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Isolation of the β-Tubulin Gene from Yeast and Demonstration of Its Essential Function In Vivo

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Summary

A DNA fragment from yeast (Saccharomyces cerevisiae) was identified by its homology to a chicken β-tubulin cDNA and cloned. The fragment was shown to be unique in the yeast genome and to contain the gene for yeast β-tubulin, since it can complement a benomyl-resistant conditional-lethal mutation. A smaller subfragment, when used to direct integration of a plasmid to the benomyl resistance locus in a diploid cell, disrupted one of the β-tubulin genes and concomitantly created a recessive lethal mutation, indicating that the single β-tubulin gene of yeast has an essential function. Determination of the nucleotide sequence reveals extensive amino acid sequence homology (more than 70%) between yeast and chicken brain β-tubulins.

Introduction

The major protein component of microtubules from eucaryotic cells is tubulin, a heterodimer of two distinct polypeptides designated α and β (Snyder and McIntosh, 1976). Tubulins appear to be conserved proteins, based on in vitro biochemical properties: co-polymerization and co-migration on two-dimensional polyacrylamide gels (Kirschner, 1978). More recently, a direct demonstration of the conservation of tubulin amino acid sequence was provided by the determination of the complete amino acid sequence for both α- and β-tubulin from pig brain (Postingl et al., 1981; Krauhs et al., 1981) and the determination of the nucleotide sequence of cDNA clones for α- and β-tubulin from chicken brain (Cleveland et al., 1980; Valenzuela et al., 1980) as well as α-tubulin from rat brain (Lemischka et al., 1981). A comparison of these sequences shows only a few amino acid changes among the tubulin proteins from these animals.

The lower eucaryote Saccharomyces cerevisiae has a simple nonmotile life cycle which has been well defined by genetic analysis (Hartwell, 1974) and electron microscopy (Byers and Goetsch, 1974). Tubulin was identified in extracts of Saccharomyces (Baum, et al., 1978; Kilmartin, 1981) and shown to co-polymerize with pig brain tubulin, suggesting functional conservation. The ability of chicken brain tubulin cDNAs to cross-hybridize with DNA from a number of organisms (Cleveland et al., 1980) led us to use these cDNAs as probes for cloning the tubulin genes from yeast.

A class of benzimidazole fungicides, including benomyl, methyl 1,2-benzimidazole carabamate (MBC), and thiabendazole was shown to have antimitotic activity in Aspergillus nidulans (Hastie, 1970; Kappas et al., 1974) and to affect nuclear migration (Oakley and Morris, 1980). These compounds appear to bind tubulin from fungi (Davidse and Flach, 1977; Baum et al., 1978; Kilmartin, 1981) and may have an effect similar to that of the mitotic inhibitors colchicine and colcemid in higher eucaryotic cells. Mutants of Aspergillus selected for their resistance to benomyl were sometimes found to have a β-tubulin protein with an altered electrophoretic mobility (Sheir-Neiss et al., 1978). These observations suggested that benomyl resistance in S. cerevisiae might also be the result of mutations in the gene(s) specifying β-tubulin.

In this report, we demonstrate that a single-copy yeast DNA fragment, identified by its homology to a chicken β-tubulin cDNA, is the gene for yeast β-tubulin and that this gene's function is indispensable for growth.

Results

Isolation of Yeast DNA Fragments Homologous to Chicken Tubulin cDNAs

The degree of conservation of tubulin genes between chicken and yeast was tested to see whether it is possible to use chicken brain α- and β-tubulin cDNAs (Cleveland et al., 1980) as hybridization probes for isolating the analogous yeast genes. Gel transfer hybridization experiments (Southern, 1975) were carried out to find hybridization conditions such that the chicken cDNA probes produce a specific and reproducible signal when hybridized to total yeast DNA. Previous experiments showed that these cDNAs do not hybridize to yeast DNA under conditions that give a strong signal against higher eucaryotic DNA (Cleveland et al., 1980). To find optimum hybridization conditions, the stringency of the hybridization reaction was changed by adding increasing amounts of formamide (0 to 20%, v/v) while holding constant salt and temperature (55°C) conditions. Sheared, sonicated E. coli DNA was used as carrier DNA. Acceptable conditions were found (15% formamide) which gave specific and reproducible patterns; an example of the best such results is shown in Figure 1. Several Eco RI fragments show homology to the cDNA probes, but the intensity of hybridization differs among them.

Using these hybridization conditions, two recombinant yeast genomic DNA libraries in bacteriophage λ were screened by plaque filter hybridization (Benton and Davis, 1977) with the two chicken cDNAs as probes. The libraries were made from DNA of two relatively unrelated yeast strains (S288C and FL100) in two different λ vectors. λ gt7 (Thomas et al., 1974; Davis et al., 1980) was used to clone Eco RI fragments of FL100 DNA and λ BF101 (a Bam HI vector derived from λ 1059 of Karn et al., 1980) was used to clone fragments (ca. 18 kb in length) from a Sau 3a partial digest of S288C DNA. Positive phage candidates were purified by four rounds of single-plaque isolation and rescreening. DNA was purified from these

phages and the Eco RI DNA restriction fragments which hybridize to the chick tubulin cDNA probes were identified by gel transfer hybridization (Southern, 1975).

Despite the several Eco RI fragments found in total yeast DNA (Figure 1) with the chicken α-tubulin cDNA probe (including ones 4.5 and 9 kb in length), among the recombinant λ phages made with S288C DNA, only phages carrying either a 4.5-kb or a 9-kb Eco RI restriction fragment homologous to the probe were found; in the case of the FL100 DNA library, only phages carrying a 4.5-kb fragment homologous to the probe were found. The characterization of these fragments will be described in detail elsewhere.

