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Sequence analysis of the glyceraldehyde-3-phosphate dehydrogenase genes from the basidiomycetes *Schizophyllum commune*, *Phanerochaete chrysosporium* and *Agaricus bisporus*

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Abstract. *GPD* genes encoding glyceraldehyde-3-phosphate dehydrogenase were isolated from the homobasidiomycetes *Schizophyllum commune*, *Phanerochaete chrysosporium* and *Agaricus bisporus*. All three species contain one transcriptionally active *GPD* gene, but *A. bisporus* also contains an inactive *GPD* gene (tandemly linked to the active gene). These genes contain 5–9 introns located at conserved positions, differing (except in one case) from intron positions in ascomycetous *GPD* genes. The predicted amino-acid sequences of the proteins encoded by the three active *GPD* genes are highly homologous. A comparison with protein sequences from filamentous ascomycetes shows a clear distinction, whereas the *GPD* genes from ascomycetous yeasts are quite distinct from both the filamentous ascomycetes and basidiomycetes. Promoter regions of ascomycetous *GPD* genes do not correspond to those of the *GPD* genes of basidiomycetes which may (partly) explain poor expression in basidiomycetes of introduced genes driven by an ascomycete *GPD* promoter.

Key words: Glyceraldehyde-3-phosphate dehydrogenase (GPD) – Basiodiomycete – Sequence – Evolution

Introduction

Glyceraldehyde-3-phosphate dehydrogenase (GPD or GAPDH, E.C. 1.2.1.12) is a key enzyme in glycolysis. The *GPD* gene is highly and constitutively expressed: 2–5% of the poly(A)$^+$ RNA of yeast may comprise GPD mRNA (Holland and Holland 1978; Edens et al. 1984), while up to 5% of the cellular protein may constitute GPD protein (Krebs 1953). The active holoenzyme is a tetramer composed of identical subunits, each with an approximate M_r of 38000.

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The development of transformation systems for homobasidiomycetes, based on selection for hygromycin B resistance and using heterologous expression signals to express the bacterial hygromycin B phosphotransferase gene, has been unsuccessful (Casselton and de la Fuente Herce 1989; Mooibroek et al. 1990; Challen et al. 1991; Royer and Horgen 1991). Only in *Laccaria laccata* have positive results been reported (Barrett et al. 1990). The isolation of strong endogenous promoters to drive the expression of heterologous genes in basidiomycetes would thus seem essential. Tho this end we have cloned the *GPD* genes from *Schizophyllum commune, Phanerochaete chrysosporium* and *Agaricus bisporus*.

Throughout the Kingdoms of organisms amino-acid sequences of GPD proteins are highly conserved (Smith 1989; Michels et al. 1991), which allows for an evolutionary analysis. The nucleotide and predicted amino-acid sequences of the homobasidiomycete *GPD*s are therefore compared with *GPD*s from several ascomycetes, the heterobasidiomycete *Ustilago maydis*, and with some plants and animals.

Materials and methods

Isolation of GPD genes. (1) *S. commune.* A genomic library of partial *Sau*3A genomic fragments of the *S. commune* monokaryotic strain 4-39 in the *Bam*HI site of λEMBL4 was screened with the 1.2 kb *Sca*I-*Sac*I fragment form pAN5-22, containing 267 codons of the *Aspergillus nidulans gpd*A gene (Punt et al. 1988). Hybridization was in 6 × SSC, 0.5% SDS, 5 × Denhardt's solution, 1.5 mg ml^{-1} denatured salmon sperm DNA at 58 °C; washings were done twice with 6 × SSC, 0.1% SDS, 0.1% NaPPi and twice with 3 × SSC, 0.1% SDS, 0.1% NaPPi at 58 °C, each for 30 min. Seven positive clones were isolated, all of which contained a 2.9 kb *Hin*dIII fragment that strongly hybridized with the *A. nidulans gpd*A gene. The 2.9 kb *Hin*dIII fragment from λ121 was subcloned (p121-9) and sequenced. (2) *P. chrysosporium.* A genomic library of the *P. chrysosporium* heterokaryotic strain ME-446, ATCC34541 in λEMBL3 (Schrank et al. 1991) was screened with the 1.4 kb *Pvu*II-*Hin*dIII fragment from p121-9, encoding the carboxy-terminal 315 aa of the *S. commune GPD* gene. Hybridization was in 0.5 M sodium phosphate pH 7.2, 7% SDS and 1% BSA (Church and Gilbert 1984) at 54 °C. Non-hybridized probe was removed by washing twice with 0.1 M

