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Phosphorylation of glucosamine-6-phosphate synthase is important but not essential for germination and mycelial growth of *Candida albicans*

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Abstract

A site-directed mutagenesis of the *GFA1* gene encoding *Candida albicans* glucosamine-6-phosphate (GlcN-6-P) synthase afforded its *GFA1S208A* version. A product of the modified gene, lacking the putative phosphorylation site for protein kinase A (PKA), exhibited all the basic properties identical to those of the wild-type enzyme but was no longer a substrate for PKA. Comparison of the *C. albicans Δgfa1/GFA1* and *Δgfa1/GFA1S208A* cells, grown under conditions stimulating yeast-to-mycelia transformation, revealed that the latter demonstrated lower GlcN-6-P synthase specific activity, decreased chitin content and formed much fewer mycelial forms. All these findings, as well as the observed effects of specific inhibitors of protein kinases, suggest that a loss of the possibility of GlcN-6-P synthase phosphorylation by PKA strongly reduces but not completely eliminates the germinative response of *C. albicans* cells.

Keywords: GlcN-6-P synthase; *Candida albicans*; Dimorphism; Protein phosphorylation; Chitin biosynthesis

1. Introduction

Human pathogenic fungi *Candida albicans* can grow as budding yeast (Y) or as septated mycelia (M), depending on the environmental conditions [1]. The latter form has been for years considered more invasive, although nowadays most agree that both forms are involved in pathogenicity [2–4]. Unfortunately, little is known about the molecular mechanism of Y → M morphological transition. It is induced by very different environmental factors, such as: *N*-acetyl-D-glucosamine (GlcNAc) as the only carbon source, elevated temperature, neutral pH, presence of serum, proteins or abundant amino acids [5,6]. A participation of several secondary messenger systems in regulation of *C. albicans* dimorphism is suggested, including those based on cAMP [7], Ca^{2+}/calmodulin or inositol phosphates [8–10] or control of intracellular pH [11]. It was demonstrated that addition of an exogenous, membrane-permeable cAMP derivative, N^6, 2′-*O*-dibutyryl-cAMP (db-cAMP), or compounds stimulating intracellular production of cAMP, including adenylate cyclase activator forskolin, could elicit a dimorphic response [12,13]. Involvement of two isoforms of protein kinase A (PKA), encoded by the *TPK1* and *TPK2* genes, respectively, in *C. albicans* morphogenesis is well documented [14,15]. On the other hand, some specific inhibitors of

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signal transduction systems decrease efficiency of Y → M transition [16]. Nevertheless, no unique system controlling *C. albicans* morphological transition has been found so far.

Metabolic changes observed in *C. albicans* cells during their morphological transformation include a substantial elevation of chitin content in the fungal cell wall [17]. This enhancement is well correlated with the increase of activity of GlcN-6-P synthase [18,19], the enzyme catalysing the first committed step in the cytoplasmic biosynthetic pathway leading to UDP-GlcNAc, a nucleotide precursor of chitin. Moreover, it is known that the morphological transition is hampered upon selective inhibition of the enzyme activity [20]. The enzyme from *C. albicans* is inhibited by UDP-GlcNAc [21] and is a substrate for PKA. The phosphorylated form of the enzyme is about fivefold more active than the non-phosphorylated one and evidence indicating a possibility that GlcN-6-P synthase molecules are phosphorylated by PKA at the onset of germ-tube formation were shown [19]. In another work, Chang et al. directly demonstrated that human GlcN-6-P synthase is phosphorylated by PKA at Ser205 [22]. We previously suggested that the *C. albicans* enzyme is likely to be phosphorylated by PKA at Ser208, i.e., a residue homologous to Ser205 of the human enzyme [23,24]. Analysis of the amino acid sequence of *C. albicans* GlcN-6-P synthase reveals that this protein contains the unique putative phosphorylation site for PKA, namely –^{205}RKGS^{208}–. The multiple alignment studies have showed that the putative PKA site is highly conserved among eukaryotic GlcN-6-P synthases but absent in the complete form from the prokaryotic sequences [24]. In the present study, a site-directed mutagenesis approach has been used to construct a modified *S208A* version of the *GFA1* gene encoding *C. albicans* GlcN-6-P synthase. We have compared the basic properties of the mutated enzyme to those of the wild-type protein, as well as the phenotypes of *C. albicans* mutant cells containing either the wild-type or the S208A version of the enzyme.

