Involvement of a Particular Species of Beta-Tubulin (Beta3) in Conidial Development in Aspergillus nidulans

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ABSTRACT Strains of Aspergillus containing the benA22 mutation are resistant to benomyl for vegetative growth but do not produce conidia. To test whether conidiation involved an additional benomyl-sensitive tubulin (i.e., was mediated by a tubulin other than the tubulins coded for by the benA locus), a collection of mutants was produced that formed conidia in the presence of benomyl, i.e., were conidiation-resistant (CR-) mutants. We analyzed the tubulins of these CR- mutants using two-dimensional gel electrophoresis and found that the mutants lacked one species of beta-tubulin (designated beta3). We have examined two of these mutants in detail. In crosses with strains containing wild-type tubulins, we found that the absence of the beta3-tubulin co-segregated perfectly with the CR- phenotype. In diploids containing both the benA22 and CR- mutations, we found that the CR- phenotype was recessive and that beta3-tubulin was present on two-dimensional gels of tubulins prepared from these diploids. In another set of crosses, these two CR- strains and seven others were first made auxotrophic for uridine and then crossed against strains that had homologously integrated a plasmid containing an incomplete internal fragment of the beta3-tubulin gene and the pyr4 gene of Neurospora crassa (which confers uridine prototrophy on transformants). If the CR- phenotype were produced by a mutation in a gene distinct from the structural gene for beta3-tubulin (designated the tubC gene), then crossing over should have produced some CR+ segregants among the uridine auxotrophic progeny of the second cross. All of the uridine auxotrophs from this type of cross, however, showed the CR- phenotype, suggesting that the mutation in these strains is at or closely linked to the tubC locus. The most obvious explanation of these results is that beta3-tubulin is ordinarily used during conidiation and the presence of this species of beta-tubulin renders conidiation sensitive to benomyl. In the CR- mutants, beta3-tubulin is absent, and in the presence of the benA22 mutation the benomyl-resistant beta1- and/or beta2-tubulin substitutes for beta3 to make conidiation benomyl resistant. We discuss these results and give two models to explain the interactions between these beta-tubulin species.

The lower eukaryote Aspergillus nidulans produces six distinguishable types of tubulin, three alpha-tubulins and three beta-tubulins. Two of the alpha-tubulins, alpha1 and alpha3, are the products of a single gene, designated tubA (8, 17). The third species of alpha-tubulin, alpha2, is the product of another structural gene (tubB) (8, 17). Similarly, two of the beta-tubulin polypeptides, beta1 and beta2, are produced by one structural gene, benA (14). The third beta-tubulin, beta3, is produced by a different structural gene (17) (this gene is now designated tubC; see accompanying paper [6]). Alpha1, alpha2, alpha3, beta1, and beta2 are distinguishable on two-dimensional gels. Beta3-tubulin is normally occluded by beta1- and beta2-tubulin, which migrate to the same electrophoretic position as beta3 in samples from strains containing wild-type tubulins. Beta3-tubulin can be distinguished, however, in strains such as benA22 in which the beta1/beta2-tubulins are electrophoretically shifted (17).

Previous work from this laboratory has established that the
beta-1- and/or beta-2-tubulins function in the mitotic spindle and in the cytoplasmic microtubules concerned with nuclear migration (8–10). Since mutations in these beta-tubulins can be suppressed by mutations in the tubA locus, it follows that the alpha-1- and/or alpha3-tubulins, the products of this gene, are also involved in these functions (10). The functions of the beta-3- and alpha2-tubulins have not been established. Evidence presented here suggests that beta3-tubulin is involved in the formation of conidia, the asexual spores of Aspergillus.

Conidial formation in Aspergillus is a regulated developmental process that requires the coordinated synthesis of a number of mRNAs that are unique to conidiation (asexual sporulation) and the formation of a specialized spore-bearing structure, the conidiophore (16). The conidiophore is a specialized hyphal element that consists of a basal foot cell; a stalk terminating in a swollen, multinucleate vesicle; and a layer of uninucleate cells (primary sterigmata or metulae); and a layer of spore-producing cells (secondary sterigmata or phialides) (16). Chains of conidia are formed by repeated mitoses of the phialide nuclei. The differentiation of the conidiophore and the formation of conidia are coordinated with the synthesis of 1,200 new polyadenylated RNA’s not found in hyphae (representing ~6% of the Aspergillus genome) (16). Recent evidence indicates that many of the genes for these sporulation-specific mRNA’s are clustered, rather than spread randomly throughout the genome (16).

