benomyl-dependent, Ben^D). Mutant 150 will be discussed in more detail in a later section.

All of the conditional-lethal Ben^R strains were crossed to an appropriate wild-type S288C strain to assess the linkage of the Ben^R and the conditional-lethal phenotypes. The resulting diploids were sporulated and the tetrads were subjected to tetrad analysis. In all cases the conditional lethality and the Ben^R phenotypes each segregated 2:2, indicating each is caused by a single nuclear lesion. Of the eight Cs mutants, only six (mutants 104, 119, 120, 158, 209 and 214) showed tight linkage of their Cs phenotype to Ben^R; only these six mutants were studied further. For each of these, at least 16 tetrads were analyzed, and no recombinants separating the two phenotypes were found. Of the three Ts mutants, one showed no linkage of the Ts and Ben^R phenotypes and was not studied further. The other two Ts mutants (207 and 216) sporulated very poorly (see below for further analysis).

Complementation tests: Complementation tests for the benomyl-resistance phenotype were performed among a number of the recessive mutations. Three recessive *ben^R* mutations were derived in the opposite mating type by crossing with an appropriate *TUB2* strain. Each of these three derivatives was crossed with 26 other recessive Ben^R mutants (including 24 mutants that displayed no conditional lethality), and diploids from the crosses were isolated. All of these pairs failed to complement for the Ben^R phenotype, suggesting that all of these *ben^R* mutations lie in the same gene. Since, as shown below, one of these *ben^R* mutations (104) is a single base change in the coding sequence of *TUB2* (identified as the β -tubulin structural gene, NEFF *et al.* 1983) we henceforth will refer to these mutations as *tub2* mutations.

Complementation for cold-sensitivity among the six *ben^R* mutations that cause a Cs phenotype was also tested. All six mutations were recessive and failed to complement for Cs and for Ben^R, except for mutation 120, which appeared to give weak Cs complementation with all other Cs mutations and is dominant for the Ben^R phenotype. Mutation 120 is the leakiest of the Cs mutations for its cold-sensitivity, and therefore its complementation was difficult to assess. We demonstrated by tetrad analysis that this mutation is tightly linked to *tub2-104*. Therefore, we suspect that its weak complementation is a

TABLE 2

Relationship of temperature and benomyl to the growth of TUB 2 and several tub2 alleles

TUB2 genotype	Growth at indicated temperature in presence of 0 or 40 μ g/ml benomyl							
	26°		30°		33°		37°	
	0	40	0	40	0	40	0	40
<i>tub2-216</i> (Ts)	+	+	+	+	-	+	-	+
<i>tub2-207</i> (Ts)	+	+	+	+	+/-	+	-	+
<i>tub2-150</i> (Ts)	-/+	+	-	+	-	+	-	+
<i>tub2-104</i> (Cs)	+	+	+	+	+	+	+	+
<i>TUB2</i>	+	-	+	-	+	-/+	+	+/-

All tests are on rich medium (YEPD) plates with 40 μ g/ml benomyl or with the equivalent amount of dimethyl sulfoxide (solvent) containing no benomyl. Degree of growth was estimated from growth on plates. Growth was estimated by eye and is represented in order of decreasing growth rate: +, +/-, -/+, -.

manifestation of the leakiness of its Cs phenotype and that it actually lies in the *TUB2* gene as well.

We also attempted to assess complementation between mutant 150 (Ben^D) and *tub2-104* for their Ben^R phenotypes. Mutant 150 is partially dominant for its benomyl resistance at 26°, our standard test condition; however, mutant 150 was found to be completely recessive for Ben^R at 17°, as is *tub2-104*. At this temperature the two mutations failed to complement each other for Ben^R, indicating that both are in the same gene. Mapping data confirm this assignment (see below).

Since the mutation conferring benomyl resistance in mutant 150 (henceforth *tub2-150*) appears by this criterion to be in the gene defined by the other *tub2* alleles, we tested complementation between *tub2-150* and the other two Ts mutants (207 and 216, which had sporulated poorly). Both of these mutants were recessive and failed to complement *tub2-150* for temperature-sensitivity. Thus, both Ts mutations are also alleles of *TUB2*.

