Molecular cloning, sequence and expression of *Aa-polB*, a mitochondrial gene encoding a family B DNA polymerase from the edible basidiomycete *Agrocybe aegerita*

Abstract  An ORF of 1716 nucleotides, putatively encoding a DNA polymerase, was characterized in the mitochondrial genome of the edible basidiomycete *Agrocybe aegerita*. The complete gene, named *Aa-polB*, and its flanking regions were cloned and sequenced from three overlapping restriction fragments. *Aa-polB* is located between the SSU rDNA (5′ region) and a gene for tRNA_{Asn} (3′ region), and is separated from these genes by two A+T-rich intergenic regions of 1048 (5′ region) and 3864 (3′ region) nucleotides, which lack repeated sequences of mitochondrial or plasmid origin. The deduced *Aa-POLB* protein shows extensive sequence similarity with the family B DNA polymerases encoded by genomes that rely on protein-primed replication (invertrons). The domains involved in the 3′→5′ exonuclease (Exo I to III) and polymerase (Pol I to Pol V) activities were localized on the basis of conserved sequence motifs. The alignment of the *Aa-POLB* protein (571 amino acids) with sequences of family B DNA polymerases from invertrons revealed that in *Aa-POLB* the N-terminal region preceding Exo I is short, suggesting a close relationship with the DNA polymerases of bacteriophages that have linear DNA. The *Aa-polB* gene was shown to be present in all wild strains examined, which were collected from a wide range of locations in Europe. As shown by RT-PCR, the *Aa-polB* gene is transcribed in the mitochondria, at a low but significant level. The likelihood of the coexistence of *Aa-POLB* and Pol γ in the *A. aegerita* mitochondrion is discussed in the light of recent reports showing the conservation of the nucleus-encoded Pol γ from yeast to human.

Key words  *Agrocybe aegerita* · Basidiomycota · Family B DNA polymerases · Mitochondrial genome · RT-PCR

Introduction

Until recently, studies on the organization, replication and expression of mitochondrial DNA in higher fungi were limited to yeasts and filamentous species of the Ascomycota division (Böckelmann et al. 1986); data on the mtDNA of Basidiomycota are still scarce (Paquin et al. 1997). Most mitochondrial proteins are nucleus encoded, and the mitochondrial genomes appear to possess a small number of conserved genes derived from the ancestral prokaryotic endosymbiont (Gray and Doolittle 1982). For example, the enzymes involved in the replication of mtDNA are nucleus-encoded DNA polymerases γ (Foury 1989), which form a highly conserved family (Lecrenier et al. 1997). However, a DNA polymerase gene (*dpo*) has been identified in the mtDNA of the chrysophyte alga *Ochromonas danica* (Coleman et al. 1991). More recently, Dohmen and Tudzynski (1994) discovered in the mtDNA of the higher plant *Secale cereale* an ORF that shows sequence similarity to the conserved domains Pol IIa to Pol V of the family B DNA polymerases from invertrons, such as the bacteriophages M2 and φ29, the adenoviruses, and the linear mitochondrial plasmids of various fungi and plants (Braithwaite and Ito 1993; Sakaguchi 1990). Interestingly, as has been described in the invertrons, the *O. danica* mtDNA possesses 2.2-kb terminal inverted repeats (Coleman et al. 1991). Three small ORFs in the mtDNA of the bryophyte *Marchantia polymorpha* are considered to represent remnants of a family B DNA polymerase gene (Weber et al. 1995). *Agrocybe aegerita* is an edible basidiomycete from which the sequences of mitochondrial genes such as the SSU rDNA (Gonzalez et al. 1997) or the *cox1* gene (Gonzalez et al. 1998) have recently been reported – the first complete sequences of these genes obtained from a
member of the Basidiomycota division. In this paper, we report the cloning and sequencing of a mitochondrial gene, named Aa-polB, encoding a family B DNA polymerase, together with the sequences of its 5' and 3' flanking regions. The distribution of the Aa-polB gene was investigated by PCR in a large population of wildtype A. aegerita strains from various regions in Europe. The pattern of its transcription was assayed by RT-PCR analysis.

**Materials and methods**

Strains and culture conditions

The 20 wild-type strains used in this study are from the collection in the Laboratory of Molecular Genetics and Breeding of Cultivated Mushrooms. Each mycelium was derived by subculture on CYM solid medium (Raper and Hoffman 1974) of a fragment of a wild basidiocarp. The strains used were SM47 (= WT-3), SM751002 (= WT-11), SM750904, SM750903, SM771, SM930901 and SM930902 (from southwestern France); SM750901, SM750902, SM760901, SM900601 and SM202002 (northwestern France); SM160, SM161 and SM162 (southeastern France); SM17027 (Spain), SM50 (Czechoslovakia), SM871106 (Scotland), SM871107 (Germany) and SM49 (Italy).

Cloning and sequencing of the mitochondrial fragments.

Cloning of the E7 mitochondrial DNA fragment, carrying the SSU rDNA and, on the opposite strand, the 5' portion of a truncated ORF showing sequence similarities with family B DNA polymerases, was previously described (Gonzalez et al. 1997). Overlapping fragments were cloned into pGEM7Zf(+) (Promega) by conventional procedures (Maniatis et al. 1982) from purified A. aegerita mtDNA. Southern and colony hybridizations used for chromosome walking, as well as Northern analyses (Maniatis et al. 1982), were carried out with probes labelled with [α-32P]dCTP using the Prime-a-Gene labelling system (Promega).

Both strands were sequenced by the method of Sanger et al. (1977), using the Sequenase II kit (United States Biochemical) and [α-35S]dATP. Nucleotide and amino acid sequences were analysed and aligned using the DNA Strider 1.2 software, and Clustal W (Thompson et al. 1994). Comparisons with sequences in the GenBank and EMBL databases were performed using the search algorithm BLAST (Altschul et al. 1990).

**Results and discussion**

Sequence analysis of the mitochondrial Aa-polB gene from A. aegerita

The E7 fragment of mtDNA from the A. aegerita wildtype strain WT-3 was previously shown to encode the SSU rRNA (Gonzalez et al. 1997); upstream of this gene, on the opposite strand, a putative ORF (1259 nt) was present, which was interrupted by the cloning site (Fig. 1). This ORF showed sequence homology to the 5' portions of genes encoding family B DNA polymerases from invertebrates. To investigate whether the complete gene is present in the A. aegerita mtDNA, sequences of three overlapping fragments, E7 (5.2 kb EcoRI fragment), Ha-pol (4.2 kb, HaeIII) and EH-pol (4.4 kb, EcoRI-HindIII) were determined from the 5' end of the SSU rDNA to the HindIII site of the EH-pol fragment (a total of 7865 bp, GenBank Accession No. AF061244).

![Fig. 1 Restriction map of the region of A. aegerita mtDNA carrying the Aa-polB gene.](image)

PCR and RT-PCR

PCR reactions were carried out using Taq DNA polymerase (Promega) according to the enzyme supplier's instructions, in a final volume of 25 μl containing 10 ng of fungal genomic DNA. The RT-PCR reaction was carried out by using the Access RT-PCR system (Promega) on RNA extracted using the RNeasy Plant kit (Qiagen), from a mitochondrial sub-cellular fraction. DNA contamination was removed by incubation with 10 units of RNase-free DNase RQ1 (Promega) for 4 h at 37°C.

**Genes**

- ORF A
- 5' Aa-polB RNA 3'
- ORF B
- ORF A
- 3' Aa-polB
- 1716 nt
- 1048 nt
- 5'
- SSU rDNA
- 3

**Determined sequence 7865 nt**