Characterization of Alpha and Beta Tubulin Genes in the Dimorphic Fungus *Histoplasma capsulatum*

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Evidence from our laboratory indicates that microtubules are involved in the differentiation of the dimorphic, pathogenic fungus *Histoplasma capsulatum*; therefore, we cloned the tubulin genes from a virulent strain of the organism. We report that the *H. capsulatum* genome contains a single alpha (*TUB1*) and a single beta (*TUB2*) tubulin gene rather than the more typical multigene family which is common in even the simplest eukaryotes. Sequence data from these genes reveal a high degree of nucleotide and protein sequence conservation relative to tubulins from other species. The coding regions of *TUB1* and *TUB2* contain five and eight intervening sequences, respectively. Field inversion gel electrophoresis of *H. capsulatum* chromosome-sized DNA fragments indicates that the *TUB1* and *TUB2* genes are unlinked. Potential regulatory elements common to both genes have been identified in the 5' promoter regions. These elements may direct the coordinate expression of *TUB1* and *TUB2* during differentiation. The cloning and characterization of alpha and beta tubulin genes from *H. capsulatum* provides the first description of gene structure in this widely distributed pathogenic fungus. Isolation of the tubulin genes will facilitate future studies of tubulin gene expression during the dimorphic phase transitions and clarify the role of microtubules in the differentiation process.

INTRODUCTION

Histoplasma capsulatum is a pathogenic, dimorphic fungus which causes the disease histoplasmosis. Histoplasmosis is one of the most common primary, systemic fungal infections in the Western Hemisphere and millions of people throughout the world have become infected with the organism (Edwards, 1971). The number of reported cases of disseminated histoplasmosis in individuals with AIDS is increasing steadily (Graybill, 1988). The organism exists in soil as multicellular, filamentous mycelia; however, the only form of the fungus found in infected tissue is unicellular, budding yeast (Emmons *et al.*, 1977). Since the mycelial phase of *H. capsulatum* is nonpathogenic and only the yeast phase is parasitic (Medoff *et al.*, 1987), factors which control the phase transition are critical for understanding pathogenicity.

The two morphological phases of the fungus can be maintained in the laboratory by appropriate manipulation of temperature and medium composition (Salvin, 1949); yeast cells grow *in vitro* at 37 °C and mycelia at 25 °C. By shifting the temperature of incubation, the transition from one phase to the other is induced. This interconversion of yeast and mycelial forms provides an attractive system for studying mechanisms and regulation of eukaryotic cell differentiation.

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Abbreviation: IVS, intervening sequence.

Cyclic AMP (cAMP) may provide the signal which triggers morphogenesis in *H. capsulatum* (Medoff *et al.*, 1981) as it does in other dimorphic fungi (Larsen & Sypherd, 1974; Paveto *et al.*, 1975; Niimi *et al.*, 1980). Exposure of *H. capsulatum* yeast cultures to dibutyryl cAMP, or to inhibitors of cAMP phosphodiesterase, induces transformation to the mycelial phase even at the non-permissive temperature of 37 °C (Maresca *et al.*, 1977). Preliminary evidence from our laboratory suggests that cAMP may induce morphological change in *H. capsulatum* at the level of microtubules (Medoff & Medoff, 1982). cAMP is known to affect the number (Porter *et al.*, 1974), organization (Kram & Tomkins, 1973), and function (Kirkland & Burton, 1972) of microtubules in intact cells, and to stimulate tubulin polymerization *in vitro* (Steiner, 1978). Fungal antimitotic agents such as methylbenzimidazole-2-yl carbamate (MBC) bind to fungal tubulin and inhibit the polymerization of tubulin monomers into microtubules (Davidse & Flach, 1977). In *H. capsulatum*, MBC reversibly inhibits the yeast-to-mycelia phase transition (Medoff & Medoff, 1982), supporting the notion that microtubules are involved in *H. capsulatum* differentiation.

To extend the study of microtubule involvement during *H. capsulatum* morphogenesis, we have cloned and characterized tubulins, the major constituent proteins of microtubules, from this fungal pathogen. We report the isolation of one alpha (*TUB1*) and one beta (*TUB2*) tubulin gene as well as the nucleotide and predicted amino acid sequence for both genes.

METHODS

Strains, media and growth conditions. The low-virulence Downs strain and the highly virulent 217B strain, mating types (–), of *H. capsulatum* were obtained from our permanent laboratory collection and maintained as previously described (Maresca *et al.*, 1977).

Isolation and purification of chromosomal and plasmid DNAs. High-molecular-mass DNA from *H. capsulatum* was prepared as described by Akins & Lambowitz (1985) for *Neurospora crassa*. DNAs isolated by this procedure were in excess of 48 kb in size. Plasmid DNAs were isolated from *Escherichia coli* DH5 alpha according to Maniatis *et al.* (1982).

