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Transcript Profiling Reveals Novel Marker Genes Involved in Fruiting Body Formation in *Tuber borchii*†

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cDNA arrays were used to explore mechanisms controlling fruiting body development in the truffle *Tuber borchii*. Differences in gene expression were higher between reproductive and vegetative stage than between two stages of fruiting body maturation. We suggest hypotheses about the importance of various physiological processes during the development of fruiting bodies.

Irrespective of their nutritional strategies, most saprotrophic and mycorrhizal fungi produce conspicuous fruiting bodies where hyphae aggregate, produce pseudotissues with differentiated compartments, develop specialized structures, and eventually differentiate meiotic spores. Among them, the ectomycorrhizal truffles (*Tuber* spp.) produce hypogeous ascocarps which are highly appreciated and commercialized for their delicate organoleptic properties. Since truffle fruiting bodies cannot yet be obtained under controlled conditions, our knowledge of the morphogenetic events leading to ascocarp development and maturation (3), as well as their underlying molecular bases (1, 4, 8, 12), is quite limited. Elucidating the spatiotemporal control of gene expression during the successive stages of the truffle life cycle will improve our knowledge of processes that initiate and coordinate the formation of hypogeous truffles. Here, we describe changes in gene expression during the formation of the ascomata of *Tuber borchii*.

Unripe (CF05; 0 to 5% mature spores) and ripe (CF70; 70 to 100% mature spores) *T. borchii* fruiting bodies were collected under hazelnut trees from a natural truffle ground near Alba in Piedmont (Italy) during the 2000 to 2001 production seasons. RNA was extracted as described by Lacourt et al. (4). cDNA libraries were constructed using the PCR–based SMART cDNA library construction kit in λTriplEx2 (Clontech, Palo Alto, CA) (2). A cDNA array containing 2,041 elements was produced, hybridized, and analyzed according to Duplessis et al. (2). Six cDNA complex probes were then prepared from total RNA of two CF05 ascomata, two CF70 ascomata, and vegetative mycelium (4).

During fruiting body development, the vast majority of genes were not significantly regulated among the different stages. However, comparisons between fruiting bodies and mycelium indicated that 69 nonredundant transcripts (i.e., 3%) showed significant changes in expression (analysis of variance, $P < 0.01$) (Table 1). In addition, inferences were only made from genes showing a differential expression ratio above 2.5 (below 0.4) between any two stages.

Genes showing the strongest changes in expression coded for homologs of proteins involved in stress metabolism (Hsp12, sterigmatocystin biosynthesis monooxygenase), lipid metabolism (isopentenyl diphosphate isomerase, acyl-coenzyme A [CoA]-dehydrogenase, hydroxymethylglutaryl [HMG]-CoA synthase) and Hmp1, which encodes a cruciform DNA binding protein. Several transcripts (34) with a differential expression coded for hypothetical proteins. Eight transcripts (Table S1 in the supplemental material) showed an increased synthesis (≥2.5) in unripe (CF05) compared to ripe (CF70) fruiting bodies, while none was more expressed in CF70 than CF05. These genes are highly similar to fungal hypothetical proteins of unknown function from other ascomycete species, e.g., *Aspergillus nidulans*, *Gibberella zeae*, and *Magnaporthe grisea*. They likely belong to a set of genes of unknown function involved in sexual development in ascomycetous fungi.

Differential expression of four differentially expressed genes representing genes related to lipid metabolism (HMG-CoA synthase, acetyl-CoA acetyltransferase [ACAT], isopentenyl diphosphate isomerase [IPPI]), and stress response (Hsp12) was validated by RNA blot analysis (Fig. S1 in the supplemental material). ACAT was selected because it operates upstream of the HMG-CoA synthase and isopentenyl diphosphate isomerase in the isoprenoid synthesis pathway. Isoprenoids are involved in the synthesis of ergosterol, related isoprenoid compounds, and several terpenic volatile aromas, which are thought to be modified during truffle formation and plant interactions (6). The observed changes in expression rates were comparable to those detected in cDNA array analysis. In addition, HMG-CoA synthase and IPPI showed an increased expression in the last stage of maturation. Similarly, the analysis of the putative ACAT showed that it was also expressed more in the mature fruiting body, whereas cDNA array anal-

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TABLE 1. List of the 69 genes with the highest (up-regulation) and lowest (down-regulation) ascoma/vegetative mycelium (M) expression ratios[a]

