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RESEARCH ARTICLE

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The α-tubulin gene *AmTuba1*: a marker for rapid mycelial growth in the ectomycorrhizal basidiomycete *Amanita muscaria*

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Abstract The apical extension of hyphae is of central importance for extensive spread of fungal mycelium in forest soils and for effective ectomycorrhiza development. Since the tubulin cytoskeleton is known to be important for fungal tip growth, we have investigated the expression of an α-tubulin gene from the ectomycorrhizal basidiomycete *Amanita muscaria* (*AmTuba1*). The phylogenetic analysis of protein sequences revealed the existence of two subgroups of α-tubulins in homobasidiomycetes, clearly distinguishable by defined amino acids. AmTuba1 belongs to subgroup1. The *AmTuba1* transcript level is related to mycelial growth rate. Growth induction of carbohydrate starved (non-growing) hyphae resulted in an enhanced *AmTuba1* expression as soon as hyphal growth started, reaching a maximum at highest mycelial growth rate. Bacterium-induced hyphal elongation also leads to increased *AmTuba1* transcript levels. In mature *A. muscaria*/*P. abies* ectomycorrhizas, where fungal hyphae are highly branched, and slowly growing, *AmTuba1* expression were even lower than in carbohydrate-starved mycelium, indicating a further down-regulation of gene expression in symbiosis. In conclusion, our analyses show that the *AmTuba1* gene can be used as a marker for active apical extension in fly agaric, and that α-tubulin proteins are promising tools for the classification of fungi.

Keywords Cytoskeleton · Ectomycorrhiza
Filamentous fungi · Tip growth · Basidiomycete

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Introduction

In filamentous fungi, such as the basidiomycete *Amanita muscaria*, apical extension is based on the intracellular transport of vesicles and growth supplies along microtubule (MT) and actin cytoskeleton. Longitudinally running MTs play a role in nuclear migration, cell- and nuclear division (Runeberg and Raudaskoski 1986; Salo et al. 1989) and are also involved in the movement of vesicles and in operation of the motile vacuolar systems (Hyde et al. 1999), both necessary for nutrient transport between different parts of a fungal colony. Inhibitor studies have suggested that microtubules are mainly responsible for long distance and actin for short distance transport in filamentous fungi (Howard and Aist 1977; Niini et al. 1993; Raudaskoski et al. 1994; Steinberg et al. 2001).

However, while actin is clearly essential for tip growth (Heath 1990), the role of MT has been controversial until recently, as MT are not an absolute requirement for apical extension. For example, germination of conidia and polarized tip growth of germ tubes occur in *Aspergillus nidulans* in the presence of anti MT drugs at concentrations that leads to fully depolymerized MT (Oakley and Morris 1980). Recent observations in *A. nidulans* and the maize pathogen *Ustilago maydis* indicate, however, that microtubules become essential in hyphal growth over extended distances, since long-distance-growth can be greatly reduced by microtubule depolymerization (Horio and Oakley 2005; Fuchs et al. 2005).

When the ECM fungus *A. muscaria* comes into contact with the soil bacterium *Streptomyces* nov. sp. AcH 505 (AcH 505), mycelial growth speed is enhanced, resulting in a higher mycorrhization rate (Maier 2003; Maier et al. 2004; Schrey et al. 2005). However, enhancement of ECM fungal growth takes place not only in response to mycorrhiza helper bacteria (Garbaye 1994), but also as a response to host plant root exudates (Melin and Rama Das 1954). Thus, promoted long distance hyphal growth is assumed to be of central

importance for mycorrhiza formation and competitiveness in the forest soils (Leake et al. 2002).

During the colonization of host plant fine roots, ectomycorrhizal (ECM) fungi change their behaviour from fast tip growth towards a highly branched structure of coenocytic, swollen hyphae (Kottke and Oberwinkler 1987). A comparable hyphal transition could be induced in the saprophyte *Schizophyllum commune* by inhibition of MT polymerization (Raudaskoski et al. 1994), suggesting that the MT cytoskeleton is involved in the modulated hyphal anatomy in ectomycorrhizas. Microscopical investigations indicated, that fungal morphogenesis is linked to the reorientation of both the MT (Timonen et al. 1993) and actin cytoskeleton (Gorfer et al. 2001), indicating that both cytoskeletal elements serve central functions during the formation and functioning of symbiotic hyphae (Raudaskoski et al. 2004).