Gel electrophoresis and hybridization methods. Genomic or plasmid DNAs were digested with restriction endonucleases (Promega Biotec), electrophoresed in 0.8% or 1.0% horizontal agarose gels in 100 mM-Tris/borate and transferred to nitrocellulose (Schleicher & Schuell) by the method of Southern (1975). Hybridization mixtures contained 1×10^6 c.p.m. ml^{-1} of [α - ^{32}P]CTP-labelled tubulin probes (Rigby *et al.*, 1977) in 50% (v/v) formamide, $5 \times$ Denhardt's solution, $5 \times$ SSC, and 25 mM-sodium phosphate, pH 6.8, with 200 μg salmon sperm DNA ml^{-1} at 42 °C [$1 \times$ Denhardt's solution is 0.02% bovine serum albumin, 0.02% Ficoll, 0.02% polyvinylpyrrolidone; $1 \times$ SSC is 0.15 M-NaCl, 0.015 M-trisodium citrate]. Low-stringency washes were performed in $2 \times$ SSC, 0.1% SDS at room temperature, and high-stringency washes in $0.2 \times$ SSC, 0.1% SDS at 50 °C.

H. capsulatum genomic library construction and screening. An *H. capsulatum* genomic library was constructed as described by Maniatis *et al.* (1982), using 13 to 20 kb fragments from a partial *Mbo*I digest of 217B DNA. The library, constructed in the bacteriophage vector EMBL3, contained 2×10^5 recombinants with an average insert size of 19 kb. A total of 10000 independent bacteriophage clones were screened *in situ* with [α - ^{32}P]CTP-labelled tubulin-specific cDNA probes from *Chlamydomonas reinhardtii* (provided by Bill Dove, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI 53706, USA) according to the method of Benton & Davis (1977).

DNA sequence analysis. The alpha (*TUB1*) and beta (*TUB2*) tubulin genes were sequenced by the dideoxy chain termination method (Sanger *et al.*, 1977). DNA sequences were analysed and compared using Sequence Version 2.0 (Delaney Software, Vancouver, BC, Canada).

Localization of tubulin genes to H. capsulatum chromosome-sized fragments. Chromosome-sized DNAs from the Downs and 217B strains were fractionated on low-percentage agarose gels by field inversion gel electrophoresis (Carle *et al.*, 1986; Steele *et al.*, 1989) using an embedded lysis technique adapted for *Histoplasma*. Resolved chromosomal DNAs were transferred to nitrocellulose membranes and hybridized as described above.

RESULTS AND DISCUSSION

Isolation of genomic clones

To identify the alpha and beta tubulin genes in the *H. capsulatum* genome, cDNA probes from *Chlamydomonas reinhardtii*, clones alpha 253 and beta 37 (Silflow & Rosenbaum, 1981), were used to screen an EMBL3 bacteriophage library containing genomic inserts from *H. capsulatum*

Fig. 1. Restriction maps of recombinant bacteriophages alpha 1.2.2.1 and beta 2.1.1.1, containing the alpha (*TUB1*) and beta (*TUB2*) tubulin genes from *H. capsulatum*, respectively. Alpha 1.2.2.1 contains a 17 kb insert of *H. capsulatum* genomic DNA cloned into the *Bam*HI site of EMBL3; *TUB1* is harboured on a 1.9 kb fragment (stippled box). Beta 2.1.1.1 contains a 15.6 kb insert also cloned into the *Bam*HI site of EMBL3; *TUB2* is harboured on a 2.3 kb fragment (stippled box). The fragments designated with an asterisk (*) were nick-translated and used as probes in subsequent experiments. B, *Bam*HI; E, *Eco*RI; Hd, *Hind*III; S, *Sal*I; St, *Sst*I; X, *Xho*I.

217B. Preliminary restriction mapping of the purified recombinant bacteriophage revealed that each set of alpha and beta phage clones contained DNAs with overlapping tubulin sequences. Thus, a single clone from each set was selected for further analysis. Figs 1(a) and 1(b) show restriction maps of the EMBL3 bacteriophage containing tubulin inserts alpha 1.2.2.1 and beta 2.1.1.1.

To test for rearrangements within the bacteriophage clones, parallel restriction digests of alpha 1.2.2.1, beta 2.1.1.1, and total *H. capsulatum* genomic DNA were compared by Southern analysis using the probes described in Fig. 1. Identical hybridization patterns were generated on the bacteriophage and genomic DNA blots, suggesting that no gross rearrangements, additions, or deletions had occurred during the cloning procedure (data not shown).

Single alpha and beta tubulin genes in the *H. capsulatum* genome

To determine the number of alpha and beta tubulin genes in the *H. capsulatum* genome, the *TUB1* and *TUB2* probes were hybridized to Southern blots of restricted *H. capsulatum* genomic DNA. Hybridization at low stringency revealed unique, strongly hybridizing bands, indicating no cross-hybridization between the alpha and beta tubulin sequences (Fig. 2). Furthermore, for each restriction enzyme digest, the alpha and beta tubulin probes hybridized to one fragment, suggesting the presence of a single gene each for alpha and beta tubulin in the *H. capsulatum* genome. Prolonged exposures of these blots did not reveal any additional hybridizing bands.