Benomyl dependent (Ben^D) phenotype: Strains carrying the *ben^D* mutation, *tub2-150*, showed an interaction between their Ben^D phenotype and growth temperature, which is summarized in Table 2. The growth pattern of strains carrying *tub2-150* is most easily described as a low-temperature spectrum Ts lethality that is rescued by benomyl. A strain carrying *tub2-150* was crossed with a *TUB2* strain to assess linkage of the Ben^D and Ts phenotypes. Although this diploid sporulated poorly and it proved very difficult to dissect tetrads, random spore analysis of the cross was performed. In 64 haploid spores that grew on benomyl, the Ts and Ben^D phenotypes always cosegregated. Linkage of *tub2-150* to *tub2-104* was also assessed by random spore analysis. When a strain carrying *tub2-150* was crossed to a strain carrying *tub2-104*, the resulting diploid was sporulated and haploid spores were tested, no benomyl-sensitive recombinants were found among 96 spore clones. Thus, *tub2-150* and *tub2-*

104 are tightly linked, and the Ben^P and Ts-lethal phenotypes of *tub2-150* appear to be due to a single mutant lesion.

Because strains carrying the mutation *tub2-150* showed an interaction between temperature and ability to grow in the absence of benomyl, it seemed possible that more detailed characterization of the other two candidates for Ts mutations in *TUB2* (mutations 207 and 216) might reveal a similar phenomenon. Therefore, we tested the effect of the presence or absence of 40 µg/ml benomyl at several temperatures on the growth of mutant strains carrying these alleles and compared them to *TUB2* strains. The results are shown in Table 2. Unlike wild type, for each of the mutants there was some temperature at which the presence of benomyl greatly improved the growth of the mutant. It should be noted that temperature also has an effect on the benomyl resistance of wild-type strains: at 26° they are strongly inhibited, but at 37° they show considerable resistance to 40 µg/ml benomyl. This tendency holds for lower temperatures as well: wild type becomes sensitive to progressively lower levels of benomyl as the temperature drops (data not shown). The cause of these temperature effects is unknown.

Benomyl resistance is a phenotype of mutations in the TUB2 gene: As shown above, the large majority of mutations to benomyl resistance occurred in a single gene. That gene was capable of mutation to either temperature- or cold-sensitivity for growth. From the precedent in *Aspergillus nidulans* (SHEIR-NEISS, LAI and MORRIS 1978), we suspected that the yeast *ben*^R mutations might lie in the gene encoding β-tubulin. The isolation, sequence analysis and identification of the yeast gene encoding β-tubulin has been described (NEFF *et al.* 1983). Correspondence between the β-tubulin gene (*TUB2*) present on cloned DNA and the Ben^R gene has been established by the genetic criteria of complementation and linkage (NEFF *et al.* 1983). Nevertheless, the final criterion for the establishment of the identity of a mutation is its localization within the coding sequence of a gene. For this reason we decided to fine-structure map several *ben*^R mutations and determine the alteration in one mutation by DNA sequence analysis.

To make this approach feasible we required two methods: the first for localizing mutations on the chromosome to small physically defined DNA intervals, the second for the recovery of the chromosomal mutations on plasmids in *E. coli*.

To localize the *tub2* mutations to physically defined intervals within the *TUB2* gene, a deletion mapping method suggested by ORR-WEAVER, SZOSTAK and ROTHSTEIN (1981) was employed. This method depends on two features of transformation of yeast with linear DNA molecules. First, when circular plasmid is cleaved within a region of yeast DNA, the ends generated are recombinogenic with the homologous sequences found on the yeast chromosome (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1981). When such a molecule is used to transform yeast, this results in repair of the double-strand break from the chromosomal sequences and integration of the plasmid. Second, when a segment of DNA has been removed by cleavage twice in a yeast sequence, this removed segment (gap) is efficiently repaired by a copy mechanism from