Similarly, although the chicken β-tubulin cDNA hybridized (Figure 1) to several Eco RI fragments (one of which is 1.6 kb in length), only recombinant phages bearing a 1.6-kb Eco RI fragment homologous to the probe were recovered from both S288C and FL100 DNA libraries. This yeast DNA fragment was isolated from a recombinant phage and used to screen a recombinant yeast genomic library in plasmid YEp24 (Carlson and Botstein, 1982) by colony hybridization (Grunstein and Hogness, 1975) in E. coli. This library had been constructed from S288C DNA partially digested with Sau 3a; one isolated plasmid (pRB120) contained a 15-kb insert of yeast DNA including the 1.6-kb fragment. A restriction endonuclease cleavage map of pRB120 was constructed and various fragments were subcloned (Figure 2) into the integrating plasmid Ylp5 (which consists of the yeast *URA3* gene inserted into the plasmid pBR322 [Struhl et al., 1979]).

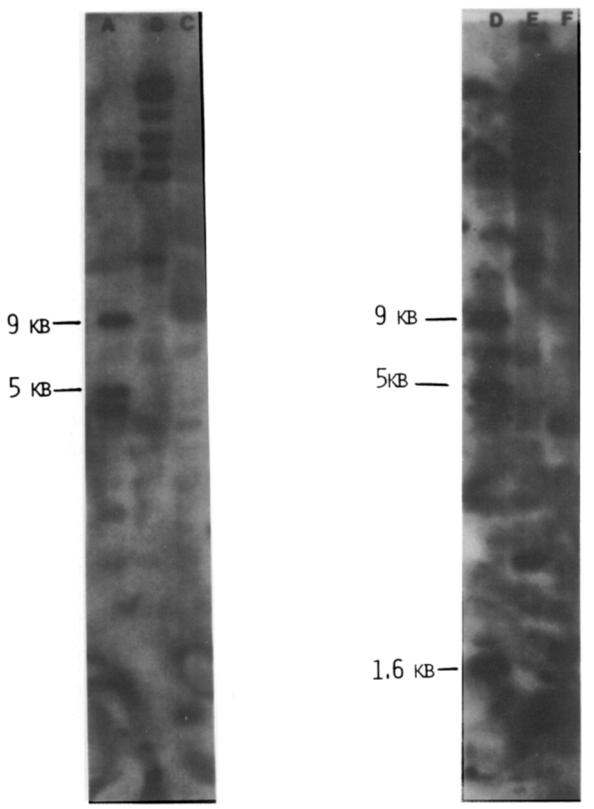

Figure 1. Gel Transfer Hybridization of Total Yeast DNA Bound to Nitrocellulose Probed with Chicken Tubulin cDNAs

Two μg of total yeast DNA were cut with restriction enzymes Eco RI (tracks A and D), Xho I (tracks B and E), and Hind III (tracks C and F). DNA fragments were separated in a 0.5% agarose gel and transferred to nitrocellulose paper. Chicken α-tubulin cDNA was used as the hybridization probe in tracks A–C; chicken β-tubulin cDNA was used as the probe in tracks D–F. The sizes of the strongly hybridizing Eco RI DNA fragments are indicated in the margins.

Genetic Relationship of the 1.6-kb Eco RI Fragment to the Benomyl Resistance Locus of Saccharomyces

Several benomyl-resistant Aspergillus nidulans strains show charge changes in their β-tubulin proteins when visualized by two-dimensional gel electrophoresis, indicating that these strains are β-tubulin mutants (Sheir-Neiss et al., 1980). A similar result has recently been obtained with S. cerevisiae strains resistant to the benomyl derivative MBC (P. Baum and J. Thorner, unpublished data). The Aspergillus results suggested to us that if benomyl-resistant yeast strains are β-tubulin mutants as well, the resistance phenotype might be used to demonstrate a genetic relationship between the 1.6-kb Eco RI fragment (cloned on the basis of homology to chicken β-tubulin cDNA) and the β-tubulin gene(s) of yeast.

Spontaneous benomyl-resistant colonies were selected from the haploid strain DBY918. The majority of these are recessive (i.e. a diploid heterozygous for the *ben*^{R} mutation is sensitive to the drug) and form a single complementation group. A few of these mutants show a concomitant conditional-lethal (cold-sensitive or temperature-sensitive in the presence or absence of benomyl) phenotype which, in crosses, remains completely linked to the drug resistance. A full genetic analysis of these mutants will be published elsewhere.

As described in detail below, two lines of evidence connecting the 1.6-kb Eco RI fragment to the benomyl resistance locus were obtained: first, the 1.6-kb fragment was shown to direct the integration of a yeast integrating plasmid to the benomyl locus; second, a larger DNA segment which includes the 1.6-kb fragment when cloned

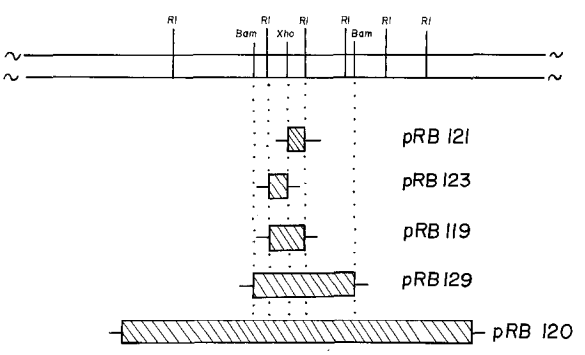

Figure 2. Yeast DNA Fragments Subcloned into Ylp5 or pNN139

The relative sizes and positions of the yeast DNA fragments used in the integration and complementation experiments are shown with the plasmid number.

onto a centromere-containing plasmid was shown to complement both the benomyl resistance phenotype and the conditional-lethal phenotype of a mutant at the benomyl locus.