Fig. 2. Southern blot analysis to determine the number of alpha and beta tubulin genes in *H. capsulatum*. Genomic DNA of *H. capsulatum* 217B was digested with the restriction endonucleases indicated on the figure, separated on 0.8% agarose gels in Tris/borate buffer, and transferred to nitrocellulose membranes. Panels (a) and (b) were hybridized respectively with the alpha (*TUB1*) and beta (*TUB2*) tubulin probes (see Fig. 1). The positions and lengths of the resulting DNA fragments are indicated on the left. The detection of one hybridizing band for each restriction digest confirms the presence of a single gene each for alpha and beta tubulin in the *H. capsulatum* genome.

Confirmation of the identities of the alpha (*TUB1*) and beta (*TUB2*) tubulin-specific sequences in *H. capsulatum* was obtained by DNA sequence analysis. It is likely that *TUB1* and *TUB2* represent the entire complement of tubulin genes in *H. capsulatum* since no additional sequences with homology to either alpha or beta tubulin have been detected, even when homologous probes were utilized under very low-stringency hybridization conditions.

In all but the simplest eukaryotes, multiple tubulin genes are required for the construction and maintenance of essential microtubules (for review, see Cleveland & Sullivan, 1985). Significantly fewer tubulin genes have been described and cloned in lower eukaryotes. Two alpha and two beta tubulin genes have been detected in the filamentous fungus *Aspergillus nidulans* (Weatherbee & Morris, 1984), and two alpha tubulin genes and one beta tubulin gene have been identified in the fission yeast *Schizosaccharomyces pombe* (Hiraoka *et al.*, 1984; Toda *et al.*, 1984), and in the budding yeast *Saccharomyces cerevisiae* (Neff *et al.*, 1983; Schatz *et al.*, 1986). The ciliated protozoan *Tetrahymena* contains a single alpha tubulin gene (Callahan *et al.*, 1984).

The presence of a single alpha (*TUB1*) and a single beta (*TUB2*) tubulin gene in *H. capsulatum* is unique among eukaryotes so far investigated, but consistent with the findings in *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*, where two alpha tubulin genes and one beta tubulin gene have been described. Transformation-mediated gene-disruption experiments have shown that although these yeasts have multiple tubulin gene families, only a single alpha and a single beta tubulin gene are required to perform all the functions that have been attributed to microtubules in the yeast life cycle (Adachi *et al.*, 1986; Schatz *et al.*, 1986). The function of the non-essential alpha tubulin gene in these fungi remains uncertain.

Fig. 3. Structural organization of the *H. capsulatum* alpha (*TUB1*) and beta (*TUB2*) tubulin genes. Each gene was sequenced by the dideoxynucleotide chain termination method to the extent, and in the direction, indicated by the arrows. Much of the sequence information for these genes was generated from a single subclone in a single direction. The *H. capsulatum TUB1* nucleotide sequence was over 75% homologous to alpha tubulins from *Saccharomyces cerevisiae* (Schatz *et al.*, 1986), *Schizosaccharomyces pombe* (Toda *et al.*, 1984) and *Chlamydomonas reinhardtii* (Silflow *et al.*, 1985). The nucleotide sequence generated for *TUB2* was 84% conserved relative to beta tubulins from *Aspergillus nidulans* (May *et al.*, 1987), *Neurospora crassa* (Orbach *et al.*, 1986), *Saccharomyces cerevisiae* (Neff *et al.*, 1983) and *Chlamydomonas reinhardtii* (Youngblom *et al.*, 1984). *TUB1* and *TUB2* coding regions are denoted by the stippled areas, and black boxes indicate the locations of the predicted intervening sequences (IVSs). Restriction endonucleases are abbreviated as in Fig. 1, plus: Hc, *HincII*; P, *PstI*.

Alpha (*TUB1*) and beta (*TUB2*) tubulin gene structure

Since *H. capsulatum* uses the products of a single alpha and a single beta tubulin gene for diverse microtubular functions as well as differentiation, the organization and sequence of its tubulin genes are of interest. *TUB1* and *TUB2* were sequenced using the strategy outlined in Fig. 3. This strategy was adopted since the primary amino acid sequence of alpha and beta tubulins from even remotely related species is highly conserved (Cleveland & Sullivan, 1985).

