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# The Intron-Containing *hsp82* Gene of the Dimorphic Pathogenic Fungus *Histoplasma capsulatum* Is Properly Spliced in Severe Heat Shock Conditions

GABRIELLA MINCHIOTTI,[1] SILVANA GARGANO,[1] AND BRUNO MARESCA[1,2]*

*International Institute of Genetics and Biophysics, Consiglio Nazionale delle Ricerche, Via Marconi, 12, 80125 Naples, Italy,[1] and Department of Internal Medicine, Division of Infectious Diseases, Washington University School of Medicine, St. Louis, Missouri 63110[2]*

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**We have isolated and characterized a heat-inducible gene, *hsp82*, from the dimorphic pathogenic fungus *Histoplasma capsulatum*, which is a filamentous mold at 25°C and a unicellular yeast at 37°C. This gene, which has a high degree of homology with other members of the *hsp82* gene family, is split into three exons and two introns of 122 and 86 nucleotides, respectively. Contrary to what has been demonstrated in *Drosophila melanogaster*, *Saccharomyces cerevisiae*, and other organisms, *hsp82* mRNA in *H. capsulatum* is properly spliced during the severe heat conditions of 37 to 40°C in the temperature-sensitive Downs strain. Splicing accuracy was also observed at 42°C in the temperature-tolerant G222B strain, which showed no evidence of accumulation of primary transcripts. Furthermore, the intron containing the β-tubulin gene is also properly spliced at the upper temperature range, suggesting that the lack of a block in splicing may be a general phenomenon in this organism.**

*Histoplasma capsulatum*, a common respiratory pathogen of humans, is a dimorphic fungus that exists in a multicellular filamentous state in nature and as unicellular budding yeasts in tissue (1). The transition between the multicellular and unicellular phases can be reversibly induced by shifting the temperature from 25°C (mycelial phase) to 37°C (yeast phase). The mycelial-to-yeast conversion is of particular interest since it is triggered by an increase in the temperature of incubation and conversion to the yeast morphology is necessary for virulence. In fact, an important pathological feature of disease is that only yeasts are found in infected tissues (25, 34). Therefore, the unique ability of dimorphic fungal pathogens to change shape in order to colonize, adapt to, and survive in host tissues is a process that parallels and may be intimately involved with this developmentally regulated morphological process (24).

Since the mycelial (saprobic)-to-yeast (parasitic) transition is induced by heat, it was suggested that the early events of the phase transition were part of a heat shock response which is followed by cell adaptation to higher temperatures (19). This notion was supported by the report of Caruso et al. (7), who cloned the *hsp70* gene from *H. capsulatum* and studied its expression in strains that differed in virulence and sensitivity to heat, and it was confirmed by the report of Shearer et al. (35), who observed the synthesis of new proteins soon after the temperature shift from 25°C to between 34 and 41°C.

To understand the effects of severe heat shock on mRNA precursor processing during the mycelium-to-yeast transition in this fungus, we cloned a heat shock 82 (*hsp82*) gene and investigated its expression in two strains that differ in sensitivity to temperature and virulence.

In *Drosophila melanogaster* cells as well as in *Saccharomyces cerevisiae*, *hsp83* mRNA processing is inhibited at the upper temperature range of the heat shock response (38, 40). In *Drosophila* cells, the block in RNA processing was not specific to *hsp83* mRNA precursor since splicing of recombinant heat-inducible alcohol dehydrogenase gene (*Adh*) transcripts was shown to be equally sensitive to heat (38). It was proposed that the block in splicing was due to the thermolability of the spliceosome and that this would represent a potential control mechanism to interrupt transcription of all intron-containing genes, while the majority of heat shock gene transcripts would escape it because of the absence of introns in their coding sequences (38, 41).

In contrast to *D. melanogaster*, which must adapt to a lower temperature in order to ensure survival (38), survival of *H. capsulatum* as a pathogen requires adaptation to higher temperatures (24). We present evidence that neither in the temperature-sensitive Downs strain, which is avirulent for mice, nor in the temperature-tolerant G222B strain, which is virulent for mice, does a block occur in *hsp82* splicing at the temperature corresponding to a severe heat shock in *H. capsulatum*. Such a block does not seem to be specific for the *hsp82* locus since the β-tubulin gene is also properly spliced under the same experimental conditions.

## MATERIALS AND METHODS

**Organisms and preparation of cells for isolation of nucleic acids.** Growth conditions and isolation of DNA from the temperature-sensitive, mouse-avirulent Downs (ATCC 38904; American Type Culture Collection, Rockville, Md.) and the temperature-tolerant, mouse-virulent G222B (ATCC 26034) strains of *H. capsulatum* have been described previously (7). Total RNA was extracted from the mycelial phase of Downs grown at 25°C and mycelia that had been incubated at a temperature of 34°C for 1, 3, 6, or 24 h after the temperature shift. Total RNA was also isolated from mycelia of strain G222B incubated at either 37 or 42°C for 1, 3, and 6 h after the temperature shift. A 1.5-ml aliquot of the cells was centrifuged and resuspended in 470 μl of lysis buffer containing 100 mM sodium acetate (pH 5.3) and 1 mM

* Corresponding author.

