Biochemical and Biophysical Research Communications 445 (2014) 138–144

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

N-acetylglucosamine kinase, *HXK1* contributes to white–opaque morphological transition in *Candida albicans*

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ARTICLE INFO

Article history:
Received 19 January 2014
Available online 31 January 2014

Keywords:
Candida albicans
N-acetylglucosamine
Hxk1
White–opaque switching
Wor1
Ras1

ABSTRACT

Morphological transition (yeast-hyphal and white–opaque) is an important biological process in the life cycle of pathogenic yeast, *Candida albicans* and is a major determinant of virulence. Earlier reports show that the amino sugar, N-acetylglucosamine (GlcNAc) induces white to opaque switching in this pathogen. We report here a new contributor to this switching phenomenon, namely N-acetylglucosamine kinase or *HXK1*, the first enzyme of the GlcNAc catabolic cascade. Microarray profile analysis of wild type vs. *hxk1* mutant cells grown under switching inducing condition showed upregulation of opaque specific and cell wall specific genes along genes involved in the oxidative metabolism. Further, our qRT-PCR and immunoblot analysis revealed that the expression levels of Wor1, a master regulator of the white–opaque switching phenomenon remained unaltered during this *HXK1* mediated transition. Thus the derepression of opaque specific gene expression observed in *hxk1* mutant could be uncoupled to the expression of *WOR1*. Moreover, this regulation via *HXK1* is independent of Ras1, a major regulator of morphogenetic transition and probably independent of *MTL* locus too. These results extend our understanding of multifarious roles of metabolic enzymes like Hxk1 and suggest an adaptive mechanism during host–pathogen interactions.

1. Introduction

Candida albicans is an opportunistic fungal pathogen and it can turn to pathogenic when the host is immunocompromised. It undergoes different morphological transitions (yeast, hyphae, white, and opaque) [1–3] in response to various environmental cues. Each morphological form provides added advantage for the pathogen in adapting to stressful conditions inside the host [4]. White to opaque transition is one of the important morphological transitions first reported by Slutsky et al. [5] and later it was also demonstrated that the switch from white to opaque was an essential step in the mating process [6,7]. Opaque cells are elongate, or bean-shaped, and are more virulent than white and are less recognized by host innate immune response system [8,9]. Each of these states is heritable for many generations, and switching between these states occurs stochastically, at low frequency. In opaque cells, WOR1 levels are high due to the existence of a positive feedback loop while in white cells its levels are undetectable [10,11].

Previously it was demonstrated that GlcNAc is a potent inducer of white to opaque switching [12] and yeast to hyphal transition in *C. albicans* [13] wherein *RAS1* was suggested to have a potent but not exclusive role in GlcNAc mediated switching. Our earlier studies has shown that Hxk1, N-acetylglucosamine kinase regulate the genes involved in the various cellular processes like filamentation, GlcNAc metabolism by modulating at chromatin level in association with Sir2 [14]. Here we report the involvement of *HXK1* in white–opaque phenotypic switching. Most importantly the *HXK1* mediated switching seemed to be independent of major regulators of morphogenetic transition like *RAS1* or *WOR1*. Although it is reported that **a**/α cells are generally switching refractory in *C. albicans*, our results probably point towards the existence of unanticipated diversity in the switching phenomenon including a *MTL* locus independent switch too.

2. Materials and methods

2.1. Yeast strains, media, and culture conditions

C. albicans strain GH1013 was used as a parent strain in this study. The other strains used in this study are listed in Supplementary Table S1. For routine growth, modified Lee's medium was used

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http://dx.doi.org/10.1016/j.bbrc.2014.01.123
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[15] with different combinations of glucose, GlcNAc or Galactose (1.25% w/v) or mannitol (2% w/v). Agar cultures were grown at a density of 40–80 colonies per 90 mm plate. Phloxine B was added to nutrient agar for opaque colony staining [16].

For all morphological studies, data has been shown as mean of three independent experiments.

2.1.1. Construction of C. albicans deletion mutants

HXK1 was deleted by a PCR product-directed disruption protocol, as described elsewhere [17]. Briefly, the HIS1 and ARG4 markers were amplified by PCR from pSN52 and pSN69, respectively. The oligonucleotide pairs HXK1DEL-F and HXK1DEL-R (Table S3) were used for PCR amplification. The *HIS1* and *ARG4* markers were sequentially transformed into the host strains GH1013, and heterozygous mutants. The null mutants were selected on SD–His–Arg plates and confirmed by PCR. For the preparation of *MTL* homozygous under *ras1* and *ras1 hxk1* double mutant back grounds, *MTL**a**1* allele was deleted by following a modified Ura-blaster method [18]. Two long primers (MTL-DEL-F, MTL-DEL-R), each containing a different 77 and 76 nucleotide sequence homologous to the gene *MTL-**a**1*, were used for PCR amplification (Supplementary Table S3). pDDB57, which contains the recyclable URA3-dpl200 marker, was used as template. The PCR product was transformed into CAN52, and HR1-4-6-2 [14]. Transformants were grown on selective synthetic defined (SD) medium SD-Ura agar plates. The transformants were confirmed by PCR by using primers (MTL**a**F, MTL**a**R, MTLαF and MTLαR, Table S3) for absence of *MTL-**a**1*.