In spite of this, little is known about the structural requirements for rapid growth rates in ectomycorrhizal fungi. Therefore we decided, to connect our analyses on nutrient-based (Nehls 2004) and mycorrhiza helper bacterium-based (Schrey et al. 2005) regulation of gene expression in *A. muscaria*, to perform a thorough analysis of gene expression of a newly isolated α-tubulin gene, *AmTuba1*.

Furthermore, α-tubulins have recently been used for phylogenetic analyses of Microsporidia and Glomeromycota (Keeling 2003; Corradi et al. 2004). Since it has been shown that usage of multigene datasets can increase the resolution of molecular phylogenetic analyses in higher fungi (e.g. Lutzoni et al. 2004; Binder et al. 2005), we also investigated the potential of Atub protein sequences in phylogenetic reconstruction.

Materials and methods

Biological material and culture conditions

Amanita muscaria (Fr.) Hooker isolate CS83, isolated from Schönbuch, Germany, was cultivated in the dark at 20°C on MMN (Modified Melin Norkrans; Marx 1969) medium with 10 gl^{-1} glucose. Fungal suspension cultures were started from actively growing mycelia from agar plates which were cut into 2×2 mm pieces, transferred to MMN medium and incubated at 20°C on a rotary shaker at 80 rpm. To determine the effect of growth rate on *AmTuba1* expression in submerged culture, mycelia were starved for carbon for 1 week. Fresh culture medium with 40 mM glucose was added, and the increase in fresh weight, and *AmTuba1* expression levels in fungal mycelia were investigated during a time course of 22 days. To determine the time point for the onset of hyphal growth after medium exchange a sample of the suspension culture was transferred to a Petri dish (containing 40 mM glucose) and hyphal growth was followed microscopically.

Seeds of Norway spruce (*Picea abies* (L.) Karst) were obtained from the Staatsklenge Nagold (Nagold, Germany). Norway spruce mycorrhizas with *A. muscaria* were synthesized according to Nehls et al. (1998). Fully developed functional mycorrhizal root tips were harvested 70 days after synthesis.

Amanita muscaria growth promoting *Streptomyces* sp. nov. 505 (AcH 505) and the control strain *Streptomyces argenteolus* 504 (AcH 504) that exerts no effect on mycelial growth in dual culture (Maier 2003), were originally isolated from the from the vicinity of mycorrhizal Norway spruce fine roots in Haigerloch, Germany (Maier 2003). The bacteria were cultivated on ISP-2 agar (Shirling and Gottlieb 1966) in the dark at 20°C. The bacterial effect on fungal growth was tested in dual culture without a direct contact between fungus and bacterium on MMN medium on Petri dishes (see Maier et al. 2004 for details). For harvest, the first cm of 9-week-old fungal cultures were cut out as a ring and immediately frozen in liquid nitrogen.

To analyse whether the bacterial effect on hyphal growth is continues throughout the whole culture period, fungal growth rate (extension of mycelia/time) was determined every 2 weeks, starting at week 1. The first 100 μm of the hyphal tips were analysed by light microscopy to investigate, if the bacteria have an influence on the lateral branching rate of the hyphae.

Molecular biology techniques

Total RNA from *A. muscaria* was extracted according to Nehls et al. (1998). The *AmTuba1* cDNA was isolated during the analysis of expressed sequence tags from a lambda-ZAP (Stratagene, Amsterdam, The Netherlands) cDNA library of *A. muscaria–Picea abies* ectomycorrhiza (U. Nehls, unpublished). Overlapping sequencing was performed using M13 universal and reverse primers as well as internal primers and the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer, Wellesley, MA, USA) according to the manufacturer's instructions.

For DNA probe synthesis, the last 280 bp of the α-tubulin cDNA were amplified by PCR from the *AmTuba1* cDNA. Northern hybridizations were performed according to Nehls et al. (1998). All Northern blot experiments were performed in triplicate.

