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Comparison of gene expression in trap cells and vegetative hyphae of the nematophagous fungus *Monacrosporium haptotylum*

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Nematode-trapping fungi enter the parasitic stage by developing specific morphological structures called traps. The global patterns of gene expression in traps and mycelium of the fungus *Monacrosporium haptotylum* were compared. The trap of this fungus is a unicellular spherical structure called the knob, which develops on the apex of a hyphal branch. RNA was isolated from knobs and mycelium and hybridized to a cDNA array containing probes of 2822 EST clones of *M. haptotylum*. Despite the fact that the knobs and mycelium were grown in the same medium, there were substantial differences in the patterns of genes expressed in the two cell types. In total, 23·3% (657 of 2822) of the putative genes were differentially expressed in knobs versus mycelium. Several of these genes displayed sequence similarities to genes known to be involved in regulating morphogenesis and cell polarity in fungi. Among them were several putative homologues for small GTPases, such as *rho1*, *rac1* and *ras1*, and a rho GDP dissociation inhibitor (*rdi1*). Several homologues to genes involved in stress response, protein synthesis and protein degradation, transcription, and carbon metabolism were also differentially expressed.

In the last category, a glycogen phosphorylase (*gph1*) gene homologue, one of the most upregulated genes in the knobs as compared to mycelium, was characterized. A number of the genes that were differentially expressed in trap cells are also known to be regulated during the development of infection structures in plant-pathogenic fungi. Among them, a *gas1* (*mas3*) gene homologue (designated *gks1*), which is specifically expressed in appressoria of the rice blast fungus, was characterized.

**INTRODUCTION**

The nematophagous fungi comprise more than 200 species of taxonomically diverse fungi that all share the ability to infect and kill living nematodes (Barron, 1977). The interest in studying these fungi arises from their potential use as biological control agents for nematodes that are parasitic on plants and animals (Larsen, 2000). Other reasons for the continued fascination with nematophagous fungi are their remarkable morphological adaptations and their dramatic infection of nematodes. The nematode-trapping fungi are particularly interesting in this respect. These fungi enter the parasitic stage by developing specific morphological structures called traps. The traps develop from hyphal branches and they can be formed either spontaneously or be induced in response to signals from the environment, including peptides and other compounds secreted by the host nematode (Dijksterhuis et al., 1994). There is a large variation in the morphology of trapping structures, even between closely related species (Ahrén et al., 1998; Barron, 1977). In some species, the trap consists of an erect branch that is covered with an adhesive material. In other species, such as in the well-studied *Arthrobotrys oligospora*, the trap is a complex three-dimensional net. A third type of trap is the adhesive knob. The knob is a morphologically distinct cell, often produced on the apex of a slender hyphal stalk. A layer of adhesive polymers covers the knob, which is not present on the support stalks. Finally, there are some species of nematode-trapping fungi that capture nematodes in mechanical traps called constricting rings (Barron, 1977).
The ultrastructure of the nematode-trapping structures has been examined extensively. These studies have shown that despite the large variation in morphology, the adhesive types of trap (branches, nets and knobs) have a unique ultrastructure that clearly distinguishes them from vegetative hyphae (Dijksterhuis et al., 1994). One feature, which is common to all of these traps, is the presence of numerous cytosolic organelles, the so-called dense bodies. Although the function of these organelles is not yet clear, the fact that they exhibit catalase and D-amino acid oxidase activity indicates that the dense bodies are peroxisomal in nature (Dijksterhuis et al., 1994). Another feature common to the trap cells is the presence of extensive layers of extracellular polymers, which are thought to be important for attachment of the traps to the surface of the nematode (Tunlid et al., 1991).

In this study we have analysed the global pattern of gene expression in knobs and mycelium of the fungus *Monacrosporium haptotylum* (syn. *Dactyliaria candida*) (Fig. 1). The advantage of using *M. haptotylum* is that during growth in liquid cultures with heavy aeration, the connections between the traps (knobs) and mycelium can be broken easily and the knobs can be separated from the mycelium by filtration (Friman, 1993). The isolated knobs retain their function as infection structures: they can ‘capture’ and infect nematodes. In order to characterize overall gene expression, we have conducted expressed sequence tag (EST) analysis with 8466 clones from four different cDNA libraries. These include libraries made from mycelium and knobs, and two libraries from knobs infecting the nematode *Caenorhabditis elegans*. Since the genome of *C. elegans* has been sequenced, transcripts expressed by the fungus were easily separated from those of the host. From the EST information assembled, we obtained a unique set of 3036 clones, of either fungal or worm origin, which was used for the construction of cDNA microarrays. Here we describe the analysis of gene expression patterns in knobs and mycelium.

**METHODS**

**Culture of organisms and infection experiments.** *Monacrosporium haptotylum* (CBS 200.50) was grown in aerated 5 l liquid cultures containing 0·01 % soya peptone, 0·005 % phenylalanine and 0·005 % (w/v) valine. The knobs were separated from the vegetative mycelium by filtering the culture through a nylon mesh (10 μm), and collected from the filtrate on a 1-2 μm pore-size membrane filter (Friman, 1993). The vegetative mycelium and knob fractions were immediately frozen in liquid nitrogen and stored at −80 °C. *C. elegans* (strain N2) was grown using standard protocols (Brenner, 1974). To partly synchronize the nematodes, they were treated with 0·25 % KOH and 2·5 % NaOCl (Emmons et al., 1979). L1 larvae were incubated on water agar plates with freshly isolated knobs from *M. haptotylum*, and the infection was followed under a light microscope. After both 4 h and 24 h, the infected nematodes were collected by floating the plates with water. The samples were centrifuged and frozen in liquid nitrogen.

