

Isolation of *mip* (Microtubule-Interacting Protein) Mutations of *Aspergillus nidulans*

CLIFFORD F. WEIL, C. ELIZABETH OAKLEY, AND BERL R. OAKLEY*

Department of Microbiology, The Ohio State University, Columbus, Ohio 43210

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We identified four mutations in two previously undescribed loci involved in microtubule function in *Aspergillus nidulans* as extragenic suppressors of *benA33*, a heat-sensitive β -tubulin mutation. Three of the four mutations map to a locus closely linked to *riboB* on linkage group VIII; we designated this locus *mipA* (for microtubule-interacting protein). We were not able to map the remaining suppressor because of chromosomal rearrangements. However, since it recombines with *riboB* at a significantly higher frequency than the *mipA* alleles, it is unlikely to be in *mipA*; thus, we designated it *mipB1*. The *mip* mutations are not allelic to the previously identified loci that encode α - and β -tubulin, and it is likely that *mipA* and *mipB* encode previously unidentified nontubulin proteins involved in microtubule function. Each of the *mip* mutations suppresses the heat sensitivity conferred by *benA33* and suppresses the blockage of nuclear division and movement conferred by this mutation at high temperatures. Interactions between *mipA* and *benA* are allele specific. All of the *mipA* mutations are cryptic in a wild-type *benA* background but cause cold sensitivity in combination with *benA33*. These mutations also confer cold sensitivity in combination with *benA31* and *benA32* and reduce the resistance conferred by these mutations to the antimicrotubule agent benomyl but do not suppress the heat sensitivity conferred by these alleles. Finally, the *mipA* alleles suppress the heat sensitivity conferred by *benA11*, *benA17*, and *benA21* but do not confer cold sensitivity in combination with these alleles.

The identification of the functional components of microtubules is a prerequisite for determining the mechanisms of microtubule-based motility. Although the major components of microtubules, α - and β -tubulin, have been known for more than a decade, the identities of other proteins required for microtubule function remain undetermined.

Microtubules have been the subject of intense biochemical study which has led to the identification of several proteins that copurify with α - and β -tubulin through several rounds of microtubule assembly and disassembly. These microtubule-associated proteins (MAPs) are the subject of an excellent recent review (13). An intrinsic limitation of biochemical studies of MAPs is that copurification of proteins with α - and β -tubulin does not demonstrate unambiguously that they are essential to microtubule function or even that they are associated with microtubules in vivo. Indeed, recent evidence suggests that at least one MAP is not associated with microtubules in vivo (1, 3, 10).

We chose to identify nontubulin components of microtubules by a genetic approach—isolating mutations that affect microtubule function in vivo, characterizing these mutations, and identifying the products of the mutant genes. We attempted to isolate such mutations as revertants of a heat-sensitive (hs^-) β -tubulin mutation of *Aspergillus nidulans*. This approach is based on the demonstration in bacteriophage P22 that reversion of some hs^- mutations is often due to mutations in other genes, the products of which interact physically with the original mutant protein (4). In addition, these extragenic suppressor mutations often cause cold sensitivity. Morris et al. (6) demonstrated that this approach can be used to isolate mutations in microtubule proteins of *A. nidulans*. They isolated extragenic suppressors of hs^- *benA* (β -tubulin) mutations and were able to identify among them mutations in an α -tubulin structural

gene which they designated *tubA*. Thomas et al. (12) have recently reported the isolation of hs^- revertants of a cold-sensitive (cs^-) β -tubulin mutation of *Saccharomyces cerevisiae*. Among them were a single hs^- β -tubulin mutation and 26 extragenic suppressors in 16 complementation groups. We took a similar approach, isolating large numbers of revertants of *benA33*, a β -tubulin mutation of *A. nidulans* that confers heat sensitivity and resistance to the antimicrotubule agent benomyl (7). This mutation inhibits mitosis and translocation of organelles at restrictive temperature but does not inhibit microtubule assembly (7). Substantial evidence suggests that this mutation hyperstabilizes microtubules such that they do not disassemble normally at high temperatures (2, 7). Among these revertants, we have identified *benA* and *tubA* mutations that confer conditional lethality (8; B. R. Oakley, C. E. Oakley, and J. E. Rinehart, submitted for publication). Here, we report the isolation and initial characterization of suppressor mutations in previously unidentified loci involved in microtubule function.

