Expression of α - and β -Tubulin Genes during Dimorphic-Phase Transitions of *Histoplasma capsulatum*

GRACE SPATAFORA HARRIS,¹* ELIZABETH J. KEATH,² AND JUDITH MEDOFF¹

Department of Biology, St. Louis University, St. Louis, Missouri 63103,¹ and Division of Infectious Diseases, Washington University School of Medicine, St. Louis, Missouri 63110²

Received 22 November 1988/Accepted 14 February 1989

Recent investigations have confirmed the presence of one α -tubulin gene (*TUB1*) and one β -tubulin gene (*TUB2*) in the dimorphic fungus *Histoplasma capsulatum*. In the present study, Northern blot (RNA blot) analyses revealed multiple α -tubulin transcripts and a single β -tubulin transcript in the yeast and mycelial phases of the high-virulence 217B strain and low-virulence Downs strain. S1 nuclease protection assays demonstrated one initiation start site and two major stop sites for the *TUB1* transcripts, suggesting that variations in 3' processing generate the α -tubulin messages of 2.5 and 2.0 kilobases. Dot blot hybridization experiments indicated that tubulin gene expression is developmentally regulated during the dimorphic phase transitions. α - and β -tubulin mRNAs increased six- to eightfold during the yeast-to-mycelium conversion and decreased two- to threefold during the reverse transition. These changes in tubulin mRNA content coincided with major morphological events associated with *H. capsulatum* development. Western blots (immunoblots) of *H. capsulatum* yeast-specific proteins resolved by two-dimensional gel electrophoresis demonstrated a single α - and a single β -tubulin isoform. Multiple tubulin polypeptides expressed in mycelia are probably products of posttranslational modifications.

Histoplasma capsulatum, a dimorphic fungal pathogen, grows in soil as multicellular filamentous mycelia and in the tissues of infected animals and humans as unicellular budding yeast cells. The mycelial and yeast phases may be maintained in vitro at 25 and 37°C, respectively. Conversion of mycelia to yeast cells is induced in the laboratory by shifting the temperature of incubation from 25 to 37°C. A temperature shift from 37 to 25°C will initiate the reverse transition.

Dimorphism in *H. capsulatum* involves elaborate changes in cell morphology, and elucidation of the mechanism involved may provide insight into differentiation and development in higher organisms. Changes in cell shape in *H. capsulatum* are likely to require alterations in cytoskeletal components; the arrangement of microtubules at the cell surface, for example, may direct subsequent morphological events. Previous studies in this laboratory suggest that tubulin, the major constituent protein of microtubules, plays an active role in the *H. capsulatum* differentiation process. Methyl benzimidazole-2-yl carbamate (MBC), an agent which binds specifically to fungal tubulins, inhibits the dimorphic phase transitions (J. Medoff and G. Medoff, Abstr. Annu. Meet. Am. Soc. Cell Biol. 1982, 95:44a). Furthermore, binding assays with [¹⁴C]MBC indicate that the level of intracellular tubulin is fourfold higher in *H. capsulatum* mycelia than in yeast cells (J. Medoff, unpublished observations).

To further investigate the role of microtubules during the morphological differentiation of *H. capsulatum* and to elucidate the possible regulatory mechanisms involved in *H. capsulatum* development, tubulin gene transcription was examined during the dimorphic phase transitions. Previous work has demonstrated the presence of a single α - and a single β -tubulin gene in the *H. capsulatum* genome (G. S.

Harris, E. J. Keath, and J. Medoff, J. Gen. Microbiol., in press). Hybridization probes from the cloned α -tubulin gene (*TUB1*) and β -tubulin gene (*TUB2*) were used in the present study to examine tubulin-specific expression during morphogenesis. The results presented here reveal that tubulin mRNA production is developmentally regulated during the dimorphic phase transitions. Overall, tubulin mRNA levels increased as yeast cells transformed to mycelia and decreased as mycelia converted to yeast cells. These changes in tubulin mRNA content may contribute to subsequent morphological events.

(The material presented here was submitted by G. S. Harris as part of a thesis in partial fulfillment of the requirements for the Ph.D. degree from St. Louis University, St. Louis, Mo., 1988.)