**RNA isolation and construction of cDNA libraries.** Total RNA was extracted from the vegetative mycelium, the knobs and infected *C. elegans* using the RNeasy plant mini kit (Qiagen) with the guanidinium isothiocyanate buffer provided. The integrity of the total RNA was checked by denaturing formaldehyde agarose gel electrophoresis. Four unidirectional cDNA libraries were constructed from total RNA using the PCR-based SMART cDNA Library Construction kit (BD Clontech). After ligation of size-fractionated cDNA (>100 bp) into the *λ*TriplEx2 arms (BD Clontech) and lambda phage packaging (GigaPack III Gold extract; Stratagene), the primary titres for the unamplified libraries were determined to be 5·9×10⁶ p.f.u. for mycelium, 1·6×10⁶ p.f.u. for knob, 2·5×10⁶ p.f.u. for the ‘early’ stage of *C. elegans* infection (4 h) and 2·9×10⁶ p.f.u. for the ‘late’ stage of *C. elegans* infection (24 h). In order to amplify the libraries, phage suspensions were used to transduce *Escherichia coli* XL-1 Blue, which resulted in titres of more than 5×10⁹ p.f.u. ml⁻¹. The *λ*TriplEx2 libraries were converted to plasmid libraries by in vivo excision in *E. coli* BM25.8 (Johansson et al., 2004). PCR amplification followed by gel electrophoresis demonstrated that more than 90 % of the randomly collected clones contained insert. Based on the analysis of 96 individual clones from each of the four libraries, the average sizes of the inserts in the mycelium, knobs, 4 h infection and 24 h infection cDNA libraries were found to be approximately 1100, 1000, 750 and 1200 bp, respectively. The size of the transcripts ranged from 120 to 2400 bp in all four libraries.

**EST analysis.** Plasmid clones were randomly collected from each of the four libraries. The colonies were transferred to 96-well plates, and the bacterial lysates were used as starting material for PCR amplification (Johansson et al., 2004). PCR was performed using pTriplEx2-specific universal primers P104 (5’-GGGAAGCGCGCC-ATTGTGTT-3’) and P105 (5’-AGTGAGCGCTGAATTCGGGC-3’) in a total volume of 10 μl, followed by assessment of the size and quality of the products by gel electrophoresis. Partial nucleotide sequences of amplified cDNA inserts were determined using the P104 primer and the dideoxy chain-termination method employing the BigDye Terminator Cycle Sequencing kit (Applied Biosystems). The sequencing reaction products were analysed using either a 377 or 3100 ABI sequencer (Applied Biosystems). cDNA clones representing genes that are discussed in more detail were verified and completely sequenced by using universal and gene-specific primers (Table 1).

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**Fig. 1.** Trapping structures of *Monacrosporium haptotylum*. The knob cell is a spherical cell formed on the apex of a hyphal branch. The picture is reproduced from Nordbring-Hertz et al. (1995), courtesy of Birgit Nordbring-Hertz and IWF, Göttingen.
Base-calling of DNA sequencer traces was conducted using the PHRED program with the quality level set to 20 (Ewing et al., 1998). Subsequently, poly(A) and vector sequence information was removed and the sequences from all three stages were assembled into contigs using the Contig Assembly Program (CAP) (Huang, 1992). After translation into all three forward frames, similarity analyses were performed by FASTA searches (Pearson, 1994) against the GenBank non-redundant (nr) protein database. Based on sequence homology, the ESTs were assigned functional roles and EC numbers (where applicable). Functional roles were classified into categories according to catalogues used for the Saccharomyces cerevisiae genome provided by the Munich Information Centre for Protein Sequences (MIPS) (Mewes et al., 2000). All EST information was stored in an SQL database (MySQL) on a Linux (Red Hat 6.2) platform, and the data were processed using the PHOREST tool (Ahren et al., 2004). Of 8466 EST clones analysed, 7115 high-quality ESTs (>99 bp) were deposited in the GenBank dbEST database and are available by accession numbers CN794415 to CN801529.

To separate fungal sequences from worm sequences, a local BLASTN search was performed against 22 055 C. elegans cDNA sequences downloaded from the ENSEMBLE website (http://www.ensembl.org).
Caenorhabditis elegans). Sequences with an E value $<1 \times 10^{-4}$ were annotated as C. elegans and sequences with an E value $>1 \times 10^{-4}$ were annotated as fungal. Sequences of $<50$ bp were annotated as unknown.

**Construction of cDNA arrays.** A single EST cDNA clone was selected to represent each assembled sequence (i.e. putatively unique transcript). For an assembled contig represented by multiple ESTs, the rule followed was to select the clone with the most extensive DNA sequencing read length. These clones were collected from the master EST library stock contained as bacterial lysate plates and aliquots were transferred into new 96-well plates. Plasmid inserts were amplified by PCR, purified and concentrated as previously described (Johansson et al., 2004). In total, 3518 clones representing approximately 3036 contigs were processed successfully. cDNA PCR products were printed on CMT-GAPSII-coated slides (Corning Glass) using a 16-pin configured MicroGrid II array printer (BioRobotics) controlled by the MicroGrid TAS Application Suite (version 2.2.0.6). The general design of the microarrays was two identical tool arrays, each containing 16 blocks (sub-arrays) and with each clone replicated twice within each block, which provided on-chip quadruplicates for each clone. Additionally, various positive and negative control reporters were replicated in various positions within each block, such as positive homologous controls, that is, M. haptotylum and C. elegans genes expected to be highly expressed. In addition, eight different heterologous and commercial PCR reporters (ArrayControl; Ambion) for which complementary RNAs were spiked into the amplification and labelling process of targets were also included in the blocks. Each control reporter was present in up to 64 per-chip replicates. DNA was cross-linked to the slides by baking at 80°C for 2 h in glass jars, followed by UV cross-linking at 90 mJ cm$^{-2}$. Spot and print quality were assessed by staining with POP03 dye (Molecular Probes), and subsequent scanning showed that no spots were missing or joined on the slides.

**Microarray analysis.** To compare the expression pattern of genes in mycelium and knobs of M. haptotylum, our microarray experiments were designed as two-samples comparisons (Kerr & Churchill, 2001; Wu, 2001) (i.e. knobs versus mycelium) using three independent biological replicates, which also included technical and dye-swapped control hybridizations. Nine cDNA microarrays were printed on-chip quadruplicates for each clone. Additionally, various positive and negative control reporters were replicated in various positions within each block, such as positive homologous controls, that is, M. haptotylum and C. elegans genes expected to be highly expressed. In addition, eight different heterologous and commercial PCR reporters (ArrayControl; Ambion) for which complementary RNAs were spiked into the amplification and labelling process of targets were also included in the blocks. Each control reporter was present in up to 64 per-chip replicates. DNA was cross-linked to the slides by baking at 80°C for 2 h in glass jars, followed by UV cross-linking at 90 mJ cm$^{-2}$. Spot and print quality were assessed by staining with POP03 dye (Molecular Probes), and subsequent scanning showed that no spots were missing or joined on the slides.