Accession no.	Clone no.	CF05/M expression ratio	CF70/M expression ratio	Similarity (species)	BLASTX E-value
DN601500	M6G10	163.6	103.1	Predicted protein (*Neurospora crassa*)	3.0E-35
CN488330	P3D05	58.9	9.78	Hypothetical protein an6633.2 (*Aspergillus nidulans*)	2.0E-09
CN488328	P1I01	41.1	15.5	Hypothetical protein fg09972.1 (*Gibberella zeae*)	1.0E-23
CN488002	M1F02	39.4	60.0	Induced by heat shock entry into stationary phase depletion of glucose, and addition of lipids (fatty acids); HSP12p (*Saccharomyces cerevisiae*)	3.0E-09
CN488054	M9E05	38.0	29.2	Hmp1 (*Ustilago maydis*)	6.0E-08
CN487953	M12B01	28.9	35.9	Ferredoxin-like iron-sulfur protein (*Paracoccidioides brasiliensis*)	6.0E-62
CN488043	M5B12	27.0	43.5		
CN488323	P12H03	24.7	7.2	Hypothetical protein fg05397.1 (*Gibberella zeae*)	7.0E-16
CN488039	M4H04	23.3	15.0		
CN487923	M11E12	22.5	34.4		
CN488178	SA1F07	19.3	20.4	Isopentenyl diphosphate isomerase (*Aspergillus nidulans*)	8.0E-66
CN488171	SA1E03	18.9	7.1	Hypothetical protein fg09455.1 (*Gibberella zeae*)	6.0E-17
CN488042	M5B09	18.7	28.7	STCW EMENI putative sterigmatocystin biosynthesis monooxygenase StcW (*Aspergillus nidulans*)	5.0E-33
CN487924	M11F01	18.4	12.7	Predicted protein (*Neurospora crassa*)	9.0E-26
CN488292	P11E09	15.3	11.5	Hypothetical protein an5480.2 (*Aspergillus nidulans*)	6.0E-25
CN488189	SA1H08	14.0	17.4	Unknown (environmental sequence)	8.0E-18
CN488278	P11B10b	13.6	5.2	Hypothetical protein an4299.2 (*Aspergillus nidulans*)	3.0E-39
CN487957	M12B08	13.5	25.1		
CN488035	M4F06	11.9	15.1	Transketolase putative (*Aspergillus fumigatus*)	3.0E-52
CN487984	M12F05	11.3	17.8		
CN488158	SA1C03	11.1	5.6	Cytochrome *c* oxidase polypeptide II (*Neurospora crassa*)	2.0E-25
CN487854	M10F07	10.0	8.0		
CN488363	SA2E04	10.0	2.9	Hypothetical protein mg08059.4 (*Magnaporthe grisea*)	3.0E-15
CN488383	SA2G07	9.8	8.0	TIP1-related; Tir3p (*Saccharomyces cerevisiae*)	2.0E-09
CN488160	SA1C09	8.7	6.2	Zinc-dependent alcohol dehydrogenase, putative (*Aspergillus fumigatus*)	1.0E-24
CN487903	M11D01	8.6	3.8		
CN487762	M08C04	8.1	6.5	Conserved hypothetical protein (*Vibrio parahaemolyticus*)	3.0E-19
CN487849	M10E11	7.8	9.7		
CN487930	M11F10	7.6	6.1		
CN487922	M11E11	7.6	5.3		
CN488311	P12D04	7.2	2.1	UPF0057 family protein; possible stress response protein (*Schizosaccharomyces pombe*)	6.0E-14
CN488364	SA2E05	6.5	2.0	Hypothetical protein fg05291.1 (*Gibberella zeae*)	4.0E-11
CN488024	M3H08	6.3	5.1	Myosin heavy chain (*Lethenteron japonicum*)	2.0E-06
CN488353	SA2C08	6.1	4.2		
CN488320	P12G07	5.8	3.3		
CN488056	M9E10	5.2	2.4	Hypothetical protein an5614.2 (*Aspergillus nidulans*)	3.0E-36
CN488012	M3B09	5.1	7.4	Probable acyl-CoA dehydrogenase (*Glomus intraradices*)	8.0E-90
CN487837	M10D09	4.4	5.6	Probable hydroxymethylglutaryl-CoA synthase (*Neurospora crassa*)	1.0E-150
CN488026	M3H11	4.2	2.5	Hypothetical protein fg01695.1 (*Gibberella zeae*)	1.0E-14
CN488027	M4A12	3.8	6.5	COG1028: dehydrogenases with different specificities (related to short-chain alcohol dehydrogenases) (*Ralstonia eutropha*)	6.0E-42
DN601486	M11G02	3.6	6.4	Glyoxysomal malate synthase (*Neurospora crassa*)	2.0E-08
CN487827	M10C08	3.5	1.8		
CN488052	M9A03	3.4	2.4	Glycine-rich RNA-binding protein GRP1 (*Triticum aestivum*)	2.0E-30
CN488167	SA1D09	3.4	4.3		
CN488350	SA2C03	3.3	2.6		
CN488360	SA2D10	3.1	1.9	DNA topoisomerase III (*Schizosaccharomyces pombe*)	2.0E-39
CN488384	SA2G08	3.1	2.2	Possible mannosylphosphorylation protein Mnn4 protein (*Aspergillus fumigatus*)	2.0E-44
CN487921	M11E10	2.9	3.9		
CN488390	SA2H06	2.9	1.9	Putative C2H2 zinc finger protein (*Podospora anserina*)	4.0E-35
CN488381	SA2G05	2.7	1.4	Rho GDP dissociation inhibitor. (*Schizosaccharomyces pombe*)	9.0E-34
CN488394	SA2H11	2.7	4.1	Putative allantoicase (EC 3.5.3.4) (*Schizosaccharomyces pombe*)	2.0E-63
CN487966	M12D01	2.7	2.5		
CN488343	SA2B04	2.5	2.7	Hypothetical protein mg07328.4 (*Magnaporthe grisea*)	2.0E-29
CN488048	M6H04	2.5	2.1	Ubiquitin, ubi1 (*Emericella nidulans*)	9.0E-80
CN488049	M6H10	2.4	2.6		
CN487944	M11H06	−2.5	−1.4	60S ribosomal protein L41 (*Quercus suber*)	4.0E-06
BM266217	VA72	−2.5	−1.4	Probable 40S ribosomal protein S5 (*Neurospora crassa*)	1.0E-98
BM266253	VL16	−3.4	−2.3		

