The Highly Divergent β-Tubulins of *Aspergillus nidulans* Are Functionally Interchangeable

Gregory S. May

Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030

Abstract. An internal 1.4-kb Bst EII fragment was used to disrupt the *benA* gene and establish heterokaryons. The heterokaryons demonstrated that the molecular disruption of *benA* results in a recessive *benA* null mutation. Conidia from a heterokaryon swell and germinate but cannot undergo nuclear division and are thus inviable. A chimeric β-tubulin gene was constructed with the *benA* promoter driving the *tubC* structural gene. This chimeric gene construction was placed on a plasmid containing a selectable marker for *Aspergillus* transformation and the gene disrupting fragment of *benA*. Integration of this plasmid at *benA* by the internal gene disrupting fragment of *benA* simultaneously disrupts the *benA* gene and replaces it with the chimeric β-tubulin gene, rescuing the *benA* null generated by the integration. Strains generated by this procedure contain only *tubC* β-tubulin for all β-tubulin functions. Strains having only *tubC* β-tubulin are viable and exhibit no detectable microtubule dysfunction though they are more sensitive than wild-type strains to the antimicrotubule drug benomyl. It is concluded that the two β-tubulin genes of *Aspergillus nidulans*, though highly divergent, are interchangeable.

MICROTUBULES form a variety of structures within cells and are involved in cellular shape and motility. Given the diversity of form and function for microtubules, it is surprising that in general microtubule ultrastructure is highly conserved. One possible source for functional diversity of microtubules are the α- and β-tubulin subunits.

The structural and functional diversity of microtubules and the existence of tubulin isotypes led Fulton and Simpson (1976) to formulate the multitubulin hypothesis. In its simplest form, the multitubulin hypothesis proposes that different microtubules are formed by different tubulins and that different genes encode the different tubulin isotypes found in the various microtubules of the cell. The existence of multiple tubulin genes, tubulin isotypes, and the diversity of microtubule structures and their functions in most eukaryotes has resulted in additional speculation about the significance of tubulin multigene families (Cleveland and Sullivan, 1985; Cleveland, 1987; Raff, 1984).

Aspergillus nidulans has two β-tubulin genes *benA* and *tubC*. The *benA* gene functions during asexual growth and participates in mitosis and nuclear movement (Oakley and Morris, 1980; 1981). The *tubC* gene appears to function only during asexual sporulation (conidiation), but is not essential for this process as demonstrated by the isolation of null mutants in *tubC* or its deliberate disruption (May et al., 1985; May and Morris, 1988; Weatherbee et al., 1985). Sequences of the *benA* and *tubC* genes predict proteins that are 17% divergent at the amino acid level (May et al., 1987). This degree of amino acid sequence divergence for β-tubulins is equal to comparing either the *benA* or *tubC* polypeptides to any known β-tubulin sequence. Thus, *A. nidulans* is an organism that allows us to test directly the functional significance of divergent β-tubulin isotypes by constructing strains capable of producing a single β-tubulin isotype. To develop strains producing *tubC* β-tubulin, a novel one step gene disruption/replacement was used. This method should be applicable to other systems that have homologous integrative recombination. It is shown in this paper that though the *benA* and *tubC* β-tubulin genes of *A. nidulans* encode highly divergent β-tubulins, they are functionally equivalent polypeptides.

1. *Abbreviations used in this paper*: DAPI, diamidino-2-phenylindole; YAG, 0.5% yeast extract, 2% glucose, 1.5% agar.

Materials and Methods

Aspergillus Strains and Culture Conditions

The strains used in this study are listed in Table I. Strains were grown on 0.5% yeast extract, 2% glucose, 1.5% agar (YAG)[1], and trace elements (Cove, 1966). Strains having the *pyrG89* mutation were grown on YAG supplemented with 5 mM uridine and 10 mM uracil. Liquid media was YAG but without agar. Transformation of *Aspergillus* was performed as described previously (Osmani et al., 1987) except that protoplasts were plated on media made osmotically stable with 1 M sucrose. Phenotypic analysis of null mutations in an essential gene by generation of heterokaryon was as reported previously (Osmani et al., 1988).

Bacterial Strains and Plasmids

Escherichia coli K-12 strain TB1 was used for routine plasmid propagation. Plasmid DNA was purified using the alkaline lysis method (Maniatis et al., 1982). General methods for plasmid construction were as described previously (May et al., 1985).

Table I. Aspergillus Strains Used

Strains	Genotype
GB20	*pyrG89 pabaA1; benA22 fwA1 uaY9*
GR5	*pyrG89; wA3; pyroA4*
R153	*wA3; pyroA4*
20.3.8	*pyrG89 biA1; argB2; methG1; benA22*
20.1.8	*argB2*
(transformant strain*)	
3.3	*pyrG89; wA3; pyroA4; benA::pbenAΔ*
K07 (transformant strain)	*pyrG89; pabaA1; benA22::pGM22K0 fwA1 uaY9*

* Additional transformant strains are presented but not phenotypically examined in detail.