A number of mutations in the benA locus (coding for beta-1- and beta-2-tubulins) of Aspergillus confer resistance to benomyl for vegetative growth. Although strains that contain these mutations grow vegetatively, they do not conidiate. This suggested that conidiation involves a benomyl-sensitive step, i.e., a step that may involve tubulin. In addition, since benA mutations do not confer benomyl resistance on this step, it seemed possible that one of the other tubulin species, for which no functions were known, might be involved. For these reasons, a number of mutants were generated that showed benomyl-resistant conidiation (designated CR- mutants for conidiation resistance or conidial revertants), and the tubulins of these mutants were analyzed on two-dimensional gels. The results presented here show that beta3-tubulin is absent from these CR- mutants. These studies suggest that beta3-tubulin is involved in conidiation and that this involvement is what ordinarily causes conidiation to be sensitive to benomyl.

MATERIALS AND METHODS

**Strains and Growth Conditions:** Mutants that could conidiate in the presence of 4.8 ug/ml of benomyl were selected from ultraviolet light-irradiated spores of strain BEN 20 plated on benomyl-containing YG medium (0.5% yeast extract, 2% glucose, trace elements).\(^1\) BEN 20, containing the mutation benA22, was originally identified as a benomyl-resistant strain BEN 20 or the conidial revertant BEN 20, CR- 2-13 plus enough benomyl in 70% ethanol to give the final concentrations indicated. Ethanol was added to equalize the final ethanol concentration in all the plates at 0.7%, a concentration that does not affect growth. The plates were incubated at 27°C for 5 d and scored. Five 0.8-cm-diam plugs were cut from each plate with a cork borer and homogenized in 5 ml of H2O containing 0.2% Tween 80. Conidia and conidiophores were counted in a Petroff-Hauser counting chamber at a magnification of 400. At least 500 conidia (where possible) and 50 conidiophores were counted for each sample. Conidial number was not affected, but conidiophore growth and maturation were severely retarded at the highest benomyl concentration.

For a comparison of the growth rates of BEN 20, CR- 2-13, and CR- 5-24, sets of each were cultured in YG containing melted agar (0.7%) at 30°C and plated in Petri dishes over YAG. The dilutions were adjusted to produce ~10 colonies/plate. The diameters of colonies were measured at 24 and 48 h. Five individual colonies were measured for each strain at each time point.

**Electrophoresis, Protein Chemistry, and Immunology:** Details of the procedure for the partial purification of Aspergillus tubulins were published previously (17). Two-dimensional gel electrophoresis was carried out according to the method of O’Farrell (11) with the modifications described previously (14). One-dimensional gel electrophoresis was carried out generally as described by Laemmli (5). The method as described by O’Farrell was determined by the method of Sedmak and Grossberg (13).

**Preparation of Total Aspergillus RNA and In Vitro Translation:** Mycelia were first grown in liquid nitrogen and RNA was prepared immediately or the frozen mycelia were stored at −70°C. Mycelia were homogenized in liquid nitrogen–cooled mortar. The powder was transferred to a 30-ml Corex tube (Corning Glass Works, Corning, NY) that contained 2.5 ml phenol and 5 ml of 50 mM Tris-HCl pH 8.0, 0.3 M NaCl, 5 mM EDTA, 2% SDS (extraction buffer) at 65°C. The contents were mixed vigorously for 2 min on a vortex mixer, 2.5-ml chloroform/isomyl alcohol (25:1) was added, and the mixture was vortexed again. The contents were centrifuged at 8,000 rpm in a Sorvall HB-4 swinging bucket rotor (DuPont)

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\(^1\) Abbreviations used in this paper: YAG, medium that contains 0.5% yeast extract, 2% glucose, 2% agar, trace elements; YG, medium that contains 0.5% yeast extract, 2% glucose, trace elements.
tubulin-enriched samples prepared from BEN 20 and one of such samples with an affinity-purified anti-tubulin antibody indicated that this spot was not a tubulin and did not represent

was that if any of the CR- mutations were in tubulin, then each of the 20 CR- mutants was distinguished by the complete absence of beta3-tubulin. Either beta3-tubulins are not being rapidly degraded, or beta3-tubulins are being produced that two-dimensional gels. This result was not obtained. Instead, two of these strains (designated CR- 2-13 and CR- 5-24) were selected for more detailed studies. Until all of the CR- strains have been completely analyzed, it will not be known whether these two strains are typical of all CR- strains.