The proposed introns for *TUB1* and *TUB2* were identified as gaps in the protein homology between the translated *H. capsulatum* genes and other highly conserved alpha and beta tubulin protein sequences. Based on this analysis, the predicted coding regions of *TUB1* and *TUB2* were interrupted by five and eight intervening sequences (IVSs) respectively (Fig. 3). Like other fungal tubulin introns, the *H. capsulatum TUB1* and *TUB2* IVSs are relatively short, ranging from 42 bp for IVS8 (*TUB2*) to 113 bp for IVS3 (*TUB1*), and contain consensus splice junctions similar to those found in other nuclear eukaryotic genes (Mount, 1982). Furthermore, twelve of the thirteen IVSs contain an adenosine residue within an internally-conserved sequence, resembling the yeast TACTAAC consensus sequence (Langford *et al.*, 1984) which mediates the splicing event (Ruskin *et al.*, 1984). In *H. capsulatum*, the internal element is located near the 3' end of each IVS, beginning 18 to 32 nucleotides upstream of the 3' splice junction.

The IVS positions within *TUB1* and *TUB2* are highly conserved when compared to the organization of other fungal tubulin genes. Five of the eight beta tubulin IVSs in *Histoplasma* (Fig. 5) occur at the same positions (after codons 4, 12 and 53, and splitting codons 21 and 35) as those of *Neurospora* (Orbach *et al.*, 1986) and *Aspergillus* (May *et al.*, 1987). The other three IVSs in *TUB2* are localized to codons 206, 318 and 438, each just one codon away from the intron positions described for the *benA* beta tubulin gene of *Aspergillus* (May *et al.*, 1987). Furthermore, one of the five alpha tubulin IVSs in *H. capsulatum* (Fig. 4) occurs at the same position as the

i Met Arg Glu Val Val Ser Leu Asn V
 ATG AGA GAG GTA GTT AAC G GTATGATGGGCTCATGGCTTA
 20
 ATTGGACTTTCAGTTTATGCGCTGTGCTGAGCTCTTCTGATGATGAAGTTGCTGTAA
 IVS1
 10
 al Gly Gln Ala Gly Gln Ile Ala Asn Ser Cys Trp
 ACAG TC GGT CAG GGC GGT GGC CAA ATC GCA AAC TCG TCG TCG
 40
 GAGGTATGCGAGCTTAATGCTAAAGGAACTAGGTGTGTTTCCATATACGCTGCATTAA
 IVS2
 CAACAGCGGATATAGATTCTATTTGATGCTAAATGCGCTTGGATTGAACCTTTTTTTA
 IVS3
 30
 Leu Tyr Cys Leu Glu His Gly Ile Gln
 TAG TTG TAT TCG CTG GAA CAT GGC ATT CAG GTAAATATATATATCC
 80
 CTCACGCTGCGATGAGATTCTATGCGGCTATGCTTATTCACACTGCGGCTTAAAG
 IVS4
 Pro Asp Gly
 AACTGACGATTGACAGCAATTTGACTAACTGTGTTCTGCTGCGCAG GGC GAC GGA
 40
 Tyr Leu Thr Glu Glu Arg Lys Ala Ala Asp Pro Asp Gln Gly
 TAC CTG ACT CAG GAA GGA GGA GGT GGC GAC GGT CAT CAA GGA
 100 120 140
 Phe Asn Thr Phe Phe Ser Glu Thr G
 TTT AAT ACA TTT TTT TCT GAA ACA G GTGCGACTCTCTTTTTCATAA
 160
 TCGAATCTTTAACTGCGCTTTTGAAAGCTTAACTTACCAAGCTCTCTCTTCCTGACATCTC
 IVS5
 60
 ly Gln Gly Lys Tyr Val Pro Arg Thr Ile Tyr Cys Asp
 TAG GC CAG GGC AAA TAC GTT CCA GGA ACC ATC TAC TCG CAT
 180 200

70 Leu Glu Pro Asn Val Val Asp Glu Val Arg Thr Gly Pro Tyr
 CTT GAG CCA AAT GTC GTG GAC GAA GTC GGC ACT GGA CCA TAT
 220 240
 Arg Ala Leu Phe His Pro Glu Gln Met Ile Thr Gly Lys Glu
 CGT GCG CTT TTC CAC CCA GAA CAG ATG ATC GCA AAG GAG
 260 280
 Asp Ala Ser Asn Asn Tyr Ala Arg Gly His Tyr Thr Val Gly
 GAT GCA TCC AAT AAC TAC GGT GGT CAT TAT ACC GTG GGT
 300 320
 Lys Glu Met Ile Asp Gln Val Leu Asp Lys Val Arg Arg Val
 AAG GAG ATG ATC GAC CAA TCC CTT GAC AAA GTC GGC GCG GTG
 340 360
 Ala Asp Asn Cys Ser Gly Leu Gln Gly Phe Leu Val Phe His
 GCA GAT AAC TCC AGC GGT CTC CAG GGC TTT TTG GTG TTC CAT
 380 400
 Ser Phe Gly Gly Gln Gly Ser Gly Phe Gly Ala Leu Leu
 TCC TTT GGA GGC GGC CAG GCA TCC GGC TTC GGT GCA CTT CTG
 420 440 460
 Met Glu Arg Leu Ser Val Asp Tyr Gly Lys Lys Thr Lys Leu
 ATG GAG GGC CTG TCC GTC GAC TAC GGC AAA AAG ACA AAG CTG
 480 500
 Glu Phe Cys Val Tyr Pro Ala Pro Gln Asn Ala Thr Ser Val
 GAA TTT TGT GTC TAC CTT GTC GGC CAG AAT GGC ACT TCA GTT
 520 540 560 580
 Val Glu Pro Tyr Asn Ser Ile Leu Thr Thr His Thr Thr Leu
 GTC GAA CCT TAC AAC TCG ATT CTC ACA ACG CAT ACT AOC CTG
 600 620
 Glu His Ser Asp Cys Gln Phe Met Val Asp Asn Glu Ala Ile
 GAG CAT TCT GAT GTG CAG TTT ATG GTG GAC AAT GAA GCT ATC