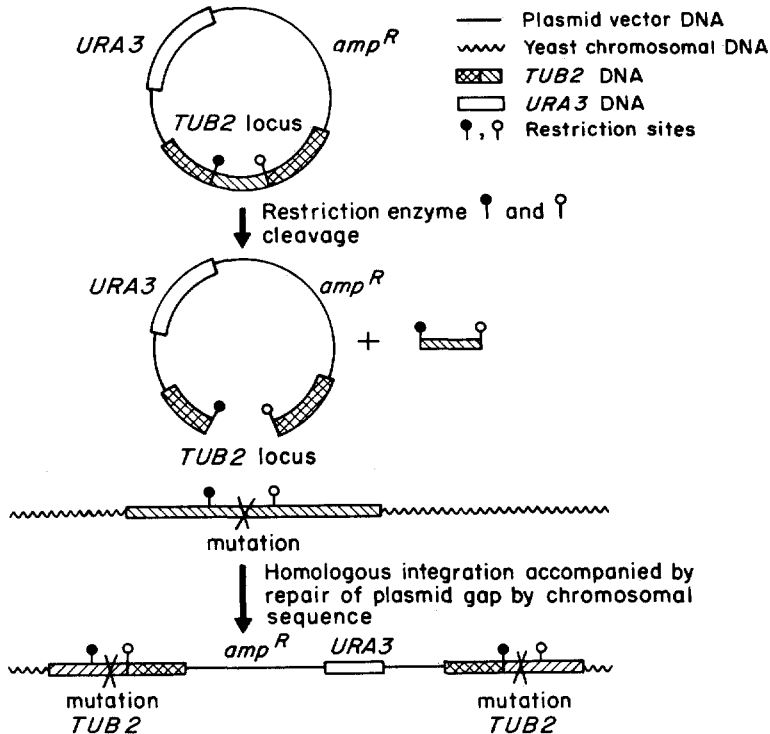


FIGURE 1.—Mechanism of repair of gapped plasmids from homologous chromosomal sequences. The plasmid pJT71 (shown in top panel) was cleaved with restriction enzymes generating a “gapped” plasmid. A representative cleavage is shown; full data on cleavage sites is given in Figure 2. The mixture of DNA fragments was used to transform a *Ura⁻* yeast strain carrying a *tub2* mutation on the chromosome selecting *Ura⁺*. In the case shown here, the mutation lies within the region on the plasmid removed by the restriction digestion. As shown by ORR-WEAVER, SZOSTAK and ROTHSTEIN (1981), the gapped plasmid transforms yeast by interacting at its cleaved ends with the homologous chromosomal sequences. The gap is repaired by a gene conversion-like event of unknown mechanism to generate an integrated copy of the plasmid that has refilled its gap from the chromosomal sequence. In this case, the mutation lies within the gapped region, so it is always copied into the gapped region during repair. If the mutation lay outside of the gapped region, the gap would frequently be repaired to wild type by the normal chromosomal sequences, resulting in a phenotypically wild-type transformant (for a recessive *tub2* mutation such as those tested in this study).

the homologous sequences on the chromosome during yeast transformation. These events are depicted in Figure 1.

Restriction enzymes can be used to remove varying amounts of the *TUB2* gene on pJT71 (carrying *TUB2* and the yeast selectable marker *URA3*), as shown in Figure 2. When each of the resulting linear molecules is used to transform yeast carrying a *tub2* mutation, selecting the plasmid *URA3* marker, the region of the plasmid *TUB2* gene that was removed will be replaced by copying the corresponding information found at the mutant *tub2* gene on the chromosome. If the *tub2* mutation lies within the interval deleted on the plasmid, it will always be copied into the plasmid gene, giving rise to no wild-type

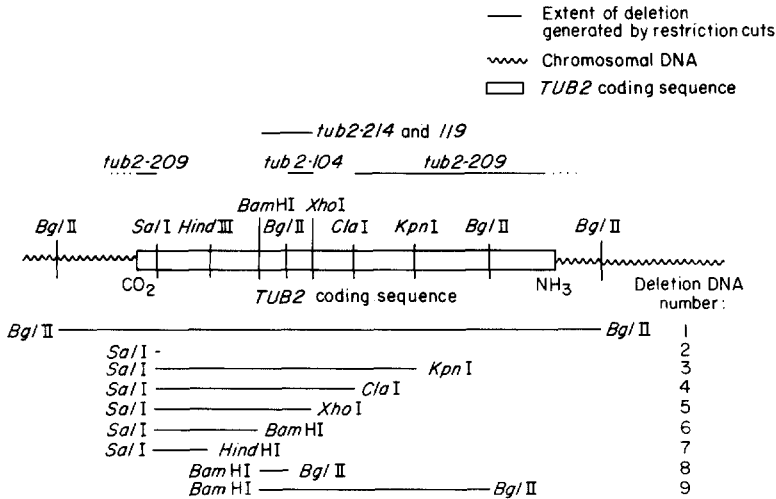


FIGURE 2.—Restriction-enzyme-generated gaps in *TUB2* and positions of four mapped *TUB2* mutations. A partial restriction map, with the relevant sites for the physical mapping experiments is shown. The extent of each deletion DNA is shown, with the restriction sites at its two termini, which were used to generate the deletion. In two cases, DNAs 8 and 9, the deletion interval terminates at a *Bgl*II site that is not unique in the *TUB2* region. These deletion intervals were generated as described in MATERIALS AND METHODS. Typical data used to infer the map positions of the four *tub2* mutations is shown in Table 3. Note that *tub2-209* probably maps near the amino terminus, but we were unable to rule out the extreme carboxyl terminus with the data obtained.

TUB2 recombinants among the Ura⁺ transformants. If, however, the *tub2* mutation lies outside the interval deleted on the plasmid, wild-type information will be used to repair the plasmid gap, resulting in frequent wild-type *TUB2* recombinants. For a more detailed discussion of these phenomena, see ORR-WEAVER, SZOSTAK and ROTHSTEIN (1981).