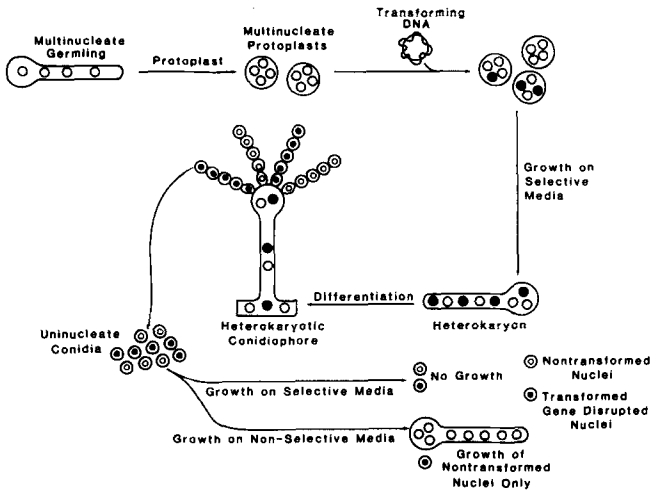


Figure 1. Diagram illustrating the method of generating heterokaryons by disruption of an essential gene. Filled in nuclei contain the integrated nutritional marker and have the disrupted essential gene. The open nuclei are auxotrophic for the nutritional marker and contain a functional copy of the essential gene. See text for a full description.

Preparation and Electrophoresis of DNA

Total genomic DNA was prepared as described previously (Osmani, 1987). Genomic Southern transfers were as described previously (May et al., 1985). Labeling of DNA probes for hybridization was performed as described (Feinberg and Vogelstein, 1983). Probes generally had specific activities of 2–5 × 10^8 cpm/μg of DNA.

Growth Studies

Radial growth studies were performed as described previously (Weatherbee et al., 1985). Ascospore viability was determined by plating dilutions of ascospores onto dialysis membrane on a plate of agar solidified media and placing sterile coverslips on top. Germination was for 9 h at 37°C, after which the coverslips were removed, fixed in 2% (vol/vol) glutaraldehyde, 50 mM potassium phosphate, pH 6.8, 0.1% (vol/vol) Triton X-100 for 10 min at room temperature and viewed using phase microscopy. Germination represents the clear extension of a germ tube and ungerminated ascospores represent the absence of a germ tube. Conidial viability was determined by plating 10^6 conidia into a Petri plate containing 25 ml liquid media and sterile coverslips. Plates were incubated for 8 h at 37°C. Coverslips were processed and analyzed as for ascospores. Cells were also stained with 4,6-diamidino-2-phenylindole (DAPI) at 20 ng/ml in the fixative, mounted in 50% (vol/vol) glycerol with 20 ng/ml DAPI and observed and photographed using epifluorescence microscopy on an axiophot (Carl Zeiss, Inc., Thornwood, NY). Images were recorded on film (Tri-X; Eastman Kodak Co., Rochester, NY) and developed as recommended by the manufacturer.

Chimeric Gene and Plasmid Constructions

The chimeric β-tubulin gene used in these studies was constructed by fusing the *benA* promoter to the *tubC* structural gene as described below. The promoter and first 12 amino acids of the *benA* gene were obtained on a 3.3-kb Pst I-Bst EII fragment. The *tubC* structural gene sequences were obtained on a 3.2-kb Bst EII-Xho I fragment. The Bst EII site of the two β-tubulin genes is conserved and lies at the start of the third exon. The amino acids encoded by the first two exons are identical for the two genes. This chimeric gene was cloned into the vector pRG3 that contains the pyr4 gene of *Neurospora crassa*, which is the selectable marker used during transformation and complements the *pyrG89* mutation of *A. nidulans*. The *benA* gene disrupting fragment was the 1387 bp Bst EII fragment of *benA* and spans amino acids 13 to 383 of the polypeptide. This fragment was made blunt by the addition of the large fragment of *Escherichia coli* DNA polymerase I and the four deoxynucleoside triphosphates. Oligodeoxynucleotides containing Eco RI restriction sites were ligated to the fragment, and the fragment was cloned into the Eco RI site of pUC19. This fragment was then cloned into the Eco RI site of pRG3 to produce the plasmid pbenAΔ and the plasmid with the chimeric gene to produce the plasmid pGM22K0.

Materials

Restriction endonucleases, DNA modifying enzymes and oligodeoxynucleotide linkers were obtained from New England Biolabs (Beverly, MA), Promega Biotec (Madison, WI), or Boehringer Mannheim Biochemicals (Indianapolis, IN), and used according to manufacturer's instructions. Agarose was from FMC Corp. (Rockland, ME) and [α-^{32}P]dATP (400 Ci/mmole) was purchased from Amersham Corp. (Arlington, IL). Nitrocellulose (BA85) and Nytran nylon membranes were from Schleicher & Schuell, Inc. (Keene, NH). All other reagents were obtained from Sigma Chemical Co. (St. Louis, MO) or Fisher Scientific Co. (Newburgh, NY).

