Ursula Kües · Timothy Y. James · Rytas Vilgalys
Michael P. Challen

The chromosomal region containing pab-1, mip, and the A mating type locus of the secondarily homothallic homobasidiomycete Coprinus bilanatus

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Abstract In this paper we describe the cloning of the DNA region containing the A1 mating type genes of the secondarily homothallic mushroom Coprinus bilanatus and compare its organization to that of heterothallic homobasidiomycetes. As in other species, the C. bilanatus A factor contains several different genes that encode two different types of homeodomain transcription factor (HD1 and HD2); and some of these genes are active in the heterologous host C. cinereus. The HD1 and HD2 genes are distributed over two closely linked subloci, Aα and Aβ. A gene coding for a mitochondrial intermediate peptidase (mip) directly flanks the Aα sublocus. The pab-1 gene, required for para-aminobenzoic acid synthase, is found 39 kb upstream of mip. The structural arrangement of this chromosomal region closely resembles the heterothallic C. cinereus. In contrast, the Aα and Aβ subloci of Schizophyllum commune are further separated, with pab-1 located between the two subloci, suggesting that a translocation event may have occurred during evolution.

Key words A Mating type genes · Homeodomain proteins · Mitochondrial intermediate peptidase gene · Para-aminobenzoic acid synthase gene

Introduction

The life cycle of a typical homobasidiomycete alternates between two mycelial forms, the monokaryon and the dikaryon. The dikaryon arises from the fusion of two compatible monokaryons, an event controlled by the mating type loci. Many basidiomycetes have two unlinked mating type loci (A and B) that segregate randomly within sexual progeny. Most fascinating in terms of evolution and population genetics, the mating type loci in basidiomycetes have developed multiple specificities. Several mating type alleles (haplotypes) have been cloned from the heterothallic Coprinus cinereus and Schizophyllum commune. In both species, the A haplotypes contain pairs of functionally redundant, paralogous genes distributed between two closely linked subloci (Aα and Aβ) previously identified from classical genetics. These genes encode two classes of homeodomain transcription factors (HD1 and HD2). Through mating, HD1 and HD2 products from allelic gene pairs are brought together to form a compatible protein interaction. There are several HD1 and HD2 products present within dikaryotic cells and compatible protein combinations derived from different haplotypes need to be discriminated from incompatible interactions from the same haplotype. The specificity domains responsible for this discrimination have been localized to the highly variable N-terminals of the proteins (Casselton and Olesnick 1998; Hiscock and Kües 1999; Kües 2000).

A consequence of allelic discrimination is that the A genes are very dissimilar in DNA sequence. Alleles of the mating type genes typically have homologies of between 65–80% (Badrane and May 1999; Stankis et al. 1992); and paralogous genes have only 50% or less identity (Kües et al. 1994a; Shen et al. 1996). This low homology does not enable paralogous genes or the alleles of a given A mating type gene to cross-hybridize with each other, unlike the DNA regions directly linked to the mating type loci (Giasson et al. 1989; Kües et al. 1992, 1994c; May et al. 1991; Pardo et al. 1996; Specht et al. 1994).
In both *C. cinereus* and *S. commune* the *Az* locus is flanked by a gene encoding a mitochondrial intermediate peptidase (*mip*), a specific metallo-endopeptidase (Caselton et al. 1995; Isaya et al. 1995). In *C. cinereus*, the *Az* sublocus and the *Ab* sublocus are separated by 7 kb of conserved non-coding DNA ('homologous hole'); and the *Ab* locus is flanked by a short conserved gene of unknown function (β-fg; Kües et al. 1992, 1994c). In this study, we have used the *C. cinereus* *mip* gene (formerly termed *az-fg*; Kües et al. 1994a, c) to identify cosmids covering the *A* mating type region of *Coprinus bilanatus*, a two-spored, secondarily homothallic species with two multi-allelic mating type loci (Kemp 1974). *C. bilanatus* and *C. cinereus* belong to section Lanatuli, but phylogenetic analyses show the two species are not closely related (Hopple and Vilgalys 1999; Kühn and Romagnesi 1978). Nevertheless, *C. cinereus* HD2 genes were previously shown to elicit *A* regulated clamp cell development when introduced into *C. bilanatus* mono-karyons (Challen et al. 1993).

**Materials and methods**

Fungal strains, cultivation and transformation

*C. bilanatus* strains were grown at 28 °C on complete yeast extract or on the minimal medium of Raper et al. (1972) and *C. cinereus* strains were grown at 37 °C on YMG/T complete medium or on minimal medium with appropriate supplements (Granado et al. 1997). Genomic DNA was isolated from *C. bilanatus* strain Ch1 (*A1 B1*; Elliott and Challen 1983). *C. bilanatus* strains R8 (*A2 B3 trap-2*) and S61 (*A3 B1 trap-2*; this study) and *C. cinereus* strains AT8 (*A43 B43 trap-3 ade-8*; Kües et al. 1992), PG78 (*A6 B42 pal-1 trap-1,1,1,6*; Granado et al. 1997), FA2222 (*A5 B6 ace-1 trap-1,1,1,6*) and LN118 (*A42 B42 ade-2 trap-1,1,1,6*; Mutasa et al. 1990) were used in transformation following published protocols (Burrows et al. 1990; Challen et al. 1994; Granado et al. 1997). For co-transformations, either 2 μg of vector pCBT2-SS containing the *C. bilanatus* trap-2*"* gene (Challen et al. 1994), 1 μg of pD38 containing the *C. cinereus* trap-3*"* gene (Burrows 1991), or 1 μg of pCc1001 containing the *C. cinereus* trap-1*"* gene (Binninger et al. 1987) were combined with 0.3–2.0 μg of test cosmid DNAs. Phenotypes of transformants were determined from colony morphology and by the presence of *A*-regulated clamp cells (Kües et al. 1992; Challen et al. 1993).

