Molecular cloning and sequence analysis of *yps-3*, a yeast-phase-specific gene in the dimorphic fungal pathogen *Histoplasma capsulatum*

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Genes specifically expressed in the parasitic yeast phase of *Histoplasma capsulatum* have been cloned to clarify the mechanisms underlying both pathogenesis and morphogenesis in this human dimorphic fungal pathogen. Previous studies have determined that the yeast-phase-specific gene, *yps-3*, is expressed differentially in two non-isogenic strains which differ in their thermotolerance and virulence. We have cloned the *yps-3* homologues from the high virulence (G217B) and low virulence (Downs) strains, and obtained a partial cDNA clone representing the expressed gene from *H. capsulatum* G217B. The Downs clone harbours a 287 bp insertion sequence that disrupts a long ORF defined by the *yps-3* G217B cDNA. Although the insertion sequence contains features reminiscent of mobile genetic elements, including 15 bp direct repeats of flanking sequence, it is not randomly distributed in the *H. capsulatum* genome. S1 nuclease analysis was utilized to map the 5' end of the expressed *yps-3* gene in G217B to potential regulatory regions which are largely homologous in both strains. This finding may point to a deficiency in a temperature inducible regulatory protein in the low virulence, temperature-sensitive Downs strain. The nucleotide sequence of the *yps-3* gene and the predicted amino acid sequence of its product represents the first report of phase-specific gene and protein sequences in this widely distributed fungal pathogen. Further analysis of the product encoded by the *yps-3* gene may provide significant insight into the pathogenic repertoire of *H. capsulatum*.

Keywords: *Histoplasma capsulatum*, *yps-3*, yeast-phase-specific genes, cloning

INTRODUCTION

Yeast-phase-specific (*yps*) genes which are potentially related to virulence or morphogenesis, or both, have been isolated and cloned from *Histoplasma capsulatum*, a dimorphic human fungal pathogen (Keath *et al.*, 1989a). The organism exists as a multicellular mycelium in rich soils and organic matter in temperate environments worldwide, and proliferates as a unicellular yeast in infected host tissues (Wheat, 1988; Maresca & Kobayashi, 1989). Clinically inapparent or mild disease can result from limited, primary site infection of *H. capsulatum* in the lungs, but an often life-threatening disseminated form of histoplasmosis can occur in immunodeficient patients,

particularly the elderly and groups with acquired immunodeficiency syndrome (AIDS) (Graybill, 1988; Wheat *et al.*, 1990).

Since the yeast phase of the organism is the parasitic form, one approach to understanding virulence and/or morphogenesis has been to clone genes expressed specifically in the yeast phase at 37 °C. Expression of the previously cloned *yps-3* gene appears to correlate with virulence, temperature sensitivity, and the speed of the hyphal-to-yeast transition in three non-isogenic *H. capsulatum* strains (Medoff *et al.*, 1986b; Keath *et al.*, 1989a). For example, the *yps-3* gene is expressed exclusively in the yeast phase of virulent strains, such as G217B, but is not transcribed in either the mycelial or yeast phases of low virulence strains, such as Downs or several similar isolates recently obtained from patients with AIDS (Spitzer *et al.*, 1990). In G217B or other class 2 strains defined by analysis of various

The GenBank accession numbers for the sequences reported in this paper are L16843 (non-expressed *yps-3*; HTOYPSA), L16844 (*yps-3* complete coding sequence; HTOYPSB) and L16845 (*yps-3* mRNA, 3' end; HTOYPSB).

restriction fragment length polymorphisms in mitochondrial (Vincent *et al.*, 1986), ribosomal (Spitzer *et al.*, 1989), or nuclear genes (Keath *et al.*, 1989b, 1992), the *yps-3* gene is expressed 1 d following an *in vitro* transition when mycelia grown initially at 25 °C are induced to form yeast by shifting the temperature of incubation to 37 °C (Keath *et al.*, 1989a).