2. Materials and methods

2.1. Materials

Gemomic DNA Prep Plus, Plasmid Miniprep Plus, DNA Gel-out and DNA Clean-up kits were provided by A&A Biotechnology, Gdańsk, Poland. DNA molecular mass markers were from DNA-Gdańsk II s.c, Poland. Trifluoperazine, db-cAMP, chitinase (*Serratia marcescens*), cytohelicase (*Helix pomatia*) and kinase A from beef heart were from Sigma. Forskolin and H-89 were from ICN.

2.2. Organisms, media and growth conditions

A list of organisms and plasmids used in this investigation is presented in Table 1. The *Escherichia coli* XL-1 Blue and the *E. coli* BL21(DE3) pLysS cells were cultured at 37 °C on LA solid medium (1.5% agar, 1% NaCl, 0.5% yeast extract and 1% tryptone) and in LB liquid medium (1% NaCl, 0.5% yeast extract and 1% tryptone) supplemented with ampicillin, 100 $\mu g\,ml^{-1}$, when required. *C. albicans* ATCC 10261 cells were grown in Yeast Extract-Peptone–Dextrose (YPD), Yeast Nitrogen Base containing 1% glucose (YNB) or YP (0.5% yeast extract and 1% peptone). *C. albicans Δgfa1* homozygous null mutant was propagated in YPD or YNB containing 2% GlcNAc.

Germ tube formation by *C. albicans* cells was induced at 37C in one of the following media: (1) 10 mM imidazole–HCl buffer, pH 6.6, containing 0.2 mM $MnCl_2$ and 5 mM GlcNAc [8], (2) and (3) 10 mM imidazole–HCl buffer, pH 7.0, containing 0.2 mM $MnCl_2$ and either 10 mM L-proline + 10 mM glucose or 2.5 mM L-gluta-

Table 1
Organisms and plasmids used in this study

Strains/plasmids	Description	Source/reference
E. coli XL-1 blue	*recA1*, *endA1*, *gyrA96*, *thi-1*, *hsdR17*, *supE44*, *relA1*, *lac* [*F′*, *proAB*, *lacI9*, *ZDM15*, *Tn10*, *(TetR)*	Stratagene
E. coli BL21(DE3) pLysS	F^-, *ompT*, $hsdS_B(r_B^-, m_B^-)$, *gal*, *dcm*, *(DE3)*, *pLysS*, Cm^r	Novagen
C. albicans CAI-4	*Δura3::imm434/Δura3::imm434*	[29]
C. albicans 10,261	wild-type	ATCC
C. albicans Δgfa1	*Δgfa1* homozygous null mutant of CAI-4	This study
C. albicans Δgfa1/GFA1	*Δgfa1* transformed with *GFA1*	This study
C. albicans Δgfa1/GFA1S208A	*Δgfa1* transformed with *GFA1S208A*	This study
pUC19	*ori pMB1*, *lacZ'*, Ap^r, rep^-, rop^-	Fermentas
pET23b	*promoter/terminator T7*, Ap^r, *lacI*, *ori f1*	Novagen
pET23b-CaGFA1	pET23b containing *GFA1*	[26]
pUC19-CaGFA1	pUC19 containing *GFA1*	[26]
pET23b-CaGFA1S208A	pET23b containing *GFA1S208A*	This study
pUC19-CaGFA1S208A	pUC19 containing *GFA1S208A*	This study

mine + 2.5 mM glucose, (4) Sabouraud medium containing 10% bovine calf serum [2], (5) Lee's amino acid medium (pH 6.8) [25], (6) Yeast Carbon Base containing 1% bovine serum albumin (YCB/BSA medium). Yeast growth was performed in the same media at 30 °C.

