Unlinked Noncomplementation: Isolation of New Conditional-Lethal Mutations in Each of the Tubulin Genes of *Saccharomyces cerevisiae*

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ABSTRACT

Mutations in genes of *Saccharomyces cerevisiae* that code for proteins that interact with β-tubulin were sought by screening for unlinked mutations that fail to complement mutations in the single β-tubulin-encoding gene (*TUB2*). Among the first three noncomplementing mutations examined, two are linked to *TUB2* while one is unlinked. The unlinked mutation was shown to be a conditional-lethal allele of the major α-tubulin-encoding gene (*TUB1*) and represents the first such mutation in that gene. The *tub1-1* mutation itself causes a cold-sensitive cell-cycle arrest, and confers supersensitivity to the antimicrotubule drug benomyl. These phenotypes occur in the presence of a wild-type copy of the minor α-tubulin-encoding gene, *TUB3*; the combination of *tub1-1* and a *tub3* null mutation is inviable in haploids. Through further application of this method, new mutations in *TUB2* and *TUB3* were isolated as unlinked noncomplementers of *tub1-1*. The noncomplementation between *tub1* and *tub2* mutations is gene specific and allele specific, suggesting that the phenotype is due to an interaction at the protein level. We conclude that isolation of unlinked noncomplementing mutations is likely to be a generally useful method for isolating mutations in interacting gene products.

MICROTUBULES are a major component of the eukaryotic cytoskeleton, and are the central constituents of the mitotic and meiotic spindles. Nevertheless, little is known about the detailed roles, especially *in vivo*, of the major proteins, α-tubulin and β-tubulin, and even less is known about the functions of the "microtubule-associated" proteins. We have taken a genetic approach to this problem, using the yeast *Saccharomyces cerevisiae*, hoping to reveal *in vivo* function in a way that is complementary to biochemical and cell biological analysis. Yeast is well suited for this endeavor as it possesses tubulin with properties similar to those of higher organisms (KILMARTIN 1981) and is manipulable using a wide range of powerful classical and molecular genetic techniques not available in higher organisms (BOTSTEIN and DAVIS 1982).

Microtubules in yeast have been observed by electron microscopy (ROBINOW and MARAK 1966; MOENS and RAPPORT 1971; BYERS and GOETSCH 1975; PETERSON and RIS 1976; BYERS 1981; KING and HYAMS 1982) and immunofluorescence (KILMARTIN and ADAMS 1984; ADAMS and PRINGLE 1984). Such studies have shown microtubules to be elements of the mitotic and meiotic spindles, and of cytoplasmic structures that resemble astral microtubules of higher eukaryotes. The effect of antimicrotubule drugs on yeast (QUINLAN, POGSON and GULL 1980; WOOD 1982; WOOD and HARTWELL 1982; DELGADO and CONDE 1984; PRINGLE *et al.* 1986) and the phenotype of tubulin mutations (UMESONO *et al.* 1983; TODA *et al.* 1983; ROY and FANTES 1983; THOMAS, NEFF and BOTSTEIN 1985; HUFFAKER, THOMAS and BOTSTEIN 1988) confirm that microtubules play essential roles in mitosis and meiosis, and in nuclear migration during cell division and mating.

S. cerevisiae has three tubulin genes. The single β-tubulin gene, *TUB2*, has been isolated, sequenced, and shown to be essential for growth (NEFF *et al.* 1983). Conditional lethal mutations have been isolated in *TUB2* by selection for drug resistance (THOMAS, NEFF and BOTSTEIN 1985) and by mutagenesis of the cloned gene (HUFFAKER, THOMAS and BOTSTEIN 1988). The two α-tubulin genes, *TUB1* and *TUB3*, have been isolated, sequenced, and analyzed genetically (SCHATZ, SOLOMON and BOTSTEIN 1986; SCHATZ *et al.* 1986). *TUB1* and *TUB3* show 10% divergence at the amino acid level, and *TUB1* is essential for growth while *TUB3* is not essential. This genetic difference does not reflect a functional difference in the proteins, however, as either can perform all the functions of α-tubulin if present at high enough copy number (SCHATZ, SOLOMON and BOTSTEIN 1986). This organization of α-tubulin genes is strikingly similar to that observed in the distantly related fission yeast *Schizosaccharomyces pombe* (ADACHI *et al.* 1986).

In this paper we describe the use of unlinked noncomplementation to isolate new mutations in the *TUB1*, *TUB2* and *TUB3* genes of yeast. This approach

was inspired by the observation that strains carrying recessive mutations in genes that are unlinked to each other, but encode proteins that are likely to be interacting components of a complex, may fail to complement (RAFF and FULLER 1984; BISSON and THORNER 1982; ATKINSON 1985; RINE and HERSKOWITZ 1987). A particularly relevant example of unlinked noncomplementation comes from studies in Drosophila of mutations that fail to complement certain β-tubulin mutations (RAFF and FULLER 1984; FULLER 1986). It has recently been demonstrated that one such unlinked noncomplementing mutation is tightly linked to a major α-tubulin locus (T. HAYS and M. FULLER, personal communication). The tubulin mutations that we have isolated cause conditional phenotypes, which can be used in further genetic and cell biological analysis of the function of microtubules *in vivo*. These results demonstrate that screening for unlinked noncomplementers is a useful alternative to more traditional genetic approaches, such as pseudoreversion analysis (JARVIK and BOTSTEIN 1975).

MATERIALS AND METHODS

Media and strains: Media for yeast growth and sporulation were as described by SHERMAN, FINK and LAWRENCE (1974), except for 5-fluoro-orotic acid (5-FOA) plates, described by BOEKE, LACROUTE and FINK (1984). Benomyl, 98.6% pure, was a generous gift from E.I. duPont de Nemours and Co., Inc., and was kept as a 10 mg/ml stock in dimethyl sulfoxide at −20°, which was thawed and added to warm YEPD medium immediately before the plates were poured. The yeast strains used in this paper were derived from a set of essentially isogenic S288C strains provided by G. R. FINK. The strains are listed in Table 1.

Genetic techniques and transformation: Yeast mating, sporulation, and tetrad analysis were performed as described by SHERMAN, FINK and LAWRENCE (1974). Growth on plates was scored by spotting suspensions of cells in sterile water onto plates using a 32- or 48-point inoculator. Yeast cells were transformed with DNA by the lithium acetate method of ITO *et al.* (1983) as modified by KUO and CAMPBELL (1983) with sonicated salmon sperm DNA as carrier. Transformants were plated on synthetic complete (SC) medium lacking the appropriate nutrient to select cells carrying the plasmid. Transformants were purified by streaking on selective medium and checked for presence of the plasmid marker. Diploid transformants were sporulated by printing directly from selective medium to sporulation plates to avoid loss of the plasmid.