FIGURE 1 Demonstration of the absence of beta3-tubulin from a strain containing the CR- mutation. Coomassie Blue-stained gels of tubulins prepared from the parental strain BEN 20 (top) and from one of the CR- mutants (CR- 5-27; bottom). All 20 of the CR- mutants show the complete absence of beta3-tubulin. The arrowhead marks a faint spot that is seen in some of the CR- mutants. This spot does not react with an anti-tubulin antibody on Western blots.

RESULTS

Strain BEN 20 is highly resistant to benomyl for vegetative growth (14) but does not conidiate when grown on medium containing 4.8 ug/ml of benomyl. We obtained a collection of CR- mutants by looking for patches of conidiation when strain BEN 20 was plated on YAG containing benomyl (4.8 ug/ml). 20 such mutants were selected, 3 as spontaneous mutations and 17 others from five separate series of platings of ultraviolet light-irradiated spores on YG containing 4.8 ug/ml of benomyl. Conidia that developed under these selective conditions were picked, streaked three times to single colony, and then grown in quantity. This collection of CR- mutants was produced in strain BEN 20 (containing the benA22 mutation) because the electrophoretic shift of the beta1- and beta2-tubulins of this strain permits visualization of all of the tubulin species on two-dimensional gels.

Tubulins from all 20 of the CR- mutants were enriched by a procedure involving DEAE-cellulose column chromatography and ammonium sulfate precipitation, which has been described previously (17). Two-dimensional gels were run on each partially purified tubulin sample. The initial expectation was that if any of the CR- mutations were in tubulin, then some of the tubulins would show electrophoretic shifts on the two-dimensional gels. This result was not obtained. Instead, each of the 20 CR- mutants was distinguished by the complete absence of beta3-tubulin. Either beta3-tubulins are not being produced, or altered beta3-tubulins are being produced and rapidly degraded, or beta3-tubulins are being produced that are sufficiently altered so that they do not co-purify with the other Aspergillus tubulins. Fig. 1 shows a comparison between tubulin-enriched samples prepared from BEN 20 and one of the CR- mutants. In some of the CR- samples, a new minor spot somewhat to the right of the beta1/beta2-tubulins was seen (arrowhead in Fig. 1). Reaction of Western blots of gels of such samples with an affinity-purified anti-tubulin antibody indicated that this spot was not a tubulin and did not represent a shifted beta3-tubulin (data not shown).

The most obvious interpretation of these results is that the CR- mutation represents an alteration in the structural gene for beta3-tubulin or an alteration in a gene that controls the net synthesis of beta3-tubulin (see Discussion). Another possibility we considered was that the CR- mutation represented a defect in a system that produced a posttranslational modification in beta3-tubulin. According to this suggestion, beta3-tubulin would appear in its usual position on two-dimensional gels only after being modified. In the CR- mutants this modification would be altered and thus beta3 would not appear in its usual position in these mutants.

We tested this hypothesis by preparing mRNA from BEN 20. This mRNA was translated in vitro using a rabbit reticulocyte lysate, and two-dimensional gels of the proteins produced were analyzed. In one series of experiments, an antibody that had been prepared against Aspergillus tubulin and purified on an affinity column containing purified pig brain tubulin was used to immunoprecipitate the products of the in vitro translation. The precipitated proteins were analyzed on two-dimensional gels (data not shown). In another series of experiments, the total protein fraction produced by in vitro translation was analyzed on gels without fractionation. The results of such an experiment are shown in Fig. 2 along with an in vitro translation of mRNA from R153, a strain that contains wild-type tubulins. Both types of experiments gave the same results. It is clear that beta3-tubulin is present in BEN 20 in its usual position. Since most posttranslational modifications would not take place under these in vitro conditions, beta3 probably does not reach this position as the result of a posttranslational modification.

As discussed previously, all of the CR- mutants that were isolated showed the same phenotype, i.e., benomyl-resistant conidiation and the absence of beta3-tubulin on two-dimensional gels. Two of these strains (designated CR- 2-13 and CR- 5-24) were selected for more detailed studies. Until all of the CR- strains have been completely analyzed, it will not be known whether these two strains are typical of all CR- strains.