2.1.2. Preparation iWOR1-GFP and hWOR1-GFP strains

Plasmid pMG2120 [19] was used as template with primers WOR-GFP-F and WOR-GFP-R and the PCR product was transformed separately in both wild type (GH1013) and *hxk1* mutant giving rise to *iWOR1-GFP* and *hWOR1-GFP* strains, respectively. Transformants were selected on YPD containing 400 μg/ml nourseothricin, and correct transformants were selected by diagnostic PCR using primers NATF and NATR to amplify the *NAT* cassette. In the background of *iWOR1-GFP* and *hWOR1-GFP*, Op4 was tagged with 13X-myc.

2.2. Microarray experiment

Wild type (GH1013) and *hxk1* mutant strains were pre-grown in Lee-Glucose liquid medium till saturation for 7 days at 25 °C, serially diluted and spread at a density of 60–80 cfus per plate on Lee Galactose + GlcNAc medium. Well isolated colonies after 3 days of growth were pooled and washed in ice cold water and RNA was isolated using Tripure Isolation Reagent (Roche). The RNA concentration was determined using Nanodrop spectrophotometer. Microarray experimentation was done as described elsewhere [14].

2.3. qRT-PCR analysis

For all qRT-PCR experiments, total RNA was treated with RNase-free DNase I (Invitrogen) to remove any residual DNA. About 500 ng of DNase I-treated RNA was used for single-stranded cDNA synthesis in 10 μl of reaction mixture using a High-Capacity cDNA Reverse Transcription kit (Applied Biosystems) and used for qRT-PCR with SYBR green PCR master mix on an ABI Prism 7000 real-time PCR apparatus (Applied Biosystems). The comparative *CT* method ($2^{-\Delta\Delta C_T}$) was used to determine the relative gene expression [20]. Control reactions without reverse transcriptase were carried out for each cDNA preparation and ascertained that no amplification was obtained as judged by high *CT* [21] values and gel analysis.

2.4. Immunoblotting

Immunoblotting was performed, as described previously [14]. For Western blot analysis, proteins from gels were electrotransferred to Hybond C Extra membrane (Amersham Biosciences). The blot was blocked using 3% skimmed milk in a buffer containing 10 mM Tris, pH 7.4, 15 mM NaCl, 0.05% Tween-20. For detection of GFP, GAPDH or myc signals, membranes were incubated with anti-GFP monoclonal antibody (Roche Diagnostics) or anti-GAPDH (MyBioSource) or anti c-myc (Roche Diagnostics), at a dilution of 1:1000 and a peroxidase conjugated secondary antibody (Amersham Biosciences, UK) at a dilution of 1:20,000. An enhanced chemiluminescence (ECL prime) detection system (Amersham Biosciences, UK) was used for antibody detection according to the manufacturer's instructions.

2.5. Microscopy

Microscopic images of yeast cells and colonies were captured using a Nikon 80i inverted microscope equipped with a Nikon Digital DXM1200C camera or Stereo microscope using ×40, ×100 or ×7 objective.

3. Results and discussion

3.1. Hxk1 regulates genes involved in white–opaque switching

During our colony morphology studies with different *hxk1* mutants on YPD plates we came across some interesting phenotypes. Sectored filamentation was observed in case of *ras1/hxk1* double mutant (*MTL* heterozygous) colonies compared with *ras1* single mutant (Fig. 1A). We also made a striking observation with *efg1/hxk1* double mutant where there was a tendency to switch to opaque/grey colonies (data not shown), the frequency of which was more when compared to that of *efg1* mutant alone as reported elsewhere [22]. These observations prompted us to explore the involvement of *HXK1* in phenotypic switching, more precisely white–opaque switching.