MATERIALS AND METHODS

Strains and culture conditions. The hypovirulent Downs strain and the high-virulence 217B strain, both mating type minus, of *H. capsulatum* were obtained from our permanent laboratory culture collection and used as sources of RNA and protein. The organisms were grown to the late logarithmic phase in GYE broth, containing 2% glucose and 1% yeast extract; yeast and mycelial cultures were grown on shaking platforms at 37 and 25°C, respectively. Growth curves for *H. capsulatum* yeast cells at 37°C and mycelia at 25°C are comparable, reaching the mid-logarithmic phase in 3 days and the stationary phase in approximately 5 days (17).

Monoclonal antibodies. YOL1/34, a monoclonal antibody raised against tubulin from *Saccharomyces cerevisiae* and specific for the α subunit was a gift from J. V. Kilmartin, Medical Research Council Laboratory of Molecular Biology, Cambridge, England. KMx-1, a β -tubulin-specific monoclonal antibody raised in mice against purified tubulin from *Physarum polycephalum* was the generous gift of Christopher Birkett, University of Kent, Canterbury, England.

Conditions for yeast and mycelial transformations. GYE broth (500 ml) was inoculated with *H. capsulatum* mycelia

* Corresponding author.

† Present address: Department of Biology, Washington University, St. Louis, MO 63130.

and grown to the mid-logarithmic phase. Cells were then subcultured 1:10 into each of six individual flasks containing 500 ml of GYE medium. The culture flasks were incubated at 25°C for 24 h and then placed at 37°C for conversion to the yeast phase. Individual cultures were harvested at 1, 3, 5, 7, 9, and 11 days following the temperature shift and used for RNA or protein isolation. The stage of transformation was confirmed by both morphological and respiratory activity criteria, as previously defined by Maresca et al. (16). Morphological changes were assessed microscopically with a Zeiss light microscope, and respiration was monitored polarographically with a KIC-oxygraph equipped with a Clark-type oxygen electrode (Gilson Medical Electronics, Inc.).

H. capsulatum yeast cultures were also seeded from a common starter culture; they were then incubated at 37°C for 24 h and shifted to 25°C to initiate the transition to mycelia. Cells were harvested at 1, 3, 5, and 7 days following the temperature shift. Morphological evidence was used to standardize the rate of yeast-to-mycelium conversion.

Isolation and purification of RNA. Yeast, mycelial, and transforming cultures of *H. capsulatum* were washed and suspended in UNET buffer, containing 8 M urea, 0.15 M NaCl, 1 mM EDTA, and 0.1 M Tris hydrochloride (pH 7.5). Buffer-saturated phenol was added to 20% immediately prior to disruption in a Braun homogenizer. Cells were disrupted for 1 to 2 min in the presence of glass beads, with CO₂ cooling. Sodium dodecyl sulfate was then added to 2%, and the slurry was extracted repeatedly with phenol and phenol-chloroform-isoamyl alcohol. RNA was collected by ethanol precipitation. Polyadenylated mRNAs were isolated by oligo(dT)-cellulose chromatography by the method of Ono et al. (20), as modified by Shen-Ong et al. (24).

Northern blot analysis. Equal quantities of poly(A)⁺-selected RNAs from 217B and Downs yeast cells and mycelia were serially diluted and fractionated on horizontal 0.8% agarose gels containing 8% formaldehyde. Quantitation of RNA was done spectrophotometrically at 260 nm. Following electrophoresis, RNAs were transferred to Nytran nylon membranes (Schleicher & Schuell, Inc.) by Northern blotting (RNA blotting) (21). The same filter was sequentially hybridized with the nick-translated α -tubulin (*TUB1*) and β -tubulin (*TUB2*) probes isolated from *H. capsulatum* (Harris et al., in press). Aqueous hybridizations were carried out by the protocol of Church and Gilbert (4). Following overnight hybridizations at 65°C, wet filters were wrapped in Saran Wrap and exposed to X-ray film (XAR-5; Eastman Kodak Co.) at -70°C for 6 to 12 h.

S1 nuclease analysis. M13 templates primed with the 17-nucleotide universal primer (Amersham Corp.) were used to generate uniformly labeled S1 probes as described by Keath et al. (12). A 1.5-kilobase *Bam*HI-*Hinc*II fragment was used for mapping the 5' end of the *H. capsulatum TUB1* transcripts, and a 1.6-kilobase *Pst*II-*Eco*RI fragment was used for mapping the 3' ends of these mRNAs (see Fig. 5). Each probe was heat denatured at 95°C for 3 min and hybridized at 45°C to 2 μ g of *H. capsulatum* poly(A)⁺-selected RNA from strain 217B mycelia. DNA-RNA hybrids were digested at 37°C for 1 h with 50 U of S1 nuclease (Boehringer Mannheim Biochemicals), and the digestion products were resolved on 6% polyacrylamide-urea sequencing gels. End-labeled *Hinf*I-restricted pBR322 markers were electrophoresed in parallel.