2.3. DNA manipulations

Isolation of plasmid DNA, elution of DNA fragments from agarose gels, DNA purification and isolation of genomic DNA were carried out as described previously [26]. DNA fragments were ligated and *E. coli* cells were transformed using the standard methods [27].

2.4. Site-directed mutagenesis

The *GFA1* gene was amplified from pUC19-CaGFA1 by PCR using two pairs of oligonucleotide primers: Kas-5: 5′-CAATAATGGGGCGCCTTTTCTAGTACCACC A-3′ and cgfaF-bam: 5′-GTGGTGGGATCCCATATG TGTGGTATTTTTGGTTACGT-3′; Kas-3:5′-CTAGA AAAGGCGCCCCATTATTGGTTGGTG-3′ and cagfa-sap: 5′-GGTGGTGGATCCTGC TCTTCCGCACTC AACAGTAACTGATTTA-3′. The mismatches with the original sequence of *GFA1* are underlined and the generated recognition sites for *Kas*I are boxed. One primer of each pair brings mutation.

The plasmid pUC19-CaGFA1 was digested using *Bgl*II and *Bsu*15I and a 3758-bp fragment was isolated. The PCR products: Kas-3/cagfa.sap (1550 bp) and Kas-5/cgfa.bam (658 bp) were digested by *Bgl*II, *Kas*I and *Bsu*15I, *Kas*I, respectively, and 690 and 380 bp fragments were isolated. The recombinant plasmid pUC19-CaGFA1S208A was constructed by ligation of 3758, 690 and 380 bp fragments. The *GFA1S208A* gene, cut off from the pUC19-CaGFA1S208A plasmid with *Nde*I and *Bam*HI, was cloned into the *Nde*I, *Bam*HI sites of pET23b affording a recombinant plasmid pET23b-CaGFA1S208A. The identities of the recombinant plasmids were confirmed by restriction analysis. Insert *GFA1S208A* of pUC19-CaGFA1S208A was sequenced using the Perkin–Elmer Api Prime system.

2.5. Overexpression of the recombinant genes and purification of the gene products

Escherichia coli BL21(DE3) pLysS cells were transformed with the recombinant plasmid pET23b-CaGFA1S208A. Conditions for overexpression and purification of the modified enzyme were the same as for the wild-type protein [26].

2.6. Disruption of GFA1

Gene disruption was carried out according to the 'ura blaster' protocol [28]. After digestion of pUC19-CaGFA1 with *Eco*T22I and *Cla*I, the resulting DNA fragment was ligated with a 3.8-kb *Bam*HI–*Bgl*II fragment carrying the *hisG-URA3-hisG* cassette, to generate pGFA1U. Thus, the *Eco*T22I–*Cla*I region of *C. albicans GFA1* was replaced by the *hisG-URA3-hisG* cassette. The resulting plasmid was then digested with *Pst*I and *Sma*I and 100 μg of thus obtained linear DNA was transfected into *C. albicans* CAI-4 cells [29] by the lithium acetate method [30]. The *Δgfa1* heterozygous mutant was isolated by selection of Ura^+ transformants. The *URA3* gene was excised by 5-fluoroorotic acid [31] and the *Δgfa1* homozygous null mutant was isolated after the second round of transformation with the same cassette, in the presence of 2% GlcNAc.

2.7. Preparation of C. albicans transformants

C. albicans Δgfa1/Δgfa1 cells were transformed with a DNA fragment comprising the mutated *GFA1* gene, excised from pUC19-CaGFA1S208A with *Kpn*I and *Bam*HI. Analogously the *C. albicans Δgfa1/GFA1* cells were obtained using the respective DNA fragment excised from pUC19-CaGFA1. Transformations were performed by the lithium acetate method [30], using YEASTMARKER Yeast Transformation System 2 (BD Bioscience, USA). Transformants of both types were screened for growth on YNB solid medium, not supplemented with GlcNAc. Additionally, the genomic DNA was isolated, DNA fragments were amplified by PCR using primers flanking the *GFA1* gene and restriction analysis of the amplified fragments was performed.

