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Enhanced activity of the *GmarMT1* promoter from the mycorrhizal fungus *Gigaspora margarita* at limited carbon supply

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Abstract

Metallothioneins are low molecular weight polypeptides, present in most eukaryotic phyla, with role in metal homeostasis and detoxification. We previously reported the identification and the characterization of a metallothionein gene (*GmarMT1*) from the arbuscular mycorrhizal fungus *Gigaspora margarita*. Here, we have used real-time quantitative RT-PCR to show that *GmarMT1* expression was turned off during the symbiotic fungal growth in the hexose-rich mycorrhizal apoplast, whereas transcripts were abundant during all other fungal growth stages, when products of lipid breakdown and the glyoxylate cycle feed carbohydrate-consuming pathways. In support of a nutritional regulation of *GmarMT1* expression, we show that transcriptional activity of *GmarMT1* promoter in yeast was strongly induced by glucose starvation (up to 20 times the basal level). We speculate that *GmarMT1*-encoded protein, with its proved metal binding ability, could regulate the homeostasis of zinc, a fundamental cofactor involved in C metabolism regulation and glucose repression.

Keywords: Plant symbiosis; Carbon metabolism; Life cycle; Mycorrhiza; Metallothionein; UAS

1. Introduction

Arbuscular mycorrhizal (AM) fungi belong to an ancient group of fungi, the Glomeromycota (Schussler et al., 2001), and form a symbiotic association with the roots of a wide range of land plants (van der Heijden and Sanders, 2002). The plant benefits from this symbiosis through an improvement of its mineral nutrition and protection against biotic and abiotic stresses whereas the fungus gains photosynthates from the plant (Smith and Read., 1997).

AM fungi rely mostly entirely on the host for their source of carbon and cannot complete their life cycle without this supply. Several studies have shown radical differences in C supply, flux and metabolism between asymbiotic and symbiotic fungal growth (Bago et al., 2003, 1999, 2000; Pfeffer et al., 1999; Saito, 1995; Shachar-Hill et al., 1995). Lipids, mainly in the form of triacylglyceride (TAG), are the main form of carbon in AM fungal spores and mobilization of lipids and activation of the glyoxylate cycle feed carbohydrate-consuming pathways during spore germination and asymbiotic growth (Bonfante, 1984). Asymbiotic fungal growth is limited and further growth is strictly dependent on symbiosis with a suitable partner (Bago et al., 1999; Beilby and Kidby, 1980; Gaspar et al., 1994). Inside the root, inter- and intracellular hyphae colonize the root cortex, and differentiated structures, such as coils and arbuscules, are produced. Since intracellular hyphae as well as arbuscules are always surrounded by plant plasma membrane and a thin layer of plant cell wall material, AM fungal colonization is apoplastic (Genre et al., 2005).

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Large fluxes of photosynthetically fixed carbon are transferred from leaves to the mycorrhizal root system during the symbiosis (Douds et al., 2000; Graham, 2000). The concentration of hexoses in these compartments is high, and up to 20% of C is transferred to the fungus, probably by virtue of a high proton gradient generated by H^+-ATPases (Karandashov and Bucher, 2005). Studies using ^{13}C-glucose nuclear magnetic resonance spectroscopy indicate glucose as a likely substrate utilized in the symbiotic stage. *In situ* hybridization analyses show high levels of transcripts involved in sucrose hydrolysis (invertase) in the arbusculated cells, but not in the surrounding cells (Blee and Anderson, 1998). The altered gene expression may create a sink for sucrose in the arbusculated cells and efflux of hexoses from these cells and the neighbouring non-colonized cells could be favoured by plant hexose transporters (Harrison, 1996). This flux is also confirmed by the absence of amyloplasts in the arbusculated cells (Bonfante, 1984).

Although substantial changes in fungal C metabolism occur during the switching from asymbiotic to symbiotic phases of the fungal life cycle, the molecular mechanisms governing such changes are largely unknown. We previously reported the characterization of a metallothionein gene (*GmarMT1*) from the AM fungus *Gigaspora margarita* (Lanfranco et al., 2002), and demonstrated lower expression of this gene in the symbiotic phase. Here, through comparative expression studies across the life cycle, we show that *GmarMT1* transcript levels maintain high in the symbiotic mycelium protruding from the roots, but drastically dropped in fungal hyphae living in the hexose-rich environments such as the mycorrhizal root apoplast. Accordingly, studies of transcriptional activity of *GmarMT1* promoter in the heterologous system *Saccharomyces cerevisiae* showed strong induction in response to glucose starvation (up to 20 times the basal level), whereas induction by metal and oxidative stresses produced only moderate or non significant changes in *GmarMT1* transcriptional activity.

