MOLECULAR AND CELLULAR BIOLOGY, June 1986, p. 2168–2178
0270-7306/86/062168-11$02.00/0

Vol. 6, No. 6

Differential Expressions of Essential and Nonessential α-Tubulin Genes in *Schizosaccharomyces pombe*

YASUHISA ADACHI, TAKASHI TODA,† OSAMI NIWA, AND MITSUHIRO YANAGIDA*
Department of Biophysics, Faculty of Science, Kyoto University, Sakyo-ku, Kyoto 606, Japan

Received 18 November 1985/Accepted 4 March 1986

The fission yeast *Schizosaccharomyces pombe* has two α-tubulin genes and one β-tubulin gene. Gene disruption experiments showed that the α1-tubulin gene (*NDA2*) is essential whereas the α2 gene is dispensable. The α2-disrupted cells missing α2 transcript and α2 polypeptide could grow and sporulate normally. The α2 gene, however, was expressed in the wild type and the α1 mutant. α2-Tubulin was distinguished as an electrophoretic band and was assembled into microtubules. The α2-disrupted cells had an increased sensitivity to an antimicrotuble drug thiabendazole, and the α1(cold-sensitive [cs]) α2 (disrupted) cells became not only cs but also temperature sensitive. Northern blot experiments indicated that α2 transcription was minor and constitutive whereas α1 transcription was major and modulated, depending on the gene copy number of the α2 gene. The amounts of α1 and α2 polypeptides estimated by β-galactosidase activities of the *lacZ*-fused genes integrated on the chromosome and by intensities of the electrophoretic bands in crude tubulin fractions, however, were comparable, indicating that α2 tubulin is not a minor subtype. We assume that the cells of *Schizosaccharomyces pombe* have no excess tubulin pool. α1 mutants would then be blocked in the cell cycle because only half the amount of functional α-tubulin required for growth can be produced by the α2 gene. On the other hand, the α2-disrupted cells became viable because the synthesis of α1 tubulin was increased by transcriptional or translational modulation or both. The real cause for essential α1 and dispensable α2 genes seems to be in their regulatory sequences instead of the coding sequences.

Tubulins are the major components of microtubules and play a fundamental role in cellular architecture, cell motility, and chromosome separation; they consist of α and β subunits. In higher eucaryotic organisms, tubulin genes are generally a multigene family whose individual members are dispersed in the genome (5, 9); many are known to show developmentally regulated and tissue-specific expression. The *Drosophila melanogaster* genome for example, has four each of α- and β-tubulin gene sequences (15, 31). One of the α-tubulins is the major transcript during oogenesis and after embryogenesis (22). One β-tubulin gene is expressed only in testis (16), and its mutants cause male sterility. The chicken genome has several α- and β-tubulin genes (19). One β-tubulin gene produces the major β-tubulin in neuronally derived tissues. Thus, the tubulin gene families appear to serve as a basis for different tubulin functions in various cells and tissues of multicellular organisms, and their expression may well be under different control mechanisms.

The fission yeast *Schizosaccharomyces pombe*, a unicellular lower eucaryote, has two α-tubulin genes and one β-tubulin gene in its haploid genome (13, 37). These genes were cloned and their nucleotide sequences were determined. One of the two α-tubulin genes (α1 gene) was originally identified by a cold-sensitive (cs) mutation (*nda2*) that blocked nuclear division and caused nuclear dislocation (38, 40). Subsequently, the α1 gene (*nda2*) was found to be a major supersensitive (ss) locus for thiabendazole and related benomyl compounds known as tubulin inhibitors (40). The other gene, α2, was identified as one of two genomic sequences which were isolated by transformation and which complemented the cs and ss phenotypes of α1 (*nda2*) mutations (37). None of the cs and ss mutants have been mapped at the α2 gene.

Nucleotide sequence determination showed that both α1 and α2 sequences encoded α-tubulin (37). Chromosomal integration of the cloned sequences proved that the α1 sequence was derived from *NDA2* but the α2 was from a locus not linked to *nda2*. The α1 gene had an intron, and its predicted amino acid sequences consisted of 455 residues, whereas the α2 gene, without intron, encoded 449 residues. The amino acid sequence homology between α1 and α2 was 85%. The homology to porcine α-tubulin was 76% in both cases. No significant homology was found in 5′ upstream sequences of α1 and α2 genes.

The present study was initiated to answer the following questions. (i) Are both α1 and α2 genes expressed? (ii) If they are expressed, then how can a cs lethal α1-tubulin mutant be obtained? (iii) Is there any difference in function or expression between α1 and α2 genes? (iv) How can the α2 gene sequence on a plasmid complement α1 mutations?

We report here that the α1 gene is essential, whereas the α2 gene is dispensable; the haploid cells in which the α2 gene was disrupted contained neither α2 transcript nor α2 polypeptide but could grow and sporulate normally. However, the α2 gene is expressed in both the wild type and α1 mutants. Its expression was detected transcriptionally, translationally, and phenotypically and differed considerably from that of α1 gene. Furthermore, the α2 polypeptide identified in this study was not a residual minor subtype but seemed to be a major one despite of its minor mRNA content. We will present a plausible explanation to the above problems based on a limited tubulin pool hypothesis combined with modulative α1 and constitutive α2 expression and

* Corresponding author.
† Present address: Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724.

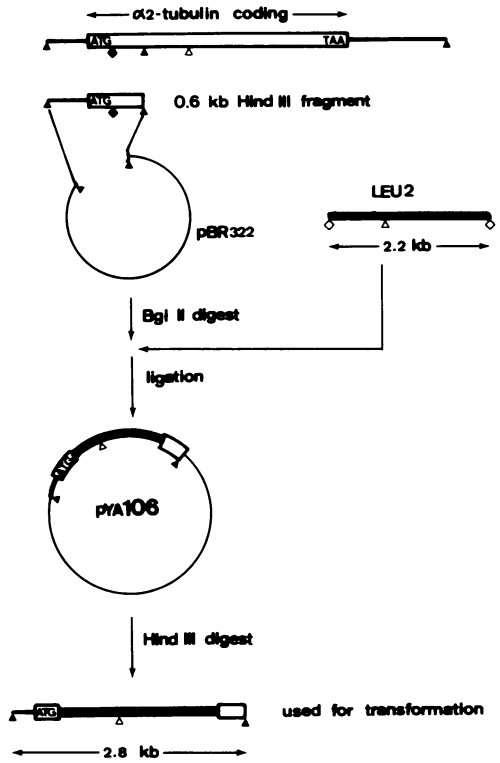

FIG. 1. Construction of pYA106 used for disruption of the α2 gene. The 0.6-kb *Hin*dIII fragment of the α2 tubulin gene (37) was cloned into pBR322 and digested with *Bgl*II, which produced a single cut within the 73th codon of α2. The linearized plasmid was ligated with the 2.2-kb *Sal*I-*Xho*I fragment of the *Saccharomyces cerevisiae LEU2* gene that had been associated with *Bam*HI linkers at both ends. The resulting plasmid pYA106 consists of pBR322 and the 0.6-kb α2 gene fragment interrupted by the *LEU2* gene. Restriction sites: ▶, *Hin*dIII; ▷, *Eco*RI; ◆, *Bgl*II; ◇, *Bam*HI.

discuss the real reason that the α1 gene is essential and the α2 gene is dispensable.

MATERIALS AND METHODS

Strains. Strains of *Schizosaccharomyces pombe* used were JP37 (h^{90} *leu1 ade6*-M210), JP38 (h^- *leu1 ade6*-M216 *nda2*-KM52), KM52-110 (h^- *leu1 nda2*-KM52), and HM123 (h^- *leu1*). h^{90} and h^- were the mating type alleles. A diploid strain for a recipient for transformation was constructed by mating JP37 with JP38 on an SPA plate (see below) for 12 h at 26°C and then streaking colonies on minimal plates containing leucine. It had homozygous *leu1* and heterozygous *nda2* markers (the genotype was h^{90}/h^- *leu1/leu1 ade6*-M210/*ade6*-M216 *nda2*-KM52/+). The diploid could be distinguished by intragenic complementation between *ade6*-M210 and *ade6*-M216 which produced white Ade^+ colonies, whereas Ade^- colonies were red (18). The constructed diploid was grown in minimal media supplemented with leucine and used for disruption of the α1 and α2 genes. Standard genetic procedures for *Schizosaccharomyces pombe* were followed (12).