To establish that the CR- mutation does not promote conidiation simply by stimulating growth, we measured the effects of benomyl on the growth and conidiation of BEN 20 and on the two "typical" CR- mutants. Fig. 3 shows a comparison of the results obtained for BEN 20 and CR- 2-13. Similar results were obtained for CR- 5-24. It is clear that the CR- mutation does not promote conidiation simply by


Figure 2 Demonstration that beta3-tubulin is present in its usual position in extracts prepared by the in vitro translation of total Aspergillus mRNA from BEN 20. This figure shows autoradiograms of the tubulin regions of two-dimensional gels of proteins produced by in vitro translation of mRNAs from R153, containing wild-type tubulins (top), and from BEN 20, containing the benA22 mutation in its beta1/ beta2-tubulins (bottom; see text for details of experimental procedures). Beta3-tubulin is present in BEN 20 at the usual position for a beta-tubulin, suggesting that migration at this position is not the result of a posttranslational modification.

stimulating growth.

To determine whether the benomyl resistance for conidiation of the CR− phenotype could possibly be caused by a new mutation at the benA locus, we crossed CR− 2-13 and CR− 5-24 against two strains, R153 and R21, that contain wild-type tubulins, and the progeny were scored for benomyl resistance and the CR− phenotype. The results of these crosses are shown in Table I. It is clear from the fact that approximately one-half of the benomyl-resistant progeny are conidiation sensitive (CR+) that the CR− mutations and benA-mediated benomyl resistance segregate independently. Therefore the CR− phenotype is not the result of a new mutation at the benA locus.

To verify that the CR− mutation co-segregated with the absence of beta3-tubulin, we randomly selected 11 of the progeny from the crosses described above. Five of these were benomyl resistant for vegetative growth but not for conidiation. Six were benomyl resistant for both vegetative growth and for conidiation, i.e., were CR−'s. Each of these 11 sub-strains was repeatedly streaked to single colony, and then tubulin was partially purified from each and analyzed on two-dimensional gels. The results are summarized in Table II. The five non-CR−'s each showed the presence of beta3-tubulin and the six CR−'s each showed the absence of beta3-tubulin.

To determine whether the CR− mutation was dominant or recessive, diploids were constructed from the progeny of the crosses described above. Three different diploids that were homozygous for benA22, heterozygous for CR− (two of these were derived from CR− 2-13 and one was derived from CR− 5-24), and heterozygous for different recessive nutritional and color markers were made (see Materials and Methods for details). These diploids grew on YAG medium containing 4.8 µg/ml of benomyl but did not conidiate. At the edges of large colonies grown on center inoculum plates, conidiating fans were occasionally seen. These represent areas of spontaneous breakdown of the diploids to haploids. Tubulin was prepared from mycelia of these three diploids grown in liquid culture on minimal medium (to maintain selective pressure). Two-dimensional gels of the tubulins prepared from these diploids showed the presence of beta3-tubulin (data not shown). Again, the presence of the CR+ phenotype corresponded with the presence of beta3-tubulin. These results show that the CR− mutation is recessive.

Finally, evidence has been obtained that strongly suggests that the CR− mutation in the two strains CR− 2-13 and CR− 5-24, as well as in at least seven others, are mutations in the structural gene for beta3-tubulin. Two DNA sequences have been cloned from a genomic library of Aspergillus by virtue of their homology to a chick beta-tubulin cDNA and cloned yeast beta-tubulin genomic DNA and sequenced sufficiently.

Figure 3 Quantification of the effect of a CR− mutation on growth and conidiation. (A) Comparison of the growth rates of the parental strain BEN 20 and a typical CR− mutant, CR2-13. O, BEN 20. ●, CR2-13. There is no significant difference in the growth rates. (B) Comparison of the production of conidia by BEN 20 and CR2-13. O, BEN 20. ●, CR2-13. At 4.8 µg/ml of benomyl, conidiation of the parental strain is almost totally inhibited, whereas conidiation of the mutant is almost unaffected.
to verify that both sequences code for proteins similar to yeast, chicken, and bovine beta-tubulins (Tsang, M. L.-S., A. Upshall, G. S. May, S. A. Fidel, and N. R. Morris, unpublished data). There is good evidence that one of these sequences is the benA gene, since plasmids carrying this sequence integrate specifically at the benA locus (see accompanying paper [6] for details). This suggested that the other sequence is the structural gene for beta3-tubulin.