Virulence in *H. capsulatum*, and its relation to morphogenesis is of particular interest. Although conversion to the yeast phase is most probably required for progressive infection (Medoff *et al.*, 1986a), the transition to the yeast form is not required for survival of the organism at an elevated temperature of 41 °C (Medoff *et al.*, 1987). Genes essential for early adaptation to elevated temperatures or for the conversion to, and maintenance of, the parasitic phase are likely to represent significant virulence determinants. Adaptation to the macrophage or tissue environment of the host and transformation to the yeast state may entail the activation of temperature-sensitive promoters or control elements which may, in turn, be strongly influenced by alteration in temperature.

To further understand the genetic and regulatory processes in *H. capsulatum*, the genomic *yps-3* gene from G217B and the non-expressed homologue from the Downs strain have been cloned and sequenced. The Downs strain contains a repetitive 287 bp sequence which interrupts a long ORF defined by a G217B *yps-3* cDNA clone. Although this sequence is flanked by two 15 bp direct repeats, it does not function as a mobile genetic element in two different classes of *H. capsulatum* as evaluated by field inversion gel electrophoresis of chromosomal sized DNAs.

METHODS

Organisms and culture conditions. Stock cultures of *H. capsulatum* G217B (ATCC 26032) and the temperature-sensitive Downs strain (ATCC 38904) were maintained in liquid medium containing 2% (w/v) glucose and 1% (w/v) yeast extract at 25 °C or 37 °C for hyphal and yeast phases, respectively. For temperature sensitivity experiments, mycelia were induced to form yeast cells at 34 °C in liquid culture.

Isolation of mRNA for Northern blot analysis and cDNA library construction. Total cellular RNA was isolated from the Downs and G217B strains of *H. capsulatum* by mechanical disruption in UNSET buffer (8 M urea, 2%, w/v, SDS, 150 mM NaCl, 100 mM Tris/HCl, pH 7.5, and 1 mM EDTA) by Braun homogenization. Nucleic acids were repeatedly extracted in phenol-chloroform-isoamyl alcohol (PCI), followed by ethanol precipitation. Following recovery, the RNA was resuspended in NSET buffer (UNSET buffer lacking urea), and digested with proteinase K (35 µg ml⁻¹, Boehringer Mannheim) for 30 min at 37 °C. The RNA was re-extracted with PCI and precipitated in ethanol. PolyA⁺ RNA was obtained from both strains and phases by oligodT cellulose chromatography (Ono *et al.*, 1980), electrophoresed with commercial RNA markers (Gibco BRL) in 1.1% (v/v) formaldehyde agarose (Rave *et al.*, 1979) and transferred to Nytran (Schleicher and Schuell) as previously described (Keath *et al.*, 1992). The blot was hybridized under aqueous conditions (7% SDS, 1% BSA, 1 mM EDTA, 0.5 M sodium phosphate buffer) with 10⁶ c.p.m. of probe per ml of hybridization solution (Church & Gilbert, 1984). Probes were prepared by nick-translation (Rigby *et al.*, 1977) and included

the previously described 1.85 kb *Hind*III fragment representing the G217B *yps-3* gene (Keath *et al.*, 1989a), and the 1.4 kb *Bam*HI-*Hind*III fragment containing the cloned *H. capsulatum* actin gene (Spitzer *et al.*, 1990). For cDNA library construction, SDS was omitted from the isolation procedure during the initial breakage and in the subsequent steps, since SDS appeared to inhibit the extent of first strand synthesis (unpublished observations).

Generation of a yeast-phase-specific subtraction library from G217B.

A subtraction library enriched for G217B yeast-phase-specific sequences was prepared in the ampicillin resistant phagemid, pTZ (Invitrogen). Individual double-stranded cDNA libraries from the yeast and mycelial phases of G217B were generated by a modified Gubler-Hoffmann protocol (Gubler & Hoffman, 1983) from 50 µg polyA⁺ RNA, and inserted into the phagemid vector in the non-palindromic *Bst*XI site. Following transformation, single-stranded (ss) phagemid DNA from each library was rescued by infection with R408 helper phage. Photo-biotinylated ss phagemid DNA from the mycelial phase was hybridized with ss yeast phagemid DNA; hybrid cDNAs common to both phases were removed with streptavidin, followed by PCI extraction. The organic phase was back-extracted three times with TE buffer (50 mM Tris/HCl, pH 7.5, 1 mM EDTA) and the aqueous phases were pooled prior to ethanol precipitation. The *yps* subtraction library was recovered following reverse transcription and transformation in *Escherichia coli* DH5α. Recombinants harbouring the *yps-3* gene were identified by colony filter hybridization (Maniatis *et al.*, 1982), and the insert sizes of five clones were determined by gel electrophoresis of *Xba*I-digested mini-prep DNAs.