2.8. Induction and monitoring of $Y \rightarrow M$ transformation

C. albicans ATCC 10261, *C. albicans Δgfa1/GFA1* and *C. albicans Δgfa1/GFA1S208A* cells were grown for 24 h in YP at 30 °C with shaking. Cells were diluted 1000× into 5 ml of the same fresh medium. The cell suspension was shaken at 30 °C until the cells reached early exponential growth phase. Yeast cells were harvested by centrifugation (3000*g*, 10 min), washed twice with sterile water, and inoculated at $1–2 \times 10^6$ cells ml^{-1} into 0.5 ml of a germ tube induction medium. Effectors, i.e., H-89, trifluoperazine, db-cAMP or forskolin were then added, if necessary, and suspensions were incubated at 30 or 37 °C. Efficiency of the morphological transformation was assessed by cell counting in a Bürker chamber.

2.9. Determination of GlcN-6-P synthase activity

Enzyme activity was determined in vitro and in situ using the previously described procedures [19]. One unit of specific activity was defined as an amount of enzyme

that catalyzed formation of 1 μmol of GlcN-6-P min^{-1} (mg protein)$^{-1}$.

2.10. Determination of chitin content

Chitin content in *C. albicans* cells was quantified using the chitinase/cytohelicase method [32]. Reaction mixtures contained 0.1 U chitinase and 850 μg of cytohelicase in a total volume of 850 μl.

2.11. Protein phosphorylation

GlcN-6-P synthase (either Gfa1p or Gfa1S208Ap), 100 μg ml^{-1}, was incubated for 30 min at 25 °C with 1 mM ATP and a catalytic subunit of cAMP-dependent protein kinase from beef heart (30 U ml^{-1}) in 0.5 ml aliquots of 50 mM Tris–HCl, pH 7.4 solution, containing 10 mM EDTA. The PKA activity was then stopped upon addition of 0.1 ml of 200 μM H-89 solution to the reaction mixtures, aliquots of 0.2 ml were withdrawn and assayed for GlcN-6-P synthase activity.

2.12. Other methods

Photomicrographs were taken using the Olympus BX 60 F5 microscope. Determination of a molecular mass and kinetic properties of the wild-type and the mutated enzyme were performed as described previously [19]. Protein was determined by the Bradford method [33].

3. Results and discussion

3.1. Construction of the GFA1S208A gene and expression plasmids

The plasmid pET23b-CaGFA1, containing the *GFA1* gene encoding *C. albicans* GlcN-6-P synthase, cloned between the *Nde*I site and the *Bam*HI site, was used for amplification with primers designed to introduce the *Kas*I site. The primers Kas-3 and Kas-5 brought mutation due to the replacement of T^{625} by G and T^{627} by C in the *GFA1* sequence. Additional replacement of T^{624} by C was necessary to introduce the *Kas*I site. The amplified mutated gene was cloned into pET23b and identity of the recombinant plasmid was confirmed by restriction analysis and sequencing.