$MgCl_2$. Cell lysis was obtained in the presence of 5 μl of diethyl pyrocarbonate by adding 25 μl of 20% sodium dodecyl sulfate (SDS). Nucleic acids were extracted with 1 volume of RFC (phenol-chloroform-isoamyl alcohol [25:24:1, vol/vol/vol]) preequilibrated at 65°C. In the presence of approximately 200 μl of glass beads, the mixture was vortexed, allowed to settle, and then centrifuged. The aqueous phase was extracted three times with RFC at 65°C and once with $CHCl_3$-isoamyl alcohol (24:1) at room temperature. RNA was precipitated with 0.3 M sodium acetate (pH 5.3) and 3 volumes of 95% ethanol at −20°C overnight.

**Molecular cloning.** A sized genomic library was prepared with nuclear DNA extracted from strain G222B, digested with *Eco*RI, and electrophoresed through agarose gel. Fragments of 5.2 kb were electroeluted from the gel (23), ligated into the *Eco*RI site of the λgt10 vector, and packaged into phage particles (16). The recombinant phages were screened with *hsp90* chicken cDNA sequence (8) and with a 1-kb *Sac*I-*Eco*RI fragment containing the 3′ end of the *hsp83* gene of *S. cerevisiae* (a gift of S. Lindquist). A total of $2 \times 10^4$ clones were screened, and three positive plaques were identified. The *Eco*RI insert of one of the phage clones was subcloned in the pUC18 vector (37) for further analysis.

**β-Tubulin probe.** A 1.1-kb fragment of the β-tubulin gene from *H. capsulatum* contained in plasmid pUC18 was used as a probe (12).

**Northern (RNA) blot analysis.** Fifteen micrograms of total RNA was precipitated in ethanol and resuspended in 1× MOPS [20 mM 3-(*N*-morpholino)propanesulfonic acid, 10 mM sodium acetate (pH 5.3), 1 mM EDTA]–50% formamide–2.2 M formaldehyde and heated 10 min at 65°C. Samples were separated by electrophoresis on 1% denaturing agarose gel in 1× running buffer (1× MOPS). RNA was then transferred onto nylon membranes in 20× SSC (1× SSC is 0.1 M NaCl plus 0.015 M sodium citrate) and fixed by UV crosslinking. Filters were then hybridized by using $10^7$ cpm of $^{32}$P-labeled probe DNA in 5× SSC–5× Denhardt's solution–50% formamide at 42°C overnight. The probe was labeled by multipriming to a specific activity of $10^9$ cpm/μg, and filters were washed under high-stringency conditions at 65°C for 40 min.

**DNA sequencing.** Sequencing was performed according to the method of Sanger et al. as modified by Hattori and Sakaki (13). Clones were sequenced on both strands.

**Transcription start site analysis by primer extension.** An antisense oligonucleotide (5′-CTCAGCCTGGAACTCG-3′) spanning from nucleotides (nt) +17 to +33 relative to the putative initiation translation site ATG was synthesized. Five nanograms of the oligomer was 5′ end labeled with [γ-$^{32}$P]ATP (specific activity, 3,000 Ci/mmol; Amersham Corp.), using 5 U of T4 polynucleotide kinase (BRL, Middlesex, United Kingdom). A mixture containing the oligomer and 30 μg of total RNA extracted from mycelia of *H. capsulatum* Downs or G222B grown at 25°C, or 15 μg of total RNA from cells that were heat shocked for 1 h at either 34 or 37°C, was denatured at 93°C for 2 min in avian myeloblastosis virus reverse transcriptase buffer (10× = 0.5 M Tris-HCl [pH 8.3], 60 mM $MgCl_2$, 0.1 M dithiothreitol, 0.1 M NaCl) and then frozen in a dry ice-ethanol bath for 1 min. The solutions were slowly thawed on ice. The primer extension reaction was carried out at 42°C for 30 min with a 200 μM concentration of a mixture containing the four cold nucleotides and 2 U of Avian myeloblastosis virus reverse transcriptase (Boehringer, Mannheim, Germany). Samples were run on a 6% polyacrylamide sequencing gel containing 7 M urea. After electrophoresis, gels were transferred to 3MM Whatman paper, dried and autoradiographed for 24 h with an intensifying screen at −70°C.