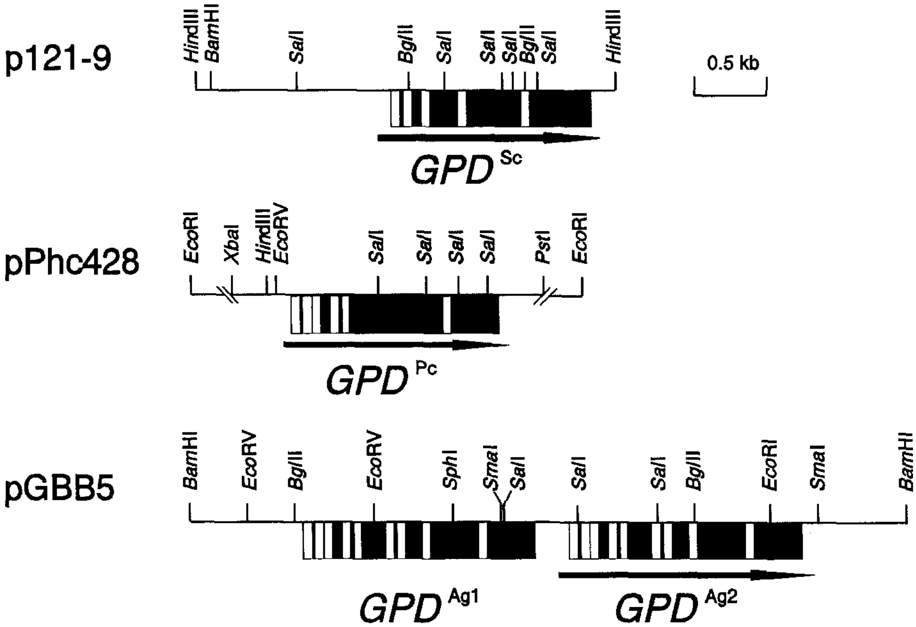

Fig. 1. Partial restriction maps of p121-9, pPhc428 and pGBB5 that contain the *GPD* gene(s) of *S. commune*, *P. chrysosporium* and *A. bisporus*, respectively. Exons and introns are indicated under each map by *black and white boxes* respectively, whereas the transcription direction is indicated by an *arrow*. The GPD^{Ag1} gene, for which no transcripts have yet been found, could be transcribed in the same direction as the GPD^{Ag2} gene

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     •-150                                                       •-100    ———                                             * •-50
ggcgcgcagt ccaggccgcc gccgatgtgt tcatcccgtt ttgtcagtat cgatcggatc tttcgggcgt gggtataaaa gcgcgccgcc cgccgtctcc ctctttctcc agcactccca tccagagcac ttccctctcc

                       •1                                             •50                                                                   •100
catcgcatcc catcacacaa ta ATG gtgagcaccg cgcgcgacgg cgagctcgca ggacgctgac atctgctcgc gatag GCC GTG AAG GTT GGA ATT AAC GGG TTC G gtgcgtacgg cccagattgg cgc
                         M                                                              A   V   K   V   G   I   N   G   F                                  10

                                         •150                                               •200
agatctcgcc ctgactccct ccgcag GT CGC ATT GGC CGT ATC GTT CTC CGT AAT GCC CTT CAG CTG GGC AAC ATC GAG GTC GTC GCC ATC AAC GA gtgagtgcag cgagctcagt ttccatcttc
                             G   R   I   G   R   I   V   L   R   N   A   L   Q   L   G   N   I   E   V   V   A   I   N   D                                 34

            •250                                                    •300                                                                          •350
aagtctgacg cgatctag C CCG TTC ATC GCC CTT GAC TAC ATG GTC TAC ATG TTC AAG TAC GAC ACC GTC CAC GGC CGC TAC AAG GGC ACC GTC GAG GTG AAG GAC GGC AAG CTC GTC
                      P   F   I   A   L   D   Y   M   V   Y   M   F   K   Y   D   T   V   H   G   R   Y   K   G   T   V   E   V   K   D   G   K   L   V     67

                                                     •400                                                     •450
GTC GAC GGC CAT GCC ATC ACC GTC TTC GCT GAG AAG AAC CCC GCC GAC ATC AAG TGG GGC TCT GCT GGC GCC GAC TAC ATC GTC GAG TCC ACC gtacgtcata tatgcgctcc catttcccgc
 V   D   G   H   A   I   T   V   F   A   E   K   N   P   A   D   I   K   W   G   S   A   G   A   D   Y   I   V   E   S   T                                   98

                       •500                                                    •550
acttcctaac ctctctcgtag GGT GTC TTC ACC ACC GTC GAG AAG GCG TCC CTG CAC TTG CAG GGC GGC GCG AAG AAG GTC GTC ATC TCG GCG CCC TCC GCC GAC GCC CCG ATG TTC GTC
                        G   V   F   T   T   V   E   K   A   S   L   H   L   N   G   G   A   K   K   V   V   I   S   A   P   S   A   D   A   P   M   F   V   131

  •600                                                   •650                                                              •700
GTC GGC GTC AAC CTC GAC AAG TAC GAC TCG AAG TAC CAG GTC ATC TCG AAC GCG TCC TGC ACG ACC AAC TGC CTG GCG CCG CTC GCG AAG GTC ATC CAC GAC AAG TAC GGC ATC GCG
 V   G   V   N   L   D   K   Y   D   S   K   Y   N   V   I   S   N   A   S   C   T   T   N   C   L   A   P   L   A   K   V   I   H   D   K   Y   G   I   A   170

                                         •750                                                  •800
GAG GGT CTC ATG ACC ACC GTG CAC GCC ACG ACC GCG ACC CAG AAG ACC GTC GAC GGC CCG TCG CAC AAG GAC TGG CGC GGT GGC CGC TCG GTC AAC AAC AAC ATC ATC CCC TCG TCG
 E   G   L   M   T   T   V   H   A   T   T   A   T   N   K   T   V   D   G   P   S   H   K   D   W   R   G   G   R   S   V   N   N   N   I   I   P   S   S   209

                       •850                                                         •900                                                      •950
ACT GGT GCC GCT AAG GCC GTC GGC AAG GTC ATC CCC TCG CTC AAC GGG CGC CTC AC gtacgtctga tctgtccaga tctccttagc ggcctcc taatctcttc cccacag C GGC CTC GCC TTC CGT
 T   G   A   A   K   A   V   G   K   V   I   P   S   L   N   G   R   L   T                                                                G   L   A   F   R   233