A strain (DBY1176) which is *MATα*, *ura3*$^-$ and carries *ben*^{R}-104, a recessive mutation which also confers cold sensitivity (i.e. failure to grow below 16°C), was transformed with plasmid pRB119 (Figure 2), a plasmid which consists of the integrating yeast vector YIp5 plus the 1.6-kb Eco RI fragment homologous to chicken β-tubulin cDNA. Integration can occur via homologous recombination (Hinnen et al., 1978) at either the *URA3* locus or at the normal chromosomal locus at which the 1.6-kb Eco RI fragment resides. Five Ura$^+$ (but still Ben^R and cold-sensitive) transformants of DBY1176 were mated with DBY947 (*MATα ura3*$^-$ *BEN*^{S}) and tetrads from each diploid were dissected. In 31 of 31 tetrads examined, the *ben*^{R} marker exhibited complete linkage to *URA3*$^+$ (4:0:0, PD:NPD:TT). This result suggested that the 1.6-kb Eco RI restriction fragment homologous to chicken β-tubulin had directed the integration of the plasmid (and thus the URA3$^+$ gene) to the benomyl resistance locus. As noted above, the transformants in DBY1176 with plasmid pRB119 were benomyl-resistant and cold-sensitive, indicating that the 1.6-kb Eco RI DNA fragment in pRB119 does not complement, and therefore seems not to contain, the entire coding region for the β-tubulin gene.

In order to test complementation, a 4.1-kb Bam HI fragment which spans the 1.6-kb Eco RI fragment was cloned into a yeast vector (pRB166; constructed by C. Mann) bearing a functional centromere (Clarke and Carbon, 1980); the resulting plasmid is called pRB129. The vector replicates autonomously in yeast due to the ARS1 sequence (Struhl et al., 1979) and carries the selectable URA3 gene as well as the centromeric DNA from chromosome III. Plasmids based on such vectors show high stability and a copy number near one in yeast (Clarke and Carbon, 1980). Plasmid pRB129 was used to transform the yeast strain DBY1176 (*ura*$^-$, *ben*^{R}-*104*), selecting Ura$^+$. All such transformants tested (12 of 12) were found to have become benomyl-sensitive and able to grow at 13°C; transformants with the vector plasmid (pRB166) showed no change in their drug resistance or cold sensitivity. Spontaneous Ura$^-$ segregants (which presumably have lost the plasmid) of all 12 pRB129 transformants were found; these invariably had become benomyl-resistant and cold-sensitive once again, indicating that the complementation was due to the plasmid. These results confirm that the 4.1-kb Bam HI fragment contains all the information required to complement the *Ben*^{R}-*104* mutation and thus apparently contains the entire β-tubulin structural gene.

These genetic arguments, combined with the biochemical data of Baum and Thorner (unpublished data) and the nucleotide sequence data described below strongly support the idea that the benomyl resistance locus is a structural gene for yeast β-tubulin. For this reason, we have named the locus *TUB2* to simplify the nomenclature.

Disruption of the *TUB2* Gene Results in a Recessive-Lethal Mutation

In order to determine more precisely the location of the β-tubulin coding region, the plasmids pRB121 and pRB123 were constructed. Each of these plasmids contains approximately one-half of the 1.6-kb Eco RI restriction fragment from pRB119. We know from the integration experiment described above that the 1.6-kb Eco RI fragment does not contain the entire coding region, and therefore must have one of its Eco RI ends within an essential part of the β-tubulin gene. It was likely that either pRB121 or pRB123 would have both of its ends within the gene. When a plasmid carrying a DNA fragment which has *both* of its endpoints in the essential region of a gene integrates into that gene by homologous recombination, the resulting plasmid structure on the chromosome will split that gene into two inactive, partially duplicated halves (Figure 3; see Shortle et al., 1982). Such a "gene disruption" will be a lethal event to a haploid cell if the gene is essential for growth. Such lethality can be detected by tetrad analysis of a diploid transformed with such a disrupting plasmid.

A diploid strain, constructed by mating DBY1176 and

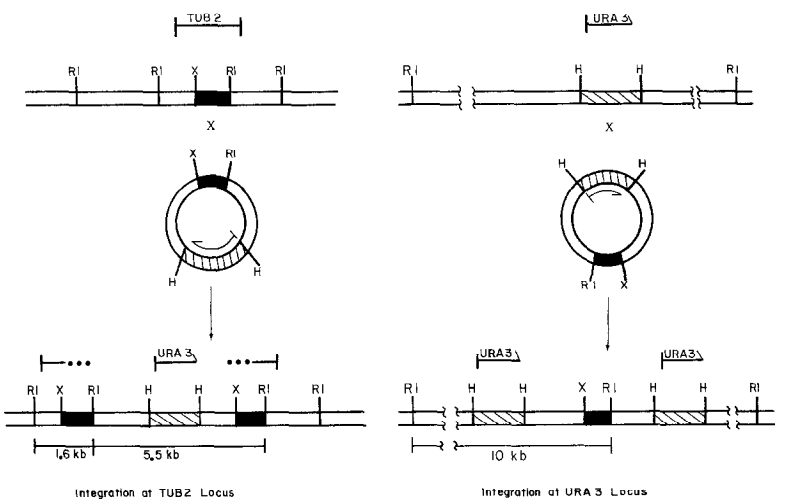

Figure 3. Gene Disruption by Integration

Integration of plasmid pRB121 at the *URA3* locus or the *TUB2* locus can be differentiated by gel transfer hybridization. The predicted chromosomal DNA structure at each locus is shown.

DBY947 (*MATa/MATα*, *ben*^{R}*104/BEN*^{S}, *ura3-52/ura3-52*) was transformed to Ura$^+$ with pRB121. The properties of these transformants indicate that pRB121 indeed has both its ends within the *TUB2* gene (Table 1). First, some of the Ura$^+$ transformants were benomyl-resistant, unlike the parent diploid, suggesting inactivation of the *BEN*^{S} allele. Second, some of the Ura$^+$, benomyl-sensitive transformants showed good spore viability (95%) while others displayed very poor (2 live:2 dead) viability in tetrads. From these preliminary findings, it appeared that at least some of the transformants in this experiment might represent instances of gene disruption.