Media and antibiotics. Culture media used for *Schizosaccharomyces pombe* were YPD (complete rich medium; 1% yeast extract, 2% polypeptone, 2% glucose; 1.7% agar was added for plates), SD (minimal medium; 0.67% yeast nitrogen base without amino acids, 2% glucose; 2% agar was added for plates), EMM2 (minimal medium [23] containing [per liter] 10 g of glucose, 5 g of NH_4Cl, 300 mg of NaH_2PO_4, 1 g of sodium acetate, 1 g of KCl, 500 mg of $MgCl_2$, 10 mg of Na_2SO_4, 10 mg of $CaCl_2$, 10 mg of inositol, 10 mg of nicotic acid, 1 mg of calcium pantothenate, 10 mg of biotin, 500 μg of H_3BO_3, 400 μg of $MnSO_4 \cdot H_2O$, 400 μg of $ZnSO_4 \cdot 7H_2O$, 200 μg of $FeCl_3 \cdot 6H_2O$, 160 μg of $H_2Mo \cdot H_2O$, 100 μg of KI, 40 μg of $CuSO_4 \cdot 5H_2O$, and 1,000 μg of citric acid; 2% agar was added for plates), and SPA (sporulation plate medium [12]; 1% glucose, 0.1% KH_2PO_4, 0.001% nicotinic acid, 0.001% inosite, 0.0001% calcium pantothenate, 0.000000001% biotin, 3% agar). *Escherichia coli* was grown in LB (0.5% yeast extract, 1% polypeptone, 1% NaCl [pH 7.5], 1.5% Bacto-Agar was added for plates). Final concentrations of antibiotics used were 40 μg of ampicillin per ml and 170 μg of chloramphenicol per ml.

Plasmids and transformation. YIp32 and YIp33 consist of pBR322 and the *Saccharomyces cerevisiae LEU2* sequences, and have an Amp^r marker (3); pDB248′ consists of pBR322, *LEU2*, and 2μm *ori* sequences (1); YIp(NDA2)1-2 and pYA112 were constructed by ligating YIp33 or YIp32 with the α1- and α2-coding regions, respectively; YIp (NDA2)1-2 contains the 905-base-pair (bp) *Bgl*II fragment spanning the coding region of α1-tubulin gene which was ligated with the *Bam*HI and *Hin*dIII sites on YIp33; pYA112 contains the 1,065-bp *Xho*II fragment (spanning the 49th through 425th amino acid residues in the α2-coding region) which was cloned into the *Bam*HI site of YIp32. pYA106 containing the 0.6-kilobase (kb) *Hin*dIII fragment of the α2-tubulin gene (37) interrupted with the *Saccharomyces cerevisiae LEU2* gene was constructed as described in Fig. 1. *Schizosaccharomyces pombe* was transformed with plasmid by the lithium acetate method described by Ito et al. (14).

Gene disruption. Methods described by Shortle et al. (33) and Rothstein (27) for *Saccharomyces cerevisiae* were followed; the *Saccharomyces cerevisiae LEU2* gene was used as a marker for integration. If a gene is essential, its gene disruption is a lethal event for a haploid cell. Lethality is rescued in a diploid used as a recipient for transformation, and the effect of gene disruption can be detected by tetrad analyses of the diploid transformants. If the disrupted gene is essential, viable and lethal spores will be in the ratio of 2:2, and viable spores will all be Leu^-. When the disrupted gene is dispensable for growth, all spores will be viable (4:0), and the segregation pattern of Leu^+:Leu^- will be 2:2.

Construction of α-tubulin–*lacZ* fusion plasmids. The α-tubulin–*lacZ* fusion plasmids were constructed by the following procedures. The 1.8- and 0.6-kb *Hin*dIII fragments of α1- and α2-tubulin genes, respectively, were cloned into a *Hin*dIII site of pDB248′ or YIp32 and were digested with *Bgl*II, which produced a single cut within the 89th codon in the α1 gene and within the 73th codon in the α2 gene (37). The linearized plasmids were further digested with *Bam*HI and ligated with the 3-kb *Bam*HI fragment derived from pMC1871, which encoded a large carboxy-terminal region of β-galactosidase (4). These α1-*lacZ* and α2-*lacZ* fused genes were expected to be in frame from the nucleotide sequences at their joining parts. The α1-*lacZ* and α2-*lacZ* fused genes contained 625 and 186 bp of the 5′ noncoding upstream sequence, respectively (Fig. 2).

Enzymes. Restriction enzymes and T4 ligase were purchased from Takara Shuzo or New England Biolabs and were used under the conditions specified by the suppliers. Bacterial alkaline phosphatase was obtained from Worthington Biochemicals (code; BAPC) and dialyzed against 50

mM Tris (pH 8.0) with 50% glycerol before use. The reaction was performed in 50 mM Tris (pH 8.0) at 65°C for 1 h.

Preparation of DNA and RNA. For *Schizosaccharomyces pombe* DNA, 100 ml of SD liquid culture (about 2×10^7 cells per ml) was harvested, washed with 50 mM Tris–50 mM EDTA (pH 8.0) and resuspended in 0.8 to 2.0 ml of the same buffer. Siliconized 0.5-mm glass beads were added to the meniscus, and the cells were vigorously agitated with a Vortex mixer six times for 15 s with 45 s of cooling in between. A 0.1 volume of 10% sodium dodecyl sulfate (SDS) and 1 mg of proteinase K per ml were added, and the suspension was incubated at 65°C for 1 h. An equal volume of phenol was added, and the suspension was mixed gently and centrifuged at 5,000 × *g* for 10 min. The aqueous phase was isolated and extracted with phenol. After ether treatment, 10 μl of RNase (10 mg/ml) was added, and the mixture was incubated at 37°C for 30 min. The solution was then extracted with phenol-chloroform-isoamyl alcohol (25:24:1), and the resulting aqueous phase was treated with ether. A 0.1 volume of 3 M sodium acetate was added, and the solution was precipitated with 2 volumes of ethanol. The pellets were washed once with 70% ethanol and suspended in 100 μl of 10 mM Tris hydrochloride–1 mM EDTA (pH 8.0). This yielded 10 to 20 μg of genomic DNA. Plasmid DNAs were prepared from *Escherichia coli* (HB101) by the alkaline lysis method. For large-scale preparation, this method was followed by centrifugation to equilibrium in a cesium chloride-ethidium bromide density gradient (20).

RNA preparation from *Schizosaccharomyces pombe*. *Schizosaccharomyces pombe* RNA was prepared by the method described by Russell and Hall (29). Poly(A)$^+$ mRNA was purified on a oligo(dT)-cellulose column (type 7; P-L Biochemicals, Inc.) by the method of Maniatis et al. (20).

Southern and Northern blotting: hybridization. Southern blots were carried out by the method described by Southern (35). Northern blots were performed by the method of Thomas (36); hybridization was carried out by the method described by Davis et al. (10).

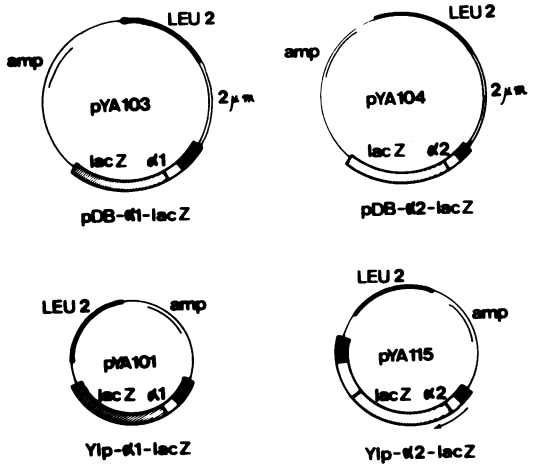

FIG. 2. α-Tubulin-*lacZ* fusion plasmids. Symbols: ■, 5′ flanking sequences of the α1 and α2 genes (625 bp for α1 and 186 bp for α2); □, coding sequences of the α1 and α2 genes (codons 1 through 89 for α1 and codons 1 through 73 for α2); ▨, *lacZ* coding region (3-kb *Bam*HI fragment [4]) in frame to the regions coding α1 and α2. pYA115 was constructed by inserting the 3-kb *Bam*HI fragment of *lacZ* into the *Bgl*II site of YIp(NDA2)2 containing the entire α2 tubulin gene to increase the frequency of integration. The arrow in pYA115 indicates the direction of transcription (orientations are the same in other plasmids).

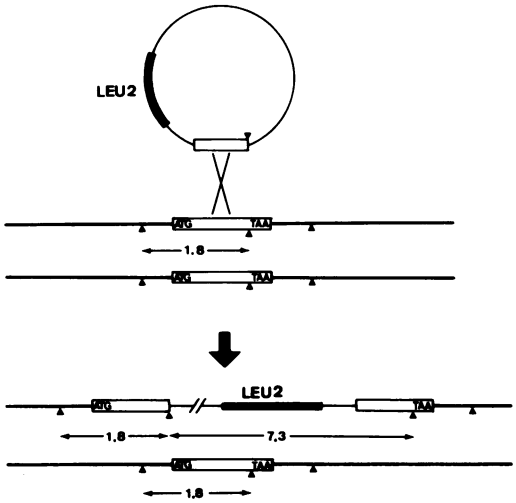

FIG. 3. α1 tubulin gene disruption by integration of YIp(NDA2)1-2. The predicted chromosomal DNA structures of *NDA2* locus (α1 tubulin gene) before (top) and after (bottom) integration of YIp(NDA2)1-2 are shown. Symbols: —, vector sequences; □, coding sequences of α1-tubulin; ■, *Saccharomyces cerevisiae LEU2* gene; ▬, chromosomal DNA; ▲, *Hin*dIII sites. Lengths of nucleotide sequences are indicated in kilobases.