Dot blot analyses of tubulin gene expression. The abundance of tubulin-specific message from *H. capsulatum* yeast, mycelial, and transforming cultures was compared by dot blot hybridization (11, 25). Initial quantitations of RNA were

done by determining A_{260} . Equivalent amounts of RNA were then denatured at 60°C for 15 min in 6 $\times$ SSC (1 $\times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate)-7.4% formaldehyde, serially diluted, and dot blotted onto nitrocellulose filters. The blots were baked at 80°C and soaked in 5 $\times$ SSC immediately prior to hybridization.

The total message bound for each time point during the phase transitions was assessed by counting [α -³²P]CTP on isolated nitrocellulose filter dots, using a radiolabeled oligo(dT) probe (10). Duplicate dot blots were hybridized with the α -tubulin (*TUB1*) or β -tubulin (*TUB2*) probe from *H. capsulatum*, and mRNA was quantitated by counting of [α -³²P]CTP on filter dots. α - and β -tubulin gene expression was expressed as a ratio of counts per minute of tubulin mRNA, to counts per minute of total mRNA.

Isolation of whole-cell protein. Cells from *H. capsulatum* yeast and mycelial phases were harvested by filtration and washed extensively, and proteins were isolated by published protocols (23).

Two-dimensional isoelectric focusing sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Isoelectric focusing gels were prepared by the method of O'Farrell (19) except that 3-(3-cholamidopropyl-dimethylammonio)-1-propane sulfonate, a dipolar ionic detergent for membrane protein solubilization, was substituted for Nonidet P-40. A pH profile (pHs 4 to 6) was generated by mixing ampholytes (LKB Instruments, Inc.) in the following proportions: 1 part 3-10, 1 part 6-8, 2 parts 5-7, and 1 part 4-6. Electrophoretic conditions were as previously described by Shearer et al. (23).

After isoelectric focusing, tube gels were equilibrated in sodium dodecyl sulfate sample buffer (14) and applied to 12.5% polyacrylamide slab gels cross-linked with 0.33% bisacrylamide. Electrophoresis proceeded at 200 V for approximately 3 h.

Western blot analysis. Tubulin-specific polypeptides from *H. capsulatum* yeast cells and mycelia, resolved by two-dimensional gel electrophoresis, were identified by Western blot (immunoblot) analysis as described by Davis et al. (5). Monoclonal antibodies YOL1/34 and KMX-1 were diluted 1:100 prior to use, and peroxidase-conjugated rabbit anti-mouse or goat anti-rat sera (Sigma Chemical Co., St. Louis, Mo.) were diluted 1:10,000.

RESULTS

Morphological analysis of the phase transitions. The yeast-to-mycelium and mycelium-to-yeast phase transitions in *H. capsulatum* were characterized by phase-contrast microscopy. Yeast cultures were induced to form mycelia at 25°C, a process requiring 7 days for completion. The first morphological evidence of this phase transition occurred 24 to 36 h after the temperature shift, when yeast cells aggregated into small clumps (Fig. 1B). Within 48 h, the extension of short germ tubes from yeast cell surfaces was obvious (Fig. 1C). By 72 h, 60% of the cells in culture displayed these mycelial projections (Fig. 1D). The filamentous elements then underwent further elongation and branching, forming typical septate hyphae by day 7 of the transition (Fig. 1E).

Unlike the yeast-to-mycelium conversion, the mycelium-to-yeast transition is a prolonged process which requires approximately 2 weeks at 37°C for completion. Three distinct stages, characterized by discrete physiological and biochemical patterns, have been delineated during this morphological transition (15). Shortly after the temperature shift, entangled mycelial clumps begin to break down into

