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Function and subcellular localization of Gcn5, a histone acetyltransferase in *Candida albicans*

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ABSTRACT

Candida albicans is an opportunistic fungal pathogen commonly found in humans. It has the ability to switch reversibly between three growth forms: budding yeast, pseudohypha, and hypha. The transition between yeast and hyphal growth forms is critical for the pathogenesis of *C. albicans*. During the yeast-to-hypha morphologic transition, gene expression is regulated by transcriptional regulators including histone modifying complexes and chromatin remodeling complexes. We previously reported that Esa1, a catalytic subunit in the histone acetyltransferase complex NuA4, is essential for the hyphal development of *C. albicans*. In this study, we analyzed the functional roles of Gcn5, a catalytic subunit in the histone acetyltransferase complex SAGA, in *C. albicans*. Gcn5 is required for the invasive and filamentous growth of *C. albicans*. Deletion of *GCN5* impaired hyphal elongation in sensing serum and attenuated the virulence of *C. albicans* in a mouse systemic infection model. The *C. albicans gcn5/gcn5* mutant cells also exhibited sensitivity to cell wall stress. Functional analysis showed that the HAT domain and Bromodomain in Gcn5 play distinct roles in morphogenesis and cell wall stress response of *C. albicans*. Our results show that the conserved residue Glu188 is crucial for the Gcn5 HAT activity and for Gcn5 function during filamentous growth. In addition, the subcellular distribution of ectopically expressed GFP-Gcn5 correlates with the different growth states of *C. albicans*. In stationary phase, Gcn5 accumulated in the nucleus, while during vegetative growth it localized in the cytoplasm in a morpha-independent manner. Our results suggest that the nuclear localization of Gcn5 depends on the existence of its N-terminal NLS and HAT domains.

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1. Introduction

Candida albicans is a commensal fungus in the skin and mucosal microbiome of humans. It is also one of the most important opportunistic human pathogens, which causes mucosal and systemic candidiasis in individuals immunocompromised due to aging, infection or cancer and HIV treatments (Odds, 1988; Pande et al., 2013; Yapar, 2014). A striking feature of *C. albicans* is that it can undergo reversible morphogenetic transitions. It grows either as an unicellular yeast or in filamentous pseudohyphal and hyphal cell forms (Whiteway and Bachewich, 2007). This unique ability to switch from yeast to hyphal cell contributes greatly to its virulence (Gow et al., 2012).

Hyphal cells in *C. albicans* form in response to many environmental cues that mimic the diverse microenvironments it encounters in its human host (Sudbery, 2011). The yeast-to-hyphal transition in *C. albicans* is tightly correlated with the transcription of hyphal specific genes which are controlled by a number of transcriptional factors, such as Cph1, Efg1, Flo8, Sfl1, Sfl2 and Brg1 (Cao et al., 2006; Liu et al., 1994; Lu et al., 2012; Nobile et al., 2012; Stoldt et al., 1997; Znaidi et al., 2013). In addition to transcription factors, dynamic changes in chromatin structure also modulate gene regulation during hyphal development. We previously reported that in *C. albicans* the histone acetyltransferase (HAT) complex NuA4, which primarily acetylates H4 and H2A, was responsible for nucleosomal H4 acetylation in the promoters of hypha-specific genes during hyphal induction in an Efg1-dependent manner (Lu et al., 2008). Further, the catalytic subunit of the NuA4 HAT complex, Esa1, is known to be essential for hyphal development (Wang et al., 2013).