Therefore, *hxk1* was mutated in a background of GH1013 which is homozygous for the **a**/**a** locus and thus has the ability to switch. Under non-inducing conditions (Lee-Glucose) all the colonies of *hxk1* mutant showed an opaque central area (Fig. 1B) after 5 days. Interestingly on Lee-mannitol plates approximately 40% of the colonies showed opaque sectors after 5 days, 20% of which completely turned opaque after 9 days of incubation and remaining all the colonies had prominent central opaque areas or sectors (Fig. 1C). Cells taken from such colonies were elongated, bean-shaped, and characteristic of opaque cells in *C. albicans* [5]. Studies by Huang et al. [12] identified GlcNAc as a potent inducer of white–opaque switching. Since the enzyme we were concerned with is a GlcNAc catabolic enzyme, its mutant was unable to grow on GlcNAc alone and hence a substitute carbon source like Galactose was added to GlcNAc to study the switching frequency of *hxk1* mutant. When this mutant was grown on Galactose + GlcNAc at 25 °C (Fig. 1C, lower panel) switching frequency was almost 80% as compared to the wild type where 30% colonies were opaque. (This reduced frequency of opaque colonies in wild type was due to the addition of Galactose which may not be a preferred sugar for switching.) Apart from this, the general increased frequency of switching in a *hxk1* mutant irrespective of the sugar used (Lee-glucose/Lee-mannitol/Lee-Gal + GlcNAc) reconfirmed the fact that a cross-talk exists between metabolic flexibility and white-opaque switching as described by Lan et al. [23].

This finding was further supported by analysis of our microarray result with *hxk1* mutant compared to wild type in