FIG. 1. *H. capsulatum* dimorphic phase transitions. The complete transformation of yeast cells (Y) to mycelia (M) required 7 days at 25°C. Within 24 to 36 h after the temperature shift, yeast cells aggregated and extended short germ tubes (B). After 72 h, 60% of the cells exhibited these mycelial projections (C). The filamentous elements continued to elongate and branch (D), forming septate hyphae by day 7 of the transition (90% transformed) (E). The reverse transition, initiated by incubating mycelia at 37°C, required 14 days for completion. Large entangled mycelial clumps broke down into smaller masses within 24 h of the temperature shift (G). After day 6 of the transition, small buds appeared on hyphal surfaces (H). Free budding yeast cells were evident on day 11 after the temperature shift (70% transformed) (I) but became most abundant on day 14 of the phase transition (90% transformed) (J).

smaller masses, and the tips of mycelial filaments become constricted (Fig. 1G to H). As morphogenesis proceeds, hyphal tips swell and separate, forming masses of budding yeast cells (Fig. 1I). Approximately 14 days after the temperature shift, the cultures are 90% transformed (Fig. 1J).

Northern blot analysis of tubulin mRNAs in *H. capsulatum* yeast cells and mycelia. To characterize α - and β -tubulin transcripts in *H. capsulatum* yeast cells and mycelia, Northern blot transfer experiments were conducted with poly(A)⁺-selected RNAs from the virulent 217B strain and hypovirulent Downs strain. The *H. capsulatum* *TUB1* α -tubulin probe hybridized to two mRNA species of 2,500 and 2,000 nucleotides in 217B yeast cells and three species of 2,500, 2,200, and 2,000 nucleotides in 217B mycelia (Fig.

FIG. 2. Northern blot analyses of *H. capsulatum* tubulin gene transcripts. Polyadenylated RNAs from yeast cells (Y) and mycelia (M) were resolved on 0.8% denaturing agarose gels and transferred to nylon membranes. RNAs from the high-virulence 217B strain were hybridized with the *TUB1* (α -tubulin) (A) and *TUB2* (β -tubulin) (B) probes previously described. (C and D) RNAs from the hypovirulent Downs strain were hybridized with the *TUB1* and *TUB2* probes, respectively. The numbers in the margins indicate the positions and lengths of the tubulin-specific RNAs. For both strains, α - and β -tubulin mRNAs were more abundantly expressed in mycelia than in yeast cells. Kb, Kilobases.

2A). In contrast, the Downs strain expressed only two α -tubulin transcripts in both morphological phases (Fig. 2C). The sizes of these mRNAs in the Downs strain were comparable to the largest and smallest of the transcripts expressed in 217B. The 2,200-nucleotide intermediate species, which was unique to the mycelial phase of 217B, was never expressed in Downs. A single 1,900-nucleotide β -tubulin species expressed in 217B yeast cells and mycelia was also seen in the yeast and mycelial phases of the Downs strain (Fig. 2B and D).

Tubulin gene expression during dimorphic phase transitions. Densitometric scanning of the autoradiographs shown in Fig. 2 indicated that α - and β -tubulin mRNAs were more abundantly expressed in *H. capsulatum* mycelia than in

FIG. 3. Quantitation of tubulin-specific RNA levels by dot blot analysis. Polyadenylated RNA samples isolated at the designated times during the strain 217B yeast-to-mycelium (Y→M) and mycelium-to-yeast (M→Y) phase transitions were serially diluted, denatured with formaldehyde, and blotted onto a series of replicate nitrocellulose filters. An oligo(dT) probe was initially used to quantitate membrane-bound tubulin message. Duplicate blots were hybridized with the *TUB1* and *TUB2* probes described elsewhere (Harris et al., in press). Radioactive dots were excised, and counts per minute were determined by liquid scintillation counting. The dot blots represent relative changes in the RNAs for α - and β -tubulin and not the absolute abundance of these messages. Dot blot hybridization experiments were similarly performed for the hypovirulent Downs strain (data not shown). myc, Mycelia; d, days.

yeast cells. Dot blot hybridization experiments using poly(A)⁺-selected RNAs from yeast, mycelial, and transforming cultures confirmed that tubulin-specific mRNA production is developmentally regulated during the dimorphic phase transitions (Fig. 3).

Due to the uncertainty in the amount of total mRNA applied to or retained by the nitrocellulose membrane, filter-bound mRNA was initially quantitated by hybridization with a radiolabeled oligo(dT) probe which binds specifically to the polyadenylated tails of eucaryotic mRNA. This quantitation provided a basis for comparing the abundance of tubulin-specific messages. Hybridization probes from the cloned α -tubulin gene (*TUB1*) and β -tubulin gene (*TUB2*) were then utilized to examine tubulin-specific gene expression during *H. capsulatum* morphogenesis. Overall, tubulin mRNA levels increased as yeast cells transformed to mycelia and decreased as mycelia converted to yeast cells (Fig. 3).