We investigated the function of this putative structural gene for beta3-tubulin by using DNA-mediated transformation to disrupt this gene by site-specific integration of a plasmid (GB20 pGM6-1) containing an internal fragment of the gene (15). In this technique, a DNA sequence that does not contain either end of a gene is used to transform a host cell. The homologous integration of such an internal gene fragment results in a gene disruption, the production of two incomplete gene sequences, neither of which is functional (15).

In the accompanying paper (6), we show that disruption of the structural gene for beta3-tubulin (tubC) in a benA22 background results in the CR- phenotype. To determine whether the CR- mutations described in the present paper are at the tubC locus, we constructed CR- strains (derived from CR- 2-13, CR- 5-24, and from seven other CR- strains) that require uridine for growth. These were crossed against a strain (GB20 pGM6-1) of BEN 20 that contains a pyr4 marker (conferring uridine prototrophy) closely linked to an internal fragment of the tubC gene homologously integrated at the tubC locus (see accompanying paper [6] for details). Since integration of the pyr4 gene and the internal tubC gene fragment results in disruption of the tubC gene and the consequent loss of beta3-tubulin, and since uridine prototrophy can only result from this integration, it follows that all uridine prototrophs among the progeny of these crosses should show the CR- phenotype. If either of these two CR- mutants contained wild-type beta3-tubulins but showed the CR- phenotype by virtue of a mutation in a gene unlinked to tubC, however, then a portion of the uridine auxotrophs among the progeny of these crosses should have shown a CR+ phenotype. Table III shows the results obtained from two of these crosses. Similar results were obtained from the other seven crosses as well. All of the uridine auxotrophs from these crosses showed the CR- phenotype, which suggests that all of these CR- mutations are in or closely linked to the tubC gene. Thus at least 9 of the collection of 20 CR- mutants are apparently tubC mutants.

**DISCUSSION**

The results presented here show that there is a perfect correspondence between the absence of beta3-tubulin and resistance of conidiation to high concentrations of benomyl. At present the most plausible interpretation of these results is that beta3-tubulin is ordinarily involved in conidiation, possibly functioning in the mitotic spindles of the conidium-generating secondary sterigmata (or phialides), having some function in directing nuclear migration, or having some other, as yet unknown, capacity. This suggestion seems at first to be contradictory, since conidiation is correlated with the absence of beta3-tubulin. The actual correlation, however, is between benomyl resistance for conidiation and the absence of beta3-tubulin.

To account for the observations presented above, we suggest that beta3-tubulin is uniquely involved in conidiation and that this involvement renders conidiation sensitive to benomyl in strains in which the beta1/beta2 benA tubulins are benomyl resistant. It is not known whether beta3 functions during vegetative growth, but this seems unlikely since BEN 20, which has a benomyl-sensitive beta3-tubulin, is resistant to benomyl for vegetative growth. We suggest also that in the CR- mutants, which lack a functional beta3-tubulin, beta1- and/or beta2-tubulin can substitute for beta3, and in the benomyl resistant benA mutant conidiation becomes resistant to benomyl. This substitution phenomenon may explain why all of the mutants obtained were apparently nulls (lacking beta3-tubulin) rather than missense mutations. This would be expected because the frequency of nonsense mutations would be much higher than the frequency of mutations leading to a benomyl-resistant gene product.

Two general models are suggested by the observations presented above. The first model suggests that beta3-tubulin functions during conidial development in microtubules that are co-polymers of beta3-tubulin and beta1- and/or beta2-tubulins, and that the presence of beta3 in these microtubules causes them to be benomyl sensitive. The CR- mutations can

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**Table I.** Segregants from Crosses between Strains Containing Wild-type Tubulin (R21 and R153) and Strains Containing BEN 20, CR- Tubulin (CR2-13 and CR5-24)

| Phenotype                        | Number of segregants |
|----------------------------------|----------------------|
| R153 x BEN 20, CR- (CR2-13)      |                      |
| Benomyl resistant, CR-           | 14                   |
| Benomyl resistant, CR+           | 12                   |
| Benomyl sensitive                | 22                   |
| R153 x BEN 20, CR- (CR5-24)      |                      |
| Benomyl resistant, CR-           | 17                   |
| Benomyl resistant, CR+           | 32                   |
| Benomyl sensitive                | 51                   |
| R21 x BEN 20, CR- (CR2-13)       |                      |
| Benomyl resistant, CR-           | 8                    |
| Benomyl resistant, CR+           | 11                   |
| Benomyl sensitive                | 27                   |
| R21 x BEN 20, CR- (CR5-24)       |                      |
| Benomyl resistant, CR-           | 13                   |
| Benomyl resistant, CR+           | 19                   |
| Benomyl sensitive                | 15                   |