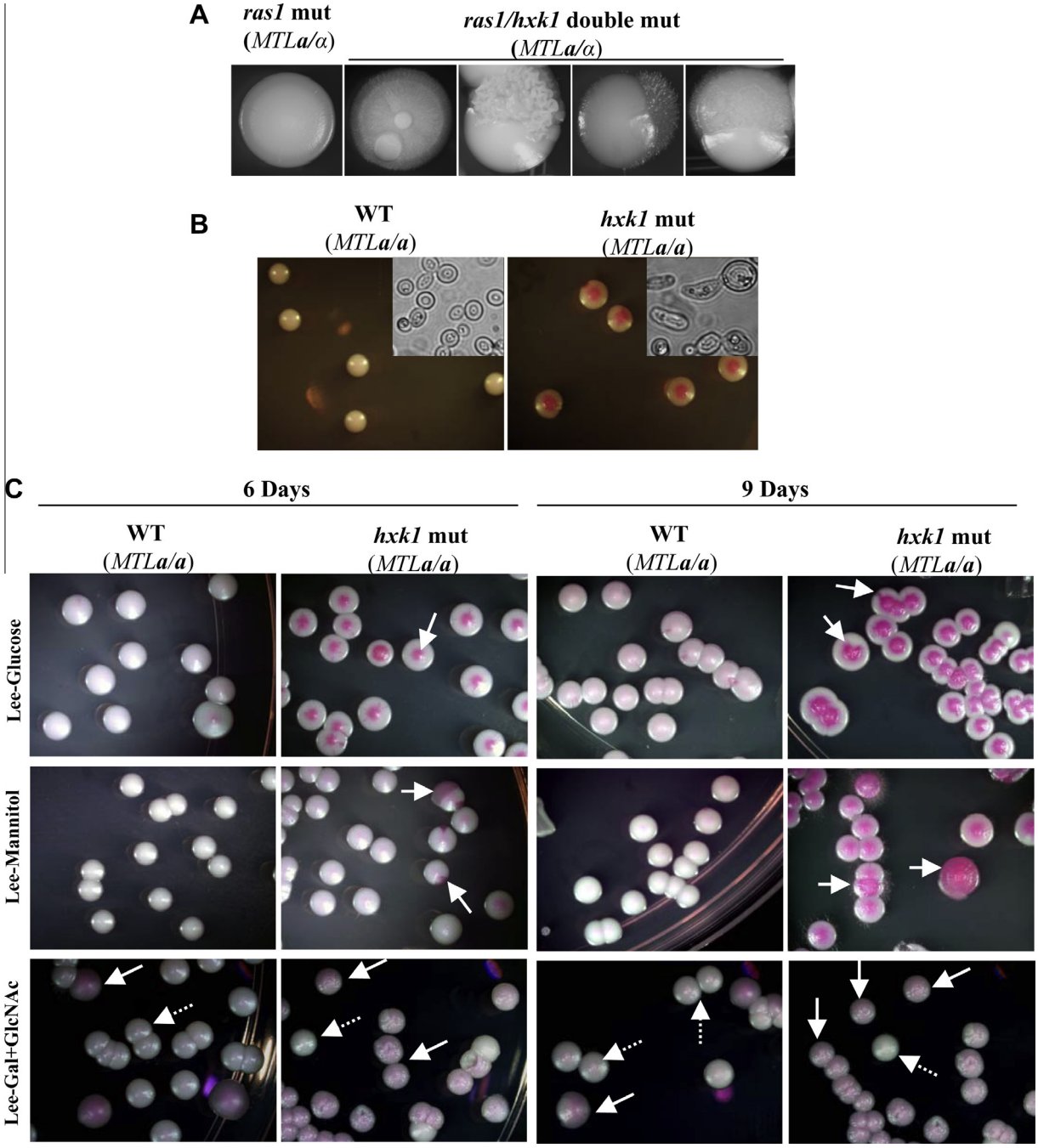


Fig. 1. Hxk1 is involved in morphogenetic transition. (A) *ras1*/*hxk1* (*MTL**a***/α) double mutant colonies showed sectored filamentation compared to *ras1* (*MTL**a***/α) mutant. *ras1* and *ras1*/*hxk1* mutants were grown on YPD plates for 7 days. (B) *hxk1* (*MTL**a***/***a***) mutant showed opaque switching in non inducing conditions (Lee-Glucose). In *hxk1* mutant the colonies showed an opaque central area under non-inducing conditions. Cells taken from Opaque colony were elongated and bean-shaped (inset). (C) White–opaque switching frequency in various media. For Lee-Glucose and Lee-Galactose + GlcNAc media cells were pregrown in Lee-Glucose liquid medium till saturation for 7 days at 25 °C, serially diluted and spread at a density of 60–80 cfus per plate [12]. In case of Mannitol the cells were pregrown in Lee-Glucose plates for 5 days, resuspended in liquid Lee media, serially diluted and spread as above [10]. Opaque sectored or complete opaque colonies are represented with white arrows, whereas white colonies are represented with dashed arrows.

Galactose + GlcNAc grown cells that showed upregulation of several opaque phase-specific genes (Fig. 2A–C). These genes had been shown to be involved in metabolic specialization during white–opaque switching by Lan et al. [23]. The common set of upregulated genes in our array analysis and their study could be broadly classified into two distinct groups viz. (1) metabolic genes – It included genes like *PXP2*, *FOX3*, *ADH3*, *ADH2*, *ACS1*, *ICL1*, *POX1-3*, *POT1*, *ALD5*, *ALD6*, *FOX2*, *FDH1* that together contribute towards the shifting of metabolic flux to morphogenetic transition by aiding the non-fermentative mode of respiration and lipid metabolism (The upregulation of genes involved in lipid metabolism is not surprising since earlier reports by Lan et al. [23] propose that in opaque cells the fatty-acid β-oxidation is more prominent since these cells are mostly present in a habitat where free sugars are absent and lipids predominate). (2) Cell wall specific genes like *PGA17*, *HGT12*, *PGA45*, *HGT13*, *HGT16*, *PGA57*, *PGA32* that reprogram the