For the yeast-to-mycelium conversion, α -tubulin messages were eightfold more abundant in mycelia than in yeast

FIG. 4. Changes in α - and β -tubulin mRNA quantities during *H. capsulatum* development, determined by relative quantitation of dot blot hybridizations. The [α -³²P]CTP signals shown in Fig. 3 were quantitated in a liquid scintillation counter, and tubulin gene expression for each time point was measured as a ratio of counts per minute of tubulin-specific message to counts per minute of total message bound. Each shaded bar represents four datum points generated from two separate experiments. Standard errors are indicated. Overall, α - and β -tubulin gene expression increased as yeast cells converted to mycelia and decreased as mycelia transformed to yeast cells. For both phase transitions, tubulin mRNAs were more abundantly expressed in *H. capsulatum* mycelia than in yeast cells (see insets). Transition endpoints were defined when cultures were 90% transformed.

cells, while β -tubulin messages were present in nearly threefold greater quantities in the mycelial phase (Fig. 4). The increase in α - and β -tubulin messages was established early during this phase transition, occurring within 24 h after the temperature shift. For the reverse phase transition, α - and β -tubulin messages were between two- and threefold more abundant in the mycelial phase. Bursts of tubulin-specific message occurred 1 and 7 days after the temperature shift from 25 to 37°C. These results, coupled with the greater abundance of tubulin protein in *H. capsulatum* mycelia than in yeast cells (J. Medoff, unpublished observations), lend further support to the notion that tubulin gene expression may be developmentally modulated in this fungal pathogen.

S1 nuclease analysis of *TUB1* transcripts. To investigate the source of multiple tubulin transcripts generated by a single

α -tubulin gene, S1 nuclease protection assays were performed using the uniformly labeled 5' and 3' *TUB1* probes shown in Fig. 5A. DNA sequence analysis of the 5' end of the *TUB1* gene (Harris et al., in press) predicts the presence of four protected fragments (270, 75, 39, and 25 base pairs) corresponding to the exons present in this region. All four fragments were detected in the S1 analysis, as well as a fifth 150-base-pair fragment corresponding to the 5' end of the *TUB1* transcript (Fig. 5B). The initiation start site for *TUB1* maps to a region within 35 nucleotides of a putative TATAA element (Harris et al., in press).

Using a 1.6-kilobase *Pst*I-*Eco*RI fragment for 3' mapping, we observed two major protected fragments of 360 and 800 base pairs (Fig. 5B). The 3' end of the *TUB1* message maps 330 and 770 nucleotides downstream from the stop codon. Since the α -tubulin transcripts share the same 5' end, it is likely that variations in transcription at the 3' end of the *TUB1* gene generated the 2,500- and 2,000-nucleotide α -tubulin mRNAs expressed in *H. capsulatum* yeast cells and mycelia.

Resolution of tubulin polypeptides on two-dimensional polyacrylamide gels. To examine the expression of tubulin-specific isotypes in the yeast and mycelial phases of *H. capsulatum*, whole-cell protein from both developmental phases was isolated and resolved by two-dimensional isoelectric focusing sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Immunoblots reacted with YOL1/34 or KMX-1 monoclonal antitubulin antibodies revealed two major polypeptides, designated α_1 and β_1 in both the yeast and mycelial phases of the fungus (Fig. 6). Additional tubulin isoforms, α_2 and β_2 , were seen only in the mycelial phase.

α_1 (pI 5.1) and β_1 (pI 5.2) have estimated molecular masses of 59,500 and 58,000 daltons, respectively. α_2 (pI 5.0) and β_2 (pI 5.1) are slightly more acidic than the constitutively expressed α_1 and β_1 subunits but migrated with molecular masses which appeared to be unaltered.

DISCUSSION

Multiple α -tubulin transcripts. Northern blot analyses indicated that *H. capsulatum* 217B mycelia expressed three α -tubulin mRNAs of 2,500, 2,200, and 2,000 nucleotides, while 217B yeast cells expressed only the 2,500- and 2,000-nucleotide species (Fig. 2A). The 2,500- and 2,000-nucleotide transcripts were also present in the yeast and mycelial phases of *H. capsulatum* Downs (Fig. 2C).