**Real-time quantitative PCR.** aRNA was reverse transcribed into cDNA using the SYBR Green RT-PCR Reagents kit (Applied Biosystems). The reverse transcription (RT) reactions were normalized to contain equivalent amounts (2 µg) of aRNA. RT reactions were primed by random hexamers and were carried out in a total volume of 10 µl according to the manufacturer’s instructions. The cDNA product was treated with 2 units of RNase H (Invitrogen) for 20 min at 37°C, followed by heat inactivation at 75°C for 10 min.

Gene-specific oligonucleotide primers were designed by using the web-based primer picking service (Primer3) (http://fokker.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) (Rozen & Skaletsky, 2000). The following criteria were applied: melting temperatures 58–60°C, primer lengths 20–22 nt, GC content 40–80%, PCR amplicon length 100–200 bp (Table 1). The PCR reactions were performed with an Mx3000P Real-Time PCR system (Stratagene) using SYBR Green PCR Master Mix (Applied Biosystems) to monitor dsDNA synthesis. The reactions contained 12.5 µl 2× SYBR Green PCR Master Mix (including AmpliTaq Gold DNA Polymerase, dNTPs with dUTP, SYBR Green I Dye, Passive Reference and optimized buffer components), 1 µl cDNA (diluted 1:10), 1 µl gene-specific forward and reverse primers (0.5 µmol l$^{-1}$ of each), in a final volume of 25 µl. The following thermal profile was used for all PCRs: an initial denaturation step at 95°C for 10 min, followed by 100 cycles at 95°C for 30 s, 58°C for 1 min, and 72°C for 1 min, and finally extended elongation at 72°C for 30 s. Reactions were set up in triplicate, including a control with no template. Amplification of gene-specific products was analysed by melting-curve analysis followed by agarose gel electrophoresis (cf. Fig. 3).

The real-time RT-PCR data were analysed using relative quantification according to the 2$^{-\Delta\Delta C_{T}}$ method (Livak & Schmittgen, 2001) (Stratagene software for the Mx3000P Real-Time PCR instrument). Briefly, the relative quantity is presented as the fold change in gene expression normalized to an internal reference (control) gene in knobs relative to the normalized expression levels in the mycelium. The

**Statistical analysis.** After scanning, data images were inspected manually and low-quality spots were excluded before further analysis. Reporters of unknown origin and related to C. elegans were excluded, and only data for 2822 reporters representing fungal genes were extracted. For those spots remaining, the raw fluorescence intensities for each channel on each slide were collected. The mean background fluorescence was calculated for each channel. After local background correction for each spot, the reporters yielding intensities below twice the mean background were excluded and the fluorescence for the remaining reporters was multiplied to give a common channel mean of 5000 fluorescence units. As a result, data for 2534 fungal genes remained in the dataset. The statistical approach we used, the mixed-model analysis of variance (ANOVA), served two purposes: (1) normalization of the data to remove systemic biases that may have affected all genes simultaneously, such as differences in the amount of RNA that was labelled for a particular replicate of a treatment; (2) assessment of the contribution of biological and experimental sources of error to the variation in expression of each individual gene (Wolfinger et al., 2001). This procedure uses differences in normalized expression levels, rather than ratios, as the unit of analysis of expression differences. We subjected the corrected log-transformed means ($y_{ijg}$) for the fungal gene $g$ (g = 1, ..., 2534) which included scores for 107 445 fungal spot measures to a normalization model of the form $y_{ijg} = \mu + A_i + D_j + (A \times D)_j + e_{ijg}$, where $\mu$ is the sample mean, $A_i$ is the effect of the ith array ($i = 1, 9$), $D_j$ is the effect of the jth dye (Cy3 or Cy5), $(A \times D)_j$ is the array–dye interaction (channel effect), and $e_{ijg}$ is the stochastic error. We then subjected the residuals from this model, which can be regarded as crude indicators of relative expression level (and which are referred to in the text as ‘normalized gene expression levels’), to 2534 general-specific models of the form $f_{ijlm} = \mu + A_i + T_j + D_j + e_{ijlm}$, where $T_j$ is the jth tissue (knob or mycelium; 1 degree of freedom, d.f.). In the gene models, which were fitted using PROC MIXED in SAS (SAS/STAT software version 8, SAS Institute), the $A_i$ variable controls for spot effects and is random (8 d.f., leaving 7 d.f. for the residual error).
purpose of the internal control gene is to establish that the RNA targets are reverse transcribed and subsequently amplified with similar efficiency in each reaction. The putative house-keeping gene glyceraldehyde-3-phosphate dehydrogenase (gpd1) served as reference. The amplification efficiency (Eff) of each target gene was compared to that of gpd1 at different template concentrations, and was found to be similar (81-6 %–95-1 %) to that of gpd1 (not shown).

**Phylogenetic analysis.** To construct the phylogenetic trees of GTPases, eGh16 homologues and glycogen phosphorylases, homologous sequences were retrieved from the GenBank nr protein database (Benson et al., 2004) using BLAST searches (Altschul et al., 1990). Protein alignments were made using CLUSTAL W (Thompson et al., 1994). Phylogeny was done using the Neighbour-Joining method (Saitou & Nei, 1987) with 500 bootstrap replications implemented in the MEGA 2.1 program (Kumar et al., 2001).

## RESULTS

### EST analysis

Four directional cDNA libraries were constructed from mycelium, knobs, and knobs infecting C. elegans for 4 and 24 h, respectively. The knobs were isolated from liquid-grown mycelium of M. haptotylum and then incubated with C. elegans on water agar plates. After 4 h of infection, a number of the knobs had adhered and started to penetrate the cuticle of C. elegans. Approximately 30–35 % of the nematodes were non-motile and were considered to have been killed by the fungus. After 24 h of infection, approximately 80–85 % of the nematodes were immobile, and fungal hyphae were growing inside the infected nematodes.

In total, 8466 ESTs were obtained from the four cDNA libraries. The sequences were assembled into 3121 contigs that putatively represent unique genes/transcripts (Table 2). Of the assembled sequences, 723 were represented in the mycelium library, 574 in the knob library, 682 in the 4 h infection library and 1531 in the 24 h infection library. Between 56 and 71 % of the contigs were singletons: they in infection library and 1531 in the 24 h infection library.

To validate the microarray data, the identities of 16 reporters were confirmed by resequencing (Table 4). In addition, the expression levels of eight of these genes were analysed by quantitative real-time RT-PCR (Fig. 3, Table 5). Although there were some differences in the estimated fold values, the overall patterns of expression were similar using the DNA microarray and real-time RT-PCR.