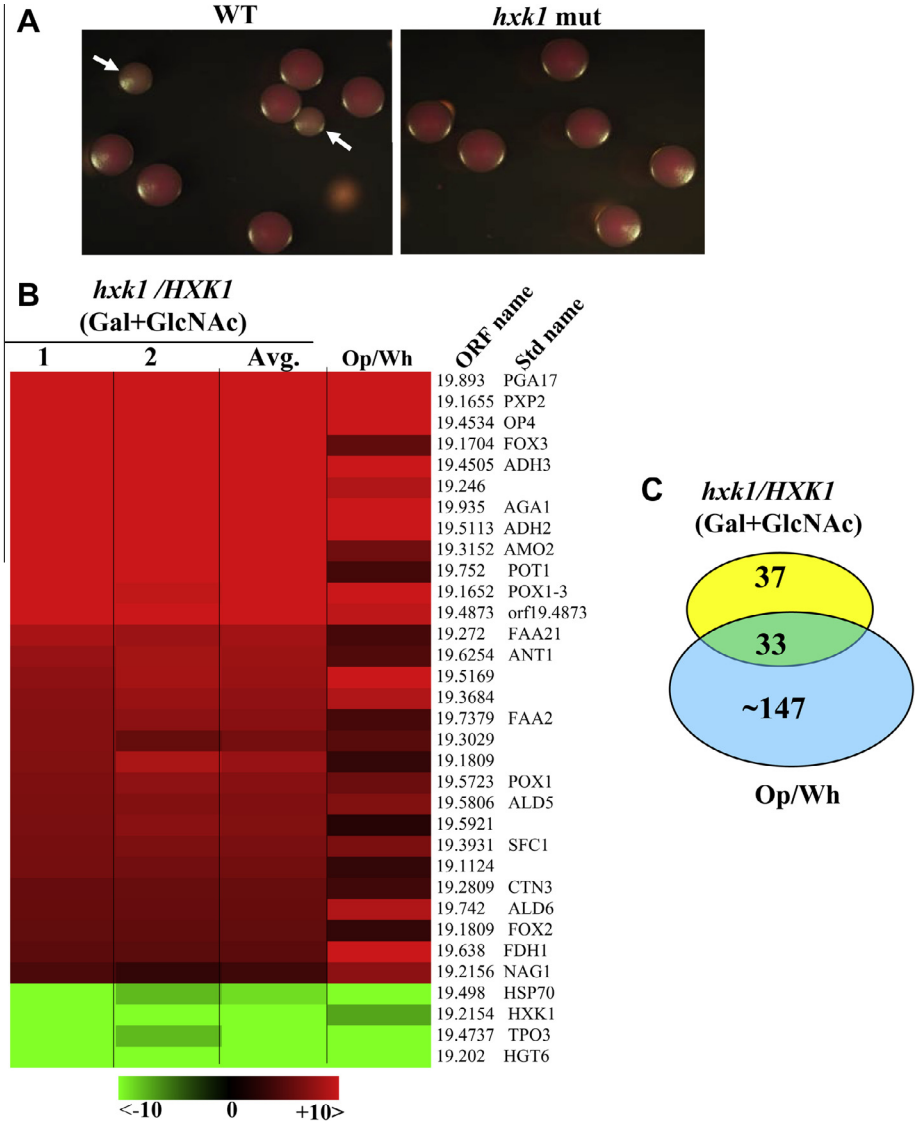


Fig. 2. Transcriptomic perspective of Hxk1 mediated white–opaque switching. (A) *hxk1* showed high frequency switching. After 5 days, 70% of the colonies turned to opaque in wild type (GH1013) when grown on Lee (Galactose + GlcNAc) agar plates, whereas all the colonies were opaque under the same conditions in an *hxk1* mutant. White colonies are represented with arrows. (B) Heat map of opaque specific genes differentially expressed in a *HXK1* dependent manner in response to GlcNAc. Two-color microarray data expressed as *hxk1*/*HXK1* ratio (1 and 2 are biological replicates with dye swap and average of 1 and 2, *Ave*) is plotted as heat map. The Op/Wh transcript profile data by [23] is also shown for comparison. The color scale at the bottom indicates the log2 ratio. (C) Numerical representation of independent and overlapping sub-sets of upregulated genes (>2-fold) between *hxk1*/*HXK1* by us and opaque specific genes as described by [23].

morphological characteristics. The unique set of genes that got differentially regulated in our expression profile analysis also mostly included genes that fell into the above two categories.

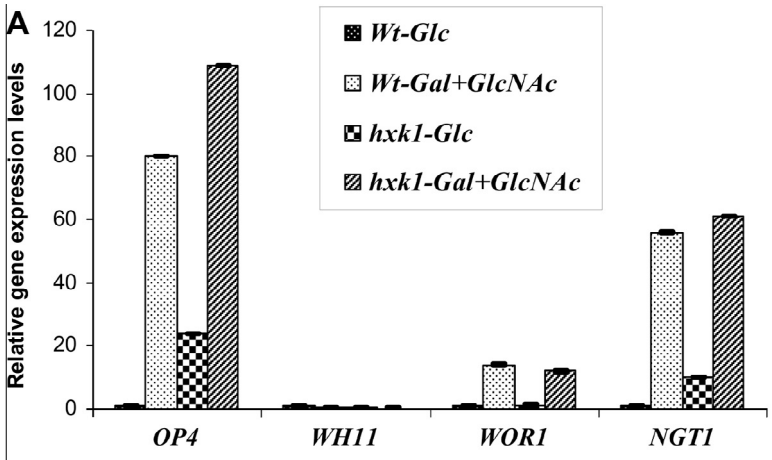
3.2. *WOR1* expression levels are unaltered in hxk1 mutant strains

Many challenges remain in understanding white-opaque switching in *C. albicans*. One known transcription factor in this switching phenomenon is *WOR1* that maintains a self-sustaining positive feed-back loop in opaque cells for several generations [11]. A qRT-PCR analysis with wild type and *hxk1* mutant under non-inducing and inducing conditions showed that *WOR1* transcript levels were almost unaltered in either condition (Fig. 3A). Expression levels of *OP4* got upregulated in opaque colony cells as expected, and these levels were considerable even in hxk1 mutant colonies grown on Lee-Glucose plate. *WH11* showed down regulation in all opaque colony cells.

To further strengthen the uncoupling of *HXK1* mediated switching from the master regulator Wor1, Western blotting analysis was performed to determine the levels of Wor1 in white or opaque cells collected from wild type and *hxk1* mutant. Interestingly, the Wor1-GFP protein levels could not be detected even in opaque colonies of *hxk1* mutant under non-inducing conditions (Lee-Glucose). Moreover, the Wor1-GFP levels were equal in opaque colonies collected from both wild type and *hxk1* mutants grown under inducing conditions (Lee-Gal + GlcNAc) (Fig. 3B). Huang et al., showed that all the identified and yet unidentified pathways of white–opaque transition converge at the point of Wor1. Our results though preliminary, open up the possibility of existence of switching pathways that can bypass Wor1.