3.2. Comparison of the properties of Gfa1p and Gfa1-S208Ap

The wild-type *GFA1* and the mutant *GFA1S208A* genes subcloned in pET23b were expressed in *E. coli*. The overexpressed gene products were purified to at least 98% homogeneity, as confirmed by SDS–PAGE analysis (not shown), and characterized. A molecular weight (MW) of the native Gfa1p, determined by size-exclusion chromatography, was 325 ± 3 kDa and that of Gfa1S208Ap was 322 ± 3 kDa. Since the MWs of denatured forms of these proteins, determined by SDS–PAGE, were 79.5 ± 0.5 and 80 ± 0.5 kDa, respectively, one may conclude that both the wild-type and the mutant enzyme are tetramers of identical subunits. Obviously, an expected slight difference in MW due to substitution of Ser^{208} for Ala, could not be detected by SDS–PAGE and gel chromatography. Both enzyme proteins exhibited practically the same kinetic parameters. K_M for L-Gln was 0.4 mM for Gfa1p and 0.54 mM for Gfa1S208Ap, while K_M values for D-Fru-6-P were 1.11 and 1.03 mM, respectively. The wild-type and the mutated GlcN-6-P synthase were inhibited by UDP-GlcNAc with inhibitory constants 3.7 and 3.5 mM. The only functional difference between both enzymatic proteins was found when they were tested as substrates for protein kinase A. Incubation of the wild-type enzyme in the presence of a catalytic subunit of PKA and ATP for 30 min led to 280% increase of GlcN-6-P synthase activity, while the activity of Gfa1S208Ap remained unchanged under the same conditions. Since we previously showed that a phosphorylated form of *C. albicans* Gfa1p is more active than its non-phosphorylated counterpart [19], the observed difference is likely to reflect a lack of the PKA phosphorylation site in Gfa1-S208Ap.

3.3. Construction of the GFA1 mutants of C. albicans

The *C. albicans Δgfa1/Δgfa1* double disruptant cells obtained by the 'ura-blaster' method, were unable to grow unless growth media contained GlcNAc. Transformation of these cells with DNA fragments excised from either pUC19-CaGFA1 or pUC19-CaGFA1-S208A plasmid afforded mutants exhibiting restored growth ability in GlcNAc-lacking media. The isolated transformants *Δgfa1/GFA1* and *Δgfa1/GFA1S208A*, contained single copies of either *GFA1* or *GFA1S208A* integrated into genomic DNA under control of the original *GFA1* promoter region, as was unequivocally confirmed by restriction analysis of DNA fragments amplified from genomic DNA. One could not therefore expect any difference between *Δgfa1/GFA1* and *Δgfa1/GFA1S208A* cells at the level of a respective gene expression and the S208A substitution was supposed to be the only difference in amino acid sequence of the gene products.

3.4. Germinative response of C. albicans transformants

The phenotypic studies of *C. albicans Δgfa1/GFA1* and *Δgfa1/GFA1S208A* transformants were performed. We compared a germinative response of the mutants to that of the wild-type *C. albicans* ATCC 10261 cells.

Appropriately prepared Y cells were grown in six different media under conditions stimulating formation of germ-tubes and supporting further mycelial growth. Data presented in Fig. 1 show that in each case an efficiency of Y → M morphological transition decreased in order: wild-type > *Δgfa1/GFA1* > *Δgfa1/GFA1S208A*. The difference between germination efficiency of *Δgfa1/GFA1* and *Δgfa1/GFA1S208A* was in most cases greater than 40% and especially high for cells incubated in a synthetic amino acid medium of Lee (5) and in media containing proteins (4) and (6). The relatively smaller difference was observed in system (1) containing GlcNAc as a sole carbon source. One may assume that exogenous GlcNAc should, to some extent, supplement any shortage of intracellular biosynthetic GlcN-6-P. Moreover, it is known that a mechanism of GlcNAc-mediated hyphal induction is different from those mediated by other factors [5], also at the level of protein kinases participating in signal transduction [34].

It must be stressed that there was a possibility of some overestimation of germ tubes and mycelia present in cultures of *Δgfa1/GFA1S208A* cells. Microscopic examination revealed that the *Δgfa1/GFA1* mutant formed exclusively true mycelia, as shown in Fig. 2(a), indistinguishable from those formed by the wild-type cells. On the other hand, the *Δgfa1/GFA1S208A* cells generated a substantial number of pseudo-hyphal forms, like those presented in Fig. 2(b). They could be possibly sometimes erroneously counted as true mycelia. Numerous bulbous forms observed in cultures of *Δgfa1/GFA1S208A* cells closely resembled those demonstrated previously for wild type *C. albicans* cells treated with inhibitors of chitin biosynthesis [35].