Results

Disruption of the benA Gene Leads to Loss of Cell Viability

The molecular disruption of an essential gene in a haploid organism such as *Aspergillus* will result in cell inviability. In *Aspergillus*, it is possible to carry a null mutation by exploit-

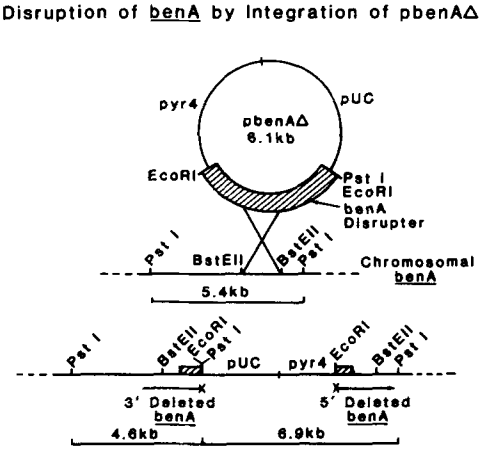


Figure 2. Structure of *benA* disrupting plasmid pbenAΔ (not drawn to scale), structure of chromosomal *benA* gene and predicted structure for integration of pbenAΔ at *benA*. The internal fragment of *benA* in pbenAΔ is the hatched box flanked by Eco RI sites, and the sequences derived from the vector pRG3, pUC19, and pyr4 are represented by the single line. The 3′ and 5′ truncated *benA* genes are indicated below. The partial restriction map and the predicted bands of hybridization for transformant DNA following Pst I digestion are also indicated below the map.

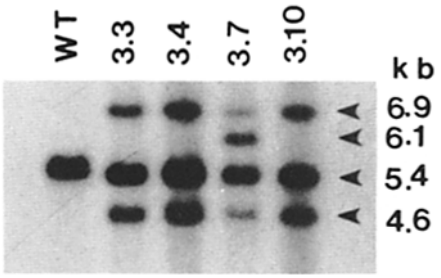


Figure 3. Autoradiograph of genomic Southern of total DNA from pbenAΔ heterokaryon transformants digested with Pst I. The filter was probed with the 1.4 kb Bst EII fragment of *benA*. The size (in kilobase) of the bands of hybridization are indicated at right.

ing some aspects of its life cycle and cell biology as illustrated in Fig. 1. *Aspergillus* grows as a syncytium (more than one nucleus in a common cytoplasm), and it will also grow as a heterokaryon (more than one genotype of nucleus in a common cytoplasm). Therefore, transformation of multinucleate protoplasts with a gene disrupting plasmid can result in heterokaryon colonies on selective media, formed from transformed nuclei containing a disrupted essential gene, but having a required nutritional marker, in the same cytoplasm with nontransformed nuclei lacking the nutritional marker but having the essential gene function. During conidiation, asexual sporulation, uninucleate conidia are formed. It is possible therefore to screen for heterokaryons of this type because conidia from such colonies are unable to grow on selective media, although the heterokaryon mycelia will grow on selective media. This strategy was first employed to generate a null mutation in the essential *bimE* gene of *Aspergillus* to determine the terminal phenotype of a null allele (Osmani et al., 1988).

A haploid strain (GR5) was transformed with pbenAΔ (Fig. 2). Eleven transformants were tested for the heterokaryon disrupted phenotype and four were found to produce conidia exhibiting no growth on selective media; these four strains were designated 3.3, 3.4, 3.7, and 3.10. The four colonies unable to grow on selective media were presumably heterokaryons composed of *benA*+, *pyr*− nontransformed nuclei and *benA*− pyr+ transformed nuclei in the same cytoplasm. To demonstrate that these heterokaryons did contain transformed nuclei with pbenAΔ integrated at *benA*, DNA from all four heterokaryons was subjected to Southern analysis. Integration of pbenAΔ at *benA* would produce a restriction endonuclease polymorphism (Fig. 2). The *benA* gene resides on a 5.4 kb Pst I fragment and integration of pbenAΔ at *benA* should result in two new fragments, one of 4.6 kb and another of 6.9 kb (Fig. 2), in addition to the 5.4-kb band, because the DNA is prepared from the heterokaryon mycelium. These fragments will all be observed following hybridization with the fragment used for the gene disruption, the 1.4-kb Bst EII fragment. Southern analysis of the pbenAΔ transformants 3.3, 3.4, 3.7, and 3.10 indicated that all contained the expected 4.6-, 5.4-, and 6.9-kb bands of hybridization for heterokaryons disrupted for *benA* (Fig. 3). The transformant 3.7 contains an additional band of 6.1 kb indicative of a tandem integration event; i.e., two copies of pbenAΔ at *benA* (Fig. 3). From these results, it can be concluded that the null mutation in *benA* generated by the disruption is recessive.