MATERIALS AND METHODS

Strains. Genotypes and sources of the *A. nidulans* strains used are listed in Table 1.

Mutagenesis. Mutagenesis of BRO2 conidiospores by UV irradiation and isolation of revertants have been previously described (8). Mutagenesis resulted in 25% survival.

Media. Complete and minimal media used were those described previously (8). Testing for the *facB* marker was on minimal medium in which 0.1 M sodium acetate was substituted for glucose. Benomyl (technical grade, 98% pure) was a generous gift from Du Pont Co., Wilmington, Del.

Light microscopy. Fixation of germlings with glutaraldehyde, staining of nuclei and mitochondria with 4',6-diamidino-2-phenylindole, and observation of nuclear and mitochondrial movement were done as previously described (8, 9).

* Corresponding author.

RESULTS

As reported elsewhere (8; Oakley et al., submitted), 2,604 non-heat-sensitive (hs^+) revertants of strain BRO2 were isolated and tested for cold sensitivity. A total of 128 revertants were cs^- . To determine whether reversion in each case was due to a mutation at the *benA* locus or to an extragenic suppressor, each cs^- revertant was crossed with a strain carrying a wild-type *benA* allele (*benA*⁺). A total of 78 revertants were crossed successfully. In 44 crosses, reversion was closely linked to *benA*; in 34 cases, reversion was due to an extragenic suppressor.

To determine, in the revertants carrying extragenic suppressors, whether cold sensitivity was due to the suppressor or to a mutation that occurred coincidentally during mutagenesis, we examined progeny from crosses between BRO2 revertants and *benA*⁺ strains for cosegregation of cold sensitivity and suppression of the heat sensitivity conferred by *benA33*. Segregants carrying *benA33* can be identified by their resistance to the antimicrotubule agent benomyl (7). If cold sensitivity and suppression of heat sensitivity are caused by the same mutation, all progeny carrying *benA33* and the suppressor of *benA33* will be cs^- hs^+ and benomyl resistant (*ben*^r). If cold sensitivity and suppression of heat sensitivity are caused by different mutations, some hs^+ *ben*^r progeny will not be cold sensitive (cs^+). Crosses of 30 revertants carrying extragenic suppressors with *benA*⁺ strains were examined. Crosses of 10 of these revertants yielded substantial numbers of cs^+ hs^+ *ben*^r progeny, but crosses of the remaining 20 revertants yielded no such segregants among 400 progeny tested from each cross, suggesting that cold sensitivity and suppression of heat sensitivity are very closely linked and almost certainly caused by the same mutation (Oakley et al., submitted).

Two revertants, BRO2RC21 and BRO2RP52, carried additional coincidental mutations. BRO2RC21 carried a mutation that reduced colony size at all temperatures, and BRO2RP52 carried a weak nutritional requirement. For subsequent studies, segregants that did not carry these additional mutations were used.

Morris et al. (6) have identified an α -tubulin mutation, *tubA1*, that suppresses the hs^- phenotype of *benA33*. We suspected that some of the extragenic suppressors might be mutations in *tubA*; consequently, we crossed each of the 20 revertants in which the *benA33* suppressor causes cold sensitivity with strain LO196, which carries *tubA4*, another *tubA* allele that suppresses the hs^- phenotype of *benA33* (Oakley et al., submitted). If, in the revertants, suppression of the hs^- phenotype is not due to a mutation of the *tubA* gene, crossovers between *tubA4* and the suppressor carried by the revertant would produce *benA33 tubA*⁺ suppressor⁺ progeny. Such progeny would be cs^+ hs^- *ben*^r. If the suppressor is a *tubA* mutation, such progeny would be extremely rare. Crosses of 16 revertants gave no more than 1 hs^- segregant among more than 400 tested. These revertants probably carry *tubA* mutations and have been reported elsewhere (Oakley et al., submitted). Crosses of the four remaining revertants (BRO2RA31, BRO2RC21, BRO2RP9, and BRO2RP52) gave significant numbers ($\approx 10\%$) of cs^+ hs^- *ben*^r progeny, demonstrating that suppression of the hs^- phenotype of *benA33* is not due to a *tubA* mutation but to a mutation at another, previously undescribed, locus.