It should be noted that the growth rates of the wild-type and mutant cells in each of the six germ-tube inducing media were practically the same, as measured by the dry weight estimation (exact data not shown). It may be thus concluded that the *GFA1S208A* mutation affects germination efficiency but not growth of *C. albicans* cells.

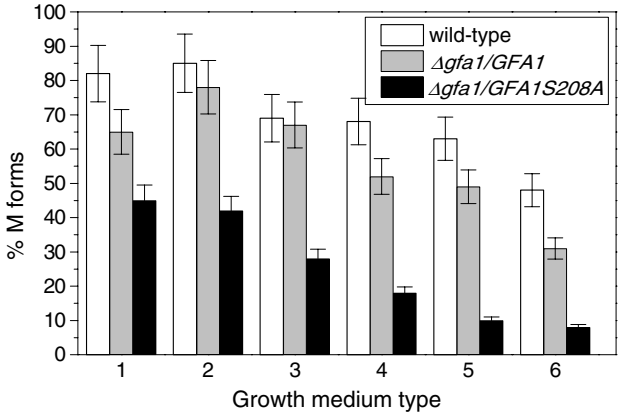

Fig. 1. Efficiency of germination of *C. albicans* wild-type and mutant cells in six different media. Cells were suspended in one of the 1–6 systems (see M&M for media composition), incubated for 3 h at 37 °C and then counted. Data are the means of three independent experiments. Bars represent SD.

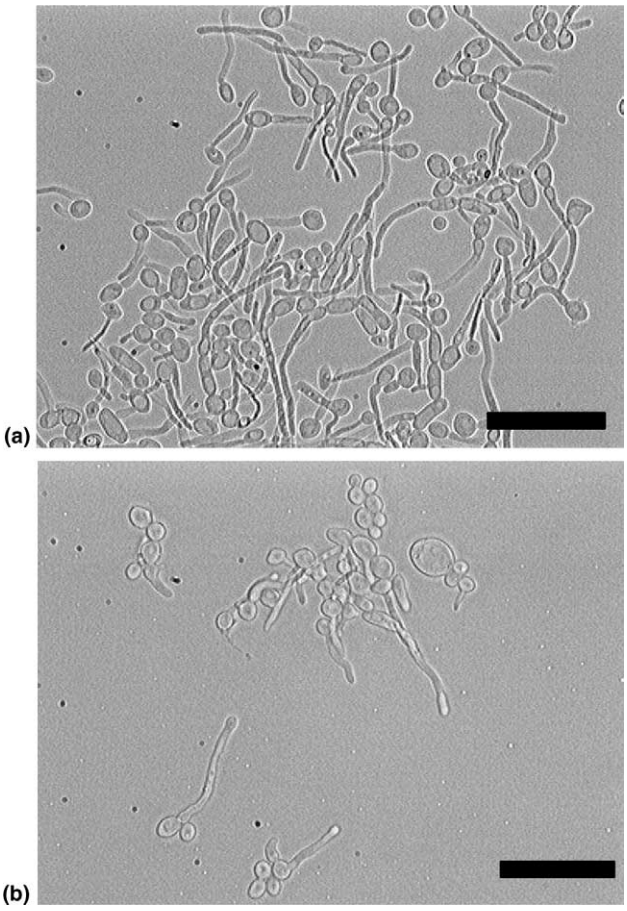

Fig. 2. Typical morphology of *C. albicans* mutants. Cells were incubated for 3 h at 37 °C in medium 2 (see M&M for composition): (a) *Δgfa1/GFA1*; (b) *Δgfa1/GFA1S208A*. Bars correspond to 30 μm.