                                               •1000                                                      •1050
GTC CCG ACC CTC GAC GTC TCC GTC GTC GAC CTC GTC GTC CGC CTC GAG AAG GAG GCG TCG TAC GAC GAG ATC GTC GCG ACC GTC AAG GAG GCG TCC GAG GGC CCG CTC AAG GGC ATC
 V   P   T   L   D   V   S   V   V   D   L   V   V   R   L   E   K   E   A   S   Y   D   E   I   V   A   T   V   K   E   A   S   E   G   P   L   K   G   I   272

                             •1100                                                      •1150
CTC GGC TTC ACC GAC GAG TCC GTC GTC TCG ACC GAC TTC ACC GGC GCG AAC GAG TCG TCC ATC TTC GAC TCC AAG GCC GGC ATC GCG ATC AGC AAG AGC TTC GTG AAG CTC ATT GCC
 L   G   F   T   D   E   S   V   V   S   T   D   F   T   G   A   N   E   S   S   I   F   D   S   K   A   G   I   A   I   S   K   S   F   V   K   L   I   A   311

           •1200                                                                        ———————————————————————————— •1300
TGG TAC GAC AAC GAG TGG GGC TAC TCC CGC CGT GTC TGC GAC CTG CTC GTC TAC GCG GCG AAG CAG GAC GGC GCG CTC TAA gcggcgaagc aagacggcgc gctctaagtg cctgatcctt
 W   Y   D   N   E   W   G   Y   S   R   R   V   C   D   L   L   V   Y   A   A   K   N   D   G   A   L   -                                                     337

                                   •1350                       •1400
tggacgcttg tactttacct ctacatagac tgggccggag agtaaaagaa gaaaggaatg taacgttgta caacatgtct aataattgta cgatcccaaa
                                            ————                                ↑
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Fig. 2. Nucleotide sequence and derived amino-acid sequence of the *GPD* gene of *S. commune*. Exon sequences are *capitalized*, conserved internal intron sequences are *underlined*. Numbering of nucleotides is with respect to the start of the coding sequence. Putative TATA and CAAT boxes are *single and double overlined*, respectively. The putative polyadenylation signal of GPD^{Sc} has been *double underlined*. The major *tsp* is marked with an *asterisk*. The last base of the transcript is marked with an *arrow*. A 27-nt repeat in the 3′ end of the gene has been *double overlined*

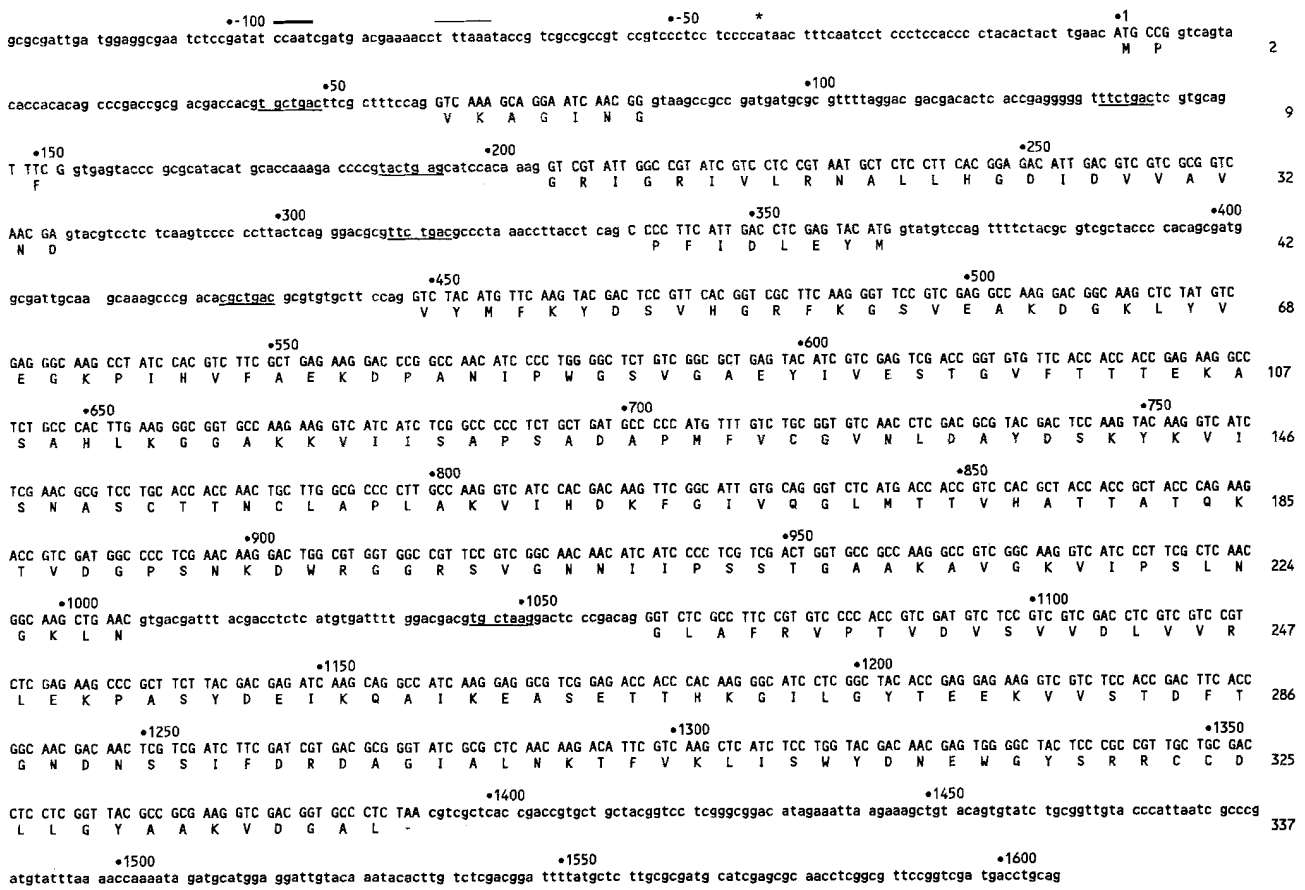

Fig. 3. Nucleotide sequence and derived amino-acid sequence of the *GPD* gene of *P. chrysosporium*. For details, see legend to Fig. 1

