Single mating type-specific genes and their 3′ UTRs control mating and fertility in Cochliobolus heterostrophus

Abstract

To determine the number of proteins required for mating type (MAT) locus-regulated control of mating in Cochliobolus heterostrophus, MAT fragments of various sizes were expressed in MAT deletion strains. As little as 1.5 kb of MAT sequence, encoding a single unique protein in each mating type (MAT-1 and MAT-2), conferred mating ability, although an additional 160 bp of 3′ UTR was needed for production of ascospores. No other mating type-specific genes involved in mating identity or fertility were found. Thus, although homologs of the C. heterostrophus MAT-1 and MAT-2 genes exist in the filamentous ascomycetes Neurospora crassa and Podospora anserina, C. heterostrophus does not appear to have mating type-specific homologs of two additional genes required by both N. crassa and P. anserina for successful sexual reproduction. Three genes were identified in the common DNA flanking the MAT locus: a gene encoding a GTPase-activating protein and an ORF of unknown function lie 5′ while a β-glucosidase encoding gene lies found 3′. None of these genes appears to be involving in the mating process.

Key words

HMG box · Transcriptional regulator · 3′ UTR · DNA-binding protein · Heterothallic ascomycete

Introduction

Comparison of the molecular organization of mating type (MAT) loci from the pyrenomycetes Neurospora crassa (Glass et al. 1990; Staben and Yano´fsky 1990) and Podospora anserina (Picard et al. 1991) with that of the loculoascomycete Cochliobolus heterostrophus (Turgeon et al. 1993) reveals both similarities and differences. In each case, MAT is a single locus with two unlike sequences, termed idiomorphs (Metzenberg and Glass 1990). In all three fungi, one of the idiomorphs encodes a single DNA-binding protein (C. heterostrophus MAT-2; N. crassa MAT a-1; P. anserina FPR1) that is a member of the High Mobility Group (HMG) of DNA-binding proteins (Wright and Dixon 1988; Sugimoto et al. 1991; Travis et al. 1991). The alternate idiomorph in C. heterostrophus encodes a single protein (MAT-1) which contains an amino acid motif (alpha box) also found in the Saccharomyces cerevisiae MAT α1 (Glass et al. 1990) and Schizosaccharomyces pombe Pc proteins (Nielsen et al. 1996). In contrast, the corresponding idiomorphs of N. crassa and P. anserina encode not one but three proteins (Coppin et al. 1997), one of which is a homolog of C. heterostrophus MAT-1 (N. crassa MAT A-1; P. anserina FMR1). One of the two additional genes (MAT A-3; SMR2) found at the N. crassa and P. anserina idiomorphs encodes a protein with a HMG motif (MAT A-3; SMR2); the remaining gene (MAT A-2; SMR1) encodes a protein with amino acid sequences shared by MAT A-2 and SMR1. These latter proteins are proposed to represent a third type of transcription factor (Arnaise et al. 1997). The small size of the C. heterostrophus idiomorphs (MAT-1 = 1297 bp; MAT-2 = 1171 bp vs. N. crassa MAT A = 5301 bp; MAT a = 3235 bp ≈ P. anserina mat− = 4.7 kb; mat+ = 3.7 kb) reflects the smaller number of genes encoded.

Transcript analysis (Leubner-Metzger et al. 1997) has shown that, in contrast to other fungi in which it has been studied, transcription of C. heterostrophus MAT-1...
and MAT-2 starts and stops in common flanking DNA (though S. cerevisiae MATα1 and α2, and S. pombe MAT1-Mn and Pce stop in flanking DNA), resulting in mRNAs that are almost twice the size (MAT-
1 = 2.2 kb; MAT-2 = 2.1 kb) of the corresponding ORFs. In both cases, transcription results in a heterogeneous population of transcripts differing in start sites and in optional splicing of two introns in the long 5' untranslated leader sequence. Both leader sequences include several short ORFs which may be involved in post-transcriptional regulation (Leubner-Metzger et al. 1997).

What functions are conferred by the C. heterostrophus MAT proteins? Are they alone sufficient for control of initiation and completion of the sexual cycle or are counterparts of the N. crassa MAT A-2 and A-3/ P. anserina SMR1 and SMR2 genes found outside the idiomorph, either in the immediate flanking region or elsewhere? Here we have used MAT deletion strains to determine by gain-of-function analysis the minimum requirements for pseudothecium formation and fertility, and conclude that only MAT-1 and MAT-2 are required. In addition, we have extended our previous investigation of the ability of transgenic MAT sequences to function at the native or at ectopic sites in the genome.