Isolation of mutants: Cells of strain DBY1034 or DBY1399 were grown in YEPD to stationary phase and mutagenized with ethyl methanesulfonate (EMS) according to SHERMAN, FINK and LAWRENCE (1974). In some experiments strains were grown in YEPGlycerol prior to mutagenesis to keep the number of ρ^- cells in the culture to a minimum. Growth in YEPGlycerol increased the sensitivity of the cells to EMS so that a shorter incubation time in the presence of the mutagen was required to obtain the desired amount of mutagenesis. Aliquots were taken at 50, 60 and 70 min and plated to determine viability; one that had between 10% and 25% viable cells was chosen and plated on YEPD to give a density of approximately 150 colonies per plate. These master plates were incubated at 26°, then mated to fresh lawns of wild-type and mutant strains of the opposite mating type on YEPD by replica-plating. These mating plates were allowed to grow overnight at 26°, then replica-plated to SC lacking the appropriate nutrients to select for diploids, and incubated in the restrictive temperature, either 16° or 14°.

The plates were examined after approximately 5 days for colonies that formed viable diploids with wild type at the restrictive temperature, but failed to do so with one or more of the tubulin mutants. Such colonies were considered to be candidate noncomplementers. The haploid noncomplementer candidates were picked from the YEPD master plate and rechecked for their noncomplementation phenotype by mating to the same tester strains on YEPD, selecting diploids on SC lacking the appropriate nutrients, and spotting the diploids onto plates at the restrictive temperature with a 32- or 48-point inoculator. Noncomplementers that retested were then subjected to tetrad analysis to determine their linkage relationship to the mutation that they fail to complement.

Assessment of cell cycle arrest: Cells were grown to a density of 5×10^6 to 1×10^7 cells/ml in liquid YEPD or SD + requirements at permissive temperature (26°). Cultures were shifted to nonpermissive temperature (11°) by incubation in a rotary shaking water bath cooled with an external refrigeration unit. The cultures were sonicated briefly and examined microscopically after 24 hr, approximately two generation times for wild-type cells at 11°. Alternatively, cells were fixed with a 5% solution of formaldehyde prior to viewing; this did not affect the distribution of cell morphologies. At least 200 cells were counted in each case.

Indirect immunofluorescence staining of yeast cells: Yeast cells were treated for immunofluorescence essentially as described by KILMARTIN and ADAMS (1984), with the exceptions that the cells were fixed by addition of formaldehyde directly to the culture medium to a final concentration of 5% formaldehyde and incubating at room temperature for 1 hr, and that cell walls were subsequently removed by incubation at 30° for 20 min in 100 μg/ml Zymolyase 60,000 in 1.2 M sorbitol, 0.1 M potassium phosphate (pH 7.5) and 25 mM 2-mercaptoethanol. All other postfixation permeabilization steps were omitted. Rat monoclonal anti-tubulin antibody, YOL 1/34, was a gift from J. KILMARTIN. FITC-conjugated goat anti-rat IgG antiserum was obtained from Cappel. DNA in the fixed and digested cells was stained with 1 μg/ml DAPI for 1 min.

Assessment of *tub1-1 tub3* double mutant lethality: To determine whether the combination of *tub1-1* and *tub3*::*TRP1* in a haploid is viable, a diploid strain with genotype *TUB1/tub1-1 TUB3/tub3*::*TRP1* (pRB316-*TUB3 URA3* 2μ plasmid) was made by mating DBY2516 to DBY2287. The wild-type *TUB3* gene on pRB316 is able to complement the defect of *tub3*::*TRP1*, and to suppress the defect of *tub1-1*. This strain was grown on SD-uracil to select cells carrying the plasmid, then inoculated on sporulation medium. Tetrads were dissected, and all segregants from this cross were incubated for 48 hr on YEPD to allow loss of the plasmid. Segregants were also transferred to SD-tryptophan medium to determine which spores were carrying the *tub*::*TRP1* mutation. The cells on the YEPD plate were transferred to 5-FOA plates to select cells that had lost the Ura^+ plasmid. The segregants were then transferred from the 5-FOA plates to plates containing 5μg/ml benomyl at 26° and to YEPD at 14° to determine which segregants were carrying the *tub1-1* mutation. At the same time they were inoculated on SD-uracil to confirm

TABLE 1

Strain list

Strain	Genotype	Source or reference
DBY1034	*MATa his4-539 lys2-801 ura3-52*	This laboratory
DBY1399	*MATα ade2-101 ura3-52*	This laboratory
DBY1384	MATa *his4-539 lys2-801 ura3-52 tub2-104*	THOMAS, NEFF and BOTSTEIN (1985)
DBY1385	*MATα ade2-101 ura3-52 tub2-104*	THOMAS, NEFF and BOTSTEIN (1985)
DBY2022	*MATα ade2-101 ura3-52 tub2-401*	HUFFAKER, THOMAS and BOTSTEIN (1988)
DBY2287	*MATa his3-Δ200 leu2-3,112 trp1-1 ura3-52 tub3::TRP1*	SCHATZ, SOLOMON and BOTSTEIN (1986)
DBY2303	*MATα ade2-101 ura3-52 tub2-402*	HUFFAKER, THOMAS and BOTSTEIN (1988)
DBY2306	*MATα ade2-101 lys2-801 ura3-52 tub2-403*	HUFFAKER, THOMAS and BOTSTEIN (1988)
DBY2307	*MATα ade2-101 lys2-801 ura3-52 tub2-404*	HUFFAKER, THOMAS and BOTSTEIN (1988)
DBY2310	*MATα ade2-101 ura3-52 tub2-405*	HUFFAKER, THOMAS and BOTSTEIN (1988)
DBY1101	*MATα his4-539 cdc45-1*	MOIR *et al.* (1982)
DBY1245	*MATα his4-539 cdc44-1*	MOIR *et al.* (1982)
DBY694	*MATα his4-539 cdc48-1*	MOIR *et al.* (1982)
DBY695	*MATα his4-539 cdc51-1*	MOIR *et al.* (1982)
AA15	*MATα his4-539 cdcY*[a]	MOIR *et al.* (1982)
CC30	*MATα his4-539 cdcZ*[a]	MOIR *et al.* (1982)
DBY2282	*MATα his3-Δ200 leu2-3,112 lys2-801 trp1-1 ura3-52 TUB1:LEU2:TUB1*[b]	SCHATZ, SOLOMON and BOTSTEIN (1986)
DBY2283	*MATa ade2-101 his3-Δ200 leu2-3,112 trp1-1 ura3-52 TUB3:URA3:TUB3*[c]	SCHATZ, SOLOMON and BOTSTEIN (1986)
DBY2284	*MATα his3-Δ200 leu2-3,112 lys2-801 trp1-1 ura3-52 TUB3:URA3:TUB3*	SCHATZ, SOLOMON and BOTSTEIN (1986)
DBY1195	*MATα ade2-101 his4-539 ura3-52 act1-1*	This laboratory
DBY2499	*MATa his4-539 lys2-801 ura3-52 tub1-1*	This study
DBY2500	*MATα ade2-101 ura3-52 tub1-1*	This study
DBY2505	*MATa his4-539 lys2-801 ura3-52 tub3-1*	This study
DBY2507	*MATa his4-539 lys2-801 ura3-52 tub1-13*	This study
DBY2509	*MATa his4-539 lys2-801 ura3-52 tub1-14*	This study
DBY2510	*MATa his4-539 ura3-52 tub2-501*	This study
DBY2511	*MATα ade2-101 ura3-52 tub2-501*	This study
DBY2512	*MATa his4-539 lys2-801 ura3-52 tub2-502*	This study
DBY2514	*MATa ade2-101 lys2-801 ura3-52 tub2-503*	This study
DBY2516	*MATα lys2-801 ura3-52 tub1-1* (pRB316-*TUB3* on YEp24)	This study
DBY2517	*MATα lys2-801 ura3-52 tub1-1* (pRB325-*TUB3* on YCp50)	This study
DBY2518	*MATα lys2-801 ura3-52 tub1-1* (pRB326-*TUB1* on YCp50)	This study
DBY2519	*MATα lys2-801 ura3-52 tub1-1*	This study