3.3. Hxk1 mediated white to opaque switching is independent of RAS1 pathway and may be independent of Mating Type Locus too

RAS1 senses the environmental cues and mediates the signalling through cAMP and MAP kinase pathways to mediate the transcriptional responses [24,25]. To test whether *HXK1* mediated switching response was under the control of this major



	OP4	*WH11*	*WOR1*	*NGT1*
Wt-Glc	1	1	1	1
Wt-Gal+GlcNAc	80.2	0.3	14.6	56.3
hxk1-Glc	24.4	0.65	1.03	9.7
hxk1-Gal+GlcNAc	109.8	0.25	12.9	61.2

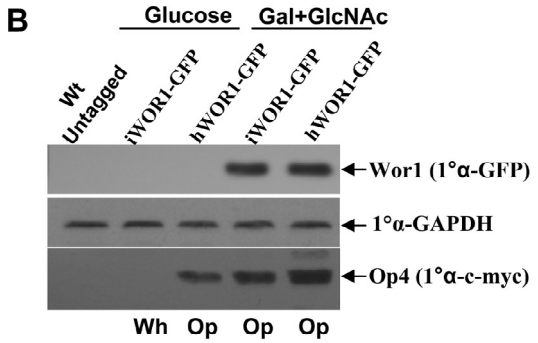


Fig. 3. Wor1 expression levels are unaltered during Hxk1 mediated switching. (A) Quantitative RT-PCR of switching specific gene transcripts from 3 days grown colonies. *OP4* and *WH11* are selected as switching specific genes. *WOR1*, the master regulator of switching, showed almost constant level of expression in similar media in either wild type or *hxk1* mutant. *NGT1* is a known GlcNAc specific induced transcript. *ACT1* has been selected as the endogenous control. The error bars represent co-efficient of variation. The average relative expression levels of transcripts from three independent experiments have been shown below the graphical representation. (B) Wor1-GFP levels remained constant at the protein level too whereas the levels of Op4 vary during white–opaque transition. The un-tagged control strain GH1013 or GFP-tagged strains iWOR1-GFP and hWOR1-GFP were grown on Lee-Glucose or Lee-Gal + GlcNAc medium for 5 days. Western blotting was performed with anti-GFP antibody. Equal loading was confirmed by reprobing the blot using anti-GAPDH antibody. The levels of Op4 protein detected with anti c-myc antibody vary in white and opaque colonies. Wh and Op at the bottom of the figure denote White and Opaque colonies, respectively.

morphogenetic regulator, we created a *MTL* homozygous *ras1* and *ras1/hxk1* double mutant (Table S1). Several attempts to select a homozygote for the *MTL* locus on sorbose plate failed; hence we deleted the *MTL**a*** allele under *ras1*and *hxk1/ras1* mutant background. Earlier work by Huang et al. [12] showed that *ras1* mutant was locked in white stage even in the presence of switching inducers like GlcNAc. Interestingly, all the colonies of *ras1/hxk1* double mutant were opaque after 10 days incubation on Lee-Gal + GlcNAc plates (Fig. 4A). Furthermore, a *MTL* heterozygous *ras1/hxk1* double mutant also gave rise to highly sectored and opaque colonies (Fig. 4B). Such an observation made us to presume that the phenomenon of white–opaque switching mediated by *HXK1* could be independent of the *MTL* locus too. This line of thought was quite in keeping with the recent report by Porman et al. [26] where they show a *MTL* independent phenotypic switching in *Candida tropicalis*.

The involvement of *HXK1* in white–opaque switching in addition to yeast–hyphal transition could be an added advantage to the pathogen via metabolic specialization. Huang et al. demonstrated Ras1 as a predominant factor involved in white–opaque switching. Our finding of switching in a *ras1/hxk1* double mutant probably opens up the possibility of existence of some minor pathways independent of *Ras1* and dependent on *Hxk1*. This presumption is further strengthened by the upregulation of several opaque specific genes in an *hxk1* mutant compared to wild type as evidenced from our microarray data. Recent studies indicate that morphological transitions are controlled at chromatin level through regulation of histone acetylases and methyltransferases [27,28]. At this point our earlier finding of Hxk1 interaction with Sir2 could be quite significant [14]. In *C. albicans SIR2* has already been reported to take part in heritable changes in the developmental pathways involved in the bud–pseudohyphae–hyphae transitions [29] and white–opaque switching. Thus, Hxk1 by interacting with Sir2, could modulate its activity at chromatin level to repress the phenomenon of switching. Moreover, this extended role of *HXK1* could be independent of *MTL* locus too as described in

