Molecular characterization and expression analysis of Acmago and AcY14 in Antrodia cinnamomea

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Mago nashi (Mago) and Y14 proteins, highly conserved among eukaryotes, participate in mRNA localization and splicing, and as such play important roles in oogenesis, embryogenesis and germ-line sex determination during animal development. Here we identified mago (AcMago) and Y14 (AcY14) homologues derived from Antrodia cinnamomea. AcMago encodes 149 amino acids and AcY14 encodes 168 amino acids. Multiple amino acid sequence alignment as well as secondary and tertiary structure prediction showed that AcMago and AcY14 have similar protein structure to the reported crystal structures of other Mago and Y14 proteins. During fungal development both AcMago and AcY14 genes were abundantly expressed in natural basidiomes. This is the first report of the molecular characterization and expression analysis of the mago and Y14 genes from fungi.

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I n t r o d u c t i o n

The mago (Mago nashi, grandchildless) gene, which is highly conserved among eukaryotes, was first identified as a strict maternal effect gene in Drosophila (Drosophila melanogaster; Boswell et al. 1991). It not only mediates germ plasm assembly in Drosophila but also regulates hermaphrodite germ-line sex determination in nematodes (Caenorhabditis elegans; Li et al. 2000; Newmark & Boswell 1994). Additionally, the mago gene is required for pollen tube growth in Arabidopsis (Arabidopsis thaliana) and root development in the conifer Taiwania (Taiwania cryptomerioides Hayata; Chen et al. 2007; Chen & Chu 2008; Johnson et al. 2004). In a yeast two-hybrid screen, the Mago protein interacted with the RNA binding protein Y14 (also called Tsunagi, connection or link; Zhao et al. 2000). Furthermore, crystal structure analysis demonstrated that they form a stable heterodimer strongly associating with spliced mRNA (Lau et al. 2003; Le Hir et al. 2001a; Shi & Xu 2003). The Mago-Y14 heterodimer is required to localize oskar mRNA and to establish the polarization of the oocyte during Drosophila oogenesis (Hachet & Ephrussi 2001; Micklem et al. 1997; Mohr et al. 2001). Moreover, the Mago-Y14 heterodimer is the core component of the exon junction complex (EJC), and associates with nonsense-mediated mRNA decay (NMD) and cytoplasmic mRNA localization (Bono et al. 2004; Fribourg et al. 2003; Kataoka et al. 2001; Kim et al. 2001; Le Hir et al. 2001b; Palacios et al. 2004; Park & Muench 2007).

Antrodia cinnamomea, a resupinate to effused-reflexed basidiomycete with porous hymenium (Chang & Chou 2004), is well-known by its Chinese name “Chang-chih” where it is...
used as a folk medicine in Taiwan. Recent reports have stated that it possesses a wide range of biological functions, including antioxidative, vasorelaxatory, anti-inflammatory, antitumor, hepatoprotective, and antiangiogenic activities (Chu et al. 2008). However, A. cinnamomea does not produce basidiomes in artificial culture and only grows on the inner surface of the heartwood cavity of the evergreen tree, Cinnamomum kraehnii Hayata, an endangered species endemic to Taiwan (Chang & Chou 2004). In order to further develop this fungus as a source of potentially useful drugs while preserving the rare trees that it grows on in the wild, we are developing an improved artificial growth system for A. cinnamomea.

To better understand the growth and development of A. cinnamomea, we examined the function of two essential genes, AcMago and AcY14. Multiple amino acid sequence alignment and secondary and tertiary structure predictions all suggest that AcMago and AcY14 have similar protein structure to the reported crystal structures of Drosophila and human Mago and Y14 proteins. Reverse Transcription-Polymerase Chain Reaction (RT-PCR) analyses confirmed that both AcMago and AcY14 genes were abundantly expressed in natural basidiomes.