sodium phosphate pH7.2, 0.2% SDS at 54 °C for 30 min. Four positive phages were isolated. Of these λPhc42 and λPhc52 contained strongly hybridizing *Eco*RI fragments of 8 and 7 kb, respectively. The 8 kb fragment from λPhc42 was subcloned (pPhc428) and relevant parts sequenced. (3) *A. bisporus*. A genomic library of partial *Sau*3A fragments of *A. bisporus* (strain Horst® U3), constructed in the *Xho*I site of λFIX II, was screened with the 1.2 kb *Sca*I-*Sac*I fragment form pAN5-22, containing the *A. nidulans gpd*A sequence (Harmsen et al. 1991). Hybridizations were done as described for the isolation of *P. chrysosporium* clones. One positive clone (λABU3-412) contained a strongly hybridizing 5 kb *Bam*HI fragment that was subcloned (pGBB5) and sequenced.

Sequence analyses. After restriction mapping a series of overlapping subclones of p121-9, pPhc428 and pGBB5 were constructed in pUC-derived vectors. Both strands from the derived subclones were sequenced at least twice by the dideoxy chain-termination method using T7 DNA polymerase (Tabor and Richardson 1987).

Transcript mapping. The transcription start points (*tsp*) of the *GPD* genes were determined using primer extension with AMV reverse transcriptase. Total ssRNA (20 µg) in 120 µl 250 mM KCl, 10 mM Tris-HCl pH 8.3, 1 mM EDTA containing 66 ng of the ^{32}P-labelled oligonucleotide was heated at 80 °C for 3 min after which annealing took place at 45 °C for 1 h. The mixture was then brought to 160 µl 188 mM KCl, 70 mM Tris-HCl pH 8.3, 7.5 mM $MgCl_2$, 6 mM DTT, 1.25 mM of each dATP, dTTP, dCTP, dGTP and 20 U of AMV reverse transcriptase. Reverse transcription was done at 42 °C for 30 min. The products were precipitated and analyzed on a 6% sequence gel alongside with the products of sequencing reactions to determine the *tsp*. The following were used as primers: 5'-ATTCCCGGGCATTATTGTGTGATGGGATG-3' for *S. commune*, 5'-GAGGACGATACGGCC-3' for *P. chrysosporium* and 5'-CATTACGGAGGACAATG-3' for *A. bisporus*. The 3' end of the *S. commune* GPD mRNA was determined by S1 nuclease protection mapping, performed as previously described (Schuren and Wessels 1990), using the 511 nt *Xho*I-*Hin*dIII fragment cloned into M13mp18.

Computer analyses. The complete nucleotide and derived amino-acid sequences were analyzed using the PC/GENE programs package (version 6.01). The amino-acid sequence and the position of introns was determined by comparison with known GPD amino-acid sequences and by comparison with intron consensus sequences. Multiple sequence alignments were done with CLUSTAL (Higgins and Sharp 1988) and the PALIGN program of PC/GENE which both use the algorithm of Myers and Miller (1988). Sequences from the *GPD* genes of *U. maydis* (Smith and Leong 1990), *A. nidulans* (Punt et al. 1988), *Aspergillus niger* (R. F. M. van Gorcom unpublished results) *Curvularia lunata* (Osiewacz and Ridder 1991), *Cryphonectria parasitica* (Choi and Nuss 1990), *Saccharomyces cerevisiae* 1 (Holland and Holland 1980), *S. cerevisiae* 2 (Holland and Holland 1979), *S. cerevisiae* 3 (Holland et al. 1983), *Kluyveromyces lactis* (Shuster 1990), *Zygosaccharomyces rouxii* (Imura et al. 1987), tobacco (Shih et al. 1986), hamster (Vincent and Fort 1990), human liver (Arcari et al. 1984), chicken (Stone et al. 1985), *Drosophila melanogaster* (Tso et al. 1985), *Homarus americanus* (Davidson et al. 1967), *Schistosoma mansoni* (Goudot-Crozel et al. 1989), *Caenorhabditis elegans* (Huang et al. 1989), maize (Brinkmann et al. 1987) and mustard (Martin and Cerff 1986) were obtained from the EMBL (Release 27) and SWPROT (Release 18) data libraries or manually entered using PC/GENE.