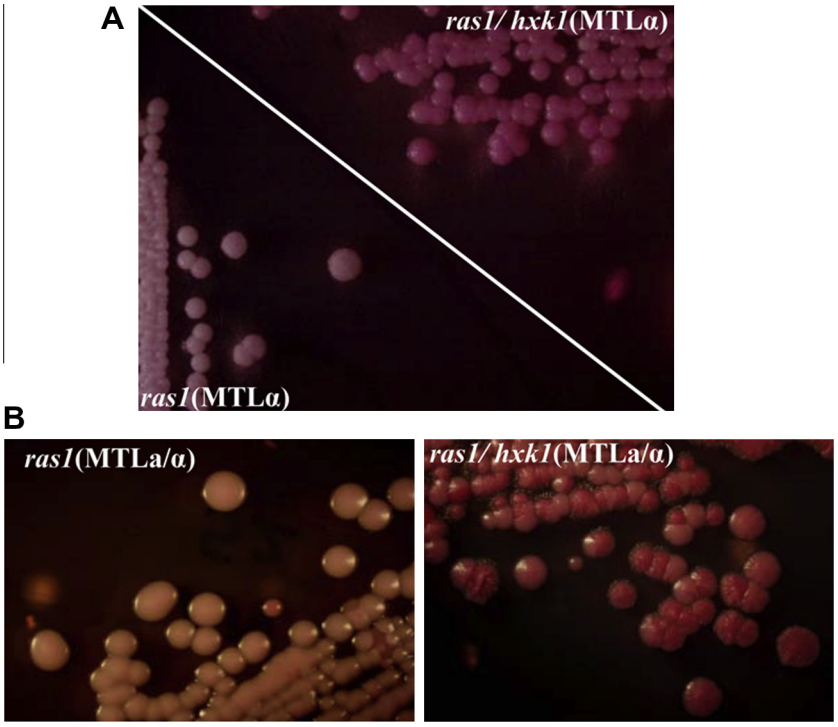


Fig. 4. *ras1hxk1* double mutant showed opaque phenotype. (A) *ras1* (*MTLα*) mutant and *ras1hxk1* (*MTLα*) double mutants were allowed to grow on Lee-Gal + GlcNAc for 10 days and photographed. All colonies turned to opaque in *ras1hxk1* double mutant. (B) *MTL***a**/α strains of *ras1* and *ras1hxk1* double mutants were grown as described above and photographed. Even when the *MTL* locus is heterozygous, all the colonies in *ras1hxk1* double mutant were opaque.

this study, which could be again a pathogen-specific adaptation keeping in mind the importance of white–opaque switching in regulating the program of sexual mating and interactions with the mammalian host.

Acknowledgments

The authors thank Drs. Judith Berman, Maryam Gerami-Nejad, Aaron P. Michell, Guanghua Huang and David Soll for generously providing the plasmids and *C. albicans* strains used in this study and providing experimental suggestions. K.H.R. and S.G. are recipients of CSIR-SRA fellowship. D.R. is a recipient of ICMR-SRF.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.01.123.

