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# Phosphatidylinositol-dependent phospholipases C Plc2 and Plc3 of *Candida albicans* are dispensable for morphogenesis and host–pathogen interaction

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**Abstract**

Phospholipases play an important role as virulence factors in human pathogens. *Candida albicans*, the major fungal pathogen of humans, encodes phospholipases of type A, B, C and D. Type B Plb2 and type D Pld1 phospholipases have been shown to contribute to virulence in this organism. We analyzed, in *C. albicans*, *PLC2* and *PLC3*, two highly conserved genes coding for phosphatidylinositol-dependent phospholipases C with homology to the known virulence factor PlcA in the human pathogen *Listeria monocytogenes*. We show that expression of *PLC2* and *PLC3* is upregulated under different filament-inducing conditions and in the constitutive filamentous mutant *tup*1Δ. In order to analyze *PLC2* and *PLC3* function in *C. albicans*, we constructed strains that carry *PLC2* or *PLC3* under a constitutive promoter and strains that lack all four *PLC2/3* alleles. These strains were not affected in their ability to produce filaments under non-inducing conditions, nor was filamentation modified under inducing conditions, suggesting that *PLC2/3* are not critical determinants of the yeast-to-hypha switch. In a cell culture model for macrophage interaction, phagocytosis of *C. albicans* and subsequent killing were not influenced by *PLC2/3*. These results demonstrate that *C. albicans PLC2* and *PLC3* are dispensable for virulence; moreover, they underline the sharp contrast with the function of *plcA* in *L. monocytogenes*.
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*Keywords: Candida albicans*; Candidiasis; Virulence; PI-PLC; Macrophage; Yeast-to-hypha switch

## 1. Introduction

Microbial diseases remain a predominant cause of mortality and morbidity. According to a study on sepsis covering the past 22 years (1979–2000) in the United States, fungal infections increased fourfold during the second period of the study, whereas infections with Gram-positive and Gram-negative bacteria have remained constant. This suggests that fungal infections take a central stage in the ever increasing number of deaths due to microbial infections [38].

Systemic fungal infections are most often the consequence of artificial or disease-associated immune suppression, and *Candida albicans* is the most frequently isolated human fungal pathogen. Whereas *C. albicans* naturally colonizes the gastrointestinal and urogenital tracts, it can become life-threatening in transplant, cancer or AIDS patients. Candidemia is also associated with predisposing factors such as diabetes, antibiotic and hormone treatments [3,10,25].

A critical component of *C. albicans* virulence lies in its ability to alternate between yeast and hyphal growth forms. Indeed, mutations that lock *C. albicans* in the yeast or hy-

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phal forms or impair the yeast-to-hypha transition are associated with a significant reduction in virulence [7,37,46]. While the filamentous form seems important for penetration of the fungus into new host niches, the yeast form appears to contribute to subsequent colonization and dissemination [5]. The yeast-to-hypha switch in *C. albicans* is under the positive control of a wide range of signals, signaling cascades and transcriptional regulators [18,55]. Interestingly, the switch is also negatively regulated by the global repressor Tup1, which acts in concert with Ssn6 and Nrg1 to prevent the expression of genes involved in virulence and hyphal formation [7,8,29,40,41,50]. Inactivation of the *TUP1*, *NRG1* or *SSN6* genes results in uncontrolled hyphal formation, and transcript profiling of *C. albicans* strains lacking either *TUP1* or *NRG1* has been used advantageously to identify targets of the Tup1–Nrg1 complex [7,8,29,40,41]. Among the genes that show strong repression by the Tup1–Nrg1 complex under conditions that promote yeast growth are known genes involved in virulence such as SAP (secreted aspartyl protease [42]) and ALS (agglutinin-like cell surface glycoprotein [27]) genes, and hyphal-specific genes such as *HWP1* or *ECE1* [6,48]. It is therefore tempting to speculate that other Tup1–Nrg1-repressed genes whose involvement in virulence and/or hyphal formation has not yet been evaluated constitute good candidates for performing important functions in one of these two processes.

