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.

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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* DH5α 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% horizontal agarose gels in 100 mm-Tris/borate and transferred to nitrocellulose (Schleicher & Schuell) by the method of Southern (1975). Hybridization mixtures contained 1 × 10⁶ c.p.m. ml⁻¹ of [α-³²P]CTP-labelled tubulin probes (Rigby et al., 1977) in 50% (v/v) formamide, 5 × Denhardt's solution, 5 × SSC, and 25 mm-sodium phosphate, pH 6.8, with 200 μg salmon sperm DNA ml⁻¹ at 42 °C [1 × Denhardt's solution is 0.02% bovine serum albumin, 0.02% Ficoll, 0.02% polyvinylpyrrolidone; 1 × SSC is 0.15 m-NaCl, 0.015 m-trisodium citrate]. Low-stringency washes were performed in 2 × SSC, 0.1%, SDS at room temperature, and high-stringency washes in 0.2 × 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 MboI digest of 217B DNA. The library, constructed in the bacteriophage vector EMBL3, contained 2 × 10⁵ recombinants with an average insert size of 19 kb. A total of 10000 independent bacteriophage clones were screened in situ with [α-³²P]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 & Neuwald (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*...
Single α and β tubulin genes in Histoplasma

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 BamHI 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 BamHI 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, BamHI; E, EcoRI; Hd, HindIII; S, SalI; St, SstI; X, XhoI.

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 protozoon *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.
Single α and β tubulin genes in Histoplasma

**TUB 1**

![Diagram of TUB 1 gene structure]

**TUB 2**

![Diagram of TUB 2 gene structure]

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* (Ohrbach 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
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.
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A 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 HSLGGGTGAGMGTLL sequence spanning amino acids 138–152 (Fig. 7) resembling a phosphate-binding pocket which is also present in other tubulins and nucleotide-binding proteins (Krauhs 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 (Krauhs 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 carboxy 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 carboxy 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.
### 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.

<table>
<thead>
<tr>
<th>Histoplasma capsulatum</th>
<th>Saccharomyces cerevisiae</th>
<th>Schizosaccharomyces pombe</th>
<th>Chlamydomonas reinhardtii</th>
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<td>I I C G A S K</td>
<td>I VN V GA G</td>
<td>I IN I V GA E</td>
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<tr>
<td>G YLTD EKEMDP D CHPF H - - - TFFSETQGQTETPFDYTECDLEPNUV</td>
<td>N EDGLSKPEGGEFCFS - N Y T A V I</td>
<td>FPTENETYHEHNSNLYHDFGF</td>
<td>- QMPST TGGGODAP R -</td>
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<tr>
<td>DEVRTEPPRBAFLPYECQMTGEDKSMYARGHTVQGKHMDQVLOK</td>
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<tr>
<td>VRRUADNCGLDQFLVFHPCCGQQQSGOFALIHERLEBVQTGKKTL</td>
<td>I KL Q D FT LT L L S L E AE S</td>
<td>I N T L T L L H M E BN</td>
<td>I KL T NAV T L L L S</td>
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<tr>
<td>P V S V V A D N T T C</td>
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<tr>
<td>LVPYPFRPMPLASTSPVUASAKRASHESHSNNNTVIQCPFPHMN</td>
<td>V L K F E S H A G</td>
<td>V I A F Q I W O Y</td>
<td>H S A I E Q Q L A I W A A A S M</td>
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<tr>
<td>V K E C P R N G E Y M A T C L L Y R G O D UVFVDUVNNAVATETKRVTFQVFDOL</td>
<td>D TO QRAVEOVH K L WC</td>
<td>T R I Q V T S I S R I WC</td>
<td>W C H K H S V I I WC</td>
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<td>K F D L T E S K RA P Y H Y V G H E E G E F</td>
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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.
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).
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 reinhardii* (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 \textit{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 \textit{H. capsulatum} morphogenetic process.

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**REFERENCES**