Fig. 4. Nucleotide and predicted amino acid sequence of the *TUB1* alpha tubulin gene from *H. capsulatum*. The nucleotide sequence is numbered from the ATG initiation codon with the numbers appearing below the nucleotides. Amino acid numbers appear above the sequence, with the initiation methionine as no. 1. The five IVSs are indicated; consensus splice junctions and internally-conserved sequences are underlined.

Fig. 5. Nucleotide and predicted amino acid sequence of the *TUB2* beta tubulin gene from *H. capsulatum*. The nucleotide sequence is numbered from the ATG initiation codon with the numbers appearing below the nucleotides. Amino acid numbers appear above the sequence, with the initiation methionine as no. 1. The eight intervening sequences (IVS) are indicated; consensus splice junctions and internally-conserved sequences are underlined. The dots within IVS1 correspond to regions of significant band compression.

single intron found in the alpha tubulin genes of *Saccharomyces cerevisiae* (Schatz *et al.*, 1986). A second intervening sequence in *TUB1* occurs after codon 21, a position very near that of the sole intron which interrupts the alpha 1 tubulin gene of *Schizosaccharomyces pombe* (Toda *et al.*, 1984).

The DNA and predicted amino acid sequences for the alpha and beta tubulin genes of *H. capsulatum* are presented in Figs 4 and 5 respectively. The sequences confirm the identity of the cloned genes, distinguish different functional regions of the tubulin molecules, and identify potential regulatory elements in the 5' promoter regions. Fig. 4 shows 1251 bp of the coding region of the *TUB1* gene. The gene was sequenced up to the *EcoRI* site (Fig. 3) and all but the last 25 to 30 amino acid residues at the carboxyl terminus have been identified (Fig. 6). The complete sequence of beta tubulin is shown in Fig. 5. The coding region of *TUB2* contains 1335 bp, which predicts a protein of 445 amino acids. The calculated molecular mass of beta tubulin is 58159 Da, a value in close agreement with other fungal beta tubulins (May *et al.*, 1987).

Significance of conserved and heterogeneous regions in the primary sequence

Sequence comparisons among ascomycetous tubulin genes indicate conserved regions in *Aspergillus* (May *et al.*, 1987), *Neurospora* (Orbach *et al.*, 1986) and *Histoplasma* tubulin genes which underscore a common functional role. All three fungi share a conserved sequence, KYVPRA, at amino acids 58–64 for beta tubulin (Fig. 7). For alpha tubulin, five of the six amino acid residues are conserved in a similar region (Fig. 6). This sequence resembles a GTP-binding domain required for the polymerization of tubulin monomers into microtubules (Weisenberg, 1972), and has been found in many tubulins and GTP-binding proteins (Leberman & Egner, 1984). The beta tubulin genes of *Aspergillus*, *Neurospora* and *Histoplasma* also share a conserved HSLGGGTGAGMGTL sequence spanning amino acids 138–152 (Fig. 7) resembling a phosphate-binding pocket which is also present in other tubulins and nucleotide-binding proteins (Krauchs *et al.*, 1981; May *et al.*, 1987). Alpha tubulin has a similar conserved sequence containing a cluster of glycines in the same region (Fig. 6) which may represent a flexible loop involved in phosphate binding (Krauchs *et al.*, 1981).

The majority of the nucleotide differences present in the *H. capsulatum* alpha and beta tubulin sequences occur at the third position of a codon and do not change the predicted amino acid. The differences in amino acids that do occur, however, appear to be clustered within the primary sequence. As seen in Fig. 6, a variable domain in the *TUB1* gene spans amino acid residues 35 to 50, near the amino terminus of the protein molecule. Another major region of heterogeneity exists at the extreme carboxyl terminus of the *TUB2* protein (Fig. 7). Serrano *et al.* (1984a) suggested that the carboxy-terminal domain of the tubulin molecule is involved in binding the microtubule-associated protein, MAP-2, which regulates tubulin assembly, yet *in vitro* experiments demonstrate that proteolytic removal of the carboxyl terminus does not prevent self-assembly (Serrano *et al.*, 1984b). Recent evidence from *Saccharomyces cerevisiae* also suggests that insertions into the amino-terminal variable region do not disrupt tubulin function *in vivo* (Schatz *et al.*, 1987). The biological significance of this and other heterogeneous domains in *H. capsulatum* remains uncertain.