Conidia from heterokaryon, 3.3, and GR5, the recipient strain, were allowed to germinate on nonselective medium, containing uracil and uridine and selective media lacking uracil and uridine, for a period of time during which wild type cells would reach the 8–32 nuclei/cell stage. The recipient strain GR5 germinated and sent out germ tubes on nonselective media typical of wild-type cells (Fig. 4 *a*), and did not swell or germinate on selective media as expected (Fig. 4 *b*). In contrast, conidia from the heterokaryon transformant 3.3 had growth characteristic of GR5 conidia on nonselective media but also had another class of slower growing cells (Fig. 4 *c*, *open arrowheads*). When conidia from transformant 3.3 were germinated on selective media, many cells did germinate but did not grow to the extent of cells on nonselective media (Fig. 4 *d*, *large arrowheads*). In addition, many swollen conidia were observed that were like those seen on nonselective media (Fig. 4 *d*, *open arrowheads*). These same cells were examined by DAPI fluorescence to determine if nuclear division had taken place in these cells (Fig. 5). GR5 cells grown on nonselective media grew and underwent nuclear division as expected (Fig. 5, *a* and *b*) but did not swell, germinate or undergo nuclear division on selective media (Fig. 5 *c*). In contrast, conidia from transformant 3.3 could germinate on nonselective media (Fig. 5, *d* and *e*) and selective media (Fig. 5 *f*), but rarely was more than a single nucleus evident. Though on nonselective media, cells like the parental strain GR5 were present as evidenced by their normal growth and multinuclear nature. Unlike GR5 though, conidia from 3.3 would swell, send out a germ tube and decondense their chromatin on selective media (compare cells Fig. 5 *f*, *center*, with the cell *upper right* in *f* and those cells in *c*). In addition, these cells rarely exhibited the interphase nuclear morphology of the wild-type cells (compare Fig. 5, *a* and *b* with *d*, *e*, and *f*). In fact, the chromatin was often diffuse (cell *right*, Fig. 5, *d*) and in some cases, may have been in a prometaphase state (cell *left* in Fig. 5 *d* and cells in *f*). Site specific integration of a plasmid carrying the internal 1.4 kb Bst EII fragment of *benA* results in loss of cell viability, due to disruption of the *benA* gene.

Disruption and Replacement of the benA Gene by pGM22K0

Knowing that disruptive integration by the internal Bst EII fragment of *benA* results in lethality, it was then possible to determine whether the *tubC* structural gene could replace *benA* by using the plasmid pGM22K0. There are three possible integrations for pGM22K0. In case I of Fig. 6, pGM22K0 integrates via the *benA* promoter sequences leading to a strain with a complete *benA* gene, a complete *tubC* gene and a chimeric gene (Fig. 6 *a*). In case II, pGM22K0 integrates via the *tubC* coding sequences producing a strain similar to that in case I but with the plasmid at *tubC* (Fig. 6 *b*). In case III, pGM22K0 integrates via the internal *benA* disrupter sequences, the same sequence as in pbenAΔ, producing a strain having a 5′ and 3′ deleted *benA* gene and a complete chimeric gene and the resident *tubC* gene (Fig. 6 *c*). In this case, a strain having only *tubC* gene product for all of its β-tubulin functions would be the result. This is possible because *A. nidulans* is haploid and integrative gene disruption of the single *benA* gene produces a strain now expressing only the chimeric gene, *tubC* structural gene driven by the *benA* promoter, and the resident *tubC* gene for all of its β-tu-

