CaMac1, a *Candida albicans* Copper Ion-sensing Transcription Factor, Promotes Filamentous and Invasive Growth in *Saccharomyces cerevisiae*

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**Abstract** Molecular mechanisms of morphogenesis share many common components between *Candida albicans* and *Saccharomyces cerevisiae*. The Kss1-associated MAPK cascade and the cAMP/PKA pathway are two important signal transduction pathways that control morphogenesis in *S. cerevisiae*. A *C. albicans* copper ion-sensing transcription factor gene, *CaMAC1*, was cloned from *C. albicans* SC5314. Ectopic expression of *CaMAC1* in *S. cerevisiae* promoted filamentous and invasive growth. In diploid cells, CaMac1 could suppress the filamentous growth defect of mutants in the Kss1-associated MAPK pathway and the cAMP/PKA pathway. In haploid strains, ectopic expression of *CaMAC1* suppressed the invasive growth defect of mutants in the MAPK pathway (*ste7*, *ste12* and *tec1*), but failed to suppress the invasive growth defect of the *flo8* mutant. Our results suggest that the activation of CaMac1 is independent of the MAPK and cAMP/PKA pathways in filament formation, but requires Flo8 factor for invasive growth. In the media containing a high concentration of CuSO₄, the yeast filamentous and invasive growth was blocked. The activating effect of CaMac1 is inhibited by copper ions.

**Key words** CaMac1; MAPK pathway; cAMP/PKA pathway; morphogenesis; *Candida albicans*; *Saccharomyces cerevisiae*

*Candida albicans* is a frequently isolated opportunistic pathogen in humans, causing both mucosal and systemic infections, especially in immunocompromised individuals [1]. The study of this fungus has been hindered by its obligate diploid genome and lack of a sexual phase [2]. *Saccharomyces cerevisiae* is a well-characterized model system. The molecular mechanisms of morphogenesis in the two yeasts share many common components. Therefore, we are able to use *S. cerevisiae* mutants to study the function of *C. albicans* genes.

The Kss1-associated MAPK cascade and the cAMP/PKA pathway work together to control the morphogenesis of *S. cerevisiae*. Mutations in the genes, such as *STE7*, *STE12*, *TEC1* and *FLO8* of the two cascades, impair the ability of diploid filamentous and haploid invasive growth in *S. cerevisiae* [3–5]. The two cascades converge on the promoter of *FLO11*, a gene encoding for a cell wall protein, which is critical for pseudohyphae formation in *S. cerevisiae* [6]. Many important components of the MAPK and cAMP/PKA pathways have been identified in *C. albicans* [7–10]. Several hyphal development regulators have been isolated in *C. albicans* by functional complementation [11,12].

Copper is an essential cofactor for a variety of enzymes, including cytochrome c oxidases, copper,zinc-SOD1 and multicopper oxidases [13]. Metal binding activator ScMac1 is a copper ion-sensing transcription factor, whose activity is inhibited by copper ions [14,15]. Loss-of-function mutants of ScMac1 are slow growing, respiratory deficient and hypersensitive to heat or exposure to cadmium. Biochemical studies have been reported to map residues and motifs, which are important to the activity of the ScMac1 protein [16,17]. However, its function in the morphogenesis of *S. cerevisiae* has not been elucidated.

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In this report, we cloned CaMAC1, a homologue of ScMAC1, from the C. albicans SC5314 genome and studied the functional role of CaMac1 in the morphogenesis of S. cerevisiae. The activation of CaMac1 in filamentous and invasive growth was analyzed.