Multiple α -tubulin transcripts could result if transcription of the *TUB1* gene originates or terminates at multiple sites. S1 nuclease protection experiments were performed to locate the 5' and 3' ends of the α -tubulin mRNAs. A single start site for *TUB1* was detected and mapped at 150 nucleotides upstream from the methionine initiation codon. S1 assays with the 3' probe, however, confirmed the presence of two major stop sites which are separated by nearly 500 nucleotides. Thus, it is likely that transcription of the *TUB1* gene from the mRNA cap site to each of the termination sites generated the 2,500- and 2,000-nucleotide α -tubulin transcripts seen on Northern blots of *H. capsulatum* yeast cells and mycelia; the predicted in vitro translation products of both isolated mRNAs could generate the same α -tubulin monomer. Recently, a single actin gene in *Schizosaccharomyces pombe* has been found to generate three transcripts differing in the lengths of their 3' ends by 610 nucleotides (18). One actin polypeptide is synthesized from these multiple transcripts.

Processive splicing of the primary transcript synthesized from the *TUB1* gene probably generated the intermediate

2,200-nucleotide species observed in 217B mycelia. This message presumably represents a precursor from which some, but not all, of the introns have been removed. In contrast, this intermediate transcript was absent in the low-virulence Downs strain. It is unclear why the *TUB1* gene generates multiple transcripts differing in their 3' untranslated regions in both strains, when each message gives rise to an identical protein product. Further identification of the DNA sequences complementary to these transcripts should resolve this issue.

***H. capsulatum* tubulin isotypes may be posttranslationally modified.** Northern blot analysis of RNAs isolated during *H. capsulatum* differentiation indicated that the α -tubulin (*TUB1*) and β -tubulin (*TUB2*) transcripts were expressed constitutively. Translation of these mRNAs was examined by Western blot analysis. Single α - and β -tubulins were observed among proteins isolated from 217B yeast cells (Fig. 6). Additional tubulin isoforms, α_2 and β_2 , were seen only in the mycelial phase. Similar results were obtained with Western blots of mycelial and yeast proteins isolated from the Downs strain. Since the four isotypes in mycelia exceed the number of known functional tubulin genes in this fungus and since the α_2 isoform was observed in Downs mycelia in the absence of the intermediate 2,200-nucleotide mRNA, α_2 and β_2 most probably represent products of posttranslational modifications. α_2 and β_2 are both slightly more acidic than their presumptive precursor subunits, α_1 and β_1 ; the observed shift in isoelectric focusing points for these polypeptides could have resulted from a phosphorylation or tyrosylation, both common posttranslational modifications of tubulin (1, 6, 7).

Expression of α - and β -tubulin mRNAs during dimorphic phase transitions. Tubulin-specific gene probes used to monitor the levels of tubulin mRNA transcripts during the *H. capsulatum* yeast-to-mycelium and mycelium-to-yeast phase transitions indicated that tubulin gene expression is developmentally regulated. A two- to threefold decrease in α - and β -tubulin mRNAs was observed during the transition from mycelia to yeast cells. α - and β -tubulin mRNAs increased six- to eightfold during the yeast-to-mycelium transition, a finding comparable with the five- to sevenfold increase in relative tubulin RNA levels observed during the *Physarum myxamoeba*-to-flagellate transformation process (9). The elevated levels of tubulin-specific message in *H. capsulatum* mycelia are also consistent with the increased abundance of mycelial tubulin observed at the protein level (data not shown). Due to overgrowth in cultures undergoing the yeast-to-mycelium phase transition, cultures containing greater than 90% mycelia were not assayed for tubulin-specific RNA content. Since it was unlikely that the RNA levels in these crowded cultures had achieved a steady state, completion of the transition was induced by subculturing in fresh medium. The resulting tubulin levels were equivalent to those observed in established mycelial cultures (data not shown).