We attempted to determine how many loci were represented among these four suppressors by performing complementation tests with stable diploids and by crossing each revertant with the other three. Our efforts were unsuccessful

TABLE 1. *A. nidulans* strains used

Strain	Genotype	Source
BRO2	<i>yA2 benA33</i>	B. R. Oakley
BRO2RA31	Revertant of BRO2	This study
BRO2RC21	Revertant of BRO2	This study
BRO2RP9	Revertant of BRO2	This study
BRO2RP52	Revertant of BRO2	This study
LO196	<i>yA2 wA3 adE20 pabaA1 proA1 pyroA4 tubA4</i>	C. E. Oakley
FGSC 154	<i>adE20 biA1 wA3 cnxE16 sC12 methG1 nicA2 lacA1 choA1 chaA1</i>	Fungal Genetics Stock Center
FGSC 442	<i>facB101 riboB2 chaA1 sE15 nirA14</i>	Fungal Genetics Stock Center
FGSC 256	<i>pabaA1 wA3 argC3 facB101 riboB2</i>	Fungal Genetics Stock Center
FGSC 449	<i>acrA1 lysB5 sD85 cnxB2</i>	Fungal Genetics Stock Center
FGSC 484	<i>acrA1 lysB5 sD85 fwA2 facC102</i>	Fungal Genetics Stock Center
LO427	<i>yA2 benA33 facB101 riboB2</i>	This study
K5A	<i>yA2 benA33 ornB7 pabaA1</i>	D. Kirsch
LO413	<i>benA33 mipA1 chaA1 riboB2</i>	This study
LO428	<i>benA33 chaA1 riboB2 facB101</i>	This study
BEN1	<i>biA1 benA3</i>	J. M. Van Tuyl
BEN2	<i>biA1 benA4</i>	J. M. Van Tuyl
BEN9	<i>biA1 benA11</i>	J. M. Van Tuyl
BEN14	<i>biA1 benA16</i>	J. M. Van Tuyl
BEN15	<i>biA1 benA17</i>	J. M. Van Tuyl
BEN17	<i>biA1 benA19</i>	J. M. Van Tuyl
BEN19	<i>biA1 benA21</i>	J. M. Van Tuyl
BEN30	<i>wA3 pyroA4 benA31</i>	B. R. Oakley
BEN31	<i>wA3 pyroA4 benA32</i>	B. R. Oakley

despite repeated attempts. No two revertants formed heterokaryons, and no crosses produced hybrid cleistothecia.

To surmount these problems, we attempted to map each suppressor to linkage group and, subsequently, to locus. To map the suppressors to linkage group, we constructed diploids from strain FGSC 154, which carries a marker on each linkage group, and segregants carrying *benA33* and each suppressor. Segregants of revertant \times *benA*⁺ crosses were used instead of revertants because the revertants did not carry the necessary nutritional markers. We induced random chromosome loss by treatment with chloral hydrate (11). Two of the suppressors mapped to linkage group VIII, and a third suppressor appeared to map to linkage group III or VIII. The data for the fourth suppressor (isolated in BRO2RP9) suggest that it is on linkage group VI or VIII, but an apparent translocation between these linkage groups, as well as additional chromosomal rearrangements, has prevented definitive assignment to linkage group. Because these rearrangements greatly complicate genetic analysis, we concentrated our efforts on the other three suppressors.