**RNase protection assay.** The appropriate cDNA fragment was subcloned into the pGEM3 vector (Promega Inc., Madison, Wis.), and radioactive antisense RNA was transcribed by using T7 RNA polymerase (23) and [α-$^{32}$P]UTP (Amersham). Total RNA was extracted from mycelia of *H. capsulatum* Downs grown in mycelial form at 25°C and from heat-shocked mycelia grown for 1 h at 34°C, for 1 h at 37°C, and for 1 h at 40°C. RNA was also isolated from *H. capsulatum* G222B grown in mycelial form at 25°C, heat shocked for 1 h at 37°C, and incubated for 1 h at 42°C. Aliquots (15 to 30 μg) of various RNA samples were mixed with a molar excess of $^{32}$P-labeled antisense RNA and denatured for 5 min at 85°C in 80% formamide–40 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES) buffer (pH 6.7) containing 0.4 M NaCl and 1 mM EDTA, and hybridization was performed at 37°C overnight. Nonhybridized single-stranded RNA was digested with RNase A (40 μg/ml) and RNase $T_1$ (2 μg/ml) at 37°C for 30 min. After inactivation of RNases by treatment with SDS and proteinase K, the protected RNA fragment was dissolved in 80% formamide, electrophoresed on a 6% acrylamide gel containing 7 M urea, dried, and exposed to a Fuji X-ray film at −70°C with an intensifying screen for 24 h.

**Nucleotide sequence accession numbers.** The sequence data reported for the *H. capsulatum hsp82* gene and its flanking regions have been assigned GenBank accession numbers M55629 and M37952.

## RESULTS

**Molecular cloning.** Total DNA purified from *H. capsulatum* G222B was digested with a variety of restriction endonucleases and probed with portions of the 5′ coding region of chicken *hsp90* (clone p9.11) and portions of the 3′ region of the *S. cerevisiae* gene. A size-selected genomic library was constructed in the λgt10 vector. Three positive recombinants were detected when $2 \times 10^4$ recombinant phages were screened by hybridization with the chicken probe under nonstringent conditions. A 2.0-kb *Sac*I fragment showing positive hybridization with chicken *hsp90* was isolated and used for further analysis and sequencing (unpublished data).

**Nucleotide and amino acid sequences and organization of the *hsp82* gene.** The entire nucleotide sequences of the putative *H. capsulatum hsp82* gene and its flanking regions have been determined. Screening against a representative protein sequence data base (Microgene; Beckman) showed a high degree of homology to *S. cerevisiae* (77%) and *D. melanogaster* (63%) genes, demonstrating that our cloned sequence corresponds to a *hsp82* gene. Similar to other heat shock proteins, the *H. capsulatum hsp82* protein sequence is highly conserved. Furthermore, though the degree of similarity is lower at the carboxy-terminal ends of the proteins, the EEVD amino acid sequence, which is highly conserved among the *hsp90* protein family of several species, is also present (4, 10, 15, 18, 27, 31). A single heat shock element with a 7-of-9-base match (CT-GAA--TTC--G) to the consensus heat shock element (2, 30) was found between nt −377 and −363 with respect to the major transcriptional start site. The *H. capsulatum hsp82* gene contains an open reading frame starting from the first ATG and is interrupted by two introns, one of 122 nt (IVS I) and the second of 86 nt (IVS II). These introns, like other fungal introns, resemble those present in all eukaryotic mRNAs, having the acceptor and donor conserved consensus sequences following the GT/AG

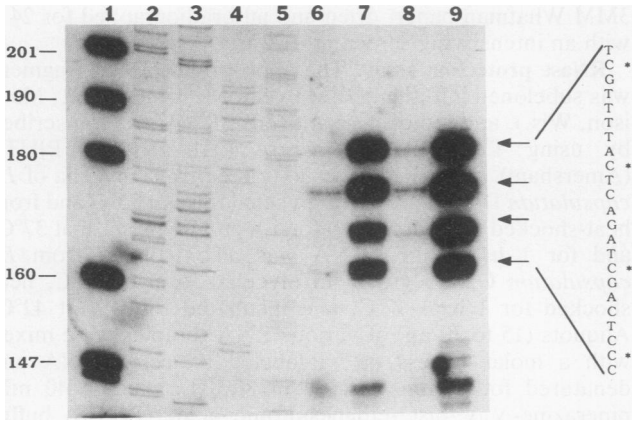


FIG. 1. Location of the 5′ end(s) of *hsp82* mRNA by primer extension. A 16-mer oligonucleotide, 5′-CTCAGCCTGGAACTCG-3′, that spans from nt +17 to +33, was end labeled with [$^{32}$P]ATP and hybridized to total RNA (see Materials and Methods) prepared from Downs grown at 25°C (lane 6) and after a shift to 34°C (lane 7) or from G222B grown at 25°C (lane 8) and after shift to 37°C (lane 9). Hybrids were isolated by ethanol precipitation, and the [$^{32}$P]DNA was extended by using avian myeloblastosis virus reverse transcriptase; the resulting fragments were analyzed for their size by electrophoresis on 6% sequencing gels; an *Msp*I digest of pBR322 was used as a molecular weight standard (lane 1). Lanes 2 to 5 are the sequence ladder of a known DNA fragment. The sequence around the initiation sites is shown on the right. The asterisks adjacent to the sequence denote the ends of the extended fragments. Sizes of standards are shown in nucleotides on the left.