Phylogenetic analyses with the AmTuba1 protein sequence

The predicted full-length protein sequence of *AmTuba1* was used for phylogenetic analyses. For alignments, all full-length sequences from other basidiomycete fungi, together with a representative selection of α-tubulins (Atubs) from other fungal groups, detected by blastx in the non-redundant database of the NCBI (http://www.ncbi.nlm.nih.gov/BLAST/) were used. The selected α-tubulins with their GenBank accession numbers were: Ascomycota: *Aspergillus nidulans* 1, P24633; *A. nidulans*

2, P24634; Basidiomycota: *Amanita muscaria* 1, AJ643287; *Coprinopsis cinerea* 1, BAC77592; *Coprinopsis cinerea* 2, BAC77593; *Cryptococcus neoformans*, AAW41563; *Schizophyllum commune* 1A, CAA60034, *Schizophyllum commune* 1B, CAA60035; *Ustilago maydis*, CAC17476; Glomeromycota: *Glomus diaphanum*, CAG3052; *Scutellospora castanea*, CAG30534; Microsporidia: *Encephalitozoon cuniculi*, NC_003234; *Nosema bombycis*, AAZ42396.

To gather more basidiomycete sequences, publicly available fungal genomic DNA sequences from Basidiomycota were screened for Atubs. Due to the lack of full-length Atubs from Zygomycota in databases, the *Rhizopus oryzae* genome sequence was screened. For both approaches, AmTuba1 was used as a template using tBLASTN. A number of putative Atubs were detected: *Cryptococcus neoformans*, 1 putative Atub; *Ustilago maydis*, 1; *Coprinopsis cinerea*, 2; *Phanerochaete chrysosporium*, 2; *Rhizopus oryzae*, 3. The Atubs of *C. neoformans*, *C. cinerea* and *U. maydis* had been previously deposited in Genbank, and the location of *P. chrysosporium* Atubs and *R. oryzae* Atubs in the respected genome sequences are listed in Table 1.

For the analysis of basidiomycete Atubs together with sequences from other fungal groups, the multiple alignment file obtained from ClustalW analysis was edited by hand. The overhanging ends were removed from both ends of the sequence to ensure that all sequences were of the same length. The analysed Atub sequence reached from Thr-25 to Tyr-407 in AmTuba1 sequence, due to the fact that from Glomeromycota no full-length Atub sequences were available. Since no significant gaps existed after the trimming between the amino acid sequences, they were directly used for phylogenetic analysis. To estimate the phylogenetic relationships, alignments were analysed using a Bayesian approach based on Markov chain Monte Carlo (MCMC, Larget and Simon 1999) as implemented in the program MrBayes 3.0b4 (Huelsenbeck and Ronquist 2001). To estimate the phylogenetic relationships, alignments were analysed using a Bayesian approach based on Markov chain Monte Carlo (MCMC, Larget and Simon 1999) as implemented in the program MrBayes 3.0b4 (Huelsenbeck and Ronquist 2001). Four incrementally heated simultaneous Monte Carlo Markov chains were run over 2×10^6 generations using random starting trees and assuming a percentage of invariable alignment sites with gamma-distributed substitution rates of remaining sites. The amino acid substitution model was sampled from the substitution models implemented in MrBayes. Trees were sampled every 100 generations resulting in an overall sampling of 20,000 trees. Stationarity of the process was controlled using the Tracer software version 1.0 (Rambaut and Drummond 2003). The first 8,000 trees were discarded (“burn in”) and the remaining 12,000 were used to compute a 50% majority rule consensus tree to get estimates of posterior probabilities. Branch lengths were averaged over the sampled trees and scaled in terms of expected numbers of amino acid substitutions per site. To test the independency of the results, the Bayesian MCMC phylogenetic analyses were performed twice for each data set. Resulting MCMC trees were rooted with *Arabidopsis thaliana* α-tubulin 1, accession P11139. The phylogenetic relationships of full-length α-tubulins inside homobasidiomycetes were further analysed by protein parsimony and neighbour joining methods in PHYLIP (Felsenstein 1993). Both methods of analysis led to the same tree topology as the Bayesian MCMC phylogenetic analysis.