When knobs and mycelium were compared, there was a large difference in the functional distribution of genes being differentially regulated (Fig. 4). A significant proportion of the genes that had putative roles in ‘transcription’, ‘cellular transport and transport mechanisms’, ‘cellular communication or signal transduction’, ‘cell rescue, defence, cell death and ageing’ and ‘cell growth, cell division and DNA synthesis’ were expressed at lower levels in the knobs than in the mycelium. Some of the genes that were differentially expressed in the two cell types are listed in Table 4, and cDNA clones representing a few of these were selected for further characterization by full-length sequencing.

## DISCUSSION

### Morphogenesis and polarity

The trap in M. haptotylum is a spherical cell, which develops at the tip of an apical growing hyphal branch (Fig. 1). This change in morphology represents a shift in the polarity of the cells. In fungi, the actin cytoskeleton provides the structural basis for establishing and modifying cell polarity.
During apical growth, the actin cytoskeleton polarizes growth by assembling into cortical patches and actin cables at the tip. During isotropic growth, the proteins of the cap are more diffusely distributed and actin cables form a meshwork. Several genes are differentially expressed in knobs and mycelium, and these display significant sequence similarities to genes known to be involved in modifying cell polarity in fungi. Among them were two down-regulated homologues to the actin-binding proteins profilin (EST clone CN795458, cDNA AY635995, average fold change on microarray $2^{1.26}$ (log2, knobs versus mycelium)) and cofilin (EST clone CN795276, cDNA AY635996, fold change on microarray $2^{1.87}$). Profilin is a small actin-binding protein, which was originally identified as an actin monomer sequestering protein that can inhibit the growth of actin filaments and prevent polymerization. Studies have also indicated that profilin can have a role in promoting actin polymerization (Ayscough, 1998). Cofilin binds to actin monomers and its activity is important for rapid turnover of actin filaments in *S. cerevisiae* (Lappalainen & Drubin, 1997).

The rho and ras family of small GTPases, including rho, rac and cdc42, play an important role in regulating the actin part of the cytoskeleton (Pruyne & Bretscher, 2000a), and these molecules act as molecular switches through their ability to hydrolyse GTP. In the GTP-bound form, these signalling proteins are active and exert a positive signal on proteins associated with polarized growth. We identified and characterized three small GTPases, including a rho1, a rac and a ras homologue (Fig. 5) that were differentially expressed in knobs and mycelium. Phylogenetic analysis showed that the EST clone CN800249 (fold change on microarray $2^{1.26}$ (log2, knobs versus mycelium))

### Table 2. Number of EST clones analysed in each cDNA library

<table>
<thead>
<tr>
<th>Library</th>
<th>Total ESTs (&gt; 1 bp)</th>
<th>Assembled sequences</th>
<th>Sequence homology (FASTA/GenBank) scores (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Contigs</td>
<td>Singletons</td>
</tr>
<tr>
<td>Mycelium</td>
<td>1937</td>
<td>723</td>
<td>508 (56%)</td>
</tr>
<tr>
<td>Knob</td>
<td>1809</td>
<td>574</td>
<td>368 (64%)</td>
</tr>
<tr>
<td>Infection (4 h)</td>
<td>1522</td>
<td>682</td>
<td>483 (71%)</td>
</tr>
<tr>
<td>Infection (24 h)</td>
<td>3198</td>
<td>1531</td>
<td>1076 (70%)</td>
</tr>
<tr>
<td>All libraries</td>
<td>8466</td>
<td>3121</td>
<td>2008 (64%)</td>
</tr>
</tbody>
</table>

Fig. 2. Volcano plot of significance against fold change in gene expression, in knobs versus mycelium. Each point represents a single gene analysed by the mixed model ANOVA (Wolfinger et al., 2001). The negative log10 of the P value is plotted against the difference between least-square means of log2-normalized expression values. Highly significant values are located towards the top and small expression differences are located at the centre of each plot. The horizontal dashed line represents the test-wise threshold of $P=0.05$ (corresponding to a Bonferroni corrected value of $-\log_{10} = 4.7$), and the solid line represents a threshold of $P=0.001$ ($-\log_{10} = 6.5$).

### Table 3. Number of genes showing significant differential regulation when knobs and mycelium of *M. haptotylum* were compared

The number of genes with a P value of less than the indicated level, as determined by mixed model ANOVA (Wolfinger et al., 2001), is shown. *P* < 0.001 corresponds to a Bonferroni value of $3.5 \times 10^{-7}$, *P* < 0.01 to $3 \times 10^{-6}$ and *P* < 0.05 to $1.8 \times 10^{-5}$. The percentage fraction of fungal genes regulated is given in parentheses (2822 reporters in total).

<table>
<thead>
<tr>
<th>Significance level (P)</th>
<th>Upregulated</th>
<th>Down-regulated</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.001</td>
<td>193 (6.8)</td>
<td>234 (8.3)</td>
<td>259 (9.2)</td>
</tr>
<tr>
<td>0.01</td>
<td>299 (10.6)</td>
<td>348 (12.3)</td>
<td>398 (14.1)</td>
</tr>
<tr>
<td>0.05</td>
<td>492 (17.4)</td>
<td>582 (20.6)</td>
<td>657 (23.3)</td>
</tr>
</tbody>
</table>
Table 4. Selected genes differentially expressed in knobs versus mycelium of *M. haptotylum*

Clones in bold have been verified by resequencing. Fold change represents the mean fold change (log2) values of expression levels in knobs (trap cells) compared to mycelium; standard error is given in parentheses. All genes are differentially regulated at a significance level of P<0.001 (Bonferroni 3·5·10⁻⁷; cf. Table 3).

