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NOTE

Cloning and characterization of the multiprotein bridging factor 1 (*YlMBF1*) gene from the dimorphic yeast *Yarrowia lipolytica*

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In order to identify *Yarrowia lipolytica* genes induced by serum, cDNA representational difference analysis was performed using a PCR-select cDNA subtraction method. One of the genes cloned from the subtraction was a gene (*YlMBF1*) homologous to *Saccharomyces cerevisiae MBF1* encoding the coactivator multiprotein bridging factor 1. Disruption of *YlMBF1* revealed that the gene was not essential for viability, and the *Ylmbf1Δ* strain did not show any distinct phenotypic change on solid serum medium. In liquid medium, however, a difference was found in the ability to maintain hyphae induced by serum. This result suggests that the YlMbf1 protein may mediate transcriptional activation of certain genes involved in the hypha formation of *Y. lipolytica*.

Key words: *Yarrowia lipolytica*, multiprotein bridging factor, MBF1, serum

Yarrowia lipolytica (formerly *Candida lipolytica*) is a dimorphic, non-pathogenic yeast that is able to switch its morphology from yeast to hyphae depending on environmental factors (Herrero *et al.*, 1999; Hurtado and Rachubinski, 1999; Kim *et al.*, 2000; Perez-Campo and Dominguez, 2001; Torres-Guzman and Dominguez, 1997). *Y. lipolytica* has a sexual cycle and is amenable to sophisticated molecular biological and genetic techniques (Barth and Gaillardin, 1997; Chang *et al.*, 1998; Juretzek *et al.*, 2000; Park *et al.*, 1997). Therefore, *Y. lipolytica* is a good model system to study mechanisms underlying dimorphic transition of yeast (Gancedo, 2001; Perez-Campo and Dominguez, 2001).

Serum, which is the most powerful inducer of hypha formation in the pathogenic yeast *Candida albicans* (Feng *et al.*, 1999; Gancedo, 2001; Lo *et al.*, 1997; Mitchell, 1998), is able to stimulate the dimorphic transition of *Y. lipolytica* (Kim *et al.*, 2000; Richard *et al.*, 2001). Serum seems to induce the morphogenetic switch in *C. albicans* through Ras-mediated signal transduction pathways, although the component(s) of serum that is mainly responsible for the induction of the morphological change is not yet known and the detailed signaling pathways are still poorly understood. Since molecular genetic studies cannot be easily carried out with *C. albicans* due to its diploid nature and unknown sexual cycle, we chose *Y. lipolytica* as an alternative organism for research related to serum-induced signaling pathways.

In an attempt to investigate the *Y. lipolytica* genes induced in response to serum, we performed representational difference analysis using a PCR-select cDNA subtraction kit (BD Biosciences, USA). mRNAs used for the subtractive hybridization were prepared with an oligotex mRNA mini kit (Qiagen, Germany) from the total RNAs that had been extracted from *Y. lipolytica* cells (SMS397A; *MATA ade1 ura3 xpr2*) (Park *et al.*, 1997) grown under various induction conditions. *Y. lipolytica* cells grown to OD_{600}=1.0 at 28°C in SM medium (0.67% yeast nitrogen base, 1% glucose, 50 mg l/L adenine, and 20 mg l/L uracil) were harvested by centrifugation at room temperature, resuspended at 10^6 cells per ml in serum induction medium [0.67% yeast nitrogen base, 10% bovine calf serum (Sigma, USA), 50 mg l/L adenine, and 20 mg l/L uracil], and incubated at 28°C for 5 h.

Subtractions were carried out according to the instructions in the subtraction kit. For forward subtraction, mRNAs extracted from *Y. lipolytica* cells grown in media with or without serum were used for tester or driver, respectively. For reverse subtraction the mRNAs for tester and driver were reversed. A subtraction library was constructed by inserting the PCR-amplified products into pGEM-T easy vector (Promega, USA). To identify the clones whose expression levels were increased upon serum induction from the library, differential screening (reverse