Localization of tubulin genes to H. capsulatum chromosomes

Electrophoretic separation of large, chromosome-sized DNA molecules has proved useful in establishing the linkage relationship between alpha and beta tubulin genes in yeast (Schwartz & Cantor, 1984). Field inversion gel electrophoresis, a technique which resolves chromosomal DNAs in an alternating electric field, was applied to *H. capsulatum*. Four chromosome-sized DNA bands were resolved by this method in the Downs strain of *H. capsulatum*, and two in the more virulent 217B strain (Fig. 8a). These large DNA molecules are chromosome-like since they migrate as discrete bands to a size range comparable with the known chromosomes of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (Schwartz & Cantor, 1984). Since classical genetics have not been performed with this fungal pathogen, however, it is unclear whether the chromosome-sized DNAs resolved at these conditions represent all of the nuclear chromosomes present in *H. capsulatum*.

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Histoplasma capsulatum      M R E V V S L N V G O A G G Q I A N S 20 C W L Y C L E H G I Q P D
Saccharomyces cerevisiae      I      I      C      G      A      S      K
Schizosaccharomyces pombe      I      V H      V      G      A      G
Chlamydomonas reinhardtii      I      I H I      I      V G      A      E

G - Y L T E E R K A A D P D Q G F N - - - T F F S E T G G G 40 K Y V P R T I Y C D L E P N V V
- H E D G L S K P K G G E E G F S - -      H      Y      F      A      V      I
F P T E N S E V H K N N S Y L N D G F G      S      V      I
- O M P S D T I G G G D D A F H - -      A      H      C      F L      T

D E V R T 80 G P Y R A L F H P E Q M I T G K E D A S 100 N H Y A R G H Y T V G K E M I D Q V L D K 120
      N      K D      L L S      A      R      I L G D      R
Q      K D      V      S      E R
      T      Q      L      S      A      F      I      I V      L A      R

V A R V A D N C S G L O G F L V F H S 140 F G G G Q G S G F G A L I M E R L S V D Y 160 G K K T K L
I K L Q D      F T      L      T      L      S      L L      E      A E      S
I      H      T      L      T      L L      N M E      S N
I K L      T      N A V      T      L      S      L L      S

E F C V Y P A P O N A T S V V E P Y N S I L T T H T T L E H S D C Q F H V D N E A I Y D I 200
      A      V S      T V      A      T      H
Q S      V S      V      A      D N      T      C
G T      S      V S      A      V      S      S L      T      V A V      L

C R R N L G I E R P S Y E N S N R L H A Q G Q S P I T A I M L L F D C A L N V D L N E F Q T H 240
X      D P      F A      L      N      I      V V      S      A S      R      G S
      D      T      L      I      S      A S      R      A G S
      S      D      T      T      L      I      I      S      A S      R      G      I T

L V P Y P R I H F P L A S Y S P V V S A G K A S H E S N S V H E V T I V C F E P N N Q H 280 300
      V      L      K S      F      S      I      N A      G
      V      I      A      F      Q      I      N Q      Y
      H      S      A      I I      E      Q      Q L      A      I      N A A      A S M

V K C D P R N G K Y H A T C L L Y R G D V V F K D V H A V A A T L K T K R T V Q F V D L Q 320 340
      D      T G      Q R A V E Q V N K      L      W C
      T      R      I      R      Q      V T S I      S R      I      W C
      H      C      H      N      S V      I      I      W C

P T G F K I G I C Y O P P T H V P N G D L A K V N R A C H L S N T T A I S E A W T A L S H 360 380
      E      A T      S Q      T      D      S      A      K R I D R
      H      G S G I      S      A      S R      D
      C      N      V      G      Q      I      S      G      I      F      S      R      D

K F D L I Y S K R A P V H W Y V G E G H E E G E F 400 420
      M      A      T E A R E D L A A L E R D Y I E V G A D
      M      S E A R E D L A A L E R D Y E E V G Q D
      M      A      S E A R E D L A A L E K D F E E V G A E

S - - - - Y A E E E E F 440
S M D N E M Y E A D E E Y
S A E G A G E G E G E E Y

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Fig. 6. Alpha tubulin protein sequence comparisons. The *TUB1* amino acid sequence from *H. capsulatum* is shown in one-letter code and compared with the alpha tubulin protein sequences of *Saccharomyces cerevisiae* (Schatz *et al.*, 1986), *Schizosaccharomyces pombe* (Toda *et al.*, 1984) and *Chlamydomonas reinhardtii* (Silflow *et al.*, 1985). Only amino acids that differ from those of the *H. capsulatum* sequence are indicated. Dashes have been inserted into the sequences to bring them into register. The *H. capsulatum* sequence underlined near amino acid residue 60 resembles a GTP-binding domain. The underlined region near amino acid residue 140 is a putative phosphate-binding pocket.