Dideoxy DNA sequencing of the genomic *yps-3* homologues from the G217B and Downs strains of *H. capsulatum*.

The Downs and G217B *yps-3* genes were sequenced by the dideoxy chain termination method (Sanger *et al.*, 1977) using Sequenase version 2.0 (USB). Clones were sequenced in both directions by a combined approach using ligation of appropriate restriction fragments into M13mp18 and M13mp19 vectors, and by the formation of a series of unidirectional nuclease *Bal*31 deletions (Maniatis *et al.*, 1982) from both the Downs and G217B clones. The pTZ *yps3.3* cDNA from strain G217B was sequenced in both directions using the *Bal*31 deletion strategy.

S1 nuclease analysis of the 5' end of the *yps-3* gene in G217B.

A 1.35 kb *Hind*III-*Sma*I fragment from the genomic *yps-3* sequence was subcloned in both orientations in M13mp18 and M13mp19 vectors. Preliminary studies (data not shown) demonstrated that only the M13mp19 construct hybridized to *yps-3* transcripts in Northern blot analysis, and this construct was used in the protection experiments. The 1.35 kb *Hind*III-*Sma*I probe was prepared by primer extension using [³²P]dATP and the Klenow fragment of DNA polymerase I (New England Biolabs). The probe was purified by separation on a urea polyacrylamide gel, and the radiolabelled fragment removed by electroelution in 1 × TBE buffer. The probe was hybridized (25000 c.p.m. per reaction) at 55 °C overnight in 20 mM Tris/HCl, pH 7.4, 0.4 M NaCl, 100 mM EDTA and 10 mM dithiothreitol to 2 µg polyA⁺ RNA from the yeast and mycelial phases of Downs and G217B. S1 nuclease (Sigma) digestion was performed at 37 °C for 1 h in 1 × S1 buffer (40 units S1 nuclease, 0.3 M NaCl, 30 mM sodium acetate, pH 4.6, 1 mM ZnSO₄, 20 µg salmon sperm DNA ml⁻¹). Samples were electrophoresed on 6% urea polyacrylamide gels followed by autoradiography. Labelled pBR322 markers prepared by digestion with *Eco*RI and *Hin*fl (Maniatis *et al.*, 1982) were electrophoresed in parallel to determine the size of the protected fragment(s).

Field inversion gel electrophoresis (FIGE) of chromosomal sized DNA molecules from *H. capsulatum*. Chromosomal sized

DNAs from the 217B and Downs strains of *H. capsulatum* were fractionated on 0.4% agarose gels by FIGE (Carle *et al.*, 1986) using an embedded lysis technique modified for *Histoplasma* (Steele *et al.*, 1989). *Histoplasma* DNAs were electrophoresed in parallel with chromosomal sized DNA obtained from *Saccharomyces cerevisiae* obtained by the method of Carle & Olsen (1985). FIGE was performed under ramping conditions for 140 h at 35 V. Initial conditions were established at 1200 s forward, 200 s reverse to a final setting of 3600 s forward, 1800 s reverse. The gel was treated with 0.1 M HCl, neutralized and then transferred to Nytran (Southern, 1975). The hybridization conditions were as described above.

RESULTS AND DISCUSSION

Expression of the *yps-3* gene in the virulent G217B and the low virulence Downs strain of *H. capsulatum*

Previous studies (Keath *et al.*, 1989a) had suggested that expression of the *yps-3* gene correlated with thermo-tolerance and virulence in the yeast phases of three non-isogenic *H. capsulatum* strains cultured at 37 °C. It was possible, however, that the absence of *yps-3* gene expression in the Downs strain was due to temperature stress in this thermosensitive strain. As a result, gene expression was evaluated in the mycelial phase and in the yeast phase achieved following a temperature-induced transition at 34 °C. As seen in Fig. 1(b), yeast phase organisms of the G217B strain cultured at 34 °C express