The set of restriction-enzyme digests used to generate the deletion intervals are shown in Figure 2. Four Cs conditional-lethal *tub2* alleles were mapped by this method. The mapping data obtained for two of the alleles are shown in Table 3. From these data, and similar data obtained for the other two mapped alleles, the map positions shown in Figure 2 were inferred. In all four cases the Ben^R as well as the Cs phenotypes were recessive and could be mapped independently. In each case the two phenotypes mapped in the same restriction interval. This strongly supports the hypothesis that both phenotypes are due to a single genetic lesion.

This mapping method has been shown to give reliable map assignments for mutations in the actin gene (SHORTLE, NOVICK and BOTSTEIN 1985). To confirm the mapping for one *tub2* mutation (*tub2-104*) we needed to establish the presence of an amino acid change in the predicted region. In order to sequence this mutation, it had to be recovered from the yeast chromosome onto a recombinant plasmid in *E. coli*. We achieved this by the method of ROEDER and FINK (1980). Briefly, a fragment of DNA flanking, but not overlapping, the mutant *tub2* gene was subcloned into a standard yeast integrating vector (see Figure 3). This plasmid was then transformed into a yeast strain carrying

TABLE 3

Fine structure physical mapping data for tub2-104 and tub2-209

Deletion DNA ^a	No. of Cs ⁺ transformants/total tested ^b	
	<i>tub2-104</i>	<i>tub2-209</i>
1	0/20	0/32
2	18 ^c /32	30/32
3	0/32	ND ^d
4	0/32	25/31
5	0/97	26/32
6	66/110	32/32
7	21/32	24/32
8	34/81	ND
9	0/220	ND

^a The region deleted in each of the deletion DNAs is shown in Figure 2. Each DNA was transformed into yeast as described in MATERIALS AND METHODS.

^b In each case, many transformants were recovered; only those tested for marker rescue of the Cs phenotype are shown in this table.

^c In this case, and in several others, only about half of the expected 100% Cs⁺ recombinants were obtained. This may be due to gene conversion of heteroduplex DNA laid down during integration, as ORR-WEAVER, SZOSTAK and ROTHSTEIN (1981) suggest occurs.

^d ND, Not done.

tub2-104, directing integration to the *tub2* locus by a restriction cleavage. Integration of the plasmid at *tub2* results in the chromosomal structure shown in Figure 3. The yeast DNA from such a transformant was purified, cut with the restriction enzyme *Sph*I, leaving the mutant *tub2* gene covalently attached to the plasmid sequences, and religated at low DNA concentrations to favor intramolecular ligation. This mixture was then transformed into *E. coli* selecting ampicillin resistance. Restriction digests of the plasmid DNA purified from *E. coli* showed that the products recovered had the expected structure and carried the DNA that should contain the *tub2-104* allele. One such plasmid was sequenced on both strands by the method of MAXAM and GILBERT (1980) across the entire restriction fragment to which mutation 104 had been mapped. In this region a single base-pair change was found, G to A in the second base of codon 241, which predicts an amino acid change from arginine to histidine (see NEFF *et al.* 1983 for the DNA sequence of *TUB2*). Since both the Cs and the Ben^R phenotypes mapped to this region, this single mutation must be responsible for both phenotypes.

Genetic mapping of TUB2: The *TUB2* gene was mapped to a chromosome by the method of 2 μ mapping (FALCO and BOTSTEIN 1983). The basis for this method is that when a plasmid carrying a particular region of the yeast 2 μ circle is integrated into a chromosome, that chromosome becomes genetically unstable. In a diploid, segregants that have lost the plasmid sequences arise at a high frequency, and among these segregants a fraction appear to have lost