In order to interpret these results unambiguously, it is necessary to determine the point of integration of the plasmid in the genome of the diploid. Two simple possibilities are anticipated: integration at the *URA3* locus and integration at the *TUB2* locus. These can be distinguished by appropriate gel transfer hybridization experiments: the expectations for an Eco RI digest probed with the 1.6-kb Eco RI fragment are diagrammed in Figure 3. Integration at the *URA3* locus results in the presence of DNA homologous to the probe on two fragments: the normal 1.6-kb fragment at the *TUB2* locus and a new band consisting of the integrated plasmid plus flanking DNA at the *URA3* locus. Integration at the *TUB2* locus, on the other hand, results in DNA homologous to the probe on a 5.5-kb Eco RI fragment resulting from the insertion of the entire plasmid at the *TUB2* locus as well as the 1.6-kb fragment from the intact *TUB2* locus on the other copy of the locus carried by the diploid strain. The results of such a gel transfer hybridization experiment with seven Ura$^+$ transformants are reproduced in Figure 4, from which it is clear that three of the diploids (lanes C, G, and H) contain the plasmid integrated at URA3 and four (lanes B, D, E, and F) contain the plasmid integrated at *TUB2*.

The genetic expectation, assuming that *TUB2* disruption is lethal, is that all the diploids in which the plasmid has integrated at the *TUB2* locus should give a 2 live:2 dead segregation in tetrad analysis. Further, all the live spores should be Ura$^-$, since the reason for lethality is the integration of the plasmid containing the *URA3*$^+$ marker. As shown in Table 1, this expectation is fulfilled. Table 1 also gives the results of analysis of the diploids containing the same plasmid integrated at the *URA3* locus, which serve as a control. They display, as they should, all spores viable and a normal Mendelian (2:2) segregation of Ura$^+$:Ura$^-$ and Ben^R:Ben^S.

Thus, it is clear that plasmid pBR121 contains a fragment of DNA which lies entirely within the essential part (presumably the coding sequence) of a gene required for growth of spores into colonies. The evidence that this gene is the β-tubulin gene comes from the observation that in half of the diploids which contain pRB121 integrated at the *TUB2* locus the integration of the plasmid has resulted in the uncovering of the recessive benomyl resistance phenotype. Thus, disruption of the gene causes not only recessive lethality, but also loss of the dominant benomyl sen-

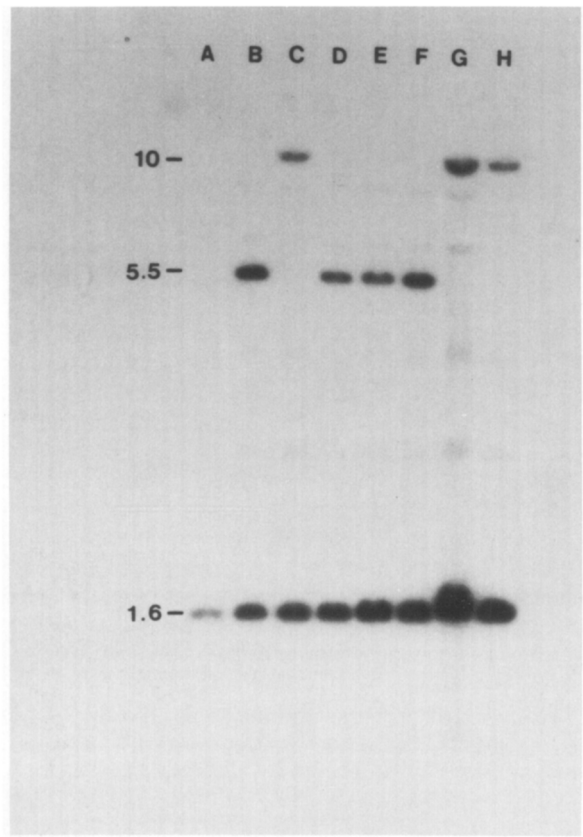

Figure 4. Gel Transfer Hybridization of Total Yeast DNA Purified from Diploid Cells Transformed with pRB121

Total yeast DNA was purified from the untransformed URA$^-$ parent diploid (trace A) and from seven URA$^+$ pRB121 transformants (tracks B–H), and cut with Eco RI. The DNA fragments were separated in a 0.5% agarose gel and transferred to nitrocellulose. The 1.6-kb Eco RI fragment from pRB119 (Figure 2) was used as the hybridization probe. The sizes of the strongly hybridizing Eco RI restriction fragments are indicated in the margins.

Table 1. Genetic Analysis of Diploids Transformed with pRB121

Transformant Phenotype	Plasmid Location	Tetrads Analyzed	Ura$^+$:Ura$^-$ Spores	Ben^R:Ben^S Spores	Live:Dead Spores
Ura$^+$ Ben^S	*URA3*	8	2:2	2:2	4:0
Ura$^+$ Ben^S	*TUB2*	10	0:2[a]	0:2	2:2
Ura$^+$ Ben^R	*TUB2*	11	0:2[a]	2:0	2:2

[a] One spore (of 20 or more) was the opposite phenotype, and presumably represents a gene conversion event.

sitivity. This interpretation is strongly supported by the observation that all viable spores from the benomyl-resistant diploids are themselves resistant, while all viable spores from the benomyl-sensitive diploids with pRB121 integrated at *TUB2* are sensitive: gene disruption affects the benomyl locus directly. The controls (diploids with pRB121 integrated at *URA3*) yield both benomyl phenotypes in Mendelian ratio, as expected.

Nucleotide Sequence of the *TUB2* Locus

The gene disruption experiment defines restriction sites within the essential (probably coding) region of the yeast β-tubulin gene. These restriction sites provided convenient starting points for sequence analysis in a region known to be of interest. The nucleotide sequence was determined using the method of Maxam and Gilbert (1980) following the strategy summarized in Figure 5. The sequence of 1850 nucleotide residues, determined from plasmids pRB119 and pRB129, is shown in Figure 6, along with the predicted amino acid sequence. The orientation and reading frame were inferred by comparison of predicted sequences with the published pig brain (Krauhs et al., 1981) and chicken brain (Valenzuela et al., 1980). The yeast β-tubulin coding sequence has no intervening sequence within it.