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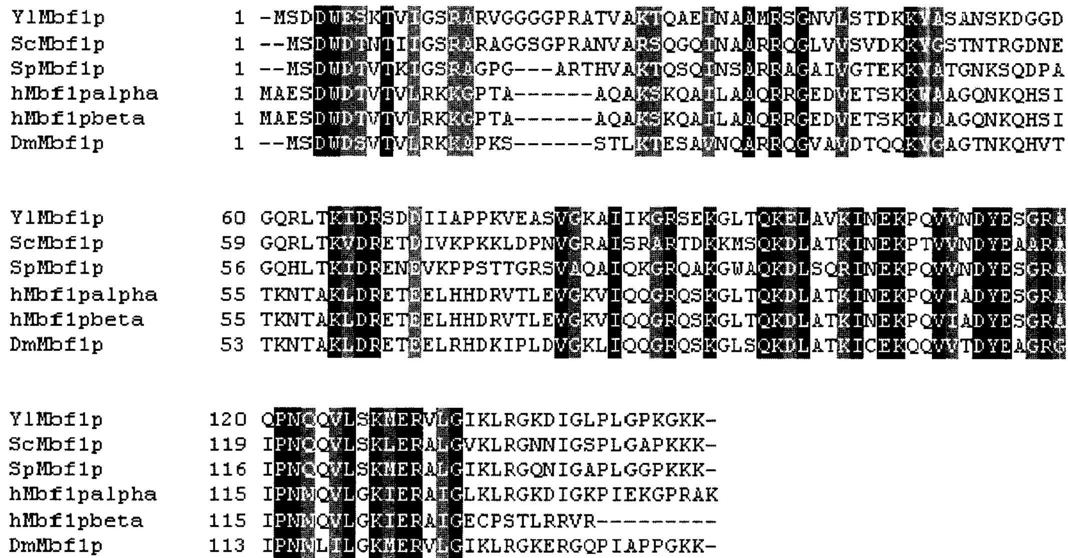

Fig. 1. Multiple sequence alignment of Mbf1 proteins from various organisms. The alignment was performed with the ClustalW1.8 program (Thompson *et al.*, 1994) and shaded using the Boxshade 3.21 program. Residues that are identical in all proteins are colored black, while similar residues are shaded gray. Sequences were retrieved from GenBank database: Sc, *S. cerevisiae* (AB017593); Sp, *S. pombe* (AL035536); Yl, *Y. lipolytica* (AF490972), h alpha, *Homo sapiens* Mbf1p alpha (AB002282); h beta, *Homo sapiens* Mbf1p beta (AB002283); Dm, *Drosophila melanoganster* (AE003527).

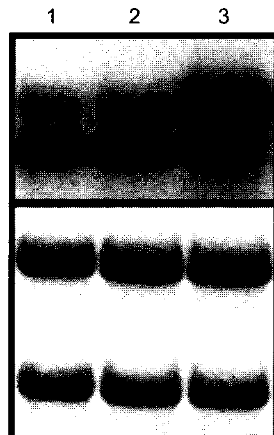

Fig. 2. Northern blot analysis of *YlMBF1* transcripts. Total RNA was electrophoresed on an agarose-formaldehyde gel, transferred to nylon membrane and hybridized to the probe (upper panel). The corresponding agarose gel was stained with ethidium bromide prior to blotting (lower panel). Lanes 1, 2, and 3 indicate RNAs extracted from the cells grown for 0, 2, and 5 h after serum induction, respectively.

Northern hybridizations) was performed with 96 randomly selected clones using forward and reverse subtracted cDNAs as probes. As a result, we selected several clones that showed about a 3-fold difference in the strength of the hybridization signal between the reverse Northern hybridizations. To further confirm the differential expression of the selected clones, Northern blot analysis was carried out; mRNAs extracted from the cells grown for 0, 2, and 5 h after serum induction were hybridized with ^{32}P-labeled probes of the selected clones. It was found that one of the selected clones showed a significantly increased hybridization signal in the sample taken 5 h after serum induction (Fig. 2). Therefore, we sequenced the clone using an ABI Model 373A automated DNA sequencer (Applied Biosystems, USA) and found that the clone was a part of the gene homologous to the *S. cerevisiae MBF1* gene encoding the coactivator multiprotein bridging factor 1.

In order to clone the complete open reading frame for the *MBF1* homolog, *Y. lipolytica* chromosomal DNA was completely digested with various restriction enzymes and Southern hybridization was performed using the DNA fragment of the *MBF1* homolog as a probe that was labeled with digoxigenin using DIG labeling kit (Roche, Germany). A partial library was constructed by purifying from an agarose gel about 2.5-3 kbp *Pst*I fragments corresponding to the position showing a strong hybridization signal, inserting the fragments into pUC19 digested with *Pst*I, and transforming the ligated DNAs into *E. coli* DH5α. Plasmid DNAs were isolated from transformants and Southern hybridization was carried out using the *MBF1* homologous DNA fragment as a probe. A plasmid showing a strong hybridization signal was isolated and the insert DNA (about 2.4 kbp) from the isolated plasmid was sequenced on both strands.