Materials and methods

Strain and growth conditions

A. cinnamomea strain TFRIB 479 was identified and provided by Dr. Tun-Tschu Chang (Taiwan Forestry Research Institute). The culture conditions for A. cinnamomea were identical to Chang and Wang’s method (Chang & Wang, 2005). Natural basidiomes were obtained from the infested wood of C. kraehnii. Liquid-cultured mycelia, solid-cultured mycelia, solid-cultured basidiomes and natural basidiomes were frozen in liquid nitrogen and stored at −80 °C until used.

RNA isolation and RT-PCR

The total RNA of each sample was isolated according to the manufacturer’s recommendations (Hopegen; Chu & Chang, 2007). For reverse transcription, each 20 μl reaction containing 1 μg total RNA, 0.5 μg oligo(dT)12–18, appropriate buffer, 0.5 mM dNTP mix, 10 mM DTT, and 200 units of Superscript II reverse transcriptase (Invitrogen) was incubated at 42 °C for 50 min. Approximately 0.3 μg of the first-strand cDNA products were used for PCR amplification. The PCR reaction was carried out for 20 cycles, with each cycle consisting of denaturation at 94 °C for 1 min, annealing at 58 °C for 30 s, and extension at 72 °C for 1 min. The TM82 gene of A. cinnamomea, obtained from microarray data (Chu et al. 2008), served as an internal control to monitor reaction efficiency and to ensure that the initial amounts of cDNA were equal. The TM82 was amplified with (5'-ACTGTGAAACTGCGAATGGCTC-3') and (5'-GACTTGGCCCTCCAATTTGGTCCT-3') primers. The AcMago and AcY14 genes were amplified using the specific primers MagoRTF (5'-GAATAGAGATGATTGGTTTTTGGTCTC-3') and MagoRTR (5'-TCCACCCAGTGCAGGAGAGACACACAG-3') and Y14RTF (5'-AGACAGTGAAGAC-3') and Y14RTR (5'-TCCACCCAGTGCAGGAGAGACACACAG-3').

Cloning of AcMago and AcY14

The partial coding regions of AcMago and AcY14 were amplified using the specific primers Mago-F (5'-ATGACCTCCTAAGACAGATGGTCTC-3') and Mago-R (5'-CATATGGGCTTTATCTTGAACTGTC-3'), and the degenerate primers Y14-F (5'-CCCTGCGCTCYATNGGARGGHTGATCT-3') and Y14-R (5'-GGCAGBACAAABGCAAAGTCTAC-3'). To obtain full-length AcMago and AcY14, 5'-RACE and 3'-RACE were performed using a SMART RACE cDNA Amplification Kit (BD Biosciences) with reverse primers Mago-5' (5'-CTCCCTTCAACACAGTGAGGACCCC-3') and Y14-5' (5'-CAGACTTGTCCATCAGATCCCTCTAGGC-3'), and forward primers Mago-3' (5'-CTGGTCGACATCCAGACAGTGAAGAC-3') and Y14-3' (5'-GGATGGATCTCGTTGGTACCTAACGTGC-3'). The PCR products were cloned using the pGEM-T Easy vector system (Promega) and sequenced on an ABI Prism 3700 DNA Analyzer (Perkin Elmer).

Homologues of AcMago and AcY14 and protein structure prediction

The homologues of AcMago and AcY14 were identified using BLAST algorithms at NCBI (http://www.ncbi.nlm.nih.gov/BLAST). The gene sequences of AcMago and AcY14 were submitted to GenBank (accession no. EF583522 for AcMago and EF583523 for AcY14). The homologues of AcMago and AcY14 are listed in supplementary Table S1, and multiple sequence alignment was carried out with the ClustalW program (http://www.ebi.ac.uk/emb/). The secondary and tertiary structures of AcMago and AcY14 proteins were predicted by the SWISS-MODEL Protein Modeling Server (http://swissmodel.expasy.org/) and the illustrations of the tertiary structure and interaction prediction were viewed and edited by the DeepView - SwissPDB-Viewer program.