[a] A cold-sensitive *cdc* mutation that has not been demonstrated to be in a new *CDC* gene.
[b] A duplication of the *TUB1* gene marked by an insertion of *LEU2*.
[c] A duplication of the *TUB3* gene marked by an insertion of *URA3*.

loss of pRB316. All strains that were deduced to be of *tub1-1 tub3::TRP1* genotype were unable to grown on 5-FOA plates.

RESULTS

We sought to isolate mutations in yeast that would fail to complement conditional mutations in *TUB2*, the single β-tubulin gene, but would nevertheless be unlinked to *TUB2*. The rationale behind this approach is that such unlinked noncomplementing mutations may identify genes that encode interacting components of the yeast microtubule system. These new mutations could then be examined for other phenotypes, such as conditional cell-cycle arrest, that would allow further analysis of the role of the gene product in that system. The presence of a simple, new phenotype is crucial to the utility of any genetic method of isolating mutations in genes that encode interacting products; it is the new phenotype, along with other genetic tests, that best allows one to determine whether the new mutation is specific to the process being studied.

The procedure that we used to isolate unlinked noncomplementers is diagrammed in Figure 1. Briefly, mutagenized haploid colonies were crossed to *TUB2* and *tub2cs* haploids, and the resulting diploids were shifted to the nonpermissive temperature. Mutants were sought that could form a Cs^+ diploid when crossed to the *TUB2* strain, but not when crossed to the *tub2cs* strain. Tetrad analysis was then performed to determine whether the mutation resulting in the failure to complement the *tub2cs* mutation was linked to the *TUB2* locus. Unlinked noncomplementing mutations were designated *tnc* for

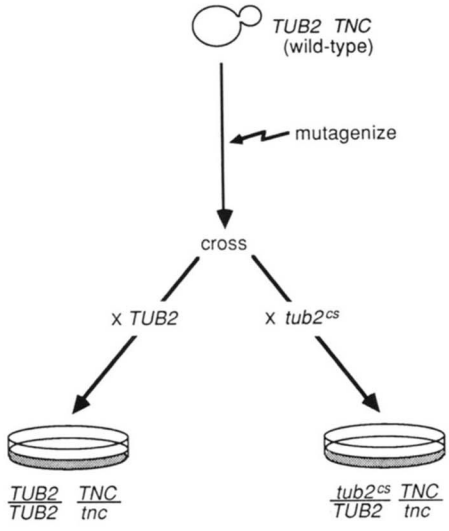

FIGURE 1.—Isolation of unlinked noncomplementers of *tub2cs* mutants. *TUB2* is the gene for β-tubulin, *TNC* is a *t*ubulin *n*oncomplementer gene. A mutant that forms a Cs^- diploid with a *tub2cs* mutant, but a Cs^+ diploid with wild-type, is a noncomplementer. The mutation responsible for the noncomplementation is then tested for linkage to the *TUB2* locus.

*t*ubulin *n*oncomplementer. A critical aspect of this screen is that there are at least three types of mutations that will give the appearance of failing to complement a given *tub2cs* mutation, yet be uninteresting for our purposes: dominant cold-sensitive mutations, sterile mutations, and nutritional marker mutations, where the mutagenized strain has acquired a mutation in one of the genes being used to select diploids. These three types of mutations can be distinguished from a true noncomplementer because they will fail to form a selectable cold-resistant diploid with a *TUB2* strain as well as with a *tub2cs* strain. In contrast, a true noncomplementer will form a selectable cold-resistant diploid with a *TUB2* strain. For this reason we screened each mutagenized clone by comparing crosses to *tub2cs* and *TUB2* in parallel.

A total of approximately 20,000 colonies grown from mutagenized cells were screened for failure to complement *tub2* mutations; 3,000 were crossed to *tub2-104* and *tub2-401*, 7,000 were crossed to *tub2-402*, and 10,000 were crossed to *tub2-403*. Three noncomplementing mutants were isolated this way, one from each of the screenings. Two of these have mutations linked to the *TUB2* locus and have interesting phenotypes of their own (see below). The third mutant fails to complement *tub2-401* at 16°, weakly complements *tub2-104* at the same temperature, and is cold-sensitive by itself. This mutation was designated *tnc1*.

The failure of *tnc1* to complement *tub2-401* segregates 2:2 in a cross to wild type, indicating that the phenotype is caused by a single nuclear mutation. Cold sensitivity also segregates 2:2 and is completely linked to the noncomplementation phenotype. To determine the linkage relationship of *tnc1* to the *TUB2* locus, the diploid resulting from the cross of *tnc1* to *tub2-104* was sporulated. This diploid exhibited a very low sporulation frequency, and poor spore viability, so *tnc1* was crossed to a strain carrying the temperature-sensitive *act1-1* mutation (SHORTLE, NOVICK and BOTSTEIN 1984). *ACT1* is the actin gene of *S. cerevisiae* (GALLWITZ and SEIDEL 1980; NG and ABELSON 1980), and is very tightly linked to the *TUB2* gene (approximately 1000 base pairs separate the *ACT1* and *TUB2* genes) (THOMAS, NEFF and BOTSTEIN 1985). The results of the cross of *tnc1* to *act1-1* in Table 2 demonstrate that *tnc1* is unlinked to the *ACT1-TUB2* locus and is therefore an unlinked noncomplementer of *tub2-401*.