Table 1 Localization of α-tubulin sequences in the genomes of *Phanerochaete chrysosporium* and *Rhizopus oryzae*

Organism	Gene	Contig	Position (bp)
P. chrysosporium	*Tuba1*	AADS01000121	46,665–48,345
P. chrysosporium	*Tuba2*	AADS01000121	44,547–46,220
R. oryzae	*Tuba1*	AACW01000348.1	1,24,334–1,25,745
R. oryzae	*Tuba2*	AACW01000422.1	1,70,325–1,71,817
R. oryzae	*Tuba3*	AACW01000088.1	14,129–15,633

Results

Phylogeny of α-tubulins

During screening of a cDNA library from Norway spruce-fly agaric ectomycorrhiza (U. Nehls, unpublished), a fungal cDNA showing high homology to α-tubulins (Atubs) was detected. The *A. muscaria* α-tubulin 1 (*AmTuba1*) cDNA comprised a full-length reading frame for a protein of 446-aa in length that revealed high homology to Atubs from other closely related homobasidiomycete fungi, *Coprinopsis cinerea*, *Phanerochaete chrysosporium*, and *Schizophyllum commune* (96, 93 and 92% identity, respectively). From all of these organisms, a second *Atub* gene was detected by blast search, showing, however, a much lower relatedness to AmTuba1 (87, 83 and 84% identity at protein level, respectively). To group the homobasidiomycete (HB) Atubs, a phylogenetic analysis was carried out. First we observed by Bayesian method, that basidiomycete Atubs clearly separate from those of other fungal phyla (Fig. 1). Then, according to Bayesian, protein parsimony and neighbour joining analyses (data not shown), the Atubs could be divided into two subgroups among the HB. AmTuba1 grouped together with its closest homologues in subgroup1. From those HB organisms where the whole genome has been sequenced (*Phanerochaete*, *Coprinopsis*) it becomes clear, that homobasidiomycete (HB) species contain two α-tubulins, one belonging to subgroup1 and a second to subgroup2. By comparison of the consensus protein sequences of each subgroup, several group-specific amino acids were identified (Table 2).