The match between the yeast sequence and the higher animal sequences is excellent (Figure 7). More than 70% (128 of 446) of the residues in the animal sequences are identical in the yeast sequence (shown boxed). No additions or deletions of residues had to be carried out in order to bring the two sequences into register. Yeast β-tubulin is apparently 12 residues longer than its chicken brain counterpart. The predicted molecular weights are 51,073 (yeast) as opposed to 49,935 (chicken; Valenzuela et al., 1980).The strong homology between the yeast and animal sequences completes the identification of the cloned genomic yeast DNA fragments as containing the structural gene for yeast β-tubulin.

Discussion

The essential yeast β-tubulin gene at the *TUB2* locus was identified by two properties: its sequence homology to the

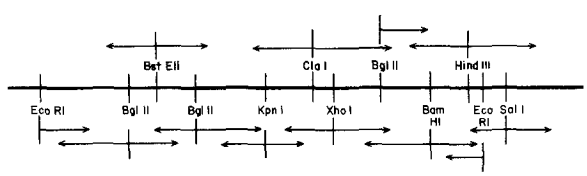

Figure 5. Sequencing Strategy for Yeast β-Tubulin

Plasmids pRB119 and pRB129 DNA were prepared and digested with the restriction enzymes indicated. Recessed 3′ ends were labeled by filling in with DNA polymerase I-large fragment (Klenow fragment). One of the dNTPs in the reaction was α-^{32}P-labeled. Otherwise, polynucleotide kinase and [α-^{32}P]ATP were used for 5′ end-labeling. Uniquely end-labeled fragments were obtained by cleavage with a second enzyme and isolation from polyacrylamide gels. The restriction enzymes used for generating the labeling sites are shown. The direction of sequencing is shown by the arrows. The distance between the two Eco RI sites is 1632 nucleotides.

β-tubulin from chicken brain and its ability to give rise to benomyl-resistant mutants. The connection between these two properties could be made by complementation, mapping, and gene disruption experiments which showed that DNA segments identified by their homology to the chicken cDNA probe in fact identified the essential functional β-tubulin gene of yeast.

The homology between the yeast and chicken sequences at the level of amino acid sequence is extremely good, given the evolutionary distance between the organisms. This homology extends throughout the protein, and evidently represents conservation at the level of function, since the DNA sequences are much more divergent. More detailed analysis of the sequence comparisons (not shown) reveals tha the codons used in yeast and chicken to specify the same amino acid are different as a rule. In the cases of arginine, serine, and leucine codons (where positions other than the third in the codon can vary), differences in two and all three bases are common.

As a consequence of the weak homology in nucleotide sequence, the chicken β-tubulin cDNA hybridizes very weakly to the yeast gene. Presumably, a few regions of reasonable match are able to form mismatched hybrids detectable under the hybridization conditions used. There appear, in fact, to be only a few regions of homology in the sequences which reach the range of length (20–50 bases) thought to be required for stable hybrid formation (McCarthy, 1967; Britten and Kohne, 1968) and which might account for the hybridization observed. Since it is clear that long regions of exact homology are not present and evidently are not required for hybrid formation, the false positives we observed are not so surprising. It should be noted that in this case it was the connection to the benomyl-resistant mutations (likely a priori to be in tubulin genes) which made it possible to determine which DNA fragments actually represented the tubulin gene. The gene disruption experiment described above indicates that the β-tubulin gene encoded at the *TUB2* locus is essential for the growth of a haploid yeast strain, and therefore cannot be substituted for by an undetected copy of this gene elsewhere in the genome. This result does not directly address the question of how many β-tubulin genes there are in a haploid yeast genome, especially since functional diversity has been shown to exist among Drosophila melanogaster β-tubulin proteins (Kemphues et al., 1979; Raff et al., 1982). However, if there are two or more β-tubulin genes, they should share at least as much DNA sequence homology to each other as the chicken cDNA does to the gene on plasmid pRB129, and probably more. Gel transfer hybridization experiments with the 1.6-kb Eco RI fragment as a probe against total yeast DNA did not reveal any strongly hybridizing fragments (Figure 4, lane A, for example). Further, all the phages isolated from two libraries using the chicken β-tubulin cDNA probe turned out to contain all or part of the 1.6-kb Eco RI fragment. These observations support the idea that there is really only one β-tubulin gene in a haploid yeast genome.

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                                                                                        -390     *        *
                                                                                        CCAATCAACCAGCAGTTTGAACAGGTAATCG

-360      *         *         *         *         *    -300      *         *         *         *         *
TACTCGCTATTCATATTAGTGCTTTTCTTGTTTTATTTATTTCAACCTGGCCTAACAGTAAAGATATCCTCCTCAAAACTGGTGCACTTAATCGCTGAATTTGTTCTGGCTTCTCTTCTT

-240      *         *         *         *         *    -180      *         *         *         *         *
TTTCTTTATTCCCCCCATGGGCCAAAAAAATAGTACTATCAGGAATTTGGCGCCGGGTCACGATATACGTGTACAGTGACCTGGCGACGCCACAAGGAAAAAGGAAAAAAAACAGAAAAA

-120      *         *         *         *         *    -60       *         *         *         *         *
ACAACAAAAACTAAAACAAACACGAAAACTTTAATAGATCTAAGTGAAGTAGTGGTGAGGCAATTGGAGTGACATAGCAGCTACTACACTACAAAAGCAAAATCTCCACAAAGTAATATA