Materials and Methods

Strains and culture conditions

The yeast strains SC5314 (wild type) [18], L5528 (MATα ura3-52 his3::hisG), HLY367 (MATα ste7::LEU2 ura3-52 leu2::hisG), HLY362 (MATα ste12::LEU2 ura3-52 leu2::hisG) [3], HLY2000 (MATα tec1:::HIS3 ura3-52) [19], HLY850 (MATα flo8::hisG ura3-52) [4], CG31 (MATα/α ura3-52/ura3-52) [20], HLY351 (MATα/α ste7::LEU2/ste7::LEU2 ura3-52/ura3-52 leu2::hisG/leu2::hisG), HLY352 (MATα/α ste12::LEU2/ste12::LEU2 ura3-52/ura3-52 leu2::hisG/leu2::hisG), HLY2002 (MATα/α tec1:::HIS3/tec1:::HIS3 ura3-52/ura3-52) and HLY852 (MATα/α flo8::hisG/flo8::hisG ura3-52/ura3-52), YPD medium (1% yeast extract, 2% peptone, 2% glucose) and synthetic medium (SD) were used for yeast growth. Synthetic low ammonia medium (SLAD) was used for pseudohyphal growth of yeast transformants. Synthetic low ammonia medium (SC-Ura) was used for selection of URA+. Complete medium (SC-Ura) was used for selection of URA+.  

Cloning of CaMAC1 and construction of plasmids

Genomic DNA of C. albicans SC5314 was extracted and used as the template for amplifying CaMAC1. Ma5 (5‘-ATCTCGAGATGATACTAAATAGATGATATCAA-3’) and Ma3 (5‘-TACTGCAGTTATTTGGTCTTTTTTGAGCAACATG-3’) were used as primers. PCR products were treated with XhoI and PstI, and inserted into the XhoI/PstI site of pVT102-U to generate the S. cerevisiae expression plasmid, pVT-CaMAC1. Sequence analysis was performed using the DNASTar and GeneDoc programs (DNASTar, Madison, USA).

Results and Discussion

Cloning of CaMAC1 and sequence analysis

A sequence (orf19.7068) which shared high similarity with ScMac1 was found by searching the C. albicans genome database (http://www-sequence.stanford.edu/group/Candida/search.html). The orf19.7068 encoded for a protein of 471 amino acids, with isoelectric point 7.39. According to the sequence similarity, the orf19.7068 was designated CaMac1. Compared with ScMac1, CaMac1 contained a conserved “copper-fist” motif in the N-terminus, which was postulated to be a DNA-binding domain. In addition, CaMac1 contained two conserved cysteine-rich sequences corresponding to the REPI and REPII motifs of ScMac1 [14]. Sequence analysis indicated that CaMac1 was a homolog of ScMac1 in C. albicans (Fig. 1).

Two primers, Ma5 and Ma3, were used to amplify CaMAC1 from genomic DNA of C. albicans SC5314. The PCR fragment was subcloned into the XhoI/PstI site of a S. cerevisiae expression vector, pVT102-U, to generate pVT-CaMAC1. The construction was confirmed by restriction digestion and sequencing (Fig. 2).

Ectopic expression of CaMAC1 promotes filamentous and invasive growth in S. cerevisiae

Many regulatory proteins responding to morphological transition are conserved between C. albicans and S. cerevisiae. We investigated the role of CaMac1 in filamentous growth in S. cerevisiae. The plasmid pVT-CaMAC1 was transformed into wild-type strain CG31. The transformants were grown on SLAD medium to demonstrate pseudohyphal colony formation. The strain trans-
formed with the vector pVT102-U was used as a control. Results showed that overexpression of CaMAC1 stimulated filamentous growth of diploid cells under nitrogen starvation condition (Fig. 3). The same plasmid was introduced into haploid strain L5528 and enhanced invasive growth under glucose starvation condition. These results suggested that CaMac1 has an activating effect on filamentous and invasive growth in S. cerevisiae.

Activation of CaMac1 in filamentous formation is independent of the Kss1-associated MAPK and cAMP/PKA pathways

Because both diploid filamentous growth and haploid invasive growth were controlled by the Kss1-associated MAPK cascade and the cAMP/PKA pathway, we were interested in examining whether or not CaMac1 plays its role in S. cerevisiae cells via the two pathways. The plasmids were introduced into the ste7, ste12, tec1 and flo8 mutants (Fig. 4). Data indicated that the activating function of CaMac1 in promoting filamentous growth bypassed both the MAPK and cAMP/PKA pathways. The transcription factor CaMac1 may act in a parallel pathway in S. cerevisiae.