Fig. 1. Expression of the *yps-3* gene in the Downs and G217B strains of *H. capsulatum* at 34 °C. PolyA⁺-selected RNAs from the Downs (low virulence) and G217B (high virulence) strains were electrophoresed in 1.1% formaldehyde agarose containing ethidium bromide (1 µg ml⁻¹) with a commercial RNA ladder (Gibco BRL, St). The ethidium bromide stained gel (a), was transferred and hybridized in (b) with the 1.85 kb *Hind*III genomic *yps-3* fragment from G217B in an aqueous buffer at 65 °C. Filters were washed once in 2×SSC/0.1% SDS at room temperature and at 65 °C in 0.1×SSC/0.1% SDS. Following exposure, the blot was stripped and re-hybridized in (c) with the 1.4 kb *Bam*HI–*Hind*III fragment containing the cloned *Histoplasma* actin gene. M, mycelial phase; Y, yeast phase. The sizes of the molecular mass standards are given in kb.

Fig. 2. Restriction analysis and DNA sequencing strategy for the genomic 1.85 kb *Hind*III G217B (a) and the 2.1 kb *Hind*III Downs (b) *yps-3* gene homologues. The restriction maps of the G217B (a) and Downs (b) clones were determined by Southern analysis. The Downs sequence carries a repetitive element designated by the black box. The G217B *yps-3* gene was sequenced by both the restriction fragment strategy [a(i)] and by *Ba*/31 unidirectional deletions [a(ii)]. The genomic Downs sequence was determined as shown in [b(i)] and [b(ii)] by the restriction fragment and deletion methods, respectively. The extent and direction of the determined sequences are indicated by arrows. A, *Ava*I; Bg, *Bgl*II; H, *Hind*III; Ha, *Hae*III; P, *Pst*I; Pv, *Pvu*II; Sm, *Sma*I.

the *yps-3* gene as three transcripts ranging in size from 0.9 to 1.3 kb. In contrast, the gene is not transcribed in either yeast (grown at 34 °C) or in mycelial forms of the Downs strain. The absence of phase-specific gene expression in Downs was not due to RNA degradation or transfer artifacts, since the staining in Fig. 1(a) is roughly equivalent for all tested RNAs, and since the 1.4 kb *Bam*HI–*Hind*III fragment containing the *Histoplasma* actin gene probe hybridizes to transcripts of 1.4 kb in all lanes (Fig. 1c).

DNA sequence analysis and comparison of the Downs and G217B genomic homologues of the *yps-3* gene

Although the *yps-3* gene was not expressed in the yeast phase of the low virulence Downs strain at either 34 °C or 37 °C, previous studies have demonstrated that the gene was not deleted from the genome but was carried on a larger 2.1 kb *Hind*III fragment (Keath *et al.*, 1989a,b). The Downs *yps-3* clone was obtained by screening a genomic

Fig. 3. For legend see facing page.

library generated from this strain (Harris *et al.*, 1989) using the 1.85 kb G217B *Hind*III fragment as a probe. Detailed restriction endonuclease analysis was performed on both the G217B and Downs clones as described in Fig. 2(a) and (b), respectively. The *Hind*III fragments from the two strains differed with respect to several restriction endonuclease cleavage sites: the 1.85 kb G217B *Hind*III fragment harboured sites for *Pvu*II and a single *Bgl*II site, while the Downs *yps-3* homologue had two *Bgl*II sites, but had recognition sites for *Pst*I. The greatest restriction fragment length polymorphisms mapped to a *Hae*III–*Ava*I fragment which was 295 bp and approximately 780 bp in the G217B and Downs homologues, respectively (Fig. 2a and b, data not shown). These preliminary findings suggested that the Downs clone might carry an insertion sequence which was absent in the G217B *yps-3* gene.

Based on these observations, a sequencing strategy was developed for the *Hind*III fragments from both strains as detailed in Fig. 2. A series of overlapping deletions were generated for both clones by nuclease *Ba*31 digestion followed by linker ligation and insertion into M13mp18 or mp19 series vectors [Fig. 2a(ii) and b(ii)], additional

sequence was confirmed by fragment subcloning in the same vectors [Fig. 2a(i) and b(i)].