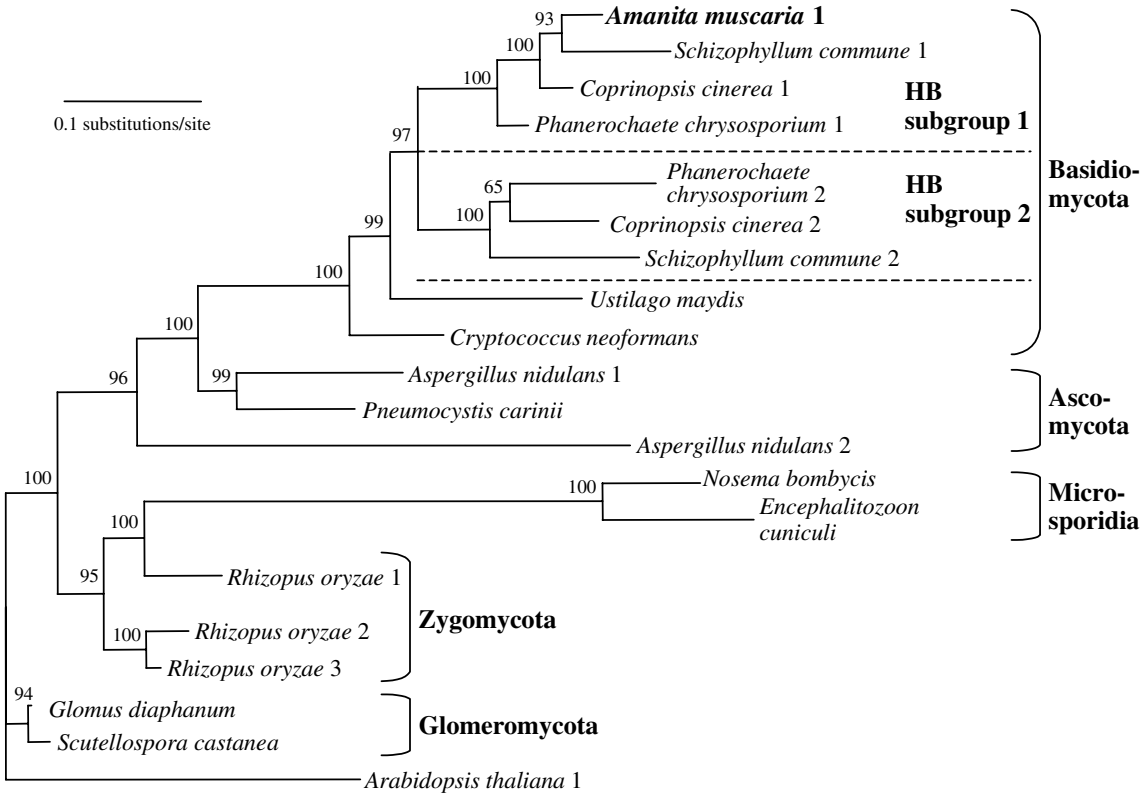

Fig. 1 Rooted tree illustrating the phylogenetic relationships between AmTuba1 and other fungal α-tubulins. All full-length basidiomycete sequences present in GenBank by September 2005 were used. From the genomes of *Coprinopsis cinerea* and *Phanerochaete chrysosporium* two and from the genome of *Rhizopus oryzae* three Tuba homologues were detected (this work) according to sequence homology to AmTuba1. Bayesian Markov chain Monte Carlo analysis was performed using partial Tuba1 sequences, reaching from position 25 to 407 in the predicted AmTuba1 protein sequence. Numbers at branches are a posteriori probabilities regarding the monophyly of the respective protein. Branch lengths are proportional to evolutionary distances. The scale bar indicates an evolutionary distance of 0.1 amino acid substitutions per position in the sequence. *Amanita muscaria, S. commune* and *P. chrysosporium* sequences were named according to the nomenclature of *C. cinerea*. The division of homobasidiomycete (HB) α-tubulins into two subgroups is highlighted. The tree was rooted with *Arabidopsis thaliana* Tuba1

Growth-dependent expression of *AmTuba1*

To determine whether a relationship between the mycelial growth rate and the *AmTuba1* expression exists, submerged fungal cultures were carbohydrate-starved for 1 week. After medium exchange and glucose (40 mM) addition, the increase of the mycelial fresh weight and *AmTuba1* gene expression were followed over a time period of 4 weeks. Eight hours after carbohydrate addition, the *AmTuba1* transcript level was six-fold increased (Fig. 2). This time point corresponded to the onset of (microscopically visible) hyphal tip growth. Strong *AmTuba1* expression was observed as long as the growth rate of the mycelium was maximal, while a reduction in growth was accompanied by the reduction of *AmTuba1* expression.

Table 2 Amino acids specific for α-tubulin subgroup1 (Tuba1) and subgroup2 (Tuba2). Shown are the positions of subgroup-specific amino acids in the predicted α-tubulin proteins

Tuba position	Aa Tuba1	Aa Tuba2
56	Ser	Gly
99	Ser	Arg
176	Ala	Ser
333	Ile	Thr
361	His	Thr
384	Ser	Ala

Mature symbiotic tissues of ectomycorrhizas contain highly branched fungal hyphae often connected by anastomosis in mantle and in Hartig net. In these hyphae tip growth has been strongly reduced (Kottke and Oberwinkler 1987). In accordance with the finding, that growth rate and *AmTuba1* expression are correlated, *AmAtub1* gene expression was further reduced (compared to slow growing hyphae) in fully developed ectomycorrhizas (Fig. 2).

The hyphal growth of *A. muscaria* was promoted by the presence of the streptomycete isolate AcH 505 in dual culture (Fig. 3a). In contrast to AcH 505, the streptomycete isolate AcH 504 does not affect mycelial growth of *A. muscaria* (Fig. 3a, Maier et al. 2004). To investigate whether bacteria-induced stimulation of mycelial growth is caused by a continuous stimulation of hyphal extension, we analysed the growth rates of *A. muscaria* mycelium, free-living and in dual culture (DC) with AcH 504