3.5. Effect of specific inhibitors and activators of protein kinases on germination of C. albicans mutants

In further experiments cells were grown in medium 2, chosen due to its relative simplicity and reproducible, unequivocal effect on *C. albicans* morphology. The wild-type and mutant cells incubated in this medium at 30 °C demonstrated exclusively yeast morphology, while at 37 °C germinated with efficiency shown in Fig. 1.

Candida albicans wild-type and mutant cells were cultivated in the presence of compounds, known to directly affect or indirectly stimulate activity of protein kinases. Results of these experiments are shown in Figs. 3(a) and (b). Data presented in Fig. 3(a) demonstrate that H-89, a specific inhibitor of PKA [16], practically stopped germ tube formation by wild type and *Δgfa1/GFA1* cells grown under conditions stimulating

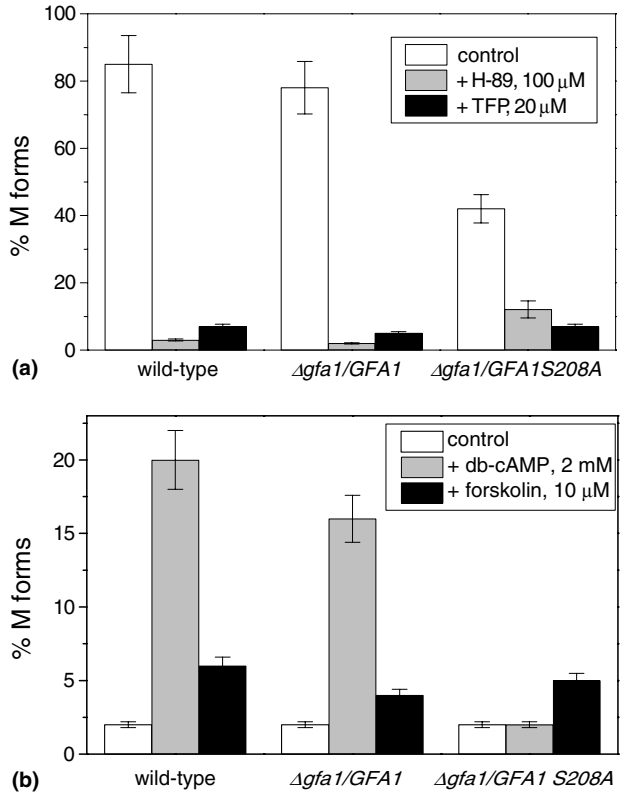

Fig. 3. Influence of specific effectors on efficiency of germination of *C. albicans* wild-type and mutants. Cells were incubated in medium 2 (M&M) for 3 h and then counted: (a) germ tube-inducing conditions (37 °C); (b) non-inducing conditions (30 °C). Data are the means of three independent experiments. Bars represent SD.

morphological transformation and further reduced already diminished germination of *Δgfa1/GFA1S208A*. Trifluoperazine, a specific inhibitor of protein kinase C and Ca^{2+}/calmodulin antagonist [13], equally strongly affected Y → M transformation of both mutants. It must be noted however that the Ca^{2+}/calmodulin complex is known to activate adenylate cyclase, so that a trifluoperazine effect may be also interpreted as a consequence of a possible indirect inhibition of cAMP formation.

Previous reports indicated that presence of a membrane-permeable cAMP analogue, db-cAMP, or an adenylate cyclase activator, forskolin, can stimulate germ tube formation by *C. albicans* cells [12,13]. We were able to confirm this phenomenon for db-cAMP acting on *C. albicans* wild-type and *Δgfa1/GFA1* cells, grown under conditions disfavoring germination, i.e., at 30 °C, although the observed stimulation was rather slight. On the contrary, virtually no stimulation was found for the *Δgfa1/GFA1S208A* mutant, as shown in Fig. 3(b). On the other hand, the forskolin effect was practically the same for both mutants. This result was surprising, since Zhou *et al.* demonstrated a strong stimulation of GlcN-6-P synthase activity in rat aortic smooth muscle cells treated with forskolin [36]. It must be noted however, that at 30 °C the forskolin-induced germination *of C. albicans* was extremely poor, since as few as 5% of cells formed germ-tubes. It seems therefore possible, that under our experimental conditions forskolin was practically not able to activate adenylate cyclase and consequently trigger activation of PKA.