Gcn5 is the first histone acetyltransferase (HAT) identified in *Saccharomyces cerevisiae* and is found to be the catalytic subunit of the HAT complexes SAGA (Spt-Ada-Gcn5-Acetyltransferase), ADA (Ada2-Gcn5-Ada3) and SLIK/SALSA (SAGA-like), which are involved in both negative and positive transcriptional regulation (Brownell et al., 1996; Daniel and Grant, 2007; Grant et al., 1997; Pray-Grant et al., 2002; Rando and Winston, 2012; Sterner et al.,

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2002). Gcn5 mainly targets N-terminal lysine residues in histones H3 and H2B respectively (Suka et al., 2001; Zhang et al., 1998). Gcn5 acts on free histones when alone and acetylates nucleosomal histones in association with other proteins (Grant et al., 1997, 1999; RuizGarcia et al., 1997). Gcn5 can also function as a lysine acetyltransferase (KAT) required for acetylation of Rsc4, a subunit of the RSC chromatin-remodeling complex, and contributed to regulation of gene expression (VanDemark et al., 2007).

Homologs of Gcn5 have been identified in many fungal species and higher eukaryotes (Spedale et al., 2012; Wang and Dent, 2014). UmGcn5, a Gcn5 homolog in *Ustilago maydis*, was reported to be involved in dimorphism and virulence. Deletion of *UmGCN5* resulted in the growth of long mycelial cells and fuzz-like colony formation in *U. maydis* (Gonzalez-Prieto et al., 2014). GcnE, a Gcn5 homolog in *Aspergillus nidulans*, is required for normal conidiophore development (Canovas et al., 2014). Mammals have two Gcn5 homologs, the Gcn5-like protein GCN5 (KAT2a) and p300/CREB-binding protein-associated factor PCAF (KAT2b) (Jin et al., 2014b; Rando and Winston, 2012; Spedale et al., 2012). GCN5 and PCAF are subunits in at least two types of multi-protein complexes, which possess global histone acetylation activity and locus-specific co-activator functions together with acetyl transferases activity on non-histone substrates (Scott et al., 2014; Weake and Workman, 2012). Together, GCN5 and PCAF can bind to many sequence-specific factors involved in cell growth and/or differentiation. Recruiting GCN5 by cytoplasmic Myc-nick induces alpha-tubulin acetylation and drives cytoplasmic reorganization and differentiation (Moore and Anderson, 2014). The acetyltransferase activity and cellular location of PCAF are regulated through the acetylation of PCAF itself (Blanco-Garcia et al., 2009; Santos-Rosa, 2003). These two acetyltransferases have distinct and redundant roles in transcriptional regulation, signaling, adipogenesis, tumorigenesis and embryogenesis (Chen et al., 2013; Jin et al., 2014a, 2011, 2014b; Love et al., 2012; Scott et al., 2014; Weake and Workman, 2012).

Despite such extensive analysis of Gcn5 homologs, the function of *C. albicans* Gcn5 (CaGcn5) is not well understood. Therefore, in this study we investigated the role of CaGcn5 in filamentous growth, invasive growth and stress response of *C. albicans*. We also examined the subcellular distribution of Gcn5 during yeast growth and hyphal growth. In addition, we analyzed the impact of Gcn5 on *C. albicans* virulence in a systemic mouse model. Our results show that the N-terminal NLS and HAT domains but not the C-terminal Bromodomain regulate the nuclear localization of Gcn5. Our data also indicate that CaGcn5 is a histone acetyltransferase required for the morphogenesis and stress response of *C. albicans*.

2. Materials and methods

2.1. Strains and culture conditions

The *C. albicans* strains used in this study are listed in Table 1. Yeast strains were routinely grown on YPD medium (1% yeast extract, 2% peptone, 2% glucose) or on synthetic complete medium with 2% glucose for selection of prototropic strains. YPD plus 10% bovine serum media were used for hyphal induction (Lane et al., 2001). Lee's media or agar plus serum were used for filamentous growth (Chen et al., 2000). YPD plates were used for invasive assay (Song et al., 2011). YPS with 1% agar was used for colony morphology assay under embedded conditions (Brown et al., 1999).

Table 1
C. albicans strains and plasmids used in this study.