A

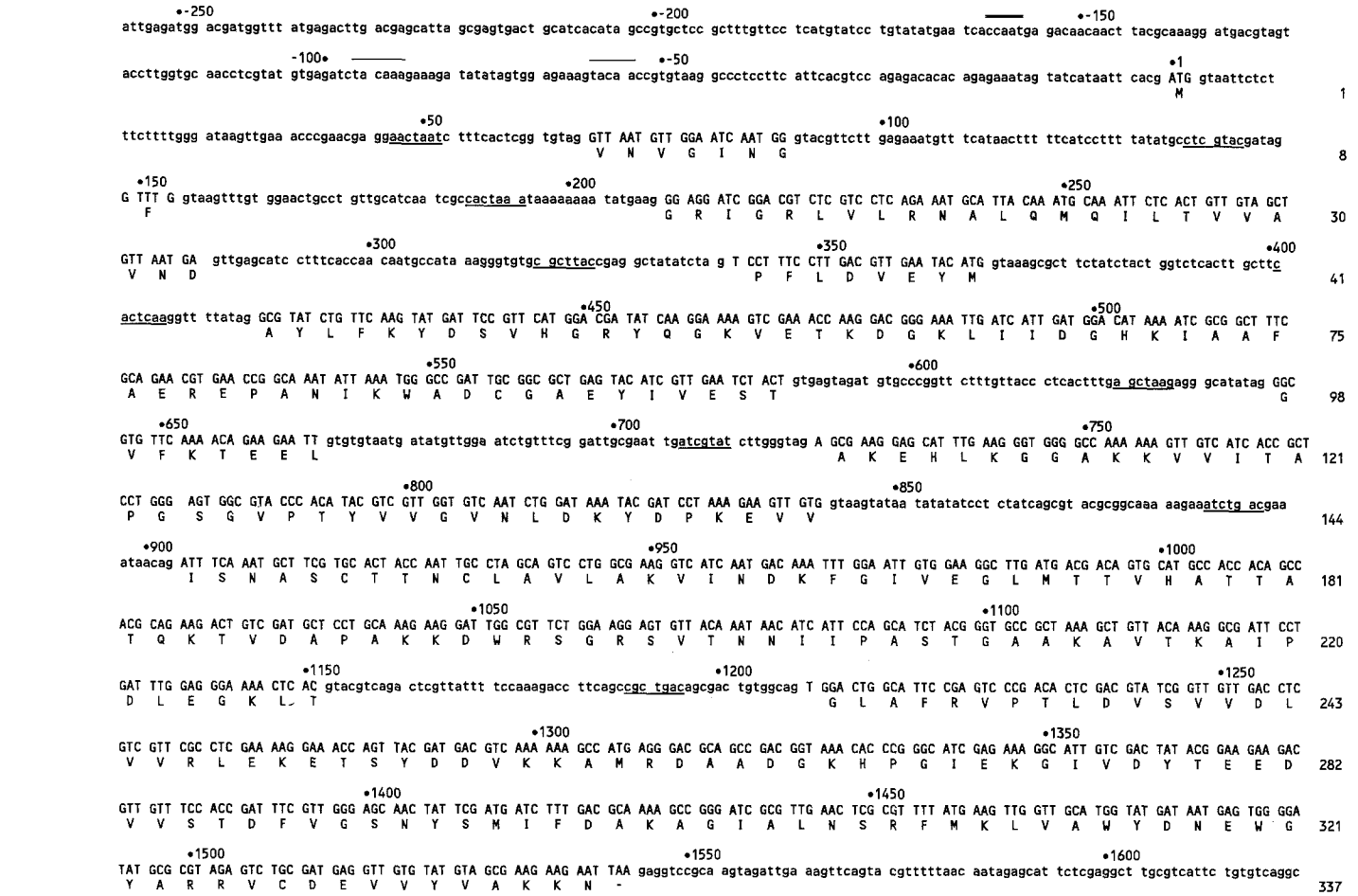

Fig. 4 A, B. Nucleotide sequence and derived amino-acid sequence of the two tandemly linked *GPD* genes of *A. bisporus;* GPD^{Ag1} (**A**) and GPD^{Ag2} (**B**). A hexameric repeat of aa in the predicted protein of GPD^{Ag2} has been *underlined* (**B**). For other details, see legend to Fig. 1

Results

GPD sequences

The *GPD* gene of *S. commune* was isolated first, using the *gpd*A gene from *A. nidulans* as a probe. Southern blots of *P. chrysosporium* DNA hybridized more strongly with the *GPD* gene from *S. commune* than with that of *A. nidulans*. The former gene was, therefore, used to isolate the *GPD* gene from *P. chrysosporium*. Both probes hybridized to the same fragments on Southern blots of *A. bisporus* DNA, but the *S. commune GPD* gene also hybridized faintly to other fragments. Consequently, the *A. nidulans GPD* gene was used to recover the *A. bisporus* gene. Unexpectedly, sequencing of the 5 kb *Bam*HI fragment from clone pGBB5 revealed the presence of two *GPD* genes on this fragment (Fig. 1). The complete sequences of the *GPD* genes of *S. commune* (GPD^{Sc}, Fig. 2), *P. chrysosporium* (GPD^{Pc}, Fig. 3) and *A. bisporus* (GPD^{Ag1} and GPD^{Ag2}, Fig. 4A, B) were determined from overlapping subclones of p121-9, pPhc428 and pGBB5, respectively. The predicted GPD proteins (shown in Figs. 2–5) all contain 337 aa residues, except for GPD^{Ag2} which encodes 338 aa. Of the two tandemly linked *GPD* genes of *A. bisporus* only GPD^{Ag2} appears highly expressed in both mycelium and fruit bodies (Harmsen et al. 1991). The mRNA lengths for GPD^{Sc}, GPD^{Pc} and GPD^{Ag2} were determined to be approximately 1.25 kb in all three cases (data not shown).