rule for 5′ and 3′ splice sites (28). In addition, each intron contains an internally conserved sequence upstream from the 3′ end that resembles the TACTAAC consensus sequence of *S. cerevisiae* needed for branch site formation and appears to be involved in splicing events (20).

**Analysis of *hsp82* expression and 5′ end extension.** A low level of *hsp82* mRNA was present in mycelia of Downs strain grown at 25°C, and maximal expression was detected 1 h after the shift to 34°C (unpublished data). Similar results were obtained with the temperature-tolerant and mouse-pathogenic strain G222B, with the difference that a temperature of 37°C was necessary to induce maximal transcription (unpublished data). In both cases, mRNA of the predicted length of 2.8 kb was detected. Furthermore, *hsp82* mRNA transcription levels were transient at 37°C in strain G222B, and its kinetics resembled that of Downs at 34°C (data not shown). Low levels of *hsp82* mRNA were also detected at 37 and 42°C in Downs and G222B, respectively. The differences in the optimal temperature of transcription as well as in the qualitative and quantitative expression of the *hsp82* gene are essentially the same as those observed for expression of the *hsp70* gene in *H. capsulatum* (7).

The location of the putative 5′ end of *hsp82* mRNA was mapped by a primer extension experiment. A 5′-end-labeled 16-mer synthetic oligonucleotide (CTCAGCCTGGAACT CG) complementary to nt +17 to +33, corresponding to a region of the 5′ portion of the first exon, was annealed to total RNA extracted from mycelia grown at 25°C or to RNA extracted after exposure of Downs to 34°C for 1 h or G222B to 37°C for 1 h. The results in Fig. 1 demonstrate the presence of at least four different extended molecules. We have in fact identified four major transcripts of the mature *hsp82* mRNA in *H. capsulatum* with bands ranging 163 to 186 nt prior to the putative translation initiation codon, thus defining a long untranslated 5′ region.

Furthermore, the same RNA start sites of the *hsp82* gene were used at 25°C or induced at higher temperatures in both strains (Fig. 1). No alterations in the RNA start sites were found when expression of the *hsp82* gene was measured by primer extension at the nonpermissive temperatures of 37 and 42°C for the Downs and G222B strains, respectively (data not shown).

**Analysis of *hsp82* mRNA processing.** To determine the capacity of the cells to properly splice the *hsp82* mRNA precursor at nonpermissive temperatures, total RNA was extracted from the temperature-sensitive Downs strain at 34, 37, and 40°C and from the temperature-tolerant G222B strain at 37 and 42°C and analyzed by Northern blots and RNase mapping. No accumulation of *hsp82* pre-mRNA was detected when RNA purified from cells exposed to the high temperature was analyzed by Northern blot. In fact, proper mRNA splicing was observed when total RNA extracted from mycelia of either strain grown at the extreme temperatures of 37°C for Downs and 42°C for G222B was hybridized with the entire cloned *hsp82* gene, pHc83.1 (data not shown). Furthermore, no signal indicating the presence of high-molecular-weight RNA that corresponded to unprocessed RNA was detected when Northern blot hybridization was carried out with a plasmid containing a portion of intron I (data not shown). A more precise determination was performed by RNase mapping to detect a putative block in *hsp82* processing under conditions of transcription at high temperatures.

We performed a higher-resolution assay to confirm the data obtained with Northern blots because of the short length of *H. capsulatum hsp82* introns and the possibility that differences in the size of the pre-mRNA compared with the spliced molecule could not be detected on a formaldehyde-agarose gel. A fragment of the *hsp82* gene was subcloned into the pGEM3 vector so that an antisense RNA was synthesized from the T7 promoter by using the T7 RNA polymerase (Fig. 2A). The generated plasmid (pGM580) contains part of exon I (from nt 83 to 261), the first intron, and a part of exon II (from nt 384 to 630). The antisense RNA was synthesized after digestion of the DNA template with *Hin*dIII and used as a probe in an RNase mapping experiment. Total RNA from the G222B mycelia grown either at 25°C or after a shift to 37 or 42°C and from Downs mycelia grown at 25°C and after a shift to 34, 37, or 40°C was hybridized to the generated antisense RNA and digested with RNases A and $T_1$. If the splicing process had been blocked by exposure to high temperatures, a protected fragment of 547 nt, complementary to exon I, intron I, and exon II, was expected, while two protected fragments of 175 nt for part of exon I and 250 nt from a portion of exon II were expected if splicing had occurred. We found that under conditions of mild heat shock (34°C for Downs and 37°C for G222B) or under more severe conditions (37°C for Downs and 42°C for G222B), *hsp82* mRNA was properly spliced (Fig. 2B and C). In fact, no accumulation of pre-mRNA was observed when mild and severe heat shock was administered to the cells. Although *hsp82* transcription decreased dramatically under severe heat shock conditions, at 37 or 40°C for Downs (Fig. 2B) and at 42°C for G222B (Fig. 2C), two protected fragments were still observed, indicating the presence of spliced RNA. In strain G222B, the sizes of the protected fragments were identical to those predicted from the restriction map of the cloned gene, whereas the RNA extracted from the temperature-sensitive Downs strain