To map these suppressors to locus, we crossed strains carrying each suppressor with strains FGSC 256, FGSC 442, FGSC 449, and FGSC 484, all of which are multiply marked on linkage group VIII (Table 1). All three mutations were found to be closely linked to the *facB* and *riboB* genes, more loosely linked to the *argC* gene, and unlinked to the *fwA*, *facC*, *cnxB*, *chaA*, *sE*, and *nirA* genes (data not shown). The data suggest that all three mutations may lie within the same

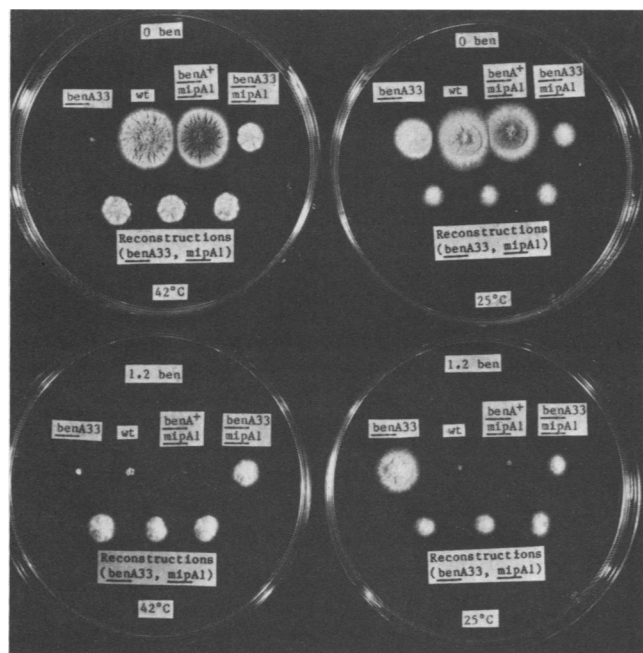


FIG. 1. Effects of benomyl concentration and temperature on strains carrying *benA33* or *mipA1* or both. The temperatures of incubation are shown at the bottom of each plate; the concentrations of benomyl used are shown at the top of each plate. The strain at the left end of the top row on each plate carries *benA33* and *mipA*⁺ and is *hs*⁻ and benomyl resistant. The wild-type strain is *hs*⁺ *cs*⁺ and benomyl sensitive. The strain carrying *benA*⁺ and *mipA1* is, like the wild-type strain, *hs*⁺ *cs*⁺ and benomyl sensitive. The revertant carrying *benA33* and *mipA1* is less heat sensitive than the strain carrying *benA33* and *mipA*⁺ and is somewhat cold sensitive. The bottom row of each plate consists of three progeny of a cross between a strain carrying *benA33* and *mipA*⁺ and a strain carrying *benA*⁺ and *mipA1*. These progeny have the same phenotype as the revertant.

gene. We designated this putative gene *mipA* (microtubule-interacting protein). We designated the suppressor alleles *mipA1*, *mipA2*, and *mipA3* (isolated from BRO2RA31, BRO2RC21, and BRO2RP52, respectively).

Three-point crosses involving the *facB* and *riboB* mutations and each *mipA* mutation yielded few or no recombinants between the *mipA* alleles and the *riboB* gene among over 400 progeny tested in each case. To determine the order of these three genes and the distance between the *mipA* alleles and the *riboB2* mutation, we plated large numbers of ascospores on medium containing benomyl and lacking riboflavin and incubated these ascospores at 25°C. These conditions selected for *mipA*⁺ *riboB*⁺ progeny obtained if crossing over occurred between *mipA* and *riboB*. We then tested the colonies which arose to determine their *facB* phenotype.