Comparative DNA sequence analysis of the Downs and G217B *yps-3* homologues (Fig. 3) indicated that the two genes were over 92% homologous in the first 1370 bp, mapping from the *Hind*III site (at position 1); the majority of differences with respect to endonuclease cleavage sites were due to point mutations which disrupted or created novel sites when the two clones were evaluated (Fig. 3).

A unique sequence was observed in the Downs *yps-3* homologue at position 1376, where an internally repetitive 15 bp element disrupted the homology shared between the two sequences. A CCCACCGACAAATA(C/T) motif was reiterated 13 times within this region. Moreover, the unique Downs sequence was flanked at the right-hand end by a 14 bp direct repeat (Fig. 3) of a CCCACGGACA-AGTA sequence preserved in G217B and by a CCCTAT-TGATAAG(T/A)A direct repeat of the G217B sequence at the left-hand end.

In this study, DNA sequence analysis was used to compare the Downs and G217B clones in this upstream, non-coding region (Fig. 3). The TATA element was preserved in both sequences at positions 1058 and 1067, respectively.

The most significant difference in the Downs strain was an additional series of nine T residues (positions 1102–1119, Fig. 3) which localized to the region immediately prior to the start site observed in G217B (see below). Several deletions (T) and point mutations (CC in G217B replaced by TT in Downs) were also apparent in this region. These differences, although slight, may be sufficient for strain-specific *ypr-3* gene expression. Alternatively, the Downs strain may be deficient in the synthesis of a temperature or stress inducible transcription factor which may recognize potential phase-specific promoters in both strains. Regulation of the expression or the levels of such transcription factors might play a significant role in the phase transition and in the morphogenic process. The discovery of additional yeast-phase-specific genes in the G217B strain which failed to be expressed in either phase of the Downs strain (unpublished observations) provides some preliminary support to this contention. However, the upstream regions of the Downs and G217B sequences may provide *in vitro* target binding sites for phase-specific

The unusual features of the Downs sequence with respect to redundancy and duplication of flanking sequence DNA suggested that the region might function as a mobile genetic element. An internal 123 bp *HincII* fragment (Fig. 3) contained within the novel sequence region of Downs was used as a probe for chromosomal sized DNA molecules separated by FIGE. Recent studies by Steele *et al.* (1989) have demonstrated the presence of three large chromosomal-like bands ranging from 2 to 7 Mb in the G217B strain inferred from parallel electrophoresis of *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*. In contrast, the Downs strain harbours five chromosomes visualized by ethidium bromide staining and chromosome specific probes (Steele *et al.*, 1989). In Fig. 4(a), the blot

Fig. 4. Distribution of the reiterated sequence element on chromosomal sized molecules obtained from the Downs and G217B strains. The FICE blot was hybridized in (a) under aqueous conditions to the 123 bp *HincII* fragment specific for the repeated sequence identified by Downs sequence analysis. No hybridization was observed in the G217B or in the *S. cerevisiae* lanes; only one of five chromosomal sized molecules in the Downs strain harboured the element. In (b) the blot was stripped and rehybridized with the 1.85 kb *HindIII* G217B *yps-3* sequence which lacked the repetitive sequence. The repetitive element and the *yps-3* homologue co-migrate in the Downs strain.

containing both chromosomal sized DNA preparations from Downs and G217B was hybridized to the 123 bp *HincII* fragment specific for the Downs insertion sequence; the probe reacted with a single chromosomal sized molecule in the Downs strain and failed to hybridize to any of the three chromosomal bands observed in G217B. Even under prolonged exposures, the probe failed to hybridize to any additional putative chromosomes in either strain, suggesting that the repetitive element was not randomly distributed among *H. capsulatum* chromosomes. To verify that the 123 bp *HincII* fragment was associated with the Downs chromosome harbouring the *yps-3* homologue, the 1.85 kb *HindIII* fragment from G217B which lacked the repeated sequence was used to probe the stripped membrane filter (Fig. 4b). The *yps-3* gene probe lacking the novel sequence hybridized to single chromosomes in both the G217B and Downs strains and the reactive band in Downs co-migrated with the chromosome identified with the repetitive element probe. These findings, as well as

additional Southern blot experiments with *EcoRI*, *BamHI*, and *XbaI* (data not shown) suggested that the 287 bp sequence found in the Downs strain was unique to this low virulence isolate, and that the element was not distributed randomly in the *H. capsulatum* genome.