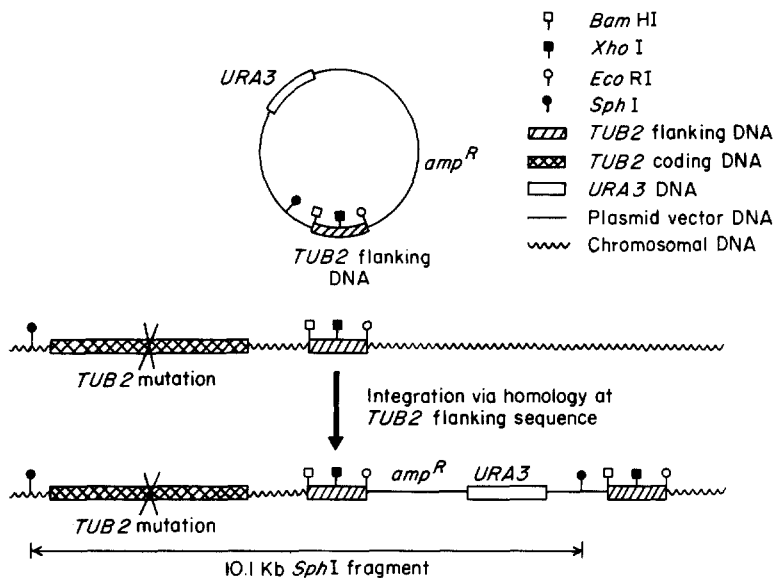


FIGURE 3.—Method for the recovery of chromosomal *TUB2* mutations on plasmids. A plasmid carrying a small region of DNA flanking the *TUB2* gene was directed to integrate at the *TUB2* locus by cleavage at the *Xho*I site shown, selecting *Ura*⁺ in a yeast strain that was *ura3* and carried the *tub2* allele of interest. The expected structure of the integrant is shown; this was not directly tested. Total DNA was prepared from two transformants, cleaved to completion with *Sph*I and religated at low DNA concentration to favor intramolecular ligation events. The recircularized 10.1-kb *Sph*I fragment, carrying the mutant *tub2* gene, was recovered in *E. coli*, as described in the text and in MATERIALS AND METHODS.

the entire chromosome in which the plasmid resided. If the diploid strain was appropriately constructed, with recessive auxotrophic markers heterozygous on each chromosome, then the identity of the lost chromosome can be established from the uncovering of one of these markers.

Plasmid pRB120 was used for the 2μ mapping. This plasmid carries a large region of genomic DNA from the *TUB2* region, a segment of the 2μ circle, and the selectable marker *URA3*, cloned into a pBR322 based vector. Integrants of pRB120 at the *TUB2* locus were isolated. Two such integrants were mated to the multiply marked auxotrophic strain DBY880 (generously provided by G. KAWASAKI). The genotype of the resulting diploids is shown in Table 4. Both diploids showed instability for *Ura*⁺, as expected (about 40% *Ura*⁻ segregants after 10 generations of growth nonselectively). About 4% of the *Ura*⁻ segregants had acquired a second growth requirement, indicating the uncovering of one or more of the marker mutations. Thirteen of 14 such segregants were analyzed and were found to be histidine auxotrophs by virtue of having uncovered the *his2* marker. This result suggests that *TUB2* is on the same chromosome as *his2*, namely chromosome VI.

To confirm this assignment and to determine the map location of *TUB2* more precisely, we needed to establish linkage of a *tub2* allele with known markers on chromosome VI by conventional tetrad analysis. Since crosses with centromere-linked markers had shown that *tub2-104* is not centromere linked

TABLE 4

Genotype of diploids for 2- μ mapping of TUB2

Chromosome	Genotype
I	<i>ade1</i> / +
II	<i>his7</i> / +
III	<i>leu2</i> +
	+ <i>his4</i>
IV	<i>trp1</i> / +
V	<i>ura3</i> / <i>ura3</i>
VI	<i>his2</i> / +
VII	<i>leu1</i> <i>ade6</i>
	+ +
VIII	<i>arg4</i> / +
IX	<i>his6</i> / +
X	<i>ilv3</i> / +
XI	<i>met14</i> / +
XII	<i>asp5</i> / +
XIII	<i>lys7</i> / +
XIV	<i>met2</i> <i>lys1</i>
	+ +
XV	<i>ade2</i> <i>arg1</i>
	+ +
XVI	<i>aro7</i> / +

Genotype of the diploids generated for 2- μ mapping of the *TUB2* locus from DBY880 \times DBY1395 (with pRB120 integrated). All of the DBY880 markers are shown above the line on each chromosome. On an unknown chromosome, pRB120 is integrated into the + homologue (below the line). Every chromosome is marked, except for chromosome V.

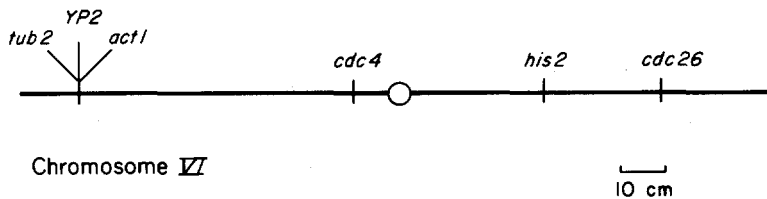


FIGURE 4.—Position of the *TUB2*-*YP2*-*ACT1* loci on chromosome VI. The position of the centromere is indicated by an open circle. The map positions are as given by MORTIMER and SCHILD 1982. The orientation of the *TUB2*, *YP2*, *ACT1* cluster with respect to the centromere is unknown.