TABLE 2

Meiotic mapping of *tnc1*

Cross	PD	NPD	TT	Distance (cM)[a]
tub2-104 × *act1-1*	57	0	0	0
tnc1 × *act1-1*	4	6	20	Unlinked
tnc1 × *TUB1:LEU2:TUB1*	24	0	0	0
tnc1 × *TUB3:URA3:TUB3*	5	0	17	39

PD = parental ditype, NPD = nonparental ditype, TT = tetratype. *tnc1* was followed in these crosses by its Cs^- phenotype, which is tightly linked to the noncomplementation phenotype. *act1-1* was followed by its Ts^- phhenotype, and the *TUB1* and *TUB3* gene duplications were followed by the inserted nutritional marker phenotype.

[a] The map distances were calculated according to MORTIMER and SCHILD (1981).

***tnc1* is a mutation in an α-tubulin gene:** Since the subunit of microtubules is a heterodimer of α-tubulin and β-tubulin (LUDUEÑA, SHOOTER and WILSON 1977), it seemed a strong possibility that *tnc1* could be a mutation in one of the two α-tubulin genes of yeast, *TUB1* and *TUB3*. Indeed, it has recently been shown that one class of mutation that fails to complement a testis-specific β-tubulin mutation in Drosophila is tightly linked to a major α-tubulin locus (T. HAYS and M. FULLER, personal communication). *TUB1* and *TUB3* are 36 cM apart on the left arm of chromosome XIII, and have been marked by integration of selectable markers (SCHATZ, SOLOMON and BOTSTEIN 1986). To determine whether *tnc1* is linked to either *TUB1* or *TUB3*, strains marked at those loci were crossed to *tnc1* and asci were dissected. The results in Table 2 show that *tnc1* is tightly linked to *TUB1*, and slightly linked (39 cM) to *TUB3*, as expected for an allele of the *TUB1* gene.

To demonstrate further that *tnc1* is a mutation in *TUB1*, complementation experiments with cloned genes were carried out. Strains carrying the *tnc1* mutation were transformed with plasmids containing

TABLE 3

Complementation and suppression of *tnc1* by plasmid-borne α-tubulin genes

Genotype	Incubation temperature (°C)			
	11	14	16	26
TNC1	+	+	+	+
tnc1	−	−	±	+
tnc1 (*TUB1* on YCp50)	+	+	+	+
tnc1 (*TUB3* on YEp24)	+	+	+	+
tnc1 (*TUB3* on YCp50)	±	+	+	+

tnc1 mutants were transformed with pRB326 (*TUB1* on YCp50), pRB325 (*TUB3* on YCp50), or pRB316 (*TUB3* on YEp24), and assayed for growth at the indicated temperatures by spotting suspensions of cells with an inoculating device. Growth was scored as follows: +, uniform growth of the spot; ±, slow uniform growth; −, no growth.

either the *TUB1* or *TUB3* genes. Table 3 shows the results of such an experiment; the *TUB1* gene in single copy complements the cold sensitivity of the *tnc1* mutation, the *TUB3* gene in multiple copies is able to suppress the cold-sensitivity of *tnc1*, and the *TUB3* gene in a single copy weakly suppresses the cold sensitivity. On the basis of the complementation, suppression, and recombination results, *tnc1* is an allele of the α-tubulin gene *TUB1*, and henceforth will be referred to as *tub1-1*.

Phenotypes of *tub1-1*: The phenotypes of strains carrying cold-sensitive mutations in the *S. cerevisiae* β-tubulin gene *TUB2* have been extensively studied (THOMAS, NEFF and BOTSTEIN 1985; HUFFAKER, THOMAS and BOTSTEIN 1988), as have the phenotypes of mutants defective in both the α-tubulin and β-tubulin genes of *S. pombe* (HIRAOKA, TODA and YANAGIDA 1984; TODA *et al.* 1984). Conditional mutations in these genes usually result in a specific cell-cycle arrest, defects in microtubule structures, and often in altered sensitivity to anti-microtubule drugs. We examined *tub1-1* mutant cells for such phenotypes.

First, we found that *tub1-1* causes super-sensitivity to the anti-microtubule drug benomyl. Benomyl is a member of the benzimidazole class of compounds that have been shown to inhibit microtubule-mediated processes in yeast *in vivo* (QUINLAN, POGSON and GULL 1980; WOOD and HARTWELL 1982; DELGADO and CONDE 1984; PRINGLE *et al.* 1986), and to inhibit microtubule assembly *in vitro* (KILMARTIN 1981). Benomyl inhibits the growth of wild-type haploid yeast at concentrations between 15 and 20 μg/ml; *tub1-1* strains are completely inhibited by 2.5 μg/ml (Table 4). A diploid with the genotype *TUB1/tub1-1* is benomyl supersensitive, demonstrating that the phenotype is dominant. One interesting aspect of the action of benomyl on yeast cells is that wild-type diploids are more sensitive to the drug than wild-type haploids (Table 4). This difference between haploids and diploids is not an effect of mating

TABLE 4

Growth of unlinked noncomplementing mutants on benomyl

Genotype	Benomyl concentration (μg/ml)					
	1	2.5	5	10	20	50
TUB1	+	+	+	+	±	−
tub1-1	+	−	−	−	−	−
TUB1/TUB1	+	+	+	±	−	−
TUB1/tub1-1	+	−	−	−	−	−
tub2-501	+	+	±	−	−	−
tub2-502	+	+	+	+	+	+
tub3-1	+	+	p	p	−	−

Cells were suspended in water and transferred to YEPD plates containing the indicated concentrations of benomyl with an inoculating device. Growth was scored as follows: +, uniform growth of the spot; ±, slow uniform growth; p, papillated growth; −, no growth.

TABLE 5

Cell morphology distribution of unlinked noncomplementing mutants at permissive and nonpermissive temperature

Diploid genotype	Incubation temperature (°C)[a]	Cell morphology distribution[b]		
		Unbudded	Small budded	Large budded
Wild type	26	32	36	32
	11	34	34	32
tub1-1/tub1-1	26	32	35	33
	11	9	20	71
tub2-501/tub2-501	26	33	35	32
	11	6	12	82

[a] Exponentially growing cultures at 26° were shifted to the indicated temperatures for approximately two generation times, 3 hr at 26°, and 24 hr at 11°.

[b] The cell morphology distributions were determined by counting at least 200 cells. Small budded refers to cells with buds <⅔ the diameter of the mother cell, large budded to cells with buds >⅔ the diameter of the mother cell.

type; a strain that is haploid except for the presence of two copies of chromosome *III*, one expressing *MATa* information, the other *MATα*, is as resistant to benomyl as a wild-type haploid of either mating type (data not shown).