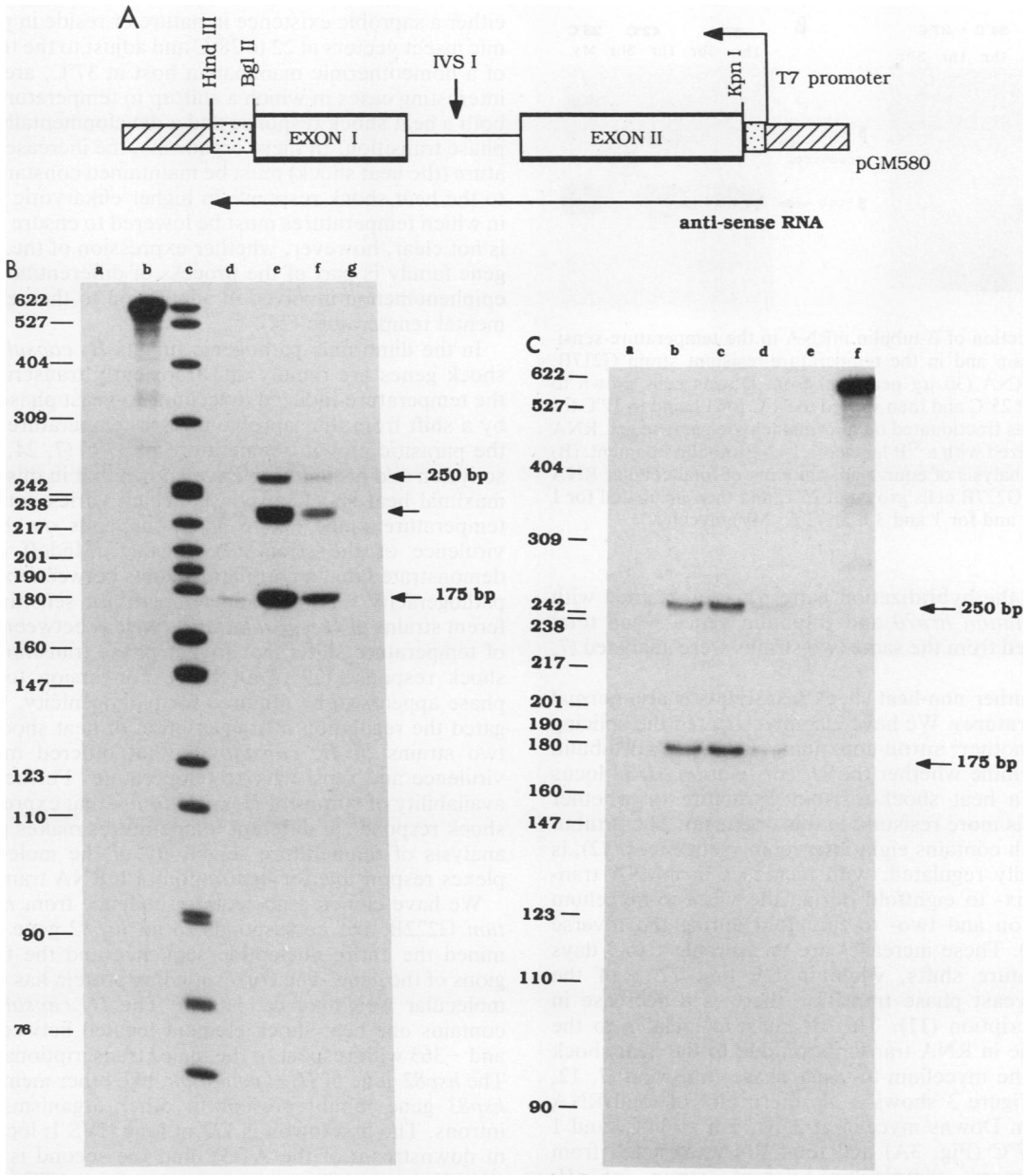


FIG. 2. RNase protection assays of *hsp82* RNA in strains Downs and G222B. The antisense RNA recognizes part of exon II, the intron (IVS I), and part of exon I of the *hsp82* transcript of *H. capsulatum* prepared from a *Hin*dIII-cut template. (A) Schematic representation of this probe, which was used in RNase protection assays with total RNA prepared from Downs (B) grown at 25°C (lane d) or after a shift to 34°C (lane e), 37°C (lane f), and 40°C (lane g). Lane a is riboprobe annealed in absence of total RNA from *H. capsulatum* and treated with RNases A and $T_1$ (Materials and Methods), showing no protected fragment. Riboprobe alone is shown in lane b. The same antisense RNA (A) was hybridized with total RNA extracted from G222B (C) grown at 25°C (lane a) and after a shift to 37°C (lanes b and c) and 42°C (lane d). Riboprobe annealed in the absence of total RNA from *H. capsulatum* and incubated with RNases A and $T_1$ (lane e) and riboprobe alone (lane f) are also shown. $^{32}$P-labeled *Msp*I-digested pBR322 was used as a molecular weight marker. The position of the splicing product is indicated and was identified on the basis of its mobility relative to that of the molecular weight marker.

showed a different pattern of protected fragments under all conditions examined, either at 25°C or after heat shock at 34, 37, or 40°C, suggesting the presence of polymorphism of the Downs strain *hsp82* gene.

**Southern blot analysis.** To detect the presence of DNA polymorphism within the region of DNA containing the first intron in Downs compared with G222B, a Southern blot experiment was performed (23). Total DNA from both Downs and G222B was digested with different restriction enzymes and, after blotting onto a nylon membrane, was hybridized with the *Bgl*II-*Kpn*I sequence derived from plasmid pGM580, containing part of exon I (from nt 83 to 261), the first intron, and part of exon II (from nt 384 to 630). Differences are seen in the hybridization patterns of the two strains (data not shown), which suggest restriction polymorphism for the enzymes tested. This could explain the different patterns of protected fragments observed in the RNase protection experiments (Fig. 2B and C). Similar

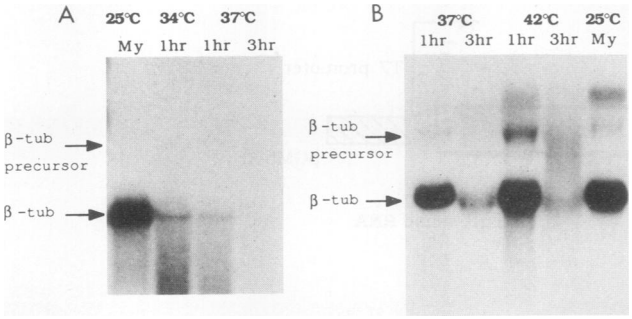