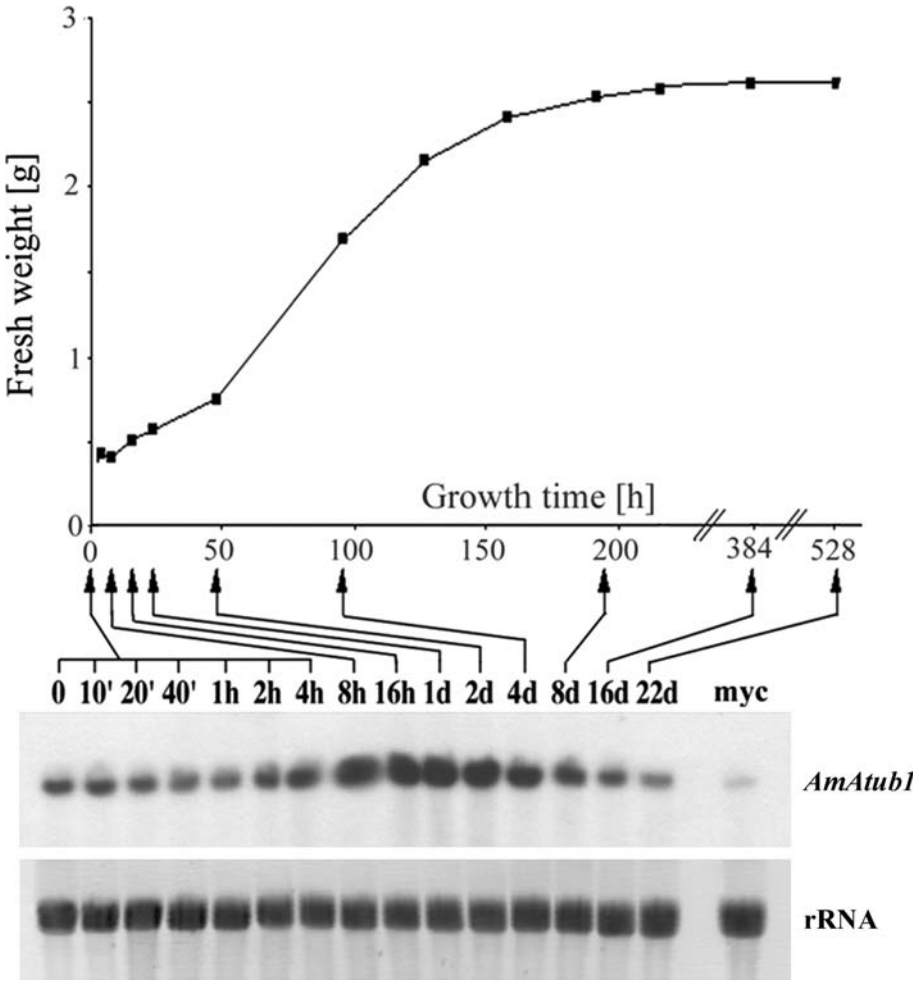

Fig. 2 *Amanita muscaria* growth rate and *AmTuba1* expression in submerged culture and in mycorrhizal root tips. Modified Melin-Norkrans medium supplemented with 40 mM glucose was added to mycelia that were carbohydrate depleted for 1 week. Mycelial samples were taken at different times. Mature Norway spruce mycorrhizas were harvested 70 days after inoculation. Total RNA was isolated and changes in the transcript levels of *AmTuba1* were analysed by Northern blot analysis using the non-coding 3′end as probe. Hybridization with *A. muscaria* 5.8S rRNA was used as loading reference

or AcH 505 (Fig. 3b). Mycelial growth rates (mm hyphal extension/2 weeks) were nearly stable over the whole growth period and higher in the presence of AcH 505 (Fig. 3b). Dual culture with neither of the streptomycete isolates provoked alterations in lateral branching rate of the fungal hyphae. When the first 100 µm of the tips of hyphae were analysed, the percentage of laterally branched front hyphae were comparable between free-living mycelium ($11.3 \pm 2.3\%$), AcH 504 DC mycelium ($10.8 \pm 2\%$), and AcH 505 DC mycelium ($12 \pm 3\%$).

To investigate if the stimulated fungal growth rate in DC with AcH 505 is associated with an increased *AmTuba1* expression, Northern blot analysis of free living mycelium and hyphae in DC with the streptomycetes was performed. While the *AmTuba1* transcript levels in free living mycelia and in dual culture with AcH 504 were comparable, gene expression increased in the presence of AcH 505 (Fig. 3c).

Discussion

According to our data, homobasidiomycete (HB) species contain two α-tubulins that could be separated by certain amino acids at defined positions. Future work has to be performed to elucidate whether these differences in amino acid composition are simply useful phylogenetic markers or indicate modulated microtubuli function. The importance of defined amino acids for proper microtubule (MT) function has been demonstrated in *Saccharomyces cerevisiae* (Bode et al. 2003; Richards et al. 2000).