1                                       11                                      21
ATG AGA GAA ATC ATT CAT ATC TCG GCA GGT CAG TAT GGT AAC CAA ATT GGT GCT GCA TTC TGG GAA ACT ATC TGT GGT GAG CAC GGT TTG
met arg glu ile ile his ile ser ala gly gln tyr gly asn gln ile gly ala ala phe trp glu thr ile cys gly glu his gly leu

31                                      41                                      51
GAT TTC AAT GGG ACA TAT CAC GGC CAT GAC GAT ATC CAG AAG GAG AGA CTG AAC GTG TAC TTC AAC GAG GCA TCT TCT GGG AAG TGG GTT
asp phe asn gly thr tyr his gly his asp asp ile gln lys glu arg leu asn val tyr phe asn glu ala ser ser gly lys trp val

61                                      71                                      81
CCA AGA TCT ATT AAC GTC GAT CTA GAA CCT TGG ACG ATT GAC GCA GTA CGC AAT TCT GCC ATC GGG AAT TTG TTT AGA CCT GAC AAT TAT
pro arg ser ile asn val asp leu glu pro trp thr ile asp ala val arg asn ser ala ile gly asn ley phe arg pro asp asn tyr

91                                      101                                     111
ATC TTT GGG CAA AGT TCT GCG GGC AAC GTG TGG GCC AAG GGT CAC TAC ACA GAA GGT GCT GAG CTT GTA GAC AGC GTC ATG GAT GTT ATT
ile phe gly gln ser ser ala gly asn val trp ala lys gly his tyr thr glu gly ala glu leu val asp ser val met asp val ile

121                                     131                                     141
AGA CGA GAG GCC GAA GGA TGC GAC TCC CTT CAA GGT TTC CAG ATC ACA CAT TCT CTT GGT GGT GGT ACC GGT TCC GGT ATG GGT ACG CTT
arg arg glu ala glu gly cys asp ser leu gln gly phe gln ile thr his ser leu gly gly gly thr gly ser gly met gly thr leu

151                                     161                                     171
TTG TTC TCG AAG ATT AAG GAA GAG TTA CCT GAT CGT ATG ATG GCC ACC TTC TCC GTC TTG CCC TCT CCG AAG ACT TCT GAC ACC GTT GTC
leu phe ser lys ile lys glu glu leu pro asp arg met met ala thr phe ser val leu pro ser pro lys thr ser asp thr val val

181                                     191                                     201
GAA CCA TAC AAT GCC ACG TTG TCT GTG CAC CAA TTG GTA GAA CAC TCT GAT GAA ACA TTC TGT ATC GAT AAC GAA GCA CTT TAT GAC ATC
glu pro tyr asn ala thr leu ser val his gln leu val glu his ser asp glu thr phe cys ile asp asn glu ala leu tyr asp ile

211                                     221                                     231
TGT CAA AGG ACC TTA AAG TTG AAT CAA CCT TCT TAT GGA GAT TTG AAC AAC TTG GTC TCG AGC GTC ATG TCT GGT GTG ACA ACT TCA TTG
cys gln arg thr leu lys leu asn gln pro ser tyr gly asp leu asn asn leu val ser ser val met ser gly val thr thr ser leu

241                                     251                                     261
CGT TAT CCC GGC CAA TTG AAC TCT GAT TTG AGA AAG TTG GCT GTT AAT CTT GTC CCA TTC CCA CGT TTA CAT TTC TTC ATG GTC GGC TAC
arg tyr pro gly gln leu asn ser asp leu arg lys leu ala val asn leu val pro phe pro arg leu his phe phe met val gly tyr

271                                     281                                     291
GCT CCA TTG ACG GCA ATT GGC TCT CAA TCA TTT AGA TCT TTG ACT GTC CCT GAA TTA ACA CAG CAA ATG TTT GAT GCC AAG AAC ATG ATG
ala pro leu thr ala ile gly ser gln ser phe arg ser leu thr val pro glu leu thr gln gln met phe asp ala lys asn met met

301                                     311                                     321
GCT GCT GCC GAT CCA AGA AAC GGT AGA TAC CTT ACC GTT GCA GCC TTC TTT AGA GGT AAA GTT TCC GTT AAG GAG GTG GAA GAT GAA ATG
ala ala ala asp pro arg asn gly arg tyr leu thr val ala ala phe phe arg gly lys val ser val lys glu val glu asp glu met

331                                     341                                     351
CAT AAA GTG CAA TCT AAA AAC TCA GAC TAT TTC GTG GAA TGG ATC CCC AAC AAT GTG CAA ACT GCT GTG TGT TCT GTC GCT CCT CAA GGT
his lys val gln ser lys asn ser asp tyr phe val glu trp ile pro asn asn val gln thr ala val cys ser val ala pro gln gly

361                                     371                                     381
TTG GAC ATG GCT GCT ACT TTC ATT GCT AAC TCC ACA TCT ATT CAA GAG CTA TTC AAG AGA GTT GGT GAC CAA TTT TCC GCT ATG TTC AAA
leu asp met ala ala thr phe ile ala asn ser thr ser ile gln glu leu phe lys arg val gly asp gln phe ser ala met phe lys

391                                     401                                     411
AGA AAA GCT TTC TTG CAC TGG TAT ACT AGT GAA GGT ATG GAC GAA TTG GAA TTC TCT GAG GCT GAA TCT AAT ATG AAT GAT CTG GTT AGC
arg lys ala phe leu his trp tyr thr ser glu gly met asp glu leu glu phe ser glu ala glu ser asn met asn asp leu val ser

421                                     431                                     441
GAA TAC CAA CAA TAC CAA GAG GCT ACT GTA GAA GAT GAT GAA GAA GTC GAC GAA AAT GGC GAT TTT GGT GCT CCA CAA AAC CAA GAT GAA
glu trp gln gln tyr gln glu ala thr val glu asp asp glu glu val asp glu asn gly asp phe gly ala pro gln asn gln asp glu

