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 ascomarp 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 prepared from 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, hydroxyethylglutaryl [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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| Accession no. | Clone no. | CF05/M expression ratio | CF70/M expression ratio | Similarity (species) BLASTX | BLASTX E-value |
|--------------|-----------|------------------------|------------------------|-----------------------------|---------------|
| DN601500     | M6G10     | 163.6                  | 103.1                  | Predicted protein (Neurospora crassa) | 3.0E-35        |
| CN488330     | P3D05     | 58.9                   | 97.8                   | Hypothetical protein an6633.2 (Aspergillus nidulans) | 2.0E-09        |
| CN488328     | P1H01     | 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 (Usitago maydis) | 6.0E-08        |
| CN487953     | M12B01    | 28.9                   | 35.9                   | Ferredoxin-like iron-sulfur protein (Paracoccidioides brasiliensis) | 6.0E-09        |
| CN488043     | M8B12     | 27.0                   | 43.5                   | Hypothetical protein fg09972.1 (Gibberella zeae) | 7.0E-23        |
| CN488323     | P12H03    | 24.7                   | 7.2                    | Hypothetical protein fg05397.1 (Gibberella zeae) | 7.0E-16        |
| CN488039     | M4H04     | 23.3                   | 15.0                   | Predicted protein (Neurospora crassa) | 9.0E-26        |
| CN487923     | M1I1E2    | 22.5                   | 34.4                   | Predicted protein (Neurospora crassa) | 9.0E-26        |
| CN488178     | SA1F07    | 19.3                   | 20.4                   | Hypothetical protein fg09455.1 (Gibberella zeae) | 6.0E-17        |
| CN488171     | SA1E03    | 18.9                   | 7.1                    | Hypothetical protein fg09455.1 (Gibberella zeae) | 6.0E-17        |
| CN488042     | M5B09     | 18.7                   | 28.7                   | Predicted protein (Neurospora crassa) | 9.0E-26        |
| CN488158     | SA1C03    | 18.1                   | 11.5                   | Hypothetical protein fg09455.1 (Gibberella zeae) | 6.0E-17        |
| CN487854     | M1I1E0    | 18.4                   | 12.7                   | Predicted protein (Neurospora crassa) | 9.0E-26        |
| 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     | SA1C02    | 8.7                    | 6.2                    | Zinc-dependent alcohol dehydrogenase, putative (Aspergillus fumigatus) | 1.0E-24        |
| CN487903     | M1I1D0    | 8.6                    | 3.8                    | Hypothetical protein fg05397.1 (Gibberella zeae) | 7.0E-16        |
| CN488167     | SA1D09    | 8.1                    | 6.5                    | Cytochrome c oxidase polypeptide II (Neurospora crassa) | 2.0E-25        |
| CN488780     | M1I1E1    | 7.8                    | 9.7                    | Hypothetical protein fg05397.1 (Gibberella zeae) | 7.0E-16        |
| CN487930     | M1I1F0    | 7.6                    | 6.1                    | Hypothetical protein fg05397.1 (Gibberella zeae) | 7.0E-16        |
| CN487922     | M1I1E1    | 7.6                    | 5.3                    | Hypothetical protein fg05397.1 (Gibberella zeae) | 7.0E-16        |
| CN488311     | P12D04    | 7.2                    | 2.1                    | UPF0057 family protein; possible stress response protein | 6.0E-14        |
| CN488364     | SA2E05    | 6.5                    | 2.0                    | (Schizosaccharomyces pombe) | 4.0E-11        |
| CN488024     | M3H08     | 6.1                    | 5.1                    | Myosin heavy chain (Lethenteron japonicum) | 2.0E-06        |
| CN488353     | SA2C08    | 5.8                    | 4.2                    | Hypothetical protein an5614.2 (Aspergillus nidulans) | 3.0E-36        |
| CN488320     | P12G07    | 5.2                    | 3.3                    | Hypothetical protein an5614.2 (Aspergillus nidulans) | 3.0E-36        |
| CN488056     | M9E10     | 5.2                    | 2.4                    | Hypothetical protein an5614.2 (Aspergillus nidulans) | 3.0E-36        |
| CN488012     | MB90      | 5.1                    | 7.4                    | Probable acyl-CoA dehydrogenase (Glomus intraradices) | 8.0E-09        |
| CN487857     | M9D09     | 4.9                    | 5.6                    | Probable hydroxymethylglutaryl-CoA synthase (Neurospora crassa) | 1.0E-15        |
| CN488026     | M3H11     | 4.2                    | 2.5                    | Hypothetical protein fg05615.1 (Gibberella zeae) | 1.0E-14        |
| CN488027     | M4A12     | 3.8                    | 6.5                    | Hypothetical protein fg05615.1 (Gibberella zeae) | 1.0E-14        |
| CN488390     | SA2H06    | 3.4                    | 2.1                    | DNA topoisomerase III (Schizosaccharomyces pombe) | 2.0E-39        |
| CN488384     | SA2G08    | 3.1                    | 2.2                    | Possible mannosylphosphorylation protein Mnp4 protein (Aspergillus fumigatus) | 2.0E-44        |
| CN487921     | M1I1E0    | 2.9                    | 3.9                    | Putative C2H2 zinc finger protein (Podpora anserina) | 4.0E-35        |
| CN488390     | SA2H06    | 2.9                    | 1.9                    | Putative C2H2 zinc finger protein (Podpora anserina) | 4.0E-35        |
| CN488381     | SA2G05    | 2.7                    | 1.4                    | Putative C2H2 zinc finger protein (Podpora anserina) | 4.0E-35        |
| CN488394     | SA2H11    | 2.7                    | 4.1                    | Putative C2H2 zinc finger protein (Podpora anserina) | 4.0E-35        |
| CN487966     | M12D01    | 2.7                    | 2.5                    | Putative C2H2 zinc finger protein (Podpora anserina) | 4.0E-35        |
| CN488343     | SA2B04    | 2.5                    | 2.7                    | Putative C2H2 zinc finger protein (Podpora anserina) | 4.0E-35        |
| CN488048     | M6H04     | 2.5                    | 2.1                    | Putative C2H2 zinc finger protein (Podpora anserina) | 4.0E-35        |
| CN488049     | M6H10     | 2.4                    | 2.6                    | Putative C2H2 zinc finger protein (Podpora anserina) | 4.0E-35        |
| CN487944     | M1I1H06   | −2.5                   | −1.4                   | Putative C2H2 zinc finger protein (Podpora anserina) | 4.0E-35        |
| BM260217     | VA72      | −2.5                   | −1.4                   | Putative C2H2 zinc finger protein (Podpora anserina) | 4.0E-35        |
| BM260253     | VL16      | −3.4                   | −2.3                   | Putative C2H2 zinc finger protein (Podpora anserina) | 4.0E-35        |