For all four *GPD* genes TATA box-like elements can be discerned (overlined in Figs. 2–4). GPD^{Sc}, GPD^{Pc}, GPD^{Ag1} and GPD^{Ag2} contain five, six, nine and nine short introns, respectively, with an average size of 56 nt. The initiator codon for Met is immediately followed by an intron, except in GPD^{Pc} where a Pro is present between Met and the first intron. In GPD^{Pc}, GPD^{Ag1} and GPD^{Ag2} another small exon of 5 nt (encoding Phe) is present. The average G+C contents of GPD^{Sc}, GPD^{Pc} and $GPD^{Ag1+Ag2}$ are 60%, 58% and 47%, respectively, similar to reported values (58%, 59% and 43–45%, respectively) for total genomic G+C composition (Dons et al. 1979; Raeder and Broda 1984; Horgen et al. 1984). The coding parts of the genes have a 5–14% higher G + C fraction than the introns and other non-coding regions.

B

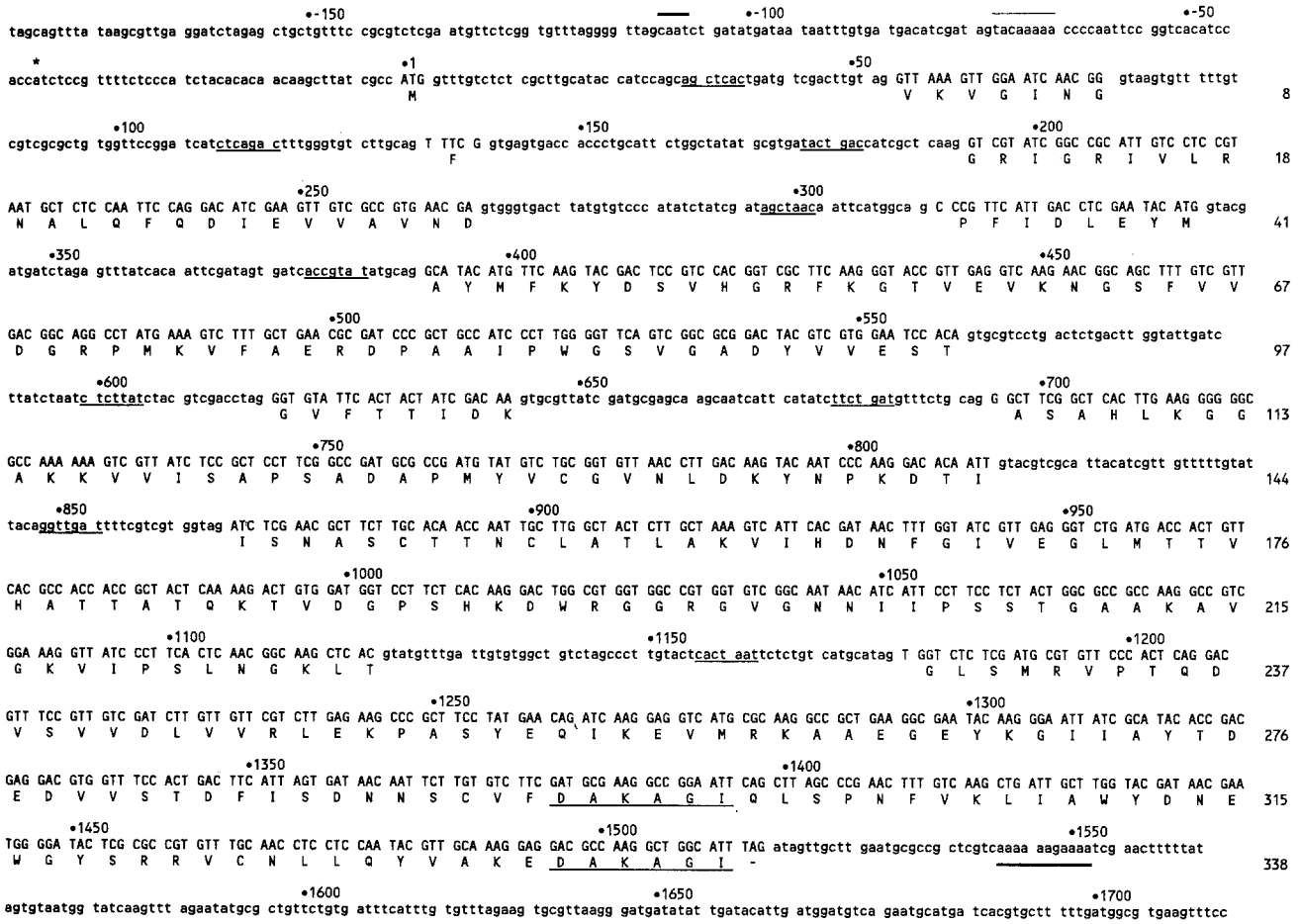

Fig. 4B (continued)

Protein and codons

The amino-acid sequences of the derived GPD proteins are highly conserved (Fig. 5). The codon usage of GPD^{Sc} and GPD^{Pc} is heavily biased with 63% and 60% C at the third position of the codons, respectively. This is less pronounced for GPD^{Ag2}, which has only 40% codons that end with C. GPD^{Ag1}, for which no transcript was found, shows no clear codon bias.