Isolation of tubulin, two-dimensional gel electrophoresis, and protein blotting. *Schizosaccharomyces pombe* tubulin was purified by the method described for *Saccharomyces* sp. by Kilmartin (17). Two-dimensional gel electrophoresis was carried out by the method described by O'Farrell and O'Farrell (25). The gels were stained with 0.1% Coomassie brilliant blue (R 250) in 25% isopropyl alcohol–10% acetic acid. Protein blotting was carried out as described by Towbin et al. (39). Rat monoclonal antibody against yeast tubulin (YL1-2) was a gift from J. Kilmartin.

Assay of β-galactosidase. For plates, the procedure of Rose and Botstein (26) were followed. β-Galactosidase in the cell extracts was measured by the procedures described by Guarente (11). Transformed *Schizosaccharomyces pombe* cells were grown in EMM2 minimal medium at 33 to 36°C. Activities are expressed as nanomoles of *o*-nitrophenyl-β-D-galactoside cleaved per min per 10^7 cells.

RESULTS

α1-Tubulin gene (NDA2) is essential. An integration vector YIp33 consists of pBR322 and the *LEU2* gene of *Saccharomyces cerevisiae* (3). *LEU2* can complement *leu1* mutation in *Schizosaccharomyces pombe* (1, 2). The 905-bp *Bgl*II-*Hin*dIII fragment spanning the coding region of the α1-tubulin gene (37) was ligated with the *Bam*HI and *Hin*dIII sites on YIp33 (Fig. 3). The resulting plasmid (YIp(NDA2)1-2) did not autonomously replicate in *Schizosaccharomyces pombe*. If it is integrated into *NDA2* locus (α1-tubulin gene), the gene will be split into two inactivated parts, one lacking the carboxy-terminal 64 amino acids and the other lacking the amino-terminal 33 amino acids.

The diploid constructed was transformed with the plasmid (see Materials and Methods), and the three Leu$^+$ diploids obtained were sporulated by nitrogen starvation (12). Results of tetrad analyses (Table 1) indicated that the α1 gene disruption was a lethal event in haploid; no ascus which contained three or four live spores was found. Furthermore,

TABLE 1. Tetrad analysis of diploid transformants integrated with the α1 plasmid YIp (NDA2) 1-2[a]

Leu+ transformants	No. of asci with the following live/dead ratio:		Segregant phenotype
	2:2	1:3	
I	2	10	All Leu− CS−
II	4	6	All Leu− CS+
III	0	7	All Leu− CS−

[a] Three diploid transformants (I, II, and III) were sporulated under nitrogen starvation, and tetrads (12 from I, 10 from II, and 7 from III) were dissected. No asci showed a live spore/dead spore ratio of 3:1 or 4:0. The number of 1:3 asci was higher than that of 2:2 asci, though the reason for this is not understood.

all of the live spores were Leu−. Therefore, the α1-tubulin gene (*NDA2*) appeared to be essential, as was expected on the basis of its cs allele. In transformants I and III, all of the live spores were cs−, indicating that the plasmid disrupted the wild-type allele. In transformant II, integration took place at the mutant allele, because all of the live spores were cs+. The genomic Southern blots of the three diploid transformants probed with the 1.8-kb *Hin*dIII fragment of the α1 gene indicated integration of the entire plasmid on one copy of the locus (data not shown).

Disruption of the α2-tubulin gene does not cause lethality in haploid cells. For α2-tubulin gene disruption, the 1,065-bp *Xho*II fragment spanning from the 49th to the 425th amino acid residue in the α2-coding region (37) was cloned into the *Bam*HI site of YIp32. The resulting plasmid (designated as pYA112) had no ARS (autonomously replicating sequence) activity in *Schizosaccharomyces pombe*. Integration of pYA112 on the chromosome would split the α2-tubulin gene into two parts (Fig. 4a).

A recipient diploid host was transformed with pYA112, three Leu+ diploid transformants were sporulated, and tetrads were dissected. In contrast to the case of α1 gene disruption, Leu+:Leu− segregated into 2:2 in all asci of the transformant examined (data not shown), and these viable Leu+ segregants grew normally, indicating that disruption of the α2 gene did not cause lethality in the haploid cells.

To confirm the α2 gene disruption in the Leu+ haploid, Southern hybridization was carried out with the mixed probe of the 0.6-kb *Hin*dIII fragment of the α2 gene and YIp32 (Fig. 4b). All three diploid transformants showed 6.6-kb *Hin*dIII bands (derived from the integrated plasmid) as well as a 0.6-kb band. Two Leu− haploid segregants, B and D, showed hybridized bands identical to the wild type, and two Leu+ segregants, A and C, showed the hybridized bands, all expected from *Hin*dIII and *Eco*RI restriction sites on the chromosomal α2-tubulin gene integrated with pYA112.

The α2 gene disrupted by the above procedures contained a normal 5′ upstream region and an incomplete coding region lacking the carboxy-terminal 24 residues (Fig. 4). A possibility considered was that this region might be transcribed

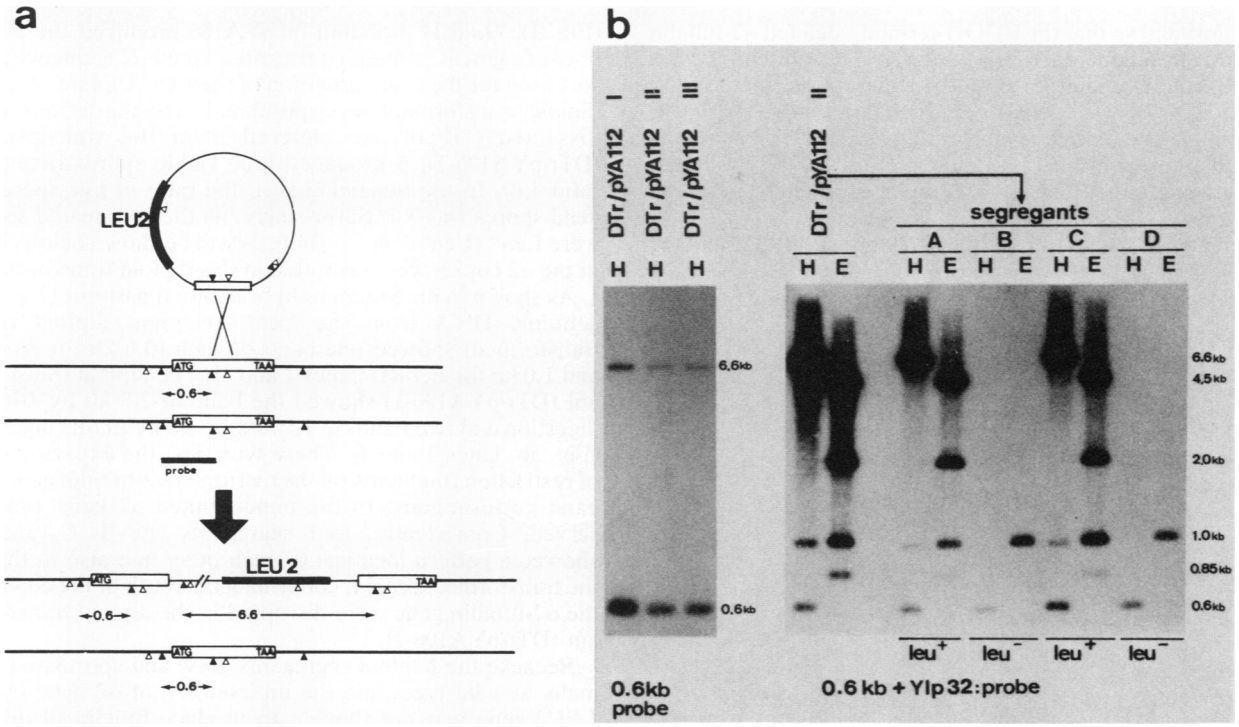

FIG. 4. α2-Tubulin gene disruption by integration of pYA112. (a) Predicted chromosomal DNA structures of the locus of α2-tubulin gene before and after the integration of pYA112 are shown. Symbols: □, coding sequence of the α2-tubulin gene; ▬, 0.6-kb *Hin*dIII fragment used as a hybridization probe; △, *Eco*RI restriction sites; ▲, *Hin*dIII restriction sites. (b) Gel transfer hybridization patterns of genomic DNAs isolated from diploid transformants with pYA112 and from haploid segregants are shown. In the three left lanes, genomic DNAs purified from the three Leu+ diploid transformants and cut with *Hin*dIII were run in a 1% agarose gel and probed with the 0.6-kb α2 gene fragment. In the 10 right lanes, genomic DNA purified from a diploid transformant II and also from its four haploid segregants (A through D lanes) and cut with *Hin*dIII (lanes H) or *Eco*RI (lanes E) were run and probed with a mixture of ^{32}P-labeled 0.6-kb *Hin*dIII fragment and YIp32. A and C lanes, Leu+ segregants; B and D lanes, Leu− segregants.