The origin of this repetitive element in the Downs strain is unclear. The sequence does not function as a mobile genetic element, and the redundant nature of the region would have minimal coding capacity. One tempting derivation for such a repetitive sequence might be as a telomere (Blackburn, 1991). Healing of chromosomes by telomerase is known to play a role in the DNA sequence rearrangements described for developmentally regulated organisms such as *Tetrahymena* (Yu & Blackburn, 1991). Native telomeric sequences have been cloned recently and described for *Histoplasma* (Woods & Goldman, 1993) and contain reiterations of a GGGTTA sequence dissimilar from the 15 bp motif observed in the Downs sequence.

DNA sequence analysis of a partial *yps-3* cDNA clone from the G217B strain

It was of interest to determine if the unusual sequence uncovered by genomic sequence and chromosome blotting techniques in the Downs strain contributed to the absence of yeast-phase-specific gene expression observed in the temperature-sensitive strain. A cDNA subtraction library enriched for yeast-phase-specific sequences from G217B in the pTZ phagemid was screened using the 1.85 kb *HindIII* fragment. Subclones of the largest pTZ *yps-3.3* cDNA were generated in M13mp18/M13mp19 vectors at relevant restriction endonuclease sites, and by a bidirectional *Bal31* deletion series. The 3' end of the *yps-3* gene of G217B was defined by a consensus poly-AAUAAA adenylation signal and a polyA tail which extended approximately 200 bases beyond the *HindIII* site (Fig. 5) of the genomic clone. The sequence of the 773 bp pTZ *yps-3* cDNA was aligned with the genomic sequence of the G217B clone (Fig. 5). The long ORF of this cDNA was in-frame with an upstream ATG beginning at position 1280 of the G217B genomic sequence and boxed in Fig. 5. A UGA stop codon would terminate the *yps-3* protein product at position 1832 in the genomic G217B sequence, prior to the *HindIII* site, predicting a *yps-3* polypeptide of approximately 17 kDa. The insertion element found in the Downs clone disrupted this ORF within a putative codon for aspartic acid (triangle in Fig. 5). Thus, the unique Downs element did not map to potential upstream promoter sequences for the *yps-3* gene and, if expressed, would have significantly disrupted the coding capacity of the *yps-3* mRNA.

5' end analysis of the expressed *yps-3* gene in G217B

The larger sizes (1.3, 1.1 and 0.9 kb) of the authentic *yps-3* transcripts detected in Northern blots (Fig. 1b) suggested that the pTZ *yps-3.3* cDNA was a partial clone which lacked the 5' end of the mRNA. To determine the precise end of the gene and thus to localize potential upstream promoter sequences in the G217B and Downs clones, S1 nuclease analysis was performed to map the 5'