Of 1.26×10^4 segregants from the cross BRO2RA31 \times FGSC 442, 41 were *cs*⁺ *ribo*⁺. Of these 41 segregants, 39 were *fac*⁻, indicating that *mipA1* maps 0.325 centimorgans (cM) to the left of *riboB2*. Of 1.49×10^4 progeny from the cross BRO2RC21 \times FGSC 442, 33 were *cs*⁺ *ribo*⁺; of these 33, 31 were *fac*⁻, showing that *mipA2* maps 0.221 cM to the left of *riboB2*. Of 9.5×10^3 spores from the cross BRO2RP52 \times FGSC 442, 26 were *cs*⁺ *ribo*⁺. All 26 colonies were *fac*⁻, showing that *mipA3* maps 0.274 cM to the left of *riboB2*.

Although chromosomal rearrangements did not allow us to

map the suppressor in BRO2RP9 precisely, recombination between this suppressor and *riboB2* occurred with a significantly higher frequency than for any of the *mipA* alleles ($\geq 6.9\%$). This value represents a minimum estimate of the map distance between *riboB* and the suppressor in BRO2RP9 since the chromosomal rearrangements can be expected to inhibit recombination. Therefore, the suppressor isolated in BRO2RP9 is unlikely to be allelic to the *mipA* mutations; thus, we designated it *mipB1*.

Phenotypes of *mip* mutations. To determine whether any of the four suppressor alleles conferred cold sensitivity in a *benA*⁺ background, we tested progeny from crosses between the BRO2 revertants and the *benA*⁺ strains for the presence of *cs*⁻ *hs*⁺ benomyl-sensitive (*ben*^s) progeny. Since benomyl resistance is conferred by *benA33* but not by *benA*⁺, all *ben*^s progeny will carry *benA*⁺. We recovered several hundred progeny from plates containing no benomyl which were incubated at 42°C. There is a significant growth advantage for *benA*⁺ spores under these conditions.

For each of the *mipA* alleles, all *ben*^s colonies were *cs*⁺ *hs*⁺ (data not shown). The absence of *benA*⁺ *cs*⁻ progeny suggests that each of the *mipA* alleles is either cryptic or lethal in a *benA*⁺ background. The close linkage of *mipA* to *riboB* allowed us to determine which of these possibilities is correct.

We first crossed each revertant carrying a *mipA* mutation to FGSC 442, which carries *facB101* and *riboB2*. Since each mutant *mipA* allele is closely linked to *riboB*⁺, the presence of substantial numbers of *ben*^s *ribo*⁺ spores indicates that the *mipA* mutation is cryptic in a *benA*⁺ background. The absence of such progeny would suggest that the *mipA* allele is lethal in a *benA*⁺ background. Each cross produced significant numbers of *ben*^s *ribo*⁺ progeny. To confirm the presence of the *mipA* mutant alleles in these *ben*^s *ribo*⁺ progeny, we crossed three such progeny from each cross to strain LO427 (*yA2 benA33 facB101 riboB2*). Each cross produced progeny which were *cs*⁻ *hs*⁺ *ben*^r, greater than 99% of which were *riboB*⁺. These progeny have the same phenotype as the original *hs*⁺ revertants (Fig. 1). Thus, each of the *mipA* mutations is cryptic in a *benA*⁺ background but *cs*⁻ in a *benA33* background.

In a BRO2RP9 \times *benA*⁺ strain cross, *benA*⁺ *cs*⁻ progeny were recovered at a frequency of approximately 20%. This result suggests that *mipB1* confers cold sensitivity even in the absence of the *benA33* mutation, but this *cs*⁻ phenotype cannot yet be rigorously assigned to the *mipB1* mutation because of the presence of chromosomal rearrangements.

Analysis of *mipA*⁻ *benA33* double mutants with respect to

TABLE 2. Effects of *mip* mutations on nuclear division, nuclear migration, and mitochondrial movement^a

Genotype	% Nuclear division at (°C):		% Nuclear movement at (°C):		% Mitochondrial movement at (°C):	
	25	37	25	37	25	37
<i>benA</i> ⁺ <i>mipA</i> ⁺	97	100	98	99	86	97
<i>benA33</i> <i>mipA</i> ⁺	94	58	88	53	99	100
<i>benA33</i> <i>mipA1</i>	82	96	84	84	76	96
<i>benA33</i> <i>mipA2</i>	99	98	93	82	99	100
<i>benA33</i> <i>mipA3</i>	94	99	89	89	98	95
<i>benA33</i> <i>mipB1</i>	88	99	94	84	100	100