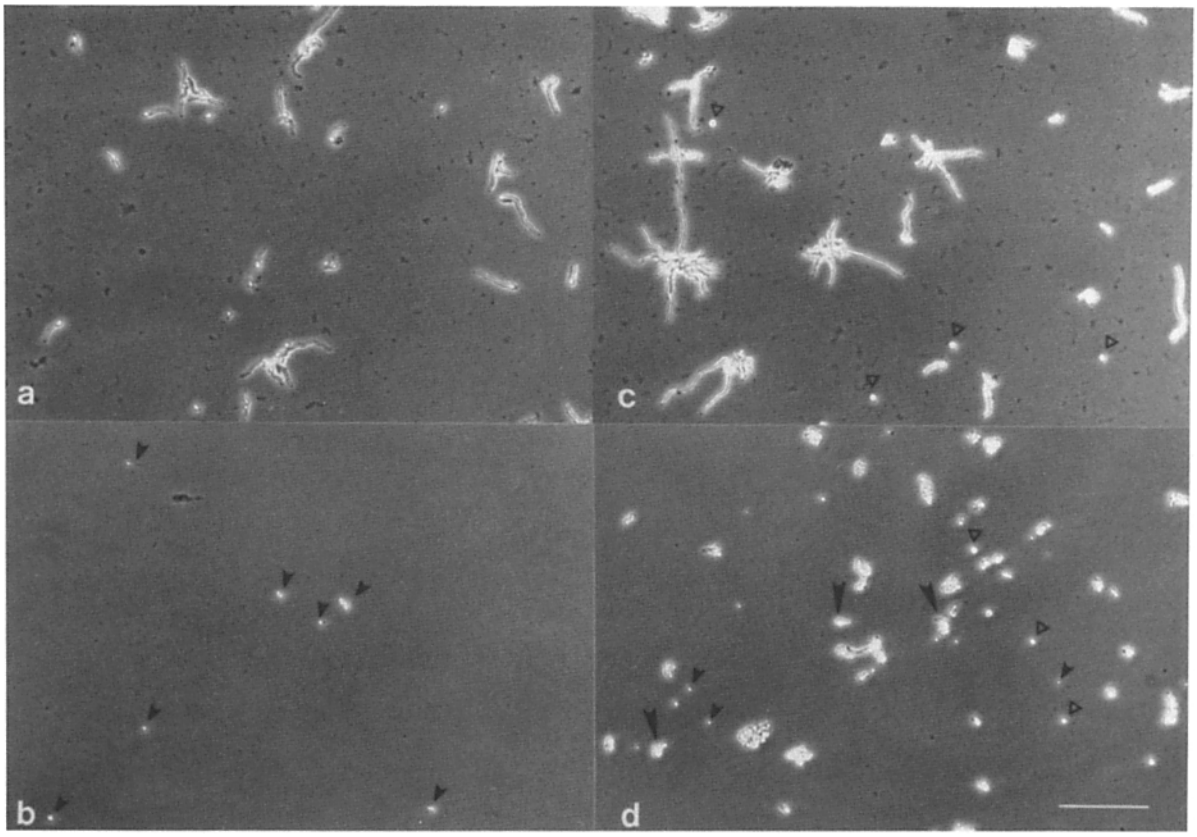


Figure 4. Phase micrographs of the recipient strain GR5 (*a* and *b*) and heterokaryon transformant 3.3 (*c* and *d*) germinated in nonselective media, YAG, plus uridine and uracil, (*a* and *c*) and selective media, YAG (*b* and *d*). The small arrowheads point to ungerminated and unswollen conidia (*b* and *d*). The open arrowheads point to swollen conidia lacking germ tubes (*c* and *d*) and the large arrowheads in *d* point to some cells having germ tubes. Bar, 100 μm.

bulin functions. These three different integration events can be distinguished from one another by restriction endonuclease digests and Southern analysis of transformant DNA (Fig. 6, *a*, *b*, and *c*). Integration via the *benA* gene disrupting fragment will produce a strain lacking the ~1.4-kb Bst EII fragment in its genome. In contrast, transformants with integrations at the *benA* locus via the *benA* promoter sequences or at *tubC* coding sequences will have this ~1.4-kb Bst EII

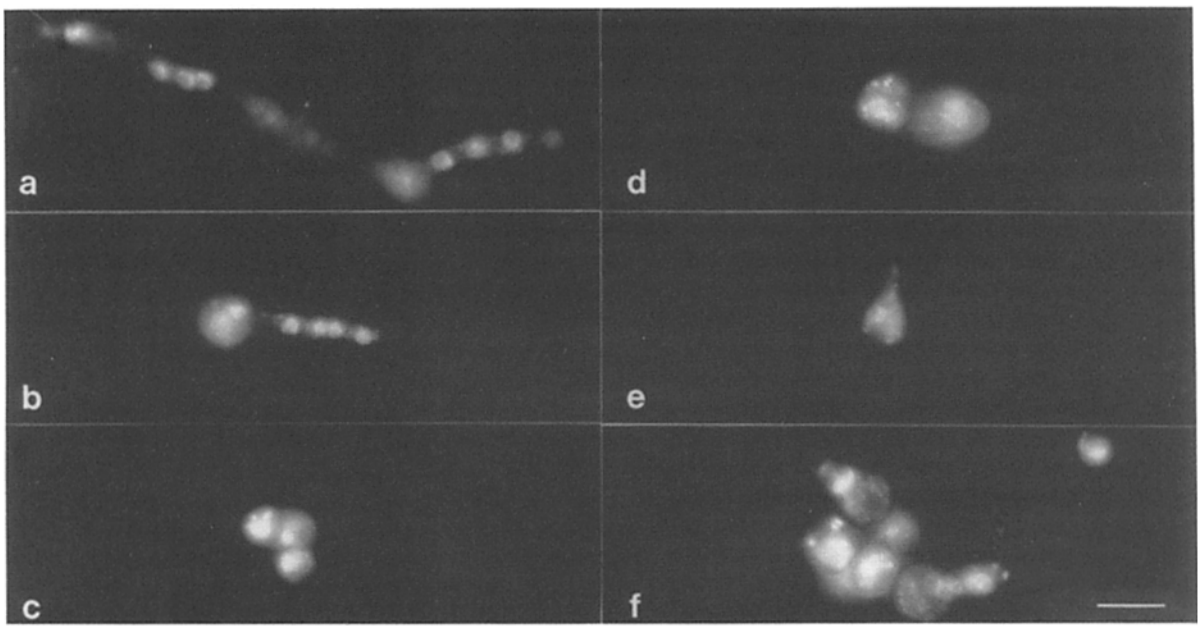
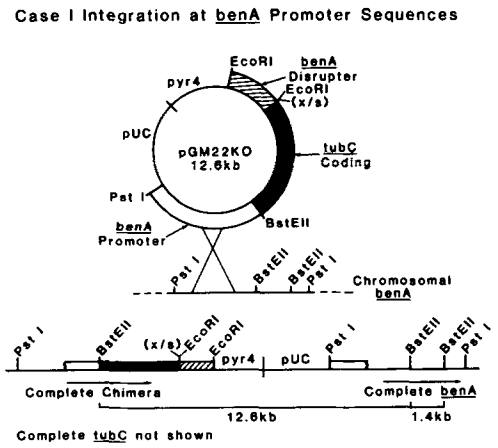
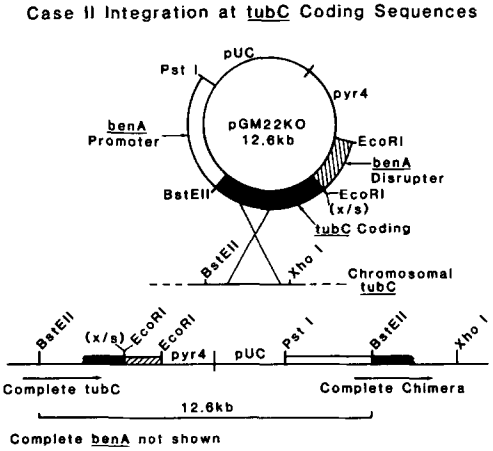