<table>
<thead>
<tr>
<th>EST clone</th>
<th>GenBank GI</th>
<th>GenBank homologue description</th>
<th>Score</th>
<th>Fold change</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>CN795465</td>
<td>113277</td>
<td>Actin gamma (<em>Emicella nidulans</em>)</td>
<td>633</td>
<td>−1·23 (0·12)</td>
<td>Actin-associated protein</td>
</tr>
<tr>
<td>CN795458</td>
<td>19114739</td>
<td>Profilin (<em>Sc. pombe</em>)</td>
<td>230</td>
<td>−1·26 (0·18)</td>
<td>Actin-associated protein</td>
</tr>
<tr>
<td>CN795276</td>
<td>11596089</td>
<td>Cofilin (<em>Zygosaccharomyces rouxii</em>)</td>
<td>235</td>
<td>−1·87 (0·26)</td>
<td>Actin-associated protein</td>
</tr>
<tr>
<td>CN794706</td>
<td>7673698</td>
<td>β-Tubulin (<em>Leptosphaeria maculans</em>)</td>
<td>409</td>
<td>−1·43 (0·25)</td>
<td></td>
</tr>
<tr>
<td>CN795822</td>
<td>729011</td>
<td>Calmodulin (<em>Neurospora crassa</em>)</td>
<td>419</td>
<td>−1·22 (0·23)</td>
<td></td>
</tr>
<tr>
<td>CN795399</td>
<td>461631</td>
<td>BM2H protein (<em>S. cerevisiae</em>)</td>
<td>414</td>
<td>−1·41 (0·17)</td>
<td>14-3-3-like protein</td>
</tr>
<tr>
<td>CN797412</td>
<td>8218222</td>
<td>Multifunctional cyclin-dependent kinase PHO85 (<em>N. crassa</em>)</td>
<td>501</td>
<td>−2·64 (0·26)</td>
<td>Cyclin-dependent protein kinase</td>
</tr>
<tr>
<td>CN798834</td>
<td>9296975</td>
<td>Glucosamine-phosphate N-acetyltransferase GNA1 (<em>Candida albicans</em>)</td>
<td>251</td>
<td>−1·27 (0·17)</td>
<td>UDP-N-acetylglucosamine biosynthesis</td>
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<tr>
<td>CN794706</td>
<td>6321670</td>
<td>Prohibitin PHB2 (<em>S. cerevisiae</em>)</td>
<td>162</td>
<td>2·06 (0·19)</td>
<td>Mitochondrial protein regulate replicative lifespan</td>
</tr>
<tr>
<td>CN794866</td>
<td>11139253</td>
<td>Mitochondrial thioredoxin peroxidase PRX1 (<em>S. cerevisiae</em>)</td>
<td>559</td>
<td>−1·42 (0·21)</td>
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<tr>
<td>CN795223</td>
<td>11135375</td>
<td>Thioredoxin (<em>Coprinus comatus</em>)</td>
<td>368</td>
<td>−2·41 (0·40)</td>
<td></td>
</tr>
<tr>
<td>CN795856</td>
<td>16416067</td>
<td>Related to hsp70 protein (<em>N. crassa</em>)</td>
<td>248</td>
<td>−3·57 (0·56)</td>
<td>HSP70 family</td>
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microarray −2.03 and by real-time RT-PCR −0.25) was a rho1 homologue, and the EST clone CN795728 (fold change on microarray −1.32) was a rac1 homologue (Fig. 5). The rho1 homologue of *M. haptotylum* (AY635989) encodes a protein of 193 aa. The deduced peptide rho1 contains all the signatures that have been found to be characteristic of the small G protein superfamily, including five GTP-binding and GTP hydrolysis domains (G1, G2, G3, G4 and G5) as well as the C-terminal prenylation site for rho GTPases (CXXL, C indicates cystein, X any amino acid and L leucin) involved in membrane associations (Bourne et al., 1991; Finegold et al., 1991). Rho1 of *M. haptotylum* shows high sequence similarity to the rho1 protein of other fungi, including that from *Aspergillus nidulans* (89% aa identity), *Schizosaccharomyces pombe* (79%), *S. cerevisiae* (76%) and *Cryptococcus neoformans* (76%).

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</table>

**Fig. 3.** Relative quantification of transcript levels for *ras1* and *gph1* in mycelium and knob tissue by the use of real-time quantitative RT-PCR. Dissociation curve profiles (temperature versus fluorescence) and amplification plots (cycle number versus fluorescence) are shown for *ras1* [(a) and (b)] and *gph1* [(c) and (d)], together with gpd1, which served as internal control for normalization (for gene identities, cf. Table 1). ●, amplification of gpd1 in mycelium samples; ○, amplification of gpd1 in knob samples; △, target genes (*ras1* or *gph1*) in knob samples; ▲, target genes in mycelium samples. Shown are mean values of triplicate cDNA samples made from aRNA. The dissociation curves show that the *Tₘ* values of the PCR products formed in the mycelium and knob samples were overlapping, indicating the specificity of the primers used. The inserted gels show that the length of the target amplicons was identical in the two tissues (M, mycelium; K, knob). In the amplification plots, the curves for the amplification of gpd1 in the knob and mycelium samples were overlapping in the log-linear phase, indicating similar levels of gpd1 transcripts in the two samples.
The switching between the GDP-bound inactive and the GTP-bound active form of rho GTPases is controlled by guanine nucleotide exchange factors (GEF) and GDP dissociation inhibitors (Koch et al., 1997). A homologue of the yeast GDP dissociation inhibitor with activity toward rho1 (rdi1) was identified among the genes being downregulated in the knobs (EST clone CN794767, fold change on microarray $\leq 0.01$). The rdi1 gene of M. haptotylum (AY635991) is predicted to encode a 196 aa polypeptide, and displays 45% aa identity to rdi1 of S. cerevisiae.

The rac1 homologue of M. haptotylum (AY635990) was predicted to encode a peptide of 194 aa, and contained the conservative G1, G2, G3, G4 and G5 domains as well as the prenylation site (CXXL) characteristic of rac1 proteins (Bourne et al., 1991). The protein showed 80% sequence identity (aa) to a rac GTPase (cflB) of the dimorphic ascomycete Penicillium marmoratus (Boyce et al., 2003). Interestingly, cflB co-localizes with actin at the tips of the vegetative hyphal cells. Deletion of cflB results in growth defects in vegetative hyphae, depolarization and inappropriate septation (Boyce et al., 2003).

The ras1 homologue of M. haptotylum (AY635992, fold change on microarray $\leq 2.67$ and by real-time RT-PCR $\leq 2.19$) was predicted to encode a 198 aa protein, and showed a significant sequence similarity to ras-like proteins from Colletotrichum trifolii (46% identity), A. nidulans (46%), S. cerevisiae (42%) and Ustilago maydis (44%), especially in regions corresponding to the GTP-binding and GTP hydrolysis domains (G1, G2, G3, G4 and G5) as well as the C-terminal prenylation site (CAAX, C indicates

### Table 5. Validation of microarray data

Presented are the mean fold change values (log$_2$) of expression levels in knobs (trap cells) compared to mycelium. Three independent biological replicates were analysed. The same source of aRNA was used for both the cDNA microarray hybridization and the real-time RT-PCR quantification.