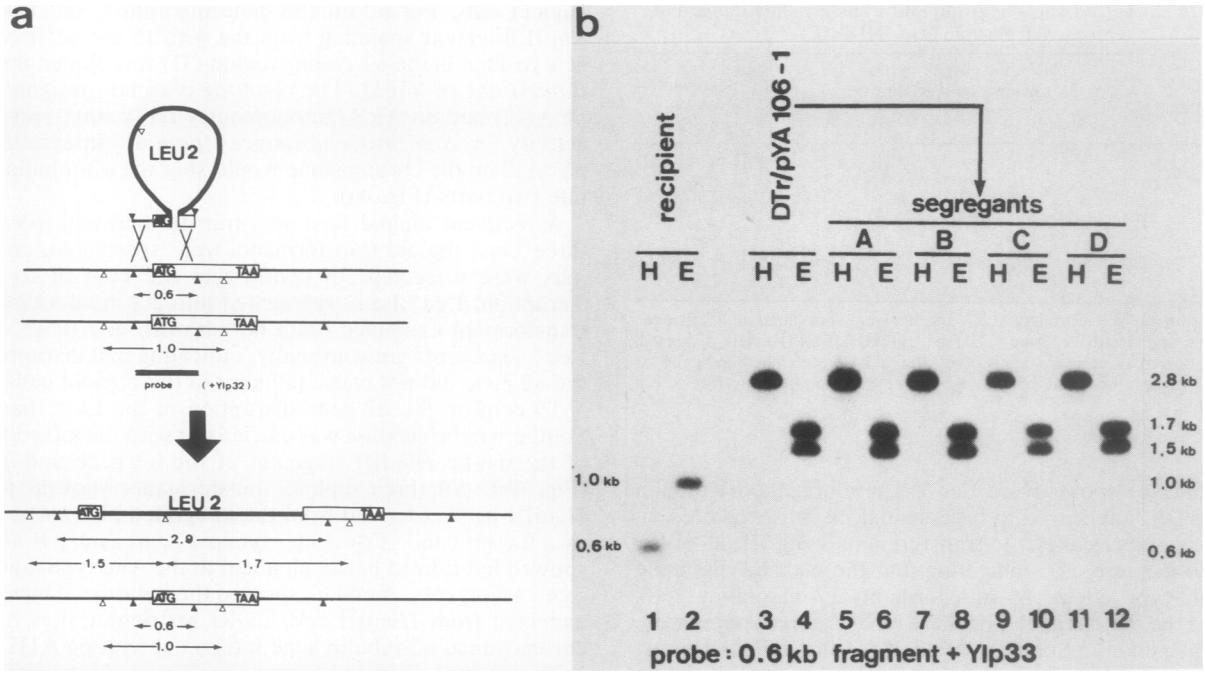

FIG. 5. α2-Tubulin gene disruption by a linear α2 fragment interrupted with the *LEU2* gene. (a) Predicted chromosomal structures of the α2-tubulin locus before and after integration. A linear fragment was derived from pYA106 plasmid cleaved with *Hin*dIII. (b) Genomic Southern hybridization patterns of a diploid transformant and its four haploid segregants. DNAs purified from the diploid and the haploid segregants (A through D lanes) were cut with *Hin*dIII (H lanes) or *Eco*RI (E lanes) and probed with the mixture of ^{32}P-labeled 0.6-kb *Hin*dIII α2 fragment and YIp32.

and translated so that the COOH-terminal-deleted α2-tubulin synthesized might function as wild-type α2-tubulin. To exclude such ambiguity, the other disruption method by Rothstein (27) was carried out. In this method, the linear α2-tubulin gene sequence interrupted by the *LEU2* gene was used to transform the Leu$^-$ diploid (Fig. 5a). In the transformants, an intact α2 gene was replaced in one step by the interrupted α2 sequence.

A chimera plasmid (pYA106) consisting of pBR322 and the 0.6-kb α2 gene fragment interrupted with the 2.2-kb *LEU2* gene was constructed as described in Materials and Methods (Fig. 1). *Hin*dIII digestion of pYA106 produced the 2.8-kb linear fragment containing the interrupted α2 sequence; this was used for the transformation of the Leu$^-$ diploid. A Leu$^+$ diploid transformant was sporulated, and the tetrads were dissected. Of 10 asci derived from the transformant (DTr/pYA106-1), 6 produced four viable spores (live/dead ratio 4:0). In the remaining asci, the ratio of live spores to dead spores was 3:1. Surprisingly, all the germinated spores were Leu$^+$ (Leu$^+$:Leu$^-$ = 34:0; as will be shown below, both of the α2 copies were disrupted in this diploid transformant).

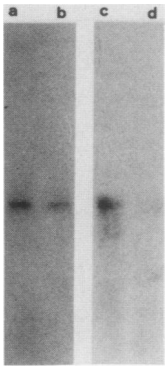

FIG. 6. RNA blot hybridization of *Schizosaccharomyces pombe* poly(A)$^+$ RNA from wild type and α2-disrupted segregant. Poly(A)$^+$ RNAs were purified from wild type (h^{90}) (lanes a and c) and from the α2-disrupted segregant A (lanes b and d), run in a 1% agarose gel and probed with an 1.8-kb *Hin*dIII α1 fragment (lanes a and b) or with a 1.3-kb *Hin*dIII α2 fragment (lanes c and d). Exposure time: lanes a and b, 24 h; lanes c and d, 72 h.

As shown in the Southern hybridization patterns (Fig. 5b), genomic DNA from the Leu$^-$ recipient diploid (nontransformed) showed one band of each (0.6 kb for *Hin*dIII and 1.0 kb for *Eco*RI) (lanes 1 and 2). The diploid transformant (DTr/pYA106-1) showed the band at 2.8 kb by *Hin*dIII digestion and two bands at 1.7 and 1.5 kb by *Eco*RI digestion (Fig. 5b, lanes 3 and 4). These were just the expected sizes of restriction fragments on the disrupted α2-tubulin gene. No band corresponding to the nondisrupted α2 gene was observed. Consistently, four segregants (A, B, C, and D) showed a pattern identical to each other and also to that of the transformed diploid, confirming that both of the copies of the α2-tubulin gene were disrupted in the diploid transformant (DTr/pYA106-1).

Because the haploid segregants grew and sporulated normally as wild type, and the intervention of α2 gene by the *LEU2* gene was not thought to produce functional tubulin polypeptides, we concluded that the α2-tubulin is not essential in mitotic and meiotic cell cycles of *Schizosaccharomyces pombe*.

Absence of α2-tubulin mRNA in the α2-disrupted transformant. The transcriptional product of the α2-disrupted gene was examined by Northern blots. RNA was extracted from h^{90} wild type as a control and from the

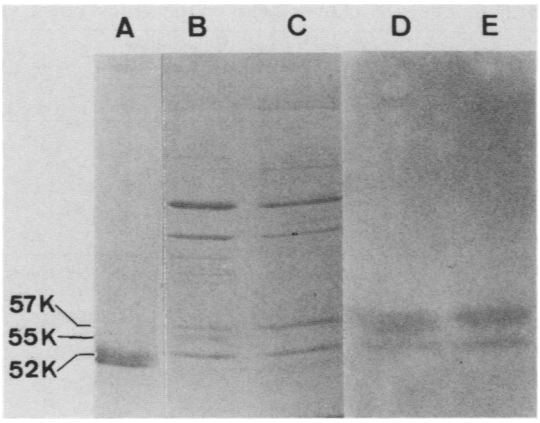

FIG. 7. SDS gel electrophoresis and immunoblots of crude tubulin fractions. Lanes A through C were stained with Coomassie blue. Lane A, Calf brain tubulin. Lanes B and C, 0.5 M KCl eluants of DEAE-Sephadex chromatography of wild type and α2-disrupted segregant A, respectively. Lanes D and E, Immunoautoradiograms of the protein transfer blots with monoclonal antibody against *Saccharomyces cerevisiae* tubulin.

segregant A, the α2 gene of which was disrupted by pYA106. Poly(A)$^+$ mRNA was purified on an oligodeoxythymidylic acid cellulose column. To detect α1 mRNA, the 1.8-kb *Hin*dIII fragment of the α1 gene was used as the hybridization probe. For α2 mRNA, the 1.3-kb *Hin*dIII fragment of the α2 gene was used.