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Histoplasma capsulatum      H R E I V H L Q T G Q C G N Q I G A A F W Q T I S G E N G L D G A G
Aspergillus nidulans      S
Neurospora crassa      A S
Saccharomyces cerevisiae      E C G F H
Chlamydomonas reinhardtii      K E V V D I P T

Y Y N G S L D I Q L E R M N V Y F H E A A E K Y V P R A V L V D L E P G T H D S V R A G P F
V T L S G N C
V T E L I S G N A
T H H D K L S S G Y S I H W I A N S A I
T H D L I T G G R I H S S Y

G Q L F R P O N F V F V Q S G A C N T W A K G H Y T E G S E L V D Q V I D V V R R E A E R C D
E G N A N V G
G H A H L G
H Y I G S V A S H I G
I T N A I S L K S

C L Q G F Q I I H S L G G G T G A G M G T L L I S K I R E E F P D R M H A T F S S V V P S P K
T
T A Y
S T S F K L L
V C S Y L I I

V S D T V V E P Y N A T L S N H E L V E N S D E T F C I D N E A L Y D I C H R T L K L S E P S
V H N
V N
T V H Q
V A C H V L F T T

- Y G D L H H L V S A V H S G V T T C L R F P G Q L H S D L R K L A V N H V P F P R L H F F H
S W
S V
S N S Y L
T F I I C C A L I

V G F A P L T S R G A Y S F R V V T V P E L T Q K H Y D P K N H M C L L D F R N G R Y L T A S
A S Q F A A S C
H H A S E F A A S C
Y A I S Q S L Q F E A A A A P V A
T S Q Q Y L T Q W A A A P H A

A I F R G K V S H K E V E D Q M R N V Q N K H H T Y F V E W I P N H V Q T A L C S I P P R G L
I S Q S I S
S S
F V E H K S S D V V A Q
L R H T D E L S S K S S V D K

K H S S T F V E N S T A I Q E L F K R V G D Q F T A H F R R K A F L H W Y T G E G H D E H E F
I G S
G I E
D A A I A S S K S L
A I G C H S E

T E A E S N H N D L V S E Y Q Q Y Q D A S I S E G E D E Y F D Y A W A H
E Y A E E E I H E G E E
G V D E E Y E E E A P L G G E E
S E T V E D D E V D E N G D F G A P O N Q D E P I T E N F E
A E E G E F E G E E E E A

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Fig. 7. Beta tubulin protein sequence comparisons. The *TUB2* amino acid sequence from *H. capsulatum* is shown in one-letter code and compared with the beta tubulin protein sequences of *Aspergillus nidulans* (May *et al.*, 1987), *Neurospora crassa* (Orbach *et al.*, 1986), *Saccharomyces cerevisiae* (Neff *et al.*, 1983) and *Chlamydomonas reinhardtii* (Youngblom *et al.*, 1984). Only amino acids that differ from those of the *H. capsulatum* sequence are indicated. The sequences underlined near amino acid residues 60 and 140 resemble a GTP-binding domain and a phosphate binding pocket, respectively.

Fig. 8. Localization of tubulin genes on *H. capsulatum* chromosome-sized molecules. Chromosome-sized DNAs were fractionated on low-percentage agarose gels by field inversion gel electrophoresis. The ethidium bromide stained gel shown in (a) permitted direct comparison of *H. capsulatum* chromosome-sized bands with chromosomes of known size from *Saccharomyces cerevisiae* (*Sac. c.*) and *Schizosaccharomyces pombe* (*Sch. p.*). Chromosomal DNAs from the Downs (b) and 217B (c) strains of *H. capsulatum* were blotted onto nitrocellulose filters and hybridized with the alpha (*TUB1*) and beta (*TUB2*) tubulin probes (see Fig. 1). The alpha and beta tubulin genes hybridized to different chromosome-sized molecules, and therefore appear to be unlinked in both strains of *H. capsulatum*.

Southern blot analysis with the cloned tubulin genes revealed that the alpha and beta genes are located on different chromosomes in both *H. capsulatum* strains. The *TUB1* probe hybridized to the lowermost chromosome-sized band in the *H. capsulatum* Downs strain, while *TUB2* clearly hybridized to a different chromosome-sized molecule (Fig. 8b). The organization of *TUB1* and *TUB2* on chromosomes in *H. capsulatum* 217B is less obvious, however, since only two chromosome-sized molecules were resolved by field inversion in this strain, and since *TUB1* and *TUB2* hybridized to a common band. This result most likely represents hybridization of the tubulin probes to different but comigrating chromosomes. Close analysis of the blots in Fig. 8(c) reveals that the *TUB1* probe hybridized to the uppermost portion of this band (3.5 cm from the origin), while *TUB2* hybridized to the lower portion (4.0 cm from the origin). Since both hybridizations were performed sequentially on the same filter, one may conclude that the alpha and beta tubulin genes are unlinked in the *H. capsulatum* 217B strain, as they are in the Downs strain. These findings are consistent with observations in *Chlamydomonas* (Brunke *et al.*, 1984), and *Physarum* (Schedl *et al.*, 1984).