2. Materials and methods

2.1. Biological materials and growth conditions

Spores of *Gigaspora margarita* (BEG 34) were collected from pot cultures of mycorrhizal *Trifolium repens* L. and sterilized with 3% (w/v) chloramine T/0.03% (w/v) streptomycin, plus four rounds of sonication. Spores were germinated in water at 26 °C for two weeks in the dark.

The plant species *Lotus japonicus* (Regel) Larsen was used for the production of mycorrhizal roots in a sandwich system (Giovannetti et al., 1993). Plants were kept in a growth chamber (Novero et al., 2002) and the roots were harvested at 1 month after inoculation with about 25 spores of *G. margarita*. About fifty 1 cm-long root segments were selected under a stereomicroscope, stained with cotton blue and used to evaluate the extent of root colonization (Trouvelot et al., 1986). Four parameters were evaluated: frequency of root segments showing internal colonization, average per cent colonization of roots segments, percentage of arbuscules in the colonized portion of the root and percentage of arbuscules in the root overall.

2.2. Screening of the genomic library

A genomic library was constructed in the phage vector lambda DASHII (Stratagene) from *G. margarita* sporal DNA purified in a CsCl gradient (Hosny et al., 1999). A total number of 500,000 primary recombinant clones was obtained. Approximately 400,000 clones were screened using a 460-bp DNA fragment covering the *GmarMT1* coding sequence (Lanfranco et al., 2002) as probe. The hybridization was performed using the ECL direct DNA nucleic acid labelling and detection kit (Amersham Biosciences) following the manufacturer's instructions. Phage DNA was prepared using the Lambda Mini Kit (Qiagen) and sequenced by GENOME express (Grenoble, France). The *GmarMT1* GenBank entry (Accession No. AJ421527) was updated.

2.3. Real-time quantitative RT-PCR

RNA was extracted from about 100 quiescent and germinated spores, extraradical mycelium collected from *L. japonicus* mycorrhizal roots, and from 50 mg mycorrhizal roots devoid of external hyphae. Samples were collected under a stereomicroscope, immediately frozen in liquid nitrogen and total RNA extracted using the SV Total RNA Isolation System kit (Promega). The RNA was precipitated by adding an equal volume of 2 M LiCl, centrifuged at 10,000 g for 30 min and resuspended in 25 µl of DEPC-treated sterile water.

All RNA samples were routinely checked for DNA contamination using the One-step RT-PCR kit (Qiagen) but omitting the RT step. Reactions were carried out in a final volume of 10 µl containing 2 µl of 5× buffer, 400 µM dNTPs, 0.6 µM of each *G. margarita* 18S rRNA specific primers (18S/283+ 5′-GAATTTCTACCTTCTGGGGAACT-3′ and 18S/388− 5′-TCAGACGTAAGCCTGCTTTG-3′), 0.25 µl of One step RT-PCR Enzyme Mix and 1 µl of total RNA. Samples were incubated for 30 min at 50 °C followed by a 15 min incubation at 95 °C. Samples corresponding to RT minus were kept on ice instead of 50 °C. Amplification reactions (92 °C for 45 s, 60 °C for 45 s, 72 °C for 45 s) were run for 35 cycles.

To obtain cDNAs from the different samples, reverse transcription reactions were performed in a final volume of 20 µl containing 2 µl 10× buffer, 0.5 mM each dNTPs, 10 µM random exanucleotides (Invitrogen), 1 µl SuperScript II RNase H^- Reverse Transcriptase (Invitrogen) and 8 µl of RNA. Samples were incubated at 42 °C for 50 min. To minimize variation in performance of the reverse transcriptase, at least two separate RT reactions were pooled for each RNA preparation. Prior to Real-Time PCR experiments, cDNAs were tested in conventional PCR experi-