Activation of CaMac1 in invasive growth requires Flo8

To investigate whether or not CaMac1 also functions in invasive growth, the plasmid pVT-CaMAC1 was transformed into S. cerevisiae wild-type, ste7, ste12, tec1 and flo8 haploid mutants. Ectopic expression of CaMAC1 suppressed the invasive growth defect of the ste7, ste12, tec1 and flo8 mutants (Fig. 4). Colony morphologies of ste7/ste7 (HLY351), ste12/ste12 (HLY352), tec1/tec1 (HLY2002) and flo8/flo8 (HLY852) strains carrying pVT102-U (left) or pVT-CaMAC1 (right) grown on synthetic low ammonia medium (SLAD) at 30 °C for 5 d.

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Fig. 5  CaMac1 enhances invasive growth in *Saccharomyces cerevisiae*

Total (before wash) and invasive (after wash) growth of wild-type (L5528), ste7 (HLY367), ste12 (HLY362), tec1 (HLY2000) and flo8 (HLY850) strains carrying pVT102-U (left) or pVT-CaMAC1 (right) after 4 d of growth on YPD.

In this paper, we reported the role of CaMac1 in filamentous and invasive growth in *S. cerevisiae*. Promotion of CaMac1 in filamentous growth did not require the Kss1-associated MAPK pathway or the cAMP/PKA pathway. Ectopic expression of CaMAC1 suppressed the invasive growth defect of mutants in the MAPK pathway (ste7, ste12 and tec1), but required Flo8. The Kss1-associated MAPK pathway and the cAMP/PKA pathway act together and converge on the promoter of *flo11* [6]. Flo11 is required both for filamentous and invasive growth in *S. cerevisiae*. Northern analysis demonstrated that the trans-

### Table 1  Copper ions inhibit filamentous and invasive growth in *Saccharomyces cerevisiae*

<table>
<thead>
<tr>
<th>Group</th>
<th>Medium</th>
<th>CuSO₄ (μM)</th>
<th>Cell growth</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
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<td>fff</td>
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<tr>
<td>L5528+Vector</td>
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<td>iiiii</td>
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</tbody>
</table>

'f' and 'i' indicates the degree of filamentous and invasive growth, respectively; −, indicates no filamentous or invasive growth. Wild-type strains used were CG31 (diploid) and L5528 (haploid). SLAD, synthetic low ammonia medium.

### Table 2  Activation of CaMac1 is repressed by copper ions

<table>
<thead>
<tr>
<th>Strain</th>
<th>Medium</th>
<th>CuSO₄ (μM)</th>
<th>Cell growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG31+Vector</td>
<td>SLAD</td>
<td>0</td>
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<tr>
<td>CG31+CaMAC1</td>
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<td>200</td>
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</tbody>
</table>

*f* indicates the degree of filamentous growth; −, indicates no filamentous or invasive growth. Wild-type strain used was CG31 (diploid). SLAD, synthetic low ammonia medium.
cription of flo11 was not enhanced by ectopic expression of CaMAC1 in ste12 and flo8 mutants (data not shown), consistent with the sequence searching that no Mac1 binding element was found in the extra long promoter of flo11. CaMac1 may not activate the expression of flo11 in these mutants, but probably works on other cell wall protein genes.

C. albicans is the most important cause of candidiasis infections. The dimorphic switch in C. albicans has been proven to be a crucial virulence factor for successful infection. C. albicans can develop into three forms: budding yeast, true hyphae and pseudohyphae. True hyphae and pseudohyphae are critical for tissue penetration and the colonization of organs [22], because they are invasive. Although S. cerevisiae is a simple system, the mechanism involved in morphogenesis could be referred by the other organisms. The phenomenon that CaMac1 promotes pseudohyphal and invasive growth of S. cerevisiae suggests that CaMac1 might play a similar role in C. albicans. The activation of CaMac1 was repressed by copper ions, reflecting a potential copper response pathway that may be involved in the regulation of morphological transition in C. albicans.

References
1 Oddes FC. Candida and Candidosis. 2nd ed. London: Bailliere Tindall 1988
4 Liu H, Styles CA, Fink GR. Saccharomyces cerevisiae S288C has a mutation in FLO8, a gene required for filamentous growth. Genetics 1996, 144: 967–978
20 Gimeno CJ, Ljungdahl PO, Styles CA, Fink GR. Unipolar cell divisions in the yeast S. cerevisiae lead to filamentous growth: Regulation by starvation and RAS. Cell 1992, 68: 1077–1090

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