Tup1–Nrg1-repressed genes include the *PLC2* gene that encodes a phosphoinositide-specific phospholipase C (PI-PLC) [1,41]. PI-PLC represents one class of phospholipases that catalyze the cleavage of (phosphorylated) membrane lipid phosphatidylinositol to produce diacylglycerol and phosphorylated *myo*-inositol [24]. *C. albicans* phospholipases have attracted significant interest because of their potential role in morphogenesis and virulence [21]. Furthermore, bacterial phospholipases have been shown to be involved in pathogenesis promoting cell lysis and tissue destruction and interfering with host cellular signaling cascades and the immune response in the host [45,47]. Phospholipases have been classified into four classes (phospholipase A, B, C or D) on the basis of their catalytic mechanisms and site of attack in phospholipids [43]. Phospholipolytic activities with features of the four different classes have been reported in *C. albicans* [21], and analysis of the *C. albicans* genome sequence [31] has consequently revealed genes encoding candidate phospholipases of all four classes (A: *IPF7423*; B: *PLB1-5*; C: *PLC1*-3; D: *PLD1*; see CandidaDB web site at http://genolist.pasteur.fr/CandidaDB [13]). The *PLB1*, *PLB2* and *PLD1* genes have already been characterized and their contribution to *C. albicans* virulence evaluated [15,26,28,35,39,51]. *PLD1* is necessary for the yeast-to-hypha switch, while both *PLB1* and *PLD1* are necessary for *C. albicans* virulence in animals, thus indicating that phospholipase B and D activities are important virulence factors for *C. albicans* [15,28,35,39].

Although the three *C. albicans* genes encoding PI-PLCs have been previously described [1,4], their contribution to morphogenesis and virulence has not been evaluated to date. *PLC1* is an orthologue of *S. cerevisiae PLC1*, whose product is involved in vacuole fusion, kinetochore function, and glucose-induced calcium influx [32,36,53]. In higher eukaryotic cells, PI–PLCs are key enzymes in most receptor-mediated signal transduction pathways [43]. *PLC2* [1] and *PLC3* encode PI-PLCs related to bacterial PI-PLCs, some of which have been previously shown to be involved in macrophage interaction [11,12]. Here we have evaluated the role of *PLC2* and *PLC3* in morphogenesis and the interaction of *C. albicans* with macrophages using *C. albicans* mutant strains that lack or overexpress these genes. Our results show that despite the upregulation of both *PLC2* and *PLC3* upon hypha differentiation, these two genes are dispensable for morphogenesis and interaction with macrophages.

## 2. Materials and methods

### *2.1.* C. albicans *strains and media*

Strains used in this study are listed in Table 1. *C. albicans* was cultured in YPD [9], SC supplemented with 80 µg uridine $ml^{-1}$ [9], Lee's medium [34], or medium 199 (Gibco/Invitrogen) buffered with 150 mM HEPES, adjusted with HCl or NaOH to the respective pH. Transformants were selected on SC drop-out media [9]. In order to induce filamentation, *C. albicans* strains were cultured in YPD at 30 °C for 24 h, washed twice with water and resuspended at a concentration of $5 \times 10^6$ cells $ml^{-1}$ in the respective inducing medium.

### *2.2. Construction of* C. albicans *strains*

Oligonucleotides used in this study are shown in Table 2. We first cloned *PLC2* and *PLC3* by PCR from genomic DNA into the *Sma*I site of pUC18 [58] using the oligonucleotide pairs 10 and 11 to generate pPLC2 and 12 and 13 to generate pPLC3. Using the oligonucleotide pairs 14 and 15 on pPLC2 and 16 and 17 on pPLC3, respectively, we amplified the pUC18 backbone with upstream and downstream homology regions to the respective *PLC* loci. The resulting products were ligated with the recyclable *URA3* module that was released from pDDB57 [56] by *Sac*I/*Hin*dIII restriction and subsequent blunt end generation using a proofreading polymerase. The resulting plasmids, pPLC2UB and pPLC3UB, were digested by *Sma*I and *Kpn*I to release the *URA3* module with flanking homology regions to the *PLC2* and *PLC3* locus, respectively, and the products were independently transformed into *C. albicans* strain BWP17 [57]. For *PLC2* we deleted 553 bp of the ORF, which bears the encoded PI-PLC-X domain, thus maintaining the 500-bp promoter region belonging to the downstream *SHE9* gene. For *PLC3* the full coding region of 885 bp was deleted. Each second allele was deleted by PCR-based gene targeting using the *ARG4* marker on pFA-ARG4 [23] with oligonucleotides 18

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