Analysis of tubulins turned out to be a promising tool for molecular phylogeny in fungi. For Basidiomycota, such analyses have thus far concentrated on β-tubulins (Begerow et al. 2004; Juuti et al. 2005; Shi and Perlin 2005). However, also α-tubulins (Atub) have recently been shown to be rather useful for phylogenetic analyses in Microsporidia and Glomeromycota (Keeling 2003; Corradi et al. 2004). The use of proteins from a wide variety of fungi in Bayesian analysis enabled us to test if our α-tubulin based phylogenetic tree had a similar topology than those based on other molecular markers. According to our data, clade-specific grouping, together with the rate of molecular variation, make α-tubulin sequences potentially useful for classification purposes. Consistent with recent results from multigene analyses of HB phylogeny (Lutzoni et al. 2004; Binder et al. 2005), the *Phanerochaete chrysosporium* (Aphyllophorales) Atub1 sequence was unambiguously placed outside the

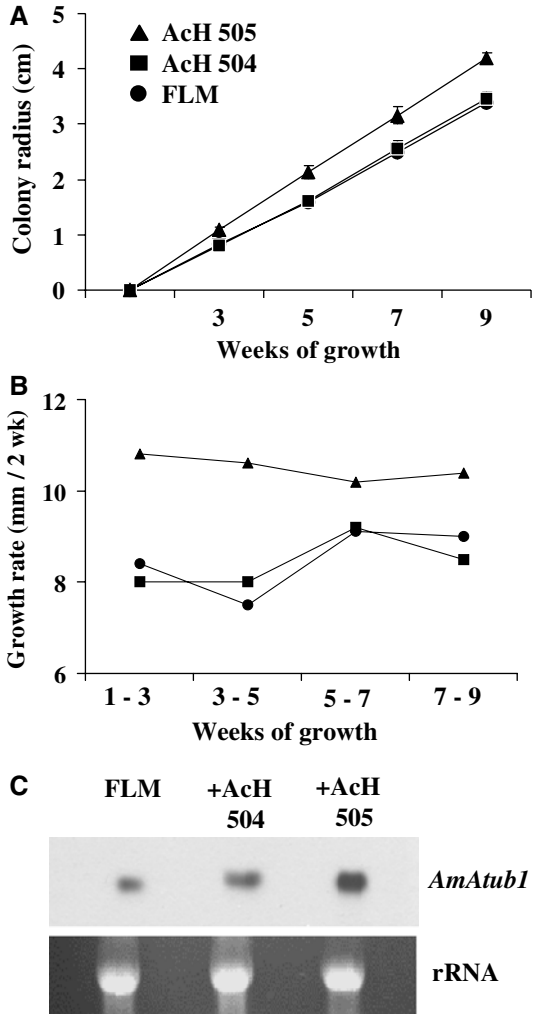

Fig. 3 *Amanita muscaria* growth and *AmTuba1* expression levels in dual culture with *Streptomyces* isolates. **a** The effect of the presence of *Streptomyces argenteolus* isolate 504 (non-growth promoting control) and the mycorrhiza helper isolate AcH 505 on mycelial growth of *A. muscaria* on agar plates is shown. Ten replicates were made from each treatment. FLM corresponds to free living mycelium. Enhanced mycelial growth was significant for AcH 505 treatment according to Student's *t* test ($P < 0.01$). **b** Growth rate of *A. muscaria* mycelia expressed as mm growth in 2 weeks. **c** Transcript levels of *AmTuba1* during *A. muscaria* growth in the absence (FLM) or presence of the bacterial isolates AcH 504 or AcH 505 on agar plates. The first cm of fungal cultures was harvested after 9 weeks culture. Total RNA was isolated and changes in the transcript levels of *AmTuba1* were analysed by Northern blot analysis using the non-coding 3′end as probe. Ribosomal RNA was used as loading control

Atub1 of Agaricales species. The isolation of three α-tubulin genes from the genome sequence of *Rhizopus oryzae* facilitated us the confirmation of a zygomycete origin of Microsporidia as suggested by Keeling (2003). *R. oryzae* and Microsporidia proteins appeared to be rather divergent, however, microsporidial sequences unambiguously placed within *R. oryzae* tubulins. That the glomeromycota group together at a very basal position in the fungal clade in the α-tubulin based phylogenetic tree is in accordance with the analysis of Corradi et al. (2004). However, a remarkable difference between our and other phylogenetic trees is the position of *Cryptococcus neoformans*. According to current molecular phylogenetic models (e.g. Weiss et al. 2004) *Ustilago maydis* (Ustilaginales) groups basal from *C. neoformans* (Hymenomycetes), while it would be the opposite according to α-tubulins. Thus, representative sampling of Atubs will be still necessary to ensure the usefulness of α-tubulins to unravel evolutionary relationships and to use them for multigene datasets in future.