451                                          *         20        *         40        *         60
CCA ATC ACT GAG AAT TTT GAA TAA TTAAGTTGCTTTCCTTTCTTTTTCTTACCTTTCTTCTTCTCTAGGATTGAAGCACTTGGAGCAAAATAGACAAAAATC  3'
pro ile thr glu asn phe glu NON
```

Figure 6. DNA Sequence of the yeast β-Tubulin Gene

The sequence of the yeast β-tubulin gene is presented along with the predicted amino acid sequence.

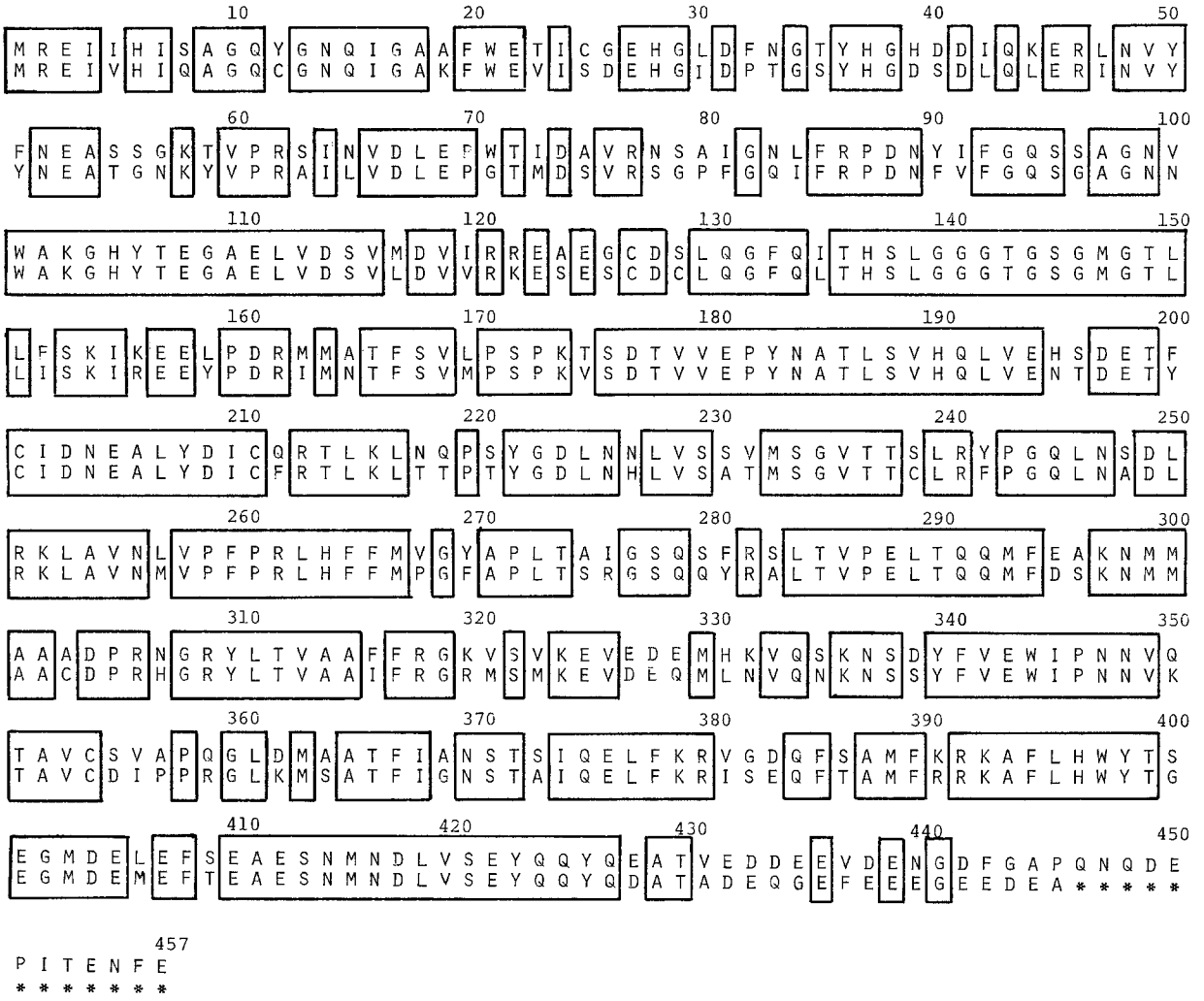

Figure 7. Comparison of Predicted Yeast and Chicken β-Tubulin Amino Acid Sequence

The yeast sequence is shown in the upper line and the chicken in the lower line. Boxes show exact amino acid homologies.

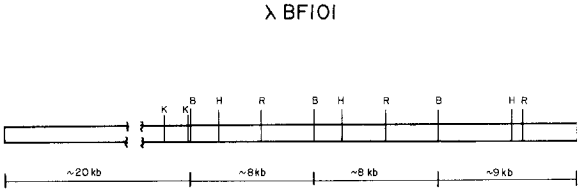

Figure 8. Restriction Map of λ Cloning Vector λ BF101

The two internal Bam HI DNA fragments can be replaced by 12–22 kb of genomic DNA.

The strong similarity between the β-tubulins of yeast and higher eucaryotes implies a similarity in the function of these proteins in their respective cells. However, yeast cells do not display all of the phenomena of higher cells in which tubulin is implicated. It seems likely that the essential function in yeast relates to mitosis and cell division, a hypothesis supported by the preliminary observation that cold-sensitive alleles of *TUB2* arrest at nonpermissive temperature with a morphology suggesting a failure in a specific step of the cell cycle (Hartwell, 1974). For this reason, it seems likely that the conserved structure of β-tubulin reflects a detailed similarity in the role of microtubules in the mitotic apparatus among all eucaryotes.

Experimental Procedures

Strains and Media

E. coli strains DB6507, derived from HB101 (Boyer and Roulland-Dussoix, 1969) (pyrF-74::Tn5), was used for bacterial transformation and plasmid growth. BNN45 (St. John and Davis, 1981) was used to grow high titer plate and liquid stocks of bacteriophage λ derivatives. NS428 and NS433 (Sternberg et al., 1977) were used to produce packaging extracts. Q358 and Q359 (Karn et al., 1980) and KRO (trp⁻ recA⁻) were used to construct the yeast genomic library in λBF101.

Bacterial media were made as described by Miller (1972), except for NZC medium (Blattner et al., 1977), used for λ growth.

Yeast strain DBY637 (*Mat ura3-2am*), a FL100 derivative, was obtained from F. Lacroute. DBY1176 (*MATa his4-38 ura3-52 ben104*) is a spontaneous benomyl-resistant derivative of DBY918 (*Matα his4-38*), an S288c strain from G. R. Fink. DBY947 (*Matα ade2-101 ura3-52*) is the result of repeated backcross of the *ura3-52* allele into S288c background and was made by B. Osmond and M. Carlson. Benomyl, 98.6%, was a gift of O. Zoebisch, E. I. DuPont de Nemours and Co., Inc., and was stored as an 8 mg/ml stock in dimethyl sulfoxide at −20°C. Standard yeast plates (Sher-

man et al., 1974) were supplemented with 40 μg/ml benomyl for selection and screening of mutants. Tetrad analysis and other standard yeast genetic techniques were performed as described by Mortimer and Hawthorne (1966).

Gel Electrophoresis and DNA Preparation

Restriction enzymes, DNA polymerase I, DNA polymerase I-large fragment, and T_4 DNA ligase were purchased from New England Biolabs and used according to the supplier's recommendations. DNA fragments were separated in horizontal agarose gels in Tris-acetate (McDonell et al., 1977) or Tris-borate buffer (Peacock and Dingman, 1968). DNA fragments were isolated from agarose by electrophoresis into hydroxylapatite (Bio-Rad Laboratories) and elution as described by Tabak and Flavell (1978).