Figure 5. DAPI fluorescence micrographs of GR5 (*a*, *b*, and *c*) and heterokaryon transformant 3.3 (*d*, *e*, and *f*) germinated on nonselective media (*a*, *b*, *d*, and *e*) and selective media (*c* and *f*). Bar, 10 μm.



a



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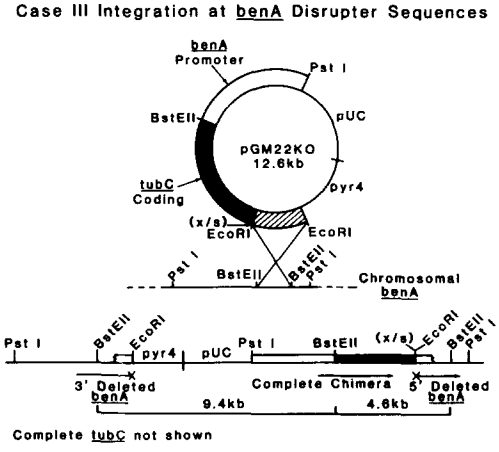

c

Figure 6. The three possible integration events for the plasmid pGM22K0 are illustrated. The vector sequences are the single line in pGM22K0 and contain pUC19 and pyr4. The hatched box represents the 1.4-kb Bst EII *benA* disrupter sequence. The filled box represents the *tubC* coding sequences and the open box represents the *benA* promoter sequences. For each case, a partial restriction map for the site of integration and the integrated sequences are shown. Below the integrated structure the functional and nonfunctional transcriptional units are indicated by the arrows or the lines with an X. In addition, the expected bands of hybridization on genomic Southerns for Bst EII digests are indicated below. Note that for case II, the complete *benA* gene will produce a 1.4-kb band of hybridization that is not shown.

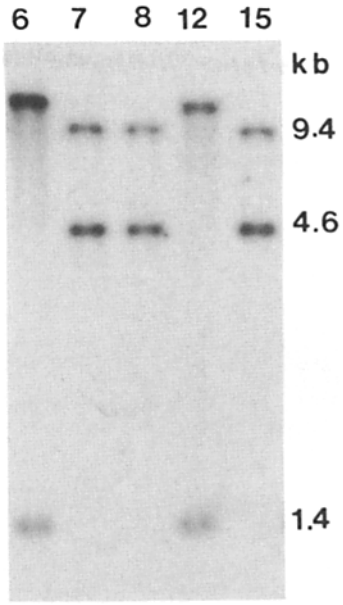

Figure 7. Autoradiograph of genomic Southern of total DNA from pGM22K0 transformants 6, 7, 8, 12, and 15 digested with Bst EII. The filter was probed with the 1.4-kb Bst EII fragment of *benA*. Transformants 6 and 12 have integrated at the 5′ flanking region of *benA* or the *tubC* coding region, leaving the *benA* gene intact as indicated by the 1.4-kb band of hybridization. The transformants 7, 8, and 15 have integrated by the 1.4-kb internal fragment of *benA*, disrupting the gene as indicated by the absence of the 1.4-kb band of hybridization.

fragment (cases I and II). The predicted fragments of hybridization for a nondisrupting integration at either *benA* or *tubC* would be 12.6 and 1.4 kb, and those for disruptive integration at *benA* would be 9.4 and 4.6 kb (Fig. 6, *a*, *b*, and *c*). Genomic Southerns of transformants indicated that both *benA* disruptive and nondisruptive classes of integrants were obtained (Fig. 7). In total, 15 transformants were analyzed by Southerns (only five of which are shown here), seven of which were found to have disrupted the *benA* gene. Four were integrations either in the promoter region of *benA* or in the *tubC* coding region, and the remaining four had ectopic integration events and were not further examined. The phenotype of one of the *benA* disrupted strains, designated K07, was examined in detail.

tubC and benA Encode Functionally Equivalent β-Tubulins

Growth in *Aspergillus* is axial and requires transport and nuclear migration towards the tip of the growing hyphum. Radial growth is therefore a sensitive measure of overall cell growth. In addition, nuclear migration into the growing hyphum is known to be a microtubule dependent process (Oakley and Morris, 1980, 1981). Microtubule function was

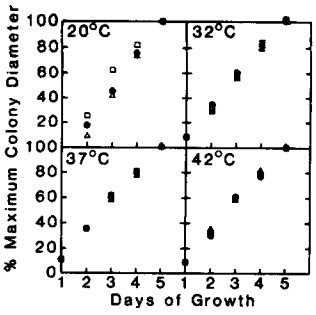

Figure 8. Comparison of growth rates plotted as percentages of maximum colony diameter on day 5 for a wild-type strain R153 (●), a benomyl resistant strain GB20 (□), and a transformant K07 (△) at 20°, 32°, 37°, and 42°C.

therefore examined by studying the radial growth of K07, GB20 the recipient strain, and the wild-type strain R153 at 20, 32, 37, and 42°C. The colony diameter for each of these strains was measured over a period of 5 d (Fig. 8). No differences in growth were observed between R153, GB20 and K07 at any of the temperatures tested. These results indicate that the *tubC* gene product β3 is capable of performing all microtubule functions and does not result in either cold or heat sensitivity of growth. We can therefore conclude that the *tubC* gene product β3 is fully capable of performing all microtubule functions in *A. nidulans* in a manner similar if not identical to that of *benA*.