Continued on following page

TABLE 1—*Continued*

Accession no.	Clone no.	CF05/M expression ratio	CF70/M expression ratio	Similarity (species)	BLASTX E-value
BM266237	VA90	−3.4	−3.1		
CN487946	M11H09	−3.7	−1.7	Related to translation initiation factor 4e (*Neurospora crassa*)	3.0E-19
BM266143	VA101	−3.9	−3.0	Hypothetical protein fg09970.1 (*Gibberella zeae*)	5.0E-13
BM266230	VA84	−4.0	−3.0	Hypothetical protein um01737.1 (*Ustilago maydis*)	8.0E-21
BM266235	VA89	−4.3	−3.3	H2A_NEUCR histone H2A (*Gibberella zeae* PH-1)	9.0E-43
CN488243	MR4P5H08	−4.4	−3.4	CS antigen (*Coccidioides posadasii*)	7.0E-28
BM266147	VA107	−6.8	−5.3	Hypothetical protein (probable V-ATPase, 20k chain) (*Neurospora crassa*)	3.0E-27
BM266192	VA48	−10.5	−9.0	Predicted protein (*Neurospora crassa*)	1.0E-05
BM266210	VA65	−27.2	−21.2	Hypothetical protein (*Plasmodium falciparum*)	5.0E-25
DN604789	VA66	−83.5	−52.9		
BM266240	VA93	−214.3	−156.9		

[a] Signal ratios of <1.0 were inverted and multiplied by −1 to aid in their interpretation.

ysis showed no change in expression levels. This discrepancy can be explained by the fact that the Northern blot probe was highly specific to the analyzed ACAT, while the complex probe hybridized to the cDNA array may cross-hybridize with transcripts of other members of the ACAT gene family.

The observed increased expression of ACAT, HMG-CoA synthase, and IPPI provides a molecular support for the observed changes in the concentration of specific volatile organic compounds synthesized during *T. borchii* fruiting body development (11). These three enzymes are also involved in the synthesis of ergosterol, a major fungal membrane component (10). The expression pattern of the stress protein Hsp12 observed by cDNA array was fully confirmed by Northern blot analysis. The high Hsp12 expression levels detected during the reproductive stage of *T. borchii* and its absence during the mycelial stage suggest that this gene could be considered a potential marker for the maturation of truffle fruiting bodies, as suggested for *Pleurotus ostreatus* (5). Moreover, Stone et al. (9) demonstrated that Hsp12 was strongly induced upon glucose deprivation and further enhanced by the addition of fatty acids. These novel results, together with our previous work on differentially expressed genes in mycelium and fruiting body of *Tuber borchii* (4), confirmed that lipid metabolism plays a key role during the reproductive stage.

The global gene expression analyses presented here add new information to existing models of fruiting body development in edible fungi (5, 7). Expression profiling showed that a moderate developmental reprogramming takes place during the time course of fruiting body formation. A marked change in gene expression was observed during fruiting body formation at multiple levels: (i) a striking induction of transcripts coding for enzymes of the isoprenoid metabolism and (ii) an activation of stress proteins. Characterization of genes that are regulated during fruiting body development is an initial step towards understanding this complex developmental mechanism. Transcript profiles provide a strong point of reference and are highly valuable for systems that have not been extensively characterized at the molecular level, such as truffles. The current data set of activated genes contained several genes coding for unknown proteins, and functional analysis of these genes will provide insights into the regulation and processes involved in truffle formation.

Nucleotide sequence accession numbers. The nucleotide sequence data reported in this paper have been submitted to the DDBJ/EMBL/GenBank databases under accession numbers CN487736 to CN488394, DN601486 to DN601509, and DN604789.

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