Yeast DNA was made by the method of Cryer et al. (1975), with the addition of an equilibrium banding in CsCl. E. coli DNA was made from frozen cells, provided by M. Gefter, by minor modifications of the method of Marmur (1961). Other recombinant DNA methods were performed as described by Davis et al. (1980).

Plasmids and λ vectors

Plasmids YIp5 and YEp24 are described in Botstein et al. (1979). Construction of the yeast genomic library in YEp24 is described in Carlson and Botstein (1982). Bacterial transformation was by the method of Mandel and Hirga (1970).

The yeast genomic library in λ gt7 (Thomas et al., 1974) was provided by M. Rose and was constructed as described in Davis et al. (1980) with DNA from DBY637.

λ 1059 (Karn et al., 1980) DNA was modified to remove the pBR322 (Bolivar et al., 1977) homology. λ 1059 DNA was cut with Bam HI, diluted to 10 μg/ml final DNA concentration, ligated with T_4 DNA ligase, and transformed into DB6507, selecting ampicillin resistance. The resulting plasmid, the 14-kb internal DNA fragment, was cut with Hind III, and the DNA fragment containing the spi (*red gamma*) region of λ was isolated, ligated with T_4 DNA ligase and digested with Bam HI. This resulting DNA fragment was added to Bam HI-cut λ 1059 DNA and ligated with T_4 DNA ligase. The ligation mixture was packaged (Sternberg et al., 1977) and plated out (10^3 plaques/100-mm plate). Plaques were screened (Benton and Davis, 1977) with plasmid pBR322 as probe. Phages not hybridizing to the probe were 2× single-plaque-purified and DNA-prepared. The resulting phage (λ BF101) carries two copies of an 8-kb internal Bam HI restriction fragment (Figure 8), arranged in a head-to-tail manner. The spi genes are arranged in the opposite orientation of λ 1059 (Figure 5). A yeast genomic library was constructed in λBF101 by partially digesting yeast from DBY939 (*MATα ade2-101 suc2am*) (Carlson and Botstein, 1982) with restriction enzyme Sau 3A, isolating 15–25-kb DNA fragments from an agarose gel and ligating the DNA fragments into Bam HI-digested λ BF101 DNA. Ligation and selection of recombinant phages were as described by Karn et al. (1980).

The centromere vector pNN139 (YRp16-5c4301) was the gift of Carl Mann (Stanford) who constructed it by inserting the 1.1-kb ClaI to Bam HI fragment containing *CEN3* (Clarke and Carbon, 1980) between the Cla I and Bam HI sites of the *TRP1 ARS1 URA3* vector YRp16 (Stinchcomb et al., 1982).

Hybridization Methods

Transfer of DNA fragments from agarose gels to nitrocellulose paper (Schleicher and Schuell) was as described by Southern (1975) except that SPE (0.18 M NaCl, 1 mM EDTA, and 10 mM sodium phosphate, pH 7) was used.

Hybridization conditions for screening the yeast genomic λ libraries with the chicken brain tubulin cDNAs as probe were: 6× SPE, 0.5% SDS (BDH), 15% formamide (MCB), 50 μg/ml E. coli carrier DNA, 55°C, for 16–18 hr with agitation in a water bath. Hybridization reactions contained 4 ml of hybridization buffer per 100-cm^2 nitrocellulose paper and approximately 10^7 cpm of ^{32}P-labeled, denatured DNA probe in a heat-sealable plastic bag. Filters were washed in a large volume of 2× SPE + 0.5% SDS a 50°C for 4 hr with agitation. Filters were dried and visualized using Kodak AR x-ray film and DuPont Cronex screens at −70°C.

When yeast DNA fragments were used as probes for plaque hybridization (Benton and Davis, 1977) or colony hybridization (Grunstein and Hogness, 1975), the hybridization conditions were the same, but the washing conditions were 2× SPE + 0.5% SDS and 55°C.

Labeling of DNA fragments for hybridization probes was by nick translation with DNA polymerase I and DNase I as described by Rigby et al. (1977), except the final enzyme reaction volume was 10 titers.

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