<table>
<thead>
<tr>
<th>Gene</th>
<th>EST</th>
<th>Description</th>
<th>cDNA microarray</th>
<th>Real-time PCR</th>
<th>Validation</th>
</tr>
</thead>
<tbody>
<tr>
<td>rho1</td>
<td>CN800249</td>
<td>GTPase Rho1</td>
<td>Down (−2.03)</td>
<td>Down (−0.25)</td>
<td>Yes</td>
</tr>
<tr>
<td>ras1</td>
<td>CN799032</td>
<td>Ras homologue</td>
<td>Up ( +2.67)</td>
<td>Up ( +2.19)</td>
<td>Yes</td>
</tr>
<tr>
<td>gks1</td>
<td>CN800713</td>
<td>GAS1 (MAS31) protein</td>
<td>Up ( +1.77)</td>
<td>Up ( +2.63)</td>
<td>Yes</td>
</tr>
<tr>
<td>gph1</td>
<td>CN795977</td>
<td>Glycogen phosphorylase</td>
<td>Up ( +4.36)</td>
<td>Up ( +2.89)</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>CN801309</td>
<td>Cuticle-degrading serine protease</td>
<td>Up ( +2.48)</td>
<td>Up ( +2.59)</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>CN800950</td>
<td>Alkaline serine protease</td>
<td>Up ( +3.08)</td>
<td>Up ( +2.79)</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>CN800820</td>
<td>Trichothecone 3-O-acetyltransferase</td>
<td>Up ( +2.02)</td>
<td>Up ( +2.17)</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>CN794863</td>
<td>Immunoreactive spherule cell wall protein</td>
<td>Down (−3.52)</td>
<td>Down (−0.27)</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**Fig. 4.** Number of down- (left) and up- (right) regulated genes in knobs versus mycelium classified into various functional categories. Data are derived from cDNA microarray analysis using a significance level of $P<0.05$ (cf. Table 3). The classification is according to MIPS (Mewes et al., 2000), and annotation of putative functional roles is based on sequence similarity to information in the GenBank nr protein database. Of a total of 398 genes down-regulated, 123 genes were annotated into the displayed functional categories, whereas the remaining genes (not shown) were annotated either as unclassified proteins (179 genes) or as orphans with no sequence homology (96 genes). Among a total number of 259 upregulated genes, 43 were assigned functional annotation, whereas 119 clones were assigned as unclassified and 97 genes as orphans.
cysteine, A an aliphatic amino acid and X any amino acid) (Bourne et al., 1991).

Comparison of gene expression in knobs and appressoria

There are several similarities in the structure and function of knobs of nematode-trapping fungi and those of appressoria formed by plant-pathogenic fungi. Like a knob, an appressorium is a specialized infection structure, which develops as a spherical cell at the tip of a hypha (germ tube). Both structures contain an adhesive layer on the outside, which binds to the surface of the host. Furthermore, both appressoria and knobs form a hypha that penetrates the host using a combination of physical force and extracellular enzymic activities (Tucker & Talbot, 2001). Comparison of data from the transcriptional profiling of knobs with that of appressoria in Magnaporthe grisea and Blumeria...
Table 6. Comparison of genes differentially regulated in knobs or appressoria in M. haptotylum, M. grisea and B. graminis, compared to mycelium, conidia and germinating conidia, respectively

For M. haptotylum knob, expression levels in knobs (trap cells) compared to mycelium are shown (Table 4). For M. grisea appressoria, expression levels in appressorium compared to differentiating conidia are shown (Takano et al., 2004). For B. graminis appressoria, a comparison of appressoria and germinated conidia calculated from serial analysis of gene expression (SAGE) is shown (Thomas et al., 2002).

<table>
<thead>
<tr>
<th>Gene*</th>
<th>Knob M. haptotylum</th>
<th>Appressoria Ma. grisea</th>
<th>Appressoria B. graminis</th>
</tr>
</thead>
<tbody>
<tr>
<td>gEgh16</td>
<td>Up</td>
<td>Up</td>
<td>Up</td>
</tr>
<tr>
<td>Chitinase</td>
<td>Down</td>
<td>Down</td>
<td>Up</td>
</tr>
<tr>
<td>Serine protease (subtilisin)</td>
<td>Down</td>
<td>Up</td>
<td>Down</td>
</tr>
<tr>
<td>Proteasome subunit</td>
<td>Down</td>
<td>Up</td>
<td>Down</td>
</tr>
<tr>
<td>Cyclophilin</td>
<td>Down/Up</td>
<td>Down</td>
<td>Down</td>
</tr>
<tr>
<td>Peptidyl-prolyl cis-trans isomerase</td>
<td>Up</td>
<td>Up</td>
<td>Up</td>
</tr>
<tr>
<td>Metallohydrolase</td>
<td>Down</td>
<td>Up</td>
<td>Down</td>
</tr>
<tr>
<td>Thioredoxin</td>
<td>Down</td>
<td>Down</td>
<td>Down</td>
</tr>
<tr>
<td>Translation elongation factor (TEF1)</td>
<td>Down</td>
<td>Up</td>
<td>Down</td>
</tr>
<tr>
<td>14-3-3-like protein (BMH2)</td>
<td>Down</td>
<td>Up</td>
<td>Down</td>
</tr>
<tr>
<td>Triose phosphate isomerase</td>
<td>Down</td>
<td>Up</td>
<td>Down</td>
</tr>
<tr>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
<td>Down</td>
<td>Down</td>
<td>Down</td>
</tr>
<tr>
<td>Enolase</td>
<td>Down</td>
<td>Down</td>
<td>Down</td>
</tr>
<tr>
<td>Fructose biphosphate aldolase</td>
<td>Down</td>
<td>Down</td>
<td>Down</td>
</tr>
<tr>
<td>GTPase Rh1</td>
<td>Down</td>
<td>Down</td>
<td>Down</td>
</tr>
<tr>
<td>Profilin</td>
<td>Down</td>
<td>Down</td>
<td>Down</td>
</tr>
</tbody>
</table>