(data not shown), we chose to test linkage with the most centromere distal markers we had readily available, *cdc26* and *cdc4*. A map of chromosome VI is shown in Figure 4. *tub2-104* was unlinked to *cdc26* (7:6:23, parental ditype (PD): nonparental ditype (NPD): tetatype (TT)), but was weakly linked to *cdc4* (16:2:59, PD:NPD:TT). Since *cdc26* is about 70 cM from *cdc4*, these data unambiguously placed *TUB2* on the other side, roughly 50 cM distal to *cdc4* on the left arm of chromosome VI (see Figure 4). This was very close to the region to which the yeast actin gene (*ACT1*) had recently been mapped (FALCO and BOTSTEIN 1983). To determine if *TUB2* was near *ACT1*, we tested linkage of *tub2-104* to a *URA3* marker integrated at *ACT1* and found it to be very

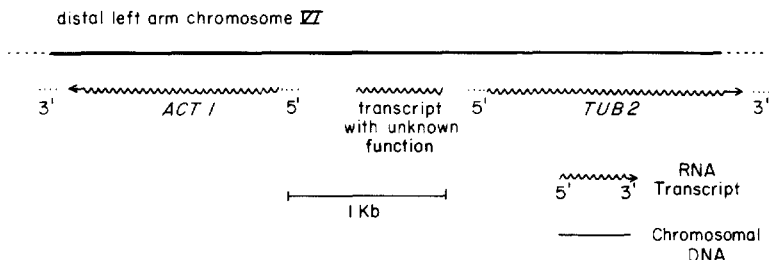


FIGURE 5.—Transcription pattern at the *TUB2-YP2-ACT1* loci. The termini of the transcripts are not known with precision, as indicated by dotted lines at the ends of the transcripts. The orientation of transcription of the “unknown transcript” (now called *YP2*, but still of unknown function, see text) has been determined; it is in the same direction as *ACT1* (GALLWITZ, DONATH and SANDER 1983).

tight (22:0:0, PD:NPD:TT). This result justified the addition of the previously obtained *ACT1-cdc4* linkage data (15:3:49, PD:NPD:TT; S. C. FALCO, personal communication) to the *TUB2-cdc4* data for a total of 31:5:108 (PD:NPD:TT). This total indicates a fairly accurate map position for both genes of 55 cM distal to *cdc4*.

TUB2 and ACT1 are nearly adjacent genes: Since *TUB2* and *ACT1* were so closely linked, it was possible that we could establish their physical linkage on cloned DNA. To test this, we compared the restriction map of a large insert containing the *TUB2* gene (pRB120; NEFF *et al.* 1983) for possible overlap with the known restriction map of the actin gene (NG and ABELSON 1980). On the basis of the comparison of many restriction sites over a 4-kb region, we were able to conclude that pRB120 contains the entire actin gene, with only about 1 kb of DNA separating *ACT1* and *TUB2* (data not shown).

However, it was possible that the structure of the cloned material did not truly reflect the genomic structure in yeast. To confirm that the pRB120 insert was an unrearranged genomic fragment, a gel-transfer hybridization experiment was performed, using pRB120 insert DNA as a probe against the homologous fragments in the genome. If the cloned insert did not correspond in structure to the genome, then the fragments detected in this experiment should differ in length from the homologous fragments mapped on the plasmid insert. On the basis of three different restriction digests, we concluded that the cloned insert corresponds precisely with the genomic structure (data not shown). The exact location of both the actin coding sequence (NG and ABELSON 1980) and the β -tubulin coding sequence (NEFF *et al.* 1983) has been determined by sequence analysis. Based on this information the exact relative positions and orientations of the two genes were determined and are shown in Figure 5. The genes encoding actin and β -tubulin are about 1 kb apart and are transcribed divergently.

In RNA gel-transfer experiments (THOMAS 1980) a third transcript, whose precise identity and function is unknown, can be detected (data not shown, and GALLWITZ, DONATH and SANDER 1983). The coding sequence of this gene, called *YP2*, shows homology to the *ras* oncogene of higher eukaryotes (GALLWITZ, DONATH and SANDER 1983). *YP2* is an essential gene, as demonstrated

TABLE 5

Restrictive arrest morphology of strains carrying tub2-104

Strain	<i>tub2</i> allele	Shift temperature	Hr after shift	Cell morphology (%)		
				No bud	Small bud	Large bud
DBY1034	Wild type	26°		41	24	35
DBY1034	Wild type	11°	26	36	24	40
DBY1373	<i>tub2-104</i> (Cs)	26°		33	29	38
DBY1373	<i>tub2-104</i> (Cs)	11°	16	4	9	87
DBY1373	<i>tub2-104</i> (Cs)	11°	24	8	1	91
DBY1373x	<i>tub2-104</i> (Cs)	26°		36	30	34
JT240	Homozygous diploid	11°	16	7	0	93

Arrests were conducted as described in MATERIALS AND METHODS. All shifts were from 26° to the temperature shown. Wild-type strains showed no significant change in cell types at any time after shift from 26° to 11° (data not shown); the 26-h time point shown is representative. About 200 cells were counted in each sample.

by gene disruption experiments (N. SEGEV and D. BOTSTEIN, unpublished results).