Strain or plasmid	Genotype or description	Source or references
Strains		
SC5314	Wild type	Fonzi and Irwin (1993))
SN152	*ura3*::imm434::*URA3*/*ura3*::imm434 *iro1*::*IRO1*/*iro1*::imm434 *his1*::hisG/*his1*::hisG *leu2*/*leu2 arg4*/*arg4*	Noble and Johnson (2005))
SN250	*ura3*::imm434::*URA3*/*ura3*::imm434 *iro1*::*IRO1*/*iro1*::imm434 *his1*/*his1*, *leu2*::CdHIS1/*leu2*::*CmLEU2*, *arg4*/*arg4*	
CPS50	*ura3*::imm434::*URA3*/*ura3*::imm434 *iro1*::*IRO1*/*iro1*::imm434 *his1*::hisG/*his1*::hisG *leu2*/*leu2 arg4*/*arg4 gcn5*::*PLP*/*gcn5*::PHP	This study
CPS82	*ura3*::imm434::*URA3*/*ura3*::imm434 *iro1*::*IRO1*/*iro1*::imm434 *his1*::hisG/*his1*::hisG *leu2*/*leu2 arg4*/*arg4 gcn5*::*PLP*/*gcn5*::*PHP ADE2*/*ade2*:: *ADH1p-GCN5-PAP*	This study
CPS194	*ura3*::imm434::*URA3*/*ura3*::imm434 *iro1*::*IRO1*/*iro1*::imm434 *his1*::hisG/*his1*::hisG *leu2*/*leu2 arg4*/*arg4 gcn5*::*PLP*/*gcn5*::*PHP ADE2*/*ade2*:: *ADH1p-PAP*	This study
CPS203	*ura3*::imm434::*URA3*/*ura3*::imm434 *iro1*::*IRO1*/*iro1*::imm434 *his1*/*his1*, *leu2*::*CdHIS1*/*leu2*::*CmLEU2*, *arg4*/*arg4 ADE2*/*ade2*:: *ADH1p-PAP*	This study
Plasmids		
pSN40	*C. maltosa LEU2* (*CmLEU2*), *Kana*^{R} in the pCR-BluntII TOPO	Noble and Johnson (2005)
pSN52	*C. dubliniensis HIS1* (*CdHIS1*), *Kana*^{R} in the pCR-BluntII TOPO	
pSN69	*C. dubliniensis ARG4* (*CdARG4*), *Kana*^{R} in the pCR-BluntII TOPO	
pHL471	GFP and *CaURA3* in the pBluescript SK	Hazan et al. (2002)
pCPC20	*C. albicans* vector containing *ADH1* promoter and *CdARG4* maker for integration at *ADE2* locus	This study
pCPC48	*LoxP-CmLEU2-LoxP* (*PLP*), *Amp*^{R} in the pUC18	This study
pCPC49	*LoxP-CdHIS1-LoxP* (*PHP*), *Amp*^{R} in the pUC18	This study
pCPC50	*LoxP-CdARG4-LoxP* (*PAP*), *Amp*^{R} in the pUC18	This study
pCPC117	*GCN5* from *ADH1* promoter with a *CdARG4* marker in the pCPC20	This study
pCPC213	Gcn5ΔHAT derived from pCPC117	This study
pCPC214	Gcn5ΔBromo derived from pCPC117	This study
pCPC218	GFP fused to N-terminus of Gcn5, derived from pCPC117	This study
pCPC219	GFP-Gcn5ΔHAT derived from pCPC218	This study
pCPC220	GFP-Gcn5ΔBromo derived from pCPC218	This study
pCPC222	GFP-Gcn5(E188A) derived from pCPC218	This study
pCPC223	GFP-Gcn5(V190A) derived from pCPC218	This study
pCPC224	GFP-Gcn5(C192A) derived from pCPC218	This study
pCPC225	GFP-Gcn5(L225A) derived from pCPC218	This study
pCPC239	GFP-Gcn5ΔNLS derived from pCPC218	This study

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