Discussion

Gene structure and expression

A consensus TATA box (TATAAAA) is present only in GPD^{Sc} (overlined in Fig 2). In GPD^{Pc} and GPD^{Ag2} this element may be represented by TTAAAT and TACAAAAA, respectively, because these sequences are located at the expected position, i.e., at −31 to −36 nt from the major *tsp* (overlined in Figs. 3 and 4B). In GPD^{Ag1}, homologs of a TATA box, TACAAA and TACAA, are found at −96 nt and −68 nt from the start codon (overlined in Fig. 4A). In the three active *GPD* genes these putative TATA elements are followed by pyrimidine-rich stretches which generally precede the *tsp* in fungal genes (Gurr et al. 1987). Sequences conserved between *A. nidulans* and *A. niger GPD* promoter regions, such as a *gpd* box, *pgk* box, *qut* box and *qa* box (Punt et al. 1990), are not present in the promoter regions of the four basidiomycetous *GPD* genes. These features, in addition to the absence of clear TATA boxes in these genes may (partly) explain why bacterial genes driven by the *A. nidulans GPD* promoter are poorly expressed in the basidiomycetes *S. commune* (Mooibroek et al. 1990) and *Coprinus cinereus* (Casselton and de la Fuente Herce 1989). Remarkably, the reverse does not seem to hold because the isocitrate lyase gene of the basidiomycete *C. cinereus* has been expressed in *A. nidulans* (Hynes 1989); similarly, the *ADE2* gene from the adenine biosynthetic pathway of *S. commune* also functions in *Neurospora crassa* (Alic et al. 1990).

The GPD^{Ag2} gene is strongly expressed in both mycelium and fruit bodies of *A. bisporus* (Harmsen et al. 1991). Only 223 bp separate the *tsp* of this gene from the last putative codon of the inactive GPD^{Ag1} gene. Neither in

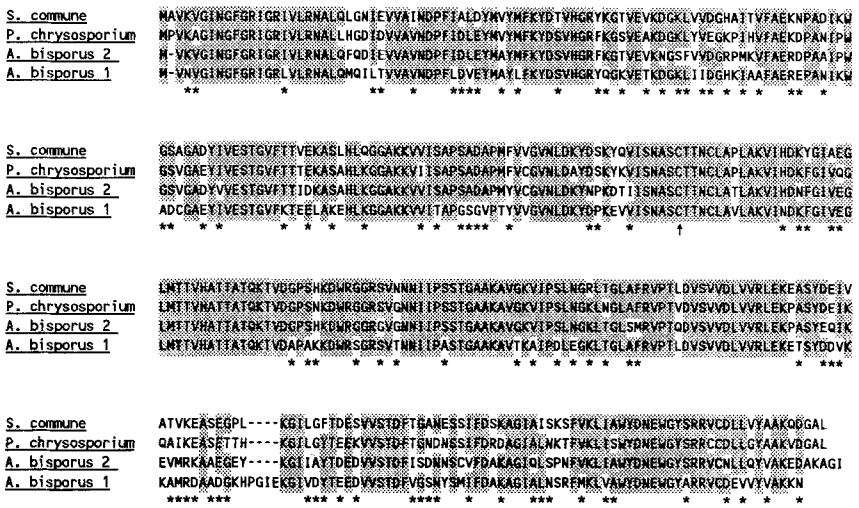

Fig. 5. Multiple sequence alignment of the aa sequences of the GPD proteins from *S. commune*, *P. chrysosporium* and *A. bisporus* (1 and 2). Identical residues in all four sequences are *shaded* and conservative substitutions are marked under the alignment with an *asterisk*. The *arrow* marks the Cys residue within the active site

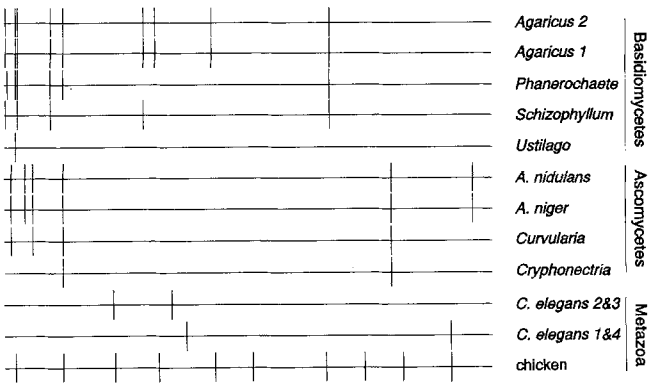

Fig. 6. Comparison of intron positions in *GPD* genes; *horizontal lines* represent the coding region (336–338 codons) of each *GPD* gene. The *vertical bars* mark the position of introns

mycelium nor in fruit bodies did we detect transcripts of GPD^{Ag1}. This suggests that all sequences necessary for efficient transcription of the GPD^{Ag2} gene are located within this relatively short stretch of DNA. The predicted protein of GPD^{Ag1} is homologous to the highly expressed GPDs of all three basidiomycetes (Fig. 5), although GPD^{Ag1} and GPD^{Ag2} do not cross-hybridize due to a large number of differences in the third base of codons which often conserve the encoded amino acid. Also the main residue defining the binding site of glyceraldehyde-3-phosphate, Cys-150 (marked with an arrow in Fig. 5), is present in the putative gene product of GPD^{Ag1}, suggesting that the gene does encode a functional GPD protein. In addition, all nucleotide sequences required for transcription and subsequent splicing appear to be present. Therefore, we suggest that the GPD^{Ag1} gene is active at some other phase of the life cycle or under particular environmental conditions, for example during anaerobiosis occurring in the lower layers of compost during the colonization phase. In maize, of three *GPD* genes coding for cytosolic proteins, one is only expressed during anaerobiosis (Russell and Sachs 1989). However, the possibility that GPD^{Ag1} is a pseudogene cannot be excluded.

