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

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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.

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), 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 TBI 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).

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

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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 × 10^6 cpm/μg of DNA.

Growth Studies

Radial growth studies were performed as described previously (Weatherbee et al., 1985). Ascospor viability was determined by plating dilutions of ascospores onto diatrizoate plates. Plates were incubated at 37°C for 10 days. Ascospore viability was determined by plating 10^5 conidia into a Petri plate containing 25 ml liquid media and observing germ tubes. The starved ascospores were spread onto a Petri plate containing 25 ml liquid media and the plates were incubated at 37°C for 9 h. The clear extension of a germ tube and ungerminated ascospores are identified as 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 incubating the plates at 37°C. Plates were incubated for 8 h at 37°C. Coverslips were processed and analyzed as for ascospores. Cells were 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 a fluorescent microscope. Germination was 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-XhoI 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 pRGl3 that contains the pyr4 gene of Escherichia coli. The pyr4 gene is on a 5.4-kb Eco RI fragment. The benA gene and predicted structural gene sequences were obtained on a 6.1-kb Pst I-Bst EII fragment. The benA gene was disrupted by introducing pbenAA (not drawn to scale), structure of chromosomal benA gene and predicted structure for integration of pbenAA at benA. The internal fragment of benA in pbenAA is the hatched box flanked by Eco RI sites, and the sequences derived from the vector pRGl3, pUC19, and pyr4 are represented by the single line. The 3' and 5' truncated benA genes are indicated. The partial restriction map and the predicted bands of hybridization for transformant DNA following Pst I digestion are also indicated below the map.

Materials

Restriction endonucleases, DNA modifying enzymes and oligodeoxynucleotide linkers were obtained from New England Biolabs (Beverly, MA), Promega Biotech (Madison, WI), or Boehringer Mannheim Biochemi-
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 non-selective 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 tubC coding sequences producing a strain similar to that in case I but with the plasmid at tubC (Fig. 6 b). In contrast, pGM22K0 integrates via the internal benA disrupter sequences, the same sequence as in pbenAA, 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-
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 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.

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.
Figure 6. The three possible integration events for the plasmid pGM22K0 are illustrated. The vector sequences are the single line in pGM22K0 and contain pUC9 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 Southern 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 Southern of transformants indicated that both benA disruptive and nondisruptive classes of integrants were obtained (Fig. 7). In total, 15 transformants were analyzed by Southern (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
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 $\beta$ 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 $\beta$ 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 benzomyl. To test this, we plated R153, GB20, and K07 on increasing concentrations of benzomyl from 0 to 1.25 $\mu$g/ml (Fig. 9). The benzomyl resistant strain GB20 was resistant to all concentrations of benzomyl used. The increased sensitivity of K07 to benzomyl relative to wild type R153 can be interpreted as indicating that the tubC gene product $\beta$ has a higher affinity for benzomyl, 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 benzomyl. 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 benzomyl. 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 $\beta$-tubulin and thus might make the cells more susceptible to benzomyl. 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, 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') and pyrG+ segregants would be rare, because the benA22 mutation, ben' marker, and the integrated plasmid, pyrG+ marker, are in repulsion in this cross. Thus, the reciprocal classes of segregants, pyrG-, ben', and pyrG+, ben', 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.

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

| pyrG | Resistant | Sensitive |
|------|-----------|-----------|
| -    | 58        | 3         |
| +    | 2         | 43        |

Table II. Analysis of Cross of K07 to 20.3.8

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

| pyrG | Resistant | Sensitive |
|------|-----------|-----------|
| -    | 1         | 33        |
| +    | 2         | 68        |

Table III. Analysis of Cross of K07 to 20.1.8
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.

### 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.

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; Weatheree 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 (Nuttle 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 post translational 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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References

Adachi, Y., T. Toda, O. Niwa, and M. Yanagida. 1986. Differential expression of essential and nonessential a-tubulin genes in Schizosaccharomyces pombe. Mol. Cell Biol. 6:2168–2178.

Bond, J. F., J. L. Fridovich-Keil, L. Pillus, R. C. Mulligan, and F. Solomon. 1986. A chicken-yeast chimeric b-tubulin protein is incorporated into mouse microtubules in vivo. Cell. 44:461–468.

Cleveland, D. W. 1987. The multitubulin hypothesis revisited: what have we learned? J. Cell Biol. 104:381–383.

Cove, D. J. 1966. The induction and repression of nitrate reductase in the fungus Aspergillus nidulans. Biochim. Biophys. Acta. 113:51–56.

Feinberg, A. P., and B. Vogelstein. 1983. A technique for radiolabeling DNA restriction fragments to high specific activity. Anal. Biochem. 132:6–13.