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AAGCTTTATTTCAGCCGCAAGAGGCTCCACTTTCTTCATCATCGAACCAATCACAGCGCGCCCCCCCCCCC
HindIII 10      20      30      40      50      60      70
CCCAGCTACCCAGATTTTGGCTACTTCCACTCGCGGCAAGGATCGCCCGATTGCATCCCCAACTCAACG
80      90      100     110     120     130     140
ACATCACCACCATCGTCAGGATGCATGGCCGAAGTTTGTATAACAATTCACGGAGGACAGTTCATGGCTA
150     160     170     180     190     200     210
TTGCTTTGCTGGTTCGTCCTCCATCGACTCTGATCCCTGCGATCCCGAGGCTGAAGCTGAAGCTGAAGCATC
220     230     240     250     260     270     280
TTGTCTCTCCAAAGCTGGCGTTGTACGGAGCTAGTCCCTGGTTTTCACCTCAAATATTTCTACGCTTCGGCT
290     300     310     320     330     340     350
ACGATCGCAAGAACTGGTGGCTACTTCCAATGGAATACATACAGGTCGTACATTCCCTCGATGTCCGCAGA
PvuII 360     370     380     390     400     410     420
TCTGGAAGTGTGTATCTTGAATCCATTCAAGCACAGATAACCCTGGCAAAATCGAATCGAGAATCCAGG
II 430     440     450     460     470     480     490
ATGACAGAATTATGTTTTTCATGGATTATCCACTTCGTGATTCGCCTTTCCCTGTACCTTTATTTAACC
500     510     520     530     540     550     560
TTTTGGCCCTAACAGATTAGGTTTTCGGAACCTTATCTCTTTCTCCATCCCTAAAACCTTTAATTCGTTTC
570     580     590     600     610     620     630
GCTTTACGACATCTTCGGTGTCTCTTGTATCGTGCATTGAGCTCATTTGAAAATCCACAGCCATGCATAGC
640     650     660     670     680     690     700
CCTTCGTAATGTGACGGGGAGCTAGTCTAGGCGTGATTTCATAGATGTTTCGATAAGTCAAGTTGCAG
710     720     730     740     750     760     770
CTGTACCACAGCGCAGTGATGCAATAATGTGTTACCCAGCTTCTTGCAACGAATGCAACTCTAAGTACT
780     790     800     810     820     830     840
GGTACACACAGCCCCATTAATTGGCTAGTTGTTTAGACAGACGTCATATATTATTTCTATTTTGAATTTG
850     860     870     880     890     900     910
ACTCTGTCTCATGTTTCTATTCTTAGCATCAGTTAGCCTGCCCATGATGAGACACTGCCTATTGAATGGA
920     930     940     950     960     970     980
CAGGTTTGTGAACATGGCCTATCAGGAACCAGGCGGGTATGATGCTAAAGAGGGGTTTAAAGCAGGCCAT
990     1000    1010    1020    1030    1040    1050
GGAGAAGGCTCGAAGATATAAAAAGGTGCGGATTTCCCTCTCTTTTATCTTCTTTTTCCTTCTTTTTT
1060    1070    1080    1090    1100    1110    1120
AACAAGCACCCGTCGTTTCGTTCCCTAAGTTGAAGATATTCAAATACTCCTTTTTCATATACATATATTT
1130    1140    1150    1160    1170    1180    1190
AATGTAATAAACTCTTCCATCATTCCTCATCGACTTGCAGTATATCAAACCCATTTTAAATCGCAGCTCAA
1200    1210    1220    1230    1240    1250    1260
TAAATAATCAACCGCAAAATGCTGAACATCAAATCGATCTCAACCCTCCTCCTTCCGACGCTCC
1270    1280    1290    1300    1310    1320    ▼
Ser Leu Leu Val Ala Ala Arg Pro Gly Glu Tyr Pro Thr Asp Lys Asp Pro
TCC CTC CTC GTC GCT GCC CGC CCG GGA GAA TAT CCC ACG GAC AAG GAC CCT
1340                                     Smal/AvaI 1360    1370
Ile Asp Lys Lys Pro Lys Pro Lys Pro Ala Pro Phe Cys Gly Thr Cys Asn
ATT GAT AAG AAG CCC AAG CCA AAG CCC GCC CCC TTC TGC GGC ACC TGC AAC
1390                                     1400    1410    1420
Pro Ile Ser Gly Lys Asn His Cys Asp Ile Thr Thr Ser Cys Ile Asn Thr
CCT ATC TCA GGC AAG AAC CAC TGT GAT ATA ACC ACT TCT TGC ATC AAC ACT
1440                                     1450    1460    1470    1480
Gly Lys Arg Phe His Cys Ala Cys Arg Ala Gly Phe Lys Ala Ser Lys Asp
GGC AAG CCG TTC CAC TGT GCC TGC CGG GCT GGC TTC AAG GCC AGC AAG GAC
1490                                     1500    1510    1520    1530
Asn Tyr Asp Ile Tyr Lys Gln Phe Arg Leu Gln Phe Lys Asn Phe Gly Phe
AAC TAC GAC ATC TAC AAG CAA TTC CGT CTG CCA TTC AAG AAC TTC GAG TTC
1540                                     1550    1560    1570    1580
Leu Val Phe Thr Pro Gly Tyr Thr Gly Cys Asp Ile Leu Cys Asp Asp Asn
CTC GTC TTC ACT CCC GAA TAT ACC GAG TGC GAT ATC CTT TGC GAC GAT AAC
1590                                     1600    1610    1620    1630
Thr Lys Pro Ala Asn Gly Leu Cys Gln Glu Val Pro Leu Tyr Lys His Cys
ACG AAG CCG GCC AAT GAG CTT TGC CAA GAG GTT CCG CTT TAT AAA CAC TGC
1640                                     1650    1660    1670    1680
Gly His Lys Pro Arg Phe Ser Cys Ser Ser Leu Thr Arg Gly Val Gly Ser
GAG CAT AAG CCG CGT TTC TCT TGT TCT TCA CTC ACT AGA GAA GTG GGA TCG
1690                                     1700    1710    1720    1730
Tyr Val Leu Gly Tyr Ser Asp Leu Phe Phe Pro Ser Ile Leu Asn Thr Leu
TAT GTT TTG GGG TAT TCG GAT TTG TTT TTT CCA ACT ATT TTG AAC ACG TTG
1740                                     1750    1760    1770    1780
Leu Ala Leu Phe Val Cys Asn Gly Glu Leu Val Gly Thr Ala Gly ***
CTT GCT TTG TTC GTT TGT AAT GGA GAG TTA GTT GGG ACA GCG GGG TGA
1800                                     1810    1820    1830
GAGAGACAATAATTTTTCGGGGTCGGGAAAGCTTCATCTATGTGCTCCAAATAGGGGTTTTTCGGGTTTC
1840    1850    1860    HindIII
CTATTGATAGCAATAAATACTGATGAAGTCGGACATAGGGAAACATCTTGTGTGCAAAATTATTTTAATAC
ATGGCTTAGACTCCTTTTGGAAAAA),100