The sequencing result indicated that the insert DNA contained one open reading frame of 152 amino acids coding for the gene homologous to *S. cerevisiae MBF1*. The gene was named *Y. lipolytica MBF1* (*YlMBF1*). The DNA sequence data of *YlMBF1* has been submitted to GenBank with the Accession Number AF490972. The deduced amino acid sequence of the *Y. lipolytica* multi-

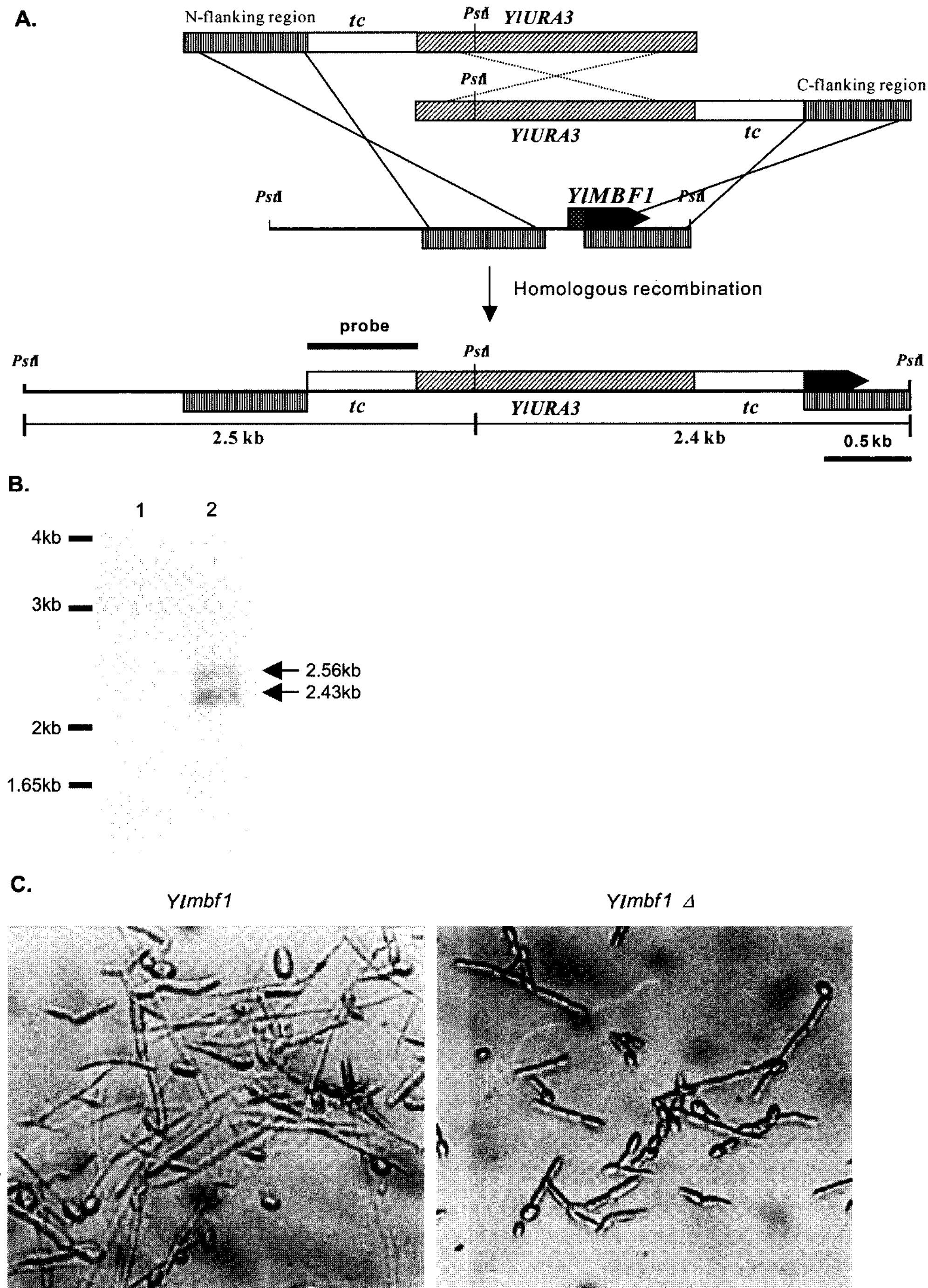

Fig. 3. Disruption of the *YlMBF1* gene and morphology of *YlMBF1* and *Ylmbf1Δ* strains in liquid serum medium. (A) Scheme of the split-marker disruption strategy using *tc*R-*YlURA3*-*tc*R cassette; see text for details. (B) Southern blot analysis of *YlMBF1* and *Ylmbf1Δ* strains. Genomic DNA was completely digested with *Pst*I and hybridized with a *tc* DNA fragment. Lane 1, *YlMBF1*; lane 2, *Ylmbf1Δ*. (C) Morphology of *YlMBF1* and *Ylmbf1Δ* strains. Strains were cultured for 20 h in liquid medium containing 10% serum. Magnification X400.