*GenBank accession numbers. gEgh16: M. haptotylum, CN800713; Ma. grisea, AI069194; B. graminis, AW792327, AW789153 and AW789327. Chitinase: M. haptotylum, CN797557; Ma. grisea, BM872118; B. graminis, AW788278 and AW792426. Serine protease (subtilisin): M. haptotylum, CN801309, CN800950, CN799628 and CN800136; Ma. grisea, AI068925; B. graminis, AW790864. Proteasome subunit: M. haptotylum, CN795966; Ma. grisea, BG810344. Cyclophilin: M. haptotylum, CN800033, CN800928, CN800871, CN797293, CN794624 and CN795925; B. graminis, AW790370. Peptidyl-prolyl cis-trans isomerase: M. haptotylum, CN799935; Ma. grisea, AI068531; B. graminis, AW791080. Metallohydrolase: M. haptotylum, CN796974; Ma. grisea, CA408249; B. graminis, AW791711. Thioredoxin: M. haptotylum, CN795223; B. graminis, AW790498. Translation elongation factor TEF1: M. haptotylum, CN794817, CN795053, CN796490, CN795963 and CN799878; Ma. grisea, AI068515. 14-3-3-like protein (BMH2): M. haptotylum, CN795822; Ma. grisea, AI069388; B. graminis, AW790531. Triose phosphate isomerase: M. haptotylum, CN800416; B. graminis, AW789921. Glyceraldehyde-3-phosphate dehydrogenase: M. haptotylum, CN796969; Ma. grisea, CA408224; B. graminis, AW788939. Enolase: M. haptotylum, CN797534; B. graminis, AW789656. Fructose biphosphate aldolase: M. haptotylum, CN797352; B. graminis, AW790281. GTPase Rh1: M. haptotylum, CN800249; B. graminis, AW792640. Profilin: M. haptotylum, CN795458; B. graminis, AW792002.

gEgh16 shows that there are also many similarities in the patterns of gene regulation in the infection structures of nematode-trapping and plant-parasitic fungi (Table 6).

Among the commonly regulated genes was a gene (EST clone CN800713, fold change on microarray +1.77 and by real-time RT-PCR +2.63) encoding a polypeptide with sequence similarity to gas1 (previously mas3) of the blast fungus Ma. grisea. The gas1 protein is specifically expressed in appressoria and belongs to a large family that in B. graminis includes the gEgh16 and gEgh16H proteins (Xue et al., 2002; Justesen et al., 1996; Grell et al., 2003). From complete cDNA sequencing of the gas1 homologue of M. haptotylum (AY635993), a 257 aa polypeptide could be predicted and the gene was designated gks1 (gEgh16 homologue expressed in knob stage). The gks1 polypeptide displayed a 40 % aa sequence identity to gas1 of Ma. grisea. A phylogenetic analysis showed that gks1 and gas1 are found in a clade with high support (bootstrap value 73) containing members of the gEgh16 subfamily of the gEgh16/gEgh16H proteins (Fig. 6). We also identified another member of the gEgh16/gEgh16H family among the EST sequences of M. haptotylum. This EST clone (CN796958) was not differentially expressed, as revealed by the microarray analysis. The predicted polypeptide of CN796958 displayed significant sequence identity with gas2 (mas1) of Ma. grisea (43 % aa identity). CN796958 and gas2 were found in a well-supported clade (bootstrap value 100) containing members of the gEgh16H subfamily of the gEgh16/gEgh16H proteins (Fig. 6). The gas1 and gas2 genes showed a similar pattern of regulation in Ma. grisea, and both genes were expressed under appressorium formation (Xue et al., 2002). In B. graminis, members of the gEgh16/gEgh16H family displayed different patterns of regulation, and several of them were differentially expressed during the development of the appressorium (Grell et al., 2003; Thomas et al., 2002).

Genes involved in stress and defence responses are one of the largest classes of genes that are differentially expressed.
Gene expression in trap cells

Fig. 6. Phylogeny of fungal gEgh16/ gEgh16H proteins. The tree (unrooted) was constructed using the neighbour-joining method (Saitou & Nei, 1987). Gks1 of M. haptotylum is found in a clade with the Gas1 of Ma. grisea and gEgh16 of B. graminis. A second Gas homologue of M. haptotylum is found in a clade containing the Gas2 of Ma. grisea and the gEgh16H1 genes of B. graminis. Genbank GI alt. accession numbers of the protein/nucleotide sequences from top to bottom are: 38107786 (hp1 Ma. grisea), 14029698, 32416532, 21333237, 51556852 (AY635993), CN796958, 38108209, 8347747, 13430279, 13430281, 38102500, 32411089 and 38102176.

Extensive biochemical and crystallographic studies of the rabbit glycogen phosphorylase have identified seven functional domains, including the active site, the glycogen storage site, the purine nucleoside inhibitor site, the cofactor (pyridoxal phosphate, PLP) binding site, the phosphorylation site, the AMP binding site, and the subunit dimerization point (Hwang & Fletterick, 1986). Four of these regions, the active site (labelled as g in Supplementary Fig. S1a with the online version of this paper at http://mic.sgmjournals.org/).

during appressoria formation in Ma. grisea (Rauyaree et al., 2004). Several such genes, including cyclophilins, peptidyl-prolyl cis-trans isomerasers, metallothionein and thioredoxins, appear to be differentially expressed in the knobs of M. haptotylum (Tables 4 and 6). Cyclophilins are a conserved family of proteins that direct and accelerate protein folding by a peptidyl cis-trans isomerase activity. In Ma. grisea, cyclophilin1 mutants showed reduced virulence and were impaired in penetration peg formation and generation of appressorium turgor pressure (Viaud et al., 2002). In B. graminis, a putative metallothionein was identified as having high transcriptional levels in both conidia and appressoria (Thomas et al., 2002). A transcript (EST clone CN796974) displaying sequence similarity to fungal Cu-binding metallothionein was differentially expressed in knobs (fold change on microarray $-3.8$).

A number of genes involved in protein synthesis (such as homologues for ribosomal proteins and translation elongation factor), protein destination and degradation (such as homologues for ubiquitin, ubiquitin-conjugating enzyme and proteasome components) were differentially expressed in both knobs and appressoria (Tables 4 and 5). This suggests that development of the infection structures in both nematode-trapping and plant-pathogenic fungi is associated with an extensive synthesis and turnover of proteins (McCafferty & Talbot, 1998).