In their expression studies of a β-tubulin gene in *Schizophyllum commune*, Russo et al. (1992) observed a high transcript level of the gene in young mycelium (large number of growing hyphal tips), but a decreased expression related to increasing age of the mycelium (small number of growing hyphal tips). This observation, and the results at hand on *A. muscaria* α-tubulin expression, indicate that tubulin genes in HB are expressed in an apical growth related manner.

When hyphal growth in *A. muscaria* was induced by glucose addition to a carbohydrate starved culture, increased *AmTuba1* expression was observed after 4 h. Compared to other *A. muscaria* genes (phenylalanine ammonium lyase, Nehls et al. 1999; cyclophilin 40 gene, S. Schrey unpublished) this is not a rapid fungal reaction to an altered environment. However, 4 h are about the lag time for non-growing *A. muscaria* hyphae after growth induction, as indicated by the parallel increase in Cdc42 gene expression (U. Nehls unpublished), a central regulator of apical growth in eukaryotes including HB fungi (Gorfer et al. 2001). We thus assume that the time needed for increased *AmTuba1* expression reflects the time that is necessary for mobilization of the machinery for apical extension.

Mycelial growth slowed down 4 days after growth induction. At this time also the *AmTuba1* transcript level was significantly reduced. However, gene expression remained above the initial transcript level when the culture entered the stationary growth phase (around day 8). Wright et al. (2005) observed two-fold higher α-tubulin expression levels in mature rhizomorphs of *Paxillus involutus* compared to mycorrhizal root tips, suggesting that enhanced α-tubulin expression levels were not only caused by rapid mycelial extension, but also to enable long distance transport within an ectomycorrhizal fungal colony. The maintenance of transport capacity inside the starved mycelia might thus be a reason for enhanced *AmTuba1* expression during the early stationary phase of *A. muscaria* mycelia.

For ectomycorrhizal fungi the symbiotic interaction with a host plant is ecologically essential. Thus, recognition and fast colonization of newly developed fine roots is necessary for survival and growth stimulation of hyphae in the rhizosphere by plant-derived growth promoting substances was often observed (Lagrange et al. 2001). In accordance with this observation, incubation with exudates of red pine roots (that have not been in contact with ectomycorrhizal fungi) causes a four-fold increase in the expression level of a *Laccaria bicolor* α-tubulin gene

(Podila et al. 2002). However, our preliminary results with *Picea abies* root extracts, give no hint for a growth promotion of *A. muscaria* hyphae and are corroborated by the fact, that *AmTuba1* expression was not increased (data not shown). This indicates, that the activation response of *L. bicolor* with red pine extract may be interaction-specific and other systems do need the recognition of a potential fungal partner before growth promoting substances are formed. In contrast to root extracts, the growth rate of *A. muscaria* hyphae is strongly enhanced by the presence of certain soil bacteria (Maier et al. 2004), indicating the ecological importance of soil bacteria for ectomycorrhizal symbiosis. This increased fungal growth rate is accompanied by an increased *AmTuba1* transcript level (this contribution).

The establishment of an ECM is accompanied by a series of developmental processes leading to the formation of the hyphal mantle and the Hartig net (Kottke and Oberwinkler 1987). As shown by Timonen et al. (1996), fungal hyphae are highly proliferating and producing actively fungal α-tubulin at the early stages of mycorrhization, whereas proliferation and α-tubulin signal are reduced at later stages (after the development of mantle and Hartig net) when hyphal growth become stagnant. In accordance with this, ectomycorrhiza development is accompanied by increased α-tubulin gene expression in developing *Paxillus involutus* mycorrhizas (Johansson et al. 2004), while in fully developed mycorrhizas both α-tubulin (Wright et al. 2005; this study) and actin (Duplessis et al. 2005; Wright et al. 2005) transcription are declined.

In conclusion, our observations suggest that *AmTuba1* expression is necessary for the maintenance of rapid tip growth in *A. muscaria*. We suggest that a moderately enhanced *AmTuba1* expression is also important for the maintenance of long distance transport in fungal hyphae. In addition, evidence was presented, that representative sampling of α-tubulins could be instrumental for inferring the phylogeny of fungi.

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