The changes in tubulin-specific message described here occurred at times of significant morphological differentiation during the phase transitions. For example, an increase in α - and β -tubulin mRNA occurred within 24 h after the temperature shift from 37 to 25°C, immediately preceding the extension of short germ tubes from yeast cell surfaces. In the reverse transition, from 25 to 37°C, two bursts of tubulin gene expression were detected; expression was elevated 1 day after the temperature shift, immediately prior to a dormant stage, and then again 7 days after the shift from 25 to 37°C. The latter burst correlated with the emergence of

FIG. 5. S1 nuclease protection analysis of the 5' and 3' ends of *H. capsulatum* α -tubulin gene transcripts. The experiments were performed with poly(A)⁺-selected mRNA from strain 217B mycelia and either the 5' *Bam*HI-*Hinc*II or 3' *Pst*I-*Eco*RI uniformly labeled probe shown in panel A (B, *Bam*HI; E, *Eco*RI; HC, *Hinc*II; HD, *Hind*III; P, *Pst*I; X, *Xho*I). Protected fragments were separated on 6% polyacrylamide-urea sequencing gels in parallel with end-labeled standard markers generated by cleavage of pBR322 with *Hinf*I. Arrowheads indicate the positions and approximate lengths of the marker DNAs and protected fragments. Kb, Kilobases.

FIG. 6. Immunoblots of *H. capsulatum* whole-cell protein isolated from yeast cells and mycelia. Proteins were resolved by two-dimensional gel electrophoresis, electroblotted onto nitrocellulose membranes, and probed with tubulin-specific monoclonal antibodies YOL1/34 (α -tubulin specific) and KMx-1 (β -tubulin specific). Tubulin migrated as two isoforms (α_1 and β_1) in the yeast phase and as four isoforms (α_1 , α_2 , β_1 , and β_2) in the mycelial phase. D, Daltons.

cells from dormancy, and within 12 h yeast cells were seen budding from the tips of hyphal elements. These changes in tubulin gene transcription immediately preceded morphological evidence of the phase transitions and therefore may be significant to the *H. capsulatum* differentiation process. The changes in tubulin content could also have been fortuitous or have reflected an increased requirement for tubulin in the mycelial phase at the slightly lower temperature of 25°C. The isolation and analysis of *H. capsulatum* mutants blocked in the differentiation process would further define the relationship between tubulin gene expression and morphogenesis.

Regulation of tubulin gene expression. Northern blot and dot blot hybridization experiments indicated that the regulated expression of α - and β -tubulin genes during *H. capsulatum* development is controlled at the level of mRNA accumulation. The regulatory signals by which tubulin-specific messages are modulated during morphogenesis, via transcriptional and/or posttranscriptional mechanisms, are unclear. Both of these regulatory features operate during the *Physarum* cell cycle (22) and in *Chlamydomonas* flagellar regeneration (2, 13), in which changes in transcription rates and mRNA stabilities result in an accumulation of tubulin-specific mRNA.

A recent study (3) has suggested that the unpolymerized pool of tubulin monomers specifically modulates tubulin gene expression by autoregulation. In vitro disruption experiments involving the first four codons of both α - and β -tubulin genes have defined a recognition sequence required for self-regulation (8). This recognition sequence is present in *H. capsulatum* and constitutes the first four codons of the *TUB1* and *TUB2* genes.

Future studies involving the isolation of tubulin-specific mutants could facilitate investigations of the regulatory

mechanisms which govern tubulin gene expression in this fungal pathogen and the role of microtubules in *H. capsulatum* development.

ACKNOWLEDGMENTS

We are grateful for the support and encouragement of Gerald Medoff, in whose laboratory this work was conducted. We also thank Paul Steele and Eric Spitzer for helpful discussions and George Kobayashi for advice and assistance.

This research was supported by Medical Mycology Grant AI-16228-09 from the National Institutes of Health.