^a The values shown are the percentages of germlings in which these processes occurred. Sample size for each value was 100. Details of fixation, staining, and scoring have been reported previously (8, 9).

other phenotypes showed that all three *mipA* alleles suppressed the inhibition of nuclear division and nuclear movement conferred by *benA33* at high temperatures (Table 2). At 25°C, the restrictive temperature of the *mipA*⁻ *benA33* double mutants, nuclear division and nuclear movement were not significantly reduced, perhaps reflecting the leakiness of the *cs*⁻ phenotype in each of the double mutants.

To determine whether the *mipA* mutations are allele-specific suppressors, we constructed a series of *mipA benA* double mutants by using different *benA* alleles. In all cases, *mipA1*, *mipA2*, and *mipA3* gave identical results. The *mipA* mutations suppressed the *hs*⁻ phenotype of the *benA11*, *benA17*, and *benA21* mutations (6; J. M. Van Tuyl, Ph.D. thesis, Agricultural University, Wageningen, The Netherlands, 1977) but did not confer cold sensitivity in the presence of these alleles (Fig. 2). With the *benA31* and *benA32* mutations (B. R. Oakley and N. R. Morris, unpublished data), the *mipA* mutations did not significantly suppress heat sensitivity but did confer cold sensitivity and also lowered the level of benomyl resistance conferred by *benA31* and *benA32* (Fig. 2). *mipA* mutations also appeared to reduce the benomyl resistance conferred by *benA33* (results not shown). *ben*^r *hs*⁺ alleles *benA3* and *benA4* and benomyl-supersensitive alleles *benA16* and *benA19* (Van Tuyl, Ph.D. thesis) were not significantly affected by any of the *mipA* alleles (data not shown).

Dominance relationships of *mipA* mutations. Stable diploids of *A. nidulans* can normally be obtained by maintaining heterokaryons, formed from haploids carrying complementing auxotrophic markers, on selective medium for several weeks. To determine whether the *mipA* mutations are dom-

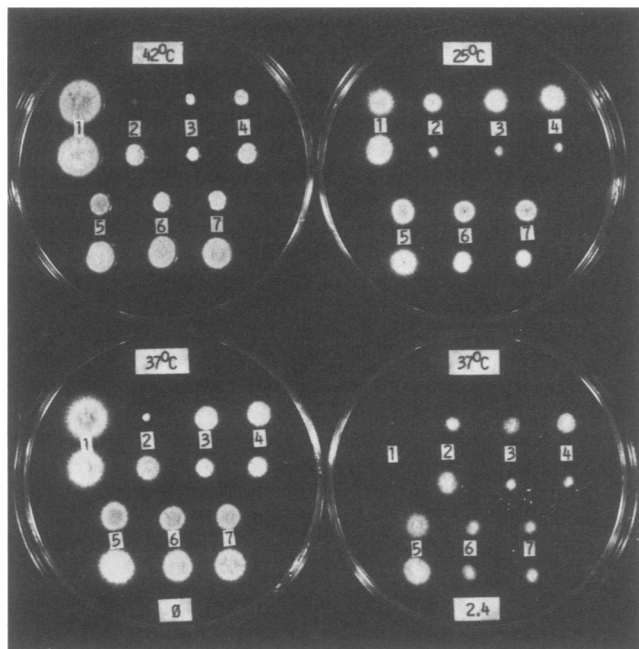


FIG. 2. Interactions between *benA* alleles and *mipA* alleles. Temperatures of incubation are given at the top of each plate. Benomyl concentrations are given at the bottom of the two bottom plates. The colonies are arranged in rows, with the colony above each number carrying *mipA*⁺ and the colony below each number carrying *mipA1*. The colonies above and below each number carry the same *benA* allele: 1, *benA*⁺; 2, *benA33*; 3, *benA31*; 4, *benA32*; 5, *benA11*; 6, *benA17*; 7, *benA21*.