In addition to measuring growth of K07 at different temperatures, we also wanted to determine whether K07 was differentially sensitive to the antimicrotubule drug benomyl. To test this, we plated R153, GB20, and K07 on increasing concentrations of benomyl from 0 to 1.25 μg/ml (Fig. 9). The *benA* disrupted strain K07 consistently grew less well than the wild-type strain R153 at all concentrations of benomyl, and GB20 was resistant to all concentrations of benomyl used. The increased sensitivity of K07 to benomyl relative to wild type R153 can be interpreted as indicating that the *tubC* gene product β3 has a higher affinity for benomyl, thus resulting in greater sensitivity to the drug. Alternatively, *tubC* may form microtubules that are inherently less stable and are thus more easily depolymerized by benomyl. In either case, it suggests that while *tubC* can substitute for *benA* in all microtubule functions, it does produce microtubules that are more sensitive to the antimicrotubule drug benomyl. These results provide some evidence for differences between the protein products of the *benA* and *tubC* genes. An alternative suggested by one of the reviewers is that the chimeric gene produces a reduced amount of β-tubulin and thus might make the cells more susceptible to benomyl. This is a formal possibility and cannot be excluded from these studies.

We also tested the ability of strain K07 to go through the sexual part of the *A. nidulans* life cycle. K07 was crossed to the 20.3.8, a *pyrG89*, *benA22*, benomyl resistant, and 20.1.8,

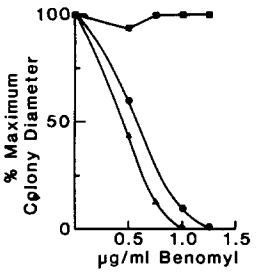

Figure 9. Comparison of growth of a wild-type strain R153 (●), a benomyl resistant strain GB20 (■), and a transformant K07 (▲) on increasing concentrations of benomyl. Growth for each strain is presented as a percentage of maximum colony diameter on media in the absence (0 μg/ml) of benomyl.

Table II. Analysis of Cross of K07 to 20.3.8

	benA	
pyrG	Resistant	Sensitive
−	58	3
+	2	43

A total of 103 segregants were analyzed from a single hybrid cleistothecium.

Table III. Analysis of Cross of K07 to 20.1.8

	benA	
pyrG	Resistant	Sensitive
−	1	33
+	2	68

A total of 104 segregants were analyzed from a single hybrid cleistothecium.

an *argB2* strain. K07 was found to be fertile in both crosses. In the cross to 20.3.8, one would expect the integrated plasmid to behave as if it were linked to *benA* if this is where the plasmid had truly integrated. Therefore, one would predict that benomyl resistant (ben^r) and $pyrG^+$ segregants, as well as benomyl sensitive and $pyrG^-$ segregants would be rare, because the *benA22* mutation, ben^r marker, and the integrated plasmid, $pyrG^+$ marker, are in repulsion in this cross. Thus, the reciprocal classes of segregants, $pyrG^-$, ben^r, and $pyrG^+$, ben^s, would be expected to be the major groups. This prediction was found to be true (Table II), and thus it is concluded that the plasmid is integrated at *benA*. Only five of 103 segregants tested were found to be in the unusual classes, $pyrG^+$, ben^r, and $pyrG^-$, ben^s. In the cross to 20.1.8, one would expect that because both parents are ben^s that all the segregants should be ben^s and that 25% of the segregants should be $pyrG^-$, because *pyrG* is unlinked to *benA*. These two predictions were found to be true (Table III). First, of the 104 segregants tested, only three were found to be ben^r and could be explained by loss of the integrated plasmid and subsequent regeneration of the *benA22* gene. Second, 34 of the 104 segregants were found to be $pyrG^-$; the Chi-Square test indicates this number is not significantly different from the expected 25%, and it is concluded that there is little marker loss during these crosses. These results indicate that the *tubC* gene product does not have a dominant effect on the normal sexual cycle in *A. nidulans* and that the plasmid in K07 is stably integrated at the *benA* locus. In addition, it was found that K07 was capable of forming self fertile cleistothecia, indicating that it was not the contribution of the *benA* gene product from the other parent that resulted in fertility.

As a final test of the *tubC* gene product to perform all β-tubulin functions, the viability of ascospores and conidiaspores was investigated. Spore viability is a very sensitive measure of microtubule function because both conidiaspores and ascospores are derived from mitotic events and any abnormal chromosome segregation during their production would lead to aneuploidy and loss of spore viability. Conidial viability was determined for a wild type strain R153, the parental strain GB20 and the transformant K07 (Table IV). For each strain, over 1,000 spores were scored for the appearance of a germ tube after 8 h at 37°C. The viability of R153 spores

Table IV. Conidial Viability

Strain	GB20	K07	R153
Germinated	1,026 (92.8)*	1,038 (92.8)	1,175 (98.7)
Ungerminated	80 (7.2)	81 (7.2)	16 (1.3)
Total	1,106 (100)	1,119 (100)	1,191 (100)

* Numbers in parentheses represent percent of total number of conidia scored.