Fulton, C., and P. A. Simpson. 1976. Selective synthesis and utilization of flagellar tubulin. The multitubulin hypothesis. In Cell Motility, R. Goldman, T. Pollard, and J. Rosenbaum, editors. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 987–1005.

Gard, D. L., and M. W. Kirschner. 1985. A polymer dependent increase in the phosphorylation of b-tubulin accompanies differentiation of a mouse neuroblastoma cell line. J. Cell Biol. 100:764–774.

Gesch, A., U. Hinz, D. Leiss, and R. Rennkowitz-Pohl. 1988. The expression of b1 and b3 tubulin genes of Drosophila melanogaster is spatially regulated during embryogenesis. Mol. & Gen. Genet. 211:8–16.

Gu, W., S. A. Lewis, and N. J. Cowan. 1988. Generation of antisera that discriminate among mammalian a-tubulins: introduction of specialized isotypes into cultured cells results in their assembly without disruption of normal microtubule functions. J. Cell Biol. 106:2011–2022.

Gunderson, G. G., M. H. Kalinoski, and J. C. Bulinski. 1984. Distinct populations of microtubules: tyrosinated and non-tyrosinated a-tubulins are distributed differently in vivo. J. Cell Biol. 98:779–789.

Huber, G., and A. Matus. 1984. Differences in the cellular distribution of two microtubule-associated proteins, MAP1 and MAP2, in rat brain. J. Neurosci. 4:151–160.

Kimble, M., J. R. Incardona, and E. C. Raff. 1989. A variant b-tubulin isoform of Drosophila melanogaster (b3) is expressed primarily in tissues of mesodermal origin in embryos and pupal, and is utilized in populations of transient microtubules. Dev. Biol. 131:415–429.

Lewis, S. A., W. Gu, and N. J. Cowan. 1987. Free intermingling of mammalian b-tubulin isotypes among functionally distinct microtubules. Cell. 49:539–548.

L'Hermault, S. W., and R. L. Rosenbaum. 1985. Reversal of posttranslational modification on Chlamydomonas flagellar a-tubulin occurs during flagellar resorption. J. Cell Biol. 100:457–462.

Maaniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

May, G. S., and N. R. Morris. 1988. Developmental regulation of a conidiation specific b-tubulin in Aspergillus nidulans. Dev. Biol. 128:406–414.

May, G. S., and N. R. Morris. 1987. The unique histone H2A gene of Aspergillus nidulans contains three introns. Gene. 58:59–66.

May, G. S., M. L.-S. Tsang, H. Smith, S. Fidel, and N. R. Morris. 1987. Aspergillus nidulans b-tubulin genes are unusually divergent. Gene. 55:231–243.

May, G. S., J. Gambino, J. A. Weatherbee, and N. R. Morris. 1985. Identification and functional analysis of b-tubulin genes by site specific integrative transformation is Aspergillus nidulans. J. Cell Biol. 101:712–719.

Natzele, J. E., and B. J. McCarthy. 1984. Regulation of Drosophila a- and b-tubulin genes during development. Dev. Biol. 104:187–198.

Oakley, B. R., and N. R. Morris. 1980. Nuclear movement is b-tubulin dependent in Aspergillus nidulans. Cell. 19:255–262.

Oakley, B. R., and N. R. Morris. 1981. A b-tubulin mutation in Aspergillus nidulans that blocks microtubule function without blocking assembly. Cell. 24:837–845.

Osmani, S. A., G. S. May, and N. R. Morris. 1987. Regulation of the mRNA levels of nimA, a gene required for the G2-M transition in Aspergillus nidulans. J. Cell Biol. 104:1495–1504.

Osmani, S. A., D. B. Engle, J. H. Dooman, and N. R. Morris. 1988. Spindle formation and chromatin condensation in cells blocked at interphase by mutation of a negative cell cycle control gene. Cell. 52:241–251.

Raff, E. C. 1984. Genetics of microtubule systems. J. Cell Biol. 99:1–10.

Roff, E. C., H. B. Diaz, H. D. Hoyle, J. A. Hutchens, M. Kimble, R. A. Raff, J. E. Rudolph, and M. A. Subler. 1987. Origins of multiple gene families: are these both functional and regulatory constraints? In Development as an Evolutionary Process. R. A. Raff and E. C. Raff, editors. 203–238. Alan R. Liss, Inc., New York.

Shatz, P. J., F. Solomon, and D. Botstein. 1986. Genetically essential and non-essential a-tubulin genes specify functionally interchangeable proteins. Mol. Cell Biol. 6:3722–3733.

Weatherbee, J. A., G. S. May, J. Gambino, and N. R. Morris. 1985. Involvement of a particular species of b-tubulin (b3) in conidal development in Aspergillus nidulans. J. Cell Biol. 101:706–711.