α1 mRNA was detected in the wild type (Fig. 6, lane a) and also in the α2-disrupted segregant A (lane b). In contrast, α2 mRNA was seen in the wild type (Fig. 6, lane c) but not in the α2-disrupted segregant A (lane d). Therefore, no complete α2 mRNA was produced in the α2-disrupted segregant. In wild type, however, both the α1 and α2 genes were transcribed as reported previously (37).

An α-tubulin polypeptide is missing in the α2-disrupted segregant. We examined the composition of tubulin polypeptides in the α2-disrupted segregant. Previously, Toda et al. (37) identified the two polypeptides as α-tubulins and one polypeptide as β-tubulin in the wild-type tubulin preparations by SDS gel electrophoresis combined with protein transfer blots. Samples (50 g) of wet pelleted cells of the α2-disrupted segregant A and the wild type (h^{90}) were broken with glass beads at 0°C under the condition that tubulins were not assembled (17), and the supernatant after centrifugation at 29,000 rpm for 90 min was applied to DEAE-Sephadex chromatography. The eluants by 0.5 M KCl were obtained as crude tubulin fractions. Tubulins could be detected in these fractions by immunoblots but not in the cell extracts, possibly due to the low amount of tubulins in fission yeast cells (37).

The 0.5 M KCl fractions of wild-type and α2-disrupted strains were run in SDS gel electrophoresis and either stained with Coomassie blue (Fig. 7, lanes b and c; lane a, calf brain tubulin) or transferred for immunoblots with rat monoclonal antibody against the *Saccharomyces cerevisiae* tubulin (lanes d and e). Two bands at apparent molecular weights of 55,000 and 57,000 correspond to α-tubulin, and a band at 52,000 corresponds to β-tubulin (37). A dye-stained band at 55,000 was missing in the α2-disrupted strain (Fig. 7, lane c). Results of the immunoblots (Fig. 7, lanes d and e) were consistent, although the bands were somewhat smeared and not clearly resolved into two in the wild type. The band of the α2-disrupted strain was narrower than that of the wild type. Note that the intensities of Coomassie-stained α-tubulin bands in the wild type were similar (in this gel pattern, the upper band was more intense than the lower band, but in other preparations, the intensity was only slightly greater than in the lower band) and that the intensity of the putative α1-tubulin band in the α2-disrupted preparation relatively increased (Fig. 7, lane c). Because *Schizosaccharomyces pombe* α- and β-tubulins have the same three residues (Glu-Glu-Tyr) at the carboxy ends (37), the antibody that is known to recognize Glu-Glu-Tyr (41) reacted not only with α-tubulin but also with β-tubulin.

We further analyzed the tubulin composition of the α2-disrupted strain by two-dimensional gel electrophoresis. Tubulins in 0.5 M KCl eluants of DEAE-cellulose chromatography were further purified by cycles of assembly and disassembly (17). Polypeptides were run in two-dimensional gel electrophoresis (Fig. 8). In the wild type, the two polypeptide spots corresponding to α-tubulins were observed (37). In the α2-disrupted segregant, however, only one polypeptide spot was seen. The missing polypeptide had

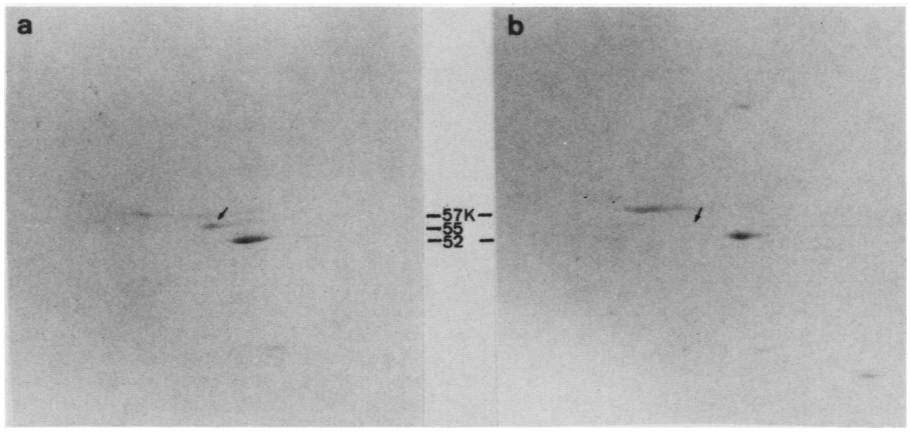

FIG. 8. Two-dimensional gel electrophoretic patterns of tubulin preparations from wild type and α2-disrupted segregant. Tubulins were purified from wild type and from the α2-disrupted segregant A and run in two-dimensional gel. Apparent molecular weights (in thousands) of the tubulin spots are indicated (the values are significantly larger than those determined by nucleotide sequences [37]. Arrows indicate the position of the putative α2 polypeptide.

a lower molecular weight, consistent with the results described above. Because the nucleotide sequence of the α2-tubulin gene showed that α2-tubulin has 6 amino acid residues less than α1-tubulin and is more acidic than α1 (37) the missing band most likely represented the product of the α2-tubulin gene. Thus, by using the α2-disrupted strain, we could identify the α1- and α2-tubulin polypeptides as different electrophoretic bands. The SDS gel electrophoretic mobilities of α1 and α2 tubulins were anomalous; molecular weights of α1 and α2 estimated from the nucleotide sequences were 51,200 and 50,600, respectively, whereas their apparent molecular weights in SDS gel electrophoresis were 57,000 and 55,000, respectively.

The results described above suggested that the α2-tubulin is not a minor, residual tubulin subtype in fission yeast cells. α2 tubulin was synthesized in the wild type and was actively assembled in vitro into microtubules. It should be emphasized that the amount of α2 polypeptide in the crude fractions as well as in the purified tubulins of wild type was similar to that of α1. In the α2-disrupted cells, meanwhile, the amounts of α1-polypeptide were approximately the same as those of β-tubulin in the crude and purified preparations (Fig. 7, lane c, and Fig. 8b). Because the direct estimates of tubulin contents in the whole-cell extracts were not successfully done by immunoblots, the amounts of synthesized α-tubulins were further investigated by using the fused genes with bacterial β-galactosidase (described below).

Phenotypes of the α2-disrupted cells. The α2-disrupted haploid cells grew normally (the generation time at 30 to 36°C in YPD medium was identical to that of wild type). The homothallic derivative sporulated normally without any apparent defect. Some of its cellular properties, however, were significantly altered. First, we found that the α2-disrupted cells became supersensitive to thiabendazole (Table 2). The wild-type cells were able to grow in 30 μg of thiabendazole per ml, whereas the α2-disrupted cells could not grow even in 10 μg of thiabendazole per ml. Similar results were obtained when the supersensitive allele of α1 gene was recombined. An α1 mutant *nda2*-KM52 was not only cs but also ss to the drug at the permissive temperature (36°C) (41) and grew only minimally in 1 μg of thiabendazole per ml. The recombined strain α1 (ss) α2 (disrupted) constructed was found to be more supersensitive and not viable in 0.5 μg of thiabendazole per ml. Thus, the disruption of the α2 gene caused the cells to become more sensitive to the drug, and the α2 gene became essential under the special circumstance.

Secondly, the cs phenotype of *nda2*-KM52 was strikingly changed when recombined with the α2-disrupted locus (Table 3). The α1 (wild) α2 (disrupted) could grow normally as wild type, and the α1 (cs) α2 (wild) showed the clear cs phenotype. In contrast, the α1 (cs) α2 (disrupted) strain did not grow at all at 22°C but grew slowly at 33°C and grew only minimally at 37°C (pin colonies were formed); the strain seemed to have both cs and temperature-sensitive pheno-

TABLE 2. Supersensitivity of α2-disrupted cells to thiabendazole[a]

Strain	Growth on the following concn (μg/ml) of thiabendazole								
	0.1	0.5	1	3	5	10	20	30	50
Wild type	+	+	+	+	+	+	+	+	−
α1 (wild) α2 (disrupted)	+	+	+	+	+	−	−	−	−
α1 (ss) α2 (wild)	+	+	−	−	−	−	−	−	−
α1 (ss) α2 (disrupted)	+	−	−	−	−	−	−	−	−

[a] Cells were plated on YPD rich medium containing different concentrations of thiabendazole and incubated at 33°C. + indicates growth. Strains used: wild type, HM123; α1 (wild) α2 (disrupted), the α2-disrupted segregant A (h^{90} *leu1 ade6*-210) α2 (disrupted); α1 (ss) α2 (wild), KM52-110; α1 (ss) α2 (disrupted), h^- *leu1 nda2*-KM52 α2 (disrupted).