protein bridging factor 1 predicts a protein with a molecular mass of 16.2 kDa. The amino acid sequence of MBF1 is highly conserved across species from *S. cerevisiae* to human (Takemaru *et al.*, 1997), and the *Y. lipolytica* MBF1 sequence showed a high similarity to the counterparts from other organisms (45-63% identity, Fig. 1). Multiprotein bridging factor 1 is a coactivator which mediates transcriptional activation by interconnecting the general transcription factor TATA-binding protein (TBP) and gene-specific activators such as the *Drosophila* nuclear receptor FTZ-F1 and the human nuclear protein Ad4BP/SF-1 (Kabe *et al.*, 1999; Takemaru *et al.*, 1997; Zhu *et al.*,

2000). In *S. cerevisiae,* Mbf1p is required for transcriptional activation of the *HIS3* gene whose expression is dependent on a yeast transcription factor GCN4 (Takemaru *et al.*, 1998).

To investigate the function of *YlMBF1,* we constructed a *YlMBF1* disrupted strain and looked for any difference in phenotype between the wild type and the *Ylmbf1Δ* strains. The *YlMBF1* gene was disrupted using a split-marker disruption strategy (de Hoogt *et al.*, 2000). Long 5' and 3' *YlMBF1*-specific regions (N- and C-flanking regions in Fig. 3A) were amplified by PCR and individually ligated to a *Y. lipolytica* URA-blaster cassette. The resulting ligation reactions were used separately as templates to generate two *YlMBF1* disruption cassettes with overlapping *URA3* marker regions (Fig. 3A). Simultaneous transformation was performed with both overlapping disruption cassettes using the lithium acetate method (Barth and Gaillardin, 1996) and efficient disruption of the *YlMBF1* allele was confirmed by Southern blotting as shown in Fig. 3B. Since expression of the *YlMBF1* gene was enhanced more than three-fold when serum was added to the culture medium, it was thought that *YlMBF1* might be related with the phenotype induced by serum. To test if the *Ylmbf1Δ* strain was defective in hypha formation that is the most distinct phenotype induced by serum, the disruptant strain was grown on solid and liquid serum media. The *Ylmbf1Δ* strain formed hyphae as well as the wild type strain on solid serum medium (data not shown). In liquid medium, however, a difference was found in the ability to maintain hyphae induced by serum; hyphae of the *Ylmbf1Δ* strain became much shorter than those of the wild type in about 20 h after serum induction (Fig. 3C). This result suggests that the YlMbf1 protein may mediate transcriptional activation of certain genes involved in the hypha formation of *Y. lipolytica.*

Mbf1p is a unique coactivator that mediates transcriptional activation by connecting the DNA-binding domain of regulators and TBP. Since the basic region within the bZIP motif of a subgroup of bZIP proteins is important for the interaction with Mbf1p, it is possible that the Mbf1 protein might serve as a coactivator for a wider spectrum of bZIP proteins. A recent study on the cellular localization of human MBF1α (hMBF1α) revealed that hMBF1α expressed in COS-1 cells normally localized in the cytoplasm, but that the majority of hMBF1α was found in the nucleus when Ad4BP/SF-1 whose distribution is restricted to the nucleus was coexpressed with hMBF1 (Kabe *et al.*, 1999). Therefore, it was proposed that temporal or specific regulation by hMBF1α could be carried out through the interaction of hMBF1 with newly synthesized regulators in the cytoplasm and then the movement of the complex to the nucleus for transcriptional activation (Kabe *et al.*, 1999). Furthermore, hMBF1α seems to be involved in the modulation of transcription during endothelial cell differentiation (Dragoni *et al.*, 1998).

The *MBF1* homologous gene of *Y. lipolytica* (*YlMBF1*) was cloned based on its characteristic that the transcription level was significantly increased in response to serum. Since the phenotype of the *YlMBF1* disrupted mutant was not distinct from that of the wild type strain, the exact function of *YlMBF1* remains to be elucidated. However, the data obtained from Mbf1 proteins of *S. cerevisiae, Bombyx mori*, and mammalian cells suggest that YlMbf1p may also mediate the interaction between certain regulator proteins and TBP (Kabe *et al.*, 1999; Takemaru *et al.*, 1998; Takemaru *et al.*, 1997). Our finding that *YlMBF1* is required to maintain the hyphal growth of *Y. lipolytica* implies that YlMbf1p may be involved in the transcriptional activation of proteins which function at the late stage of hyphal growth in *Y. lipolytica*, although *YlMBF1* may mediate the connection between TBP and other transcriptional activators with the bZIP domain.

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