References

[1] J. Berman, P.E. Sudbery, *Candida albicans*: a molecular revolution built on lessons from budding yeast, Nat. Rev. Genet. 3 (2002) 918–930.
[2] P. Sudbery, N. Gow, J. Berman, The distinct morphogenic states of *Candida albicans*, Trends Microbiol. 12 (2004) 317.
[3] S. Biswas, P.V. Dijck, A. Datta, Environmental sensing and signal transduction pathways regulating morphopathogenic determinants of *Candida albicans*, Microbiol. Mol. Biol. Rev. 71 (2007) 348–376.
[4] K. Pande, C. Chen, S.M. Noble, Passage through the mammalian gut triggers a phenotypic switch that promotes *Candida albicans* commensalism, Nat. Genet. 45 (2013) 1088–1091.
[5] B. Slutsky, M. Staebell, J. Anderson, L. Risen, M. Pfaller, D.R. Soll, "White–opaque transition": a second high-frequency switching system in *Candida albicans*, J. Bacteriol. 169 (1987) 189–197.
[6] M.G. Miller, A.D. Johnson, White–opaque switching in *Candida albicans* is controlled by mating-type locus homeodomain proteins and allows efficient mating, Cell 110 (2002) 293–302.
[7] S.R. Lockhart, C. Pujol, K.J. Daniels, M.G. Miller, A.D. Johnson, M.A. Pfaller, D.R. Soll, In *Candida albicans*, white–opaque switchers are homozygous for mating type, Genetics 162 (2002) 737–745.
[8] M.B. Lohse, A.D. Johnson, Differential phagocytosis of white versus opaque *Candida albicans* by *Drosophila* and mouse phagocytes, PLoS One 3 (2008) e1473.
[9] J. Geiger, D. Wessels, S.R. Lockhart, D.R. Soll, Release of a potent polymorphonuclear leukocyte chemoattractant is regulated by white–opaque switching in *Candida albicans*, Infect. Immun. 72 (2004) 667–677.
[10] G. Huang, H. Wang, S. Chou, X. Nie, J. Chen, H. Liu, Bistable expression of WOR1, a master regulator of white–opaque switching in *Candida albicans*, Proc. Natl. Acad. Sci. USA 103 (2006) 12813–12818.
[11] R.E. Zordan, D.J. Galgoczy, A.D. Johnson, Epigenetic properties of white–opaque switching in *Candida albicans* are based on a self-sustaining transcriptional feedback loop, Proc. Natl. Acad. Sci. USA 103 (2006) 12807–12812.
[12] G. Huang, S. Yi, N. Sahni, K.J. Daniels, T. Srikantha, D.R. Soll, N-acetylglucosamine induces white to opaque switching, a mating prerequisite in *Candida albicans*, PLoS Pathog. 12 (2010) e1000806, http://dx.doi.org/10.1371/journal.ppat.1000806 (Erratum. In: PLoS Pathog. 2010;6(3). doi: 10.1371/annotation/ed4d1473-cf80-4e85-ad9d-7a390be260f6).
[13] N. Simonetti, V. Strippoli, A. Cassone, Yeast-mycelial conversion induced by N-acetyl-D-glucosamine in *Candida albicans*, Nature 250 (1974) 344–346.
[14] K.H. Rao, S. Ghosh, K. Natarajan, A. Datta, N-acetylglucosamine kinase, HXK1 is involved in morphogenetic transition and metabolic gene expression in *Candida albicans*, PLoS One 8 (1) (2013) e53638, http://dx.doi.org/10.1371/journal.pone.0053638.
[15] G.W. Bedell, D.R. Soll, Effects of low concentrations of zinc on the growth and dimorphism of *Candida albicans*: evidence for zinc-resistant and sensitive pathways for mycelium formation, Infect. Immun. 26 (1979) 348–354.
[16] J.M. Anderson, D.R. Soll, Unique phenotype of opaque cells in the white–opaque transition of *Candida albicans*, J. Bacteriol. 169 (1987) 5579–5588.
[17] R.B. Wilson, D. Davis, A.P. Mitchell, Rapid hypothesis testing with *Candida albicans* through gene disruption with short homology regions, J. Bacteriol. 181 (1999) 1868–1874.
[18] R.B. Wilson, D. Davis, B.M. Enloe, A.P. Mitchell, A recyclable *Candida albicans* URA3 cassette for PCR product-directed gene disruptions, Yeast 16 (2000) 65–70.
[19] M. Gerami-Nejad, A. Forche, M. McClellan, J. Berman, Analysis of protein function in clinical *C. albicans* isolates, Yeast 29 (2012) 303–309.
[20] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ method, Methods 25 (2001) 402–408.
[21] T.D. Schmittgen, K.J. Livak, Analyzing real-time PCR data by the comparative C_T method, Nat. Protoc. 3 (2008) 1101–1108.
[22] S.A. Lachke, T. Srikantha, D.R. Soll, The regulation of *EFG1* in white–opaque switching in *Candida albicans* involves overlapping promoters, Mol. Microbiol. 48 (2003) 523–536.

[23] C.Y. Lan, G. Newport, L.A. Murillo, T. Jones, S. Scherer, et al., Metabolic specialization associated with phenotypic switching in *Candida albicans*, Proc. Natl. Acad. Sci. USA 99 (2002) 14907–14912.
[24] E. Leberer, D. Harcus, D. Dignard, L. Johnson, S. Ushinsky, D.Y. Thomas, K. Schröppel, Ras links cellular morphogenesis to virulence by regulation of the MAP kinase and cAMP signalling pathways in the pathogenic fungus *Candida albicans*, Mol. Microbiol. 42 (2001) 673–687.
[25] H. Liu, Transcriptional control of dimorphism in *Candida albicans*, Curr. Opin. Microbiol. 4 (2001) (278-235).
[26] A.M. Porman, M.P. Hirakawa, S.K. Jones, N. Wang, R.J. Bennett, MTL-independent phenotypic switching in *Candida tropicalis* and a dual role for Wor1 in regulating switching and filamentation, PLoS Genet. 9 (2013) e1003369, http://dx.doi.org/10.1371/journal.pgen.1003369 [Epub 2013 Mar 21].
[27] P.K. Mishra, M. Baum, J. Carbon, DNA methylation regulates phenotype-dependent transcriptional activity in *Candida albicans*, Proc. Natl. Acad. Sci. USA 108 (2011) 11965–11970.
[28] J.S. Stevenson, H. Liu, Regulation of white and opaque cell-type formation in *Candida albicans* by Rtt109 and Hst3, Mol. Microbiol. 8 (2011) 1078–1091.
[29] J. Pérez-Martín, J.A. Uría, A.D. Johnson, Phenotypic switching in *Candida albicans* is controlled by a *SIR2* gene, EMBO J. 8 (1999) 2580–2592.