FIG. 3. Detection of β-tubulin mRNA in the temperature-sensitive Downs strain and in the temperature-resistant strain G217B. Total cellular RNA (30 μg per lane) from Downs cells grown to mid-log phase at 25°C and then shifted to 34°C for 1 h and to 37°C for 1 and 3 h (A) was fractionated on a formaldehyde-agarose gel. RNA was blot hybridized with a $^{32}$P-labeled 1.1-kb β-tubulin fragment. (B) Northern blot analysis of equivalent amounts of total cellular RNA extracted from G217B cells grown at 25°C and then incubated for 1 and 3 h at 37°C and for 1 and 3 h at 42°C. My, mycelia.

differences in the hybridization pattern were obtained with the *H. capsulatum hsp70* and β-tubulin genes when total DNAs prepared from the same two strains were analyzed (7, 11).

**Splicing of other non-heat shock transcripts is also normal at high temperatures.** We have also investigated the splicing activity of another intron-containing gene, the β-tubulin gene, to determine whether the *H. capsulatum hsp82* locus had evolved a heat shock-resistant structure or whether splicing itself is more resistant in this organism. The β-tubulin gene, which contains eight intervening sequences (12), is developmentally regulated, with increases in mRNA transcription of six- to eightfold during the yeast-to-mycelium phase transition and two- to threefold during the reverse transition (11). These increases are measurable 1 to 3 days after temperature shifts, while in the first 12 h of the mycelium-to-yeast phase transition there is a decrease in mRNA transcription (11). This decrease correlates to the general decline in RNA transcription due to the heat shock condition of the mycelium-to-yeast phase transition (7, 12, 21, 22, 35). Figure 3 shows a Northern blot of total RNA extracted from Downs mycelia at 25°C, 1 h at 34°C, and 1 and 3 h at 37°C (Fig. 3A) and total RNA extracted from G217B (a more temperature-tolerant strain of *H. capsulatum*) mycelia at 25°C, 1 and 3 h at 37°C, and 1 and 3 h at 42°C (Fig. 3B) hybridized to a $^{32}$P-labeled β-tubulin fragment. In both cases, no evidence of accumulation of precursor mRNA is detectable. Similar results were obtained with strain G222B (data not shown).