Table V. Ascospore Viability

Strain	GB20	K07
Germinated	107 (97.2)*	128 (94.5)
Ungerminated	3 (2.8)	7 (5.5)
Total	110 (100)	135 (100)

* Numbers in parentheses represent percent of total number of ascospores scored.

was 98.7%, and for GB20 and K07 it was 92.8%. Although the viability of R153 spores was higher than that of GB20 or K07, there was no difference between the transformant K07, and its parental strain GB20, suggesting that the *tubC* gene product does not result in a high degree of spore inviability and therefore abnormal chromosome segregation. Similarly, when ascospores from self-fertile cleistothecia were examined for GB20 and K07, there did not appear to be a large number of inviable ascospores for either strain (Table V). GB20 ascospores were 97.2% viable and for K07 ascospores viability was 94.5%. This further suggests that the *tubC* gene product is also fully functional during ascospore production.

Discussion

The experiments presented in this paper have demonstrated that a null mutation in the *benA* gene generated by a disruption of the gene using an internal fragment from *benA* is recessive and leads to cell inviability. Secondly, such a null mutation can be rescued by the incorporation of chimeric β-tubulin gene composed of the *benA* promoter driving the *tubC* structural gene. Using these transformants, I have directly tested the ability of the *tubC* gene product β3 to function in place of the *benA* gene products β1 and β2 when *tubC* is up regulated by expression from the *benA* promoter. This type of experiment is only possible in an organism like *A. nidulans* where there are two highly divergent and differentially expressed β-tubulin genes, and it is possible to replace one structural gene with another using integrative transformation (May et al., 1985; May et al., 1987; Weatherbee et al., 1985). It can be concluded that although the *benA* and *tubC* β-tubulins of *A. nidulans* are highly divergent, with regard to their amino acid sequences, they are functionally equivalent. If one is willing to consider differential sensitivity to the antimicrotubule drug benomyl of strains with varying tubulin constitutions as a measure of difference between the *tubC* and *benA* gene products, then it is possible to say that they are different.

Previous experiments designed to test the functional interchangeability of different tubulin isotypes have made use of transfected animal cells in culture (Bond et al., 1986, 1987; Gu et al., 1988; Lewis et al., 1987), and the conclusion from all of these experiments was that if a cell made a particular isotype, it used it in all discernible microtubules of the cell. Alternative approaches have been used to examine the interchangeability of the two α-tubulins in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (Adachi et al., 1986; Schatz et al., 1986). In each of these yeasts, the essential α-tubulin gene could be replaced by the increased expression of the nonessential gene, indicating the functional interchangeability of the two α-tubulins. The experiments presented here, and those just discussed, indicate that tubulin isotypes, whether α-tubulins or β-tubulins, are functionally equivalent. I conclude that if there are isotype specific functional differences, they are subtle.

Another question is, why maintain two functionally equivalent yet highly divergent β-tubulins in *Aspergillus*? As suggested by others (Cleveland, 1987; Raff et al., 1987), different β-tubulins may be functionally equivalent and multiple genes may exist to allow greater control over total β-tubulin levels in cells and to provide additional temporal and spatial regulation over multiple genes for fine tuning of the abundance of these gene products. These questions have been most carefully examined in *Drosophila melanogaster*, which has four α-tubulin and four β-tubulin genes (Natzle and McCarthy, 1984), and the spatial and temporal regulation of these genes has been studied (Gasch et al., 1988; Kimble et al., 1989). The conclusion from such studies is generally that multiple β-tubulin genes exist primarily to ensure the presence of and proper amount of β-tubulin in cells, although in one study (Kimble et al., 1989), there was some suggestion that β3 was not distributed uniformly within some cells. Such an observation, as the authors suggest, could indicate a specialized function. It was a similar set of observations that led to the experiments presented here, and yet we have not been able to detect isotype specific differences in function. It will be of interest to see whether in *D. melanogaster* similar rescue of cell viability by chimeric genes will lead to the conclusion reached in this study.

The apparent promiscuity of tubulin isotype participation in the formation of microtubules leads one to believe that functional differences between various microtubule structures or classes of microtubules may be dependent on factors other than tubulin primary structure. Such factors would include microtubule-associated proteins, which are known to exhibit differential distribution in cells, and posttranslational modifications of tubulin proteins, such as phosphorylation, tyrosination, and acetylation (Gard and Kirschner, 1985; Gundersen et al., 1984; Huber and Matus, 1984; L'Hernault and Rosenbaum, 1985). If the isotype composition of microtubules is truly homogeneous within a given cell, the mechanism by which different proteins become associated with or modify different microtubules is the central paradox of microtubule biology.

Finally, a method to disrupt a gene and replace it simultaneously with another has been developed. The method can be used, as shown here, to place one member of a multigene family under the control of the regulatory element of another member. It could also be used to introduce in vitro–generated mutants. This disruptive replacement avoids the need for multiple selectable markers and vectors.

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