Fig. 1 Genetic organization of the MAT-1 region. The MAT-2 region (not shown) is virtually identical, except for the idiomorph (striped bar), which is 125 bp shorter than the MAT-1 idiomorph, starting at bp 5100 and ending at bp 6271. The scale (center, in kb) indicates the position of the MAT-1 idiomorph and relevant restriction enzyme sites. The SalI and NcoI sites in bold type delimit the smallest and largest deletions used in this study (see Fig. 2). The six possible reading frames are shown (above), together with exons (open boxes) and introns (diagonal lines), which would be spliced out to create each ORF. Numbers refer to positions in the MAT-1 sequence deposited in Genbank (Accession No. AF029913) and indicate the first and last nucleotides of each complete ORF. The coding capacities (in amino acids) of ORFs, after removal of introns, are indicated by the ovals (below); direction of transcription is indicated by the arrowheads. GAP, GTPase activating protein; ORF1 is a homolog of a yeast gene of unknown function (YLR456W). Positions and sequences of the genes flanking MAT are identical in the MAT-1 and MAT-2 regions, except that the β-glucosidase gene is shifted 5' by 125 bp in MAT-2.

Materials and methods

Strains, media, crosses, and transformation

C. heterostrophus strains C4 (MAT-2), C5 (MAT-1), CB9 (MAT-1; alb1) and CB12 (MAT-2; alb1), as well as conditions for fungal growth, storage and mating were described previously (Leach et al. 1982; Wirsel et al. 1996). Production of pseudothecia, asc and asci was scored with a stereo microscope. MAT-2 deletion strains (Wirsel et al. 1996) included ΔSalMAT-2 (isolation no. C4-
31.3; hygR) deleted for a 2.5-kb SalI fragment, ΔKpnI/SphI MAT-2 (isolation no. C4-35.2; hygR) and ΔNcoI MAT-2 (isolation no. C4-41.7; hygR) deleted for a 6.3-kb NcoI fragment; note that ΔSalMAT-2 and ΔNcoIMAT-2 were previously referred to as MAT-0[pMDS] and MAT-0[pMDN] (Wirsel et al. 1996). C. heterostrophus was transformed (Turgenev et al. 1987) with 15 μg of DNA, or co-transformed with 12.5 μg of a MAT-1 plasmid and 12.5 μg of the vector pBG (Straubinger et al. 1992) carrying the selectable marker bar for resistance to bialaphos (Meiji Seika Kaisha, Yokohama, Japan). Co-transformed protoplasts were embedded in minimal medium-regeneration agar, incubated overnight and overlaid, as previously described (Turgenev et al. 1987; Wirsel et al. 1996), with 1% agar containing bialaphos at a final concentration in the plate of 100 μg/ml.

Plasmids

Plasmids pM1.4.50, which carries MAT-1 on an 8.3-kb BglII fragment, and p56HB, which carries MAT-2 on an 8.2-kb (partial digest) BglII fragment, were described earlier (Wirsel et al. 1996), together with an additional group of plasmids to which names were not previously assigned (Turgenev et al. 1993). The latter include pdB11, which carries MAT-1 on a 2.2-kb PstI fragment; p46HB, carrying MAT-2 on a 6.1-kb KpnI-BglII fragment; p47HB, carrying MAT-2 on a 5.0-kb BglII-SalI fragment; p48HB, carrying MAT-2 on a 5.5-kb HindIII-BglII fragment and p73HB carrying MAT-1 on a 3.3-kb EcoRV fragment. New plasmid constructions included pC9HSM1 and pC9HSM2, carrying the 1.6-kb HindIII-SphI MAT-1 and 1.5-kb HindIII-SphI MAT-2 fragments, respectively, in pC9HS (Turgenev et al. 1993); pSCM1B, which carries the 1.6-kb HindIII-EcoRI MAT-1 fragment from pC9HSM1 in the corresponding sites of pBG; pSCM2B, which carries the 1.5-kb HindIII-Smal MAT-2 fragment from pC9HSM2 in the HindIII/EcoRV sites of pBG; and pASM1, which carries the 3.4-kb EcoRV MAT-1 fragment from pM1.4.50 in the EcoRV site of pBG. Idiomorph-specific PCR products were amplified as described previously (Sharon et al. 1996; Wirsel et al. 1996), cloned in pUC18 and designated pMAT1PCR or pMAT2PCR (Christiansen et al. 1997).