Glycogen and carbon metabolism

One of the most upregulated genes in the knobs was a gene (EST clone CN795977, fold change on microarray $+4.36$ and by real-time RT-PCR $+2.49$) showing similarity to glycogen phosphorylases ($\alpha$-$\beta$-glucosyltransferase, EC 2.4.1.1). Complete sequence analysis of this cDNA clone (AY635994) revealed an ORF corresponding to a putative protein of 874 aa. The entire aa sequence of M. haptotylum phosphorylase (gph1) showed $59\%$ identity to the S. cerevisiae glycogen phosphorylase (Hwang & Fletterick, 1986) and $45\%$ identity to rabbit muscle glycogen phosphorylase (Nakano et al., 1986). Multiple sequence alignment showed extensive sequence similarity in regions corresponding to the catalytic domain and C-terminal domain, while the regulatory or N-terminal domain appeared more variable (Supplementary Fig. S1a).

Extensive biochemical and crystallographic studies of the rabbit glycogen phosphorylase have identified seven functional domains, including the active site, the glycogen storage site, the purine nucleoside inhibitor site, the cofactor (pyridoxal phosphate, PLP) binding site, the phosphorylation site, the AMP binding site, and the subunit dimerization point (Hwang & Fletterick, 1986). Four of these regions, the active site (labelled as g in Supplementary Fig. S1a (1 substitution in 17 residues), the glycogen storage site (s) (2 substitutions in 8 residues), the purine nucleoside inhibitor site (c) (1 substitution in 4 residues) and the PLP binding site (v) (1 substitution in 8 residues) were highly conserved between the M. haptotylum and the rabbit enzymes. The N-terminal regulatory domain is important for allosteric regulation, and carries the regulatory phosphorylation site (p) of glycogen phosphorylases. In the rabbit enzyme, this domain is located within the first 80 aa of the N-terminal region (Palm et al., 1985). Apart from the phosphorylation site, the N-terminal region of the phosphorylases contains the AMP binding site (a) and the subunit dimerization point (d). Considerable amino acid sequence identity (76%) was observed in the N-terminal region of the yeast and the M. haptotylum phosphorylases, whereas the rabbit enzyme appeared more divergent. The yeast enzyme is phosphorylated at a specific threonine residue (Thr-19) by a phosphorylase kinase, and additionally by a cyclic AMP-dependent protein kinase (Hwang & Fletterick, 1986). The Thr-19 residue, as well as adjacent residues, were well conserved between the yeast and the M. haptotylum phosphorylase.

A phylogenetic analysis of phosphorylases from different organisms showed that the gph1 protein of M. haptotylum
was found in a clade with high bootstrap support (value 100), including a putative glycogen phosphorylase from several ascomycetes, including a putative glycogen phosphorylase from the human pathogen *Aspergillus fumigatus* and hypothetical proteins from the rice blast fungus *Ma. grisea* (Supplementary Fig. S1b).

Glycogen is an important storage carbohydrate in eukaryotes, and glycogen phosphorylase catalyses and regulates the degradation of glycogen to glucose 1-phosphate (Fletterick & Madsen, 1980). Glycogen is accumulated in incipient appressoria of *Ma. grisea*, but is rapidly degraded before generating the turgor pressure needed for penetration of the plant cuticle (Thines et al., 2000). The turgor pressure results from a rapid accumulation of glycerol, and there are different lines of evidence suggesting that the production of glycerol is achieved by the mobilization of energy reserves, such as glycogen and neutral lipids (Thines et al., 2000). The glucose 1-phosphate generated from the degradation of glycogen is metabolized in the glycolytic pathway. Glycerol can be synthesized from several of the intermediates in this pathway. Unfortunately, we did not identify any putative homologues in the *M. haptotylum* EST database of enzymes known to be involved in these pathways in *S. cerevisiae* (see references in Thines et al., 2000). Thus, whether or not the genes encoding these enzymes are regulated in the knobs is not known. Alternatively, the breakdown of glycogen can lead to the formation of pyruvate and the production of energy (ATP). However, this part of the pathway does not appear to be upregulated in the knobs. The homologues of enzymes in this part of the glycolytic pathway that were identified, including glyceraldehyde-3-phosphate dehydrogenase (CN796696) and enolase (CN797534), were downregulated in the knobs compared to mycelium (fold change on microarray, $-1.59$ and $-2.07$, respectively) (Table 4). Studies have also shown that the degradation of glycogen in *Ma. grisea* is under the control of the cAMP-dependent protein kinase A (PKA) and MAP kinase (MAPK) pathway (Thines et al., 2000). In yeast, phosphorylase activity is regulated by reversible phosphorylation/dephosphorylation mediated by the cAMP regulatory cascade (Hwang & Fletterick, 1986). The fact that the N-terminal domain, which contains regions important for allosteric regulation and carries the regulatory phosphorylation site (Thr-19), was well conserved between the yeast and *M. haptotylum* enzymes, suggests that the two phosphorylases may be regulated by similar mechanisms.

**Peroxisomal associated proteins**

The presence of numerous dense bodies, which are related to peroxisomes, is typical for traps of nematode predatory fungi (Dijksterhuis et al., 1994). Interestingly, one transcript (CN796915, fold change on microarray +0.72) with similarity to a peroxisomal membrane protein in *Candida boidinii* (PMP30B) was significantly upregulated. There is a homologue in *S. cerevisiae* (PMP27, PEX11, peroxin) which is involved in peroxisomal proliferation. Deletion of PMP27 generates a phenotype containing a few large peroxisomes, as if peroxisomal fission was inhibited (Erdmann & Blobel, 1995). Other genes that were regulated in knobs and displayed sequence similarities to genes encoding proteins associated with peroxisomes included homologues to D-amino acid oxidase (CN796046, fold change on microarray $-3.09$), 2,4-dienoyl-CoA reductase (SPS19, *S. cerevisiae*) (CN797536, fold change on microarray $+1.05$), and fatty acid transporter and very long-chain fatty acyl-CoA synthetase (FAT1, *S. cerevisiae*) (CN796073, fold change on microarray $-2.30$).

**ACKNOWLEDGEMENTS**

This paper is dedicated to Professor Birgit Nordbring-Hertz to honour her pioneer work on the morphology and physiology of nematode-trapping fungi. Custom microarrays were produced at the SWEGENE DNA Microarray Resource Center at the BioMedical Center B10 in Lund, and DNA sequencing was performed at the SWEGENE Center of Genomic Ecology at the Ecology Building in Lund, supported by the Knut and Alice Wallenberg Foundation through the SWEGENE consortium. The study was supported by grants from the Swedish Natural Science Research Council. We wish to thank Björn Canbäck and Balaji Rajashekar for their kind help on bioinformatics issues.

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Gene expression in trap cells