Cell-division-cycle arrest of a tub2 mutant: To test whether β -tubulin function is required throughout the vegetative cell-division cycle or only at a single point, a strain carrying a Cs β -tubulin allele (*tub2-104*) was shifted to its non-permissive temperature and was observed over a time course. We used 11° for this study because this temperature gave a more uniform terminal arrest morphology than our standard 14°, presumably because the mutant defect is more severe at this lower temperature.

Asynchronous cultures grown at 26° were shifted to 11°, incubated for up to 24 hr and viewed by phase contrast microscopy. Control experiments with DBY1034 and DBY1374 (both *TUB2*) showed that this temperature shift does not cause a lag in growth or significantly alter the distribution of cells in the cell cycle. The doubling time of these wild-type strains at 11° was about 11.5 hr. However, a strain carrying *tub2-104* arrested as 90% large-budded cells after only 16 hr (1.4 generation times). Detailed cell counts are shown in Table 5. Very similar results were obtained for the independently isolated Cs allele *tub2-214* (data not shown). This result suggests that β -tubulin is required for progression through only one part of the cell cycle and that the *tub2-104* allele causes arrest on the first cell cycle after temperature shift. Thus, *TUB2* qualifies as a *CDC* gene as defined by HARTWELL, CULOTTI and REID (1970; HARTWELL 1974; PRINGLE and HARTWELL 1981). Since no previously defined *CDC* gene had been reported to map in the same region of chromosome VI, *TUB2* is probably not an allele of any of the reported *CDC* genes.

DISCUSSION

The central result reported in this paper is the finding that a conditional-lethal mutation, *tub2-104*, in a gene known to specify a major cytoskeletal protein (β -tubulin) exhibits a uniform terminal arrest phenotype typical of cell-division-cycle mutations as defined by HARTWELL, CULOTTI and REID (1970).

This terminal phenotype is consistent with the fact that β -tubulin is a major protein component of microtubules that comprise the mitotic spindle, because arrest with a large bud suggests that the defect might well be due to failure of the mitotic spindle to form and/or function. It previously had been found (QUINLAN, POGSON and GULL 1980; WOOD and HARTWELL, 1982) that methylbenzimidazole-2-yl carbamate (MBC) causes a uniform cell cycle arrest in yeast (MBC is a close chemical relative of benomyl). All of 50 Ben^R mutants tested were highly cross-resistant to MBC (data not shown), suggesting that the cell-cycle arrest induced by MBC results from an effect on tubulin.

The *tub2* mutations described here were all isolated on the basis of their ability to confer resistance to the antimicrotubule drug benomyl. However, the conditional-lethal phenotype of *tub2-104* has been shown to result from the same amino acid substitution that causes the drug resistance. Further, T. HUFFAKER (personal communication) has isolated mutations in *tub2* by *in vitro* mutagenesis that confer cold-sensitivity without conferring benomyl resistance. These mutations confer a cell-cycle phenotype very similar to the one described here for *tub2-104*.

The basis of the Ben^R phenotype: We isolated 173 independent mutations that confer benomyl resistance in yeast. All of the mutations tested appear to fall in a single gene; however, the majority of the mutations were not assigned to any gene (including all of those that confer dominant benomyl resistance and no conditional lethality). Although the remaining mutations might define other genes, our results indicate that, unlike the case in *A. nidulans*, only a single yeast gene mutates to Ben^R at a high frequency. Nine of the *ben^R* mutations were shown to have conditional-lethal phenotypes that are probably due to the same mutation that is responsible for Ben^R. An interesting finding is that all three of the Ts mutations can be rescued for growth at some temperature by benomyl. The basis of the relationship between growth and benomyl resistance is uncertain, but there is an interesting possible explanation. Benomyl acts by destabilizing microtubules (QUINLAN, POGSON and GULL 1980), and cold temperature is known to have the same effect on yeast microtubules *in vitro* (KILMARTIN 1981). Thus, it is possible that high temperature directly antagonizes the action of benomyl by shifting the equilibrium between free dimer and polymer toward the polymerized state. The benomyl-dependent mutations described here fit this model well. They could have a defect that renders their microtubules more stable than wild type, as has been demonstrated for a tubulin mutation in *A. nidulans* (OAKLEY and MORRIS 1981). Such a defect could be compensated for by a chemical destabilizing agent, such as benomyl, or by a physical one, such as low temperatures.