3.6. GlcN-6-P synthase activity and chitin content in C. albicans *transformants*

The GlcN-6-P synthase activity was determined *in situ* in *C. albicans Δgfa1/GFA1* and *Δgfa1/GFA1S208A* cells collected at the onset and after 3 h of their cultivation. The initial level of 0.02 units practically did not changed after 180 min incubation of both cell types at 30 °C, i.e., under conditions promoting yeast growth. When the cells were incubated at 37 °C, the enzyme specific activity increased to 0.08 units in *Δgfa1/GFA1* but only to 0.03 units in *Δgfa1/GFA1S208A* cells. Although we were not able to measure Gfa1p or Gfa1-S208Ap concentration by Western blotting since the respective antibodies were not available, any difference between the mutants at the gene expression level seems unlikely. Our strategy of mutants construction ensured identical level of respectively *GFA1* or *GFA1S208A* expression in mutant cells and on the other hand, there is very little chance for any difference at the transcriptional level. This is therefore most likely that the markedly enhanced GlcN-6-P synthase activity observed in germinating *Δgfa1/GFA1* but not *Δgfa1/GFA1S208A* cells was entirely due to the difference in properties of the existing GlcN-6-P synthase molecules. According to expectations, a differential level of GlcN-6-P synthase activity in wild-type and mutant cells affected their chitin content. Data shown in Fig. 4 clearly demonstrate that *Δgfa1/GFA1* cells cultivated for 3 h at 37 °C contained about fourfold more chitin than the same cells grown at 30 °C. In the case of *Δgfa1/GFA1S208A* cells, the re-

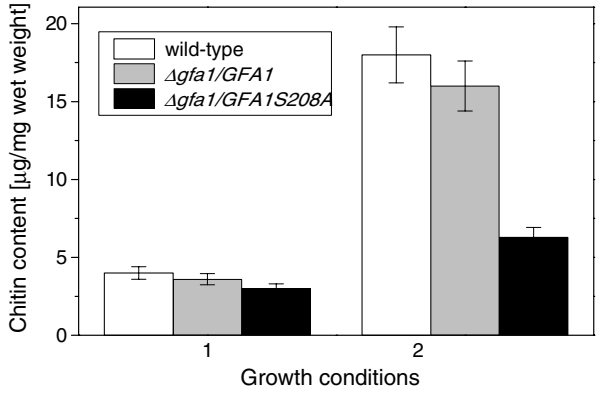

Fig. 4. Chitin content in *C. albicans* wild-type and mutants. Cells were grown in medium 2 (M&M) for 3 h: (1) non-inducing conditions (30 °C); (2) germ-tube inducing conditions (37 °C). Data are the means of three independent experiments. Bars represent SD.

spective enhancement of chitin content was lower than twofold.

4. Conclusions

Our results have provided evidence that *C. albicans* cells containing GlcN-6-P synthase lacking the putative PKA phosphorylation site, germinate with much lower efficiency than their counterparts containing the wild-type enzyme. When cultivated under conditions promoting Y → M transition, the S208A mutant cells form a substantial number of pseudo-hyphae instead of true mycelia, exhibit relatively low GlcN-6-P synthase activity and a decreased chitin content in comparison to the wild-type cells. All these findings, as well as the observed effects of specific inhibitors and activators of protein kinases, suggest that a loss of the possibility of GlcN-6-P synthase phosphorylation by PKA strongly reduces but not completely eliminates the germinative response of *C. albicans*. It is therefore clear that enhancement of GlcN-6-P synthase activity triggered upon phosphorylation of the PKA site of this enzyme is an important part of morphogenesis leading to formation of *C. albicans* mycelia.

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