Although the number of introns in the four homobasidiomycetous *GPD* genes differ, their positions are strongly conserved (Fig. 6); even to the nucleotide where a codon is interrupted (cf. Figs. 2–4). The position of the single intron in the *GPD* gene of the heterobasidiomycete *U. maydis* is also conserved among the three investigated homobasidiomycetes. Within the ascomycetous species that have been studied (Fig. 6), intron positions in the *GPD* genes are also conserved; contrary to an earlier report (Osiewacz and Ridder 1991) all intron positions in the *Curvularia lunata GPD* gene are also present in the *Aspergillus GPD* genes. On the other hand, only the position of one intron is found to be conserved between basidiomycetes and ascomycetes (Fig. 6). An intron is also found at this position in chicken but the other introns in metazoan *GPD* genes are located differently from those in fungi. This comparison suggests that there is a clear boundary between ascomycetes and basidiomycetes with respect to intron positions within the *GPD* genes.

Amino-acid sequence comparisons

Table I shows the large extent of homology between GPD proteins in various organisms. Except for GPD^{Ag1}, the similarity of GPD proteins within the filamentous fungi (ascomycetes and basidiomycetes) is higher than between filamentous fungi and yeasts. The unique position of the yeasts is further illustrated by the construction of a tree representing amino-acid sequence divergence between GPDs predicted with the CLUSTAL program (Fig. 7). The tree is essentially similar to the phylogenetic trees constructed by Smith (1989), using an evolutionary parsimony method (Lake 1987) on *GPD* nucleotide sequences, and Michels et al. (1991), who examined GPD amino-acid sequences using a distance matrix method (Fitch and Margoliash 1967). However, we have expanded the number of fungal GPDs. Our analysis reveals that basidiomycetes and ascomycetes are two diverged groups of fungi, which agrees well with the analysis of intron positions (Fig. 6). This is in accordance with the classification

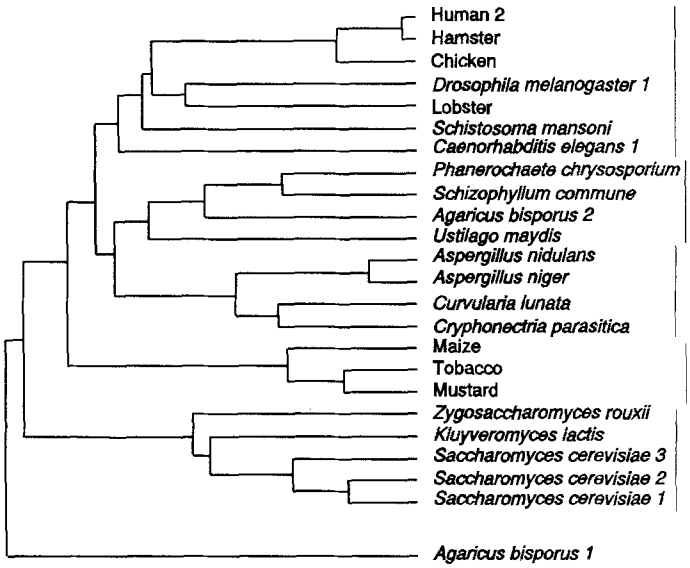

Fig. 7. Phylogenetic tree of similarities in amino-acid sequences of *GPD* genes from various organisms. The GPD^{Ag1} gene product differed greatly from all other known sequences

Table 1. Comparison of GPD amino-acid sequences with the Myers and Miller (1988) algorithm: percentage amino-acid identitiy between all analyzed fungal species, a plant and a mammalian species

Species	Basidiomycetes					Filamentous ascomycetes				Yeast-like ascomycetes					Other	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1 *A. bisporus* 1	100	70	69	70	64	63	64	59	62	62	61	61	64	59	62	63
2 *A. bisporus* 2		100	76	77	70	69	69	67	70	62	63	63	66	63	67	70
3 *P. chrysosporium*			100	81	75	71	71	70	69	60	62	62	62	62	68	71
4 *S. commune*				100	72	69	69	69	67	64	64	64	67	62	69	70
5 *Ustilago maydis*					100	72	72	73	69	63	65	63	64	67	68	73
6 *Aspergillus nidulans*						100	90	81	79	63	66	65	64	64	67	70
7 *Aspergillus niger*							100	80	77	64	67	66	65	67	69	72
8 *Curvularia lunata*								100	82	62	64	63	64	65	69	69
9 *Cryphonectria parasitica*									100	64	65	64	66	65	68	69
10 *Saccharomyces cerevisiae* 1										100	95	87	80	79	64	65
11 *Saccharomyces cerevisiae* 2											100	87	82	80	67	66
12 *Saccharomyces cerevisiae* 3												100	81	78	66	64
13 *Kluyveromyces lactis*													100	78	68	65
14 *Zygosaccharomyces rouxii*														100	68	64
15 *Nicotiana tabacum* (tobacco)															100	68
16 *Cricetulus griseus* (hamster)																100

based on morphological characteristics. However, the classification of the analyzed ascomycetous yeasts within the ascomycetes is not reflected in GPD similarities. It is conceivable that this divergence from the filamentous ascomycetes reflects the unicellular mode of growth of yeasts and/or their facultative fermentative metabolism. With respect to unicellular growth, a mutation in a single nucleus could more readily be fixed in a population than in a multicellular filamentous fungus, thus allowing for a more rapid rate of evolution. Another conclusion from Fig. 7 is that the *GPD*s from filamentous fungi appear more closely related to the *GPD*s from metazoa than to those from plants.

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