Second, we found that *tub1-1* causes a cold-sensitive cell-division-cycle arrest at mitosis. Strains expressing the *tub1-1* phenotype grow at a rate comparable to wild-type at the permissive temperature of 26°, but fail to grow at the nonpermissive temperature of 14°. Mutants affected in chromosome segregation often arrest in the cell-cycle as large-budded cells at the nonpermissive temperature (PRINGLE and HARTWELL 1981). A temperature shift experiment was carried out to determine whether *tub1-1* causes such a cell-cycle arrest in the cold. Diploid strains with the genotype *TUB1/TUB1* or *tub1-1/tub1-1* were grown to exponential phase at 26°, then shifted to 11° for 24 hr, approximately 2 generation times for a wild-type strain. Examination of the distribution of cell

morphologies after the temperature shift indicates that *tub1-1* strains arrest as large-budded cells (Table 5), although the arrest is not as complete as in true cell-division-cycle (*cdc*) mutants (HARTWELL, CULOTTI and REID 1970). It is worth noting that in *tub1-1* mutants there is an intact *TUB3* gene that encodes a functional α-tubulin, possibly accounting for the lack of a tight cell-division-cycle arrest.

Third, we found that *tub1-1* causes the formation of an aberrant spindle at the nonpermissive temperature. The spindle of yeast can be visualized by indirect immunofluorescence with anti-tubulin antibodies (KILMARTIN, WRIGHT and MILSTEIN 1982; KILMARTIN and ADAMS 1984), and the DNA of the nucleus and mitochondria can be stained with DAPI (WILLIAMSON and FENNELL1975). *TUB1/TUB1* and *tub1-1/tub1-1* cultures were grown as described for the cell-cycle experiment above and prepared for immunofluorescence. The results are shown in Figure 2. Wild-type cells grown at 26° show characteristic staining: in large-budded cells (approximately 30% of the population) the nucleus has divided (Figure 2b), and the spindle is either elongated or has already elongated and is breaking down just prior to cytokinesis (Figure 2a). In contrast, *tub1-1/tub1-1* cells arrested at 11°, which are mostly large-budded and considerably larger in size than the wild-type cells, have an undivided nucleus (Figure 2d). The single nucleus has often moved to the neck of the cell, or proceeded to enter the neck. The spindle has not elongated and there are long cytoplasmic microtubules extending from the spindle pole bodies (Figure 2c). Wild-type cells grown at 11° and *tub1-1* cells grown at 26° are indistinguishable from wild-type cells grown at 26° (data not shown).

Specificity of the noncomplementation interaction: The observation that two mutations display a novel type of genetic interaction is often interpreted to mean that the gene products interact in some direct way. While this is known to be true by biochemical means for the case of α-tubulin and β-tubulin (LUDUEÑA, SHOOTER and WILSON 1977), it need not be true that the *tub1-1* and *tub2-401* mutant gene products interact in a specific way that causes the failure of the complementation test. An alternative model is that the *tub1-1* mutation in heterozygous condition constitutes a "weak link" in the pathway of chromosome segregation, and that the additional stress caused by being heterozygous for any of a number of other mutations in the same pathway would cause the cell to arrest in the cell cycle. To investigate this, we tested the gene-specificity and allele-specificity of the noncomplementation phenotype.

Gene specificity was tested by crossing *tub1-1* to six different cold-sensitive *cdc* mutants: *cdc44-1, cdc45-1, cdc48-1, cdc51-1*, Y, and Z (MOIR *et al.* 1982; *Y* and *Z* do not have *cdc* numbers because they have not been demonstrated to be different from previously isolated temperature-sensitive *cdc* mutants). These mutants all arrest as large-budded cells upon shifting to the nonpermissive temperature, and thus have defects in the same general part of the cell cycle as *tub1-1*. Strains expressing *tub1-1* complemented all of these mutants at the nonpermissive temperature, thus the noncomplementation is gene specific.

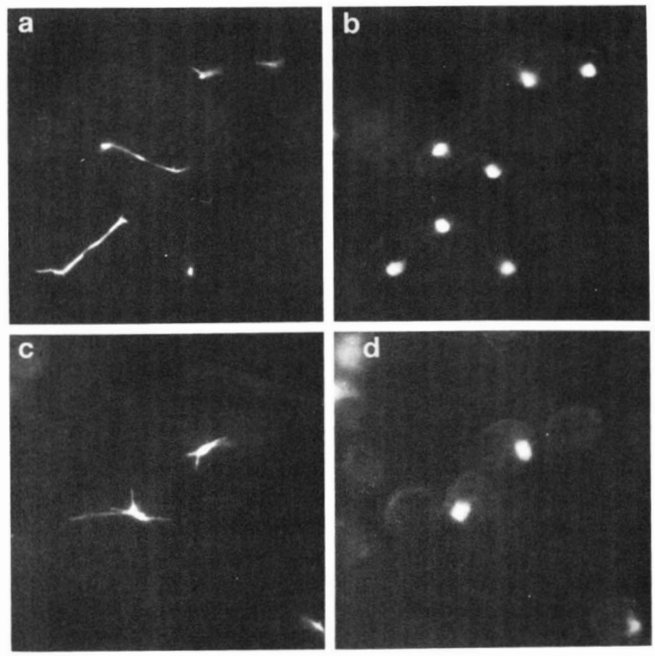

FIGURE 2.—Fluorescence micrographs of *TUB1/TUB1* and *tub1-1/tub1-1* cells. *TUB1/TUB1* wild-type cells grown at 26° (a and b) and *tub1-1/tub1-1* mutant cells grown at 26°, then shifted to 11° for 24 hr (c and d). Left (a and c), indirect immunofluorescence of tubulin. Right (b and d), the same cells stained with DAPI to reveal the location of the DNA.

Allele specificity was tested by crossing *tub1-1* to six recessive cold-sensitive β-tubulin mutants *tub2-104, tub2-401, tub2-402, tub2-403, tub2-404* and *tub2-405*. These alleles of *TUB2* have been characterized as to their cold sensitivity, benomyl resistance or supersensitivity, and microtubule structures present after a shift to the nonpermissive temperature (THOMAS, NEFF and BOTSTEIN 1985; HUFFAKER, THOMAS and BOTSTEIN 1988). At 16° the *tub1-1* mutation fails to complement the *tub2-401* and *tub2-402* mutations, but complements other alleles of *TUB2* (Table 6). At lower temperatures (14° and 11°) *tub1-1* also fails to complement *tub2-405*. The *TUB1/tub1-1 TUB2/tub2-104* double heterozygote grows poorly at all temperatures. This allele specificity is not simply due to the differing severity of the *TUB2* mutants examined; *tub2-401* is the most cold sensitive of the alleles tested, and shows little or no microtubule structure left after a shift to the cold, but *tub2-402* is one of the leakier mutants, and has a considerable amount of structure in the cold (HUFFAKER, THOMAS

TABLE 6

Allele specificity of the unlinked noncomplementation interaction[a]

Diploid genotype	Incubation temperature (°C)			
	11	14	16	26
tub1-1 / + + / *tub2-104*	±	±	±	±
tub1-1 / + + / *tub2-401*	−	−	−	+
tub1-1 / + + / *tub2-402*	−	−	−	+
tub1-1 / + + / *tub2-403*	+	+	+	+
tub1-1 / + + / *tub2-404*	+	+	+	+
tub1-1 / + + / *tub2-405*	−	±	+	+

[a] See the legend to Table 4 for experimental details.

and BOTSTEIN 1988). *tub2-403* has a phenotype very similar to that of *tub2-401* in terms of cold sensitivity and microtubule morphology, but is complemented by *tub1-1*. It is clear from these results that the noncomplementation phenotype that *tub1-1* displays is gene specific and allele specific, and that the interaction is more complex than would be indicated by a simple model based on the additive severity of the mutations involved.