LITERATURE CITED

- Argarana, C. E., H. S. Barra, and R. Caputto. 1978. Release of ^{14}C tyrosine from tubulinyl- ^{14}C tyrosine by brain extract. Separation of a carboxy peptidase from tubulin tyrosine ligase. *Mol. Cell. Biochem.* 19:17-22.
- Baker, E. J., J. A. Schloss, and J. L. Rosenbaum. 1985. Rapid changes in tubulin RNA synthesis and stability induced by deflagellation in *Chlamydomonas*. *J. Cell Biol.* 99:2074-2081.
- Ben Ze'ev, A., S. R. Farmer, and S. Penman. 1979. Mechanisms of regulating tubulin synthesis in cultured mammalian cells. *Cell* 17:319-325.
- Church, G. M., and W. Gilbert. 1984. Genomic sequencing. *Proc. Natl. Acad. Sci. USA* 81:1991-1995.
- Davis, L. G., M. D. Digner, and J. F. Battey. 1986. Basic methods in molecular biology, p. 311-314. Elsevier Science Publishing, Inc., New York.
- Eipper, B. A. 1972. Rat brain microtubule protein: purification and determination of covalently bound phosphate and carbohydrate. *Proc. Natl. Acad. Sci. USA* 69:2283-2287.
- Forgue, S. T., and J. L. Dahl. 1979. Rat brain tubulin: subunit heterogeneity and phosphorylation. *J. Neurochem.* 32:1015-1025.
- Gay, D. A., T. J. Yen, J. T. Y. Lau, and D. W. Cleveland. 1987. Sequences that confer beta tubulin autoregulation through modulated mRNA stability reside within exon 1 of a beta tubulin mRNA. *Cell* 50:671-679.
- Green, L. L., and W. F. Dove. 1984. Tubulin proteins and RNA during the myxamoeba-flagellate transformation of *Physarum polycephalum*. *Mol. Cell. Biol.* 4:1706-1711.
- Harley, C. B. 1987. Hybridization of oligo(dT) to RNA on nitrocellulose. *Gene Anal. Tech.* 4:17-22.
- Kafatos, F. C., C. W. Jones, and A. Efstratiadis. 1979. Determination of nucleic acid sequence homologies and relative concentrations by dot hybridization procedure. *Nucleic Acids Res.* 7:1541-1552.
- Keath, E. J., A. Kelekar, and M. D. Cole. 1984. Transcriptional activation of the translocated c-myc oncogene in mouse plasmacytomas: similar RNA levels in tumor and proliferating normal cells. *Cell* 37:521-528.
- Keller, L. R., J. A. Schloss, C. D. Silflow, and J. L. Rosenbaum. 1984. Transcription of alpha and beta tubulin genes *in vitro* in isolated *Chlamydomonas reinhardtii* nuclei. *J. Cell Biol.* 98:1138-1143.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* 227:680-685.
- Maresca, B., A. M. Lambowitz, V. B. Kumar, G. A. Grant, G. S. Kobayashi, and G. Medoff. 1981. Role of cysteine in regulating morphogenesis and mitochondrial activity in the dimorphic fungus *Histoplasma capsulatum*. *Proc. Natl. Acad. Sci. USA* 78:4596-4600.
- Maresca, B., G. Medoff, D. Schlessinger, G. S. Kobayashi, and J. Medoff. 1977. Regulation of dimorphism in the pathogenic fungus *Histoplasma capsulatum*. *Nature (London)* 266:447-448.
- Medoff, J., E. Jacobson, and G. Medoff. 1981. Regulation of dimorphism in *Histoplasma capsulatum* by cyclic adenosine 3'-5'-monophosphate. *J. Bacteriol.* 145:1452-1455.
- Mertins, P., and D. Gallwitz. 1987. A single intronless actin gene in the fission yeast *Schizosaccharomyces pombe*: nucleotide

- sequence and transcripts formed in homologous and heterologous yeast. *Nucleic Acids Res.* **15**:7369–7379.
19. O'Farrell, P. H. 1975. High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* **250**:4007–4021.
 20. Ono, M., M. D. Cole, A. T. White, and R. C. C. Huang. 1980. Sequence organization of cloned intracisternal A particle genes. *Cell* **21**:465–473.
 21. Rave, N., R. Crkvenjakov, and H. Boedtker. 1979. Identification of procollagen mRNAs transferred to diazobenzyloxymethyl paper from formaldehyde agarose gels. *Nucleic Acids Res.* **6**:3559–3567.
 22. Schedl, T., T. G. Burland, K. Gull, and W. F. Dove. 1984. Cell cycle regulation of tubulin RNA level, tubulin protein synthesis, and assembly of microtubules in *Physarum*. *J. Cell Biol.* **99**: 155–165.
 23. Shearer, G., C. H. Birge, P. D. Yuckenberg, G. S. Kobayashi, and G. Medoff. 1987. Heat-shock proteins induced during the mycelial to yeast transitions of strains of *Histoplasma capsulatum*. *J. Gen. Microbiol.* **133**:3300–3308.
 24. Shen-Ong, G. L. C., E. J. Keath, S. P. Piccoli, and M. D. Cole. 1982. Novel myc oncogene RNA from abortive immunoglobulin gene recombination in mouse plasmacytomas. *Cell* **31**:443–452.
 25. White, B. A., and F. C. Bancroft. 1982. Cytoplasmic dot hybridization. *J. Biol. Chem.* **257**:8569–8572.