## DISCUSSION

All cells respond to a sudden increase in temperature by synthesizing heat shock proteins and partially suppressing the synthesis of housekeeping proteins (3, 6, 21, 22). Heat shock proteins have been strongly conserved in structure through evolution, indicating that they play a vital role in survival of organisms by protecting cells against environmental insults (33).

While the response to heat shock is a transient phenomenon in many biological systems, dimorphic fungi like *H. capsulatum* (24) and parasitic protozoa like *Trypanosoma brucei* and *Leishmania major* (36), which shift between either a saprobic existence in nature or reside in poikilothermic insect vectors at 22 to 28°C and adjust to the temperature of a homeothermic mammalian host at 37°C, are especially interesting cases in which a shiftup in temperature results in both a heat shock response and a developmentally regulated phase transition. In these organisms, the increase in temperature (the heat shock) must be maintained constant, contrary to the heat shock response in higher eukaryotic organisms, in which temperatures must be lowered to ensure survival. It is not clear, however, whether expression of the heat shock gene family is part of the process of differentiation or is an epiphenomenon involved in adaptation to the new environmental temperature (24).

In the dimorphic pathogenic fungus *H. capsulatum*, heat shock genes are rapidly and transiently transcribed during the temperature-induced mycelium-to-yeast phase transition by a shift from the saprobic growth temperature of 25°C to the parasitic growth temperature of 37°C (7, 24, 35). Transcription and protein data have shown that in this fungus the maximal heat shock response, which varies within a large temperature range (34 to 37°C), depends on the level of virulence of the strain (7). In fact, Medoff et al. (26) demonstrated that a correlation exists between the degree of pathogenicity for mice and temperature sensitivity in different strains of *H. capsulatum* as well as between the extent of temperature shifts that trigger phase transition and heat shock response (19, 29a). Since conversion to the yeast phase appears to be required for pathogenicity, we investigated the regulation of transcription of heat shock genes in two strains of *H. capsulatum* that differed markedly in virulence and sensitivity to temperature. Furthermore, the availability of strains of *H. capsulatum* that express the heat shock response at different temperatures makes possible an analysis of temperature sensitivity of the molecular complexes responsible for maturation of mRNA transcripts.

We have cloned a nucleotide sequence from *H. capsulatum* G222B that corresponds to an *hsp82* gene and determined the entire nucleotide sequence and the flanking regions of the gene. The corresponding protein has a predicted molecular weight of ca. 82,000. The *H. capsulatum* gene contains one heat shock element located between nt −377 and −363 with respect to the major transcriptional start site. The *hsp82* gene of *H. capsulatum*, like other members of the *hsp83* gene family present in other organisms, contains introns. The first intron is 122 nt long (IVS I; located at 261 nt downstream of the ATG), and the second is 86 nt long (IVS II; located 988 nt downstream of the ATG). In the first 1 to 3 h after the temperature shift, maximal transcription of a 2.8-kb transcript of the *hsp82* gene occurred at 34°C in the temperature-sensitive Downs strain, while a temperature of 37°C was necessary to induce maximal transcription in the temperature-tolerant G222B strain.

It is well known that heat shock affects RNA metabolism, including RNA processing as well as mRNA degradation (32). Yost et al. (38, 41) examined the expression of the intron-containing *Drosophila hsp83* gene, as well as the intron containing *Adh* gene, and found that they were induced strongly during moderate heat shock conditions (29 to 33°C) but only poorly under severe conditions (37 to 39°C). At these temperatures, mature mRNA was not produced and intron-containing pre-mRNAs accumulated in the cytoplasm in this organism. Such a block in splicing may persist for several hours after the cells are returned to the permissive temperature (25°C). More recently, inhibition of mRNA maturation has been demonstrated in chicken cells (5), in *Caenorhabditis elegans* (17), in trypanosomes (*trans-*

splicing of *hsp70* mRNA, while the *trans*-splicing pathway is sensitive to disruption by severe heat shock [29]), in HeLa cells (5, 32), and in *S. cerevisiae* (40). It was also shown that when *Drosophila* cells were first exposed to mild heat pretreatments (temperatures that induce heat shock proteins) prior to a shift to higher temperatures, mRNA precursor splicing continued under nonrestrictive conditions (38). Even though it has not yet been demonstrated directly that the synthesis of heat shock proteins has a direct effect on mRNA maturation, these data strongly suggested that induction of thermotolerance somehow protects the splicing machinery from disruption by stabilizing the pre-mRNA–spliceosome complex in higher eukaryotic cells (39).