TABLE 3. Effect of α2 (disrupted) on the α1 (cs) phenotype

Strain[a]	Growth at the following temperature (°C)			Phenotype
	20	33	37	
α1 (wild) α2 (disrupted)	+	+	+	cs^+
α1 (cs) α2 (wild)	−	+	+	cs^-
α1 (cs) α2 (disrupted)	−	+[b]	−	cs^- ts^-

[a] α1 Strains used: (wild) α2 (disrupted), α2-disrupted haploid segregant A (h^{90} *leu1 ade6*-M210 α2-disrupted); α1 (cs) α2 (wild), KM52-110 (h^- *leu1 nda2*-KM52); α1 (cs) α2 (disrupted), h^- *leu1 nda2*-KM52 α2 (disrupted).
[b] Slow growth.

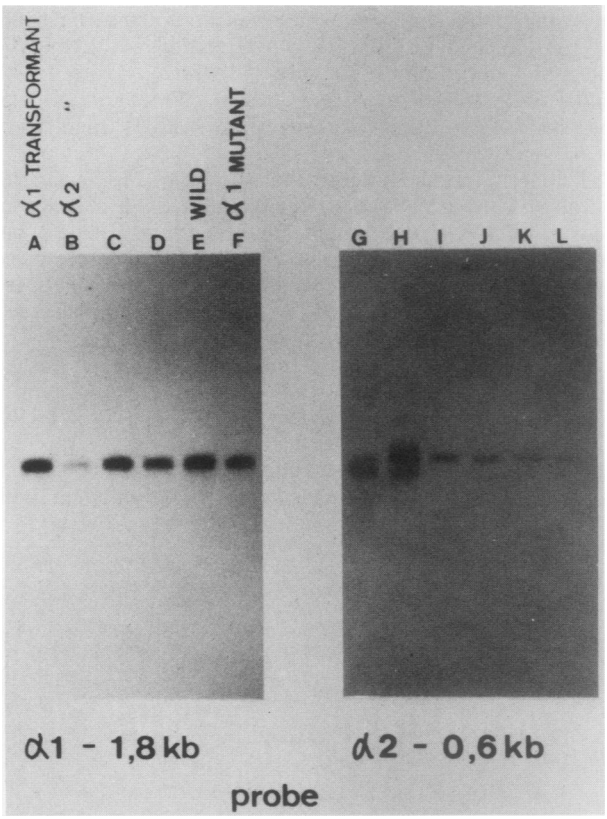

FIG. 9. RNA blot hybridization of α1 and α2 mRNAs. Poly(A)$^+$ RNAs were prepared from the cells of *nda2*-KM52 *leu1* transformed with α1 plasmid [pDB(NDA2)1-2] grown at 22°C (lanes A and G); from *nda2*-KM52 *leu1* transformed with α2 plasmid [pDB(NDA2)2-4] grown at 22°C (lanes B and H); from nontransformed wild type grown at 36°C (lanes C and I) and at 22°C (lanes E and K); from nontransformed *nda2*-KM52 *leu1* grown at a permissive temperature, 36°C (lanes D and J); and from *nda2*-KM52 incubated at a nonpermissive temperature, 22°C, for 5 h (lanes F and L). Samples were run in agarose gel electrophoresis and probed with a ^{32}P-labeled 1.8-kb *Hin*dIII α1 fragment (lanes A through F) or with a 0.6-kb *Hin*dIII α2 fragment (lanes G through L).

TABLE 4. β-Galactosidase activity in cells transformed with α1-*lacZ* or α2-*lacZ* plasmid

Strain	β-Galactosidase activity in cells containing[a]:				
	Multicopy plasmid			Integrated gene	
	pDB248	α1-*lacZ*	α2-*lacZ*	α1-*lacZ*	α2-*lacZ*
HM123	0.4	38.0	18.0	ND[b]	5.8
KM52	0.4	32.4	16.0	5.8	8.7

[a] Extracts were prepared from transformed cells grown in EMM2 minimal medium at 33°C for HM123 and at 36°C for KM52-110. β-Galactosidase activities are expressed as nanomoles of *o*-nitrophenol-β-D-galactoside cleaved per minute per 10^7 cells. Activities of the transformants with pDB-α1-*lacZ* and pDB-α2-*lacZ* are shown in the columns of multicopy plasmid, and activities of the transformants integrated with YIp-α1-*lacZ* and YIp-α2-*lacZ* are shown in the columns of integrated genes. Integration of the fused genes was confirmed by the stability of the Leu$^+$ marker in the transformants through mitosis and meiosis.

[b] ND, Not determined.

types. Thus, the clear cs phenotype of α1 was lost, indicating that it was dependent upon the expression of the α2 gene. In other words, the clear cs phenotype of α1 (*nda2*) could only be attained in the presence of the normal α2 gene.

The α1 (cs) α2 (disrupted) could be considered semilethal. Even at the permissive temperature (33°C), the cells were very abnormal; many were elongated, branched, or bent. Fluorescence microscopy by 4′-6-diamidino-2-phenylindole staining showed that their nuclei were frequently displaced from the center of the cell (data not shown). These aberrations in the slowly growing α1 (cs) α2 (disrupted) cells at 33°C were similar to those of the single *nda2* mutant at 22°C (40). We therefore concluded that the normal growth of α1 (cs) α2 (wild) at 36°C was supported by the expression of the α2 gene.

Differential amounts of α1 and α2 mRNA. Previously we showed by Northern blots with the specific 3′ probes of α1 and α2 genes that both α1- and α2-tubulin genes were transcribed (37). The amount of α1 transcript in the wild type appeared to be severalfold more than that of α2 transcript, and was strikingly reduced in a transformant with multicopy plasmid containing the α2 gene.

Similar experiments were done with the 5′ probes (Fig. 9). The probes for α1 and α2 mRNA were the 1.8- and 0.6-kb *Hin*dIII fragments, respectively. These probes were weakly cross-hybridized with each other (37), but under a stringent condition (65°C in 5× SSPE [10] with 0.3% SDS); hybridization was negligible. Poly(A)$^+$ mRNAs were purified from the following strains: transformants of *nda2*-KM52 with multicopy α1 plasmid (Fig. 9, lanes A and G), or with multicopy α2 plasmid (lanes B and H) grown at 22°C, the wild type grown at 36°C (lanes C and I) or at 22°C (lanes E and K), *nda2*-KM52 at 36°C (lanes D and J) or at 22°C (lanes F and L).

Consistent with the previous results, the amount of α1 mRNA appeared to be much more than those of α2 in the wild type and in the *nda2*-KM52 mutant at 36 and 22°C. The amount of α1 mRNA was strikingly reduced in the transformant with α2 plasmid. The amount of α1 transcript in the transformant with α1 multicopy plasmid, however, was the same level as in the wild type. In contrast, the amount of α2 mRNA was not reduced by the copresence of the α1 multicopy plasmid but increased severalfold by α2 multicopy plasmid. Different sizes of the RNA bands hybridized with the α2 probe were found in the transformants. These additional bands were not observed when the 3′ α2 probe was used (37) and might be due to incorrect transcriptional products on plasmid DNAs.

S1 nuclease mapping experiments (Y. Asakura, unpublished results) were consistent with the above results and showed that the initiation of α1 transcript starts at position −118 (A) from the initiation codon, whereas the α2 site was very weak and was located at multiple sites −62, −64, and −100 from the initiation codon.

In short, striking differences existed in the quantities of α1 and α2 mRNAs. α1 mRNA was predominant over that of α2 mRNA in the wild-type cells, and the amount of α1 mRNA was modulated, depending on the gene copy number of α2, but α2 mRNA seemed to be unaffected by that of the α1 gene. It looked as if the amount of α1 mRNA was reduced with increasing α2 gene copy number so as to keep the total amounts of α1 and α2 mRNA constant, whereas the amount of α2 mRNA increased additively.

Translational products of α-tubulin–*lacZ* fused genes. α-Tubulin–β-galactosidase hybrid genes were constructed as described in Materials and Methods (Fig. 2) to quantify the translational product of α1 and α2 genes. The resulting fused genes were in frame at the joining parts between α1- or α2-tubulin and *lacZ* sequences. The blue-colored colonies of the transformants were obtained on the minimal plates containing X-gal. Haploid strains HM123 (*h*$^-$ *leu1*) and KM52-110 (*h*$^-$ *leu1 nda2*-KM52) were used as recipients for transformation.

β-Galactosidase was detected in the extracts of transformants with the α1-*lacZ* or α2-*lacZ* fused genes but was negligible in a control transformant with pDB248 (Table 4). Transformants with multicopy plasmid α1-*lacZ* had about twofold-higher activity than those with α2-*lacZ*. The transformants with multicopy plasmids contained enzyme activities two to five times higher than those integrated chromosomally with YIp-α1-*lacZ* or YIp-α2-*lacZ*. The en-

TABLE 5. Comparison of α1- and α2-tubulins

Tubulin	Mol wt[a]	Chromosomal locus	Gene disruption	Transcription	Translated products[b]	Assembly into microtubules	Defective phenotype
α1	51,200 (57,000)	*nda2*	Lethal	Major (modulative)	Major	Yes	Nuclear division arrest Absence of spindle Displaced nucleus TBZ supersensitivity[c]
α2	50,600 (55,000)	ND[d]	Viable	Minor (constitutive)	Major	Yes	TBZ supersensitivity

[a] Determined by predicted amino acid sequences and by SDS gel electrophoresis (in parentheses).