Isolation of unlinked noncomplementers of *tub1-1*: The cold sensitivity of *tub1-1* allowed us to initiate another noncomplementation screening, this time using *tub1-1* as the starting point. We crossed 15,000 colonies grown from mutagenized cells to a *tub1-1* strain and to a wild-type strain. We found two unlinked noncomplementers; *tnc2*, which causes cold sensitivity and benomyl supersensitivity, and *tnc3*, which causes benomyl supersensitivity, but is not by itself cold sensitive. To determine whether *tnc2* is an allele of any of the tubulin genes, a *tnc2* strain was crossed to strains marked at the tubulin loci; the resulting diploids were scored for complementation, and sporulated to test linkage. The *tnc2* mutation fails to complement all *tub2cs* alleles tested, and the tetrad results indicate that *tnc2* is tightly linked to the *TUB2* locus (28 PD: 0 NPD: 0 TT). On the basis of the complementation and recombination tests, *tnc2* is an allele of the *TUB2* gene, and will be referred to as *tub2-501*. Cells carrying the *tub2-501* mutation arrest as large-budded cells at 14° (Table 5) and are sensitive to 10 μg/ml of benomyl (Table 4). The phenotype of *tub2-501* mutants is similar in these respects to that of previously characterized *tub2* mutations (HUFFAKER, THOMAS and BOTSTEIN 1988).

Strains carrying the *tnc3* mutation are sensitive to 5 μg/ml of benomyl, but papillate to Ben$^+$ (approximately wild-type benomyl resistance) at a high frequency. This phenotype is similar to that of *TUB3* null alleles constructed by gene disruption (SCHATZ, SOLOMON and BOTSTEIN 1986). The papillation of *TUB3* null alleles to Ben$^+$ is most likely caused by gain of an extra copy of chromosome *XIII*, which, in a *tub3::TRP1* strain, contains both the wild-type *TUB1* gene and *tub3::TRP1*; the extra copy of *TUB1* suppresses the Ben^{SS} phenotype of the *tub3::TRP1* mutation.

To determine whether *tnc3* is an allele of *TUB3*, a strain carrying *tnc3* was crossed to a *tub3::TRP1* strain, and complementation of the Ben^{SS} phenotype was scored. The diploid formed was Ben^{SS} and papillated to Ben$^+$, like the parental haploid strains. In contrast, the diploid formed between a *tnc3* strain and a wild-type strain is resistant to wild-type levels of benomyl. To test linkage of *tnc3* to *TUB3*, a *tnc 3* strain was crossed to a strain containing a duplication of the *TUB3* locus, marked by an insertion of the *URA3* gene. The results of tetrad analysis of this diploid establish that *tnc3* is tightly linked to the *TUB3* locus (12 PD: 0 NPD: 0 TT), and it will be referred to as *tub3-1*.

Since *tub3-1* and *tub3::TRP1* have similar phenotypes, it seemed possible that the *tub3::TRP1* null allele may also fail to complement *tub1-1*. To investigate this, *tub1-1* strains were crossed to *tub3-1* and *tub3::TRP1* and the resulting diploids were scored for their ability to grow at 14°. The interesting result is that both *tub3-1*, presumably a point mutation, and *tub3::TRP1*, a null mutation, fail to complement *tub1-1*. This means that the failure to complement in this case is not due to a specific protein-protein interaction. Instead, it seems that a reduction in the total level of functional α-tubulin in the double heterozygote causes the failure to grow at 14°.

Linked noncomplementers: Mutations within the same gene fail to complement in most cases; therefore the expected result from a noncomplementation screen as performed here is the isolation of more mutations in the gene being screened against. This expectation was realized in the experiments described in this paper, but, surprisingly, such linked noncomplementers were not found any more frequently than the unlinked noncomplementers that we sought. Two new *TUB2* mutants were isolated while searching for unlinked noncomplementers of *TUB2*. One of these, *tub2-502*, is resistant to high concentrations of benomyl (Table 4). It has previously been shown that when spontaneous mutations conferring resistance to high concentrations of benomyl are selected, the mutation causing the resistance is invariably found to be in the *TUB2* gene (THOMAS, NEFF and BOTSTEIN 1985); this presumably reflects the mode of action of benomyl. The other *TUB2* mutant, *tub2-503*, is of

interest because it fails to complement *tub2-403* and *tub2-405* at 14°, but does complement the other *tub2* mutants, including *tub2-401*, which is completely inhibited at 14° as a haploid. If the temperature is lowered to 11°, *tub2-503* fails to complement all of the *tub2* mutant alleles tested. The fact that there is intragenic complementation between *tub2-503* and some other of the *tub2* alleles is not surprising given the known polymeric nature of the microtubule, and the likely interaction of β-tubulin molecules within that polymer. Another explanation for the observed intragenic complementation is that the β-tubulin mollecule may have more than one role in the cell, such as a role in regulation as well as the established role as a subunit of microtubules (BEN-ZE'EV, FARMER and PENMAN 1979; CLEVELAND *et al.* 1981).

Two new *TUB1* mutants, *tub1-13* and *tub1-14* were isolated while screening for unlinked noncomplementers of *tub1-1*. Each of these two mutations confers only a slight cold sensitivity at the nonpermissive temperature but completely fails to complement a *tub1-1* mutation. *tub1-13* also causes a slightly increased sensitivity to benomyl.

Lethality of *tub1-1 tub3* double mutants: In the course of mapping *tub3-1*, a cross of *tub3-1* to *tub1-1* was analyzed. In this cross there was a pattern of spore inviability that suggested that a *tub1-1 tub3-1* double mutant is inviable at a temperature that is permissive for either single mutant. Tetrads that had four viable spores were parental ditype with respect to *TUB1* and *TUB3*, and most tetrads that had three viable spores could be inferred to be tetratype (no nonparental ditypes were observed in the nine tetrads dissected, consistent with the 36-cM distance between *TUB1* and *TUB3*). The tetratype tetrads consisted of one wild-type spore, one *tub1-1* spore, and one *tub3-1* spore, with the presumed double mutant failing to grow. The same result is obtained if *tub1-1* and *tub3*::*TRP1* are crossed. These experiments are complicated, however, by the observation that *tub3* null mutants have reduced spore germination even in the presence of wild-type *TUB1* (SCHATZ, SOLOMON and BOTSTEIN 1986).