We have demonstrated by Northern blot and RNase analyses that splicing of the *hsp82* mRNA precursor, unlike that of the *Drosophila hsp83* gene, is not blocked in either strain of *H. capsulatum* at the upper temperature range of the heat shock response specific for each of these organisms (up to 42°C). We have also analyzed mRNA maturation of another intron-containing gene during severe heat shock conditions in *H. capsulatum*. Under the conditions that we used, unspliced β-tubulin RNAs never accumulated when cells were shifted directly from 25 to 37°C in the temperature-sensitive Downs strain and to 42°C in the more temperature-resistant G217B strain, suggesting not only that the *hsp82* locus had evolved a heat shock resistant structure but possibly that the spliceosome itself is a heat-resistant structure in this organism.

The difference in mRNA splicing of the *H. capsulatum hsp82* gene during severe heat shock conditions compared with that of intron-containing genes of other eukaryotic cells can be explained by at least two considerations. First, in *Drosophila* cells, the latter experiments were performed on eukaryotic cells under nonphysiological conditions, i.e., exposure to a sudden change to a very high temperature followed by a rapid shift to a low temperature in order to ensure survival of the organism. Since conductivity of heat from air to animal tissue is slow, it is very unlikely that any organisms would experience such dramatic environmental changes in nature. For these organisms, one would predict that the response would be gradual, e.g., in plants exposed to sun, febrile response in mammals, or increase of water temperature for marine life. Under these circumstances, as the temperature rises, there would be a steady and persistent induction of heat shock proteins. Thus, thermotolerance plays a central physiological role in the maintenance of functional cellular properties, including splicing activity in eukaryotic cells, and there would be no need for these cells to have a functional temperature-resistant spliceosome machinery at elevated temperatures. Parasitic organisms like *H. capsulatum*, on the other hand, are organisms that face a sudden and drastic environmental temperature change, e.g., from one that is poikilothermic to one that is homeothermic. Parasites such as these must adapt to dramatic shifts in temperatures and environmental stresses (changes in the redox state, nutrition, induction of new metabolic processes, etc.) in order to invade and survive in mammalian tissues. In these organisms, each of these changes requires a response at the gene level, and it is reasonable to postulate that several intron-containing DNA sequences play a vital role in adaptation to the new environment. Therefore, in dimorphic organisms like *H. capsulatum*, the heat shock response is not an artificial phenomenon, and the mRNA maturation machinery must remain functional after infection in order to allow the fungus to undergo morphogenesis and survive.

Recently, Yost and Lindquist demonstrated that in the *S. cerevisiae ssa1 ssa2* double mutant carrying mutations in two members of an *hsp70* gene family, unspliced RNA never accumulated when cells were directly shifted from 25 to 41°C (39). This is consistent with the short half-lives of yeast mRNAs (14) and with the consideration that non-heat shock genes have reduced transcription rates under heat shock conditions (14, 41). In this particular mutant, which is about 100-fold more thermotolerant than the wild-type cells, high constitutive expression of heat shock proteins at the normal temperature has been correlated with the ability to survive lethal temperatures and to protect mRNA processing. It was also suggested that HSP70 may be one of the heat shock proteins involved in this protection, since in this double mutant this protein is highly expressed at 25°C, while, for example, it is missing at normal temperatures in *Drosophila* cells. It is reasonable to postulate that in dimorphic organisms, the mechanisms that are normally activated by mild pretreatment are already functional at the normal growth temperature of mycelia (25°C). This could be achieved either by evolution of temperature-resistant structures or by the existence of a constitutive thermotolerant state before host invasion, which, by permitting proper mRNA processing, would allow adaptation to the host environment and subsequent morphogenesis. Consistent with the hypothesis of Yost and Lindquist, it is intriguing that also in *H. capsulatum* HSP70 is expressed at a very high level in mycelial cells at 25°C (7, 35).

We have not measured directly the rate of transcription of the *hsp82* gene in the first hour of temperature shiftup. However, it has been demonstrated that incorporation of mRNA precursors in *H. capsulatum* continues at a constant rate for at least the first 2 h of the mycelium-to-yeast transition, as compared with mycelia growing at 25°C (9). Furthermore, it has been demonstrated that in yeast cells there were no significant differences in the mRNA decay rates measured in heat-shocked and non-heat-shocked cells and that for most mRNAs, comparable relative decay rates with a half-life up to 45 min were observed (14). Moreover, under heat shock conditions, heat shock genes are transcriptionally induced generally up to 3 h, with an increase up to a 1,000-fold of their mRNA concentration, while there is a gradual and almost total cessation in the synthesis of most of the other genes (21). We think that under the conditions used, the lack of accumulation *hsp82* precursor mRNAs was a measure of true mRNA maturation and that transcription continued in a nonrestrictive mode.

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