[b] Estimated by the β-galactosidase activity of fused genes and by Coomassie blue-stained band intensities in the DEAE-cellulose fraction.

[c] TBZ, Thiobendazole.

[d] ND, Not determined.

zyme activities in the integrated transformants with α1-*lacZ* and α2-*lacZ* were about the same level despite a great difference in the amounts of α1 and α2 mRNA. These results suggested that roughly equal amounts of α1- and α2-tubulin subunits are synthesized in the wild-type cells.

Complementation of α1 mutation by two chromosomal α2 genes. α2 multicopy plasmid can complement α1 mutations (37). To determine the gene copy number of α2 required for the complementation, we integrated the α2 gene on the chromosome of an α1 mutant KM52-110 (*h*$^-$ *nda2*-KM52 *leu1*) by YIp32 plasmid containing the yeast *LEU2* and the α2 gene (1.9-kd *Hin*dIII fragment). Eight Leu$^+$ transformants were obtained, all of which were found to be cs$^+$, indicating that the α1 cs mutation was complemented by integration of an additional α2 gene.

To confirm the integration, two transformants were crossed with Leu$^-$ α1$^+$ α2$^+$ strain JY6 (*h*$^+$ *leu1 his2*), and the segregation of Leu$^+$:Leu$^-$ was examined. All of them showed 2$^+$:2$^-$ segregation, confirming the integration of α2 gene. The segregation of cs$^+$:cs$^-$ was also examined in 10 tetrads. Two showed 4$^+$:0$^-$ segregation (corresponding to the parental ditype), seven showed 3$^+$:1$^-$ (tetratype), and one showed 2$^+$:2$^-$ (nonparental ditype). Therefore, cs$^-$ *nda2* mutation was suppressed by an extra α2 gene that was integrated on a chromosomal locus not linked to α1. Consistently, all of the cs$^-$ segregants were Leu$^-$.

A similar experiment was carried out with JY6 as a recipient strain for transformation, and the Leu$^+$ transformants were crossed with KM52-110. Segregation of Leu$^+$:Leu$^-$ was 2:2 in all of the six tetrads examined. On the other hand, the segregation of cs$^+$:cs$^-$ was 2:2 in two tetrads, 3:1 in three, and 4:0 in one, and all of the cs$^-$ segregants were Leu$^-$. These results demonstrated that integration of an additional α2 gene complemented the α1 cs mutation and that the integrated locus was not linked to the α1 gene. Thus, the α1 mutation can be complemented by two copies of the chromosomal α2 gene.

DISCUSSION

The conclusion obtained in this study from gene disruption experiments is that α1-tubulin gene (*NDA2*) is essential, whereas the α2-tubulin gene is dispensable. This is consistent with the presence of a cs lethal allele in the α1 gene. Definitive evidence for the dispensability of the α2 gene was obtained by analyses of tubulin polypeptides in the α2-disrupted cells. One of the two α-tubulin polypeptides present in the wild type was missing in the α2-disrupted segregant that grows normally as wild type. Homothallic (h^{90}) α2-disrupted segregants could sporulate and produce normal tetrads, indicating that the α2 gene is also nonessential in the meiotic process. Chromosomal mapping of α2 gene is being conducted, using the integrated *LEU2* gene as a marker. None of the supersensitive mutants, including SH3, SH6, and SH7 (40), nor the resistant *ben2* (42) and *ben4* (28) were linked to the α2 gene. The locus has been silent so far in mutant screening.

Another conclusion obtained in the present and previous (37) studies is that the patterns of transcription and translation seem to differ strikingly between the α1 and α2 genes. α1 mRNA was predominant over α2 mRNA in the wild-type cells. The amount of α1 mRNA was reduced by increasing the gene copy number of α2. The amount of α2 mRNA was increased by the α2 multicopy plasmid but unaffected by the α1 multicopy plasmid. These suggested that α1 transcription is major and can be modulated whereas α2 transcription is minor and constitutive. The amounts of α1- and α2-tubulins estimated by the *lacZ*-fused genes or by gel electrophoresis, however, appeared to be at a similar level; α2-tubulin is not a negligible, minor tubulin subtype. Therefore, α1 mRNA may be short-lived, or α2 mRNA may be more stable in translation. Alternatively, efficiency in translation may differ between the two mRNAs.

Although the α2-tubulin gene is dispensable, it is expressed, and its expression could be detected phenotypically. The α2-disrupted strain was more sensitive to thiabendazole than was wild type; the expression of α2 gene apparently made the cells more resistant to the drug. The phenotype of α1 mutant (*nda2*-KM52) was altered when the α2 gene was disrupted; the α1(cs) α2(disrupted) strain was ts as well as cs; the clear cs phenotypes of *nda2*-KM52 were attained only in the presence of α2 gene. These results showed that the α2 gene product functions in cooperation with the α1 gene product, possibly through direct interaction. Differences between α1- and α2-tubulins are summarized in Table 5.

A characteristic of this two α-tubulin gene family is that one member is essential whereas the other is dispensable, although both members are expressed. What causes such difference? α1 and α2 polypeptides differ 14% in their amino acid sequences (37). Many of the variable residues reside in two discrete regions (one between the 31st and 54th residues and the other near the carboxy end). One possibility is that only the α1 polypeptide plays an essential role; the protein structures of α1 and α2 might confer essential or dispensable functions. Because the α2 plasmid has the ability to complement mutations of the α1 gene, however, we considered this unlikely, although we have no direct evidence against it.

Previously we postulated that a large difference in the amount of α1 and α2 mRNAs may account for the essential nature of the α1 gene (37). Minor α2 mRNA might produce a residual α-tubulin subtype that could not suppress mutations of the major α1 subtype. The increase of α2 mRNA (and the decrease of α1 mRNA) by multicopy gene dosage might explain the ability of the α2 multicopy plasmid to complement the cs *nda2* mutation. An apparent discrepancy in this hypothesis is the finding presented in this paper that the amount of α2 polypeptide in the wild-type cells is comparable to that of α1 polypeptide.

If, however, the cells of *Schizosaccharomyces pombe* have no excess pool of tubulin, the lack of functional α1 gene product might lead to the arrest of cell growth, because the constitutive synthesis of α2-tubulin would produce only about half the amount of functional α-tubulin required for growth. Our finding that two copies of the chromosomal α2-tubulin genes can complement the cs α1 mutation is consistent with this hypothesis. On the other hand, the α2-disrupted cells are viable because α1-tubulin synthesis may be modulated in the absence of α2 gene so as to increase the amount of α1-tubulin to the total of α1 and α2 tubulins found in the wild type.

If the above limited-pool hypothesis is correct, the real reason that α1 is essential and α2 is dispensable is in the regulatory sequences instead of the coding sequences in the two α-tubulin genes. The α2 gene is dispensable because the α1 gene can modulate the tubulin synthesis by recognizing the absence of the α2 gene, and the α1 gene becomes essential because the α2 gene is independent of the α1 gene and cannot modulate the tubulin synthesis in the absence of the α1 gene. The essentiality of the α1 gene and dispensability of the α2 gene may be intricately related to the ability and inability of the two α-tubulin genes to regulate the synthetic

level of α-tubulin. Such regulation in the α1 gene, at the level of transcription or translation or both, may be essential for dynamic microtubular architecture during the cell cycle. Autoregulatory control of tubulin synthesis not through a transcriptionally regulated mechanism has been demonstrated in cultured animal cells by Cleveland and co-workers (6–8). The increased levels of tubulin mRNA were found in the flagellar assemblyof *Chlamydomonas* sp. (21, 34) and the spindle formation of *Physarum* sp. (32).

Why, then should there be the α2-tubulin gene, which has a product amount comparable to that of α1? What is its role? We have no satisfactory answer for these questions, but a clue may be obtained from the recent findings by Schatz and Botstein (P. J. Schatz and D. Botstein, Abstracts for UCLA Symposia for Yeast Cell Biology, abstract no. 9C, 1985) that *Saccharomyces cerevisiae* also has two α-tubulin genes (*TUB1* and *TUB3*) and one β-tubulin gene (TUB2 [24]) and that *TUB3* is nonessential. Furthermore, the *TUB3*-disrupted strain has an increased sensitivity to the antimicrotubule drug benomyl. These striking similarities in organization and expression of the tubulin genes in two distantly related organisms are surprising and suggest a conserved role for the α2 gene. We suggest that constitutive α2-tubulin synthesis causes the retention of a basal amount of α-tubulin, which may become essential under a certain natural environment.