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Fig. 5. Nucleotide and predicted amino acid sequence of the partial pTZ yps-3.3 cDNA aligned with the genomic G217B sequence. The long ORF of the 773 bp cDNA was oriented with respect to the genomic sequence, beginning at position 1328. The translated product was in-frame with the boxed ATG start codon at position 1280 of the genomic sequence. The predicted transcription start site inferred from S1 analysis is designated by an arrow; a TATAA element, and a putative ribosome binding site are found at positions 1067 and 1207, respectively. A consensus polyadenylation signal located downstream of the 3' *Hind*III sequence is underlined and the putative stop codon is designated by asterisks.

Fig. 6. S1 nuclease analysis of the 5' end of the *yps-3* gene. A 1.35 kb *HindIII*–*SmaI* fragment was subcloned in M13mp19 for the nuclease analysis (see Fig. 2a). The single-stranded probe was hybridized to mycelial and yeast polyA⁺ RNAs from the Downs and G217B strains. A major protected fragment of 230 nucleotides was obtained following S1 digestion in the G217B yeast sample. The full length probe fragment was observed in the control lane.

end of the *yps-3* gene. A 1.35 kb *HindIII*–*SmaI* fragment from the genomic G217B clone was uniformly labelled by primer extension within M13mp19 (Fig. 6a), processed by electroelution, and hybridized to mycelial and yeast polyA⁺ RNAs from the Downs and G217B strains of *H. capsulatum*. The major start site of the gene mapped 230 nucleotides upstream of the *SmaI* site exclusively in the G217B yeast tract (Fig. 6b), at position 1121 in the genomic G217B sequence. The putative purine start site was preceded in the genomic sequence by a region rich in CT bases, and was localized approximately 50 bases from a potential TATA box at positions 1067–1072 (Fig. 5). A consensus ribosome binding site (CCATCA) was located upstream of the putative ATG codon (Fig. 5) at position 1207. Neither the nucleotide sequences of the *yps-3* gene

nor the predicted protein product of the cDNA are homologous to sequences currently in GenBank.

The *yps-3* gene itself, expressed at high levels in the virulent G217B strain, may represent an important virulence determinant. Although the gene is not required for the transition from hyphal to yeast forms, it may play a role in facilitating recovery of the virulent strains from environmental or thermal stress. Further analysis of the *yps-3* gene product will be necessary to ascertain its role in virulence in G217B and other *H. capsulatum* strains, since only the yeast phase is known to survive within tissues or within macrophages (Eissenberg & Goldman, 1991).

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