**DNA techniques**

Cosmid DNAs were prepared using the protocol of Little (1987) and fungal genomic DNA was prepared by the method of Zolan and Pukkila (1986). DNA manipulation and Southern blot analysis were performed by routine methods (Sambrook et al. 1989). Co-smid DNA was subcloned using pBlueScript KS"*" (Stratagene) and *Escherichia coli* hosts XL1-Blue or DH5α (Sambrook et al. 1989). Agarose gel-purified fragments or plasmid clones were labelled with [γ-32P]CTP using a nick-translation kit (BRL). Hybond-N membranes (Amersham) were used for Southern blotting. Hybridization with homologous probes was carried out at 65 °C and stringency was reduced (57 °C) for heterologous probes. Filters of pooled cosmid DNA or colony hybridization of microtitre-ordered *E. coli* clones were prepared as previously described (Botitti et al. 1999). To sequence the *C. bilanatus* *Az* sublocus (a 4.6-kb EcoRV–SpII fragment; GenBank accession number AF271164), shotgun subfragment libraries were generated in pUC119 or pZZERO-2 (Invitrogen) following Zhou et al. (1988). Double-strand DNA sequences were generated using dye terminator chemistries on ABI 373 or 377 DNA sequencers (Perkin Elmer). Sequences were edited and assembled using the Sequencer package (Gene Codes).

**C. cinereus** mating-type and *pab*-1 plasmids

The following pBluescript KS"*" subclones were used in hybridizations: (1) pHH5 and pH7 containing the *S*‘ and 3‘ ends of the *C. cinereus* *mip* gene on 2.4-kb and 1.1-kb EcoRI fragments, respectively, (2) pAMT6 containing a 4.0-kb HindIII fragment and pAMT7 containing a 1.9-kb HindIII fragment from the 7-kb conserved region (‘homologous hole’) separating the *C. cinereus* *Az* and *Af* subloci, (3) pAMT1 containing a 3.0-kb HindIII fragment with the HD1 gene *b1*-1 which overlaps the ‘homologous hole’ at its 3′ end, and (4) PK6 containing HD1 gene *d1*-1 and gene β-fg on a 4.8-kb SalI fragment (Kües et al. 1992). pST17 contains the *C. cinereus* *pab*-1 gene on a 5.6-kb PstI fragment (Granado et al. 1997; Mutasa et al. 1990) and was kindly provided by L.A. Casselton.

Cosmid cloning, mapping and characterization

The genomic library of *C. bilanatus* DNA of strain Cb.M8 (*A1 B1*) in cosmid LoristX has been previously described (Challen et al. 1994). The *C. cinereus* *mip* insert of pHH5 was used to probe Southern blots of the EcoRI-digested library, pooled in 46 lots of 96 different cosmids. Subsequently, individual cosmids were identified with the same probe in colony filter hybridization of the positive pools. Overlapping cosmids were detected by hybridization with a 2.7-kb BamHI fragment and a 1.8-kb HindIII that directly flanked the Lorist backbone in cosmid 28D4 and cosmid 38F10, respectively.

All cosmids were crudely mapped by comparing them in restriction digestes using BamHI and HindIII, individually and in double-digests. DNA–DNA hybridizations further defined the order of fragments. The following *C. bilanatus* probes were used: the 1.8-kb Lorist flanking HindIII fragment of 38F10, cosmid C28H generated through HindIII restriction and re-ligation of cosmid 28D4, an 11.5-kb BamHI fragment from cosmid 28D4 overlapping cosmid C28H by 3 kb, and the 7.5-kb HindIII fragment with the *mip* gene and its adjacent 3.7-kb and 7.7-kb HindIII fragments. Conserved genes were localized by hybridization with *C. cinereus* homologues. All probes were used against *BglII*, *ClaI*, *EcoRI*, *PstI*, *SalI*, *XbaI*, and *XhoI* single and double cosmid digestes with HindIII to enable more precise mapping.

**Results**

Isolation of cosmids containing *C. bilanatus* *A* mating type DNA

The 3′ end of the *C. cinereus* *mip* gene from pHH7 detected a 3.6-kb fragment in EcoRI-digested *C. bilanatus* genomic DNA, while the 5′ end in pHH5 hybridized to the same 3.6-kb fragment and a 2.2-kb fragment. Both probes hybridized to a 10-kb band in HindIII-digested DNA (not shown), indicating the presence of a single *mip* gene in *C. bilanatus*. The pHH5 *mip* probe was further used to isolate three individual *C. bilanatus* cosmids (28D4, 38F10 and 45A8) from the Lorist library (Fig. 1A). Through transformation of various *C. bilanatus* and *C. cinereus* host strains (Table 1), we recovered transformants exhibiting the *A*-regulated clamp cell development. As in earlier experiments (Challen et al. 1993), the morphology of these transformants was changed from the normal condensed growth of mono-karyons to the fluffy, less dense colony mycelia. These