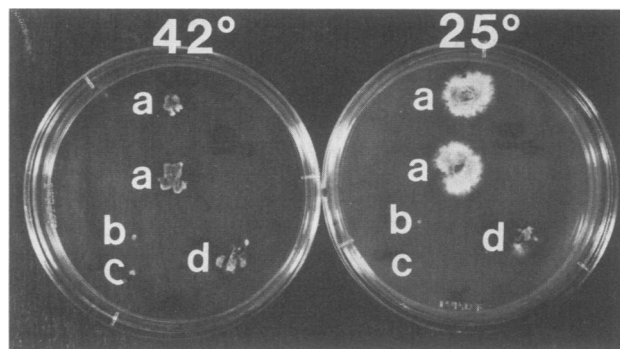


FIG. 3. Dominance relationship of *mipA1*. The plate at the left was incubated at 42°C; the plate at the right was incubated at 25°C. Colonies are designated by letters to their left. The two colonies designated by the letter a are identical heterokaryons formed from two strains, one carrying *benA33* and *mipA*⁺ and the other carrying *benA33* and *mipA1*. These heterokaryons grew at 25°C but not at 42°C, indicating that the mutation is recessive. The letters b and c designate the two parental strains which did not grow in this case because they carry nutritional markers which do not allow growth on the minimal medium used. The letter d designates a control heterokaryon constructed from two strains carrying *benA33* and *mipA*⁺.

inant or recessive, we attempted to make diploids between *benA33 mipA*⁻ strains and *benA33 mipA*⁺ strains. Heterokaryons formed at 28°C but not at 25 or 42°C, the restrictive temperatures of each of the respective parents. In all three cases, the heterokaryons were *hs*⁻ *cs*⁺, compared with wild-type and *benA33 mipA*⁺/*benA33 mipA*⁺ controls (Fig. 3). These data suggest that all three *mipA* mutations are recessive. In all heterokaryons in which both parents carried the *benA33* mutation, stable diploids did not form, even after 8 weeks, although the heterokaryons persisted if subcultured weekly. Streaking the heterokaryons to single colonies on nonselective medium after this time produced haploids of the expected parental types.

DISCUSSION

We isolated, among extragenic suppressors of *benA33*, four mutations at two new loci. Three of the suppressor mutations map to less than 0.4 cM to the left of the *riboB2* mutation on linkage group VIII. The close linkage of these mutations suggests strongly that they are allelic, and we have assigned them to a new gene, *mipA*. Reconstruction experiments demonstrated that these mutations are cryptic in a *benA*⁺ background but confer a *cs*⁻ *hs*⁺ phenotype in the presence of the normally *cs*⁺ *hs*⁻ mutation, *benA33*.

Despite the remarkable phenotypic similarities of the three *mipA* alleles, we are confident that they are the result of three separate mutational events. The mutagenesis was of uninucleate conidiospores, each revertant was isolated from a separate plate, and two of the three carried different coincidental mutations.

Although each of the *mipA* mutations is closely linked to the *riboB2* mutation, we do not believe that they are mutations in the *riboB* gene itself for two reasons. None of the *mipA* alleles confers riboflavin auxotrophy, and the presence of *cs*⁺ *hs*⁻ *ribo*⁻ progeny from crosses of the original *riboB*⁺ revertants to *riboB*⁻ strains demonstrates that riboflavin auxotrophy does not suppress the *hs*⁻ phenotype of *benA33*. However, it is still possible that the *mipA* mutations are in

the *riboB* gene but that they do not affect riboflavin biosynthesis.