The two α-tubulin genes of the fission yeast *Schizosaccharomyces pombe* appear to constitute an interesting gene family that consists of an essential and a dispensable member. They are different from families such as histone genes, in which each gene member carries basically the same function (30) and, in most cases, encodes identical proteins. These two genes also do not belong to any class of developmentally regulated or tissue specific gene family because they exist in a unicellular organism and also because the α2-disrupted strain did not show any defect in meiosis and germination. No cell cycle-specific transcription was found in these two tubulin genes (Y. Adachi, T. Toda, O. Niwa, M. Yanagida, and P. Nurse, unpublished results).

ACKNOWLEDGMENTS

This work was supported by grants from the Ministry of Education, Science and Culture of Japan and the Mitsubishi Foundation.

LITERATURE CITED

1. **Beach, D., and P. Nurse.** 1981. High frequency transformation of the fission yeast *Schizosaccharomyces pombe*. Nature (London) **290**:140–142.
2. **Beach, D., M. Piper, and P. Nurse.** 1982. Construction of a *Schizosaccharomyces pombe* gene bank in a yeast bacterial shuttle vector and its use to isolate genes by complementation. Mol. Gen. Genet. **187**:326–329.
3. **Botstein, D., S. C. Falco, S. E. Stewart, M. Brennan, S. Scherer, D. T. Stinchcomb, K. Struhl, and R. W. Davis.** 1979. Sterile host yeasts (SHY): a eukaryotic system of biological containment for recombinant DNA experiments. Gene **8**:17–24.
4. **Casadaban, M. J., A. Martinez-Avis, S. K. Shapiro, and J. Chou.** 1983. β-Galactosidase gene fusions for analyzing gene expression in *Escherichia coli* and yeast. Methods Enzymol. **100**:293–308.
5. **Cleveland, D. W.** 1983. The tubulins: from DNA to RNA to protein and back again. Cell **34**:330–332.
6. **Cleveland, D.W., and J. C. Havercroft.** 1983. Is apparent autoregulatory control of tubulin synthesis nontranscriptionally regulated? J. Cell Biol. **97**:919–924.
7. **Cleveland, D. W., and M.W. Kirschner.** 1981. Autoregulatory control of the expression of α- and β-tubulins: implications for microtubule assembly. Cold Spring Harbor Symp. Quant. Biol. **44**:173–183.
8. **Cleveland, D. W., M. F. Pittenger, and J. R. Feramisco.** 1983. Elevation of tubulin levels by microinjection suppresses new tubulin synthesis. Nature (London) **305**:738–740.
9. **Cleveland, D. W., and K. F. Sullivan.** 1985. Molecular biology and genetics of tubulin. Annu. Rev. Biochem. **54**:331–365.
10. **Davis, R. W., D. Botstein, and J. Roth.** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
11. **Guarente, L.** 1980. Yeast promoters and *lacZ* fusions designed to study expression of cloned genes in yeast. Methods Enzymol. **101**:181–191.
12. **Gutz, H., H. Heslot, U. Leupold, and N. Loprieno.** 1974. *Schizosaccharomyces pombe*, p. 395–446. *In* R. C. King (ed.), Handbook of genetics, vol. 1. Plenum Publishing Corp., New York.
13. **Hiraoka, Y., T. Toda, and M. Yanagida.** 1984. The NDA3 gene of fission yeast encodes β-tubulin: a cold-sensitive *nda3* mutation reversibly blocks spindle formation and chromosome movement in mitosis. Cell **39**:349–358.
14. **Ito, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153**:163–168.
15. **Kalfayan, L., and P. C. Wensink.** 1981. α-Tubulin genes of *Drosophila*. Cell **24**:97–106.
16. **Kemphues, K. T., T. C. Kaufman, R. A. Raff, and E. C. Raff.** 1982. The testis-specific β-tubulin subunit in *Drosophila melanogaster* has multiple functions in spermatogenesis. Cell **31**:655–670.
17. **Kilmartin, J.** 1981. Purification of yeast tubulin by self-assembly *in vitro*. Biochemistry **20**:3629–3633.
18. **Leupold, U., and H. Gutz.** 1964. Proc. Int. Congr. Genet. **2**:31–35.
19. **Lopota, M. A., J. C. Havercroft, L. T. Chow, and D. W. Cleveland.** 1983. Four unique genes required for β-tubulin expression in vertebrates. Cell **32**:713–724.
20. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular Cloning. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Minami, S., P. S. Collins, E. E. Young, and D. P. Weeks.** 1981. Tubulin induction in *C. reinhardii*: requirement for tubulin mRNA synthesis. Cell **24**:89–95.
22. **Mischke, D., and M. L. Pardue.** 1982. Organization and expression of α-tubulin genes in *Drosophila melanogaster*: one member of the α-tubulin multi-gene family is transcribed in both oogenesis and later embryonic development. J. Mol. Biol. **156**:446–466.
23. **Mitchison, J. M.** 1970. Physiological and cytological methods for *Schizosaccharomyces pombe*, p. 131–165. *In* D. H. Prescott (ed.), Methods in cell physiology, vol. 4. Academic Press, Inc., New York.
24. **Neff, N. F., J. H. Thomas, P. Grisaf, and D. Botstein.** 1983. Isolation of β-tubulin gene from yeast and demonstration of its essential function in vivo. Cell **33**:211–219.
25. **O'Farrell, P. H., and P. Z. O'Farrell.** 1977. Two-dimensional polyacrylamide gel electrophoretic fractionation. Methods Cell Biol. **17**:407–420.
26. **Rose, M., and D. Botstein.** 1983. Construction and use of gene fusions to *lacZ* (β-galactosidase) that are expressed in yeast. Methods Enzymol. **101**:167–180.
27. **Rothstein, R. J.** 1983. One-step gene disruption in yeast. Methods Enzymol. **101**:202–211.
28. **Roy, D., and P. A. Fantes.** 1983. Benomyl-resistant mutants of *Schizosaccharomyces pombe* cold-sensitive for mitosis. Curr. Genet. **6**:195–202.
29. **Russell, P. R., and B. D. Hall.** 1982. Structure of the *Schizosaccharomyces pombe* cytochrome *c* gene. Mol. Cell. Biol. **2**:106–116.
30. **Rykowski, M. C., J. W. Wallis, J. Choe, and M. Grunstein.** 1981. Histone H2B subtypes are dispensable during the yeast cell cycle. Cell **25**:477–487.
31. **Sanchez, F., J. E. Natzle, D. W. Cleveland, M. W. Kirschner, and B. J. McCarthy.** 1980. A dispersed multi-gene family

encoding tubulin in *Drosophila melanogaster*. Cell **22**:845–854.
32. **Schedl, T., T. B. Burland, K. Gull, and W. F. Dove.** 1984. Cell cycle regulation of tubulin RNA level, tubulin protein synthesis, and assembly of microtubules in *Physarum*. J. Cell. Biol. **99**:155–165.
33. **Shortle, D., J. Harber, and D. Botstein.** 1982. Lethal disruption of the yeast actin gene by integrative DNA transformation. Science **217**:371–373.
34. **Silflow, C. D., and J. L. Rosenbaum.** 1981. Multiple α- and β-tubulin genes in *Chlamidomonas* and regulation of tubulin mRNA levels after deflagellation. Cell **24**:81–88.
35. **Southern, E.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **158**:157–179.
36. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Nat. Acad. Sci. USA **77**:5200–5205.
37. **Toda, T., Y. Adachi, Y. Hiraoka, and M. Yanagida.** 1984. Identification of the pleiotropic cell division cycle gene NDA2 as one of two different α-tubulin genes in *Schizosaccharomyces pombe*. Cell **37**:233–242.
38. **Toda, T., K. Umesono, A. Hirata, and M. Yanagida.** 1983. Cold-sensitive nuclear division arrests mutants of the fission yeast *Schizosaccharomyces pombe*. J. Mol. Biol. **168**:251–270.
39. **Towbin, H., T. Stachelin, and I. Gordon.** 1979. Electrophoretic transfer for proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Nat. Acad. Sci. USA **76**:4350–4354.
40. **Umesono, K., T. Toda, S. Hayashi, and M. Yanagida.** 1983. Two cell division cycle genes NDA2 and NDA3 of the fission yeast *Schizosaccharomyces pombe* control microtubular organization and sensitivity to antimitotic benzimidazole compounds. J. Mol. Biol. **168**:271–284.
41. **Wehland, J., H. C. Schroder, and K. Weber.** 1984. Amino acid sequence requirements in the epitope recognized by the α-tubulin specific rat monoclonal antibody YL1-2. EMBO J. **3**:1295–1300.
42. **Yamamoto, M.** 1980. Genetic analysis of resistant mutants to antimitotic benzimidazole compounds in *Schizosaccharomyces pombe*. Mol. Gen. Genet. **180**:231–234.