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**Table III.** Scoring of Uridine Auxotrophs for the CR- or CR+ Phenotype in Crosses of Two CR- Strains (CR- 2-13 and CR- 5-24) with a Strain Containing a Disrupted Beta3-Tubulin Gene Linked to a pyr4 Marker (GB20 pGM6-1)

| Number of uridine auxotrophs | CR- 2-13 x GB20 pGM6-1 | CR- 5-24 x GB20 pGM6-1 |
|-----------------------------|------------------------|------------------------|
| CR-                         | 46                     | 42                     |
| CR+                         | 1                      | 1                      |
be explained as mutations that do not produce beta3-tubulin or that produce a defective beta3-tubulin mRNA or polypeptide that is rapidly degraded. In the absence of beta3-tubulin, the microtubules consisting of beta1- and/or beta2-tubulin are now benomyl resistant and conidiation can occur.

The second model suggests some type of feedback or switch mechanism. This model proposes that beta1- and/or beta2-tubulin is synthesized and functional during vegetative growth but normally repressed during conidiation. Beta3, on the other hand, is absent or present at a low level during vegetative growth but is induced during the early stages of conidiation. The presence of beta3-tubulin or some other factor that appears during conidiation could activate a switch that turns off synthesis of beta1- and beta2-tubulins or inactivates them in some other way. The CR- mutation could then be explained either as a mutation that does not produce beta3-tubulin, leading to a failure of repression of beta1/beta2, or, alternately, the CR- mutation could represent a defect in the switch that turns on the synthesis of beta3 and turns off the synthesis of beta1- and beta2-tubulin or inactivates them. Under either of these circumstances, beta1- and/or beta2-tubulin would be synthesized and active during conidiation and could substitute well enough for beta3 to produce benomyl-resistant conidiation. Both of these models are plausible and make a number of predictions that should be easily testable.

Although these models can explain the results obtained here, they leave and raise a number of questions. One question is, Why is there any need for beta3-tubulin if beta1- and/or beta2-tubulin can function in conidiation? This of course is the fundamental question posed by the observations that many organisms have multiple tubulins (3). There are at least two reasonable answers to this question that could apply here. One is that various species of tubulins have differentiated from a prototypic form of tubulin and have become specialized for specific functions. The second is that different tubulin genes have arisen to permit independent control of expression of these genes.

Another question raised by the second model proposed above concerns the level at which beta1- and beta2-tubulin might be prevented from functioning when beta3 is present. That is, Is control exerted at the transcriptional, translational, or posttranslational level? This question can be investigated by determining whether mRNA's for beta1 and beta2 are being produced during conidiation and by determining whether beta1- and beta2-tubulin polypeptides are present in conidia. This information should also allow a distinction to be made between the two models.

A third question raised by the second model concerns the nature of the CR- mutation. As discussed above, these mutations could be mutations in the structural gene for beta3-tubulin or mutations that control the induction or repression of beta3. It is not yet known how many loci are represented in the present collection of 20 CR- mutants. However, the nine strains containing CR- mutations that have been analyzed so far all appear to be in the beta3 structural gene.

A possible objection to the suggestion of the second model that the presence of beta3-tubulin prevents the synthesis or functioning of beta1- and/or beta2-tubulin comes from the observation that mycelia grown in suspension culture show the presence of all three species of beta-tubulins even though Aspergillus does not produce conidia under these conditions. There are two possible explanations for this apparent discrepancy. First, although beta3-tubulin is detectable under these conditions, it clearly represents a very minor tubulin species in comparison to the beta1- and beta2-tubulin (17). Perhaps a constitutive amount of beta3 is produced even during vegetative growth. A second possibility is suggested by the observation that in suspension cultures of Aspergillus part of the mycelium sticks to the walls of the culture flask. This portion of the culture may enter the early stages of conidial development (which is stimulated by contact with air in surface cultures), and the presence of the low levels of beta3-tubulin observed could be the result of a contribution from these cells.

All of the results presented above suggest that beta3-tubulin is involved in conidiation. Two models have been proposed that suggest a unique role for beta3 in conidiation and a control mechanism that switches on synthesis of beta3-tubulin during conidial development. The predictions made by these models and the questions they raised are being investigated.

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