Phylogenetic analysis

The homologues of AcMago and AcY14 used for phylogenetic analysis are listed in supplementary Table S1. The analysis was performed using phylip3.6 software. One hundred BS replicates were generated using SEQBOOT, distances were calculated using PROTDIST and trees inferred using NJ and BS values calculated using CONSENSE. The dendrogram was obtained using TREEVIEW.

Results and discussion

Molecular characterization of AcMago and AcY14

The partial sequences of AcMago and AcY14 obtained by the degenerate primers were used for designing 5'-RACE and 3'-RACE primers. The sequence thus obtained for AcMago was 535 bp long, consisting of a 450 bp coding region encoding 149 amino acids, and a 85 bp 3'-UTR (GeneBank accession no. EF583522). AcY14 was 898 bp long, comprising a 288 bp 5'-UTR, a 507 bp coding region encoding 168 amino acids, and a 103 bp 3'UTR (GeneBank accession no. EF583523). In comparison with
other organisms, including fungi and some model organisms listed in supplementary Table S1. *Acmago* and *AcY14* had higher similarities to the homologous genes of *Coprinopsis cinerea* (93% and 71% identities, respectively) than the others. *Acmago* shared 71–93% identity with other basidiomycetes and was also highly conserved among different organisms with 33–96% identity. As shown by the alignment analysis, Mago proteins were highly conserved among eukaryotic

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**Fig 1 – Multiple amino acid sequence alignment and secondary structure prediction of AcMago.** The conserved leucine residues that constitute a potential leucine zipper motif in the C-terminus are indicated with black dots. Identical and similar residues are shaded in black and light grey respectively. The black arrows show the β-strands while the grey bars refer to α-helices. The abbreviations and the accession numbers are as given in Supplemental Table S1.
kingdoms. A conserved leucine zipper motif was identified in the C-terminal portion of the AcMago protein.

Y14 proteins, however, appeared to be more varied among different organisms, and AcY14 only shared 23–91% identity between homologues. Y14 proteins had well-conserved RNA binding motif (RNP1 and RNP2) in the middle of the protein (Figs 1 and 2), and two arginine-serine dipeptide repeats that were characteristics of the SR splicing factors were found in the C-terminal portion of the AcY14 protein (Fu 1995).

Protein structures and interaction predictions between AcMago and AcY14

The predicted protein structure (Fig 3A) of AcMago shows six β-strands (β1-β6) and three α-helices (α1-α3), which is consistent with the reported crystal structures of the Drosophila (Shi & Xu 2003) and the human Mago proteins (Lau et al. 2003). The predicted secondary structure of AcY14 protein consisted of seven β-strands (β1-β7) and two α-helices.
(α1-α2), and the RNP2 and RNP1 sequence motifs located on β2 and β5 respectively (Fig 3B), again similar to the crystal structures of the Drosophila (Shi & Xu 2003) and the human Y14 proteins (Lau et al. 2003). Moreover, some residues in AcY14, such as Lys102 and Tyr104 located at β5 (RNP1), were predicted to interact with Leu139 and Asp131 located at α3 of AcMago (Fig 3C; Fribourg et al. 2003).

Expression of the Acmago and AcY14 during different stages

The variation of Acmago and AcY14 expression in different A. cinnamomea samples, namely liquid-cultured mycelia, solid-cultured mycelia, solid-cultured basidiomes and natural basidiomes, was analyzed using RT-PCR with specific primers. A 389-bp DNA fragment, corresponding to the partial coding region and the 3′-noncoding region of the Acmago cDNA, and a 316-bp DNA fragment, corresponding to the partial coding region of the AcY14 cDNA, were obtained. RT-PCR analysis indicated that the Acmago and AcY14 genes were strongly expressed in natural basidiomes (Fig 4). There has been no previous report associating mago and Y14 genes with development in fungi. The particular roles of these genes and the associated protein interactions in fungal development merit further examination.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi: 10.1016/j.mycres.2009.01.012.

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