The fact that the *mip* mutations suppress the heat-sensitive blockage of microtubule function conferred by *benA33* demonstrates that the products of these genes interact with the β -tubulin encoded by the *benA* gene and are involved in the functioning of microtubules. However, it should be pointed out that whereas the most obvious type of interaction between the *mip* gene products and β -tubulin is a direct, physical one, such as the interaction between a MAP and tubulin, two other equally interesting modes of interaction are possible and consistent with our data. One is that *mip* genes encode products that interact specifically but indirectly with β -tubulin. It is likely that there are classes of proteins that are essential to microtubule function but that do not interact physically with microtubules. To choose one of many possible examples, such proteins might be involved in a posttranslational modification that affects the functioning of a MAP. Another possibility is that one or both of the *mip* genes encode a protein involved in regulating the cellular environment in a way that affects microtubule function. A *mip* product could be involved in the regulation of Ca^{2+} levels or pH, for example.

Our results do not allow us to distinguish unambiguously among these possibilities, but they suggest that, for *mipA*, regulating the cellular environment is the least likely possibility. If *mipA* mutations caused significant alterations in the cellular environment, one would expect that they would have some effects on phenomena that are not microtubule mediated. Since the *mipA* mutations have no detectable effects in a *benA*⁺ background, the alterations that they cause must be very subtle or very specific. The fact that different combinations of cold sensitivity, heat sensitivity, and benomyl resistance can be constructed by combining different *benA* alleles with the same *mipA* allele indicates that the interactions between the products of *mipA* and *benA* are allele specific. This specificity can be explained if the interactions between the *mipA* gene product and the β -tubulin encoded by *benA* are specific. For example, a mutation in a MAP that affected a site where the MAP binds to β -tubulin might cause the mutant MAP to bind with different affinities to β -tubulins encoded by different *benA* alleles, thus giving different phenotypes. It is much more difficult to explain how general alterations in the cellular environment could produce such allele-specific interactions.

The specificity of the interactions between the *mipA* and *benA* mutations also provides an explanation for the differences between our results and those of Thomas et al. (12). Thomas et al. (12) found, among *hs*⁻ revertants of a *cs*⁻ β -tubulin mutation of *S. cerevisiae*, a single intragenic suppressor and 26 extragenic suppressors in 16 complementation groups. We found, among a significantly larger number of *cs*⁻ revertants of an *hs*⁻ β -tubulin mutation, that most carry intragenic suppressors and, although we also found a large number of extragenic suppressors, they define only three loci, *mipA*, *mipB*, and *tubA*. It appears that the number of genes in which mutations can be isolated which both suppress the heat sensitivity of *benA33* and confer cold sensitivity is small. These data also suggest that new mutations in previously undiscovered genes involved in microtubule function can be isolated more easily among revertants of other *benA* alleles than among additional revertants of *benA33*.

Two-dimensional gel electrophoresis has shown that, in *A. nidulans*, there is a β -tubulin produced in minor amounts in addition to the major β -tubulin encoded by *benA* (14). May

et al. (5) have recently cloned the gene encoding this minor β -tubulin and designated it *tubC*. Their evidence also suggests that there are no β -tubulin genes other than *benA* and *tubC*. Since the *benA33* allele is recessive with respect to heat sensitivity, a mutation that resulted in overproduction of the *tubC* β -tubulin might overcome the heat sensitivity conferred by *benA33*. However, *tubC* has recently been mapped to linkage group I (G. May and N. R. Morris, personal communication), and since neither *mipA* nor *mipB* is on linkage group I, neither can be *tubC*. We believe it is more likely that *mipA* and *mipB* encode nontubulin proteins involved in microtubule function.

The results we report here do not permit the identification of the products of the *mip* genes or the determination of the function of these products. However, they do suggest an approach to the identification and characterization of the product of *mipA* which we are currently using. The close linkage of *mipA* to *riboB* suggests that, if *riboB* can be cloned, it should be possible to identify a clone carrying *mipA* with a very short chromosome walk. The cloned *mipA* gene could be used to identify the *mipA* gene product, and in vitro mutagenesis could be used to create the *mipA* mutations that have obvious phenotypes in *benA*⁺ backgrounds. We have recently cloned a sequence complementing *riboB2* (unpublished results), and we hope to be able to identify a clone carrying *mipA* soon.

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