To determine whether *tub1-1 tub3*::*TRP1* double mutant is incapable of growing mitotically or is simply unable to germinate to form a colony, we repeated the experiment, this time including a plasmid bearing *TUB3* to complement the *tub3*::*TRP1* defect. The presence of this plasmid should allow the double mutant segregants to germinate and grow into colonies; cells that had lost the complementing plasmid could then be selected to determine the ability of the double mutants to grow without *TUB3*. A strain carrying *tub3*::*TRP1* was crossed to two *tub1-1* strains, one with a 2μ-based plasmid containing *TUB3* and the selectable marker *URA3*, the other with no plasmid, but otherwise isogenic. The two diploid strains resulting from these crosses were sporulated and dissected. The diploid with the *TUB3* plasmid produced 38/46 tetrads with all four spores viable. The remaining eight tetrads had three viable spores. The diploid without the plasmid gave the characteristic pattern of spore inviability described above for the cross of *tub1-1* × *tub3-1*. The segregants from the diploid carrying the plasmid were incubated on YEPD plates for several generations, then on plates containing 5-FOA to select cells that had lost the plasmid (BOEKE, LACROUTE and FINK 1984). The segregants were also analyzed to determine their genotype with respect to *TUB1* and *TUB3*. In all cases, the segregants that were deduced to be double mutants were unable to grow on the 5-FOA plates, and thus are assumed to require the *TUB3* plasmid for mitotic growth. These results indicate that a *tub1-1 tub3*::*TRP1* double mutant is unable to grow mitotically at the permissive temperature for either *tub1-1* or *tub3*::*TRP1*. The combination of these two mutations in a haploid therefore is a synthetic lethal (DOBZHANSKY 1946; STURTEVANT 1956); the double mutant is inviable under conditions that are permissive for either of the single mutants alone.

DISCUSSION

The complementation test classically defines the gene (BENZER 1955): when strains bearing recessive mutations in different genes are crossed, they usually complement; when strains bearing recessive mutations in the same gene are crossed, they usually fail to complement. There are exceptions, of course, and in the experiments described in this paper we have exploited the exceptional case of unlinked noncomplementation, where two recessive mutations in different genes fail to complement. We have demonstrated that strains bearing mutations in unlinked genes that encode interacting proteins may fail to complement, and we have used this property of unlinked noncomplementation to isolate several new mutations in the α-tubulin and β-tubulin genes of yeast. These mutations cause phenotypes, such as conditional lethality or altered drug resistance, that allow further analysis of the role that these proteins and their interactions play *in vivo*. The method that we have used is generally applicable, and has several advantages over more traditional methods of isolating mutations in interacting gene products.

New tubulin mutants: The *tub1-1* mutation was isolated as an unlinked noncomplementer of *tub2-401*. This is the first conditional-lethal α-tubulin mutation reported in *S. cerevisiae*. The *tub1-1* mutation causes cold-sensitivity, cell-division-cycle arrest at mitosis at the nonpermissive temperature, and benomyl supersensitivity. Previous work on *TUB1* and *TUB3*, the other α-tubulin gene of yeast, dem-

onstrated that the two genes encode α-tubulins that are 90% homologous (SCHATZ *et al.* 1986), and that *TUB1* is an essential gene, while *TUB3* is not essential (SCHATZ, SOLOMON and BOTSTEIN 1986). The genetic difference between *TUB1* and *TUB3* is not reflective of a functional difference in the proteins however; either gene alone can perform all of the *in vivo* roles of α-tubulin, although *TUB3* must be supplied in more than single copy to suppress a *tub1* null mutation (SCHATZ, SOLOMON and BOTSTEIN 1986). By isolating the cold-sensitive lethal *tub1-1* mutation, we have shown that the phenotype of a *tub1* mutant is a block in mitosis, even in the presence of wild-type *TUB3*. This phenotype is similar to that of conditional lethal β-tubulin mutants (THOMAS, NEFF and BOTSTEIN 1985; HUFFAKER, THOMAS and BOTSTEIN 1988). This situation is very similar to that observed in *S. pombe* where the *NDA2* gene encoding α-1 tubulin is essential and was originally identified as a cold-sensitive mutation that blocks mitosis (TODA *et al.* 1983). This conditional lethality occurs even in the presence of a wild-type copy of the gene encoding the α-2 tubulin, which is not essential (ADACHI *et al.* 1986).

The use of *tub1-1* as the mutation to be complemented in a new round of screening for unlinked noncomplementers resulted in the isolation of *tub2-501* and *tub3-1*. These are mutations in β-tubulin and the minor α-tubulin, respectively, that have conditional phenotypes of their own. The success of this "chain of unlinked noncomplementers" is analogous to that of the "chain of revertants" described by JARVIK and BOTSTEIN (1975) and clearly demonstrates that several new conditional-lethal mutations can be generated from a single starting mutation by sequential rounds of unlinked noncomplementation analysis.

In addition to the unlinked noncomplementers that we sought, we also found linked noncomplementers, as expected. Two new *tub2* mutants were isolated by screening against known *tub2cs* mutants, and two new *tub1* mutants were isolated by screening against *tub1-1*. It is interesting that unlinked noncomplementers, normally considered to be an exceptional class, were found as often as linked noncomplementers. This does not seem to be an intrinsic property of the tubulin genes, as a similar approach has been applied to conditional-lethal actin mutants with similar results (D. DRUBIN and D. BOTSTEIN, personal communication).

Synthetic lethality: We attempted to combine *tub1-1* with *tub3::TRP1*, a null mutation in the minor α-tubulin gene that has only subtle phenotypes of its own. This would allow us to assess the phenotype of *tub1-1* in the absence of the α-tubulin encoded by *TUB3*. The combination of these two mutations in a haploid results in a synthetic lethal phenotype, however; the double mutant is not viable even under conditions that are permissive for either a *tub1-1* or *tub3::TRP1* single mutant. This indicates that the mutant α-tubulin encoded by *tub1-1* is not sufficient, in either quantity, quality, or both, to perform all of the required functions of α-tubulin. Recent experiments have shown that additional plasmid-borne copies of *tub1-1* allow a *tub1-1 tub3::TRP1* strain to survive at the permissive temperature, but do not alleviate the cold-sensitivity of the *tub1-1* mutation (T. STEARNS and D. BOTSTEIN, unpublished results).