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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 IPP synthase 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 contains 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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**REFERENCES**

1. Balestrini, R., D. Mainieri, E. Swaghi, L. Garnero, S. Rollino, A. Viotti, S. Ottonello, and P. Bonfante. 2000. Differential expression of chitin synthase III and IV mRNAs in ascomata of *Tuber borchii* Vittad. Fungal Genet. Biol. 31:219–232.

2. Duplessis, S., P. E. Court, D. Tagu, and F. Martin. 2005. Transcript patterns associated with ectomycorrhiza development in *Eucalyptus globulus* L. and *Pisolithus microcarpus*. New Phytol. 165:599–611.

3. Hall I. R., W. Yun, and A. Amicucci. 2003. Cultivation of edible ectomycorrhizal mushrooms. Trends Biotechnol. 21:433–438.

4. Lacourt, L., S. Duplessis, S. Abbà, P. Bonfante, and F. Martina. 2002. Isolation and characterization of differentially expressed genes in the mycelium and fruit body of *Tuber borchii*. Appl. Environ. Microbiol. 68:5788–5788.

5. Lee, S. H., B. G. Kim, K. J. Kim, J. S. Lee, D. W. Yun, J. H. Hahn, G. H. Kim, K. H. Lee, D. S. Suh, S. T. Kwon, C. S. Lee, and Y. B. Yoo. 2002. Comparative analysis of sequences expressed during the liquid-cultured mycelia and fruit body stages of *Pleurotus ostreatus*. Fungal Genet. Biol. 35:115–134.

6. Menotta, M., A. M. Gioacchini, A. Amicucci, M. Buffalini, D. Sisti, and V. Stocchi. 2004. Headspace solid-phase microextraction with gas chromatography and mass spectrometry in the investigation of volatile organic compounds in an ectomycorrhiza synthesis system. Rapid Commun. Mass Spectrom. 18:206–210.

7. Ospina-Giraldo, M. D., P. D. Colloly, C. P. Romaine, and D. J. Royse. 2000. Classification of sequences expressed during the primordial and basidiose stages of the cultivated mushroom *Agaricus bisporus*. Fungal Genet. Biol. 29:81–94.

8. Pieraletti, R., M. Buffalini, L. Vallorani, C. Guidi, S. Zeppa, C. Sacconi, P. Pucci, A. Amoresano, A. Casbarra, and V. Stocchi. 2004. *Tuber borchii* fruit body: 2-dimensional profile and protein identification. Phytochemistry 65: 813–820.

9. Stone, R. L., V. Matarese, B. B. Magee, P. T. Magee, and DA Bernlohr. 1990. Cloning, sequencing and chromosomal assignment of a gene from *Saccharomyces cerevisiae* which is negatively regulated by glucose and positively by lipids. Gene 2:171–176.
10. Weete, J. D., and S. R. Gandhi. 1996. Biochemistry and molecular biology of fungal sterols, p. 421–438. In R. Brambl and G. Marzluf (ed.), The mycota III, biochemistry and molecular biology. Springer, Berlin, Germany.

11. Zeppa, S., A. M. Gioacchini, C. Guidi, M. Guescini, R. Pierleoni, A. Zambonelli, and V. Stocchi. 2004. Determination of specific volatile organic compounds synthesized during Tuber borchii fruit body development by solid-phase microextraction and gas chromatography/mass spectrometry. Rapid Commun. Mass Spectrom. 18:199–205.

12. Zeppa, S., C. Guidi, A. Zambonelli, L. Potenza, L. Vallorani, R. Pierleoni, C. Sacconi, and V. Stocchi. 2002. Identification of putative genes involved in the development of Tuber borchii fruiting body by mRNA differential display in agarose gel. Curr. Genet. 42:161–168.