TUB1

ATGTGATTGCAGAAGCTTGCTGCCCAGCCAACCCGTCGTTCCCCGCTAGTAGAGATCGTCCGCTGCAGAGTGTACTGCACTCTGGCTCCGTTA
 CGGGATACAATGCCATACAGAGTACACCACGTTACGCACCTCTTCCCAACAAGACGAACCTGAGGTCTATTATTCTTGACCAGCTGTTCCAT
 CTCATCCCATTCTGCTTACATTAGTTAGCTCTCAACACTAACTTGTGGTAATTGCCCTATCTA*TA*CTGCTCTAACCGTCATTCTACTTTCTT
 TGTGTGAGATAGGTTTCGGCTCTAACCAGAAAGTGCAGCAGCTCCAGGTTTTGCTTGTGTCGAAGCTTCGCGAACTCCCCATTCTTACCGTTGGG¹
 CTCATCTCTCCACTCAATCCTCATCACCTAAACGTTACTCGCAAAGCCTCCACAACCATCCACC ATG AGA GAG GTA GTT....

TUB2

TCACGTGGTGTTTCAGACAGCGACGTTAGTTCAACTGAGACTAGTGCAACAACACAGAGCCCCGTCCTCAACCTGGTTTGTCTCTCTCTGTTCT
 TCTGTTTCTACCGCTTTCTAATCCATCCACCACACACCACCACCTCCAACCACTACCACTACGAGATACATCTCCCATCTCGCTGCTTGACA
 TTCTGATATTGTCTCTCAGCTCTTTTCATTCTCCGTATCTA*CTA*CTCCACAACCAACA ATG CGT GAA ATC

Fig. 9. Potential regulatory elements are evident in the 5' flanking regions of *TUB1* and *TUB2*. A TATA-like element (asterisks) is present 194 nucleotides upstream of the *TUB1* initiation codon, and 20 nucleotides upstream of the *TUB2* initiator. Also upstream of *TUB1* and *TUB2* is a 7 bp element (brackets) which occurs in multiple copies both intermittently and in overlapping arrangements. These repeated heptanucleotides may be involved in regulating tubulin gene expression during *H. capsulatum* morphogenesis.

Potential regulatory elements which control the coordinate expression of unlinked tubulin genes in H. capsulatum

The arrangement of alpha and beta tubulin genes on different chromosomes in *H. capsulatum* raises the question of how tubulin gene expression is controlled during differentiation. The analysis of tubulin-specific mRNAs during *H. capsulatum* morphogenesis reveals that alpha and beta tubulin gene expression is induced coordinately in response to temperature shift (Harris *et al.*, 1989). Coordinate gene regulation in this fungus may imply the presence of common regulatory features which direct the synchronous response. Intermittent and overlapping stretches of repetitive elements can be noted in the upstream region of the *H. capsulatum TUB2* gene, which are shared in the 5' region of the *TUB1* gene. The putative regulatory element upstream of *TUB2* (CCACCAC) is repeated six times with few nucleotide substitutions (Fig. 9). A similar 7 bp element is repeated five times in the upstream regulatory region of the *TUB1* gene. The nucleotide sequence of the *TUB1* element is variably divergent, however, at the third, fifth and sixth base positions (Fig. 9). The tight clustering and frequent overlapping of these putative regulatory sequences in the 5' flanking regions of the *TUB1* and *TUB2* genes of *H. capsulatum* is reminiscent of the promoter region associated with the coordinately-regulated tubulin genes of *Chlamydomonas reinhardtii* (Brunke *et al.*, 1984).

Other elements with putative regulatory significance in *H. capsulatum* include a TATA-like element which is located 194 bp upstream of the *TUB1* initiation methionine, and 20 bp upstream of the *TUB2* initiator.

Concluding remarks

The environmentally-controlled interconversion of *H. capsulatum* yeast and mycelial forms provides an attractive system for studying eukaryotic cell differentiation and modes of gene expression. As a first step toward investigating the role of microtubules during the morphological differentiation of *H. capsulatum*, the alpha (*TUB1*) and beta (*TUB2*) tubulin

genes have been cloned and characterized. The finding that conserved and overlapping sequences are common in the upstream regions of the single alpha and beta tubulin genes of *H. capsulatum* suggests a potential mechanism for coordinate gene expression. Future investigations will focus on elucidating the regulatory mechanisms that underlie specific programs of tubulin gene expression during the *H. capsulatum* morphogenetic process.

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