Why do unlinked noncomplementers fail to complement? Two types of unlinked noncomplementation were observed. The first is noncomplementation between alleles of *TUB1* and *TUB2*. It is known that the α-tubulin and β-tubulin molecules form an α/β heterodimer that is the subunit of the polymerized microtubule (LUDUEÑA, SHOOTER and WILSON 1977). This association of α-tubulin and β-tubulin into a functional heterodimer is likely to be sensitive to changes in the physically interacting regions of the proteins and it may be these regions that are altered by the cold-sensitive *tub1* and *tub2* mutations. Cold-sensitive mutations in general are thought to be due to defects in protein-protein interaction (GUTHRIE, NASHIMOTO and NOMURA 1969; TAI, KESSLER and INGRAHAM 1969). Microtubules themselves are inherently cold sensitive (WEISENBERG 1972), possibly making them susceptible to subtle changes in protein-protein interactions.

The allele specificity of the noncomplementation between *tub1-1* and the *tub2* mutations suggests that the noncomplementation phenotype is due to a specific interaction between mutant gene products at the protein level. Two models could explain how unlinked noncomplementation works in this case. One model would be that the combination of either mutant α-tubulin or β-tubulin with the corresponding wild-type subunit would result in a heterodimer that is nonfunctional at the nonpermissive temperature, and that the double mutant heterodimer is also nonfunctional. Assuming that the mutant subunits compete equally with the wild-type subunits, this would result in the situation diagrammed in Figure 3a. Allele specificity in this model might be due to differences in the ability of the mutant subunits to compete with the wild-type subunits. This "subunit level" model, in its simplest form, assumes that the cell is capable of growing with one-half of the normal amount of functional tubulin heterodimer, but is incapable of growing with only one-quarter of the normal amount. An alternative, the "poison subunit" model, would be that the double mutant heterodimer has a unique property that prevents proper functioning of the entire microtubule assembly of which it is a part (Figure 3b). A possible type of poisoning would be that addition of the double mutant heterodimer to the end of a microtubule prevents addition of

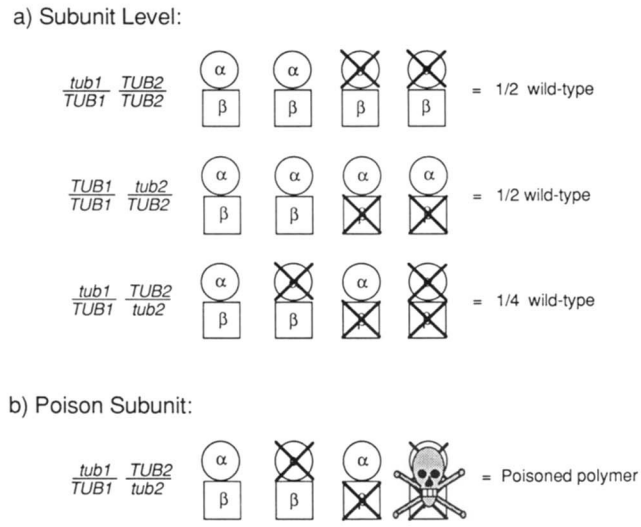

FIGURE 3.—Models for the mechanism of unlinked noncomplementation.

more subunits. This model predicts that simple addition of more wild-type heterodimer would not relieve the mutant phenotype.

An experiment that might clearly demonstrate the validity of the assumption that the noncomplementation phenotype is due to interactions between mutant subunits, and thus that the above models are reasonable, would be to cross a *tub1* null allele to the *tub2* mutants. If the noncomplementation is due to specific interactions between mutant subunits, then the *tub1* null allele would be expected to complement all of the *tub2* mutations. Unfortunately, this experiment would be quite difficult to perform and interpret, as *tub1* is an essential gene in halpoids, and diploid strains heterozygous for a *tub1* null allele show dominant phenotypes and rapidly become aneuploid (SCHATZ, SOLOMON and BOTSTEIN 1986).

The second type of unlinked noncomplementation is the case of mutations in *TUB3* failing to complement *tub1-1*. Both *TUB1* and *TUB3* encode α-tubulins, and both assemble into microtubules (SCHATZ *et al.* 1986). The failure to complement in this case appears to be allele nonspecific; a null allele of *TUB3* fails to complement *tub1-1* to the same extent that *tub3-1* fails to complement. It is likely that this failure to complement is due to a reduced level of functional α-tubulin in cells that are shifted to the nonpermissive temperature, and that it is this reduced level that prevents the cells from completing some essential step of mitosis.

Advantages of the unlinked noncomplementation approach: It is useful to compare the unlinked noncomplementer approach that we describe in this paper to the more commonly used method of isolating interacting gene products, selection of pseudorevertants. In this method, revertants of a conditional mutation are selected, then screened to identify those that have new mutations, unlinked to the original, that confer a phenotype of their own. That these new mutations will often be found in physically interacting elements of the system has been demonstrated (JARVIK and BOTSTEIN 1975; MORRIS, LAI and OAKLEY 1979).

As a genetic tool, screening for unlinked noncomplementing mutations in yeast as described here has several advantages over pseudorevertant selection. First, the haploid mutants, saved before mating, are available for analysis as single mutants. This allows the phenotype of the noncomplementing mutation itself to be readily assessed. This is particularly important in the case of the intragenic mutations isolated as a part of the screen; these are isolated as single mutants, while intragenic mutations identified in pseudorevertant selections are isolated as double mutants with the original mutation, and are difficult to separate from the original mutation. Second, the assessment of the noncomplementation is done in a diploid, allowing for complementation of any extraneous recessive mutations induced by the mutagenesis. This is a significant problem when selecting pseudorevertants because recessive mutations that reduce the growth rate are often picked up as weak nonspecific pseudorevertants (T. HUFFAKER, T. STEARNS and D. BOTSTEIN, unpublished results). Third, unlinked noncomplementation analysis is nonselective. Typically, pseudorevertants are selected for growth under nonpermissive conditions, which can create problems if the nonpermissive condition itself is mutagenic. In contrast, unlinked noncomplementation analysis involves screening by replica-plating, so that the original mutant is never exposed to nonpermissive conditions.

Although these advantages are most apparent in an organism such as *S. cerevisiae* that can be stably grown as a haploid or diploid, there are also advantages to this type of analysis in obligate diploid organisms, mainly because complementation can be assayed in the F_1 generation, unlike recessive suppressors, which must be made homozygous before they can be detected.

We suspect that each case in which genetic approaches are used to isolate mutations in interacting gene products will be different in terms of the relative ease with which such mutations are isolated, and thus it is advisable to make use of a number of different approaches when possible. As an example, the success of the unlinked noncomplementer approach with the tubulin mutations in this paper can be contrasted with attempts to isolate pseudorevertants of β-tubulin mutations in yeast. Although there have been some promising results, very many of the pseudorevertants appear to suppress by some indirect means, and do not have phenotypes that suggest an involvement in the tubulin system (THOMAS 1984). The results presented in this paper demonstrate that a screen for

unlinked noncomplementers is a viable alternative to pseudoreversion analysis, that the interaction between the mutations involved can be highly allele specific, and that the mutations isolated in such a screen often have useful phenotypes of their own.

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