The dominance of some nonconditional-lethal *ben^R* mutations deserves some comment. Assuming that benomyl indeed acts to destabilize microtubules, one hypothesis might be that subunits specified by a dominant resistant allele can, after assembly, affect benomyl binding by wild-type subunits. Alternatively, one might speculate that destabilization occurs primarily at microtubule ends, and the presence of one or more resistant subunits at an end improves stability even in the presence of the drug. To explain recessive resistance, lowered

binding of the drug by the mutant subunits, without any special effect on wild-type subunits seems the simplest hypothesis.

Complementation behavior: The genetic properties of β -tubulin mutations are of interest because they are a substantial set of mutations in a gene product that forms a linear polymer. Because it might be possible to incorporate mutant subunits into a microtubule, where they would fail to function properly and would lead to a dominant toxic effect, some conditional-lethal alleles might be expected to be dominant to wild type. The nine conditional-lethal alleles described here are largely recessive for their conditional lethality, although the Ts mutations may not be completely recessive. This result is consistent with two simple explanations: (1) failure of the mutant subunits to be incorporated into microtubules or (2) structural compensation by the wild-type subunits for the defect conferred by the mutant subunits that are incorporated into microtubules. This could result in microtubules that are functional despite the presence of mutant subunits.

Complementation patterns between defective subunits of multimeric proteins is often complex (for example, CULBERTSON and HENRY 1975; MAJUMDER *et al.* 1981) and might be expected to be especially so for a protein like β -tubulin that is part of a complex structure. However, for the mutations described here, there is no indication of intragenic complementation for either conditional lethality or drug resistance. It is possible that this is due to the fact that β -tubulin is a protein required in stoichiometric amounts for the construction of microtubules. Whereas intragenic complementation that restores only a small fraction of the activity of an enzymatic function may be sufficient to permit growth (see MAJUMDER *et al.* 1981), nearly complete restoration of activity might be required for proteins such as actin or tubulin.

Significance of the linkage of TUB2 and ACT1: The close proximity of the actin and β -tubulin genes in yeast is interesting, although difficult to interpret. Genes of similar functions are very rarely clustered in yeast (MORTIMER and SCHILD 1982). Furthermore, any apparent relationship between actin and tubulin as important components of the cytoskeleton may simply reflect the interests of the investigators. In this connection, it seems worth mentioning that conditional-lethal mutations in actin do not display a simple *CDC*-gene phenotype (NOVICK and BOTSTEIN 1985). The function of the *YP2* gene, which lies between *ACT1* and *TUB2*, is unknown, as is any relationship among the expression of any of the three genes.

Fine-structure mapping: The fine-structure physical mapping method we describe here is simple and rapid. It should be a useful tool in the future for fine-structure mapping with the purpose of establishing the sequence of mutations. It is worth noting that linker insertions at the ends of the transforming fragment do not interfere with recombination with the chromosome and seem to be removed from the transforming DNA fragment at some point during transformation. This observation indicates that the fine-structure mapping method should be useful even when convenient restriction sites are not available (by insertion of new restriction sites via molecular cloning).

In our case, three of the four alleles mapped fell into a single interval of

the *TUB2* gene. One of these was cloned and sequenced, revealing a single amino acid change from wild type in the expected interval. We do not know for certain whether or not the other two alleles mapped to this interval are identical mutations. At least one of the alleles is unlikely to be, because it has a significantly different temperature spectrum for growth from *tub2-104*. It is possible that a hot spot for spontaneous mutation or a region likely to mutate to Ben^R Cs explains the clustering.

The isolation of conditional mutations in yeast β -tubulin makes possible a number of experiments. More detailed phenotypic analysis (in particular, using antibodies to stain *in vivo* microtubules and the electron microscope to visualize individual microtubules in mutant strains) should reveal a more detailed picture of the functional role of microtubules in yeast. Mutagenesis of the cloned *TUB2* gene *in vitro* and introduction of the mutations into the chromosome is under way and has already revealed new classes of mutations with different phenotypes (T. HUFFAKER, personal communication). Reversion of *tub2* mutations may allow identification of genes whose products interact *in vivo* with β -tubulin and, thus, can suppress its defects (MOIR *et al.* 1982). Such genes could include α -tubulin, as has been demonstrated in *A. nidulans* (MORRIS, LAI and OAKLEY 1979) and in other, as yet unknown, genes whose products contribute to microtubule function.

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